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Editors

The Prokaryotes

Alphaproteobacteria and Betaproteobacteria

Fourth Edition

Springer Reference

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Eugene Rosenberg (Editor-in-Chief)
Edward F. DeLong, Stephen Lory, Erko Stackebrandt and Fabiano Thompson (Eds.)

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With 90 Figures and 263 Tables

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Foreword

The purpose of this brief foreword is unchanged from the first edition; it is simply to make you, the reader, hungry for the scientific feast that follows. These 11 volumes on the prokaryotes offer an expanded scientific menu that displays the biochemical depth and remarkable physiological and morphological diversity of prokaryote life. The size of the volumes might initially discourage the unprepared mind from being attracted to the study of prokaryote life, for this landmark assemblage thoroughly documents the wealth of present knowledge. But in confronting the reader with the state of the art, the Handbook also defines where more work needs to be done on well-studied bacteria as well as on unusual or poorly studied organisms.

This edition of *The Prokaryotes* recognizes the almost unbelievable impact that the work of Carl Woese has had in defining a phylogenetic basis for the microbial world. The concept that the ribosome is a highly conserved structure in all cells and that its nucleic acid components may serve as a convenient reference point for relating all living things is now generally accepted. At last, the phylogeny of prokaryotes has a scientific basis, and this is the first serious attempt to present a comprehensive treatise on prokaryotes along recently defined phylogenetic lines. Although evidence is incomplete for many microbial groups, these volumes make a statement that clearly illuminates the path to follow.

There are basically two ways of doing research with microbes. A classical approach is first to define the phenomenon to be studied and then to select the organism accordingly. Another way is to choose a specific organism and go where it leads. The pursuit of an unusual microbe brings out the latent hunter in all of us. The intellectual challenges of the chase frequently test our ingenuity to the limit. Sometimes the quarry repeatedly escapes, but the final capture is indeed a wonderful experience. For many of us, these simple rewards are sufficiently gratifying so that we have chosen to spend our scientific lives studying these unusual creatures. In these endeavors, many of the strategies and tools as well as much of the philosophy may be traced to the Delft School, passed on to us by our teachers, Martinus Beijerinck, A. J. Kluyver, and C. B. van Niel, and in turn passed on by us to our students.

In this school, the principles of the selective, enrichment culture technique have been developed and diversified; they have been a major force in designing and applying new principles for the capture and isolation of microbes from nature. For me, the "organism approach" has provided rewarding adventures. The organism continually challenges and literally drags the investigator into new areas where unfamiliar tools may be needed. I believe that organism-oriented research is an important alternative to problem-oriented research, for new concepts of the future very likely lie in a study of the breadth of microbial life. The physiology, biochemistry, and ecology of the microbe remain the most powerful attractions. Studies based on classical methods as well as modern genetic techniques will result in new insights and concepts.

To some readers, this edition of *The Prokaryotes* may indicate that the field is now mature, that from here on it is a matter of filling in details. I suspect that this is not the case. Perhaps we have assumed prematurely that we fully understand microbial life. Van Niel pointed out to his students that—after a lifetime of study—it was a very humbling experience to view in the microscope a sample of microbes from nature and recognize only a few. Recent evidence suggests that microbes have been evolving for nearly 4 billion years. Most certainly, those microbes now domesticated and kept in captivity in culture collections represent only a minor portion of the species that have evolved in this time span. Sometimes we must remind ourselves that evolution is actively taking place at the present moment. That the eukaryote cell evolved as a chimera of certain prokaryote parts is a generally accepted concept today. Higher as well as lower eukaryotes evolved in contact with prokaryotes, and evidence surrounds us of the complex interactions between eukaryotes and prokaryotes as well as among prokaryotes. We have so far only scratched the surface of these biochemical interrelationships. Perhaps the legume nodule is a pertinent example of nature caught in the act of evolving the "nitrosome," a unique nitrogen-fixing organelle. The study of prokaryotes is proceeding at such a fast pace that major advances are occurring yearly. The increase of this edition to four volumes documents the exciting pace of discoveries.

To prepare a treatise such as *The Prokaryotes* requires dedicated editors and authors; the task has been enormous. I predict that the scientific community of microbiologists will again show its appreciation through use of these volumes—such that the pages will become "dog-eared" and worn as students seek basic information for the hunt. These volumes belong in the laboratory, not in the library. I believe that a most effective way to introduce students to microbiology is for them to isolate microbes from nature, that is, from their habitats in soil, water, clinical specimens, or plants. *The Prokaryotes* enormously simplifies this process and should encourage the construction of courses that contain a wide spectrum of diverse topics. For the student as well as the advanced investigator, these volumes should generate excitement.

Happy hunting!

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Preface

During most of the twentieth century, microbiologists studied pure cultures under defined laboratory conditions in order to uncover the causative agents of disease and subsequently as ideal model systems to discover the fundamental principles of genetics and biochemistry. Microbiology as a discipline onto itself, e.g., microbial ecology, diversity, and evolution-based taxonomy, has only recently been the subject of general interest, partly because of the realization that microorganisms play a key role in the environment. The development and application of powerful culture-independent molecular techniques and bioinformatics tools has made this development possible. The fourth edition of *the Handbook of the Prokaryotes* has been updated and expanded in order to reflect this new era of microbiology.

The first five volumes of the fourth edition contain 34 updated and 43 entirely new chapters. Most of the new chapters are in the two new sections: Prokaryotic Communities and Bacteria in Human Health and Disease. A collection of microorganisms occupying the same physical habitat is called a "community," and several examples of bacterial communities are presented in the Prokaryotic Communities section, organized by Edward F. DeLong. Over the last decade, important advances in molecular biology and bioinformatics have led to the development of innovative culture-independent approaches for describing microbial communities. These new strategies, based on the analysis of DNA directly extracted from environmental samples, circumvent the steps of isolation and culturing of microorganisms, which are known for their selectivity leading to a nonrepresentative view of prokaryotic diversity. Describing bacterial communities is the first step in understanding the complex, interacting microbial systems in the natural world.

The section on Bacteria in Human Health and Disease, organized by Stephen Lory, contains chapters on most of the important bacterial diseases, each written by an expert in the field. In addition, there are separate general chapters on identification of pathogens by classical and non-culturing molecular techniques and virulence mechanisms, such as adhesion and bacterial toxins. In recognition of the recent important research on beneficial bacteria in human health, the section also includes chapters on gut microbiota, prebiotics, and probiotics. Together with the updated and expanded chapter on Bacterial Pharmaceutical Products, this section is a valuable resource to graduate students, teachers, and researchers interested in medical microbiology.

Volumes 6–11, organized by Erko Stackebrandt and Fabiano Thompson, contain 265 chapters in total on each of the ca. 300 known prokaryotic families, in some cases even higher taxa. Each chapter presents both the historical and current taxonomy of these taxa, mostly above the genus level; molecular analyses (e.g., DDH, MLSA, riboprinting, and MALDI-TOF); genomic and phenetic properties of the taxa covered; genome analyses including nonchromosomal genetic elements; phenotypic analyses; methods for the enrichment, isolation, and maintenance of members of the family; ecological studies; clinical relevance; and applications.

As in the third edition, the volumes in the fourth edition are available both as hard copies and as eReferences. The advantages of the online version include no restriction of color illustrations, the possibility of updating chapters continuously and, most importantly, libraries can place their subscribed copies on their servers, making it available to their community in offices and laboratories. The editors thank all the chapter authors and the editorial staff of Springer, especially Hanna Hensler-Fritton, Isabel Ullmann, Daniel Quiñones, Alejandra Kudo, and Audrey Wong, for making this contribution possible.

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Eugene Rosenberg holds a Ph.D. in biochemistry from Columbia University (1961) where he described the chemical structures of the capsules of *Hemophilus influenzae*, types B, E, and F. His postdoctoral research was performed in organic chemistry under the guidance of Lord Todd in Cambridge University. He was an assistant and associate professor of microbiology at the University of California at Los Angeles from 1962 to 1970, where he worked on the biochemistry of *Myxococcus xanthus*. Since 1970, he has been in the Department of Molecular Microbiology and Biotechnology, Tel Aviv University, as an associate professor (1970–1974), full professor (1975–2005), and professor emeritus (2006–present). He has held the Gol Chair in Applied and Environmental Microbiology since 1989. He is a member of the American Academy of Microbiology and European Academy of Microbiology. He has been awarded a Guggenheim Fellowship, a Fogarty International Scholar of the NIH, the Pan Lab Prize of the Society of Industrial Microbiology, the Proctor & Gamble Prize of the ASM, the Sakov Prize, the Landau Prize, and the Israel Prize for a "Beautiful Israel."

His research has focused on myxobacteriology; hydrocarbon microbiology; surface-active polymers from *Acinetobacter*; bioremediation; coral microbiology; and the role of symbiotic microorganisms in the adaptation, development, behavior, and evolution of animals and plants. He is the author of about 250 research papers and reviews, 9 books, and 16 patents.

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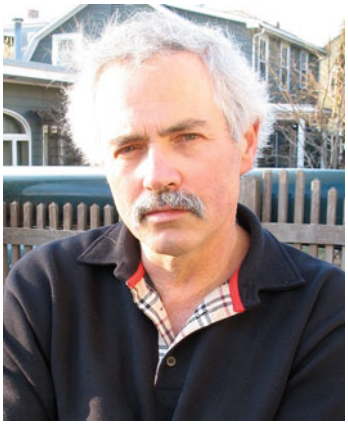
Edward DeLong received his bachelor of science in bacteriology at the University of California, Davis, and his Ph.D. in marine biology at Scripps Institute of Oceanography at the University of California, San Diego. He was a professor at the University of California, Santa Barbara, in the Department of Ecology for 7 years, before moving to the Monterey Bay Aquarium Research Institute where he was a senior scientist and chair of the science department, also for 7 years. He has worked for the past 10 years as a professor at the Massachusetts Institute of Technology in the Department of Biological Engineering, and the Department of Civil and Environmental Engineering, and in August 2014 joined the University of Hawaii as a professor of oceanography. DeLong's scientific interests focus primarily on central questions in marine microbial genomics, biogeochemistry, ecology, and evolution. A large part of DeLong's efforts have been devoted to the study of microbes and microbial processes in the ocean, combining laboratory and field-based approaches. Development and application of genomic, biochemical, and metabolic approaches to study and exploit microbial communities and processes is his other area of interest. DeLong is a fellow in the American Academy of Arts and Science, the U.S. National Academy of Science, and the American Association for the Advancement of Science.



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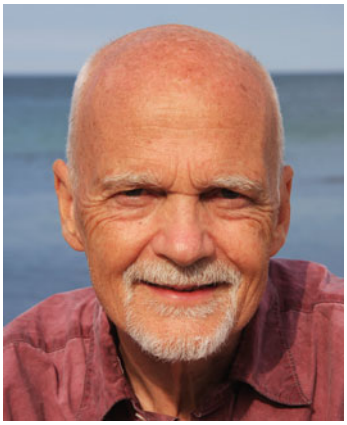
Stephen Lory received his Ph.D. degree in microbiology from the University of California in Los Angeles in 1980. The topic of his doctoral thesis was the structure-activity relationships of bacterial exotoxins. He carried out his postdoctoral research on the basic mechanism of protein secretion by Gram-negative bacteria in the Bacterial Physiology Unit at Harvard Medical School. In 1984, he was appointed assistant professor in the Department of Microbiology at the University of Washington in Seattle, becoming full professor in 1995. While at the University of Washington, he developed an active research program in host-pathogen interactions including the role of bacterial adhesion to mammalian cells in virulence and regulation of gene expression by bacterial pathogens. In 2000, he returned to Harvard Medical School where he is currently a professor of microbiology and immunobiology. He is a regular reviewer of research projects on various scientific panels of governmental and private funding agencies and served for four years on the Scientific Council of Institute Pasteur in Paris. His current research interests include evolution of bacterial virulence, studies on post-translational regulation of gene expression in *Pseudomonas*, and the development of novel antibiotics targeting multi-drug-resistant opportunistic pathogens.



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Erko Stackebrandt holds a Ph.D. in microbiology from the Ludwig-Maximilians University Munich (1974). During his postdoctoral research, he worked at the German Culture Collection in Munich (1972–1977), 1978 with Carl Woese at the University of Illinois, Urbana Champaign, and from 1979 to 1983 he was a member of Karl Schleifer's research group at the Technical University, Munich. He habilitated in 1983 and was appointed head of the Departments of Microbiology at the University of Kiel (1984–1990), at the University of Queensland, Brisbane, Australia (1990–1993), and at the Technical University Braunschweig, where he also was the director of the DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (1993–2009). He is involved in systematics, and molecular phylogeny and ecology of Archaea and Bacteria for more than 40 years. He has been involved in many research projects funded by the German Science Foundation, German Ministry for Science and Technology, and the European Union, working on pure cultures and microbial communities. His projects include work in soil and peat, Mediterranean coastal waters, North Sea and Baltic Sea, Antarctic Lakes, Australian soil and artesian wells, formation of stromatolites, as well as on giant ants, holothurians, rumen of cows, and the digestive tract of koalas. He has been involved in the description and taxonomic revision of more than 650 bacteria taxa of various ranks. He received a Heisenberg stipend (1982–1983) and his work has been awarded by the Academy of Science at Göttingen, Bergey's Trust (Bergey's Award and Bergey's Medal), the Technical University Munich, the Australian Society for Microbiology, and the American Society for Microbiology. He held teaching positions in Kunming, China; Budapest, Hungary; and Florence, Italy. He has published more than 600 papers in refereed journals and has written more than 80 book chapters. He is the editor of two Springer journals and served as an associate editor of several international journals and books as well as on national and international scientific and review panels of the German Research Council, European Science Foundation, European Space Agency, and the Organisation for Economic Co-Operation and Development.

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Fabiano Thompson became a professor of the Production Engineer Program (COPPE-UFRJ) in 2014 and the director of research at the Institute of Biology, Federal University of Rio de Janeiro (UFRJ), in 2012. He was an oceanographer at the Federal University of Rio Grande (Brazil) in 1997. He received his Ph.D. in biochemistry from Ghent University (Belgium) in 2003, with emphasis on marine microbial taxonomy and biodiversity. Thompson was an associate researcher in the BCCM/LMG Bacteria Collection (Ghent University) in 2004; professor of genetics in 2006 at the Institute of Biology, UFRJ; and professor of marine biology in 2011 at the same university. He has been a representative of UFRJ in the National Institute of Metrology (INMETRO) since 2009. Thompson is the president of the subcommittee on the Systematics of Vibrionaceae–IUMS and an associate editor of *BMC Genomics* and *Microbial Ecology*. The Thompson Lab in Rio currently performs research on marine microbiology in the Blue Amazon, the realm in the southwestern Atlantic that encompasses a variety of systems, including deep sea, Cabo Frio upwelling area, Amazonia river-plume continuum, mesophotic reefs, Abrolhos coral reef bank, and Oceanic Islands (Fernando de Noronha, Saint Peter and Saint Paul, and Trindade).

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Alphaproteobacteria

1 The Family *Acetobacteraceae*

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Abstract

The family *Acetobacteraceae* is taxonomically included in the order *Rhodospirillales* of the class *Alphaproteobacteria*, and 32 genera are validly described. The genera are basically classified into two groups, an acetous group and an acidophilic group, in the light of application, ecology, and phylogeny. The acetous group comprises genera in acetic acid bacteria like *Acetobacter, Gluconacetobacter, Gluconobacter, Asaia, Granulibacter, and Komagataeibacter*. The acidophilic group consists of acidophilic and neutrophilic genera like *Acidiphilium* and *Roseomonas*. In the 1960s, taxonomy of acetic acid bacteria was significantly affected by the chemotaxonomic study with G+C content of DNA, quinone systems, cellular fatty acid composition, and DNA-DNA similarity. Further, data of phylogenetic analysis based on 16S rRNA gene sequences have had a profound impact on the systematics of acetic acid bacteria and other bacteria over all. Membrane-bound dehydrogenases are responsible for the oxidation of alcohols and sugars in acetic acid bacteria. The dehydrogenases are located in the periplasmic side of the cytoplasmic membrane of the bacteria. The direct electron acceptor of the dehydrogenases is ubiquinone in the respiratory chain of the acetic acid bacteria. Production of acetic acid from ethanol and of D-gluconate, 2-keto-D-gluconate, 5-keto-D-gluconate, and 2,5-diketo-D-gluconate from D-glucose is due to the membrane-bound dehydrogenases. Acetic acid bacteria are widely distributed in alcoholic and acidic environments, and they are isolated from vinegar, wine, beer, sake, cider, fermented foods, fruits, flowers, and other alcoholic materials. *Asaia* strains are isolated from flowers and even from mosquitoes and other insects. *Granulibacter* is known for its pathogenicity for humans. Acetic acid bacteria are widely used for production of vinegar. D-Gluconic acid and keto-D-gluconic acids are produced from D-glucose by *Gluconobacter* strains, which are used in the food, pharmaceutical, and chemical industries. L-Sorbose is produced from sorbitol by *Gluconobacter* strains, which is further converted to 2-keto-L-gulonic acid as a penultimate intermediate in the industrial production of vitamin C.

Taxonomy, Historical and Current

A.ce.to.bac.te.ra'ce.ae. M. L. masc. n. *Acetobacter* the type genus of the family; *-aceae* ending to denote a family; M. L. pl. n. *Acetobacteraceae* the *Acetobacter* family.

The family *Acetobacteraceae* basically consists of two groups, an acetous group and an acidophilic group, in the light of application, ecology, and phylogeny. The former includes the so-called acetic acid bacteria, which are physiologically and biochemically homogeneous, and the latter includes other acidophilic and neutrophilic bacteria, which have heterogeneous natures physiologically and biochemically.

At the beginning of research in acetic acid bacteria, a few phenotypic characteristics were used for classification, identification, and differentiation. A brief history in the early stage on the taxonomy of acetic acid bacteria is outlined according to Asai's description (Asai 1968).

In 1894, Hansen classified acetic acid bacteria on the basis of pellicle formation and an iodine reaction. In his group I, *Bacterium aceti*, the pellicle of which was easily separable from media and not stained with iodine, and *Bacterium pasteurianum* and *Bacterium küzingianum*, the pellicles of which were easily separable from media and stained with iodine, were included. On the other hand, *Bacterium xylinum* in his group II produced thick and leathery pellicle. At that time, a few species of acetic acid bacteria were known. Beijerinck in 1898 introduced the utilization of an inorganic nitrogen source with pellicle formation and the

production of viscous colonies on sucrose-containing beer gelatin. Henneberg in 1898 considered natural habitats as a differential characteristic and used the terms of malt infusion or wort acetic acid bacteria, beer vinegar bacteria, wine vinegar bacteria, and quick vinegar bacteria. In the next year, Hoyer followed Beijerinck's classification and used a sucrose inversion for the differentiation of acetic acid bacteria. In 1916, Janke classified acetic acid bacteria on the basis of utilization of inorganic ammonium salts as a nitrogen source and acetic acid as a carbon source, along with pellicle formation, motility, and production of acid from carbohydrates. Kluyver and de Leeuw introduced the utilization of ethanol, glucose, and mannitol in 1925. Following the above classifications, biochemical and physiological characteristics were taken into account of the classification of acetic acid bacteria. Visser't Hooft in the same year used catalase, growth on Hoyer's medium, the production of D-gluconic and 5-keto-D-gluconic acids from D-glucose, and pigment formation for the classification. In 1942, Vaughn developed Visser't Hooft's system and classified acetic acid bacteria into two groups, viz., acetate oxidizers and non-acetate oxidizers. Later, the Vaughn system was dealt with in *Bergey's Manual of Determinative Bacteriology*, 7th ed., 1957. Shimwell revised the Vaughn system in 1948 and introduced the oxidation of acetic acid to carbon dioxide, the utilization of ammoniac nitrogen, the formation of leathery cellulosic pellicle on liquid medium, pigment formation, and temperature for growth. Frateur divided the genus *Acetobacter* into four groups in 1950, the *peroxydans* group, the *oxydans* group, the *mesoxydans* group, and the *suboxydans* group, based on the presence or absence of catalase, the oxidation of acetic acid, the oxidation of lactic acid, ketogenic activities, and the production of D-gluconic acid. More detailed history of classification in acetic acid bacteria can be seen in the Asai's descriptions (Asai 1968).

The generic name *Acetobacter* was first introduced for acetic acid bacteria by Beijerinck (1898). The type species of the genus was *Acetobacter aceti*. *Acetobacter pasteurianus* and *Acetobacter peroxydans* were subsequently described (Beijerinck and Folpmers 1916; Visser't Hooft 1925).

The new genus *Gluconobacter* was proposed for the acetic acid bacteria by Asai (1934, 1935), the strains of which were isolated from a variety of fruit, the new type of isolation sources. Most of isolates produced a large amount of D-gluconic acid from D-glucose and generally oxidized ethanol to acetic acid. The strains of the genus *Gluconobacter* interestingly neither assimilated nor oxidized acetic acid and were different in this respect from the strains of the genus *Acetobacter*, which oxidized acetic acid to carbon dioxide and water. In addition, the genera *Acetobacter* and *Gluconobacter* were subdivided into two subgenera, "*Euacetobacter*" and "*Acetogluconobacter*" and "*Eugluconobacter*" and "*Gluconoacetobacter*," respectively (Asai 1935). However, the new generic name *Gluconobacter* was hardly recognized, since the Asai's articles were published in Japanese.

Almost 20 years later from the publications of Asai's articles, the genus "*Acetomonas*" was proposed for strains characterized by polar flagellation and no acetate oxidation (Leifson 1954). In contrast, strains assigned to the genus *Acetobacter* had peritrichous flagellation and the capability of oxidizing acetic acid to carbon dioxide and water. The proposals of the two generic names, *Gluconobacter* and "*Acetomonas*," brought about confusion on naming of similar microorganisms (Asai and Shoda 1958; Shimwell 1958; Shimwell and Carr 1959).

However, the priority of the generic name *Gluconobacter* over the name of "*Acetomonas*" was recognized (De Ley 1961) and *Gluconobacter oxydans* was designated as the type species, because Asai (1935) did not designate the type species (De Ley 1961; Asai et al. 1964; De Ley and Frateur 1970; Buchanan 1970).

In the Approved Lists of Bacterial Names (Skerman et al. 1980), the three species *Acetobacter aceti*, *Acetobacter pasteurianus*, and *Acetobacter peroxydans* are listed in the genus *Acetobacter*, and the only one species *Gluconobacter oxydans* is listed in the genus *Gluconobacter*. The family *Acetobacteraceae* was introduced for the two genera of acetic acid bacteria (Gillis and De Ley 1980).

The two types of intermediate strains were additionally presented in acetic acid bacteria (Asai et al. 1964). One was of peritrichous flagellation, and the other was of polar flagellation. The genera *Acetobacter* and *Gluconobacter* were clearly distinguished by the presence of major ubiquinone homologues, viz., Q-9 for *Acetobacter* and Q-10 for *Gluconobacter* (Yamada et al. 1968b, 1969). Of interest was that the peritrichously flagellated intermediate strains, which were once recognized as pigment-producing strains of *Acetobacter aceti* (Carr and Shimwell 1960; Kimmit and Williams 1963), had Q-10, as found in the strains of the genus *Gluconobacter* (Yamada et al. 1968b, 1969). On the other hand, the polarly flagellated intermediate strains had Q-8, which was never found in the strains of the genera *Acetobacter* and *Gluconobacter* (Yamada et al. 1969, 1976a). The strains were later classified as a new genus and a new species, *Frateuria aurantia* (Swings et al. 1980).

The peritrichously flagellated, Q-10-equipped intermediate strains, viz., the pigment-producing strains of *Acetobacter aceti*, as well as the Q-10-equipped, nonmotile *Acetobacter xylinus* strains, were distinguished chemotaxonomically from the Q-9-equipped *Acetobacter* strains at the subgeneric level, and the subgenus *Gluconacetobacter* corrig. was proposed (Yamada and Kondo 1984). The genus *Acidomonas* was then introduced for the methanol-assimilating *Acetobacter methanolicus* strains (Urakami et al. 1989). However, the proposals of the two taxa, viz., the subgenus *Gluconacetobacter* and the genus *Acidomonas*, were not recognized (Swings 1992). The same conclusion was derived for the genus *Acidomonas* based on 16S rRNA gene sequence analysis (Sievers et al. 1994b). However, there was a different opinion that the name of *Acidomonas* was acceptable from the data obtained by 5S rRNA sequencing (Bulygina et al. 1992).

The subgenus *Gluconacetobacter* was phylogenetically discussed on the basis of the partial 16S rRNA sequences, along with the genus *Acidomonas*, and elevated to the generic level to the genus *Gluconacetobacter*, the type species of which was designated as *Gluconacetobacter liquefaciens*, with a concomitant recognition of the generic name of *Acidomonas* (Yamada et al. 1997). The genus *Gluconacetobacter* was later subdivided into two groups, viz., the *Gluconacetobacter liquefaciens* group and the *Gluconacetobacter xylinus* group, and the two groups

were suggested to be phylogenetically, phenotypically, and ecologically different from each other at the generic level (Yamada and Yukphan 2008). For the latter group, the genus *Komagataeibacter* was newly introduced with the type species, *Komagataeibacter xylinus* (Yamada et al. 2012a, b).

Thus, the four genera, *Gluconobacter*, *Acidomonas*, *Gluconacetobacter*, and *Komagataeibacter*, were additionally established for the species that were once classified in the genus *Acetobacter*. This indicates that the genus *Acetobacter* Beijerinck 1898 had an extremely large generic circumscription.

The genus *Acidiphilium* was first proposed for an acidophilic bacterium (Harrison 1981). The type species *Acidiphilium cryptum* grows at pH 1.9–5.6. However, the species of other genera such as *Roseococcus thiosulfatophilus* and *Erythrobacter longus* cannot grow at such acidic pHs but grow at pH 6–9 and 6.5–9, respectively (Yurkov 2006).

To the family *Acetobacteraceae*, 16 genera are nowadays accommodated to the acetous group, and 16 genera are accommodated to the acidophilic group.

Molecular Analyses

Phylogenetic Structure of the Family and Its Genera

The family *Acetobacteraceae* is a member of the order *Rhodospirillales* in the class *Alphaproteobacteria* (Sievers and Swings 2005a). In 1980, the genera *Acetobacter* and *Gluconobacter* were genetically separated on the basis of DNA-rRNA hybridizations and united in the family *Acetobacteraceae* (Gillis and De Ley 1980). Moreover, the genera *Acetobacter* and *Gluconobacter* were shown to constitute one large rRNA superfamily, which was shown as the alpha subclass of the *Proteobacteria* (Stackebrandt et al. 1988), later named the class *Alphaproteobacteria* (Garrity et al. 2005), together with the genera *Agrobacterium*, *Rhizobium*, *Zymomonas*, etc. On the basis of 5S rRNA gene sequencing, the genus *Acidomonas* was recognized to form an independent lineage apart from the genera *Acetobacter* and *Gluconobacter* in the family *Acetobacteraceae* (Bulygina et al. 1992). The genus *Gluconacetobacter* was phylogenetically distinguished from the genera *Acetobacter* and *Gluconobacter* by the partial 16S rRNA gene sequence analysis (Yamada et al. 1997). Meanwhile, acetic acid bacteria were indicated to relate to acidophilic bacteria including the genera *Acidiphilium*, *Acidocella*, and *Rhodopila* (Kishimoto et al. 1995; Sievers et al. 1994a). Bacteriochlorophyll *a*-containing bacteria such as the genera *Rhodopila* and *Roseococcus* were moderately related to the genus *Acidiphilium* (Yurkov et al. 1994). Therefore, the family *Acetobacteraceae* mainly consists of acetic acid bacteria, acidophilic bacteria, and bacteriochlorophyll *a*-containing bacteria.

Currently, at least 32 genera are thought to be the member of the family *Acetobacteraceae*. The phylogenetic relationships of the family *Acetobacteraceae* are shown in ❯ *Fig. 1.1*. The 30 genera except for the genera *Stella* and *Zavarzinia* form a supra-group within the order *Rhodospirillales*. This lineage is clearly and completely separated from the family *Rhodospirillaceae* with a high bootstrap value (95 % or more) by the phylogenetic tree constructed with neighbor-joining (NJ) and maximum-likelihood (ML) methods. The family *Acetobacteraceae* is divided into three major sublineages. One sublineage consists of acetic acid bacteria with 99 % of bootstrap value in the family *Acetobacteraceae*. Furthermore, the acetic acid bacteria are subdivided into three major groups: one group with the genera *Acetobacter*, *Gluconobacter*, *Neokomagataea*, and *Saccharibacter*; the second group with the genera *Asaia*, *Kozakia*, *Neoasaia*, and *Swaminathania*; and the third group with the genera *Gluconacetobacter* and *Komagataeibacter*. *Gluconacetobacter entanii* is phylogenetically affiliated with the genus *Komagataeibacter*. However, *G. entanii* was not listed as a new combination according to Rule 27 of the Bacteriological Code (Tindall et al. 2006) because the type strain of *G. entanii* was not available in any culture collection (Yamada et al. 2012b).

The genera *Acidomonas*, *Endobacter*, and *Granulibacter* form an independent sublineage each with a single species in the sublineage of the acetic acid bacteria. The second sublineage consists of acidophilic bacteria although bootstrap probability with the sublineage of the acetic acid bacteria is insufficiently supported by the phylogenetic analysis. This lineage includes the genera *Acidicaldus*, *Acidiphilium*, *Acidisoma*, *Acidisphaera*, and *Acidocella*. The third sublineage mainly consists of neutrophilic bacteriochlorophyll *a*-containing bacteria including the genera *Belnapia*, *Craurococcus*, *Humitalea*, *Paracraurococcus*, *Roseococcus*, *Roseomonas*, and *Rubritepida* with 84 % of bootstrap value. The genera *Rhodopila* and *Rhodovarius*, acidophilic phototrophic bacteria and neutrophilic bacteriochlorophyll-not-containing bacteria, respectively, form an independent lineage each with a single species in the family *Acetobacteraceae*. The phylogenetic location of the genera consisted of a single species, for instance, *Acidicaldus, Craurococcus*, *Rhodopila*, and *Saccharibacter*, was unstable due to scarce representation in the sequence dataset. The genera *Stella* and *Zavarzinia* are described as the member of the family *Acetobacteraceae* in *Bergey's Manual of Systematic Bacteriology* 2nd ed. 2005 (Garrity et al. 2005). However, the genus *Stella* is phylogenetically located near to the genera *Azospirillum* and *Skermanella* in the family *Rhodospirillaceae* on the phylogenetic tree based on 16S rRNA gene sequence. The genus *Stella* should be regarded as the member of the family *Rhodospirillaceae*. The genus *Zavarzinia* is phylogenetically located between the members of the family *Acetobacteraceae* and of the family *Rhodospirillaceae*. The phylogenetic position of the genus *Zavarzinia* is required to be discussed in detail because a bootstrap probability is insufficient at the corresponding nodes.

Genome Analyses

Approximately 20 complete and draft genome sequences of the branching within the 16S rRNA gene tree of the family *Acetobacteraceae* have been reported in public. The genome

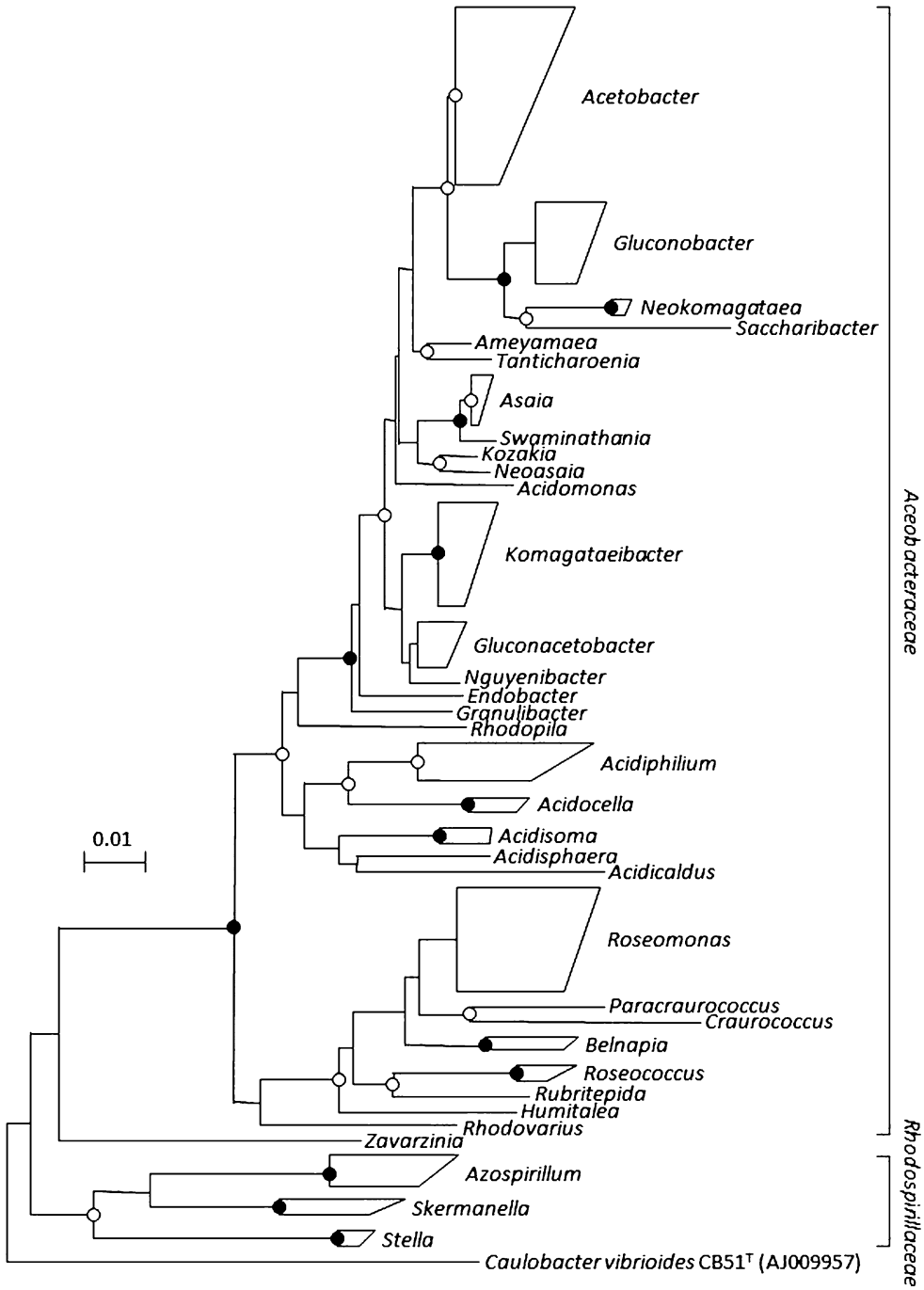

Fig. 1.1

Neighbour-joining tree of the family *Acetobacteraceae* and related bacteria based on 16S rRNA gene sequences. Phylogenetic analysis was based on alignment of 1,219 bp 16S rRNA gene sequences of 114 strains. Solid circles at branching nodes indicate supporting probabilities above 95 % by two phylogenetic analysis methods (NJ and ML), and open circles indicate probabilities above 85 % by either analysis. Bar, 0.01 substitutions per nucleotide position

of *Acetobacter aceti* NBRC 14818 is 3,577,688 bp with a G+C content of 57.3 mol% (Sakurai et al. 2011). The transcriptome profile in the *A. aceti* cells indicated that the TCA cycle (tricarboxylic acid cycle, citric acid cycle, citrate cycle) genes showed higher expression levels in the cells grown on acetate or D-glucose, and the glyoxylate pathway genes were significantly induced by ethanol or acetate. Many SOS-response gene, superoxide dismutase and catalase genes, a gene for one of type I NADH dehydrogenase, genes for bo_3-type (BO3), and cyanide-insensitive types (CIOs) for quinol oxidases were highly expressed or downregulated by acetate, ethanol, and D-glucose. These results indicate that energy conservation efficiency is fine-tuned by changing the respiratory components according to the growth conditions in the *A. aceti* cells.

Genomes of *Acetobacter pasteurianus* NBRC 3283, SKU1108, and IFO 3191 consist of 2,907,495 bp, 3,018,312 bp, and 2,888,200 bp with G+C contents of 50.7 mol%, 52.68 mol%, and 53.08 mol%, respectively, showing 100 % sequence identity by 16S rRNA gene sequence (Azuma et al. 2009; Matsutani et al. 2012). Genes involved in translation, transcription, and signal transduction are highly conserved among the each genome of these strains. Strain IFO 3191 increased mutation rates compared with the thermotolerant strains SKU1108 and NBRC 3283. Some of these genes might be correlated with the thermosensitivity of IFO 3191.

Acetobacter tropicalis SKU1100 is a thermotolerant acetic acid bacterium. The strain is able to grow above 40 °C and can perform amino acid substitutions from large to small residue and Lys to Arg in many orthologous genes compared with a mesophilic acetic acid bacterium, *A. pasteurianus* IFO 3283 (Matsutani et al. 2011). In the orthologous proteins, *A. tropicalis* SKU1100 increased the number of Arg-based salt bridges than *A. pasteurianus* IFO 3283. This fact strongly suggests that the increased number of Arg-based salt bridges may contribute to the thermotolerance of *A. tropicalis* SKU1100 because Arg-based salt bridges were reported to be an important factor for thermostability of protein structure.

Gluconacetobacter diazotrophicus Pal5 is an endophytic diazotrophic bacterium and lives in association with sugarcane plants. Its genome is composed of a circular chromosome of 3,944,163 bp with an average G+C content of 66.19 mol% and two plasmids of 16.6 and 38.8 kb, respectively (Bertalan et al. 2009). The relation to the endophytic lifestyle such as nitrogen fixation, plant growth promotion, sugar metabolism, transport systems, synthesis of auxin, and the occurrence of bacteriocins was revealed based on 3,938 coding sequences. Gene clusters for *gum*-like polysaccharide biosynthesis, *tad* pilus biosynthesis, quorum sensing, modulation of plant growth by indole acetic acid, and mechanisms involved in tolerance to acidic conditions may be related to the sugarcane endophytic and plant growth-promoting traits of *G. diazotrophicus*. An accessory component of at least 851 genes was most likely acquired by horizontal gene transfer, distributing in genome islands.

Gluconacetobacter hansenii ATCC 23769 is a model organism for cellulose biosynthesis, and its genome consists of 3,547,122 bp with a G+C content of 59 mol% (Iyer et al. 2010). The genome contains 3,351 genes, of which 3,308 are protein-encoding genes, accounting for 84 % of the genome. The genes encoding proteins involved in cellulose synthesis are in an operon consisting of *ascAB*, *acsC*, and *acsD*. The genome also contains three genes encoding diguanylate cyclase, which catalyzes the formation of cyclic di-GMP, the second messenger in bacteria that functions as an allosteric activator of cellulose synthase *AcsAB*.

The genome of *Gluconacetobacter xylinus* NBRC 3288, which is a cellulose-not-producing strain isolated from vinegar in Japan, consists of a single circular chromosome of 3,136,818 bp with a G+C content of 60.92 mol% and seven distinct plasmids (2,218–255,866 bp) (Ogino et al. 2011). The genome includes 3,195 protein-encoding ORFs, five copies of rRNA operons, and 60 genes encoding tRNAs. The genome of *G. xylinus* NBRC 3288 includes 11 genes related to cellulose synthesis within two operons. The cellulose-synthesizing operon contains the endoglucanase and the cellulose synthase catalytic subunit. On the other hand, a nonsense mutation causes splitting of *bcsB* gene that is indispensable for cellulose production. This single mutation might affect the cellulose synthesis of this strain. *Gluconacetobacter xylinus* NBRC 3288 was reidentified as *Gluconacetobacter medellinensis* NBRC 3288^{T} (= LMG 1693^{T}) (Castro et al. 2013).

Gluconobacter oxydans is known for its incomplete oxidation of a wide range of alcohols, sugars, and acids in a bioprocess. The chromosome of *G. oxydans* 621H consists of 2,702,173 bp with a G+C content of 60.8 mol% and contains 2,432 ORFs (Prust et al. 2005). In addition, five plasmids are identified that comprised 232 ORFs. *G. oxydans* 621H contains many membrane-bound dehydrogenases that are part of a strategy to thrive and to survive in nutrient-rich environments. The enzymes rapidly form sugars or sugar acids that are difficult to assimilate for the most microorganisms, whereas *G. oxydans* can easily take advantage of these substrates. The oxidized compounds are taken up and reduced in the cytoplasm, the reactions being catalyzed by a soluble set of oxidoreductases. Furthermore, incomplete oxidation of D-glucose and other aldoses leads to the formation of sugar acids and to a decrease in the pH value, thereby preventing propagation of many other microorganisms. The respiratory chain is designed to accelerate this process because proton-translocating abilities are limited. This prevents an increase in the electrochemical membrane potential that would otherwise lead to the inhibition of membrane-bound redox reactions. However, the low energy-transducing efficiency results in very low growth yields. The inability to degrade D-glucose and other sugars via the Embden-Meyerhof pathway and the incomplete citrate cycle contribute to the inadequate utilization of the substrates. Although the respiratory chain of *G. oxydans* is found to be rather simple, the organism contains many membrane-bound dehydrogenases that are critical for the incomplete oxidation of biotechnologically important substrates.

The complete genome of *Gluconobacter oxydans* H24 consists of a circular chromosome and a plasmid (Ge et al. 2013). The chromosome is composed of 3,602,424 bp with a G+C content of 56.25 mol%. The plasmid contains 213,808 bp with a G+C content of 56.14 %. There are a total of 3,732 putative ORFs (3,469 in the chromosome and 263 in the plasmid). The most significant feature of *G. oxydans* H24 is its high L-sorbose productivity. Two different membrane-bound and one cytoplasmic sorbitol dehydrogenases are identified from the genome of *G. oxydans* H24. The gene cluster responsible for the synthesis of the cofactor pyrroloquinoline quinone (PQQ) and several genes encoding sorbose dehydrogenase, sorbose reductase, sorbosone dehydrogenase, glucose dehydrogenase, and other enzymes were annotated.

The complete genome of *Gluconobacter oxydans* WSH-003 contains a single circular chromosome of 3,364,884 bp with a G+C content of 56.77 mol% (Gao et al. 2012). The draft genome harbors 3,705 predicted protein-coding sequences (CDSs); a total of 48 tRNA genes and one 16S-23S-5S rRNA are identified. The gene clusters encoding D-sorbitol dehydrogenase

and responsible for synthesis of the cofactor pyrroloquinoline quinone are identified. In addition, several genes encoding aldehyde dehydrogenase, alcohol dehydrogenase, glucose dehydrogenase, formaldehyde dehydrogenase, etc., are annotated.

The genome of *Granulibacter bethesdensis* CGDNIH1, an emerging human pathogenic acetic acid bacterium, is composed of a single circular chromosome of 2,708,355 bp with a G+C content of 59.07 mol% (Greenberg et al. 2007). Among 2,437 putative ORFs identified, 1,470 of which share sequence similarity with ORFs in the nonpathogenic but related *G. oxydans* genome. The 967 ORFs are unique to *G. bethesdensis* and potentially important for virulence, adherence, DNA uptake, and methanol utilization. Comparison of *G. bethesdensis* to other known chronic granulomatous disease (CGD) pathogens demonstrated conservation of some putative virulence factors, suggesting possible common mechanisms involved in pathogenesis in CGD. Genotyping of the four patient isolates by the use of a custom microarray demonstrated genome-wide variations in regions encoding DNA uptake systems and transcriptional regulators and in hypothetical ORFs.

The genome of *Acidiphilium* sp. PM consists of a chromosome of 3.98 Mbp with a G+C content of 68 % and nine plasmids (San Martin-Uriz et al. 2011). Metabolic construction revealed the presence of a complete Entner-Doudoroff pathway, instead of the classical Embden-Meyerhof pathway. The pentose phosphate pathway, the tricarboxylic acid cycle, and the Calvin-Benson-Bassham cycle were also found to be complete. This preliminary genome analysis revealed that *Acidiphilium* sp. PM has versatile respiratory metabolisms.

Acidocella sp. MX-AZ02 was isolated from a naturally acidic (pH 2.3) and a heavy metal-containing shallow lake. Its genome was estimated to be 3.6 Mbp with a G+C content of 64.1 mol%, and it carried 3,553 open reading frames (ORFs) (Servín-Garcidueñas et al. 2013). The genome codes for arsenic, chromium, copper, and cobalt-zinc-cadmium transporters, as well as heavy-metal sensor signal transduction histidine kinases and chaperones. Carbonic anhydrases are also encoded. The enzyme may provide to cope with the low CO_2 levels in acidic waters.

Roseomonas sp. B5 degrades *N*-acylhomoserine lactone. Its genome contains 471,409,596 bp with a mol% G+C content of 70.5 % (Chen et al. 2012). The genome includes 4,365 ORFs; 51 tRNAs, one complete rRNA operon, and one copy each of a 5S rRNA gene, 23S rRNA gene, and 16S rRNA gene are identified. One predicted protein-CDS encoding 263 amino acids shows 84 % similarity to the reported *attM* gene, and an *N*-acylhomoserine lactones (AHL)-degrading gene is detected.

Phages

A bacteriophage A-1 was isolated from *Gluconobacter oxydans* strain E and characterized to be of type A phage according to Bradley's morphological classification (Bradley 1967; Jucker and Ettlinger 1981; Robakis et al. 1985a; Schocher et al. 1979). Abnormalities in the microbial process for the oxidation of D-sorbitol to L-sorbose by *G. oxydans* were indicated to be caused by probable phage infection (Schocher et al. 1979). Two bacteriophages GW6210 and JW2040 were isolated from decaying apples using *G. oxydans* ATCC 621 and VPI 204JW, respectively (Robakis et al. 1985b). Phages GW6210 and JW2040 belonged to groups A and C of Bradley's classification, respectively. Phage GW6210 is unusually large and has a head diameter of 170 nm. Both the phages contain a double-stranded DNA. The G+C content of the DNA of phage GW6210 is 29.3 mol% (*Tm*), and the size of the genome is approximately 250–300 kb. The size of the DNA of phage JW2040 is 37 kb, and the G+C content is 56.5 mol% (Tm).

Bacteriophages were demonstrated in industrial vinegar fermentation (Sellmer et al. 1992; Stamm et al. 1989; Teuber et al. 1987). Phages with hexagonal and icosaedric heads belong to types A and C according to Bradley's classification, respectively. Seven types are morphologically identified based on head sizes, tail lengths, and diameters (Sellmer et al. 1992). Head sizes vary between 60 and 110 nm. The corresponding tail lengths ranged from 99 to 360 nm with tail diameters from 14 to 31 nm.

A bacteriophage, termed ΦAc1, infects strains of the genus *Acidiphilium*. Several of its properties were characterized (Ward et al. 1993). The virion has a lambdoid morphology and is larger than λ. The sedimentation coefficient of the virion is approximately 615S. The nucleic acid of ΦAc1 is a double-stranded DNA, approximately 102 kb in length. ΦAc1 is a temperate phage, and the plaques are turbid. ΦAc1 prophage DNA integrates into the bacterial genome during the temperate growth phase.

A prophage Acm1 was found in *Acidomonas methanolica* strains (Mamat et al. 1995). A 262-bp DNA fragment of phage Acm1 contains three very short open reading frames of 21, 24, and 36 bp. The fragment codes for the synthesis of a trans-acting RNA molecule of 97 nucleotides, designated lbi (lipopolysaccharide biosynthesis-interfering) RNA. This RNA has the ability to form intracellularly rRNA hybrid duplexes with mRNA.

Biochemical Properties

Bioenergetics

Acetic acid bacteria, the obligate aerobes, are biochemically quite unique. The Embden-Meyerhof pathway is not operative for strains assigned to the genera *Acetobacter* and *Gluconobacter*, but instead, the pentose phosphate cycle is functioning (Hauge et al. 1955; Cheldelin 1961; De Ley 1960; Asai 1968). In addition, the strains of the genus *Gluconobacter* lack the TCA cycle, differing from the strains of the genus *Acetobacter*, and the pentose phosphate cycle acts as the only terminal oxidative catabolic pathway. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reduce NAD, except for NADP, in the cells of the acetic acid bacteria, and the two dehydrogenases seem to play an important role in the respiratory chain phosphorylation (Cheldelin 1961).

Acetic acid bacteria are also known to accumulate a large quantity of acetic acid from ethanol; dihydroxyacetone from glycerol; D-gluconic acid, 2-keto-D-gluconic acid, and

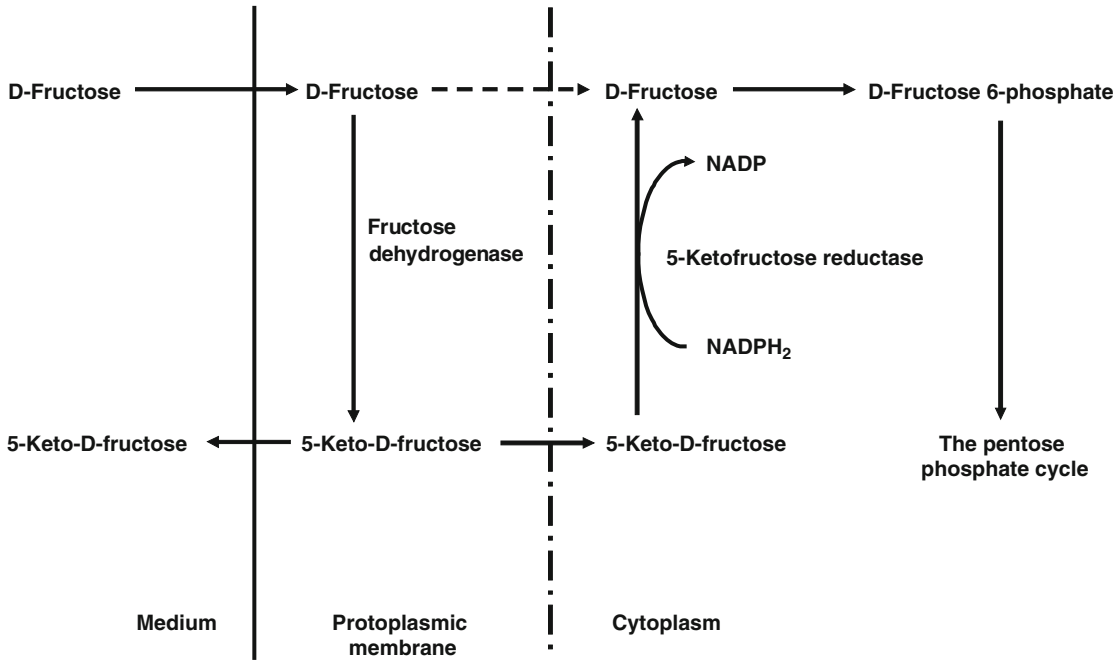

Fig. 1.2

A presumed pathway for the catabolism of D-fructose in *Gluconobacter* species. 5-Ketofructose reductase is active only for reduction, and the reverse reaction is scarcely found. The pH optimum of the reductase is 7.0 (The figure was cited from Yamada et al. (1967b))

5-keto-D-gluconic acid from D-glucose; D-fructose from D-mannitol; and L-sorbose from D-sorbitol. Such a partial or incomplete oxidation of alcohols and sugars is traditionally called "oxidative fermentation" and carried out by the membrane-bound dehydrogenases that are linked to the respiratory chain (De Ley 1960; Cheldelin 1961; Asai 1968).

The production of a large amount of dicarbonyl hexose from D-fructose was found, and the chemical structure of the product was elucidated as 5-keto-D-fructose (Terada et al. 1960). Concerning the catabolism of D-fructose and 5-keto-D-fructose in acetic acid bacteria, the existence of 5-ketofructose reductase [EC 1.1.1.124] and fructose 5-dehydrogenase [EC 1.1.99.11] was reported (Aida and Yamada 1964; Yamada et al. 1966). The membrane-bound fructose 5-dehydrogenase was supposed to be linked to ubiquinone-10 (Yamada et al. 1967a, 1968a). Within the cells of *Gluconobacter* species, the D-fructose was produced again from 5-keto-D-fructose by 5-ketofructose reductase, which was speculated to flow into the pentose phosphate cycle after the phosphorylation of D-fructose (*Fig. 1.2*; Yamada et al. 1967b; Avigad et al. 1966; Asai 1968).

In acetic acid bacteria, several kinds of membrane-bound dehydrogenases are responsible for the oxidation of alcohols and sugars (*Table 1.1*). The dehydrogenases are located in the periplasmic side of the cytoplasmic membrane of the microorganisms (Matsushita et al. 2004; Yakushi and Matsushita 2009, 2010). The direct electron acceptor of the dehydrogenases is ubiquinone in the respiratory chain of the acetic acid bacteria. Alcohol dehydrogenase [EC 1.1.5.5] was first isolated from "*Gluconobacter suboxydans*" IFO 12528 as a homogeneous state and composed of three subunits, in which a cytochrome *c* component was contained (Adachi et al. 1978). The alcohol dehydrogenase isolated from the acetic acid bacterium was then proved to contain three subunits of 72 Kd [pyrroloquinoline quinone (PQQ), heme *c*], 48 Kd (3 hemes *c*), and ~15 Kd (*Fig. 1.3*; Matsushita et al. 1994). On the other hand, glucose dehydrogenase [EC 1.1.5.2] was a monomer of 80 Kd equipped with only PQQ (Matsushita et al. 1994). Glycerol dehydrogenase [EC 1.1.99.22] exceptionally showed a wide range of substrate specificity and oxidized glycerol, D-arabitol and D-sorbitol, respectively, to dihydroxyacetone, D-xylulose, and L-sorbose under the Bertrand-Hudson law. The dehydrogenase had two subunits comprised of 80 Kd (PQQ) and ~15 Kd. Fructose 5-dehydrogenase [EC 1.1.99.11] was a trimer composed of three subunits of 67 Kd [FAD (*Table 1.1*)], 51 Kd (3 hemes *c*), and ~20 Kd (Ameyama et al. 1981; Kawai et al. 2013).

Several types of terminal oxidases were reported in the respiratory chain of acetic acid bacteria (Matsushita et al. 2004). "*Gluconobacter suboxydans*" NBRC 12528 (= IFO 12528; now *Gluconobacter oxydans*) was reported to have the respiratory chain branched to the energy-producing cytochrome *o* (bo_3), a quinol oxidase, which consisted of four nonidentical subunits and contained heme *b*, heme *o*, and copper atom as the prosthetic group, as well as to the less energy-producing cyanide-insensitive quinol oxidase (*Fig. 1.3*; Matsushita et al. 1987). The purified cyanide-insensitive quinol oxidase consisted of two nonidentical subunits, contained heme *b* and heme *d* but no Cu atom, and had the pH optimum of 5–6, in contrast to the pH optimum of 7 of cytochrome *o* (bo_3), and the apparent

Table 1.1
Membrane-bound primary dehydrogenases in acetic acid bacteria

Dehydrogenase	Substrate	Product	Subunit structure and prosthetic group	Acceptor	Species
Alcohol dehydrogenase [EC 1.1.5.5]	Ethanol	Acetaldehyde	72 Kd (PQQ/heme c), 48 Kd (3 hemes c), ~15 Kd	Ubiquinone	*'G. suboxydans'* (1) *A. aceti* (1)
Aldehyde dehydrogenase [EC 1.2.99. 7][a]	Acetaldehyde	Acetic acid	84 Kd (Iron sulfur?/ molybdopterin cytosine dinucleotide?), 49 Kd (3 hemes c), ~17Kd	Ubiquinone[b]	*'G. suboxydans'* (1) *'A. polyoxogenes'* (1), *A. europaeus* (8)
Glucose dehydrogenase [EC 1.1.5.2]	D-Glucose	D-Glucono-δ-lactone	80 Kd (PQQ)	Ubiquinone	*'G. suboxydans'* (1)
Gluconate 2-dehydrogenase [EC 1.1.99.3][a]	D-Gluconate	2-Keto-D-gluconate	64 Kd (FAD), 45 Kd (3 hemes c), ~21 Kd	Ubiquinone	*'G. dioxyacetonicus'* (1)
2-Keto-D-gluconate dehydrogenase [EC 1.1.99.4][a]	2-Keto-D-gluconate	2,5-Diketo-D-gluconate	61 Kd(FAD), 47 Kd (3 hemes c), ~25 Kd	Ubiquinone	*'G. melanogenes'* (1)
Fructose 5-dehydrogenase [EC 1.1.99. 11][a]	D-Fructose	5-Keto-D-fructose	67 Kd (FAD[c]), 51 Kd (3 hemes c), ~20 Kd	Ubiquinone	*'G. industrius'* (1, 7)
L-Sorbose 5-dehydrogenase [EC 1.1.99.12]	L-Sorbose	5-Keto-D-fructose		Ubiquinone?	*'G. suboxydans'* (2)
L-Sorbose 1-dehydrogenase [EC 1.1.99.32][a]	L-Sorbose	L-Sorbosone	58 Kd (FAD)	Ubiquinone	*G. oxydans* (1), *'G. melanogenus'* (5)
L-Sorbosone dehydrogenase	L-Sorbosone	2-Keto-L-gulonate	48 Kd?	Ubiquinone?	
Sorbitol dehydrogenase [EC 1.1.99. 21][a]	D-Sorbitol	L-Sorbose	63 Kd (FAD), 51 Kd (3 hemes c), ~17 Kd	Ubiquinone	*'G. suboxydans'* (1)
Glycerol dehydrogenase [EC 1.1.99. 22][a]	Glycerol, *meso*-Erythritol, D-Arabitol, D-Sorbitol, etc.	Dihydroxyacetone, L-Erythrose, D-Xylulose, L-Sorbose, etc.	80 Kd (PQQ), ~14 Kd	Ubiquinone	*'G. industrius'* (3), *'G. suboyxdans'* (4, 6), *Gluconobacter* (9).

For more details, see Matauahita et al. (2004), and Yakushi and Matsushita (2009, 2010)
[a]The electron acceptor was proved to be ubiquinone. Therefore, the dehydrogenases will be classified as EC class 1.1.5
[b]Unpublished data of Yakushi et al. (from Dr. Matsushita)
[c]The prothsthetic group is assumed to be FAD, but it is not proved biochemically
References: (1), Matsushita et al. (1994); (2), Sato et al. (1969b); (3), Ameyama et al. (1985); (4), Adachi et al. (2001); (5), Sugisawa et al. (1991); (6), Sugisawa and Hoshino (2002); (7), Kawai et al. (2013); (8), Thurner et al. (1997); and (9), Matsushita et al. (2003)

maximum velocity of the cyanide-insensitive quinol oxidase was much 17-fold higher than that of cytochrome *o* (bo_3) (Miura et al. 2013). The cyanide-insensitive quinol oxidase may play some important roles in the rapid oxidation of substrates under acidic growth conditions. The other types of cytochromes in acetic acid bacteria were cytochrome *a*1 (ba_3), cytochrome *d* (*bd*), and cytochrome *co* (cbb_3) (Matsushita et al. 2004). The cytochrome *a*1 is characterized as cytochrome *ba*-type ubiquinol oxidase, which consisted of four subunits and contained one mol each of heme *a*, heme *b*, and Cu atom. Interestingly, *Acetobacter aceti* IFO 3284 produced either one of the different terminal oxidases under the different culture conditions (Matsushita et al. 1990; Sievers and Swings 2005b). The cells of rough colony type of the strain predominantly produce cytochrome *a*1 (ba_3) under shaking culture, while the cells of smooth colony type of the strain predominantly produce cytochrome *o* (bo_3) under static culture.

On the oxidative fermentation of 5-keto-D-fructose from D-sorbitol by "*Gluconobacter suboxydans*" strain 1, L-sorbose and 5-keto-D-fructose were accumulated, and the biphasic

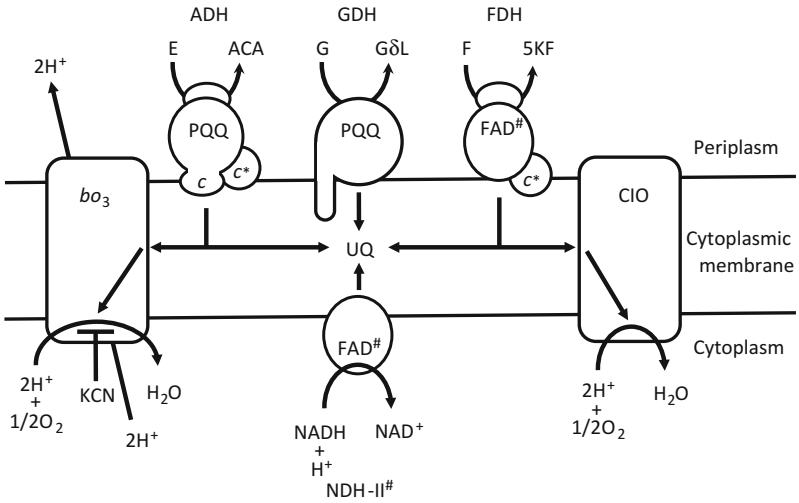

Fig. 1.3

The membrane-bound dehydrogenases and the respiratory chain of *'Gluconobacter suboxydans'*. The dehydrogenases, e.g., alcohol dehydrogenase, glucose dehydrogenase and fructose 5-dehydrogenase that contain prosthetic groups such as PQQ, FAD and heme *c* generally reduce ubiquinone, an electron acceptor. The resulting electrons are then transferred either to the energy-producing cytochrome *o* (*bo_3*), a quinol oxidase or to the less energy-producing cyanide-insensitive quinol oxidase. For more details, see the references (Matsushita et al. 2004; Yakushi and Matsushita 2009, 2010). Abbreviations: *ADH* alcohol dehydrogenase, *GDH* glucose dehydrogenase, *FDH* fructose 5-dehydrogenase, *E* ethanol, *ACA* acetaldehyde, *G* D-glucose, *GδL* D-glucono-δ-lactone, *F* D-fructose, *5KF* 5-keto-D-fructose, *FAD#* the presence of FAD is assumed but not proved biochemically, *NDH-II#* Type-II NADH dehydrogenase, which is not purified; *bo_3*, cytochrome *bo_3*; *CIO* cyanide-insensitive terminal quinol oxidase, *c* heme *c*; *c cytochrome c**

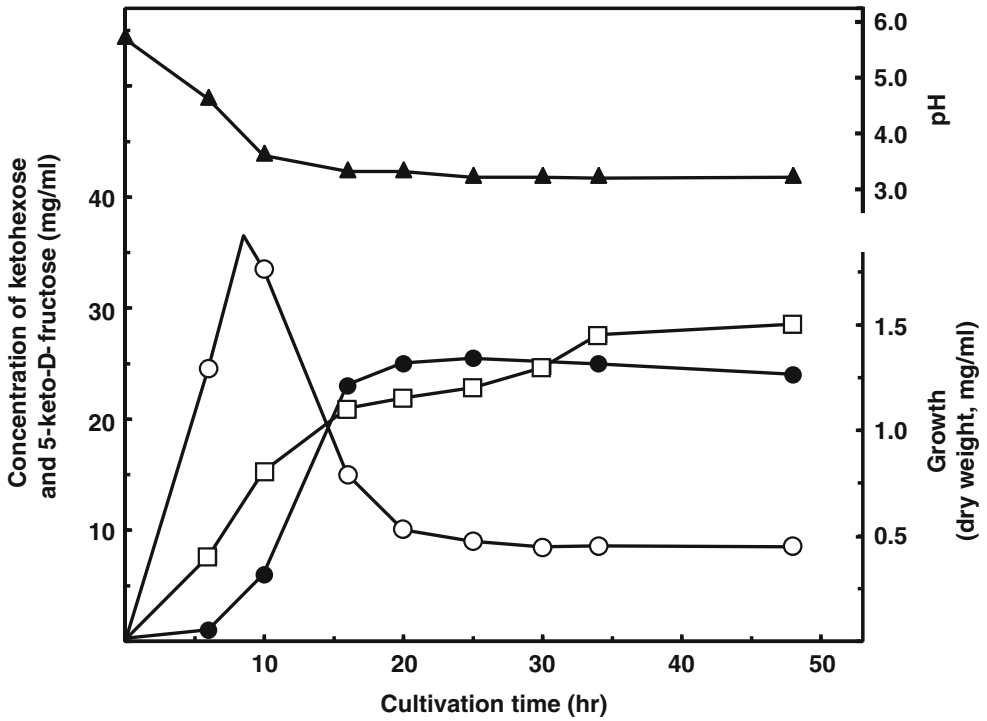

Fig. 1.4

The chemical changes during the oxidation of D-sorbitol by *'Gluconobacter suboxydans'*. '*Gluconobacter suboxydans*' strain 1 was cultured in 500 ml of a medium containing 4.0 % D-sorbitol and 2.0 % defatted soybean extract in a 5-l conical flask. The cultivation was carried out at 30 °C on a rotary shaker at 160 rpm (The figure was cited from Sato et al. (1969a). ○, L-sorbose; ●, 5-keto-D-fructose, □, growth; ▲, pH)

growth was generally seen (Fig. 1.4; Sato et al. 1969a). L-Sorbose accumulation reached the maximum 10 h later after inoculation, and then the amount of L-sorbose decreased. The production of 5-keto-D-fructose started 7 h later. After 16 h, the first growth of the organism mostly stopped, and the organism showed a temporarily stationary phase for approximately 10 h. Of the two steps of the D-sorbitol oxidation, glycerol dehydrogenase [EC 1.1.99.22] but not sorbitol dehydrogenase [EC 1.1.99.21] first appeared to function (Soemphol et al. 2008), and L-sorbose 5-dehydrogenase [EC 1.1.99.12] was secondly concerned (Sato et al. 1969b), viz., L-sorbose production from D-sorbitol and 5-keto-D-fructose production from L-sorbose. The second growth began after 25 h from inoculation. The first growth is due to the oxidations of D-sorbitol to L-sorbose and then to 5-keto-D-fructose, which seems to be coupled with the respiratory chain phosphorylation, and the second growth is assumed to be due to the reduction of 5-keto-D-fructose to D-fructose, followed by phosphorylation to D-fructose 6-phosphate and by flowing into the pentose phosphate cycle, the terminal oxidative catabolic pathway of the organism. The biphasic growth based on the oxidation of ethanol to acetic acid and then to carbon dioxide and water can be seen in vinegar production by an *Acetobacter* strain (Matsushita et al. 2004).

Nitrogen Fixation

Acetic acid bacteria capable of fixing molecular nitrogen seemed to be restricted only to those of the three genera, *Gluconacetobacter*, *Swaminathania*, and *Acetobacte*r (Pedraza 2008).

Acetobacter diazotrophicus (= *Gluconacetobacter diazotrophicus*) was first reported as a nitrogen-fixing acetic acid bacterium (Gillis et al. 1989; Cavalcante and Döbreiner 1988). Strains of the species were found inside sugarcane plant tissues. As the second examples, *Gluconacetobacter johannae* and *Gluconacetobacter azotocaptans* were found to be associated with coffee plants (Fuentes-Ramírez et al. 2001). *Swaminathania salitolerans* was described as a salt-tolerant, nitrogen-fixing, and phosphate-solubilizing bacterium (Loganathan and Nair 2004). *Acetobacter peroxydans* and *Acetobacter nitrogenifigens* were isolated, respectively, from wetland rice plants and kombucha tea (Muthukumarasamy et al. 2005; Dutta and Gachhui 2006).

Recently, *Asaia* species, including *Asaia bogorensis* and *Asaia siamensis*, were reported to have the capability of nitrogen fixation (Samaddar et al. 2011). *Nguyenibacter vanlangensis* found in rhizosphere or root of Asian rice plants showed the growth on nitrogen-free LGI medium. The strains of the species would be candidates able to fix molecular nitrogen (Vu et al. 2013).

The nitrogenase of *Gluconacetobacter diazotrophicus* was immunochemically investigated from the viewpoint of the protection against molecular oxygen (Ureta and Nordlund 2002). The nitrogenase in the organism could be protected by conformational mechanisms involving a putative FeSII protein operating when cells were subjected to sudden increases in oxygen pressure, together with increased respiration of the organism under diazotrophic condition.

Different genes involved in the N_2-fixing process and their regulation such as *nifHDK*, *nifA*, *nifB*, *nifV*, *nifE*, and *ntrBC* were identified in the nitrogenase of *Gluconacetobacter diazotrophicus* (Sevilla et al. 1997). As the results of sequence analysis, the genes were organized into eight transcriptional units, and the overall arrangement of genes was most likely that of the *nif-fix* cluster in *Azospirillum brasilense*, while the individual gene products were more similar to those in the species of *Rhizobiaceae* or in *Rhodobacter capsulatus* (Lee et al. 2000).

Swaminathania salitolerans strains reduced acetylene to ethylene and showed the expected 450-bp amplification product corresponding to *nifD* (Loganathan and Nair 2004). In the type strain of *Acetobacter nitrogenifigen*s, a 336-bp region product encoding dinitrogenase reductase, *nifH* was detected (Dutta and Gachhui 2006). Likewise, strains of *Asaia* species, including *Asaia bogorensis* and *Asaia siamensis*, were confirmed to have *nifH* (Samaddar et al. 2011).

Phenotypic Analyses

Currently, the family *Acetobacteraceae* is basically consists of two groups, an acetous group and an acidophilic group. Additionally, the genus *Frateuria* is listed in this section. The genus resembles biochemically the genera in acetic acid bacteria, but it locates in the *Gammaproteobacteria* (Swings and Sievers 2005).

The Acetous Group

Acetic acid bacteria are included in the family *Acetobacteraceae* on the basis of 16S rRNA sequence analysis, considering phenotypic and chemotaxonomic characteristics (Sievers and Swings 2005a). Thus, acetic acid bacteria can be summarized as follows: Acetic acid bacteria are Gram negative, rod-shaped, peritrichously or polarly flagellated when motile, and strictly aerobic. Catalase is positive (negative for *Acetobacter peroxydans*), and oxidase is negative. The bacteria are able to oxidize sugars, sugar alcohols, and alcohols to corresponding acids. However, phenotypic characteristics of acetic acid bacteria are diverse among the genera. Further, the table consisting of the phenotypic characteristics and species names is not always filled with data based on common characteristics (Table 1.2). Differentiation of the species based on phenotypic characteristics comes into being incomplete. Therefore, analysis of ribosomal RNA (16S rRNA and others) sequences and determination of DNA-DNA similarity are recommended for correct identification of strains. The genus *Acetobacter* has ubiquinone 9 (Q-9), but other genera have Q-10. The cellular fatty acid composition consists of $C_{18:1}\omega7c$ as a major acid and $C_{16:0}$ and $C_{14:0}$ 2-OH as minor acids (Yamada et al. 1981a; Iino et al. 2012b; Vu et al. 2013).

Several strains of acetic acid bacteria are used for the production of vinegar, gluconic acid, vitamin C, etc. The bacteria are widely distributed in vinegar and other acetous substances (wine, beer, sake, and other fermented products), fruits and sugary substances, and flowers.

Table 1.2
Characteristics of the genera in the acetous group

	Acetobacter	*Acidomonas*	*Ameyamaea*	*Asaia*	*Endobacter*	*Gluconacetobacter*	*Glucono-bacter*	*Granulibacter*
Type species	*A. aceti*	*A. methanolica*	*A. chiangmai-ensis*	*A. bogorensis*	*E. medicaginis*	*G. liquefaciens*	*G. oxydans*	*G. bethesdensis*
Cells	Ellipsoidal to rods	Rods	Rods	Rods	Coccoid to rods	Ellipsoidal to rods	Rods	Coccobacillus to rods
Size (μm)	0.4–1.0 × 1.0–3.0	0.5–0.8 × 1.5–2.0	0.6–0.8 × 1.0–1.8	0.4–1.0 × 0.8–2.5		0.5–0.9 × 1.0–2.0	0.6–1.0 × 1.0–3.0	
Motility	+/−	+	+	+	+	+	+	-
Flagella	Peritrichous	Polar	Polar	Peritrichous to lateral	Subpolar	Peritrichous	Polar	
Catalase	+	+		+	+	+		+
Oxidase		−			−			−
Growth on								
Glutamate agar		_	+w	+	+	+	+	+
Mannitol agar		+	+	+	+	+	+	+w
Colonial appearance	Beige, creamy to brown	Convex, circular, smooth, entire, beige to pink	Smooth, entire, creamy	Smooth, raised, entire, pink to dark pinkish	Mucoid, white	Light brown to brownish	Raised, smooth, entire, shiny, white or pink or brown	Convex, smooth, entire, yellow (non-diffusible)
Oxidation of								
Acetate	+	+	+	+/+W		+		+
Lactate	+	−/+w	+w	+/+w	−	+		+w
Acetic acid from ethanol	+	+w	+	−/+	+	+	+	+w
Production of DHA	−	−	+w	+/+W	−		+	−
Production of								
D-Gluconate	+	+						
2-Keto-D-gluconate	+/−	*	+	+		+	+	
5-Keto-D-gluconate	−	*	+	+		−	+	
2,5-Diketo-D-glucoante	−	*	+	−		+/−	+/−	
Acid from								
L-Arabinose	+/−	+	+	+			+	
L-Sorbose	−		−	+			+/−	
D-Xylose	+/−	+	+	+	+		+	−

Table 1.2 (continued)

	Acetobacter	*Acidomonas*	*Ameyamaea*	*Asaia*	*Endobacter*	*Gluconacetobacter*	*Glucono-bacter*	*Granulibacter*
L-Rhamnose	–	–	+	+/–			–/+	
D-Fructose	–	–	–	+			+	
D-Galactose	+/	+	+	+			+	
D-Glucose	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+			+	
Lactose			–				–	
Maltose		–	+	+/+W	–		–/+	–
Melibiose		v		+			+	
Sucrose		–	–	+	–		+/–	–
Trehalose		–						
Raffinose		–		+/–			+/–	
Dulcitol		–	–	–/+				
meso -Erythritol							+/+W	
myo -Inositol		–						
D-Mannitol	–	–	–	+	–		+/+W	–
meso -Ribitol								
D-Sorbitol	–	–	–	+	–		+	–
Xylitol								
Glycerol	–	+	+	+	+		+	V
Ethanol	+	+	+	–/+	+	+	+	+
1-Propanol		+						
2-Propanol		+						
1-Butanol		+						
Acetate						+		
Growth on methanol		+			–	–		+
Assimilation of ammoniac nitrogen				+	+ (D-glucose)	+		+ (D-glucose)
Growth on N_2 free medium						+/–		
Production of cellulose								
Production of water-soluble pigment(s)						+/–	+/–	
Growth in the presence of 0.35 % acetic acid		+		–				

Nutritional requirement		Pantothenic acid					Nicotinic acid/no	
Requirement of acetic acid								
Growth on 30 % glucose	−/+	+	+	−		+		
Growth on 1.0 % KNO_3								
Growth at 37 °C	+					−		
Range of growth temperature				10–37 °C	20–37 °C	15–30 °C		25–37 °C
Opt. temperature					28 °C	20–30 °C		35–37 °C
Max. temperature				Not grow at 42 °C				Not grow at 42 °C
Min. temperature								
Growth at pH 3.0				+		+	+	
Range of growth pH		3.0–8.0		3.0–8.5				4.0–7.5
Opt. pH					5.0–7.0			5.0–6.0
Max. pH					3.5			Not at pH 3.5
Min. pH								
Pathogenicity								To humans
Major cellular fatty acids	$C_{18:1}$ ω7c	$C_{18:1}$ω7c	$C_{18:1}$ω7c	$C_{18:1}$ω7c; $C_{16:0}$	$C_{18:1}$ω7c; $C_{19;0}$cycloω8c; $C_{16:0}$	$C_{18:1}$ω7c	$C_{18:1}$ω7c	$C_{18:1}$ω7c; $C_{16:1}$
Major isoprenoid quinone	Q-9	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10
G+C content of DNA	52–61	62–63	66	60–61		62–67	54–62	
G+C content of type strain		62			60.3			59.1
Isolation sources	Beer, cider, curd of tofu, fermented cocoa beans, fermented rice flour, banana, mango, nata de coco, Kombucha, rotten apple, spoiled red wine, sugar cane, canna flower, tempeh, guava	Type strain was isolated from a septic methanol/yeast process. abundantly isolated from activated sludge	Flower of red ginger (*Alpinia purpurea*)	Orchid tree, astilbes, Asian dayflower, heliconia, spider lily, self-heal crown flower, African tulip tree, fermented glutinous rice in Indonesia	A surface-sterilized nodule of alfalfa (*Medicago sativa*)	Kaki, roots and stem of sugarcane, rhizosphere of coffee plants, Kitora tumulus Japan	Dahlia, Chinese bayberry, strawberry, rambutan, kaki, grape, spoiled jackfruit	Lymph node culture of a chronic granulomatous patient
Remarks	*A. nitrogenifigens* has polar flagella	*not accumulated in culture media						

Table 1.2 (continued)

	Komagataeibacter	*Kozakia*	*Neoasaia*	*Neokomagataea*	*Nguyenibacter*	*Saccharibacter*	*Swaminathania*	*Tanticha-roenia*
Type species	*K. xylinus*	*K. baliensis*	*N. chiangmai-ensis*	*N. thailandica*	*N. vanlangensis*	*S. floricola*	*S. salitolerans*	*T. sakaeratensis*
Cells	Coccoid to rods	Rods	Rods	Rods	Rods	Straight rods	Straight rods	Rods
Size (μm)	0.6–0.8 × 1.0–3.0	0.6–0.8 × 2.0–3.0	0.8–1.0 × 1.0–2.0	0.6–0.8 × 1.0–1.6	0.6–0.8 × 1.0–1.6	0.8–1.0 × 2.5–4.0	0.7–0.9 × 1.9–3.1	0.6–0.8 × 1.0–1.6
Motility	−	−	−	−	+	−	+	−
Flagella					Peritrichous		Peritrichous	
Catalase	+	+			+	+	+	
Oxidase						−		
Growth on								
Glutamate agar	+	−	+	+	+	−	+	+w
Mannitol agar	+	+	+	+	+	+	+	+
Colonial appearance	Raised, convex to umbonate, smooth to rough, entire to irregular, butyrous		Raised, smooth, entire, shiny, pink	Smooth, entire, creamy	Smooth, entire, transparent, creamy to brown	Circular, entire, pale in color	Raised, smooth, entire, yellowish to dark orange	Smooth, entire, creamy
Oxidation of								
Acetate	+	+	−	−	+		+w	−
Lactate	+	+w	−	−	−	+w	+w	−
Acetic acid from ethanol	+	+w	+	+w	−	Negligible or no	+	+
Production of DHA	+	−	+w		−			
Production of								
D-Gluconate	+	+				+		
2-Keto-D-gluconate	+	+	+	+	+	+		+
5-Keto-D-gluconate	+/−	+	+	+	+	+		+
2,5-Diketo-D-Gluconate	−	−		+				+
Acid from								
L-Arabinose	+	+		+/+w	+	+	+	+
L-Sorbose	−	−	−	−	−	V		−

D-Xylose	+	+	+	+	+	+		+
L-Rhamnose		−	+			−		
D-Fructose	−	−	+d	+	+	+	+w	+w
D-Galactose	+	+	+	+/+W	+w	+	+	+
D-Glucose	+	+	+	+	+	+	+	+
D-Mannose		+	+	+w/-		+w	+	+
Lactose								−
Maltose	−		−	−	+	−		−
Melibiose			+	+/−	+	+		+
Sucrose	+/−	V	+	+	+	+		+w
Trehalose								
Raffinose		+	+	+/−	+w	+		+w
Dulcitol		−	+w		−			−
meso - Erythritol		+	+	+w/-				+
D-mannitol	−	−	+w	−	+		−	
D-sorbitol	−	−	+d	−	+	−	+	−
Glycerol	+/−	+	+	−	−	−	+	+
Ethanol	+		+	+w/−	−	−	+	+
1-Propanol								
2-Propanol								
1-Butanol								
Growth on								
L-Sorbose			+	−	+w			−
D-Xylose	+/−		+	−	+w			+
L-Rhamnose			−	+/+W				
D-Fructose	+		+	−	+w			+
D-Galactose	+/−		+	−	+			
D-Glucose	+		+	+	+			+
D-Mannose			+w	−				−
Lactose				−	−			−
Maltose	+		−	−	+			−
Melibiose			−	−	+w			−
Sucrose	+		+	+	+			+
Trehalose	+							
Raffinose			+	+/−	+			−
Dulcitol			+	−	−			−

Table 1.2 (continued)

	Komagataeibacter	*Kozakia*	*Neoasaia*	*Neokomagataea*	*Nguyenibacter*	*Saccharibacter*	*Swaminathania*	*Tanticha-roenia*
meso - Erythritol			+	−				+
D-Mannitol	+		+	−	+w			+
D-Sorbitol	+		+	−	+w			+
Glycerol	+		+	−	+			+
Ethanol	+/−		−	−	−			−
1-Propanol								
2-Propanol								
1-Butanol								
Acetate								
Growth on methanol		−					−	−
Assimilation of ammoniac nitrogen	+	−	+w	−	+ (D-mannitol)			−
Growth on N_2 free medium					+		+	
Production of cellulose	+/−							
Production of water-soluble pigment(s)					+		+	+
Growth in the presence of 0.35 % acetic acid	+	+	+	−	+w		+	−
Nutritional requirement								
Requirement of acetic acid	−/+							
Growth on 30 % glucose	+	−	+	+	+w	+**		+
Growth on 1.0 % KNO_3					−		+	
Growth at 37 °C			−					

Range of growth temperature	25–37 °C					20–33 ° C		
Opt. temperature						25–30 ° C		
Max. temperature								
Min. temperature								
Growth at pH 3.0		+						
Range of growth pH						4.0–7.5		
Opt. pH						5.0–7.0		
Max. pH						above 8.0		
Min. pH						below 4.0		
Pathogenicity								
Major cellular fatty acid(s)	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$; $C_{16:1}$	$C_{18:1}7c$; $C_{16:0}$	$C_{18:1}\omega7c$; $C_{16:0}$ $C_{18:1}$ 2-OH $C_{18:0}$	$C_{18:1}\omega7c$	$C_{16:0}$ 2-OH, $C_{18:1}\omega7c$	$C_{18:0}\omega7c/\omega9t/\omega l2t$	
Major isoprenoid quinone	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10
G+C content of DNA	56–64	57		51–57	68–69	52–53	58–60	65–66
G+C content of type strain			63.1					
Isolation sources	Many kinds of vinegar, Kombucha, nata de coco, cider, apple juice, beet juice, cherry	Palm brown sugar, ragi	Flower of red ginger (*Alpinia purpureus*)	Flower of lantana (*Lantana camera*), candle bush *(Senna alata*)	Rhizosphere and roots of Asian rice	Pollens of flowers	Wild rice (*Proteresia coarctata*)	Soil in Thailand
Remarks		Production of fructan from sucrose				Osmophilic. *no growth on ordinary glutamate agar, but grown on the medium supplemented with 7 % glutamate.	Fix nitrogen and solubilize phosphate	

Symbols: +, positive in most species; +w, weakly positive; +/−, positive in more than half species; −/+, negative in more than half species; v, variable; and −, negative in most species

Acetobacter Beijerinck 1898

A.ce.to.bac'ter. L. n. *acetum*, vinegar; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Acetobacter*, vinegar rod.

The genus *Acetobacter* is the oldest in the history of acetic acid bacteria. The role of acetic acid bacteria could date back to the study in 1822, in which a living organism was recognized on the film formed on acetifying wine, and was named *Mycoderma* (Persoon 1822). Further, the relationship was mentioned between the film and the process of acetification and the organism was identified as *Ulvina aceti*, but it was an alga (Kützing 1837). Moreover, the relationship was established between the microbial film, oxygen, and acetification, but its microbial characteristics were failed to recognize and the organism was described as "little plant", a microscopic fungus, *Mycoderma aceti*. The results were presented in a lecture given in Orleans, which was also published as "Etudes sur la vinaigre" (Pasteur 1868). Following these studies, a cellulose-forming species *Bacterium xylinum* was described (Brown 1886), and *Bacterium aceti*, *Bacterium pasteurianum*, and *Bacterium küzingianum* were isolated from vinegar (Hansen 1894) (see Adams 1998; Asai 1968).

The following comment was described in The Index Bergeyana: [Beijerinck used the vernacular name azijnbacterien for the acetic acid bacteria. Apparently at some time before 1900 the vernacular name was rendered into neo-Latin as *Acetobacter* and finally used in publication. There is no record of its formal proposal as a genus. Kluyver (in) 1940 states: "... It is surprising that neither Beijerinck nor Hoyer proposed in their publications the creation of a new genus for the acetic acid bacteria... There can be no doubt that, in any case morally, but probably also according to the code of Botanical Nomenclature, Beijerinck is to be considered as the author of the genus *Acetobacter* as it occurs today..." The nomenclatural status of *Acetobacter* Beijerinck has been referred to the Judicial Commision of the International Commitee] (Buchanan et al. 1966). Kluyver express the same comment in the biography of Beijerinck (Kluyver 1983). The Approved Lists of Bacterial Names cited the genus *Acetobacter* as *Acetobacter* Beijerinck 1898 (Skerman et al. 1980).

Asai divided acetic acid bacteria into two genera: One group comprised the species oxidizing ethanol more actively than D-glucose and was motile with peritrichous flagella and Asai named them the genus *Acetobacter*, and the other consisted of the species oxidizing D-glucose more strongly than ethanol and was motile with polar flagella and Asai named them the genus *Gluconobacter* (Asai 1935, 1968).

Since then, the two genera have been the core in acetic acid bacteria. The genus *Acetobacter* is clearly differentiated from the genus *Gluconobacter* by quinone systems, abovementioned biochemical behaviors, and oxidation of acetate and lactate. The genus *Acetobacter* is the type genus of the family *Acetobacteraceae* (Sievers and Swings 2005b) (details are shown below in the section *Gluconobacter*).

The type species is *Acetobacter aceti* (Pasteur 1864) Beijerinck 1898. The closest neighbors are the genera *Gluconobacter, Neokomagataea*, and *Saccharibacter* (*Fig. 1.1*). Currently, the genus contains 23 species.

Acetobacter Beijerinck 1898

Cells are Gram negative, ellipsoidal to rod-shaped, measuring 0.4–1.0 by 1.2–3.0 μm, rarely longer cells. Cells occur singly or short chains and occasionally long chains. Some strains in *Acetobacter cerevisiae* produce involution forms like swollen cells. Peritrichously flagellated when motile. Exceptionally, *Acetobacter nitrogenifigens* has polar flagella. Endospores are not produced. Colonial appearance varies with cultural conditions. Generally colonies are circular, smooth, entire, convex, cream color to beige, opaque, and butyrous on glucose-yeast extract-peptone agar.

Strictly aerobic. Catalase positive (negative in *Acetobacter peroxydans*), and oxidase negative. Acetic acid is produced from ethanol. Acetate and lactate are oxidized. Usually dihydroxyacetone is not produced from glycerol, but produced by a few species. D-Gluconate and 2-keto-D-gluconate are produced from D-glucose by most of the species, and 5-keto-D-gluconate is produced by a few species. 2,5-Diketo-D-gluconate is not produced.

Generally *Acetobacter* species produce acid from a rather limited number of sugars, sugar alcohols, and alcohols. Acid production is variable with species and strains on L-arabinose, D-xylose, and D-galactose. Acid is not produced from D-fructose, lactose, maltose, melibiose, sucrose, trehalose, raffinose, D-mannitol, and sorbitol. Usually ammoniac nitrogen is not utilized. Oxidation of methanol is described by some species, but the precise test is needed (Suzuki et al. 2009). Real cellulose is not produced. The activity of nitrogen fixation is described only about *A. nitrogenifigens*.

Optimal growth temperature is around 30 °C. Most of species are able to grow at 37 °C, but not at 45 °C. They are able to grow between pH 3.5 and 8.0. Most of species are unable to grow in the presence of 30 % glucose. Growth does not occur in the presence of 10 % ethanol. The major cellular fatty acid is $C_{18:1}\omega7c$, and minor components are $C_{16:0}$, $C_{16:0}$ 2-OH, $C_{14:0}$, $C_{14:0}$ 2-OH, and $C_{19:0}$ cyclo ω8c. The major ubiquinone is Q-9. The G+C content of DNA is 53.5–60.7 mol%.

Acetobacter strains are widely distributed in vinegars, fermented foods (wine, beer, sake, nata de coco, pickles, palm wine, moromi, curd of tofu, tempeh, etc.), fruits (guava, sapodilla, star fruit, mangosteen, banana, papaya, durian, etc.), and flowers (canna, etc.). Generally, *Acetobacter lovaniensis*, *A. indonesiensis*, *A. orleanensis*, *A. pasteurianus*, and *A. tropicalis* were isolated in descending order from fermented foods, fruits, and flowers in Indonesia (Lisdiyanti et al. 2001).

Acetobacter aceti (Pasteur 1864) Beijerinck 1898

Data of this species are fragmentally recorded. Therefore, the characteristics are mainly cited from those described for the type strain of this species (Lisdiyanti et al. 2000), referring to other papers (Gosselé et al. 1983b).

Type strain: ATCC 15973^T (= CCUG 18122^T = CIP 103111^T = DSM 3508^T = ICMP 8807^T = JCM 7641^T = LMG 1261^T = LMG 1504^T = NCCB 23001^T = NBRC 14818^T = NCIMB 8621^T), isolated from beechwood

shavings of a vinegar plant. The G+C content of DNA of the type strain is 57.2 mol%.

Acetobacter cerevisiae Cleenwerck, Vandemeulebroecke, Janssens, and Swings 2002

Characteristics are the same as those described for the species (Cleenwerck et al. 2002).

Type strain: LMG 1625^T (= ATCC 23765^T = DSM 14362^T = JCM 17273^T = LMG 1625^T = NCIMB 8894^T), isolated from beer (ale) in storage at Toronto, Canada (Kozulis and Parsons 1958). The G+C content of DNA of the type strain is 57.6 mol%.

Acetobacter cibinongensis Lisdiyanti, Kawasaki, Seki, Yamada, Uchimura, and Komagata 2002

Characteristics are the same as those described for the species (Lisdiyanti et al. 2001).

Type strain: 4H-1^T (= CIP 107380^T = DSM 15549^T = JCM 11196^T = NBRC 16605^T), isolated from mountain soursop (*Annona montana*) in Indonesia. The G+C content of DNA of the type strain is 54.5 mol%.

Acetobacter estunensis (Carr 1958) Lisdiyanti, Kawasaki, Seki, Yamada, Uchimura, and Komagata 2001

Characteristics are the same as those described for the species (Lisdiyanti et al. 2000).

Type strain: NBRC 13751^T (= ATCC 23753^T = DSM 4493^T = JCM 21172^T = LMG 1626^T = NCIMB 8935^T), isolated from cider, Bristol. The G+C content of DNA of the type strain is 59.7 mol%.

Acetobacter fabarum Cleenwerck, Gonzalez, Camu, Engelbeen, De Vos, and De Vuyst 2008

Characteristics are the same as those described for the species (Cleenwerck et al. 2008).

Type strain: 985^T (= R-36330^T = DSM 19596^T = LMG 24244^T), isolated from a Ghanaian cocoa heap fermentation. The G+C content of DNA of the type strain is 57.6 mol%.

Acetobacter farinalis Tanasupawat, Kommanee, Yukphan, Muramatsu, Nakagawa, and Yamada 2011

Characteristics are the same as those described for the species (Tanasupawat et al. 2011a).

Type strain: G360-1^T (= BCC 44845^T = NBRC 107750^T = PCU 319^T), isolated from fermented rice flour. The G+C content of DNA of the type strain is 56.3 mol%.

Acetobacter ghanensis Cleenwerck, Camu, Engelbeen, De Winter, Vandemeulebroecke, De Vos, and De Vuyst 2007

Characteristics are the same as those described for the species (Cleenwerck et al. 2007).

Type strain: R-29337^T (= 430A^T = DSM 18895^T = LMG 23848^T), isolated from a traditional heap fermentation of Ghanaian cocoa beans. The G+C content of DNA of the type strain is 57.3 mol%.

Acetobacter indonesiensis Lisdiyanti, Kawasaki, Seki, Yamada, Uchimura, and Komagata 2001

Characteristics are the same as those described for the species (Lisdiyanti et al. 2000).

Type strain: 5H-1^T (= JCM 10948^T = LMG 19824^T = NBRC 16471^T = NRIC 0313^T), isolated from fruit of zirzak (*Annona muricata*) in Indonesia. The G+C content of DNA of the type strain is 53.7 mol%.

Acetobacter lovaniensis (Frateur 1950) Lisdiyanti, Kawasaki, Seki, Yamada, Uchimura, and Komagata 2001

Characteristics are the same as those described for the species (Lisdiyanti et al. 2000).

Type strain: NBRC 13753^T (=ATCC 12875^T= DSM 4491^T = JCM 17121^T = LMG 1579^T = LMG 1617^T = NCIMB 8620^T), isolated from sewage on soil by Frateur, 1929. The G+C content of DNA of the type strain is 58.6 mol%.

Acetobacter malorum Cleenwerck, Vandemeulebroecke, Janssens, and Swings 2002

Characteristics are the same as those described for the species (Cleenwerck et al. 2002).

Type strain: LMG 1746^T (= DSM 14337 = JCM 17274^T), isolated from a rotten apple in Ghent, Belgium (Gosselé et al. 1983a, b). The G+C content of DNA of the type strain is 57.2 mol%.

Acetobacter nitrogenifigens Dutta and Gachhui 2006

Characteristics are the same as those described for the species (Dutta and Gachhui 2006).

Type strain: RG1^T (= LMG 23498^T = MTCC 6912^T), isolated from kombucha tea. The G+C content of DNA of the type strain is 64.1 mol%.

Acetobacter oeni Silva, Cleenwerck, Rivas, Swings, Trujillo, Willems, and Velázquez 2006

Characteristics are the same as those described for the species (Silva et al. 2006).

Type strain: B13^T (= CECT 5830^T = LMG 21952^T), isolated from spoiled red wine of the Dão region, Portugal. The G+C content of DNA of the type strain is 58.1 mol%.

Acetobacter okinawensis Iino, Suzuki, Kosako, Ohkuma, Komagata, and Uchimura 2013

Characteristics are the same as those described for the species (Iino et al. 2012a).

Type strain: 1-35^T (= JCM 25146^T = LMG 26457^T = NRIC 0658^T), isolated from a piece of the stem of sugarcane, Okinawa, Japan. The G+C content of DNA of the type strain is 59.3 mol%.

Acetobacter orientalis Lisdiyanti, Kawasaki, Seki, Yamada, Uchimura, and Komagata 2002

Characteristics are the same as those described for the species (Lisdiyanti et al. 2001).

Type strain: 21 F-2^T (= CIP 107379^T = DSM 15550^T = JCM 11195^T = NBRC 16606^T = NRIC 0481^T), isolated from canna flower (*Canna hybrida*) in Indonesia. The G+C content of DNA of the type strain is 52.3 mol%.

Acetobacter orleanensis (Henneberg 1906) Lisdiyanti et al. 2001

Characteristics are the same as those described for the species (Lisdiyanti et al. 2000).

Type strain: NBRC 13752^T (= ATCC 12876^T = CCUG 18126^T = DSM 4492^T = JCM 7639^T = LMG 1583^T = NCCB 31003^T = NCIMB 8622^T), isolated from beer by Frateur in 1929. The G+C content of DNA of the type strain is 58.6 mol%.

Acetobacter papayae Iino, Suzuki, Kosako, Ohkuma, Komagata, and Uchimura 2013

Characteristics are the same as those described for the species (Iino et al. 2012a).

Type strain: 1-25^T (= JCM 25143^T = LMG 26456^T = NRIC 0655^T), isolated from a papaya fruit, Okinawa, Japan. The G+C content of DNA of the type strain is 60.5 mol%.

Acetobacter pasteurianus (Hansen 1879) Beijerinck and Folpmers 1916

Data of this species are fragmentally recorded. Therefore, the characteristics are mainly cited from those described for the type strain of this species (Lisdiyanti et al. 2000), referring to other paper (Gosselé et al. 1983b).

Type strain: LMG 1262^T (=ATCC 33445^T = CCUG 18125^T = CIP 103108^T = DSM 3509^T = JCM 7640^T = LMD 22.1^T = LMG 1262^T = NCCB 22001^T), isolated from beer, Netherlands. The G+C content of DNA of the type strain is 52.7 mol%.

Acetobacter peroxydans Visser't Hooft 1925

Data of this species are fragmentally recorded. Therefore, the characteristics are mainly cited from those described for the type strain of this species (Lisdiyanti et al. 2000), referring to other paper (Gosselé et al. 1983b).

Type strain: NBRC 13755^T (=ATCC 12874^T = JCM 25077^T = LMG 1635^T = NCCB 88049^T = NCCB 89009^T), isolated from ditchwater, Delft, Netherlands. The G+C content of DNA of the type strain is 60.3 mol%.

Acetobacter persici Iino, Suzuki, Kosako, Ohkuma, Komagata, and Uchimura 2013

Characteristics are the same as those described for the species (Iino et al. 2012a).

Type strain: T-120^T (= JCM 25330^T = LMG 26458^T), isolated from a peach fruit, Okinawa, Japan. The G+C content of DNA of the type strain is 58.7 mol%.

Note: The original spelling of the specific epithet, *persicus*, has been corrected on validation according to Rule 61.

Acetobacter pomorum Sokollek, Hertel, and Hammes 1998

Characteristics are the same as those described for the species (Sokollek et al. 1998).

Type strain: LTH 2458^T (= CIP 105762^T = DSM 11825^T = LMG 18848^T), isolated from a submerged cider vinegar fermentation at a factory in the southern part of Germany. The G+C content of DNA of the type strain is 50.5 mol%. *A. pomorum* DSM 11825^T did not indicate the presence of the *maxF* gene and did not grow on methanol (Suzuki et al. 2009).

Acetobacter senegalensis Ndoye, Cleenwerck, Engelbeen, Dubois-Dauphin, Guiro, Van Trappen, Willems, and Thonart 2007

Characteristics are the same as those described for the species (Ndoye et al. 2007).

Type strain: CWBI-B418^T (= DSM 18889^T = LMG 23690^T), isolated from mango fruit in Senegal (sub-Saharan Africa). The G+C content of DNA of the type strain is 56.0 mol%.

Acetobacter syzygii Lisdiyanti, Kawasaki, Seki, Yamada, Uchimura, and Komagata 2002

Characteristics are the same as those described for the species (Lisdiyanti et al. 2001).

Type strain: 9H-2^T (= CIP 107378^T = DSM 15548^T = JCM 11197^T = NBRC 16604^T = NRIC 0483^T), isolated from fruit of Malay rose apple (*Syzygium malaccense*) in Indonesia. The G+C content of DNA of the type strain is 55.3 mol%.

Acetobacter tropicalis Lisdiyanti, Kawasaki, Seki, Yamada, Uchimura, and Komagata 2001

Characteristics are the same as those described for the species (Lisdiyanti et al. 2000).

Type strain: Ni-6b^T (= JCM 10947^T = LMG 19825^T = NBRC 16470^T = NRIC 0312^T), isolated from coconut (*Cocos nucifera*) in Indonesia. The G+C content of DNA of the type strain is 55.9 mol%.

Acidomonas Urakami, Tamaoka, Suzuki, and Komagata 1989. emend. Yamashita, Uchimura, and Komagata 2004

A.ci.do.mo'nas. L. adj. *acidus*, sour, acid; L. fem. n. *monas*, a unit, monad; N.L. fem. n. *Acidomonas*, acidophilic monad.

Establishment of this genus can be traced back to the isolation of methanol-utilizing bacteria from a non-sterile fermentation process for the production of single-cell protein (SCP) from methanol with *Candida* species (Steudel et al. 1980). The strains were identified as a new species, *Acetobacter methanolicus*, on the basis of the growth on methanol and ethanol at a low initial concentration (Uhlig et al. 1986). Later, the genus *Acidomonas* was created for acidophilic, facultatively methylotrophic bacteria, with incorporating *A. methanolicus*, on the basis of DNA-DNA similarity that showed difference from those of *Acetobacter*, *Gluconobacter*, *Acidiphilium*, and *Thiobacillus* (Urakami et al. 1989).

The phylogenetic relationship of the genus *Acidomonas* to acetic acid bacteria (the family *Acetobacteraceae*) was first suggested by 5S rRNA sequencing (Bulygina et al. 1992) and subsequently confirmed by 16S rDNA sequences (Yamada et al. 1997; Boesch et al. 1998; Yamashita et al. 2004). The revival of *Acetobacter methanolicus* was once proposed (Sievers et al. 1994b), but a distant relation was recognized between the genus *Acidomonas* and other genera in the family *Acetobacteraceae* (Yamada et al. 1997, 2000; Yamashita et al. 2004).

However, this taxon was problematic because some characteristics differed from the previous descriptions (Uhlig et al. 1986; Urakami et al. 1989). Cells of *Acidomonas methanolica* had a single polar flagellum (or occasionally polar tuft flagella); this differed from a previous study that described peritrichous flagella (Yamashita et al. 2004). A few *Acidomonas methanolica* strains were available from culture collections worldwide. This low number of strains was not enough to verify the diversity of the species. Therefore, novel *Acidomonas* strains were isolated from activated sludge samples of sewage treatment plants, and the genus *Acidomonas* was recharacterized by using the new isolates and strains that were obtained from culture collections and with newly obtained 16S rDNA sequences, DNA-DNA similarities, quinone systems, and phenotypic characteristics. Thus, the genus *Acidomonas* was emended (Yamashita et al. 2004). The genus *Acidomonas* is a methanol-utilizing bacterium in the acetic acid bacteria (Suzuki et al. 2009).

The type species is *Acidomonas methanolica* (Uhlig et al. 1986) Urakami et al. 1989 emend. Yamashita, Uchimura and Komagata 2004. The genus *Acidomonas* is phylogenetically rather distant from known genera in the family *Acetobacteraceae* (*Fig. 1.1*). Currently, the genus comprises a single species, *Acidomonas methanolica*.

Acidomonas Urakami et al. 1989. emend. Yamashita et al. 2004

Cells are Gram negative and short rods with rounded ends, measuring 0.5–0.8 by 1.5–2.0 μm, and are occasionally up to 4 μm in length. Cells occur singly, in pairs, or (rarely) in short chains and are either motile with a single polar flagellum or nonmotile. Cells with polar tuft flagella are found very rarely. Endospores are not produced. Colonies are shiny, smooth, circular, convex, entire, beige to pink, and 1–3 mm in diameter on PYM agar (pH 4.5) after 5 days at 30 °C. Pellicles are produced in PYM broth, but they are not real cellulose.

Aerobic. Catalase positive. Oxidase negative. Acetic acid is produced from ethanol. Acetate is oxidized, but lactate is not or only weakly oxidized. Dihydroxyacetone is not produced from glycerol. D-Gluconate is produced from D-glucose, but 2-keto-, 5-keto-, or 2,5-diketo-D-gluconate does not accumulate in culture media. Acid is produced from L-arabinose, D-xylose, D-ribose, D-galactose, D-mannose, D-glucose, glycerol, *n*-propanol, *n*-butanol, 2-methylpropan-1-ol, ethanol, and methanol, but not from L-rhamnose, D-fructose, sucrose, maltose, lactose, raffinose, trehalose, D-mannitol, inositol, D-sorbitol, dulcitol, or soluble starch. Production of acid from D-arabinose, D-ribose, melibiose, and 2-methylpropan-1-ol varies with strains. Methanol, ethanol, D-glucose, D-mannose, glycerol, and succinic acid are utilized as sole sources of carbon, but D-fructose, L-arabinose, maltose, trehalose, inositol, and D-mannitol are not utilized. Pantothenic acid is an essential requirement for growth. Urease negative.

Growth occurs in the presence of 30 % glucose and 0.35 % acetic acid. Growth occurs at the same extent between pH 3.0 and 8.0; acidotolerant. Growth occurs at 30 °C, but not at 45 °C.

Major cellular fatty acids are $C_{18:1}$ and $C_{16:0}$, and major hydroxyl acids are $C_{16:0}$ 2-OH and $C_{16:0}$ 3-OH. Major ubiquinone is Q-10. The G+C content of DNA ranges from 62 to 63 mol%.

The type strain was isolated from a non-sterile fermentation process for the production of single-cell protein (SCP) from methanol with *Candida* species. *Acidomonas* strains were most abundantly isolated from activated sludge samples, but not from vegetables, fruit, decayed wood and leaves, manure, and paddy soil (Yamashita et al. 2004).

Acidomonas methanolica Urakami, Tamaoka, Suzuki, and Komagata 1989. emend. Yamashita, Uchimura, and Komagata 2004

Characteristics are the same as those described for the emended genus (Yamashita et al. 2004). The type strain: MB 58^T (= DSM 5432^T = IMET 10945^T = JCM 6891^T = LMG 1668^T = NRIC 0498^T), isolated from a non-sterile fermentation process for the production of single-cell protein (SCP) from methanol with *Candida* species. Cells of the type strain are nonmotile, and the G+C content of DNA is 62 mol%.

Ameyamaea Yukphan, Malimas, Muramatsu, Takahashi, Kaneyasu, Potacharoen, Tanasupawat, Nakagawa, Hamana, Tahara, Suzuki, Tanticharoen, and Yamada 2010

A.me.ya.ma'e.a. N. L. fem. n. named after Dr. Minoru Ameyama, Professor Emeritus of Yamaguchi University, Yamaguchi, Japan, who contributed studies of acetic acid bacteria, especially their biochemical and systematic studies.

Ameyamaea strains were isolated from the flowers of red ginger (*Alpinia purpurea*) collected in Chiang Mai, Thailand. They are motile with polar flagella. The strains intensely oxidize acetate to CO_2 and water and weakly oxidize lactate. Gene restriction analysis of 16S rRNA with two restriction endonucleases, TaqI and Hin6I, discriminates the strains from those of other genera in acetic acid bacteria. Thus, the genus *Ameyamaea* was established (Yukphan et al. 2009).

The type species is *Ameyamaea chiangmaiensis* Yukphan et al. 2010. The closest neighbor is the genus *Tanticharoenia* (Fig. 1.1). Currently, the genus contains a single species, *Ameyamaea chiangmaiensis*.

Ameyamaea Yukphan et al. 2010

Cells are Gram negative, rod-shaped, measuring 0.6–0.8 by 1.0–1.8 μm, and motile with polar flagella. The strains grow on glutamate agar (weakly positive) and mannitol agar. Colonies are creamy and smooth with entire margin on glucose-ethanol-peptone-yeast extract-calcium carbonate agar.

Acetic acid is produced from ethanol. Acetate is intensely oxidized to carbon dioxide and water, but lactate is weakly oxidized. Dihydroxyacetone is weakly produced from glycerol. 2-Keto-D-gluconate and 5-keto-D-gluconate are produced from D-glucose.

Acid is produced from D-glucose, D-mannose, D-galactose, D-xylose, D-arabinose (weakly positive), L-arabinose, L-rhamnose, *meso*-erythritol, glycerol (weakly positive), melibiose, and ethanol, but not from D-fructose, L-sorbose, D-mannitol, D-sorbitol, dulcitol, maltose, lactose, sucrose, or raffinose. Growth occurs on D-glucose, D-mannose (very weakly positive), D-galactose, D-xylose, L-arabinose, L-rhamnose, D-fructose, L-sorbose, D-mannitol, D-sorbitol, dulcitol, *meso*-erythritol, glycerol, and melibiose (very weakly positive), but not on D-arabinose, maltose, lactose, sucrose, raffinose, or ethanol. Growth is weak on methanol. Ammoniac nitrogen is very weakly assimilated in the presence of D-glucose, D-mannitol, or ethanol as a carbon source. A water-soluble brown pigment is not produced on glucose-peptone-yeast extract-calcium carbonate agar.

Growth occurs in the presence of 0.35 % acetic acid (w/v). Growth does not occur on 30 % D-glucose (w/v). A major isoprenoid quinone is Q-10. The G+C content of DNA is 66.0–66.1 mol% G+C, with a range of 0.1 mol%.

Ameyamaea chiangmaiensis Yukphan, Malimas, Muramatsu, Takahashi, Kaneyasu, Potacharoen, Tanasupawat, Nakagawa, Hamana, Tahara, Suzuki, Tanticharoen, and Yamada 2010

Characteristics are the same as those described for the species (Yukphan et al. 2009). The type strain is AC04^T (= BCC 15744^T = NBRC 103196^T), isolated from a flower of red ginger (*Alpinia purpurea*) in Chiang Mai, Thailand. The G+C content of DNA of the type strain is 66.0 mol%.

Asaia Yamada, Katsura, Kawasaki, Widyastuti, Saono, Seki, Uchimura, and Komagata 2000

A.sa'i.a. N.L. fem. n. named after Prof. Toshinobu Asai, a Japanese bacteriologist who contributed to the systematics of acetic acid bacteria.

Asaia strains were first isolated from flowers of orchid tree (*Bauhinia purpurea*) and plumbago (*Plumbago auriculata*) and fermented glutinous rice in Indonesia. The strains produced no or only limited amounts of acetic acid from ethanol and did not grow in the presence of 0.35 % acetic acid. Further, they were distant from the known genera in the family *Acetobacteraceae* on the basis of 16S rRNA gene sequences. Therefore, the bacterial strains were classified in a new genus, *Asaia*, on the basis of phenotypical characteristics and the phylogenetic position. The type strain was named *Asaia bogorensis* after the isolation site, Bogor, Indonesia (Yamada et al. 2000).

Since the description of *A. bogorensis*, *A. siamensis* (Katsura et al. 2001), *A. krungthepensis* (Yukphan et al. 2004b), *A. lannensis* (Malimas et al. 2008c), *A. spathodeae*

Table 1.3
Differential characteristics of *Asaia* species

	A. bogorensis	*A. astilbis*	*A. krungthepensis*	*A. lannensis*	*A. platycodi*	*A. prunellae*	*A. siamensis*	*A. spathodea*
Flagella	Peritrichous	Lateral	Peritrichous	Peritrichous	Lateral	Lateral	Peritrichous	Peritrichous
Acetic acid from ethanol	−(w)	−	−	+	v	−	−(w)	+
Acid from								
L-Rhamnose		−	+	+	+	−	−	−
Dulcitol	+	−	+	+(w)	−	−	+	−
Growth in the presence of 0.35 % acetic acid	−	−		−	−	−	−	w
Growth on 30 % glucose		+		+	+	+		+
Growth at 37 °C		−			−	+	+	
Growth pH 3.0	+	+	+	+	+	+		+
Isoprenoid quinone	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10
G+C content of DNA of the type strain	60.2	58.9	60.3	60.8	60.0	58.9	59.3	59.7

Symbols: +, positive in most strains; w, weakly positive; v, variable with strains; and −, negative in most strains

(Kommanee et al. 2010), *A. astilbis* (Suzuki et al. 2010), *A. platycodi* (Suzuki et al. 2010), and *A. prunellae* (Suzuki et al. 2010) have been reported to date. *Asaia* strains were isolated not only from tropical countries, Indonesia, Thailand, and the Philippines, but also from a temperate region, Japan. Therefore, *Asaia* species are thought to be cosmopolitan. Further, the strains were isolated from clinical specimens and an Asian malarial mosquito vector, *Anopheles stephensi* (Favia et al. 2007). Species in the genus *Asaia* are barely differentiated with phenotypic characteristics; no or little production of acetic acid from ethanol, the growth in the presence of 0.35 % acetic acid, the growth on 30 % glucose, the acid production from a considerable number of sugars and sugar alcohols, and the production of pink to red colonies on agar plates would be possible features. DNA-DNA similarity and 16S rRNA gene sequences are reliable for the identification of the species. Possible differential characteristics are shown in *Table 1.3*.

The type species is *Asaia bogorensis* Yamada et al. 2000. The closest neighbor is the genus *Swaminathania* (*Fig. 1.1*). Currently, the genus *Asaia* comprises eight species.

Asaia Yamada et al. 2000

Cells are Gram negative, rod-shaped, measuring 0.4–1.0 by 1.0–2.5 (rarely 4.5) μm, and motile with peritrichous or lateral flagella. Endospores are not produced. Colonies are smooth, entire, raised, shiny, and light brown, pink to dark pinkish on GYP or GESA. Grows on glutamate agar and mannitol agar.

Aerobic. Catalase positive. Oxidase negative. Produces no or a limited amount of acetic acid from ethanol. Oxidizes acetate and lactate to carbon dioxide and water. Production of dihydroxyacetone from glycerol is generally positive. Produces 2-keto-D-gluconate and 5-keto-D-gluconate from D-glucose, but not 2,5-diketo-D-gluconate. Acid is produced from D-glucose, D-galactose, D-fructose, and other sugars and sugar alcohols, but not from lactose or maltose (with an exception). Grows on D-glucose, D-fructose, and D-mannitol, but not on lactose. Ammoniac nitrogen is assimilated on D-glucose and D-mannitol.

Grows on 30 % glucose, but not in the presence of 0.35 % acetic acid. Growth occurs between 10 °C and 30 °C, but not at 37 °C. However, *Asaia prunellae* is able to grow at 37 °C. Growth occurs between pH 3.0 and 8.5. Major cellular fatty acids are $C_{18:1}\omega7c$ and $C_{16:0}$ (*Asaia spathodeae*). Major quinone is Q-10. The G+C content of DNA is 58.6–61.0 mol%.

Asaia strains are isolated from a variety of such flowers as orchid tree, plumbago, astilbe, Asian dayflower, heliconia, spider lily, balloon flower, self-heal, crown flower, ixora, African tulip tree, etc.

Asaia bogorensis Yamada, Katsura, Kawasaki, Widyastuti, Saono, Seki, Uchimura, and Komagata 2000

Characteristics of the species are the same as those described for the species (Yamada et al. 2000).

Type strain: 71^T (= JCM 19569^T = NRIC 0311^T), isolated from orchid tree (*Bauhinia purpurea*) in Bogor, Indonesia. The G+C content of DNA of the type strain is 60.2 mol%.

Asaia astilbis Suzuki, Zhang, Iino, Kosako, Komagata, and Uchimura 2010

Characteristics of the species are the same as those described for the species (Suzuki et al. 2010).

Type strain: T-6133^T (= DSM 23030^T = JCM 15831^T), isolated from astilbe (*Astilbe thunbergii* var. *congesta*), Yamanashi, Japan. The G+C content of DNA of the type strain is 58.9 mol%.

Note: The original spelling of the specific epithet, *astilbes* (sic), has been corrected on validation according to Rule 61.

Asaia krungthepensis Yukphan, Potacharoen, Tanasupawat, Tanticharoen, and Yamada 2004

Characteristics of the species are the same as those described for the species (Yukphan et al. 2004b).

Type strain: AA08^T (= BCC 12978^T = NBRC 0535^T = TISTR 1524^T), isolated from a heliconia flower (*Heliconia* sp.) in Bangkok, Thailand. The G+C content of DNA of the type strain is 60.3 mol%.

Asaia lannensis Malimas, Yukphan, Takahashi, Kaneyasu, Potacharoen, Tanasupawat, Nakagawa, Tanticharoen, and Yamada 2008

Characteristics of the species are the same as those described for the species (Malimas et al. 2008c).

Type strain: AB92^T (= BCC 15733^T = NBRC 102526^T), isolated from a flower of spider lily (*Crinum asiaticum*) in Chiang Mai, Thailand. The G+C content of DNA of the type strain is 60.8 mol%.

Note: The specific epithet, *lannaensis* (sic), has been corrected on validation according to Rule 61.

Asaia platycodi Suzuki, Zhang, Iino, Kosako, Komagata, and Uchimura 2010

Characteristics of the species are the same as those described for the species (Suzuki et al. 2010).

Type strain: T-683^T (= JCM 25414^T = DSM 23029^T), isolated from balloon flower (*Platycodon grandiflorum*) in Akita, Japan. The G+C content of DNA of the type strain is 60.0 mol%.

Asaia prunellae Suzuki, Zhang, Iino, Kosako, Komagata, and Uchimura 2010

Characteristics of the species are the same as those described for the species (Suzuki et al. 2010).

Type strain: T-153^T (= DSM 23028^T = JCM 25354^T), isolated from self-heal (*Prunella vulgaris*) in Akita, Japan. The G+C content of DNA of the type strain is 58.9 mol%.

Asaia siamensis Katsura, Kawasaki, Potacharoen, Saono, Seki, Yamada, Uchimura, and Komagata 2001

Characteristics of the species are the same as those described for the species (Katsura et al. 2001).

Type strain: S60-1^T (= JCM 10715^T = NBRC 16457^T = NRIC 0323^T), isolated from a flower of crown flower (*Calotropis gigantea*), in Bangkok, Thailand. The G+C content of DNA of the type strain is 59.3 mol%.

Asaia spathodeae Kommanee, Tanasupawat, Yukphan, Malimas, Muramatsu, Nakagawa, and Yamada 2010

Characteristics of the species are the same as those described for the species (Kommanee et al. 2010).

Type strain: GB23-2^T (= BCC 36458^T = NBRC 105894^T = PCU 307^T), isolated from a flower of the African tulip (*Spathodea campanulata*) in Thailand. The G+C content of DNA of the type strain is 59.7 mol%.

Endobacter Ramírez-Bahena, Tejedor, Martín, Valázques, and Peix 2013

En.do.bac'ter. Gr. pref. *endo* within; N.L. masc. n. *bacter* a rod; *Endobacter* a rod isolated from the inside of a root nodule of *Medicago sativa*.

An *Endobacter* strain was isolated from a surface-sterilized alfalfa (*Medicago sativa*), as an endophyte, in Zamora, Spain. The results of *recA* gene analysis supported the classification of the strain in a new genus in the family *Acetobacteraceae*. Further, ITS fragment analysis showed the phylogenetic divergence of the strain with respect to other species in the family *Acetobacteraceae*. The results of *recA* gene and ITS fragment analysis of the strain agreed with those from 16S rRNA gene sequence analysis and supported the strain in a member of a new genus

within the family *Acetobacteraceae*. Thus, the genus *Endobacter* was established (Ramírez-Bahena et al. 2013).

The type species is *Endobacter medicaginis* Ramírez-Bahena et al. 2013. The genus *Endobacter* is phylogenetically rather distant from known genera in the family *Acetobacteraceae* (Fig. 1.1). Currently, the genus contains a single species, *Endobacter medicaginis*.

Endobacter Ramírez-Bahena et al. 2013

Cells are Gram negative and coccid to rod-shaped. Motile with subpolar flagella. Colonies are white and mucoid on YMA agar. Growth occurs on glutamate agar and mannitol agar.

Aerobic. Catalase positive. Oxidase negative. Urease negative. Acetate and lactate are not oxidized. Acetic acid is produced from ethanol. Dihydroxyacetone is produced from glycerol. Acid is produced from D-xylose, D-glucose, glycerol, and ethanol, but not from lactose, maltose, sucrose, dulcitol, D-mannitol, or D-sorbitol. Ammoniac nitrogen is assimilated on D-glucose.

Growth occurs between 20 °C and 37 °C with an optimum temperature for growth of 28 °C. Optimal pH for growth ranges from 5.0 to 7.0, but the growth occurs at pH 3.5.

Major cellular fatty acids are $C_{18:1}$ ω7*c* (39.94 %), $C_{19:0}$cyclo ω8*c* (12.15 %), and $C_{16:0}$ (13.40 %). The lipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, two aminophospholipids, three aminolipids, four glycolipids, two phospholipids, and one lipid. Major quinone is Q-10. The G+C content of DNA of the type strain is 60.3 mol%.

An *Endobacter* strain was isolated from a surface-sterilized nodule of alfalfa (*Medicago sativa*), Spain.

Endobacter medicaginis Ramírez-Bahena et al. 2013

Characteristics of the species are the same as those described for the species (Ramírez-Bahena et al. 2013).

Type strain: M1MS02^T (= CECT 8088^T = LMG 26838^T), isolated from a surface-sterilized nodule of alfalfa (*Medicago sativa*), Spain. The G+C content of DNA of the type strain is 60.3 mol%.

Gluconacetobacter Yamada, Hoshino, and Ishikawa 1998

Glu.con.a.ce.to.bac'ter. N. L. n. *acidum gluconicum*, gluconic acid; L. n. *acetum*, vinegar; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Gluconacetobacter*, gluconate-vinegar rod.

The genus *Gluconacetobacter* Yamada et al. 1998 (*Gluconoacetobacter* [sic]) was introduced into the family *Acetobacteraceae* by the elevation of the subgenus *Gluconacetobacter* (ex Asai 1935) Yamada and Kondo 1984 on the basis of partial 16S rRNA sequences with the type species of *Gluconacetobacter liquefaciens* (Asai 1935) Yamada et al. 1998 (Yamada et al. 1997). Thus, five *Acetobacter* species, *Acetobacter diazotrophicus*, *A. europaeus*, *A. hansenii*, *A. liquefaciens*, and *A. xylinus*, were transferred to the genus *Gluconacetobacter* as new combinations.

Further, *Gluconacetobacter liquefaciens*, *G. diazotrophicus*, and newly described *Gluconacetobacter sacchari* were positioned distantly from *Gluconacetobacter xylinus*, *G. europaeus*, and *G. hansenii* by 16S rRNA sequence similarities, and two groups were suggested in the genus *Gluconacetobacter* (Franke et al. 1999). The two groups or two subclusters were recognized in the genus *Gluconacetobacter* according to phylogenetic analyses based on 16S rRNA sequences and phenotypical and ecological point of view (Dellaglio et al. 2005; Lisdiyanti et al. 2006; Yamada and Yukphan 2008; Yamada et al. 2012a). The two groups were referred to as the *Gluconacetobacter liquefaciens* group and the *Gluconacetobacter xylinus* group in the genus *Gluconacetobacter*.

Differentiation between *G. liquefaciens* strains and *G. xylinus* strains was beforehand reported based on the G+C content of DNA, DNA-DNA similarity, and oxidation products from D-glucose (Navarro and Komagata 1999). In addition, the two groups were confirmed by multilocus analyses of housekeeping genes, *dnaK*, *groEL*, and *rpoB* as well (Cleenwerck et al. 2010). Consequently, the genus *Gluconacetobacter* was divided into two genera, the genus *Gluconacetobacter* for the *Gluconacetobacter liquefaciens* group and a new genus *Komagataeibacter* for the *Gluconacetobacter xylinus* group with 12 new combinations on the basis of the taxonomic characteristics (Yamada et al. 2012b). Some species have the activity of nitrogen fixation. Further, *Gluconacetobacter asukensis* and *G. tumulicola* were isolated from the Kitora Tumulus in Japan (Tazato et al. 2012).

The type species is *G. liquefaciens* (Asai 1935) Yamada et al. 1998. The closest neighbors are the genera *Komagataeibacter* and *Nguyenibacter* (Fig. 1.1). Currently, the genus *Gluconacetobacter* comprises seven species.

Gluconacetobacter Yamada et al. 1998

Cells are Gram negative, ellipsoidal to rod-shaped, measuring 0.5–0.8 by 1.0–3.0 μm, and motile with peritrichous flagella when motile. Occurring singly or in pairs (*Gluconacetobacter azotocaptans* and *G. johannae*). Long involution forms are found (*G. diazotrophicus*). Colonial color is light brown to brownish (*G. azotocaptans* and *G. johannae*). Growth occurs on glutamate agar and mannitol agar.

Aerobic. Catalase positive. Oxidase negative. Acid is produced from ethanol. Oxidizes acetate and lactate. A few species produce dihydroxyacetone from glycerol. 2-keto-D-gluconate is produced from D-glucose. Most of species produce 2,5-diketo-D-gluconate, and a few species produce 5-keto-D-gluconate. Acid is produced from D-glucose, D-fructose, and sucrose. Growth occurs on D-fructose, D-glucose, sucrose, D-mannitol, and

ethanol, but not on methanol. Ammoniac nitrogen is used as a sole nitrogen source. Most of the species produce water-soluble pigment(s). Most of the species have the activity of nitrogen fixation.

Most of the species grow on 30 % D-glucose. Growth occurs between 15 °C and 30 °C, but not at 37 °C. Optimum growth temperature is around 30 °C. Growth occurs between pH 3.0 and 6.8 (*G. asukensis* and *G. tumulicola*). Optimum growth pH is about 5.5 and no growth at pH 7.0 (*G. diazotrophicus*). Major cellular fatty acid is $C_{18:1}$ ω7*c*. A major quinone is Q-10. The G+C content of DNA ranges from 58 to 65 mol%.

Gluconacetobacter strains are isolated from vinegar, fruits, dried fruits, rhizosphere of coffee plants, pink sugarcane mealybug, and even in a tumulus in Japan.

Gluconacetobacter liquefaciens (Asai 1935) Yamada et al. 1998

The characteristics of this species are rather fragmentally described. Following papers are relating to phenotypical characteristics (Asai et al. 1964; Gosselé et al. 1983a), quinone systems (Yamada and Kondo 1984), the G+C content of DNA (Navarro and Komagata 1999), and methanol utilization (Suzuki et al. 2009).

Type strain: Asai G-1^T (= ATCC 14835^T = CCUG 18124^T = CIP 103109^T = DSM 5603^T = JCM 17840^T = LMG 1381^T = LMG 1382^T = NBRC 12388^T = NCCB 76052^T), isolated from dried persimmon. The G+C content of DNA of the type strain is 64.9 mol%.

Gluconacetobacter asukensis Tazato, Nishijima, Handa, Kigawa, Sano, and Sugiyama 2012

Characteristics are the same as those described for the species (Tazota et al. 2012).

Type strain: K8617-1-1b^T (= JCM 17772^T = NCIMB 14759^T), isolated from a brown viscous gel on the northeast area of the ceiling in the stone chamber of the Kitora Tumuli in Asuka village, Nara Prefecture, Japan. The G+C content of DNA of the type strain is 65.4 mol%.

Gluconacetobacter azotocaptans Fuentes-Ramírez, Bustillos-Cristales, Tapia-Hernández, Jiménez-Salgado, Wang, Martínez-Romero, and Caballero-Mellado 2001

The characteristics are the same as those described for the species (Fuentes-Ramírez et al. 2001).

Type strain: CFN-Ca54^T (= ATCC 700988^T = CIP 107161^T = DSM 13594^T), isolated from the rhizosphere of coffee plants. The G+C content of DNA of the type strain is 64.0 mol%.

Gluconacetobacter diazotrophicus (Gillis et al. 1989) Yamada, Hoshino, and Ishikawa 1998

The characteristics are the same as those described for the species (Gillis et al. 1989).

Type strain: PA 5^T (= ATCC 49037^T = CCUG 37298^T = CIP 103539^T = DSM 5601^T = LMG 7603^T = NCCB 89154^T), isolated from roots and stems of sugarcane in Alagoas, Brazil. The G+C content of DNA of the type strain is 61 mol%.

Gluconacetobacter johannae Fuentes-Ramírez, Bustillos-Cristales, Tapia-Hernández, Jiménez-Salgado, Wang, Martínez-Romero, and Caballero-Mellado 2001

The characteristics are the same as those described for the species (Fuentes-Ramírez et al. 2001).

Type strain: CFN-Cf55^T (= ATCC 700987^T = CIP 107160^T = DSM 13595^T), isolated from the rhizosphere of coffee plants. The G+C content of DNA of the type strain is 57.95 mol%.

Gluconacetobacter sacchari Franke, Fegan, Hayward, Leonard, Stackebrandt, and Sly 1999

The characteristics are the same as those described for the species (Franke et al. 1999).

Type strain: SRI 1794^T (= CIP 106693^T = DSM 12717^T), isolated from the leaf sheath of sugarcane and from the pink sugarcane mealybug. The G+C content of DNA of the type strain is 65 mol%.

Gluconacetobacter tumulicola Tazato, Nishijima, Handa, Kigawa, Sano, and Sugiyama 2012

The characteristics are the same as those described for the species (Tazato et al. 2012).

Type strain: K5929-2-1b^T (= JCM 17774^T = NCIMB 14760^T), isolated from a black viscous substance in a plaster hole at the center of the ceiling in the stone chamber of the Kitora Tumulus in Asuka village, Nara Prefecture, Japan. The G+C content of DNA of the type strain is 64.7 mol%.

Gluconobacter Asai 1935

Glu.co.no.bac'ter: N.L. n. *acidum gluconicum*, gluconic acid; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Gluconobacter* gluconate rod.

The generic name *Gluconobacter* appeared for the first time in 1935 by Asai. He focused isolation sources of acetic acid bacteria on a various kinds of fruit and others. As a result, 67 isolates were classified into two groups: One group consisted of the isolates from

fruit and oxidized D-glucose more vigorously than ethanol; and the other group comprised the isolates from vinegar and related materials and oxidized ethanol more actively than D-glucose. The two groups differed clearly from each other on the oxidation of acetate and growth temperature as well. The former was named the genus *Gluconobacter* and the latter the genus *Acetobacter* (Asai 1935). Asai's study showed an important thing, which the genus *Gluconobacter* was established on the basis of biochemical characteristics different from those of the genus *Acetobacter*, and the isolation sources of acetic acid bacteria were focused on the other materials than vinegar and related alcoholic materials. However, his study did not become widely known in the West because his papers were written in Japanese.

Subsequently Leifson proposed the division of acetic acid bacteria into two genera, *Acetobacter* and "*Acetomonas*," on the basis of flagellation and oxidation of acetate and lactate (Leifson 1954). Surprisingly the genera *Gluconobacter* and "*Acetomonas*" agreed to each other on taxonomic characteristics. Later, De Ley and Frateur concluded after detailed investigation in 1970 that the genus *Gluconobacter* was validly and legitimately published 19 years before Leifson (De Ley and Frateur 1970). Thus, the name of *Gluconobacter* was listed in Approved Lists of Bacterial Names 1980 (Skerman et al. 1980). The genus *Gluconobacter* with ubiquinone 10 (Q-10) was distinguished from the genus *Acetobacter* with Q-9 (Yamada et al. 1968b; Yamada et al. 1969). This criterion is still useful for the differentiation of both the genera. In general, the species in the genus *Gluconobacter* is divided into two groups based on the G+C content of DNA and nutritional requirement: The species with the high G+C content of DNA around 60 mol% require pantothenic acid for growth, and the species with the low G+C content of DNA around 55 mol% do not require pantothenic acid. This relationship shows a good correlation with the phylogenetic relationship based on the analysis of 16S rRNA gene sequences and 16S-23S rRNA gene ITS sequences (Tanasupawat et al. 2011b).

The type species is *Gluconobacter oxydans* (Henneberg 1897) De Ley 1961. The closest neighbors are the genera *Neokomagataea* and *Saccharibacter* (Fig. 1.1). Currently, the genus *Gluconobacter* comprises 13 species.

Gluconobacter Asai 1935

Cells are Gram negative, ellipsoidal to rod-shaped, measuring 0.4–1.2 by 1.0–3.0 μm, and polarly flagellated when motile. Colonies are smooth, raised to convex, entire, and glistening on agar media containing ethanol-glucose-yeast extract-$CaCO_3$. Some strains produce pink-colored colonies. Growth occurs on mannitol agar, but not on glutamate agar.

Strictly aerobic. Catalase positive. Oxidase negative. Acetic acid is produced from ethanol. Acetate and lactate are not oxidized. Dihydroxyacetone is produced from glycerol. 2-Keto-D-gluconate and 5-keto-D-gluconate are produced from D-glucose. Some strains produce D-gluconate, and a few strains produce 2,5-diketo-D-gluconate. Acid is produced from L-arabinose, D-xylose, D-fructose, D-galactose, D-glucose, D-mannose, melibiose, D-mannitol, glycerol, and ethanol, but not from lactose or dulcitol. Acid production varies with species from D-arabinose, L-sorbose, L-rhamnose, maltose, sucrose, raffinose, L-arabitol, *meso*-erythritol, D-mannitol, and *meso*-ribitol. Growth occurs on D-glucose and D-mannitol, but does not on lactose or maltose. Growth on other sugars and sugar alcohols differs with the species. Growth on *meso*-ribitol and L-arabitol was once used for differentiation of *Gluconobacter oxydans* from *Gluconobacter cerinus* and *Gluconobacter frateurii* (Mason and Claus 1989; Katsura et al. 2002; Sievers and Swings 2005d). However, this relation is not clear because some strains show a weak or very weak reaction toward one of the polyols. The production of water-soluble pigment is found in the strains of *G. oxydans*, *G. kanchanaburiensis*, *G. sphaericus*, *G. uchimurae*, and *G. wancherniae*. Strains in several species require nicotinic acid for growth.

Optimum temperature for growth is between 25 °C and 30 °C. Majority of species grow at 35 °C, and a few species grow at 37 °C. Optimum pH for growth is around pH 5.5. Majority of species grow at pH 3.0 or 3.5. Major cellular fatty acid is $C_{18:1}\omega7c$. Major ubiquinone is Q-10. The G+C content of DNA ranges from 54.0 to 61.5 mol%.

Gluconobacter strains are isolated from a variety of fruits, including kaki (persimmon), jujube, peach, a variety of citrus fruits, strawberry, cherry, apple, apricot, grape, fig, etc., and flowers and other sugar-rich environments.

Gluconobacter oxydans (Henneberg 1897) De Ley 1961

The characteristics are the same as those described for the species (Asai et al. 1964; Gosselé et al. 1983a; Katsura et al. 2002; Mason and Claus 1989; Sievers and Swings 2005d; Tanaka et al. 1999; Yamada and Akita 1984). Characterization of *Gluconobacter oxydans* is rather fragmentally, and the description of the species is needed to be compiled from data based on detailed research.

Type strain: ATCC 19357^T (= CCUG 18132^T = CIP 103106^T = DSM 3503^T = DSM 7145^T = ICMP 12533^T = JCM 7642^T = LMG 1408^T = NBRC 14819^T = NCCB 75005^T = NCIMB 9013^T), isolated from beer by J. G. Carr. The G+C content of DNA of the type strain is 60 mol%.

Gluconobacter albidus (ex Kondo and Ameyama 1958) Yukphan, Takahashi, Potacharoen, Tanasupawat, Nakagawa, Tanticharoen, and Yamada 2005

The characteristics are the same as those described for the species (Yukphan et al. 2004a).

Type strain: NBRC 3250^T (= BCC 14434^T = JCM 20271^T), isolated from a flower of dahlia by Kondo and Ameyama (1958). The G+C content of DNA of the type strain is 60.0 mol%.

Gluconobacter cerinus (ex Asai 1935) Yamada and Akita 1984 emend. Katsura et al. 2002

The characteristics are the same as those described for the species (Yamada and Akita 1984; Katsura et al. 2002).

Type strain: NBRC 3267^T (= ATCC 19441^T = CIP 103150^T = DSM 9533^T = DSM 9534^T = LMG 1368^T = NRRL B-4241^T), isolated from cherry (*Prunus* sp.). The G+C content of DNA of the type strain is 56 mol%.

Gluconobacter frateurii Mason and Claus 1989

The characteristics are the same as those described for the species (Mason and Claus 1989; Katsura et al. 2002; Tanaka et al. 1999).

Type strain: Kondo 40^T (= ATCC 49207^T = CIP 104735^T = DSM 7146^T = LMG 1365^T = NBRC 3264^T), isolated from strawberry (*Fragaria ananassa*). The G+C content of DNA of the type strain is 55 mol%.

Gluconobacter japonicus Malimas, Yukphan, Takahashi, Muramatsu, Kaneyasu, Potacharoen, Tanasupawat, Nakagawa, Tanticharoen, and Yamada 2009

The characteristics are the same as those described for the species (Malimas et al. 2009b).

Type strain: Kondo 7^T (= BCC 14458^T = NBRC 3271^T), isolated from a fruit of Chinese bayberry. The G+C content of DNA of the type strain is 56.4 mol%.

Gluconobacter kanchanaburiensis Malimas, Yukphan, Lundaa, Muramatsu, Takahashi, Kaneyasu, Potacharoen, Tanasupawat, Nakagawa, Suzuki, Tanticharoen, and Yamada 2009

The characteristics are the same as those described for the species (Malimas et al. 2009a).

Type strain: AD92^T (= BCC 15889^T = NBRC 103587^T), isolated from a spoiled fruit of jackfruit (*Artocarpus heterophyllus*). The G+C content of DNA of the type strain is 59.5 mol%.

Gluconobacter kondonii Malimas, Yukphan, Takahashi, Kaneyasu, Potacharoen, Tanasupawat, Nakagawa, Tanticharoen, and Yamada 2007

The characteristics are the same as those described for the species (Malimas et al. 2007).

Type strain: Kondo 75^T (= BCC 14441^T = NBRC 3266^T), isolated from strawberry. The G+C content of DNA of the type strain is 59.8 mol%.

Gluconobacter nephelii Kommanee, Tanasupawat, Yukphan, Malimas, Muramatsu, Nakagawa, and Yamada 2011

The characteristics are the same as those described for the species (Kommanee et al. 2011).

Type strain: RBY-1^T (= BCC 36733^T = NBRC 10606^T = PCU 318^T), isolated from rambutan (*Nephelium lappaceum*). The G+C content of DNA of the type strain is 57.2 mol%.

Gluconobacter roseus (ex Asai 1935) Malimas, Yukphan, Takahashi, Muramatsu, Kaneyasu, Potacharoen, Tanasupawat, Nakagawa, Tanticharoen, and Yamada 2008

The characteristics are the same as those described for the species (Malimas et al. 2008a).

Type strain: Asai G-2^T (= BCC 14456^T = JCM 20293^T = NBRC 3990^T), isolated from a fruit of kaki (persimmon, *Diasporas kaki*). The G+C content of DNA of the type strain is 60.5 mol%.

Gluconobacter sphaericus (Ameyama 1975) Malimas, Yukphan, Takahashi, Muramatsu, Kaneyasu, Potacharoen, Tanasupawat, Nakagawa, Tancharoen, and Yamada 2008

The characteristics are the same as those described for the species (Ameyama 1975; Malimas et al. 2008b).

Type strain: NBRC 12467^T (= BCC 14448^T = CIP 106061^T = LMG 1414^T), isolated from fresh grapes by Ameyama. The G+C content of DNA of the type strain is 59.5 mol%.

Gluconobacter thailandicus Tanasupawat, Thawai, Yukphan, Moonmangmee, Itoh, Adachi, and Yamada 2004

The characteristics are the same as those described for the species (Tanasupawat et al. 2004).

Type strain: F-149 T (= BCC 14116^T = JCM 12310^T = NBRC 100600^T = PCU 225^T = TISTR 1533^T), isolated from a flower of Indian cork tree (*Millingtonia hortensis*), Bangkok, Thailand. The G+C content of DNA of the type strain is 55.8 mol%.

Gluconobacter uchimurae Tanasupawat, Kommanee, Yukphan, Moonmangmee, Muramatsu, Nakagawa, and Yamada 2011

The characteristics are the same as those described for the species (Tanasupawat et al. 2011b).

Type strain: ZW160-2^T (= BCC 14681^T = NBRC 100627^T =PCU 264^T), isolated from rakam fruit (*Salacca wallichiana*). The G+C content of DNA of the type strain is 60.5 mol%.

Gluconobacter wancherniae Yukphan, Malimas, Lundaa, Muramatsu, Takahashi, Kaneyasu, Tanasupawat, Nakagawa, Suzuki, Tanticharoen, and Yamada 2010

The characteristics are the same as those described for the species (Yukphan et al. 2010).

Type strain: AC42^T (= BCC 15775^T = NBRC 103581^T), isolated from unknown seed. The G+C content of DNA of the type strain is 56.6 mol%.

Granulibacter Greenberg, Porcella, Stock, Wong, Conville, Murray, Holland, and Zelazny 2006

Gra.nu.li.bac'ter. L. n. *granulum*, grain; N. L. masc. n. *bacter*, rod; N.L. masc. n. *Granulibacter*, a rod that causes granules or granuloma formation.

A *Granulibacter* strain was first isolated from cervical and supraclavicular lymph nodes of a chronic granulomatous disease (CGD) patient in Bethesda, USA. This organism was subsequently isolated from the same patient and then from other two CGD patients (Greenberg et al. 2006a). The strains were characterized by a multilocus analysis on the 16S rRNA gene, the internal transcribed spacer (ITS) region, and the RecA protein, and they were assigned with a new genus *Granulibacter* in the family *Acetobacteraceae* (Greenberg et al. 2006b). Further, the organism was isolated from a child with CGD (López et al. 2008). The genome sequence of a *G. bethesdensis* strain was determined, in which methanol dehydrogenase genes were recognized (Greenberg et al. 2007; Greenberg et al. 2010). However, the strain grew poorly on methanol compared with that of *Acidomonas methanolica*.

The type species is *Granulibacter bethesdensis* Greenberg et al. 2006. This genus is phylogenetically rather independent from other genera in the family *Acetobacteraceae* (*Fig. 1.1*). Currently, the genus contains a single species, *Granulibacter bethesdensis*.

Granulibacter Greenberg et al. 2006

Cells are Gram negative, coccobacillus to rod-shaped, and nonmotile. Colonies are convex, entire, smooth, and yellow (nondiffusible) on a modified glucose-yeast extract-$CaCO_3$. Growth occurs on glutamate agar and on mannitol agar.

Strictly aerobic. Catalase positive. Oxidase negative. Little acetic acid is produced from ethanol. Lactate and acetate are oxidized to carbon dioxide and water, but the activity of the latter is weak. Dihydroxyacetone is not produced from glycerol. Acid is produced from D-glucose and ethanol and variably from glycerol (weak or a negative reaction), but not from D-mannitol, D-sorbitol, dulcitol, lactose, sucrose, maltose, or D-xylose. Methanol can be used as a sole carbon source. Ammoniac nitrogen is assimilated on glucose medium. Urease variable.

High concentration of glucose [e.g., 5 % (w/v) glucose] is preferable for growth. Optimum temperature for growth is 35–37 °C. Growth does not occur at 42 °C. Optimum pH for growth is pH 5.0–6.5. Growth occurs at pH 3.5. The major cellular fatty acids are a straight-chain unsaturated acid ($C_{18:1}\omega7c$) and saturated acid ($C_{16:0}$). A cyclopropane acid ($C_{19:0}$ cyc. 11–12) is contained in a relatively high concentration. A major quinone is Q-10 (Yukphan et al. 2009). The G+C content of DNA is 59.1 mol%.

Granulibacter strains are isolated from lymph nodes of CGD patients.

Granulibacter bethesdensis Greenberg, Porcella, Stock, Wong, Conville, Murray, Holland, and Zelazny 2006

Characteristics are the same as those described for the species (Greenberg et al. 2006b). The type strain: CGDNIH1^T (= ATCC BAA-1260^T = DSM 17861^T), isolated from lymph node culture from a granulomatous disease patient in Bethesda, MD, USA, in 2003. The G+C content of DNA of the type strain is 59.1 mol%.

Komagataeibacter Yamada, Yukpan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Ko.ma.ga.ta.e.i.bac'ter. N. L. fem. n. *Komagataea*, Komagata (the name of a Japanese microbiologist); N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Komagataeibacter*, a rod, which is named in honor of Dr. Kazuo Komagata, Professor of The University of Tokyo, Japan, who contributed to the bacterial systematics, especially acetic acid bacteria.

The genus *Gluconacetobacter* Yamada et al. 1998 (*Gluconoacetobacter* [sic]) was divided into two genera, the genus *Gluconacetobacter* and the genus *Komagataeibacter*, on the basis of 16S rRNA gene analysis and phenotypic characteristics, and 12 species in the previous *Gluconacetobacter* were transferred to the genus *Komagataeibacter* as new combinations (Yamada et al. 2012b) (see *Gluconacetobacter*). In addition, *Gluconacetobacter kakiaceti* (Iino et al. 2012b), *Gluconacetobacter medellinensis* (Castro et al. 2013), and *Gluconacetobacter maltaceti* (Slapšak et al. 2013) were described. The three species were included into the genus *Komagataeibacter* on the basis of the phylogenetic relationships (Yamada 2014).

The type species is *Komagataeibacter xylinus* (Brown 1886) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat and Nakagawa 2013. The closed neighbors are the genera *Gluconacetobacter* and *Nguyenibacter* (Fig. 1.1). Currently, this genus comprises 13 species.

Komagataeibacter Yamada et al. 2013

Cells are Gram-negative rods, measuring 0.5–0.8 by 1.0–3.0 μm, occurring singly, in pairs, or in chains. Nonmotile (a *K. xylinus* strain is motile with peritrichous flagella) (Asai et al. 1964). Colonies are circular, smooth, or rough, raised to convex or umbonate, entire, glistening, and white-creamy to beige. Growth occurs on glutamate agar and mannitol agar. *K. kakiaceti* strains do not grow on glutamate agar.

Aerobic. Catalase positive. Oxidase negative. Acetic acid is produced from ethanol. Acetate and lactate are oxidized to carbon dioxide and water. Dihydroxyacetone is generally produced from glycerol. D-Gluconate, 2-keto-D-gluconate, and/or 5-keto-D-gluconate is produced from D-glucose, but 2,5-diketo-D-gluconate is not produced. *K. kakiaceti* strains do not produce ketogluconates. Acid is produced from L-arabinose, D-xylose, D-galactose, D-glucose, and ethanol, but not from L-sorbose, D-fructose, D-mannitol, lactose, maltose, or D-sorbitol. Growth occurs on D-fructose, D-glucose, maltose, sucrose, and D-mannitol, but not on lactose. Ammoniac nitrogen is generally assimilated on D-mannitol. Cellulosic materials are produced by the strains of *K. xylinus* and other species. A water-soluble brown pigment is not produced on glucose-yeast extract-calcium carbonate medium. γ-Pyrone compounds are not produced.

Growth occurs in the presence of 0.35 % acetic acid (v/v) in general. Some species require acetic acid for growth. Growth occurs at pH 3.0. A major cellular fatty acid is $C_{18:1}\omega7c$. Additionally, *K. rhaeticus* shows the presence of $C_{14:0}$ 2-OH and $C_{16:0}$ 2-OH. A major quinone is Q-10. The G+C content of DNA ranges from 58 to 64 mol%.

A number of strains of this genus are isolated from acetous materials including a variety of vinegar, kombucha, fruits, and a variety of fruit juice.

Komagataeibacter xylinus (Brown 1886) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics of this species are rather fragmentally described. Following papers are relating to phenotypical characteristics (Gosselé et al. 1983a; Lisdiyanti et al. 2006; Navarro and Komagata 1999; Sievers and Swings 2005c; Yamada 1983), quinone systems (Yamada et al. 1976b), cellular fatty acid composition (Yamada et al. 1981a), and the G+C content of DNA (Navarro and Komagata 1999). The G+C content of the type strain is 62 mol%. A few strains of this species produce cellulose from sugars, but cellulose-less strains are known (Yamada 1983).

Type strain: NCIMB 11664^T (= BCC 49175^T = DSM 6513^T = JCM 7644^T = LMG 1515^T = NBRC 15237^T), isolated from mountain-ash berry by Prof. G. Bertrand. The G+C content of DNA of the type strain is 62 mol%.

Gluconacetobacter entanii Schüller, Hertel, and Hammes 2000

Characteristics are the same as those described for the species (Schüller et al. 2000).

Type strain: LTH 4560^T (= BCRC 17196^T = DSM 13536^T = LMG 20950^T = LMG 21788^T), isolated from submerged high-acid industrial vinegar fermentations. The G+C content of DNA of the type strain is 58 mol%.

The type strain is not available in any culture collection (Yamada et al. 2012b). Therefore, this species cannot be listed as a new combination according to Rule 27 of the Bacteriological Code (Tindall et al. 2006).

Komagataeibacter europaeus (Sievers, Sellmer, and Teuber 1992) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics are the same as those described for the species (Sievers et al. 1992).

Type strain: DES11^T (= BCC 36446^T = DSM 6160^T = JCM 16935^T), isolated from a submerged culture vinegar generator at a factory in Esslingen in the southern part of Germany. The G+C content of DNA of the type strain is not recorded. The range of the species is described from 56.2 to 57.3 mol%.

Komagataeibacter hansenii (Gosselé, Swings, Kersters, Pauwels, and De Ley 1983) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics are the same as those for the species (Gosselé et al. 1983b; Lisdiyanti et al. 2006).

Type strain: NCIMB 8746^T (= BCC 6318^T = DSM 5602^T = JCM 7643^T = LMG 1527^T = NBRC 14820^T), isolated from a local vinegar in Jerusalem, Israel. The G+C content of DNA of the type strain is 59 mol%.

Komagataeibacter intermedius (Boesch, Trček, Sievers, and Teuber 1998) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics are the same as those for the species (Boesch et al. 1998; Yamada 2000).

Type strain: TF2^T (= DSM 11804^T = JCM 16936^T = BCC 36447^T = LMG 18909^T), isolated from a commercially available tea fungus beverage (kombucha) in Switzerland. The G+C content of DNA of the type strain is 61.55 mol%.

Gluconacetobacter kakiaceti Iino, Suzuki, Tanaka, Kosako, Ohkuma, Komagata, and Uchimura 2012

Characteristics are the same as those described for the species (Iino et al. 2012b).

Type strain: G5-1^T (= JCM 25156^T = LMG 26206^T = NRIC 0798^T), isolated from a traditional Japanese kaki vinegar. The G+C content of DNA of the type strain is 63.6 mol%.

Gluconacetobacter maltaceti Slapšak, Cleenwerck, De Vos, and Trček 2013

The characteristics are the same as those described for the species (Slapšak et al. 2013).

Type strain: Walker strain A^T (= LMG 1529^T = NBRC 14815^T = NCIMB 8752^T), isolated from malt vinegar brewery acetifier. The G+C content of DNA of the types strains is 62.5 mol%.

Gluconacetobacter medellinensis Castro, Cleenwerck, Trček, Zuluaga, De Vos, Caro, Aguirre, Putaux, and Gañán 2013

Characteristics are the same as those described for the species (Castro et al. 2013).

Type strain: Kondo 51^T (= LMG 1693^T = NBRC 328^T), isolated from vinegar. The G+C content of DNA of the type strain is 60.7 mol%.

Komagataeibacter nataicola (Lisdiyanti, Navarro, Uchimura, and Komagata 2006) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics are the same as those described for the species (Lisdiyanti et al. 2006).

Type strain: LMG 1536^T (= BCC 36443^T = JCM 25120^T = NRIC 0616^T), isolated from nata de coco in the Philippines. The G+C content of DNA of the type strain is 62 mol%.

Komagataeibacter oboediens (Sokollek, Hertel, and Hammes 1998) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics are the same as those described for the species (Sokollek et al. 1998; Yamada 2000).

Type strain: LTH 2460^T (= BCC 36445^T = DSM 11826^T = JCM 16937^T = LMG 18849^T), isolated from a submerged red wine vinegar fermentation at a factory in the southern part in Germany. The G+C content of DNA of the type strain is 59.9 mol%.

Komagataeibacter rhaeticus (Dellaglio, Cleenwerck, Felis, Engelbeen, Janssens, and Marzotto 2005) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics are the same as those described for the species (Dellaglio et al. 2005).

Type strain: DST GL02^T (= BCC 36452^T = DSM 16663^T = JCM 17122^T = LMG 22126^T), isolated from apple juice in South Tyrol region, Italy. The G+C content of DNA of the type strain is 63.4 mol%.

Komagataeibacter saccharivorans (Lisdiyanti, Navarro, Uchimura, and Komagata 2006) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics are the same as those described for the species (Lisdiyanti et al. 2006).

The type strain: LMG 1582^T (= BCC 36444^T = JCM 25121^T = NRIC 0614^T), isolated from beet juice in Germany in 1927. The G+C content of DNA of the type strain is 61 mol%.

Komagataeibacter sucrofermentans (Toyosaki, Kojima, Tsuchida, Hoshino, Yamada, and Yoshinaga 1996) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

Characteristics are the same as those described for the species (Toyosaki et al. 1995; Cleenwerck et al. 2010).

Type strain: LMG 18788^T (= BCC7227^T = DSM 15973^T = JCM 9730^T), isolated from a cherry. The G+C content of DNA of the type strain is 62.7 mol%.

Komagataeibacter swingsii (Dellaglio, Cleenwerck, Felis, Engelbeen, Janssens, and Marzotto 2005) Yamada, Yukphan, Vu, Muramatsu, Ochaikul, Tanasupawat, and Nakagawa 2013

The characteristics are the same as those described for the species (Dellaglio et al. 2005).

Type strain: DST GL01^T (= BCC 36451^T = DSM 16373^T = JCM 17123^T = LMG 22125^T), isolated from apple juice in South Tyrol region, Italy. The G+C content of DNA of the type strain is 61.7 mol%.

Kozakia Lisdiyanti, Kawasaki, Widyastuti, Saono, Seki, Yamada, Uchimura, and Komagata 2002

Ko.za'ki.a. N. L. fem. n. named after Kozaki, to honor the Japanese microbiologist Michio Kozaki, Professor Emeritus of Tokyo University of Agriculture, in recognition of his contributions to the study of microorganisms in tropical regions, especially Southeast Asia.

Kozakia strains were isolated from palm brown sugar and ragi (a starter for fermentation in southeastern Asia) collected in Indonesia by an enrichment culture approach for acetic acid bacteria. They were tentatively identified as *Gluconacetobacter* (Yamada et al. 1999). Later, the strains were included in a new genus *Kozakia*, which was established on phenotypic and chemotaxonomic characteristics, DNA-DNA similarity, and 16S rRNA sequences (Lysdiyanti et al. 2002). The strains produced a large amount of mucous polysaccharide from a sucrose-containing medium. The polysaccharide was determined as fructan (Jakob et al. 2013).

The type species is *Kozakia baliensis*. The closest neighbor is the genus *Neoasaia* (Fig. 1.1). Currently, the genus contains a single species, *Kozakia baliensis*.

Kozakia Lisdiyanti et al. 2002

Cells are Gram negative, rod-shaped, measuring 0.6–0.8 by 2.0–3.0 μm, and nonmotile. Growth occurs on mannitol agar, but not on glutamate agar. Nonpigmented.

Strictly aerobic. Catalase positive. Oxidase negative. Acetic acid is produced from ethanol. Acetate and lactate are oxidized to carbon dioxide and water, but the activity is weak. Dihydroxyacetone is produced from glycerol. D-Gluconate, 2-keto-D-gluconate, and 5-keto-D-gluconate are produced from D-glucose, but 2,5-diketo-D-gluconate is not produced. Acid is produced from L-arabinose, D-xylose, D-glucose, D-galactose, D-mannose, melibiose, raffinose, *meso*-erythritol, glycerol, and ethanol, but not from L-rhamnose, D-fructose, L-sorbose, lactose, D-mannitol, D-sorbitol, or dulcitol. Acid production from D-arabinose and sucrose is variable depending on the strain. Methanol is not utilized. Ammoniac nitrogen is not assimilated on D-glucose, mannitol, and ethanol medium without vitamins. Levan-like mucous substance(s) is produced from sucrose or D-fructose. Water-soluble brown pigment(s) is not produced from D-glucose or on $CaCO_3$-containing agar slants. γ-Pyrone is produced from D-fructose but not from D-glucose. Gelatinase, H_2S, indole, or ammonia is not produced from L-arginine, and nitrate is not reduced.

Growth is not inhibited by 0.35 % acetic acid at pH 3.5. Growth does not occur on 30 % D-glucose. Growth occurs at pH 3.0 and 30 °C. Major ubiquinone is Q-10. The G+C content of DNA is from 56.8 to 57.2 mol%.

Kozakia strains are isolated from palm brown sugar and ragi.

Kozakia baliensis Lisdiyanti, Kawasaki, Widyastuti, Saono, Seki, Yamada, Uchimura, and Komagata 2002

Characteristics are the same as those described for the species (Lisdiyanti et al. 2002).

Type strain: Yo-3^T (= DSM 14400^T = JCM 11301^T = NBRC 16664^T= NRIC 0488^T), isolated from palm brown sugar collected in Bali, Indonesia in 1996. The G+C content of DNA of the type strain is 57.2 mol%.

Neoasaia Yukphan, Malimas, Potacharoen, Tanasupawat, Tanticharoen, and Yamada 2006

Ne.o.a.sa'i.a. Gr. adj. *neos*, new; N. L. fem. n. *Asaia*, a bacterial name; N.L. fem. n. *Neoasaia*, a new *Asaia*.

A *Neoasaia* strain was isolated from a flower of red ginger (*Alpinia purpurata*) collected in Chiang Mai, Thailand, by the use of a glucose-ethanol-acetic acid (0.3 %, w/v) medium at pH 3.5. The strain was assigned with the new genus *Neoasaia*, which was established on 16S–23S rRNA internal transcribed spacer (ITS) region sequences (Yukphan et al. 2005).

The type species is *Neoasaia chiangmaiensis*. The closest neighbor is the genus *Kozakia* (Fig. 1.1). Currently, this genus contains a single species, *Neoasaia chiangmaiensis*.

Neoasaia Yukphan et al. 2006

Cells are Gram negative, rod-shaped, measuring 0.8–1.0 by 1.0–2.0 μm, and nonmotile. Colonies are smooth, raised, entire, shiny, and pink. Growth occurs on glutamate agar and mannitol agar.

Aerobic. Acetic acid is produced from ethanol. Acetate and lactate are not oxidized. Dihydroxyacetone is weakly produced from glycerol. 2-Keto-D-gluconate and 5-keto-D-gluconate are produced from D-glucose. Acid is produced from D-arabinose (weakly positive), L-arabinose, D-xylose, L-rhamnose (weakly positive), D-fructose (delayed), D-galactose, D-glucose, D-mannose, melibiose, sucrose, raffinose, dulcitol (weakly positive), *meso*-erythritol, D-mannitol (weakly positive), D-sorbitol (delayed), glycerol, and ethanol, but not from L-sorbose, lactose, or maltose.

Growth occurs in a liquid medium containing D-arabinose (weakly positive), L-arabinose, L-sorbose, D-xylose, D-fructose, D-galactose, D-glucose, D-mannose (weakly positive), sucrose, raffinose, dulcitol, *meso*-erythritol, D-mannitol, D-sorbitol, and glycerol, but not from L-rhamnose, lactose, maltose, melibiose,

or ethanol. Ammoniac nitrogen is hardly assimilated in the presence of D-glucose or D-mannitol as a carbon source. A water-soluble brown pigment is not produced on a glucose-peptone-yeast extract-$CaCO_3$ medium, and a levan-like polysaccharide is not produced on a sucrose medium. However, the production of fructan is reported by *Neoasaia chiangmaiensis* NBRC 101099 (Jakob et al. 2013).

Growth occurs on 30 % D-glucose (w/v) and in the presence of 0.35 % acetic acid (w/v), but not in the presence of 1.0 % KNO_3 (w/v). Major cellular fatty acids are $C_{18:1}$ ϖ7c and $C_{16:0}$. Major ubiquinone is Q-10. The G+C content of DNA is 63.1 mol% G+C.

Neoasaia strains are isolated from red ginger (*Alpinia purpurata*).

Neoasaia chiangmaiensis Yukphan, Malimas, Potacharoen, Tanasupawat, Tanticharoen, and Yamada 2006

Characteristics are the same as those described for the species (Yukphan et al. 2005).

The type strain: AC28^T (= BCC 15763^T = NBRC 101099^T), isolated from a flower of red ginger (*Alpinia purpurata*) in Chiang Mai, Thailand, in September 2002. The G+C content of DNA of the type strain is 63.1 mol%.

Neokomagataea Yukphan, Malimas, Muramatsu, Potacharoen, Tanasupawat, Nakagawa, Tanticharoen, and Yamada 2011

Ne.o.ko.ma.ga.ta'ea. N. L. fem. n. new Komagata, named after Dr. Kazuo Komagata, a Japanese microbiologist who contributed to bacterial systematics and phylogeny, especially of acetic acid bacteria.

Two *Neokomagataea* strains were isolated from flowers collected in Thailand. One strain was isolated from a flower of lantana (*Lantana camera*) by the use of an enrichment culture medium containing 2.0 % glucose. The other strain was isolated from a flower of candle bush (*Senna alata*) by the use of an enrichment culture medium containing 10 % glucose. In phylogenetic trees based on 16S rRNA gene sequences, the two strains formed an independent cluster in the family *Acetobacteraceae*. The two isolates were able to grow in the range between 1.0 % and 30 % glucose. Therefore, the isolates are osmotolerant but not osmophilic. A new genus *Neokomagataea* was proposed, and two species, *Neokomagataea thailandica* and *N. tanensis*, were described on the basis of the difference of the G+C content of DNA and acid production from melibiose and raffinose (Yukphan et al. 2011).

The type species is *Neokomagataea thailandica*. The closest neighbor is the genus *Saccharibacter* (❯ *Fig. 1.1*). Currently, this genus contains two species, *N. thailandica* and *N. tanensis*.

Neokomagataea Yukphan et al. 2011

Cells are Gram-negative rods, measuring 0.6–0.8 by 1.0–1.6 μm, and nonmotile. Colonies are smooth, entire, and creamy on glucose-ethanol-peptone-yeast extract-calcium carbonate agar. Growth occurs on glutamate agar or mannitol agar.

Acetic acid is weakly produced from ethanol. Acetate and lactate are not oxidized. Dihydroxyacetone is not produced from glycerol. 2-Keto-D-gluconate, 5-keto-D-gluconate, and 2,5-diketo-D-gluconate are produced from D-glucose. Acid is produced from L-arabinose (weakly), D-xylose, D-fructose, D-glucose, D-galactose (weakly), and sucrose, but not from D-arabinose, D-mannose, lactose, maltose, melibiose, raffinose, dulcitol, *meso*-erythritol, D-mannitol, D-sorbitol, glycerol, or ethanol (*N. tanensis* produces acid from melibiose and raffinose).

Growth occurs on L-rhamnose (weakly), D-glucose, and sucrose, but not on D-arabinose, L-arabinose, L-sorbose, D-xylose, D-fructose, D-galactose, D-mannose, lactose, maltose, melibiose, raffinose, dulcitol, *meso*-erythritol, D-mannitol, D-sorbitol, glycerol, or ethanol (*N. tanensis* grows on raffinose). Methanol is not utilized as a carbon source. Ammoniac nitrogen is assimilated on D-glucose (very weakly) or ethanol as a source of carbon (*N. tanensis* does not grow on D-glucose, D-mannitol, or ethanol). Water-soluble brown pigment is not produced.

Growth occurs on between 1.0 % and 30 % D-glucose (w/v). Osmotolerant. Growth does not occur in the presence of 0.35 % acetic acid (w/v) or in the presence of 1.0 % or 2.0 % NaCl (w/v) or 1.0 % KNO_3 w/v. Major cellular fatty acids are $C_{18:1}$ϖ7c, $C_{18:1}$2OH, and $C_{18:0}$. The major quinone is Q-10. The G+C content of DNA is 51.2–56.8 mol% (the G+C content of DNA of the type strain *N. thailandica* is 56.8 mol%, and that of *N. tanensis* is 51.2 mol%).

Neokomagataea strains are isolated from a flower of lantana (*Lantana camera*) and a flower of candle bush (*Senna alata*).

Neokomagataea thailandica Yukphan, Malimas, Muramatsu, Potacharoen, Tanasupawat, Nakagawa, Tanticharoen, and Yamada 2011

Characteristics are the same as those described for the species (Yukphan et al. 2011).

The type strain: AH11^T (= BCC 25710^T = NBRC 106555^T), isolated from a flower of lantana (*Lantana camera*) at Tan Island, Hat Khanom-Mu Ko Thale Tai National Park, Nakhon Si Thammarat, Thailand in 2007. Does not produce acid from melibiose and raffinose. The G+C content of DNA of the type strain is 56.8 mol%.

Neokomagataea tanensis Yukphan, Malimas, Muramatsu, Potacharoen, Tanasupawat, Nakagawa, Tanticharoen, and Yamada 2011

Characteristics are the same as those described for the species (Yukphan et al. 2011).

The type strain: AH13^T (= BCC 25711^T = NBRC 106556^T), isolated from a flower of candle bush (*Senna alata*) at Tan Island, Hat Khanom-Mu Ko Thale Tai National Park, Nakhon Si Thammarat, Thailand in 2007. Produces acid from melibiose and raffinose. The G+C content of DNA of the type strain is 51.2 mol%.

Nguyenibacter Vu, Yukphan, Chaipitakchonlatarn, Malimas, Muramatsu, Bui, Tanasupawat, Duong, Nakagawa, Pham, and Yamada 2013

Ngu.ye.ni.bac'ter. N.L. masc. n. *Nguyenius* Nguyen (the name of a famous Vietnamese microbiologist); N. L. masc. n. *bacter*, a rod; N.L. masc. n. *Nguyenibacter* a rod, which is named in honor of Dr. Dung Lan Nguyen, Professor, Institute of Microbiology and Biotechnology, Vietnam National University – Hanoi, Hanoi, Vietnam – who contributed to the study of microorganisms, especially of strains isolated in Vietnam.

Two *Nguyenibacter* strains were isolated by an enrichment culture with N_2-free medium (Cavalcante and Döbreiner 1988) from the rhizosphere and roots of Asian rice collected in Vietnam. The results of 16S rRNA gene sequence and so-called partial 16S rRNA gene 800R-region sequence analyses supported the strains in a member of a new genus within the family *Acetobacteraceae*. Thus, the genus *Nguyenibacter* was established (Vu et al. 2013).

The type species is *Nguyenibacter vanlangensis*. The closest neighbor is the genus *Gluconacetobacter* (Fig. 1.1). Currently, this genus consists of a single species, *Nguyenibacter vanlangensis*.

Nguyenibacter Vu et al. 2013

Cells are Gram-negative rods, measuring 0.6–0.8 by 1.0–1.6 μm. Motile with peritrichous flagella. Colonies are smooth, entire, transparent, and creamy to brownish. Growth occurs on glutamate agar and mannitol agar.

Aerobic. Catalase positive. Oxidase negative. Acetic acid is not produced from ethanol. Acetate is oxidized to carbon dioxide and water, but lactate is not oxidized. Dihydroxyacetone is not produced from glycerol. 2-Keto-D-gluconate and 5-keto-D-gluconate are produced from D-glucose. Acid is produced from L-arabinose, D-xylose, D-fructose, D-galactose (weakly), D-glucose, maltose, melibiose, sucrose, and raffinose (weakly), but not from D-arabinose, L-sorbose, L-rhamnose, lactose, dulcitol, *myo*-inositol, D-mannitol, D-sorbitol, glycerol, or ethanol. Growth occurs on L-arabinose (weakly), L-sorbose (weakly), D-xylose (weakly), D-fructose (weakly), D-galactose, D-glucose, maltose, melibiose (weakly), sucrose, raffinose, D-mannitol (weakly), D-sorbitol (weakly), and glycerol, but not from D-arabinose, L-rhamnose, lactose, dulcitol, *myo*-inositol, or ethanol. Ammoniac nitrogen is utilized on D-mannitol, but not on D-glucose or ethanol. Growth occurs on N_2-free medium. γ-Pyrone compound is weakly produced. Water-soluble pigment is produced. Levan-like polysaccharides are produced from sucrose.

Growth occurs weakly on 30 % glucose and weakly in the presence of 0.35 % acetic acid. Growth does not occur on 1.0 % KNO_3. Major cellular fatty acid is $C_{18:1}\varpi7c$. Major quinone is Q-10. The G+C content of DNA ranges from 68.1 to 69.4 mol%.

Nguyenibacter strains were isolated from the rhizosphere and roots of Asian rice collected in Vietnam.

Nguyenibacter vanlangensis Vu, Yukphan, Chaipitakchonlatarn, Malimas, Muramatsu, Bui, Tanasupawat, Duong, Nakagawa, Pham, and Yamada 2013

Characteristics are the same as those described for the species (Vu et al. 2013).

The type strain: TN01LGI^T (= BCC 54774^T = NBRC 109046^T = VTCC-B-1198^T), isolated from the rhizosphere of Asian rice collected at Long Thanh Trung Commune, Hoa Thanh District, Tay Ninh Province, Vietnam. The G+C content of DNA of the type strain is 69.4 mol%.

Saccharibacter Jojima, Mihara, Suzuki, Yokozeki, Yamanaka, and Fudo 2004

Sac.cha.ri.bac'ter. L. neut. n. *saccharum* or *saccharon*, a kind of sugar; N. L. masc. n. *bacter*, rod; N. L. masc. n. *Saccharibacter*, a sugar rod, a rod that grows well in a sugar-rich environment.

Saccharibacter strains were isolated from the pollen of flowers in Japan by the use of an enrichment medium containing 20 % glucose, as producers of xylitol that is a promising low-calorie sweetener. They have rather unusual characteristics for the acetic acid bacteria, such as a negligible or weak capacity to produce acetic acid from ethanol and an osmophilic growth profile. The osmophilic growth property distinguishes the strains from other acetic acid bacteria. Major cellular fatty acids are a 2-hydroxy acid ($C_{16:0}$2-OH) and an unsaturated straight-chain acid ($C_{18:1}\varpi7c$). Phylogenetic analyses of the 16S rRNA gene sequences demonstrated that the isolates formed a new cluster within the family *Acetobacteraceae* in the *Alphaproteobacteria*. The genus *Saccharibacter* was established on the basis of unique biochemical characteristics and phylogenetic profile (Jojima et al. 2004).

The type species is *Saccharibacter floricola*. The closest neighbor is the genus *Neokomagataea* (Fig. 1.1). Currently, this genus contains a single species, *Saccharibacter floricola*.

Saccharibacter Jojima et al. 2004

Cells are Gram negative and straight rods, measuring 0.8–1.0 by 2.5–4.0 μm, and nonmotile. Endospores are not produced.

Colonies are circular, entire, and pale in color on YGP agar. Growth occurs on mannitol and glutamate agar supplemented with 7 % (w/v), but not on glutamate agar supplemented with 1 % (w/v). No growth occurs on ordinary mannitol agar and glutamate agar.

Strictly aerobic. Chemoorganotrophic. Catalase positive. Oxidase negative. Produces negligible or very little acetic acid from ethanol. Acetate is not oxidized to carbon dioxide and water, and lactate is weakly oxidized. Dihydroxyacetone is not produced from glycerol. D-Gluconic acid, 2-keto-D-gluconic acid, and 5-keto-D-gluconic acid are produced from D-glucose. Acid is produced from L-arabinose, D-xylose, D-glucose, D-galactose, D-mannose, melibiose, sucrose, and mannitol, but not from D-arabinose, L-rhamnose, raffinose, D-sorbitol, dulcitol, glycerol, or ethanol. Production of acid from L-sorbose is variable. Methanol is not utilized. Ammoniac nitrogen is not assimilated on Hoyer-Frateur medium with glucose, mannitol, or ethanol. Cellulosic pellicles and water-soluble mucous substances are not produced. Not pigmented.

Growth occurs in the glucose range between 2 % and 40 % (w/v), with an optimum around 10 % (w/v). High glucose concentration [e.g., 10 % (w/v) glucose] is preferable for the growth. Osmophilic. No growth occurs in 0.35 % acetic acid (v/v) at pH 3.5. Temperature for growth ranges from 20 °C to 33 °C and the optimum around 25–30 °C. The growth pH ranges from pH 4.0 to 7.5, and the optimum pH is around pH 5.0–7.0. No growth is observed above pH 8.0 or below pH 4.0. The major cellular fatty acids are $C_{16:0}$ 2-OH (31.1–41.0 %) and $C_{18:1}$ ϖ7*c* (22.0–29.8 %). The major quinone is Q-10. The G+C content of DNA is between 52 and 53 mol%.

Saccharibacter strains are isolated from pollens of flowers.

Saccharibacter floricola Jojima, Mihara, Suzuki, Yokozeki, Yamanaka, and Fudo 2004

Characteristics are the same as those described for the species (Jojima et al. 2004).

The type strain: S-877^T (= AJ 13480^T = DSM 15669^T = JCM 12116^T), isolated from pollen collected in Kanagawa Prefecture, Japan. The G+C content of DNA of the type strain is 52.3 mol%.

Swaminathania Loganathan and Nair 2004

Swa.mi.na.tha'nia. N. L. fem. n. after Swaminathan, Indian biologist, the father of the Green Revolution in India.

Swaminathania strains were isolated from the rhizosphere, roots, and stems of salt-tolerant, mangrove-associated wild rice (*Porteresia coarctata* Tateoka) in Pichavaram, Tamil Nadu, India, with nitrogen-free, semisolid LGI medium at pH 5.5 (Cavalcante and Döbreiner 1988) supplemented with 250 mM NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that these strains were related to the genera *Acidomonas*, *Asaia*, *Acetobacter*, *Gluconacetobacter*, *Gluconobacter*, and *Kozakia*. The strains were able to fix nitrogen and solubilize phosphate in the presence of NaCl. Based on overall analysis of the tests and comparison with the characteristics of the members of the family *Acetobacteraceae*, a novel genus *Swaminathania* was established (Loganathan and Nair 2004).

The type species is *Swaminathania salitolerans*. The closest neighbor is the genus *Asaia* (Fig. 1.1). Currently, this genus contains a single species, *Swaminathania salitolerans*.

Swaminathania Loganathan and Nair 2004

Cells are Gram negative and straight rods with round ends, measuring approximately 0.7–0.9 by 1.9–3.1 μm, and motile with peritrichous flagella. Colonies are initially yellowish, becoming dark orange later on, smooth, and raised, with entire margin on LGI medium (Cavalcante and Döbreiner 1988). Growth occurs on mannitol agar and glutamate agar.

Aerobic. Catalase positive. Oxidase negative. Acetic acid is produced from ethanol in neutral and acidic conditions. Glucose is oxidized to acetic acid. Acetate and lactate are oxidized to carbon dioxide and water, but the activity was weak. Acid is produced from L-arabinose, D-galactose, D-glucose, D-mannose, sorbitol, glycerol, and ethanol, but not from L-rhamnose or D-mannitol. Methanol is not utilized. Water-soluble brown pigments are produced on D-glucose- and $CaCO_3$-containing agar plates and GYC agar medium. Brown pigmentation is observed on yeast extract-, D-glucose-, and $CaCO_3$-containing medium. Gelatin and starch are not hydrolyzed. The strains are able to fix nitrogen, which is determined by acetylene reduction assay and PCR amplification of *nifD* gene. Solubilization of phosphate is recognized, which is determined by spotting on Pikovskaya medium and generation of clearing zones (Pikovskaya 1948).

Good growth occurs in the presence of 0.35 % acetic acid at pH 3.5 and 3 % NaCl using 1 % KNO_3 as a nitrogen source.

Major cellular fatty acids are $C_{18:1}$ ϖ7c/ϖ9t/ϖ12t (30.41 %), $C_{13:1}$ AT 12-13 (0.53 %), and $C_{19:0}$ cyclo ϖ8c (11.84 %). The major quinone is Q-10. The G+C content of DNA is between 57.6 and 59.9 mol%.

Swaminathania strains are isolated from mangrove-associated wild rice (*Porteresia coarctata* Tateoka).

Swaminathania salitolerans Loganathan and Nair 2004

Characteristics are the same as those described for the species (Loganathan and Nair 2004).

The type strain: PA51^T (= LMG 21291^T = MTCC 3852^T), isolated from mangrove-associated wild rice in Pichavaram, Tamil Nadu, India. The G+C of DNA of the type strain is 59.9 mol%.

Tanticharoenia Yukphan, Malimas, Muramatsu, Takahashi, Kaneyasu, Tanasupawat, Nakagawa, Suzuki, Potacharoen, and Yamada 2008

Tan.ti.cha.ro.e'nia. N. L. fem. n. named after Dr. Morakot Tanticharoen, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand, who contributed to studies of acetic acid bacteria and especially to their systematic study.

Tanticharoenia strains were isolated from soil collected at Sakaerat, Nakhon Ratchasima, in Thailand by an enrichment approach with glucose-ethanol-acetic acid medium. Those are included within a lineage comprising the genera *Asaia*, *Kozakia*, *Swaminathania*, *Neoasaia*, *Acetobacter*, *Gluconobacter*, and *Saccharibacter* in a phylogenetic tree based on 16S rRNA gene sequences but formed a quite different and independent cluster. The strains did not oxidize acetate and lactate and grew on 30 % glucose (w/v). A new genus, *Tanticharoenia*, was established on the basis of biochemical activities and the phylogenetic position (Yukphan et al. 2008).

The type species is *Tanticharoenia sakaeratensis*. The closest neighbor is the genus *Ameyamaea* (Fig. 1.1). Currently, this genus comprises a single species, *Tanticharoenia sakaeratensis*.

Tanticharoenia Yukphan et al. 2008

Cells are Gram negative, rod-shaped, measuring 0.6–0.8 by 1.0–1.6 μm, and nonmotile. Colonies are creamy and smooth with entire margin when grown on glucose-ethanol-peptone-yeast extract-calcium carbonate agar. Growth occurs on glutamate agar (weakly positive) and mannitol agar.

Acetic acid is produced from ethanol. Acetate and lactate are not oxidized. Dihydroxyacetone is produced from glycerol. 2-Keto-D-gluconate, 5-keto-D-gluconate, and 2,5-diketo-D-gluconate are produced from D-glucose. Acid is produced from L-arabinose, D-xylose, D-fructose (weakly positive), D-galactose, D-glucose, D-mannose, melibiose, sucrose (weakly positive), raffinose (weakly positive), *meso*-erythritol, glycerol, and ethanol, but not from D-arabinose, L-sorbose, L-rhamnose, lactose, maltose, dulcitol, D-mannitol, or D-sorbitol. Growth occurs on D-arabinose, D-xylose, D-fructose, D-glucose, D-galactose, *meso*-erythritol, D-mannitol, D-sorbitol, glycerol, or sucrose, but not on D-arabinose, L-sorbose, L-rhamnose, D-mannose, lactose, maltose, melibiose, raffinose, dulcitol, or ethanol. Ammoniac nitrogen is not assimilated in the presence of D-glucose, D-mannitol, or ethanol as a carbon source. Growth does not occur on methanol. A water-soluble brown pigment is intensely produced on glucose-peptone-yeast extract-calcium carbonate agar.

Growth occurs in the presence of 0.35 % acetic acid (w/v), but not of 1 % KNO_3. Growth occurs on 30 % D-glucose (w/v). The major quinone is Q-10. The G+C content of DNA ranges from 64.5 to 65.6 mol%.

Tanticharoenia strains are isolated from soil.

Tanticharoenia sakaeratensis Yukphan, Malimas, Muramatsu, Takahashi, Kaneyasu, Tanasupawat, Nakagawa, Suzuki, Potacharoen, and Yamada 2008

Characteristics are the same as those described for the species (Yukphan et al. 2008).

Type strain: AC37^T (= BCC 15772^T = NBRC 103193^T), isolated from soil collected at Sakaerat, Nakhon Ratchasima, Thailand. The G+C content of the type strain is 65.6 mol%.

The Acidophilic Group

The acidophilic group is said aerobic phototrophic bacteria in another way (Yurkov 2006). Photosynthesis in purple bacteria has been widely assumed to be an anaerobic metabolic process, active in light and anaerobic conditions. However, bacteriochlorophyll (BChl) *a* was found in aerobic heterotrophic bacteria in spite of no growth of the bacteria in anaerobic and phototrophic conditions (Sato 1978; Harashima et al. 1978). The properties of BChl-protein complexes in aerobic phototrophic bacteria were shown to be similar to those in typical anaerobic photosynthetic bacteria although photochemical reactions supported only under aerobic conditions (Yurkov and Beatty 1998). The majority of the aerobic phototrophic bacteria are phylogenetically associated with the member of the class *Alphaproteobacteria*. Among the aerobic phototrophic bacteria, the acidophilic group belonging to the family *Acetobacteraceae* has heterogeneous natures as shown in Table 1.4, e.g., acidophilic to slightly alkaliphilic, mesophilic to moderately thermophilic, and phototrophic or non-phototrophic. The acidophilic group mostly consists of the species with being catalase positive and oxidase positive, which differs from the acetous group.

Acidicaldus Johnson, Stallwood, Kimura, and Hallberg 2006

A.ci.di.cal'dus. N. L. n. *acidum* an acid; N. L. adj. *caldus*, warm; a (moderately) thermophilic acid-requiring microorganism.

Acidicaldus strains are facultatively anaerobic, moderately thermophilic, acidophilic bacteria and were isolated from acidic geothermal springs and soils in Yellowstone National Park, Wyoming (Johnson et al. 2003, 2006). Those formed an independent lineage near the genus *Acidisphaera* in a phylogenetic tree based on 16S rRNA gene sequences but formed an independent lineage (Fig. 1.1). Currently, this genus contains a single species, *Acidicaldus organivorus*.

Acidicaldus Johnson et al. 2006

Cells are Gram negative, non-sporulating, motile, and rod-shaped. Obligately heterotrophic, acidophilic, and moderately thermophilic. Facultative anaerobes, growing by ferric iron

Table 1.4

Characteristics of the genera in the acidophilic group

	Acidicaldus	*Acidiphilium*	*Acidisoma*	*Acidisphaera*	*Acidocella*	*Belnapia*	*Craurococcus*	*Humitalea*
Cell form	Rods	Rods	Coccobacilli	Cocci, short rods	Rods	Cocci	Cocci	Rods
Motility	+	V	–	–	V	–	–	–
Colonial color								
White, cream, pink, light brown	+	+	+	+	+	+	+	+
Red, purple	–	+	–	–	–	+	–	–
Anaerobic phototrophy	NA	–	–	–	–	–	–	–
Bacteriochlorophyll *a*	NA	V	_	+	–	-	+	+
Carotenoids	NA	V	NA	+	–	NA	NA	+
Optimum temperature	Thermophilic	Mesophilic	Mesophilic	Mesophilic	Mesophilic	Mesophilic	Mesophilic	Mesophilic
Optimal growth pH	Acidophilic	Acidophilic	Acidophilic	Acidophilic	Acidophilic	Neutrophilic	Neutrophilic	Neutrophilic
Oxidase	NA	V	+	+	–	V	+	+
Major fatty acids	NA	$C_{18:1}$	Cyclo $C_{19:0}$ ϖ8*c*	NA	$C_{18:1}$	$C_{16:0}$, $C_{18:1}$ϖ7c/$C_{18:1}$ϖ6c	$C_{18:1}$	$C_{18:1}$ϖ7c
Major quinone (s)	NA	Q-10	Q-10	Q-10	Q-10	Q-9	Q-10	NA
DNA G+C content (mol %)	72	63–70	60.5–61.9	69.1–69.8	58.7–65.6	70–75	70.5	68.2

	Paracraurococcus	*Rhodopila*	*Rhodovarius*	*Roseococcus*	*Roseomonas*	*Rubritepida*	*Stella*	*Zavarzinia*
Cell form	Cocci	Cocci	Short rods	Cocci, short rods	Cocci to rods	Short rods	Flat star	Rods
Motility	–	+	–	+	v	+	–	v
Colonial color								
White, cream, pink, light brown	–	+	–	+	+	–	–	NA
Red, purple	+	+	+	–	–	+	–	NA
Anaerobic phototrophy	–	+	–	–	NA	–	–	NA
Bacteriochlorophyll α	+	+	–	+	NA	+	NA	NA
Carotenoids	NA	+	+	+	NA	+	NA	NA
Optimum temperature	Mesophilic	Mesophilic	Mesophilic	Mesophilic	Mesophilic	Thermophilic	Mesophilic	Mesophilic
Optimal growth pH	Neutrophilic	Acidophilic	Neutrophilic	Slightly alkaliphilic	Neutrophilic	Neutrophilic	Neutrophilic	Neutrophilic
Oxidase	+	NA	+	+	VA	+	+	NA
Major fatty acids	$C_{18:1}$	$C_{18:1}$	$C_{16:0}$, $C_{19:1}$ϖ7c, Cyclo $C_{19:0}$ ϖ8*c*	$C_{18:1}$ϖ7c	$C_{16:0}$, $C_{18:1}$ϖ7c	$C_{16:0}$, $C_{18:1}$ ϖ7*c*, Cyclo $C_{19:0}$ 2-OH C_{11-12}	NA	$C_{16:0}$, $C_{16:0}$ 3-OH, $C_{18:1}$, C18:0 3-OH
Major quinone (s)	Q-10	Q-9/10, MK-9/10, RQ-9/10	Q-10	NA	Q-10	Q-9	NA	Q-10
DNA G+C content (mol %)	70.3–71.0	66.3	72.4	69.1–70.4	65–73	70.2	69.3–73.5	66.1

Symbols: +, positive; –, negative; v, variable; and NA, data not available

respiration in the absence of oxygen. Colonies are round to convex and off-white. Bacteriochlorophyll (BChl) *a* and carotenoids are absent.

Grows on simple organic compounds such as sugars and small molecular weight alcohols, but aliphatic acids inhibit growth at millimolar concentrations. Capable of dissimilatory oxidation of elemental sulfur, but not of autotrophic growth on sulfur in organic-free media. The G+C content of the genomic DNA is ca. 72 mol%. The type species is *Acidicaldus organivorus*.

Acidicaldus organivorus Johnson, Stallwood, Kimura, and Hallberg 2006

The characteristics of the species are the same as those described for the species (Johnson et al. 2006).

Type strain: Y008^T (= ATCC BAA-1105^T = DSM 16953^T), isolated from acidic geothermal springs and soils in Yellowstone National Park, Wyoming. The G+C content of DNA of the type strain is 71.8 ± 0.9 mol%.

Acidiphilium Harrison 1981 emend. Kishimoto, Kosko, Wakao, Tano, and Hiraishi 1996

A.ci.di.phi.li'um. N.L. n. *acidum* (from L. adj. *acidus*, sour), an acid; N.L. neut. adj. *philum* (from Gr. neut. adj. *philon*), friend, loving; N.L. neut. n. *Acidiphilium*, acid lover.

Acidiphilium strains are aerobic, mesophilic, acidophilic bacteria and were isolated from acidic drainage waters and mine refuse from mines (Harrison 1981; Kishimoto and Tano 1987; Lobos et al. 1986; Wakao et al. 1988; Wichlacz and Unz 1981). The closest neighbor is the genus *Acidocella* (*Fig. 1.1*). Currently, this genus contains six species.

Acidiphilium Harrison 1981

Cells are Gram negative, non-sporulating, and rod-shaped, 0.6–3.8 μm long. Motile or nonmotile; motile cells have one polar flagellum or two lateral flagella. Aerobic. Chemoorganotrophic or heterotrophic, mesophilic, and acidophilic. Colonies are white to cream, yellow, pink, red, or brown.

Catalase positive. Oxidase variable. Some strains form BChl *a* and carotenoid. Sugars, sugar alcohols, organic acids, and amino acids are used as a carbon and energy sources. Sodium acetate inhibits the growth of some strains.

The major cellular fatty acid is $C_{18:1}$. The major quinone is Q-10. The G+C content of the genomic DNA is between 63 and 70 mol%. The type species is *Acidiphilium cryptum*.

Acidiphilium cryptum Harrison 1981

The characteristics of the species are the same as those described for the species (Harrison 1981).

Type strain: Lhet2^T (= ATCC 33463^T = DSM 2389^T = JCM 21277^T = NBRC 14242^T =NCIMB 11690^T), isolated from a culture of *Thiobacillus ferrooxidans* from coal mine water in Pennsylvania. The G+C content of DNA of the type strain is 69.8 mol%.

Acidiphilium acidophilum (Harrison 1981) Hiraishi, Nagashima, Matsuura, Shimada, Takaichi, Wakao, and Katayama 1998

The characteristics of the species are based on information from the previous data (Guay and Silver 1975; Harrison 1983; Hiraishi et al. 1998; Katayama-Fujimura et al. 1982, 1983, 1984; Mason et al. 1987; Norris et al. 1986; Pronk et al. 1990).

Type strain: ATCC 27807^T (= CIP 104483^T = DSM 700^T), isolated from an iron-oxidizing culture of *Thiobacillus ferrooxidans*. The G+C content of DNA of the type strain is 63.5 mol%.

Acidiphilium angustum Wichlacz, Unz, and Langworthy 1986

The characteristics of the species are the same as those described for the species (Wichlacz et al. 1986).

Type strain: KLB^T (= ATCC 35903^T = NCCB 86036^T), isolated from acidic waters discharged from abandoned coal mines in central Pennsylvania. The G+C content of DNA of the type strain is 67 mol%.

Acidiphilium multivorum Wakao, Nagasawa, Matsuura, Matsukura, Matsumoto, Hiraishi, Sakurai, and Shiota 1995

The characteristics of the species are the same as those described for the species (Wakao et al. 1994).

Type strain: AIU 301^T (= DSM 11245^T = JCM 8867^T = NBRC 100785^T = NBRC 100883^T), isolated from pyritic acid mine drainage. The G+C content of DNA of the type strain is 69.8 mol%.

Acidiphilium organovorum Lobos, Chisolm, Bopp, and Holmes 1986

The characteristics of the species are the same as those described for the species (Lobos et al. 1986).

Type strain: ATCC 43141^T (= NCCB 86037^T), isolated from a *Thiobacillus ferrooxidans* culture. The G+C content of DNA of the type strain is 64 mol%.

Acidiphilium rubrum Wichlacz, Unz, and Langworthy 1986

The characteristics of the species are the same as those described for the species (Wichlacz et al. 1986).

Type strain: OP^T (= ATCC 35905^T), isolated from acidic waters discharged from abandoned coal mines in central Pennsylvania. The G+C content of DNA of the type strain is 63 mol%.

Acidisoma Belova, Pankratov, Detkova, Kaparulina, and Dedysh 2009

A.ci.di.so'ma. M.L. n. *acidum* in acid; Gr. neut. n. *soma* body; M.L. neut. n. *Acidisoma* an acid(-requiring) body.

Acidisoma strains are aerobic, mesophilic, and acidophilic and were isolated from acidic Sphagnum-dominated tundra and Siberian wetlands in Russia (Belova et al. 2009). The strains phylogenetically belong to the class *Alphaproteobacteria* but were distantly related to the type strains of acidophilic bacteria: *Acidisphaera rubrifaciens* (93.4–94.3 % 16S rRNA gene sequence identity), members of the genera *Acidiphilium* (91.3–93 %), and *Acidocella* (91.8–92.4 %). The closest neighbors are the genera *Acidisphaera* and *Acidicaldus* (Fig. 1.1). Currently, this genus contains two species.

Acidisoma Belova et al. 2009

Cells are Gram negative, non-sporulating, nonmotile, and coccobacilli. Strictly aerobic, chemoorganotrophic, psychrotolerant, and moderately acidophilic. Colonies are circular, smooth, convex slimy, and white to cream and pinkish.

Catalase positive. Oxidase positive. BChl *a* is absent. Sugars, polyalcohols, and some organic acids are used as carbon sources. The major cellular fatty acid is cyclo $C_{19:0}\omega 8c$. The major quinone is Q-10. The major polar lipids are phosphatidylcholine, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and unknown aminolipids. The G+C content of the genomic DNA is between 60 and 62 mol%. The type species is *Acidisoma tundrae*.

Acidisoma tundrae Belova, Pankratov, Detkova, Kaparulina, and Dedysh 2009

The characteristics of the species are the same as those described for the species (Belova et al. 2009).

Type strain: WM1^T (= DSM 19999^T = VKM B-2488^T), isolated from an acidic oligotrophic Sphagnum-dominated wetland in the Vorkuta region of northern Russia. The G+C content of DNA of the type strain is 61.9 mol%.

Acidisoma sibiricum Belova, Pankratov, Detkova, Kaparulina, and Dedysh 2009

The characteristics of the species are the same as those described for the species (Belova et al. 2009).

Type strain: TPB606^T (= DSM 21000^T = VKM B-2487^T), isolated from the Sphagnum peat bog Bakchar in the Tomsk region of western Siberia. The G+C content of DNA of the type strain is 60.5 mol%.

Acidisphaera Hiraishi, Matsuzawa, Kanbe, and Wakao 2000

A.ci.di.sphae'ra. M.L. n. *acidum* an acid; M.L. fem. n. *sphaera* a sphere; M.L. fem. n. *Acidisphaera* acid-requiring coccoid microorganism.

Acidisphaera strains are aerobic, mesophilic, and acidophilic and produce BChl *a*. The strains were isolated from surface water and sediment from an acidic hot spring and pyritic mine drainage (Hiraishi et al. 2000). Those were phylogenetically clustered in the acidophilic group which includes the genera *Acidiphilium* and *Rhodopila*. The closest neighbor is the genus *Acidicaldus* with 93.4 % of 16S rRNA gene sequence similarity (Fig. 1.1). Currently, this genus contains a single species, *Acidisphaera rubrifaciens*.

Acidisphaera Hiraishi et al. 2000

Cells are Gram negative, non-sporulating, nonmotile, and cocci or coccobacilli, 0.8–1.2 μm long. Obligately aerobic, chemoorganotrophic, facultative photoorganotroph mesophilic, and obligately acidophilic. Colonies are circular, smooth, convex, and salmon-pink to red.

Catalase positive. Oxidase positive. Produces Bchl *a* and carotenoid. The major quinone is Q-10. The G+C content of the genomic DNA is between 69.1 and 69.8 mol%. The type species is *Acidisphaera rubrifaciens*.

Acidisphaera rubrifaciens Hiraishi, Matsuzawa, Kanbe, and Wakao 2000

The characteristics of the species are the same as those described for the species (Hiraishi et al. 2000).

Type strain: HS-AP3^T (= JCM 10600^T), isolated from surface water from an acidic hot spring in Hakone, Kanagawa Prefecture, Japan. The G+C content of DNA of the type strain is 69.3 mol%.

Acidocella Kishimoto, Kosako, Wakao, Tano, and Hiraishi 1995

A.ci.do.cel'la. Gr. n. *acidum* an acid; L. fem. n. *cella* a cell; N.L. fem. n. *Acidocella*, acid (-requiring) cell.

Acidocella strains are aerobic, mesophilic, and acidophilic. The strains were isolated from acidic mine drainage and mud (Kishimoto and Tano 1987; Wichlacz et al. 1986). *Acidocella* was distinguished from *Acidiphilium* by 16S rRNA gene sequence analysis with less than 94.2 % similarity (Kishimoto et al. 1995). The closest neighbor is the genus *Acidiphilium* (Fig. 1.1). Currently, this genus contains three species.

Acidocella Kishimoto et al. 1995

Cells are Gram negative, non-sporulating, and rod-shaped, 1.0–1.8 μm long. Motile or nonmotile; motile cells have a polar flagellum. Strictly aerobic. Chemoorganotrophic. Mesophilic. Acidophilic. Colonies are circular, smooth, opaque, and white to pale light brown. Catalase positive. Oxidase negative. BChl and carotenoids are absent.

The major cellular fatty acid is $C_{18:1}$. The major quinone is Q-10. The G+C content of the genomic DNA is between 63 and 70 mol%. The type species is *Acidocella facilis*. The G+C content of the genomic DNA is between 58.7 and 65.6 mol%.

Acidocella facilis (Wichlacz, Unz and Langworthy 1986) Kishimoto, Kosako, Wakao, Tano, and Hiraishi 1996

The characteristics of the species are the same as those described for the species (Kishimoto et al. 1995; Wichlacz et al. 1986).

Type strain: PW2^T (= ATCC 35904^T = NCCB 86038^T), isolated from acidic waters discharged from abandoned coal mines in central Pennsylvania. The G+C content of DNA of the type strain is 65 mol%.

Acidocella aluminiidurans Kimoto, Aizawa, Urai, Ve, Suzuki, Nakajima, and Sunairi 2010

The characteristics of the species are the same as those described for the species (Kimoto et al. 2010).

Type strain: AL46^T (= NBRC 104303^T = VTCC-D9-1^T), isolated from a waterweed, *Panicum repens*, grown in a highly acidic swamp (pH 3) at an actual acid sulfate soil area of Vietnam. The G+C content of DNA of the type strain is 65.6 mol%.

Acidocella aminolytica (Kishimoto, Kosako, and Tano 1994) Kishimoto, Kosako, Wakao, Tano, and Hiraishi 1996

The characteristics of the species are the same as those described for the species (Kishimoto et al. 1993, 1995).

Type strain: 101^T (= ATCC 51361^T = DSM 11237^T = JCM 8796^T), isolated from acidic mine drainage. The G+C content of DNA of the type strain is 58.7 mol%.

Belnapia Reddy, Nagy, and Garcia-Pichel 2006

Bel.na'pi.a. N.L. fem. n. *Belnapia* after J. Belnap, in honor of her contributions to the study of biological soil crusts (BSCs).

Belnapia strains are aerobic, mesophilic, neutrophilic, and facultatively fermentative. The strains were isolated from biological soil crusts, forest soil, and grass soil (Jin et al. 2012, 2013; Reddy et al. 2006). Those were phylogenetically distantly related BChl-producing bacteria *Paracraurococcus ruber* (<94.9 %), *Craurococcus roseus* (<92.2 %), and *Roseococcus thiosulfatophilus* (<92.3 %) and non-BChl-producing bacteria *Roseomonas rosea* (<94.9 %), *Roseomonas gilardii* (<94.2 %), and *Roseomonas mucosa* (<93.8 %). Currently, this genus contains three species.

Belnapia Reddy et al. 2006

Cells are Gram negative, non-sporulating, nonmotile, and cocci. Aerobic, facultative fermentative, mesophilic, and neutrophilic. Colonies are circular, smooth or rough, convex, and red to pink. Catalase positive. Oxidase variable. BChl is absent, and carotenoid is produced.

The major cellular fatty acids are $C_{18:1}\omega7c/C_{18:1}\omega6c$ and $C_{16:0}$. The major quinone is Q-9. The G+C content of the genomic DNA is between 70 and 75 mol%. The type species is *Belnapia moabensis*.

Belnapia moabensis Reddy, Nagy, and Garcia-Pichel 2006

The characteristics of the species are the same as those described for the species (Reddy et al. 2006).

Type strain: CP2C^T (= ATCC BAA-1043^T = DSM 16746^T), isolated from biological soil crusts in the Colorado Plateau, USA. The G+C content of DNA of the type strain is 75 mol%.

Belnapia rosea Jin, Liu, Wei, Li, Zhang, and Yu 2012

The characteristics of the species are the same as those described for the species (Jin et al. 2012).

Type strain: CPCC 100156^T (= DSM 23312^T = CGMCC 1.10758^T), isolated from a forest soil from Hainan Island, South China. The G+C content of DNA of the type strain is 70.3 mol%.

Belnapia soli Jin, Lee, No, Ko, Kim, Ahn, and Oh 2013

The characteristics of the species are the same as those described for the species (Jin et al. 2013).

Type strain: PB-K8^T (= JCM 18033^T = KCTC 23765^T), isolated from grass soil in Daejeon, Republic of Korea. The G+C content of DNA of the type strain is 72.1 mol%.

Craurococcus Saitoh, Suzuki, and Nishimura 1998

Cra.u.ro.coc'cus. Gr. adj. *crauros* fragile; Gr. n. *coccus* a grain, berry; M.L. masc. n. *Craurococcus* fragile coccus.

A Craurococcus strain is aerobic, mesophilic, neutrophilic, and heterotrophic and contains BChl. The strain was isolated from soil (Saitoh and Nishimura. 1996). Phylogenetic analysis revealed that *Craurococcus* formed a distinct lineage with *Paracraurococcus ruber* (94.2 %) in the class *Alphaproteobacteria*.

Craurococcus Saitoh et al. 1998

Cells are Gram negative, nonmotile, and cocci, 0.8–2.0 μm in size. Aerobic, heterotrophic, mesophilic, and neutrophilic. No growth occurs anaerobically in the light. Colonies are irregular and pink. Catalase positive. Oxidase positive. BChl *a* is present. Currently, this genus contains a single species, *Craurococcus roseus*.

The major cellular fatty acid is $C_{18:1}$. The major quinone is Q-10. The G+C content of the genomic DNA is 70.5 mol%. The type species is *Craurococcus roseus*.

Craurococcus roseus Saitoh, Suzuki, and Nishimura 1998

The characteristics of the species are the same as those described for the species (Saitoh et al. 1998).

Type strain: NS130^T (= CIP 105707^T = JCM 9933^T), isolated from soil. The G+C content of DNA of the type strain is 70.5 mol%.

Humitalea Margesin and Zhang 2013

Hu.mi.ta'le.a. L. n. *humus* soil; L. fem. n. *talea* a rod, stick; N.L. fem. n. *Humitalea* a rod from soil.

Humitalea strain is aerobic, mesophilic, and neutrophilic and produces BChl. The strain was isolated from soil (Margesin and Zhang 2013). The strain was phylogenetically related to members of the genera *Roseococcus* and *Rubritepida* (<92.8 %) but formed a distinct lineage in the family *Acetobacteraceae* (Fig. 1.1). Currently, this genus contains a single species, *Humitalea rosea*.

Humitalea Margesin and Zhang 2013

Cells are Gram negative, nonmotile, and rod-shaped, 1.4–1.7 μm long. Obligately aerobic, mesophilic, and neutrophilic. Colonies are smooth, round, glossy, convex, circular with entire margins, and pale pinkish. Catalase positive. Oxidase positive. BChl *a* is present.

The major cellular fatty acids are $C_{18:1}\omega7c$ and $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylchloline, and unidentified aminolipids. The G+C content of the genomic DNA is 68.2 mol%. The type species is *Humitalea rosea*.

Humitalea rosea Margesin and Zhang 2013

The characteristics of the species are the same as those described for the species (Margesin and Zhang 2013).

Type strain: W37^T (= CIP 110261^T = LMG 26243^T), isolated from soil. The G+C content of DNA of the type strain is 68.2 mol%.

Paracraurococcus Saitoh, Suzuki, and Nishimura 1998

Pa.ra.cra.u.ro.coc'cus. Gr. Prep. *Para* like, along side of; Gr. adj. *crauros* fragile; Gr. n. *coccus* a grain, berry; M.L. masc. n. *Paracraurococcus* coccus like *Craurococcus*.

Paracraurococcus strains are aerobic, mesophilic, neutrophilic, and heterotrophic and contain BChl. The strains were isolated from soil (Saitoh and Nishimura. 1996). Phylogenetic analysis revealed that *Paracraurococcus* formed a distinct lineage with *Craurococcus roseus* (94.2 %) in the class *Alphaproteobacteria* (Fig. 1.1). Currently, this genus contains a single species, *Paracraurococcus ruber*.

Paracraurococcus Saitoh et al. 1998

Cells are Gram negative, nonmotile, and cocci, 0.8–1.5 μm in size. Aerobic, heterotrophic, mesophilic, and neutrophilic. No growth occurs anaerobically in the light. Colonies are irregular and red. Catalase positive. Oxidase positive. BChl *a* is present.

The major cellular fatty acid is $C_{18:1}$. The major quinone is Q-10. The G+C content of the genomic DNA is 70.3–71.0 mol%. The type species is *Paracraurococcus ruber*.

Paracraurococcus ruber Saitoh, Suzuki, and Nishimura 1998

The characteristics of the species are the same as those described for the species (Saitoh et al. 1998).

Type strain: NS89^T (= CIP 105708^T = JCM 9931^T), isolated from soil. The G+C content of DNA of the type strain is 70.3 mol%.

Rhodopila Imhoff, Trüper, and Pfennig 1984

Rho.do.pi'la. Gr. n. *rhodon* rose; M.L. fem. n. *pila* ball or sphere; M.L. fem. n. *Rhodopila* red-colored spherical bacterium.

A *Rhodopila* strain is anaerobic, mesophilic, acidophilic, and phototrophic and produces BChl. The strain was isolated from the acidic water and sludge sample from a warm sulfur spring of pH 3.0 near the Gibbon River in the Yellowstone Park, Wyoming, USA (Pfennig 1974). The strain was once named *Rhodopseudomonas globiformis* but reidentified as *Rhodopila globiformis* on the basis of amino acid sequence of cytochromes *c*, DNA-rRNA hybridization, 16S rRNA oligonucleotide catalogs, and major components of the polar lipids (Imhoff et al. 1984; Pfennig and Trüper 1983). Currently, this genus contains a single species, *Rhodopila globiformis*.

Rhodopila Imhoff, Trüper et al. 1984

Cells are Gram negative, spherical to ovoid, 1.6–1.8 µm in diameter, and motile by means of a polar flagellum. Photoheterotrophic, growing either anaerobically in the light or microaerobically in the dark. Mesophilic and acidophilic. Cells growing anaerobically in the light are red, and those growing microaerobically are pink. BChl *a* and carotenoids are present.

The major cellular fatty acids are $C_{18:1}$ and $C_{16:0}$. The G+C content of the genomic DNA is 66.3 mol%. The type species is *Rhodopila globiformis*.

Rhodopila globiformis (Pfennig 1974) Imhoff et al. 1984

The characteristics of the species are the same as those described for the species (Pfennig 1974; Imhoff et al. 1984).

Type strain: 7950^T (= ATCC 35887^T = DSM 161^T = LMG 4312^T), isolated from the acidic water and sludge sample from a warm sulfur spring at the Gibbon River, Yellowstone Park, Wyoming, USA. The G+C content of DNA of the type strain is 66.3 mol%.

Rhodovarius Kämpfer, Busse, Rosselló-Mora, Kjellin, and Falsen 2004

Rho.do.va'ri.us. Gr. n. *rhodon*, the rose; L. adj. *varius*, diverse, varied; M.L. masc. n. *Rhodovarius*, the varying red-colored one.

A *Rhodovarius* strain is aerobic, mesophilic, and neutrophilic. The strain was isolated from a Rodac medium during a routine industrial hygiene control (Kämpfer et al. 2004). The strain was formed a phylogenetically distinct lineage in the class *Alphaproteobacteria* (Fig. 1.1). Currently, this genus contains a single species, *Rhodovarius lipocyclicus*.

Rhodovarius Kämpfer et al. 2004

Cells are Gram negative and short rods, 1.0–1.2 µm long. Long filaments are formed in liquid culture. Strictly aerobic, chemoorganotrophic, mesophilic, and neutrophilic. Colonies are red. Catalase positive. Oxidase positive. BChl is absent, but carotenoids are present.

The major cellular fatty acids are cyclo $C_{19:0}\omega 8c$ and cyclo $C_{17:0}$. The major quinone is Q-10. The major polar lipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidyl di-methylethanolamine, phosphatidylcholine, and unknown aminolipids. The G+C content of the genomic DNA is 72.4 mol%. The type species is *Rhodovarius lipocyclicus*.

Rhodovarius lipocyclicus Kämpfer, Busse, Rosselló-Mora, Kjellin, and Falsen 2004

The characteristics of the species are the same as those described for the species (Kämpfer et al. 2004).

Type strain: CCUG 44693^T (= CIP 108310^T), isolated from Rodac medium during a routine industrial hygiene control submitted to CCUG for identification. The G+C content of DNA of the type strain is 72.4 mol%.

Roseococcus Yurkov, Stackebrandt, Holmes, Fuerst, Hugenholtz, Golecki, Gad'on, Gorlenko, Kompantseva, and Drews 1994

Ro.se.o.coc'cus. M.L. adj. *roseus* rose, pink; Gr. n. *coccus* sphere or spheroidal shape; M.L. masc. n. *Roseococcus* pink spherical bacterium.

Roseococcus strains are aerobic, mesophilic, slightly alkaliphilic, and facultatively photoheterotrophic and produce BChl. The strains were isolated from freshwater cyanobacterial mat of a thermal alkaline sulfide spring and a low-mineralized steppe soda lake (Boldareva et al. 2009; Yurkov et al. 1994). Those were phylogenetically moderately related to *Rubritepida flocculans* with less than 95.0 % 16S rRNA gene sequence similarity. Currently, this genus contains two species.

Roseococcus Yurkov et al. 1994

Cells are Gram negative, cocci or short rods, 1.2–1.7 µm long, and motile by means of a single polar flagellum. Obligately aerobic, chemoorganotrophic, facultatively photoheterotrophic, mesophilic, and neutrophilic to alkaliphilic. Colonies are pinkish. BChl *a* and carotenoids are present.

The major cellular fatty acid is $C_{18:1}\omega 7c$. The G+C content of the genomic DNA is between 69.1 and 70.4 mol%. The type species is *Roseococcus thiosulfatophilus*.

Roseococcus thiosulfatophilus Yurkov, Stackebrandt, Holmes, Fuerst, Hugenholtz, Golecki, Gad'on, Gorlenko, Kompantseva, and Drews 1994

The characteristics of the species are the same as those described for the species (Yurkov et al. 1994).

Type strain: RB3^T (= ATCC 700004^T = DSM 8511^T), isolated from freshwater cyanobacterial mat of a thermal alkaline sulfide spring. The G+C content of DNA of the type strain is 70.4 mol%.

Roseococcus subuntuyensis Boldareva, Tourova, Kolganova, Moskalenko, Makhneva, and Gorlenko 2009

The characteristics of the species are the same as those described for the species (Boldareva et al. 2009).

Type strain: SHET^T (= DSM 19979^T = VKM B-2453^T), isolated from the low-mineralized steppe soda lake (Eastern Siberia). The G+C content of DNA of the type strain is 69.1 mol%.

Roseomonas Rihs, Brenner, Weaver, Steigerwalt, Hollis, and Yu 1998

Ro.se.o.mo'nas M.L. adj. *roseus* rosy, rose colored, or pink; Gr. n. *monas* a unit; M.L. n. *Roseomonas*, a pink-pigmented bacterium.

Roseomonas strains are aerobic, mesophilic, neutrophilic, and chemoorganotrophic. The genus *Roseomonas* is associated with pathogenic for humans, causing bacteremia, wound, urinary tract, and other infections, usually as a secondary or opportunistic pathogens. *Roseomonas* strains are isolated from the blood and cervix of patients, air, drinking water, water of estuary and pond, soil, and freshwater sediment. The closest neighbors are the genera *Paracraurococcus* and *Craurococcus* (*Fig. 1.1*). Currently, this genus contains 16 species and 2 subspecies.

Roseomonas Rihs et al. 1998

Cells are cocci, coccobacilli, or rod-shaped, 0.6–3.0 μm long. Motile or nonmotile; motile cells have one polar flagellum. Gram negative, non-sporulating, aerobic, chemoorganotrophic, mesophilic, and neutrophilic. Colonies are white, yellow, orange, or pink. Catalase positive. Oxidase variable.

The major cellular fatty acid is $C_{18:1}$ 2-OH. The major quinone is Q-10. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, and/or unidentified aminolipids. The G+C content of the genomic DNA ranges 65–73 mol%. The type species is *Roseomonas gilardii*.

Roseomonas gilardii subsp. *gilardii* (Rihs, Brenner, Weaver, Steigerwalt, Hollis, and Yu 1998) Han, Pham, Tarrand, Rolston, Helsel, and Levett 2003

The characteristics of the species are the same as those described for the species (Han et al. 2003; Rihs et al. 1993). *R. gilardii* corresponded to *Roseomonas* genomospecies 1 defined by Rihs et al. (1998) and Wayne et al. 1987).

Type strain: 5424^T (= ATCC 49956^T = CCUG 33005^T = CIP 104026^T), isolated from potable water around 1980. The G+C content of DNA of the type strain is 67.6 mol%.

Roseomonas gilardii subsp. *rosea* Han, Pham, Tarrand, Rolston, Helsel, and Levett 2003

The characteristics of the species are the same as those described for the subspecies (Han et al. 2003).

Type strain: MDA5605^T (= ATCC BAA-691^T = NCTC 12390^T), isolated from a patient's blood in 1999.

Roseomonas aerilata Yoo, Weon, Noh, Hong, Lee, Kim, Kwon, and Go 2008

The characteristics of the species are the same as those described for the species (Yoo et al. 2008).

Type strain: 5420S-30^T (= DSM 19363^T = KACC 12521^T), isolated from air sampled at an urban site in Suwon city, Republic of Korea. The G+C content of DNA of the type strain is 72.1 mol%.

Roseomonas aerophila Kim, Weon, Ahn, Hong, Seok, Whang, and Kwon 2013

The characteristics of the species are the same as those described for the species (Kim et al. 2013).

Type strain: 7515 T-07^T (= KACC 16529^T = NBRC 108923^T), isolated from air sampled in the Taean region, Republic of Korea. The G+C content of DNA of the type strain is 73.0 mol%.

Roseomonas aestuarii Venkata Ramana, Sasikala, Takaichi, and Ramana 2010

The characteristics of the species are the same as those described for the species (Venkata Ramana et al. 2010).

Type strain: JC17^T (= CCUG 57456^T = KCTC 22692^T = NBRC 105654^T), isolated from estuary water in India. The G+C content of DNA of the type strain is 66.2 mol%.

Roseomonas aquatica Gallego, Sánchez-Porro, García, and Ventosa 2006

The characteristics of the species are the same as those described for the species (Gallego et al. 2006).

Type strain: TR53^T (= CECT 7131^T = JCM 13556^T), isolated from drinking water. The G+C content of DNA of the type strain is 68.6 mol%.

Roseomonas cervicalis Rihs, Brenner, Weaver, Steigerwalt, Hollis, and Yu 1998

The characteristics of the species are the same as those described for the species (Rihs et al. 1993). *R. cervicalis* is corresponded *Roseomonas* genomospecies 2 defined by Rihs et al. (1998) and Wayne et al. (1987).

Type strain: E7107^T (= ATCC 49957^T = CIP 104027^T), isolated from the cervix of a woman in New Jersey in 1980. The G+C content of DNA of the type strain is 70.6 mol%.

Roseomonas frigidaquae Kim, Baik, Park, Rhee, Oh, and Seong 2009

The characteristics of the species are the same as those described for the species (Kim et al. 2009).

Type strain: CW67^T (= JCM 15073^T = KCTC 22211^T), isolated from a cooled water from an oxygen-producing plant in Gwangyang, Republic of Korea. The G+C content of DNA of the type strain is 69.5 mol%.

Roseomonas lacus Jiang, Dai, Wang, Zhou, and Liu 2006

The characteristics of the species are the same as those described for the species (Jiang et al. 2006).

Type strain: TH-G33^T (= CGMCC 1.3617^T = JCM 13283^T), isolated from freshwater lake sediment. The G+C content of DNA of the type strain is 71.9 mol%.

Roseomonas ludipueritiae (Kämpfer, Andersson, Jäckel, and Salkinoja-Salonen 2003) Sánchez-Porro, Gallego, Busse, Kämpfer, and Ventosa 2009

The characteristics of the species are the same as those described for the species (Kämpfer et al. 2003; Sánchez-Porro et al. 2009).

Type strain: 170/96^T (= CIP 107418^T = DSM 14915^T), isolated from non-water-damaged building material of a children's day-care center. The G+C content of DNA of the type strain is 65.8 mol%.

Roseomonas mucosa Han, Pham, Tarrand, Rolston, Helsel, and Levett 2003

The characteristics of the species are the same as those described for the species (Han et al. 2003).

Type strain: MDA5527^T (= ATCC BAA-692^T = CCUG 48654^T = NCTC 13291^T), isolated from patient's blood in 1999.

Roseomonas pecuniae Lopes, Espírito-Santo, Grass, Chung, and Morais 2011

The characteristics of the species are the same as those described for the species (Lopes et al. 2011).

Type strain: N75^T (= CIP 110074^T = LMG 25481^T), isolated from the surface of a 50 Euro cent copper-alloy coin. The G+C content of DNA of the type strain is 72.5 mol%.

Roseomonas riguiloci Baik, Park, Choe, Kim, Moon, and Seong 2012

The characteristics of the species are the same as those described for the species (Baik et al. 2012).

Type strain: 03SU10-P^T (= JCM 17520^T = KCTC 23339^T), isolated from freshwater from Woopo wetland in Gyeongnam Province, Republic of Korea. The G+C content of DNA of the type strain is 68 mol%.

Roseomonas rosea (Kämpfer, Andersson, Jäckel, and Salkinoja-Salonen 2003) Sánchez-Porro, Gallego, Busse, Kämpfer, and Ventosa 2009

The characteristics of the species are the same as those described for the species (Kämpfer et al. 2003; Sánchez-Porro et al. 2009).

Type strain: 173/96^T (= CIP 107419^T = DSM 14916^T), isolated from non-water-damaged building material of a children's day-care center. The G+C content of DNA of the type strain is 68.9 mol%.

Roseomonas stagni Furuhata, Miyamoto, Goto, Kato, Hara, and Fukuyama 2008

The characteristics of the species are the same as those described for the species (Furuhata et al. 2008).

Type strain: HS-69^T (= DSM 19981^T = JCM 15034^T = KCTC 22213^T), isolated from pond water of Nomori Pond in Shizuoka, Japan. The G+C content of DNA of the type strain is 72.0 mol%.

Roseomonas terrae Yoon, Kang, Oh, and Oh 2007

The characteristics of the species are the same as those described for the species (Yoon et al. 2007).

Type strain: DS-48^T (= JCM 14592^T = KCTC 12874^T), isolated from soil. The G+C content of DNA of the type strain is 69.3 mol%.

Roseomonas vinacea Zhang, Yu, Wang, Liu, Sun, Jiang, Zhang, and Li 2008

The characteristics of the species are the same as those described for the species (Zhang et al. 2008).

Type strain: CPCC 100056^T (= CCM 7468^T = KCTC 22045^T), isolated from a soil from the Qinghai-Tibet Plateau, northwest China. The G+C content of DNA of the type strain is 67.3 mol%.

Rubritepida Alarico, Rainey, Empadinhas, Schumann, Nobre, and Da Costa 2002

Ru.bri.te'pi.da. L. adj. *rubrus* red; L. adj. *tepidus* warm; N.L. fem. adj. *Rubritepida* the red-warm one.

Rubritepida strains are aerobic, moderately thermophilic, and neutrophilic and produce BChl *a*. The strains were isolated from a hot spring (Alarico et al. 2002). The strain was phylogenetically most closely related to *Roseococcus thiosulfatophilus* with 93.2 % of 16S rRNA gene sequence similarity (Fig. 1.1). Currently, this genus contains a single species, *Rubritepida flocculans*.

Rubritepida Alarico et al. 2002

Cells are Gram negative, short rods, 1.4–1.7 μm long, and motile by means of a single polar flagellum. Strictly aerobic, chemoorganotrophic, moderately thermophilic, and slightly alkaliphilic. Colonies are red. Catalase positive. Oxidase positive. BChl *a* and carotenoids are present.

The major cellular fatty acids are $C_{16:0}$ and $C_{18:1}\varpi 7c$. The major quinone is Q-9. The major polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and two unidentified aminolipids. The G+C content of the genomic DNA is 70.2 mol%. The type species is *Rubritepida flocculans*.

Rubritepida flocculans Alarico, Rainey, Empadinhas, Schumann, Nobre, and Da Costa 2002

The characteristics of the species are the same as those described for the species (Alarico et al. 2002).

Type strain: H-8^T (= ATCC BAA-385^T = DSM 14296^T), isolated from the hot spring at Egerszalók in Hungary. The G+C content of DNA of the type strain is 70.2 mol%.

Stella Vasilyeva 1985

Stel'la. L. fem.n. *stella* star, denoting the star-shaped morphology of the cells.

Stella strains are aerobic, mesophilic, and neutrophilic. The strains were isolated from soils, freshwater sediments, sewage and sludge, and horse manure (Vasilyeva 1985). Currently, this genus contains two species. *Stella* is phylogenetically affiliated to the family *Rhodospirillaceae* on the phylogenetic tree based on 16S rRNA gene sequence as shown in phylogenetic analysis (Fig. 1.1), but described in this chapter.

Stella Vasilyeva 1985

Cells are Gram negative, flat, six-pronged stars, 0.7–3.0 μm in diameter, and nonmotile. Aerobic or microaerophilic, chemoorganotrophic, mesophilic, and neutrophilic to slightly alkaliphilic. Catalase positive. Oxidase positive. The G+C content of the genomic DNA ranges from 69.3 to 73.5 mol%. The type species is *Stella humosa*.

Stella humosa Vasilyeva 1985

The characteristics of the species are the same as those described for the species (Vasilyeva 1985).

Type strain: AUCM B-1137^T (= ATCC 43930^T = DSM 5900^T = VKM B-1137^T), isolated from virgin chernozem on a layer, Krasnodar region, USSR. The G+C content of DNA of the type strain is 72.7 mol%.

Stella vacuolata Vasilyeva 1985

The characteristics of the species are the same as those described for the species (Vasilyeva 1985).

Type strain: 229^T (= ATCC 43931^T = DSM 5901^T = INM 229^T = VKM B-1552^T), isolated from sewage sludge from pig farm, Leningrad region, USSR. The G+C content of DNA of the type strain is 73.5 mol%.

Zavarzinia Meyer, Stackebrandt, and Auling 1994

Za.var.zi'ni.a. N.L. fem. n. *Zavarzinia*, named for Georgi Alexandrovich Zavarzin, the Russian microbiologist who with his co-workers made the first thorough investigation of mesophilic, Gram-negative carboxydotrophic strains.

A *Zavarzinia* strain was isolated from mud of the Moskva River (Meyer et al. 1993). The strain had been once allocated to the genus *Pseudomonas* but was allocated as a member of the class *Alphaproteobacteria* due to their quinone, polyamine, fatty acid patterns and the signature oligonucleotides in their 16S rRNA gene catalogs (Auling et al. 1988; Meyer et al. 1993). Currently, this genus contains a single species, *Zavarzinia compransoris*. The phylogenetic position of *Zavarzinia* is unclear in the order *Rhodospirillales* as shown in phylogenetic analysis (*Fig. 1.1*) but is described in this chapter.

Zavarzinia Meyer et al. 1994

Cells are Gram negative, rod-shaped, 1.0–1.8 μm long, and motile by means of a single polar flagellum. Colonies are very thin and translucent. Catalase positive. Organic acids and amino acids are used as sole carbon and energy sources.

The major cellular fatty acid is $C_{18:1}$. The major quinone is Q-10. The G+C content of the genomic DNA is 66.1 mol%. The type species is *Zavarzinia compransoris*.

Zavarzinia compransoris Meyer, Stackebrandt, and Auling 1994

The characteristics of the species are the same as those described for the species (Meyer et al. 1993).

Type strain: Z-1155^T (= ATCC 51430^T = CCUG 15334^T = DSM 1231^T = LMG 5821^T = LMG 8357^T), isolated from mud of the Moskva river. The G+C content of DNA of the type strain is 66.1 mol%.

A Genus in the *Gammaproteobacteria*

Frateuria Swings, Gillis, Kersters, De Vos, Gosselé and De Ley 1980 emend. Zhang 2011

Fra.teur'i, a. N. L. fem. n. *Frateuria*, named after Joseph Frateur (1903–1974), eminent Belgian microbiologist.

Four strains of acetic acid bacteria were isolated from lily flowers (*Lilium aurantum*), and the strains produced brown pigment on the media containing glucose and calcium carbonate, oxidized D-glucose to D-gluconic acid, produced a large amount of acid from pentoses, and utilized ammonium salt. These characteristics resembled those of acetic acid bacteria, and the strains were named "*Acetobacter aurantium*" (sic) (Kondo and Ameyama 1958). Later, the strains were regarded as an intermediate type between the genera *Acetobacter* and *Gluconobacter* on the basis of different phenotypic characteristics from those of both genera and tentatively named "polarly flagellated intermediate strains" (Asai et al. 1964). Additional strains were isolated from rose (*Rose hybrida*), saffron (*Crocus sativus*), and ladybell (*Adenophora triphylla*) and recognized polar flagellation and a negative response to gelatin liquefaction (Ameyama and Kondo 1967). Further, three other strains were obtained from raspberry (*Rubus parvifolius*) and recognized polar flagellation, growth on mannitol and glutamate agar, oxidation of acetate and lactate, and the production of dihydroxyacetone from glycerol (Yamada et al. 1976a). Prior to the work, quinone systems of the polarly flagellated intermediate strains were found to be Q-8, which is clearly different from Q-9 of the genus *Acetobacter* and Q-10 of the genus *Gluconobacter* (Yamada et al. 1969).

Recharacterization of "*A. aurantium*" was carried out for the strains obtained from IFO by phenotypic characteristics, protein electrophoreogram, and DNA-rRNA hybridization. As a result, positive reactions were found for oxidation of lactate but not for that of acetate, for the production of dihydroxyacetone from glycerol, for the production of 2-keto-D-gluconate and 2,5-diketo-D-gluconate from D-glucose but not 5-keto-D-gluconate, and for the production of H_2S, and negative reactions were found for gelatin liquefaction and oxidation of acetate. Further, the strains were circumscribed by the protein electrophoreogram and DNA-rRNA hybrids, and a new genus and a new species *Frateuria aurantia* were established with phenotypic characteristics (Swings et al. 1980; Swings and Sievers 2005).

Cellular fatty acid composition of acetic acid bacteria was determined, and the peculiar composition was recognized for *Frateuria aurantia* strains. The representatives of the genera *Acetobacter* and *Gluconobacter* contained the straight-chain unsaturated acid of $C_{18:1}$ as a major constituent. In contrast, *Frateuria aurantia* strains had *iso*-branched saturated acid of *i*-$C_{15:0}$ as a main component (Yamada et al. 1981a). This discriminates the genus *Frateuria* from the genera *Acetobacter* and *Gluconobacter* and the other genera in acetic acid bacteria. Further, DNA base composition and DNA-DNA similarity were determined for acetic acid bacteria. *Frateuria aurantia* strains showed a range of 62.8–65.8 mol% of G+C content of DNA and were discriminated on the basis of DNA-DNA similarity from the other members of the genera *Acetobacter*, *Gluconobacter*, and *Pseudomonas* (including *Pseudomonas maltophilia*, now *Stenotrophomonas maltophilia*) (Yamada et al. 1981b). On the basis of the differences in a major isoprenoid quinone comprising Q-8 and a major *iso*-branched saturated fatty acid comprising *i*-$C_{15:0}$, the *Frateuria* strains were said to be "pseudoacetic acid bacteria" (Yamada 1979; Yamada et al. 1981a; Lisdiyanti et al. 2003a).

Further, 16 *Frateuria aurantia* strains were isolated from fruits of mango, rambai, jackfruit, and kemaris and coconut flower collected in Indonesia. The isolates were determined for phenotypic characteristics, DNA base composition, DNA-DNA similarity, quinone systems, cellular fatty acid composition, and phylogenetic relationships on the basis of 16S rRNA sequences (Lisdiyanti et al. 2003a). The cellular fatty acid composition and quinone systems were confirmed the same as those previously reported (Yamada et al. 1969, 1981a). The DNA base composition on the new isolates fell into a range of the composition as previously reported (Swings et al. 1980; Yamada et al. 1981b). Phylogenetic analysis with 16S rRNA sequences revealed the allocation of the isolates and the reference strains of *F. aurantia* to the *Gammaproteobacteria*.

Recently, a membrane-bound, pyrroloquinoline quinone (PQQ)-dependent alcohol dehydrogenase (ADH) was purified from *Frateuria aurantia* LMG 1558, and its similarity to ADH of *Gluconacetobacter europaeus* V3 (LMG 18494) (*Alphaproteobacteria*) was found. The similarity of ADH over the class suggests the possibility of horizontal gene transfer between two groups of genera (Trček and Matsushita 2013).

Problematic features were solved with the recharacterization of the new isolates, which were the oxidation of acetate, ketogenesis, the utilization of ammoniac nitrogen, and the production of H_2S (Lisdiyanti et al. 2003a). In conclusion, acetate was not oxidized as the previous study (Swing et al. 1980), and a more reliable method was needed for the test of ketogenesis. Ammoniac nitrogen was utilized when D-mannitol was served as a carbon source, but not ethanol or D-glucose. The production of H_2S was reported to be useful for differentiation of the genus *Frateuria* from the other genera in acetic acid bacteria (Swing et al. 1980). When the production of H_2S was tested with lead acetate paper strips, the isolates and reference strains of *F. aurantia* blackened the tip of the lead acetate paper strips, and *Pseudomonas* and *E. coli* strains did as well. However, all the abovementioned strains showed a negative response with TIS agar. The lead acetate paper strips were much more sensitive than TIS agar and would give a false-positive reaction. Therefore, the production of H_2S was not recognized for applicable to differentiation of the genus *Frateuria* from the other genera in acetic acid bacteria (Lisdiyanti et al. 2003a). *Frateuria terrea* was described, and the description of the genus *Frateuria* was emended (Zhang et al. 2011). Type species is *Frateuria aurantia*. Currently, the genus comprises two species, *F. aurantia* and *F. terrea*.

Frateuria Swings et al. 1980 emend. Zhang 2011

Cells are Gram negative, rod-shaped, measuring 0.4–0.8 by 0.8–2.0 μm, singly or in pairs, motile with a single polar or subpolar flagellum when motile (*F. terrea* shows a subpolar flagellum). Endospores are not produced. Luxuriant growth on glucose-yeast extract-$CaCO_3$ agar. Colonies are flat or circular, and medium turns to brown. Grows on glutamate agar and mannitol agar.

Aerobic. Catalase positive or negative (*F. terrea* is for negative). Oxidase negative or positive (*F. terrea* is for positive). Oxidizes lactate but not acetate. Production of dihydroxyacetone from glycerol is generally positive. Produces D-gluconate, 2-keto-D-gluconate, and 2, 5-diketo-D-gluconate from D-glucose, but not 5-keto-D-gluconate. Acid is produced from D-arabinose, L-arabinose, D-ribose, D-xylose, L-rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, glycerol, and ethanol, but not from L-sorbose, sucrose, D-mannitol, or D-sorbitol. Growth does not occur on methanol. Assimilates ammoniac nitrogen with mannitol. Produces water-soluble brown pigment. No growth in the presence of 0.35 % acetic acid. Growth occurs on 20 % D-glucose, at 34 °C, and at pH 3.5. A major cellular fatty acid is *iso*-$C_{15:0}$. A major quinone is Q-8. The G+C content is from 62 to 64 mol %. *Frateuria* strains are isolated from flowers of lily, rose, ladybell, and coconut and fruits of mango, rambai, and jackfruit.

Frateuria aurantia Swings, Gillis, Kersters, De Vos, Gosselé, and De Ley 1980

Characteristics are the same as those described for the species (Swings et al. 1980; Lisdiyanti et al. 2003a).

Type strain: G-6^T (= Kondô 67 = ATCC 33424 = DSM 6220 = NBRC 3245 = LMG 1558), isolated from flowers of lily. The G+C content of DNA of the type strain is 63 mol%.

Frateuria terrea Zhang, Liu, and Liu 2011

Characteristics are the same as those described for the species (Zhang et al. 2011).

Type strain: VA24^T (= CGMCC 1.7053 = NBRC 104236), isolated from forest soil of the Changbai Mountains, Heilongjiang Province, China. The G+C content of DNA of the type strain is 67.4 mol%.

Isolation, Enrichment, and Maintenance Procedures

Sugary and alcoholic materials are widely used for isolation of acetic acid bacteria. Recently, many acetic acid bacteria are found in a wide variety of natural environments, and the kinds of isolation sources greatly increased (see the sections of the respective species concerned and the ecology).

A medium for the enrichment procedure and isolation of acetic acid bacteria, designated as the medium with pH 3.5 (Yamada et al. 1999), is composed of 1.0 % glucose, 0.5 % ethanol (99.8 %) v/v, 0.3 % peptone, 0.2 % yeast extract, and 0.01 % cycloheximide and adjusted pH to 3.5 with a diluted hydrochloric acid solution. LGI medium is used for isolation of acetic acid bacteria capable of fixing atmospheric nitrogen (Cavalcante and Döbereiner 1988). The medium contains 10.0 % sucrose, 0.06 % KH_2PO_4, 0.02 % K_2HPO_4, 0.02 % $MgSO_4$, 0.002 % $CaCl_2$, 0.001 % $FeCl_3$, and 0.0002 % Na_2MoO_4 and adjusted pH to 6.0. When microbial growth is found in the LGI medium; the culture is transferred to the medium with pH 3.5 (Vu et al. 2013). The culture in the medium with pH 3.5 is streaked onto agar plates to obtain candidates of acetic acid bacteria. The medium is composed of 2.0 % glucose, 0.5 % ethanol (99.8 %) v/v, 0.3 % peptone, 0.3 % yeast extract, 0.7 % calcium carbonate (precipitated), and 1.5 % agar (Yamada et al. 1976a, 1999). Colonies dissolving calcium carbonate on the agar plates are picked up and transferred onto the agar slants with the same composition as the agar plates. The cultures are preserved for the further study. The isolates are examined again for the growth in the medium with pH 3.5.

Enrichment culture approach and selective isolation of acetic acid bacteria can be done by changing the medium composition, especially the carbon source. In fact, strains of *Asaia bogorensis*, *Asaia siamensis*, and *Asaia krungthepensis* were first isolated with D-sorbitol or dulcitol as a carbon source instead of D-glucose (Yamada et al. 2000; Katsura et al. 2001; Yukphan et al. 2004b).

Four to six kinds of media were employed for the enrichment culture (Lisdiyanti et al. 2003b; Huong et al. 2007; Suzuki et al. 2010). The medium with pH 4.5 is also useful for isolation of acetic acid bacteria (Yamada et al. 1976a; Tanasupawat et al. 2004).

The genera *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, *Asaia*, and *Komagataeibacter* are not monotypic and include 8–20 more species. Brief identification can be predicted at the genus level from the combination of oxidation of acetate and lactate and production of acetic acid from ethanol (Yamada and Yukphan 2008).

Acetobacter strains show a deep blue color fast and clearly on the oxidation of acetate and lactate (Asai et al. 1964). On the other hand, *Gluconobacter* strains show a clear yellow color in the acetate and lactate oxidation tests. In *Gluconacetobacter* and *Komagataeibacter* strains, the color change to blue is not so vigorous compared with that of *Acetobacter* strains. The two conventional tests described above are very useful particularly when a large number of isolates are classified or identified routinely at the generic level. *Gluconacetobacter* and *Komagataeibacter* strains can be discriminated from each other by the production of a water-soluble brown pigment and cell motility. *Gluconacetobacter* strains generally produce a water-soluble brown pigment and are motile. In contrast, *Komagataeibacter* strains do not produce the pigment and are nonmotile. *Asaia* strains show no or little acetic acid production from ethanol, differing from the above four genera, and the color change is very slow on the oxidation of acetate and lactate.

Aerobic phototrophic bacteria in the acidophilic group can be isolated by the enrichment procedure, which are preferable to be under similar conditions to isolation sources (Yurkov 2006).

Most acetic acid bacteria and their relatives can be generally maintained at 4 °C for 1 month on agar slants containing the appropriate medium. Long-term preservation of acetic acid bacteria and related organisms can be achieved by lyophilization or by storage in liquid nitrogen or by cryoconservation at −80 °C with low-temperature refrigerators and appropriate cryoprotectants (Kersters et al. 2006).

Ecology

The genera *Acetobacter* and *Gluconobacter* were the core genera in acetic acid bacteria for a long time. Members of the genus *Acetobacter* have niches in alcoholic and acidic environments, and they are isolated from vinegar, wine, beer, sake, cider, fruits, and other alcoholic materials. The main substance of vinegar is acetic acid, and its concentration ranged from approximately 4 % to 6 %. Malt vinegar contains 4.3–5.9 % of acetic acid, cider vinegar 3.9–9.0 %, wine vinegar 4.4–7.4 %, spirit vinegar 11.5–12.5 %, rice vinegar 4.00–5.24 %, and Balsamic vinegar 6.25–14.88 % (Adams 1998). By contrast, the members of the genus *Gluconobacter* are isolated from sugary materials, fruits, flowers, foods, and other sources. However, the concept of acetic acid bacteria has extended to all of the bacteria in the family *Acetobacteraceae*. Consequently, sources of the bacteria become diversified, and the sources have extended beyond the limits (see the section of Phenotypic Analyses).

Sources of Isolation

Members of the genus *Gluconobacter* were first isolated from kaki (*Diospyros kaki* Thunb.) and other fruits (Asai 1934), and isolation sources extend to a variety of flowers and fruits (Kondo and Ameyama 1958). The genus *Komagataeibacter* is separated from the genus *Gluconacetobacter* and has niches in acetous materials such as a variety of vinegar, kombucha, and fruit juice. *Gluconacetobacter* strains were reported to be isolated from vinegar, fruits, dried fruits, rhizosphere of coffee plants, pink sugarcane mealybug, and even in a tumulus in Japan.

Asaia strains are widely distributed in such flowers as orchid tree, plumbago, astilbe, Asian dayflower, heliconia, spider lily, balloon flower, self-heal, crown flower, ixora, and African tulip tree. Further, an *Asaia* strain was isolated from spoiled fruit-flavored bottle water (Moore et al. 2002). *Acidomonas methanolica* strains were abundantly isolated from activated sludge samples, but not from vegetables, fruits, decayed wood and leaves, manure, and paddy soil (Yamashita et al. 2004).

Strains of the genera *Ameyamaea*, *Neoasaia*, and *Neokomagataea* were isolated from a variety of flowers. A *Tanticharoenia sakaeratensis* strain was isolated from soil. An osmophilic acetic acid bacterium, *Saccharibacter floricola*, was isolated from pollens in Japan.

Flora of Acetic Acid Bacteria in Southeast Countries

The study of distribution of acetic acid bacteria is rather limited to certain sources, and systematic research is sporadically reported. However, microbiologists are interested in acetic acid bacteria in the tropical region from the viewpoint of the diversity of the bacteria. For example, the microbiological study was conducted on microflora of acetic acid in Indonesia. In this study, 64 strains of acetic acid bacteria were isolated from flowers, nata de coco, brown sugar, fruits, ragi, and mash of soybean paste collected in Indonesia. An enrichment medium was used, which consisted of 1 % glucose, 0.5 % ethanol, 0.3 % acetic acid, 1.5 % peptone, 0.8 % yeast extract, and 100 ppm cycloheximide (adjusted pH to 3.5 with diluted hydrochloric acid). The isolates were classified on the basis of cell morphology, quinone systems, oxidation of acetate and lactate, DNA base composition, and DNA-DNA hybridization, and *Acetobacter* strains accounted for 70 %, *Gluconacetobacter* strains 13 %, and *Gluconobacter* strains 17 % (Yamada et al. 1999). This led to the further study of the diversity of acetic acid bacteria and resulted in the descriptions of five new species, *Acetobacter indonesiensis*, *A. tropicalis*, *A. syzygii*, *A. cibinongensis*, and *A. orientalis*, of which strains were isolated from flowers, fruits, and fermented foods collected in Indonesia; and three new

combinations, *Acetobacter orleanensis*, *A. lovaniensis*, and *A. estunensis*, were described, of which strains were reference strains (Lisdiyanti et al. 2000, 2001).

Further, a survey was carried out on acetic acid bacteria in Thailand, and 40 strains were isolated from 23 kinds of fruits and other samples. Of the 40 strains isolated, 31 strains were identified as *Acetobacter pasteurianus*, three strains as *A. orientalis*, and six strains as *Gluconacetobacter liquefaciens* (Seearunruangchai et al. 2004). In addition, 13 strains were isolated from 12 kinds of flowers collected in Thailand, and nine strains were identified as *Asaia bogorensis*, two strains as *A. siamensis*, and two strains as *A. krungthepensis* (Yukphan et al. 2006). Moreover, of 44 Thai isolates from flowers and fruits, 16 strains were identified as *Gluconobacter oxydans* and six strains as *G. frateurii*, and the rests were remained unidentified (Huong et al. 2007).

Besides the above studies, 26 strains of acetic acid bacteria were isolated from 13 kinds of fruits including apple, banana, guava, Kaffier lime, longan, mango, Manila tamarind, muskmelon, peach, plum mango, sapodilla, and sugar apple and four other samples collected at markets in Bangkok and other sites in Thailand. The isolates were identified as *Acetobacter tropicalis* (one strain), *A. orientalis* (four strains), *A. pasteurianus* (five strains), *A. syzygii* (one strain), *A. ghanensis* (three strains), *Gluconobacter frateurii* (four strains), *G. japonicus* (six strains), *Gluconobacter* sp. (one strain), and *Asaia* sp. (one strains) based on phenotypic characterization, 16S rRNA sequence analysis, and 16S-23S rRNA gene internal spacer (ITS) restriction analysis (Tanasupawat et al. 2009). So far as reported, a considerable number of genera and species of acetic acid bacteria are expected to be in fruits and flowers in the tropical region.

A Comprehensive Survey of Acetic Acid Bacteria in Southeast Asia

As mentioned above, acetic acid bacteria show a wide distribution in various kinds of vinegar, wine, beer, cider, sake, a variety of fruits and flowers, fermented foods, and environments. Nevertheless, a few comprehensive studies were conducted in the environmental and ecological points of view. The distribution of the acetic acid bacteria was studied in the light of isolation sources, the sources from three different countries, Indonesia, Thailand, and the Philippines, and different enrichment media. A total of 331 strains including 196 new isolates and 135 strains previously reported were subjected to the study of the diversity of acetic acid bacteria (Lisdiyanti et al. 2003b).

First, the diversity of acetic acid bacteria is described from the viewpoint of isolation sources. Of 331 isolates, 118 strains were isolated from fermented foods, followed by 98 strains from fruits, and 99 strains from flowers. At the generic level, 83 *Acetobacter* strains were obtained from fermented foods, followed by 60 from fruits. Most of *Gluconobacter* strains were isolated from fruits and flowers, followed by fermented foods. Interestingly, *Asaia* strains were mostly isolated from flowers. *Asaia bogorensis* strains were mostly isolated from flowers as well (*Table 1.5*).

Secondly, of 331 isolates, 208 strains were isolated from Indonesian samples, followed by 51 strains from Thai samples and 72 strains from the Philippine samples (*Table 1.6*; Lisdiyanti et al. 2003b). This figure does not indicate the relevance to the specialty of the country because the number of the samples varies with country to country.

Thirdly, enrichment media were designed for isolation (*Table 1.7*; Lisdiyanti et al. 2003b). Medium I (ME I) is good for a wide variety of acetic acid bacteria. Medium V (ME V) is designed for isolation of *Acidomonas* because it contains methanol as a carbon source. Medium VI (ME VI) is prepared for isolation of *Asaia* strains (Suzuki et al. 2010), which will be mentioned below. Of 331 isolates of acetic acid bacteria, 139 were isolated with EM I, followed by 103 with EM II, 38 with EM III, 34 with EM IV, and 17 with ME V (*Table 1.8*; Lisdiyanti et al. 2003b). EM I is appropriate for isolation of *Acetobacter* strains because it contains both D-glucose and ethanol as carbon sources. Members of *Gluconacetobacter* and *Kozakia* are also enriched with EM I, and EM II is suitable for isolation of the genus *Gluconobacter* as well. EM II and EM IV are good for isolation of *Asaia* strains because D-sorbitol and dulcitol serve carbon sources for *Asaia*. Further, *Asaia* and *Frateuria* strains are not enriched with EM I and EM III because acetic acid inhibits the growth of those bacteria. No *Acidomonas* strains are isolated even with EM V in which methanol is supplied. Modification of the enrichment media is helpful for the isolation of targeted microorganisms in nature.

Fourthly, the relevancy was discussed between the species isolated and detailed isolation sources (*Table 1.9*; Lisdiyanti et al. 2003b). *Acetobacter* strains are isolated from fermented foods such as palm vinegar, palm wine, nata de coco, and pickles as well as fruits such as guava, mango, zirzak, etc. It is worthy of remark that *Acetobacter pasteurianus* strains were isolated only from fermented foods such as palm vinegar, palm wine, rice wine, pickles, water of nata de coco, fermented rice, and tape cassava. Further, *A. pasteurianus* strains are not isolated from fruits and flowers. Most of the *Gluconobacter* strains inhabit fruits and flowers. *Gluconacetobacter* strains are mostly found in nata de coco. This tells us that acetic acid bacteria have particular niches for each genus and even for each species.

Flora of Acetic Acid Bacteria in Japan

The genus *Asaia* was assumed to be a genus with specific niches in the tropical countries because the *Asaia* species had been isolated flowers and fruits in the tropics. Therefore, a survey was made on the bacterial flora of acetic acid bacteria in Japan, particularly focusing on *Asaia* strains (Suzuki et al. 2008). Japan extends approximately 3,000 km from the north to south and covers isolation sites from Hokkaido in the northern area to Okinawa in the southern area. Two media are used: Medium I (ME I) is designed for isolation of diverse acetic acid bacteria, and Medium VI (ME VI) is aimed to isolate *Asaia* strains, which contains D-sorbitol and dulcitol as

Table 1.5
Diversity of acetic acid bacteria relevant to isolation sources

		Sources				
Genera	Species	Fermented foods	Fruits	Flowers	Others	Total
Acetobacter	*A. pasteurianus*	41				41
	A. orleanensis	9	16	1		26
	A. lovaniensis	14	15		2	31
	A. indonesiensis	8	14	4	1	27
	A. tropicalis	7	7		1	15
	A. syzygii	2	2	2		6
	A. cibinogensis		1		1	2
	A. orientalis	2	5	1	1	9
		83	**60**	**8**	**6**	**157**
Gluconobacter	*G. oxydans*	2	8	9	2	21
	G. frateurii	12	16	14	4	46
		14	**24**	**23**	**6**	**67**
Gluconacetobacter	*G. xylinus* (*K. xylinus*)	8				8
	G. hansenii	5	1			6
	Gluconacetobacter sp.	6				6
		19	**1**	**0**	**0**	**20**
Asaia	*A. bogorensis*	2		49		51
	A. siamensis			13		13
	Asaia sp.			3		3
		2	**0**	**65**	**0**	**67**
Kozakia	*K. baliensis*				4	4
		0	**0**	**0**	**4**	**4**
Frateuria	*F. aurantia*		13	3		16
		0	**13**	**3**	**0**	**16**
Total		**118**	**98**	**99**	**16**	**331**

Reproduced and modified data of Lisdiyanti et al. (2003b)

sources of carbon (*Table 1.7*). Thus, 345 strains were isolated from 776 samples consisting of 684 from flowers, 58 from fruits, and 34 from fermented foods (Suzuki et al. 2008).

The isolates were classified into the genera *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, *Asaia*, *Saccharibacter*, and *Frateuria* based on phenotypic characteristics, 16S rRNA sequence analysis, and DNA-DNA hybridization (Suzuki et al. 2008). *Asaia* strains are most abundantly isolated, followed by *Acetobacter* strains, *Gluconobacter* strains, and *Gluconacetobacter*. A few strains of the genera *Saccharibacter* and *Frateuria* are isolated (*Table 1.10*). No *Acidomonas* strains are found. The *Asaia* strains are obtained by the use of ME VI and from flowers as expected (*Table 1.10*). Further, acetic acid bacteria are distributed evenly across Japan (*Fig. 1.5*). In addition, the strains are isolated from Akita, Niigata, Tokyo, and Yamanashi located in the temperate zone in Japan. Three new species of the genus *Asaia* were identified based on the characterization of the isolates from flowers collected in the northern area (Suzuki et al. 2010). Consequently, the genus *Asaia* is not a particular acetic acid bacterium, and members of the genus are distributed widely and not limited to tropical countries. Thus, the genus can be said to be a cosmopolitan bacterium. Besides, *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter* strains are isolated by the use of ME I and from fermented foods, followed by fruits, and flowers, as shown in "A comprehensive survey of acetic acid bacteria in Southeast Asia." The abovementioned figures indicate that a variety of isolation sources and enrichment media are essential for the study of distribution of targeted microorganisms.

Insects

Mosquitoes

A mosquito, *Anopheles stephensi*, is a vector of *Plasmodium* sp., the pathogen of Asian malaria. The total number of cases of malaria is estimated to be 300–500 million in the world, and two

Table 1.6

Diversity of acetic acid bacteria relevant to country origins

		Country			
Genera		Indonesia	Thailand	The Philippines	Total
Acetobacter	*A. pasteurianus*	9	11	21	41
	A. orleanensis	11	3	12	26
	A. lovaniensis	27		4	31
	A. indonesiensis	20		7	27
	A. tropicalis	9	6		15
	A. syzygii	4		2	6
	A. cibinogensis	2			2
	A. orientalis	9			9
		91	**20**	**46**	**157**
Gluconobacter	*G. oxydans*	17	4		21
	G. frateurii	35	8	3	46
		52	**12**	**3**	**67**
Gluconacetobacter	*G. xylinus* (*K. xylinus*)	8		3	11
	G. hansenii			6	6
	Gluconacetobacter sp.			3	3
		8	**0**	**12**	**20**
Asaia	*A. bogorensis*	25	17	9	51
	A. siamensis	9	2	2	13
	Asaia sp.	3			3
		37	**19**	**11**	**67**
Kozakia	*K. baliensis*	4			4
		4	**0**	**0**	4
Frateuria	*F. aurantia*	16			16
		16	**0**	**0**	**16**
Total		**208**	**51**	**72**	**331**

Reproduced and modified data of Lisdiyanti et al. (2003b)

to three million deaths are caused by malaria. Approximately 90 % of malaria occurs in Africa. Therefore, malaria is the most important disease of the tropical parasite disease. In the above circumstances, *Asaia* strains were found in *Anopheles stephensi* and dominated the microflora of the mosquito. Therefore, 16S rRNA gene libraries were made from DNA of the abdomen of the laboratory-fed mosquitoes. Analysis of the 16S rRNA gene sequence revealed that the libraries dominated with sequences related to the genus *Asaia*, which showed more than 90 % of nucleotide identity with those of *Asaia bogorensis* and *Asaia siamensis*. Further, *Asaia* strains were isolated from *Anopheles stephensi* bodies, with $9.8 \times 10^{4-5}$ cells/individual (Favia et al. 2007). Thus, *Asaia* strains are recognized to be normally associated with larvae and adults of A*nopheles stephensi*. A green fluorescent protein (Gfp)-tagged *Asaia* strain effectively logged in male and female reproductive organs and salivary glands, which are sites crucial for *Plasmodium* sp. development and transmission. Further, *Asaia* strains were found to transmit from the mother to offspring and undergo paternal transmission to the progeny (Damiani et al. 2008; Favia et al. 2007).

In addition, *Asaia* strains were present in the body of the mosquito *Aedes aegypti*, which is a vector of yellow fever and dengue fever, and in the leafhopper *Scaphoideus titanus*, which is a vector of grapevine flavescence dorée phytoplasma. Cross-colonization patterns of the body of *Aedes aegypti*, *Anopheles stephensi*, and *Scaphoideus titanus* were recognized with *Asaia* strains isolated from *Anopheles stephensi* or *Aedes aegypti*. This indicated the cross-colonization in insects of phylogenetically distant genera and orders (Crotti et al. 2009).

Further, the ability of *Asaia* strains was recognized in colonizing Afro-tropical malaria vector *Anopheles gambiae* mosquitoes (Damiani et al. 2010). In addition, 281 *Asaia* strains were isolated from *Anopheles stephensi*, *Anopheles gambiae*, *Aedes aegypti*, and *Aedes albopictus* and clustered into two groups, one clustering with *Asaia krungthepensis* and the other clustering *Asaia bogorensis* (Chouaia et al. 2010). Wild *Aedes*

Table 1.7

Enrichment media for acetic acid bacteria

Ingredient	Enrichment media at pH 3.5					
	ME I	ME II	ME III	ME IV	ME V	ME VI
D-Glucose	1.0[a]				0.15	
D-Sorbitol		2.0				1.0
D-Mannitol			2.0			
Dulcitol				2.0		1.0
Methanol					2.0	
Ethanol	0.5					
Peptone	1.5	0.5	0.5	0.5	0.5	0.5
Yeast extract	0.8	0.3	0.3	0.3	0.3	0.3
Acetic acid	0.3		0.2			
Cycloheximide	0.001	0.01	0.001	0.001	0.001	0.001

[a]The concentration of ingredients is expressed in (w/v) % or (v/v) %

Reproduced and modified data of Lisdiyanti et al. (2003b) and Suzuki et al. (2010)

Table 1.8

Diversity of acetic acid bacteria relevant to enrichment media

Genera		Enrichment media					
		ME I	ME II	ME III	ME IV	ME V	Total
Acetobacter	*A. pasteurianus*	23	6	12			**41**
	A. orleanensis	21	1	4			**26**
	A. lovaniensis	16	7	7		1	**31**
	A. indonesiensis	15	8	4			**27**
	A. tropicalis	12	1	2			**15**
	A. syzygii	5		1			**6**
	A. cibinogensis	1		1			**2**
	A. orientalis	1	6	2			**9**
		94	**32**	**33**	**0**	**1**	**157**
Gluconobacter	*G. oxydans*	11	8	2			**21**
	G. frateurii	10	24	3	6	3	**46**
		21	**32**	**5**	**6**	**3**	**67**
Gluconacetobacter	*G. xylinus* (*K. xylinus*)	8					**8**
	G. hansenii	6					**6**
	Gluconacetobacter sp.	6					**6**
		20	**0**	**0**	**0**	**0**	**20**
Asaia	*A. bogorensis*		32		17	2	**51**
	A. siamensis		5		6	2	**13**
	Asaia sp.		2		1		**3**
		0	**39**	**0**	**24**	**4**	**67**
Kozakia	*K. baliensis*	4					**4**
		4	0		0	**0**	**4**
Frateuria	*F. aurantia*		3		4	9	**16**
		0	**3**	**0**	**4**	**9**	**16**
Total		**139**	**103**	**38**	**34**	**17**	**331**

Reproduced and modified data of Lisdiyanti et al. (2003b)

Table 1.9
Habitats of acetic acid bacteria

Genera		Fermented foods	Fruits	Flowers	Others
Acetobacter	*A. pasteurianus*	Palm vinegar, palm wine, rice wine, pickles, water of nata, fermented rice, tape cassava			
	A. orleanensis	Nata de coco, rice wine	Guava, sapodilla, star-fruit	Flower	
	A. lovaniensis	Nata de coco, mash of soy sauce, palm wine, tape cassava, pickles	Coconut, star fruit, markisa, mangosteen, mango, sapodilla, java grape		Palm seed, coconut juice
	A. indonesiensis	Palm wine	Banana, papaya, zirzak, mango, durian, coconut, star fruit	Hibiscus	Palm seed
	A. tropicalis	Palm wine, rice wine	Lime, orange, guava, coconut		Coconut juice
	A. syzygii	Vinegar	Malay rose apple, star fruit	Flower	
	A. cibinogensis		Montana, coconut		Soybean curd
	A. orientalis	Tempeh	Coconut, star fruit	Canna flower	Soybean curd
Gluconobacter	*G. oxydans*	Nata de coco	Sapodilla, papaya, orange, soursop, mangosteen	Flower of pea, hibiscus, chili flower, bougenvillea	Coconut juice, ragi, palm brown sugar
	G. frateurii	Nata de coco, mash of soy sauce	Sapodilla	Ixora, palm flower, African tulip	Cincau
Gluconacetobacter	*G. xylinus* (*K. xylinus*)	Nata de coco, vinegar			
	G. hansenii	Nata de coco	Pineapple		
	Gluconacetobacter sp.	Nata de coco			
Asaia	*A. bogorensis*	Tape ketan		Bauhinia, plumbago, ixora, lantana, African tulip, coconut flower, alamanda	
	A. siamensis			Crown flower, spider lily, ixora, lantana, rose	
	Asaia sp.			Jude vine, New Guinea creeper	
Kozakia	*K. baliensis*				Ragi, palm brown sugar
Frateuria	*F. aurantia*		Lime, menteng, cempedak, kemaris	Coconut flower	

Reproduced and modified data of Lisdiyanti et al. (2003b)

albopictus and *Aedes aegypti* mosquito specimens were collected in three different regions in Madagascar. Culturing methods and denaturing gradient gel electrophoresis (DGGE) and sequencing of the *rrs* amplicons revealed *Proteobacteria* and *Firmicutes* as major phyla. Isolated genera were dominated by *Bacillus*, followed by *Acinetobacter*, *Agrobacterium*, and *Enterobacter*. Common DGGE bands belonged to *Acinetobacter*, *Asaia*, *Delftia*, *Pseudomonas*, *Enterobacteriaceae*, and uncultured *Gammaproteobacteria* (Zouache et al. 2011).

Further, prevalence of the bacteria in *Aedes albopictus* was surveyed in field populations in Madagascar, and both genera were detected at relatively high frequencies, 46 % of *Asaia* and

Table 1.10
Relationships between enrichment media and isolation sources

	Enrichment media			Isolation sources			
	Me I	Me II	Me VI	Flowers	Fruits	Fermented foods	Total
Genus							
Acetobacter	56	19	21	24	29	53	96
Gluconobacter	24	0	22	25	21	0	46
Gluconacetobacter	25	3	13	7	16	18	41
Asaia	38	0	119	152	5	0	157
Saccharibacter	1	0	0	0	1	0	1
Frateuria	1	0	3	3	1	0	4
Total	145	22	178	211	63	71	345 strains

Cited from data of Suzuki et al. (unpublished)

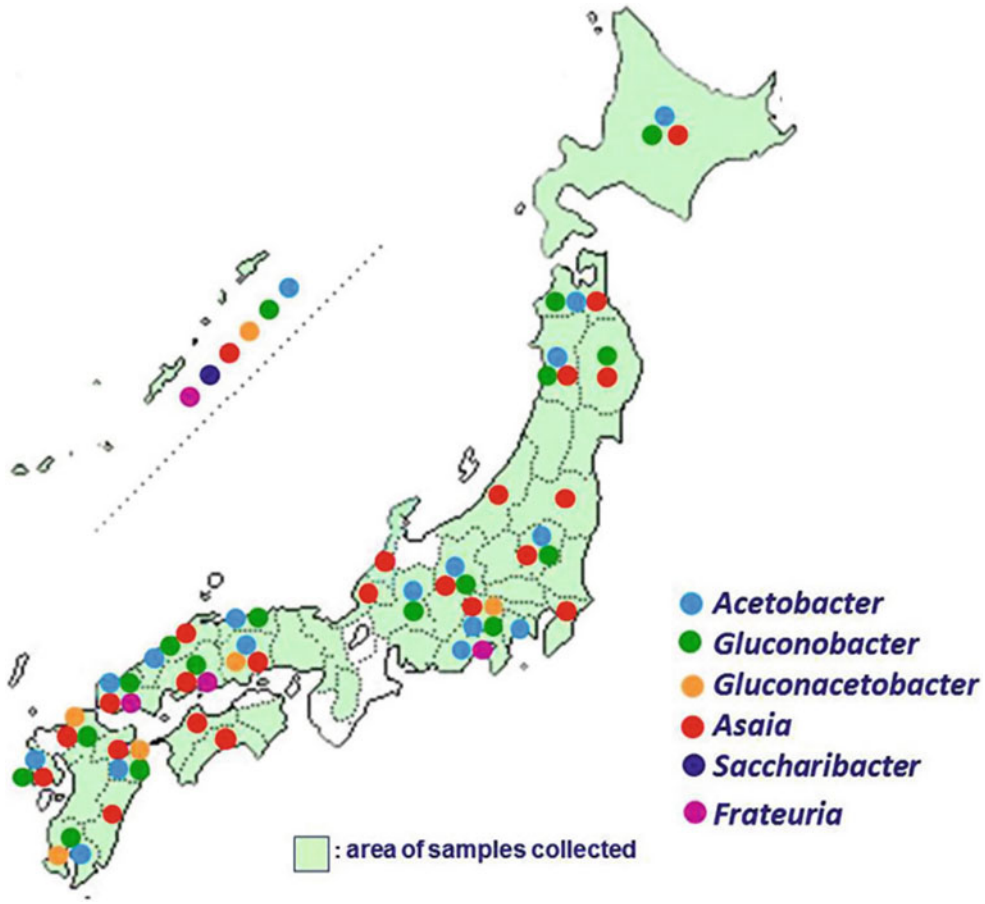

Fig. 1.5
Distribution of acetic acid bacteria in Japan Suzuki et al. (2008)

74 % of *Acinetobacter*. The prevalence of the genus *Acinetobacter* correlated with mosquito gender and the prevalence of the genus *Asaia* with the interaction between mosquito gender and the sampling sites. Male mosquitoes were more infected than female mosquitoes for each bacterial genus (Minard et al. 2013a). This study emphasizes the need to gain a global view on the bacterial partners on the mosquito vectors. Since the genus *Asaia* was regarded as tools for control of mosquito-borne disease, particularly malaria, *Asaia* spp. have been investigated on circulating among humans. All human sera and whole blood samples were negative for *Asaia* spp. This suggested that *Asaia* spp. bacteria could be regarded as opportunistic

pathogens and could be utilized as a malaria control in the future (Epis et al. 2012).

A review entitled "Diversity and function of bacterial microbiota in the mosquito holobiont" was presented (Minard et al. 2013b). According to the review, bacterial phyla and genera found in *Culicidae* mosquitoes are recorded as follows: *Proteobacteria*, 73 genera; *Bacteroidetes*, 11 genera; *Actinobacteria*, 17 genera; *Firmicutes*, *Tenericutes*, and *Fusobacteria*, 16 genera; *Cyanobacteria*, 2 genera; and *Deinococcus* and *Thermus*, 1 genus each. In addition, the genera *Asaia* and *Gluconobacter* are isolated from *Aedes*, *Anopheles*, *Culex*, and *Mansonia* mosquitoes, the genus *Acetobacter* from *Aedes* and *Mansonia* mosquitoes, and the genus *Roseomonas* from *Aedes* and *Anopheles* mosquitoes.

Flies

Drosophila melanogaster (fruit fly) is widely used in research in genetics and developmental biology. In view of bacterial community in the fly, adult flies were collected from 11 sites in the USA, and the DNAs from bacteria were isolated from male flies at each of the 11 different locations. Bacterial 16S rRNA gene sequences libraries recovered were compared, and the bacteria from these sequences were grouped into 74 different taxa, spanning the phyla *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*. In addition, many groups were found in the genus *Gluconacetobacter* in the order *Alphaproteobacteria* (Corby-Harris et al. 2007). However, conflicts are reported on the bacterial taxa, particularly the genera *Gluconobacter* and *Gluconacetobacter*, which were found in *Drosophila* samples. The samples were collected around the world, and after extraction of DNA, the 16S rDNA PCR amplification and sequencing were done. As a result, the family *Acetobacteraceae* was not a major family, and both the groups were not detected in the samples (Chandler et al. 2011).

Further, commensalism with microorganisms in gut of the *Drosophila* has attracted attention to biologists. In the light of interests, bacteria were examined by DGGE fingerprints of PCR-amplified 16S rRNA fragments for the midgut of reared wild-type flies and Cad-RNAi flies that carried the *UAS-Cad-RNAi* construct to mimic the loss of function via RNA interference (RNAi). *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Acetobacter pomorum* were commonly detected in both the wild-type flies and Cad-RNAi flies. However, a bacterium in the family *Acetobacteraceae* EW911 (*A911*) [a dominant commensal member in wild-type flies, controls, 1.5×10^5 colony-forming units (CFUs) per gut] was markedly diminished and maintained at very low level in the *Cad-RNAi* gut (900 CFUs per gut), whereas *Gluconobacter* sp. EW707 (*G707*) (a minor commensal member in the control gut, 800 CFUs per gut) emerged as a dominate commensal in the *Cad-RNAi* gut (1.7×10^4 CFUs per gut) (Ryu et al. 2008).

Olive fruit fly (olive fly, *Bactrocera oleae*) is one of the major pests of the olive tree. Wild specimens of *B. oleae* collected in field as pupae from 10 locations in Greece and a population reared at the laboratory were used for study. Bacterial 16S rRNA gene libraries revealed the presence of several bacterial taxa associated with this insect, among which *Acetobacter tropicalis* was dominant. Using an *A. tropicalis*-specific PCR assay, the bacterium was detected in all insects originating from laboratory stocks or field collected. *A. tropicalis* was successfully established in cell-free medium, and typing analyses, carrying out on a collection of isolates, revealed that differed strains are present in fly populations (Kounatidis et al. 2009).

Mealybug

Pink sugarcane mealybug (*Saccharococcus sacchari*) is common to all countries that grow sugarcane and almost the extensive species of mealybug found on commercially grown sugarcane. A small number of acetic acid bacteria were isolated from pink sugarcane mealybugs and were identified as *Gluconobacter oxydans* (Ashbolt and Inkerman 1990). In addition, *Gluconacetobacter sacchari*, *G. liquefaciens*, and *G. diazotrophicus* strains were isolated from pink sugarcane mealybugs collected in Australia and Hawaii (Franke et al. 1999).

Bee

Honeybees (*Apis mellifera*) are domesticated bees reared in apiaries and are used for producing honey and for pollination commercial crops. Isolation of *Gluconobacter* strains was attempted with enrichment approach from not only 411 samples, mostly bees, but also flowers visited bees, honey, and other materials from the hive and some wasps in three regions in Belgium. As a result, 53 strains were isolated, and the strains were studied based on phenotypic characteristics. Further, the strains were classified into two groups, Group A and Group B with polyacrylamide gel electrophoresis of soluble cell proteins. As a result, 37 strains in Group A were identified as "*Gluconobacter oxydans* subsp. *suboxydans*" (now *Gluconobacter oxydans*) and 15 strains in Group B as a member of the genus *Gluconobacter* (Lambert et al. 1981). Bacteria associated with worker adults of two subspecies of *Apis mellifera* in Southern Africa, *Apis mellifera capensis* and *Apis mellifera scutellata*, were screened by analysis with high-fidelity PCR of 16S rRNA sequences. As a result, the genus *Gluconacetobacter* was detected in the abdomen of the workers with the genera *Lactobacillus*, *Bifidobacterium*, *Serratia*, and three other genera (Jeyaprakash et al. 2003).

In addition, the structural diversity of bacteria found in the gut of three different bee species, *Apis mellifera carnica* (honeybee), *Bombus terrestris* (bumblebee), and *Osmia bicornis* (red mason bee), was studied for understanding how specific insects select for their own bacterial communities. The bees were collected at a flowering oilseed rape field for three successive years

in Germany. Total DNA was extracted from gut materials, and the bacterial diversity was analyzed by genetic profiling with single-strand conformation polymorphism of PCR-amplified partial 16S rRNA gene sequences. Sequencing of the PCR products revealed a dominance of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, and *Firmicutes* in all bee species. Further, *Gluconobacter* sp. was exclusively detected in adults of *Apis mellifera* collected in 2001 (Mohr and Tebbe 2006).

Leafhopper

Flavescence dorée (FD) is a grapevine disease that afflicts several wine production area in Europe. FD is caused by "*Candidatus* Phytoplasma vitis" and the insect vector of a leafhopper, *Scaphoideus titanus*, which is widespread in vineyards. Focusing on *Asaia* sp. and *S. titanus*, the association with the bacteria and the leafhopper was studied in the light of better understanding of a possible role of the bacteria.

S. titanus adult individuals were collected in vineyards with heavy symptoms of FD from different area in Piedmont region in Italy. Further, feeds that were fed on 300 µl of sucrose 5 % in TE pH 8.0 with or without rifampicin (200 µg ml^{-1}) were used in order to simulate the natural situation of insects feeding on plant. Quantitative real-time PCR was performed on insects or feeds DNA with appropriate primers.

Data demonstrated that *Asaia* sp. was a significant part of the insect microflora. The distributing pattern of the bacteria inside the insect body was investigated through the use of *Asaia* expressing a green fluorescent protein. *Asaia* strain SF2.1 tagged with Gfp was able to colonize efficiently salivary glands and female and male reproduction organs of *S. titanus* (Crotti et al. 2008).

Plant Hopper

The brown plant hopper (brown rice plant hopper, *Nilaparvata lugens*) is a monophagous insect herbivore of rice. The insect feeds on rice plants, sucks sap from the rice leaf sheath, and makes rice plant dry out. This gives a serious damage to rice crops. Since little was known about bacteria in *N. lugens*, the bacteria are examined.

Insects from three populations of *N. lugens* were used, which were maintained at Wuhan University in China, and designated biotypes 1, 2, and 3. Three rice varieties were used for the biotypes: TMC (a susceptible variety) for biotype 1, Mudgo (carrying the resistance gene to *N. lunges*, *Bph1*) for biotype 2, and ASD 7 (carrying resistance gene to *N. lunges*, *Bph2*) for biotype 3.

DNA was isolated from individual insects and used for construction of 16S rRNA gene libraries. 16S rRNA gene sequences were amplified from three biotype libraries. The restriction patterns of the PCR products were examined by ARDRA using *Hha*I, *Hae*II, and *Rsa*I and then sequenced. Resulted restriction patterns were assigned to four phyla, *Proteobacteria* (72 %), *Firmicutes* (11 %), *Actinobacteria* (11 %), and *Bacteroidetes* (11 %). *Asaia* was detected only in biotype 2 and located in front of the ovipositor sheath in biotype 2 females (Tang et al. 2010).

Medical and Clinical Specimens

Recently, acetic acid bacteria draw attention of medical and clinical microbiologists (see the section of pathogenicity of acetic acid bacteria). A Gram-negative rod was isolated from cervical and lymph nodes of a patient with the chronic granulomatous disease (CGD). The rod-shaped bacterium was identified as a new genus and a new species, *Granulibacter bethesdensis*. Introducing the bacterial strain into mouse models of CGD brought about evidence that the organism cause similar disease in the patient, and the organisms was re-isolated from the mouse. This is the first evidence with the pathogenicity of acetic acid bacteria (Greenberg et al. 2006a, b).

Prior to the establishment of the genus *Granulibacter*, *Asaia bogorensis* strains were isolated from the peritoneal dialysis fluid of a patient with a medical history of end-stage renal disease (Snyder et al. 2004) and from the blood of a man with a history of intravenous drug abuse (Tuuminen et al. 2006). These cases suggest the potential opportunistic pathogen of *Asaia bogorensis*. Further, *Asaia lannensis* strains were isolated from a bloodstream infection on a child with cancer and bone transplantation (Abdel-Haq et al. 2009), and *Gluconobacter* spp. were isolated from human blood and sputa (Alauzet et al. 2010). In addition, an *Acidomonas methanolica* strain was isolated from an excised lymph node of a boy with chronic granulomatous disease (Chase et al. 2012). *Roseomonas* strains were isolated from human blood, cerebrospinal fluid, sputum, genitourinary site, and other clinical specimens (Gilardi and Faur. 1984; Wallace et al. 1990; Struthers et al. 1996; Han et al. 2003).

Pathogenicity, Clinical Relevance

Acetic acid bacteria play roles in vinegar production, and the oldest record on vinegar would date back to 5,000 B.C. Babylonian people are said to prepare vinegar from juice or sap of date palm, date wine, and raisin wine by acetous fermentation (Conner and Allgeier 1976). However, it is only 150 years ago that Pasteur made clear the role of acetic acid bacteria in vinegar production. The bacteria can be said to be the genus *Acetobacter* in modern bacterial taxonomy. They grow well in acidic conditions and produce acetic acid from ethanol. Further, *Acetobacter* strains are widely distributed in nature, particular in vinegar, wine, beer, sake, and other alcoholic materials, and the members of some other genera in acetic acid bacteria are found in a variety of fruits, flowers, and sugary materials.

Therefore, the pathogenicity of acetic acid bacteria to humans has been ignored entirely until recently. Acetic acid bacteria clearly differ from generally known bacteria of medical and clinical importance in the light of growth responses and phenotypes

and further in phylogenetic relationships. Advances in bacterial taxonomy on acetic acid bacteria, particularly 16S rRNA sequence analysis, brought about rearrangement of classification of all the bacteria, and acetic acid bacteria were allocated in the family *Acetobacteraceae*. Thus the bacteria other than typical acetic acid bacteria are allocated in the family, and a number of new genera have come into being described. More recently, acetic acid bacteria have been isolated from clinical specimens, and the pathogenicity is recognized in the genus *Granulibacter*. Sources and routes of infection of acetic acid bacteria are still unknown, but removal of the suspected indwelling device is suggested (Alauzet et al. 2010). In addition, acetic acid bacteria are resistant to almost all antibiotics and establishment of susceptibility guidelines are required for clinicians. Therefore, acetic acid bacteria draw attention to medical and clinical microbiologists as newly emerging opportunistic pathogens.

Granulibacter bethesdensis

Scientists in an American group isolated a novel Gram-negative rod from cervical and supercervical lymph nodes of a patient with the chronic granulomatous disease (CGD). The disease is a rare inherited disease of the phagocyte NADPH oxidase system, which leads to defective production of superoxide and hydrogen peroxide. Normally, the phagocyte kills bacteria and fungi ingested by reactive oxygen compounds produced, but the phagocyte of the patient with the disease is unable to kill the bacteria and fungi because of deficiency of the phagocyte. Therefore, the patient with the disease suffers from recurrent life-threatening infection with bacteria and fungi. The scientists attempted to make clear the pathogenicity of the strains and introduced the bacteria into mouse models of CGD. As a consequence, they obtained evidence that the organism causes the similar disease in the patient, and the organism was re-isolated from the mouse. Therefore, Koch's postulates were fulfilled as a new pathogen (Greenberg et al. 2006a).

Further, this organism was isolated from the same patient three more times, and a similar Gram-negative rod was isolated from other two patients. The group was unable to identify the strains by conventional methods and commercial identification kits at that time. Therefore, the scientists employed phylogenetic analysis of 16S rRNA sequence, IT region, and *RecA* protein-encoding gene. Thus, they identified the bacterium as a new genus and a new species and named them *Granulibacter bethesdensis* in the family *Acetobacteraceae* (Greenberg et al. 2006b).

The *Granulibacter bethesdensis* strains have phenotypic characteristics common to acetic acid bacteria, produce acid from D-glucose and ethanol, and oxidize lactate and acetate weakly. Further, the strains prefer high concentrations of D-glucose (e.g., 5 % of D-glucose) and grow on glutamate agar and mannitol agar. Interestingly, they assimilate methanol (Greenberg et al. 2006b). Further, genomic sequences revealed genes relevant to methanol utilization. Therefore, *Granulibacter* strains have the characteristics of both acetic acid bacteria and facultative methylotrophs (Greenberg et al. 2007). This study should be emphasized as the first case to evidence the pathogenicity of acetic acid bacteria. In addition, more cases of *Granulibacter* infections are reported (Greenberg et al. 2010), and other case of the isolation of this bacterium was described in Spain from a child patient with CGD (López et al. 2008) (detailed characteristics of the genus *Granulibacter* are shown in the Section of Phenotypic Analyses).

Asaia bogorensis

Scientists at the University of Pennsylvania isolated a bacterium from the peritoneal dialysis fluid of a patient with a medical history of end-stage renal disease secondary to diabetes mellitus, but they were unable to identify the organism by conventional biochemical tests. Therefore, the scientists performed 16S rRNA gene sequencing of the organism and identified it as *Asaia bogorensis*. They concluded that the organism from multiple samples made it unlikely that the organisms represented a contaminant and may cause peritonitis (Snyder et al. 2004).

Other case was presented on *Asaia bogorensis* as well. A man with a history of intravenous drug abuse was transferred to University of Helsinki Hospital, Finland, who was suspected to have systemic infection. A Gram-negative rod was isolated from his blood and not identified by conventional routine tests and commercial identification systems. However, this bacterium was identified as *Asaia bogorensis* on the basis of 16S rRNA gene analysis (Tuuminen et al. 2006).

These reports suggest the potential opportunistic pathogens of *Asaia bogorensis*. Both groups of Pennsylvania University and Helsinki University emphasized the usefulness of sequencing of 16S rRNA gene for the identification of unusual bacteria from the clinical specimen.

Asaia lannensis

Following the isolation of *Asaia bogorensis*, Gram-negative bacilli were isolated from a bloodstream infection on a child with cancer and bone marrow transplantation. However, the bacteria were unable to be identified with commercial identification systems but were identified as *Asaia lannensis* according to 16S rRNA gene sequencing. This would be the first case of bacteremia caused by *Asaia lannensis* (Abdel-Haq et al. 2009).

Further, two cases of nosocomial infection with *Asaia lannensis* were reported. Case 1 is of a 15-month-old female and Case 2 is of a 5-year-old female both with congestive heart failure due to idiopathic dilated cardiomyopathy. In Case 1, the patient was presumed to have bacteremia. Isolates from blood cultures of both patients showed the same micro- and macroscopic features. The organisms were strictly aerobic, catalase positive, and oxidase negative and produced acid from D-xylose, D-glucose, and D-mannitol. However, three commercial identification systems failed to identify the bacteria, and the bacteria were identified as *Asaia lannensis* according to 16S rRNA gene sequencing (Juretschko et al. 2010).

Acetobacter cibinogensis and *Acetobacter indonesiensis*

The genus *Acetobacter* is one of the typical genera in acetic acid bacteria, and the member of this genus has been isolated from vinegar, wine, beer, cider, and other alcoholic materials. However, a strain of *Acetobacter cibinogensis* was isolated from blood samples of a man who had been receiving chronic hemodialysis for end-stage renal failure and had intravenous drug use. Two samples were drawn through a subclavian catheter and through the arteriovenous fistula. A Gram-variable rod was isolated. The strain grew on 0.7 % calcium carbonate agar plates with 7 % ethanol (pH 3.5) and cleared calcium carbonate. Commercial identification systems failed to identify it. Therefore, 16S rRNA gene sequencing was employed, and the sequence matched with *Acetobacter cibinogensis* (Gouby et al. 2007). In other case, Gram-negative, catalase-positive, and oxidase-negative bacilli were isolated from sputum samples of a man with cystic fibrosis. Commercial identification systems failed to identify the bacteria. However, a strain of the bacteria was identified by matching the sequence of 16S rRNA gene with that of *Acetobacter indonesiensis* (Bitter et al. 2008).

Gluconobacter spp.

Members of the genus *Gluconobacter* are widely distributed in fruits and flowers. However, several strains were found in human blood and sputa (Alauzet et al. 2010). A Gram-negative rod was isolated from peripheral blood samples of a male patient with a history of intravenous drug abuse. Conventional methods and commercialized systems failed to identify it. However, the strain was identified as *Gluconobacter* sp. by sequencings of 16S rRNA gene, 16S-13S tRNA gene, and *dnaK* gene. In other case, four Gram-negative, catalase-positive, and oxidase-negative rods were isolated from sputa of a boy suffering from cystic fibrosis (CF). Phenotypic identification was unsuccessful for the strains, but the strains were identified as *Gluconobacter* spp. according to the above molecular means. In addition, a Gram-negative, catalase-positive, and oxidase-negative strain was isolated from the sputum of a girl with CF. The strain was not identified by phenotypic methods while identified as *Gluconobacter* sp. by the molecular means. In addition, strains of *Gluconobacter* spp. were isolated from bloodstream infection associated with endocardial lesions in a female intravenous drug abuser (Bassetti et al. 2013). However, the abovementioned *Gluconobacter* strains were unable to be identified at the level of species.

Acidomonas methanolica

The genus *Acidomonas* comprises one species, *Acidomonas methanolica*, which is a known methanol-utilizing acetic acid bacterium. Until now, the strains are most abundantly isolated from sewage samples (Yamashita et al. 2004). However, an *Acidomonas methanolica* strain was isolated from an excised lymph node of a boy with chronic granulomatous disease (CGD). Identification was carried out by sequencing 16S rRNA gene. *Acidomonas methanolica* is described to be a pathogen associated with adenitis in the patient with CGD (Chase et al. 2012).

Roseomonas spp.

The genus *Roseomonas* includes Gram-negative coccobacilli characterized by pink-pigmentation and their inability to utilize methanol as a carbon source (Rihs et al. 1993). Strains of this genus have since been isolated from human blood, cerebrospinal fluid, sputum, genitourinary site, and other clinical specimens (Gilardi and Faur 1984; Wallace et al. 1990; Struthers et al. 1996; Han et al. 2003). Cases of catheter-related bacteremia due to *Roseomonas gilardii* and *Roseomonas mucosa* were reported (Alcalá et al. 1997; Vasallo et al. 1998; Bard et al. 2010). Further, blood isolates were obtained from patients with clinically significant underlying disease who had central venous catheters in place, and the majority was associated with polymicrobial catheter infections (Lewis et al. 1997). Cases of peritonitis caused by *Roseomonas gilardii* and *Roseomonas fauriae* were reported in patients undergoing continuous ambulatory peritoneal dialysis (Sandoe et al. 1997; Bibashi et al. 2000). In addition, peritonitis caused by *Roseomonas mucosa* was reported in an adolescent infected with HIV on continuous cycling peritoneal dialysis (Boyd et al. 2012). *Roseomonas* species were reported to have an overall low pathogenic potential for humans, but the species, in particular *R. gilardii*, may be significant pathogens in persons with underlying medical complications (Struthers et al. 1996). A case of chronic postoperative endophthalmitis due to *Roseomonas* spp. was reported, which might result in misdiagnosis and delayed treatment and caused ocular damage and severe visual loss. This was related to a case with postoperative endophthalmitis secondary to *Roseomonas* infection (Chen et al. 2009). Natural reservoir of *Roseomonas* strains is not known. However, the isolates from water were described (Rihs et al. 1993), and domestic water supplies were noticed for *Roseomonas* infection (Sandoe et al. 1997; Bibashi et al. 2000). In addition, *Roseomonas vinacea* was isolated from a soil sample collected from the Qinghai-Tibet Plateau, China (Zhang et al. 2008).

Application

Vinegar

The word "vinegar" is derived from "vin," "aigre," and "vinaigre" in French, literally meaning "sour wine" (Kersters et al. 2006). In the past, wine making and vinegar preparation were always linked. As early as 4000 B.C., vinegar was already mentioned in Babylonian writings, as a product of the date palm, the culture of which was highly developed (Nickol 1979). In Babylon, "fruit honey," viz., sugary syrup made from figs, apricots, grapes, and dates, was used in the kitchen and for

the preparation of wine (Kersters et al. 2006). After the process of wine making was completed, the extracted dates, together with their stones, were used for making a drink composed of a kind of lemonade and vinegar. Vinegar was also a side product, mostly as the unwanted material, in beer brewing. Originally, beer, wine, and vinegar were homemade (4000–3000 B.C.), but later they were prepared in breweries (3000–2000 B.C.). The Babylonian and the Assyrian breweries started mixing barley and dates. Raisin wine and raisin vinegar also became very popular in Babylon from approximately 1000 B.C.

In the Greek settlements in Southern Italy, wine production and trade were important from the eighth century B.C. The Roman took over the Greek knowledge of vine growing and wine making. In Rome, good cooking was an art for the rich, who eagerly accepted the Greek cooking heritage. For example, one of the principal characteristics of the classical Latin taste was its preference for sweet sour (honey vinegar) combinations (André 1961). Uncooked vegetables were eaten with a sort of French dressing ("vinaigrette"), the *acetaria* (Kersters et al. 2006). Several kinds of vinegar were known, e.g., *acetum piperatum*, vinegar containing pepper; *oxygarum*, made from vinegar, garum, and herbs; *oxymeli*, made by boiling vinegar, honey, water, and salt; and *oxysporium*, vinegar containing spices (André 1961).

In ancient China, vinegar seemed to be produced from fruit in the later Shang or Yin period (c. 1300–1046 B.C.) (Yanagida 1990a). In the subsequent Zhou period (1046–256 B.C.), the vinegar was widely used among noblemen. According to a Chinese old book, alcoholic beverages were mainly produced from grains. The vinegar seemed to be produced first from huángjiŭ (a yellow-colored alcoholic beverage), which is at present made from rice and widely brewed. In the old China, there were three kinds of saccharification methods for making alcoholic beverages. The first was by *Aspergillus oryzae*, the second was by *Rhizopus* species, and the third was by the use of malt. However, the malt technique for brewing was lost before the Tang period (A.D. 618–907) except for candy making, for which malt is still utilized in the Chinese countryside (Yanagida 1990a). In the Yuan period (A.D. 1271–1368), there was a record that vinegar was made by *Monascus* species.

In Japan, the sake brewing technique using rice koji by *Aspergillus oryzae* was supposed to come from China in the third century. In China, however, the technique appeared to be already developed in the third century B.C. (Ohtsuka 1990). Sake or rice wine is easily spoiled and turns to rice vinegar. In the Nara period (710–794), the rice vinegar was brewed extensively in Japan. In the middle to late Edo period (c. 1693–1868), many kinds of "sushi" were made using rice vinegar or sake lees vinegar (Ohtsuka 1990).

In Asian countries, the rice vinegar is also produced in Thailand (Yanagida 1990b). In the Philippines, coconut vinegar is particularly made from fermented coconut water. Sugarcane vinegar, which is made from either sugarcane juice or molasses, is most popular also in the Philippines. When the sugarcane vinegar is produced from molasses, the fermented material is distilled, and the resulting alcohol is adequately diluted to be subjected to vinegar fermentation (Yanagida 1990b). Chinese black vinegar is an aged product, which is made from rice, wheat, millet, and sorghum or their combinations. A lighter form of black vinegar is made from rice in Japan. It is called "kurosu" and used as a healthful drink. Date vinegar is produced from dates and a traditional product of the Middle East.

The first commercial vinegar process used wooden casks, and the wine was oxidized on the surface of the liquid by the mother of the vinegar, which contained the acetic acid bacteria (Kersters et al. 2006). The vinegar process was known as the Orleans process or the French method. In Japan, the commercial production of vinegar started in the middle Edo period (around 1690) (Masai 1980). The raw materials were rice, sake, and sake lees. The lees is a by-product of the sake production. Up to the end of World War II, the surface fermentation in wooden shallow trays was the most common process in Japan (Masai 1980). In the traditional fermentation of rice vinegar in Japan, strains with more than 99 % similarity to *Acetobacter pasteurianus* in 16Sr RNA gene sequence analysis dominated all the stages of fermentation, indicating that appropriate strains were spontaneously established almost pure cultures during a century of the fermentation (Nanda et al. 2001).

The German method or the quick process was first introduced from Germany at the beginning of the nineteenth century (Kersters et al. 2006). The system was composed of the generator, consisting of a tank packed with beechwood shavings. Wine was dropped at the top and allowed to trickle through the generator. The acetic acid bacteria grow on the surface of the beechwood shavings and catalyze the oxidation of ethanol to acetic acid. Air is introduced from the holes of the bottom of the tank.

In addition to the surface culture of acetic acid bacteria for vinegar production, the submerged fermentation processes were introduced. After World War II, the techniques for penicillin production were extensively developed. The techniques were applied to vinegar production. The acetator, which was composed of a stainless tank equipped with the aeration and the cooling systems, was put on the market in 1954 by Frings, Germany (Yanagida 1990c). The cavitator of Yeoman, USA, was also utilized for submerged vinegar fermentation. On the other hand, the acetic acid bacteria useful for producing high-acid vinegar were isolated, viz., "*Acetobacter polyoxogenes*," *Acetobacter europaeus* (= *Komagataeibacter europaeus*), and *Gluconacetobacter entanii* (Entani et al. 1985; Sievers et al. 1992; Schüller et al. 2000). The combination of such submerged fermentations and the different types of acetic acid bacteria made possible to produce vinegar with high acidity.

Recently, the quorum-sensing system was investigated for *Gluconacetobacter intermedius* (= *Komagataeibacter intermedius*) NCI 1051 and its mutants (Iida et al. 2008a, b, 2009). Acetic acid production was intensely enhanced with either the *glnI*: :Km mutant harboring pMV24 or the *gltA*::Km mutant harboring pMV24, in comparison with the wild-type strain NCI 1051 which also harbored the plasmid vector pMV24. The final yields of acetic acid with the mutants were twice, when compared with the wild-type strain (Iida et al. 2009).

Alcoholic Beverages

Starting in the Pasteur's time, it was known that acetic acid bacteria were involved in the acetification of wine (Vallery-Radot 1924). The microbiologists at that time already knew that these bacteria could be isolated from the pellicle that formed on wine when it was exposed to air. They were also aware of the fact that the acetification required oxygen (Behrens 1896; Fuhrmann 1905; Wermischeff 1893). The high alcohol tolerance of acetic acid bacteria was already observed. Many acetic acid bacteria were isolated from wine must, but not from either intact or injured grapes (Fuhrmann 1905). *Acetobacter pasteurianus* (75 % of the isolated strains) and *A. aceti* (19 %) strains were isolated from wines in southern France (Dupuy 1957). Acetic acid bacteria were present in about half of the samples of ripe grapes, and they multiplied on injured grapes (Peynaud and Domercq 1961).

Gluconobacter strains occurred profusely on ripe grapes of the Bordeaux region (73 % of all strains isolated), whereas *Acetobacter* and *Pseudomonas* strains constituted, respectively, only 12 and 15 % of the isolates (Blackwood et al. 1969). *Gluconobacter* strains were present on intact and injured grapes but not during the fermentation. The ketogenesis by *Gluconobacter* strains on grapes led to a higher requirement for SO_2 in the sulfitation process. *Gluconobacter* but no *Acetobacter* strains were isolated from dried and mature grapes and vines (Passmore and Carr 1975). *G. oxydans* strains were isolated from grapes (Ameyama 1975). *G. oxydans* was reported as the dominating acetic acid bacteria in fresh grape must, and *A. pasteurianus* and *Gluconacetobacter liquefaciens* were dominated during the fermentation process in South Africa (Du Toit and Lambrechts 2002).

Palm wine is a typical tropical beverage from the fermentation of sugary palm sap and a whitish, effervescent, acidic alcoholic beverage, which is the product of a mixed alcoholic, lactic acid, and acetic acid fermentation. It is popular beverages in Africa, South America, and the Far East. As a first step, the sugar of the sap is fermented to ethanol within 8–12 h by *Zymomonas* strains and yeasts, thus creating a medium highly suitable for the development of acetic acid bacteria. A rather complex flora was present in palm wines (Swings and De Ley 1977). During palm sap fermentation, the acetic acid bacteria appear after 2–3 days. Acetic acid bacteria utilizing D-glucose and/or sucrose might be present in earlier stages of the palm sap fermentation (Okafor 1975). *Acetobacter* species were isolated from palm wines (Faparusi 1973; Okafor 1975) and from the immature spadix of the palm tree (Faparusi 1973). *A. pasteurianus, A. lovaniensis, A. indonesiensis,* and *A. tropicalis* were isolated from palm wine (Simonart and Laudelout 1951; Lysdiyanti et al. 2003b) and *Gluconacetobacter xylinus* from the leaflets of the palm tree and the surrounding air (Faparusi 1973). *Gluconobacter oxydans* was found on the floret of the palm tree, in the tap hole, and in palm sap (Faparusi 1973, 1974).

Acetic acid bacteria occasionally cause deterioration of alcoholic beverage. *Acetobacter pasteurianus* strains were isolated from hiochi sake samples with the smell of acetic acid, applying "hiochi" to sake altered after fermentation, clarification, and pasteurization (Takahashi 1907). *Acetobacter* infection in tequila was described, which occurred in developed cloudiness upon standing and seasonally during summer (Greene and Breazeale 1952).

Cocoa Fermentations

Cocoa is one of many foods that need microbial fermentation or microbial curing for flavor development. The main reason for the fermentation of cocoa is to induce biochemical transformation within the cacao beans that leads to the formation of the color, the aroma, and the flavor of precursors of cocoa. These properties are further developed during drying, roasting, and final processing of the well-fermented cocoa beans to produce cocoa powder and chocolate (Hansen et al. 1998; Thompson et al. 2001; Cleenwerck et al. 2007; Romero-Cortes et al. 2012).

Of the microbial flora in the fermentation, yeasts, lactic acid bacteria, acetic acid bacteria, and spore-forming bacteria were identified (Schwan and Wheals 2004). During the fermentation of cocoa beans, yeasts dominate for the first 24 h. Since the pulp of cocoa beans disappears and acetic acid bacteria start to dominate, the temperature rises to 50 °C, and the resulting heat and the resulting acid are due to chemical reactions in the cocoa beans known as the microbial fermentation or the microbial curing (Schwan 1998). The effect of acetic acid produced and rise in temperature cause the death of the seed embryo as well as the end of fermentation (Cleenwerck et al. 2007). In the cocoa fermentation process, *Acetobacter ghanensis* and *Acetobacter tropicalis* strains were reported (Cleenwerck et al. 2007; Romero-Cortes et al. 2012).

Cocoa wine is made by fermentation of cocoa seeds and is a popular drink in Nigeria (Bassir 1968; Kersters et al. 2006). The cocoa beans are ground and boiled in sugared water and allowed to cool to approximately 50 °C, before inoculum is added. The alcohol concentration of the cocoa wine is 9.5–11.6 % and is significantly higher than that of palm wine (Bassir 1968). *Acetobacter* and *Gluconobacter* strains were isolated from cocoa wine (Bassir 1968). To improve the reliability and quality of the cocoa fermentation process, a defined and mixed starter culture including *Acetobacter* and *Gluconobacter* strains was successfully used (Schwan 1998).

Nata

Nata is a white- to creamy-colored gelatinous or agar-like film grown on surface of juice made from coconut, pineapple, sugarcane, or other fruit wastes in the Philippines, Thailand, Indonesia, and other Southeast Asian countries. Nata de coco is produced from coconut water or coconut milk. The following ingredients are added to coconut water: to be final concentrations 2 % of glacial acetic acid; 15 % of sucrose; 0.5 % of ammonium dihydrogen phosphate, giving pH 5.0–5.5; and 10 % of 48 h culture inoculum. After fermentation at 28–31 °C

for 12–15 days, 5–7 cm-thick layer of bacterial cellulose is formed. Washing it with water and removing acetic acid, the film is cut into cubes. Nata de coco is a popular dessert in the Southeast Asian countries (Campbell-Platt 1987) and was once exported to other countries (Librero and Tidon 1994). Mother liquor in which acetic acid bacteria inhabit can be used (Librero and Tidon 1994). Cultures of *Komagataeibacter xylinus* (*Gluconacetobacter xylinus*) were considered a major cellulose-producing bacterium. Analysis of 16S rRNA sequence revealed that *K. xylinus and K. hansenii* (*Gluconacetobacter hansenii*) concerned with the production of nata de coco (Bernardo et al. 1998). In addition, *A. pasteurianus*, *A. orleanensis*, *A. lovaniensis*, *Gluconobacter oxydans*, and *G. frateurii* strains were also found in the process of nata production (Lysdiyanti et al. 2003b).

Kombucha and "Tea Fungus"

Kombucha is a slightly acidulous and effervescent beverage obtained by the fermentation of sugared black tea with a symbiotic culture of acetic acid bacteria and yeasts. Several names have been given to the beverage and the organisms responsible for acetic acid fermentation of tea infusions: kombucha, tea fungus, miracle fungus, fungus of charity, fungus of a long life, remedy for immortality, Ma Gu, Chinese or Japanese fungus, Japanese or Indian tea fungi, Hongo, Haipao, Teeschwamm, Teekwasspilz, indischer Weinpilz, Wunderpilz, and Wolgapilz (Dinslage and Ludorff 1927; Stadelmann 1957; Kraft 1959; Abadie 1962). These names, often referring to a fungus, are confusing, in fact, which mean an association of yeasts with acetic acid bacteria, forming a thick film on the surface of a tea infusion.

Kombucha and Japanese tea fungus were cited as follows: The Japanese word "kombu" describes an edible fungus (*Laminaria japonica*) that is used for the production of tea (Japanese "cha" = tea) (Caesar 1990; Mayser et al. 1995). However, kombu (*Laminaria japonica*) is a kind of seaweeds in Japanese but not "edible fungi," and "cha" means tea. "Kombucha" in Japanese is a powdery material produced from the dried seaweed (*Laminaria japonica*) and a small amount of sodium chloride. It has been used as a drink and a seasoning in Japan. Therefore, "kombucha" in Japanese clearly differs from kombucha used in Europe and the USA.

Approximately one hundred years ago, the tea fungus was already used in Japan, China, and India; then in Russia, Poland, and the Baltic States starting in about 1915; in the Balkan, Germany, and Eastern Europe in about 1925; and in Spain, Italy, France, and Switzerland in about 1955 (Kraft 1959).

Recipes of kombucha are almost identical to one another. One example is shown (Teoh et al. 2004). The tea medium was prepared traditionally by boiling 1 l of distilled water, 100 g white sugar, and 5.4 g black tea leaves for 5 min and allowing the mixture to steep for 15 min. The sweetened tea was strained to remove the leaves, during transfer to a sterile glass jar (2 l). Once cooled to the ambient laboratory temperature (20–22 °C), the tea was inoculated with the kombucha culture (pellicle). At the completion of the first fermentation, the pellicle, covering the entire surface area of the liquor, was used to inoculate the next (second) fermentation. During subsequent fermentations, a "daughter" pellicle similar to that of the "parent" was used as the inoculum. For each of the second and subsequent fermentations, 100 ml of beverage was added from the previous fermentation, in addition to the pellicle. For each fermentation, the jar was covered with paper towel, secured with rubber band. Fermentation was conducted in a temperature-controlled laboratory (20–22 °C) for up to 14 days. The pH of the beverage decreased rapidly within the first 24 h., decreasing to pH 2.2 by the completion of fermentation.

Hermann (1928a, b) isolated "*Bacterium gluconicum*" (*Gluconobacter oxydans*) and "*Bacterium xylinus*" (*Komagataeibacter xylinus*) from kombucha. *Acetobacter xylinum* (*Komagataeibacter xylinus*) (Sievers et al. 1995), *Acetobacter aceti* and *A. pasteurianus* (Liu et al. 1996), *Acetobacter intermedius* (*Komagataeibacter intermedius*) (Boesch et al. 1998), *Acetobacter nitrogenifigens* (Dutta and Gachhui 2006), and *Gluconoacetobacter kombuchae* (Dutta and Gachhui 2007; later reidentified as *Gluconacetobacter hansenii*, Cleenwerck et al. 2009) have also been identified in kombucha beverage. The following yeasts have been identified in kombucha: the genera *Brettanomyces*, *Zygosaccharomyces* and *Saccharomyces* (Mayser et al. 1995); *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Brettanomyces bruxellensis* (Liu et al. 1996); and *Zygosaccharomyces kombuchaensis, Z. bailii*, *Z. bisporus*, and *Pichia fluxuum* (Kurtzman et al. 2001).

Kombucha was made with pure cultures of *Acetobacter* sp. NRRL B-2357 and two yeast strains, NRRL Y-4810 (later identified *as Zygosaccharomyces kombuchaensis*; Kurtzman et al. 2001) and NRRL Y-4882 (later identified as *Pichia fluxuum*; Kurtzman et al. 2001) (Hesseltine 1965). When all three organisms were used together, normal fermentation occurred. The fermentation with three organisms appeared to be exactly similar to that which occurred when the original culture was used, which contained a number of a few different organisms. When an *Acetobacter* strain was used alone, a film formed with some gas. When the yeasts were used singly or in combination, no film was formed. When the three microorganisms were used in combination, a thicker film was formed rapidly, no gas accumulated, and a typically normal fermentation occurred.

The process during kombucha fermentation was described (Sievers et al. 1995). *Zygosaccharomyces* yeasts in kombucha converted sucrose into D-glucose and D-fructose. D-Fructose was metabolized prior to D-glucose. Further, D-glucose and D-fructose were turned to ethanol and carbon dioxide. *Acetobacter xylinus* produced acetic acid from ethanol and D-gluconic acid from D-glucose. The pH value of kombucha decreased during fermentation from pH 3.75 to 2.42. Sucrose was completely hydrolyzed after 40 days, and D-fructose was entirely metabolized. After 20 days, 7.0 g/l of ethanol was produced, and after 40 days, 2.0 g/l of alcohol remained. Further, 28.4 g/l acetic acid was

D-Glucose → D-Sorbitol → L-Sorbose → Diacetone-L-sorbose → Diacetone-2-keto-L-gulonic acid → L-Ascorbic acid

Fig. 1.6
The Reichstein-Grüssner process for the production of L-ascorbic acid. The biological oxidation of D-sorbitol to L-sorbose is catalyzed by *G. oxydans*. The other processes are no biological steps

D-Sorbitol → L-Sorbose → L-Sorbosone → 2-Keto-L-gulonic acid → L-Ascorbic acid

Fig. 1.7
The conversion of D-sorbitol to 2-keto-L-gulonic acid by *G. oxydans*. The conversion of 2-keto-L-gulonic acid to L-ascorbic acid is a no biological step to yield vitamin C

produced after 40 days, and 13.5 g/l gluconic acid remained. In the first period (6–8 days), the taste of kombucha was fruit-sour-like; with prolonged incubation, the tea fungus developed a vinegar-like taste. The liquid portion is used for a drink. Nata de coco is basically the same as kombucha, and a cellulose pellicle is taken for a dessert.

The claimed health effects of kombucha have been discussed (Dufresne and Farnworth 2000; Greenwalt et al. 2000).

Production of D-Gluconate and Keto-D-gluconates

D-Gluconic acid and its lactone, salts, and keto-forms have a wide range of applications in the food, pharmaceutical, and chemical industries. *Gluconobacter* strains oxidize D-glucose and D-gluconic acid to keto-D-gluconic acids (2-keto-D-gluconic acid, 5-keto-D-gluconic acid, and 2,5-diketo-D-gluconic acid). 2-Keto-D-gluconate and 5-keto-D-gluconate are interesting precursors for isoascorbic acid synthesis and tartaric acid production, respectively (Klasen et al. 1992). 2,5-Diketo-D-gluconate can be converted to 2-keto-L-gulonic acid as a penultimate intermediate in the industrial production of vitamin C.

Production of L-Sorbose and Vitamin C

Gluconobacter strains are used for the oxidative conversion of D-sorbitol to L-sorbose, which is an important intermediate in the production of L-ascorbic acid (vitamin C) (estimated world production, more than 100,000 t per year at the time of writing). Vitamin C is important for human and animal nutrition and is used as an antioxidant in the food industry. It is currently produced by the Reichstein-Grüssner process, involving a series of chemically based unit operations and a single biological oxidative step, where D-sorbitol is regioselectively oxidized to L-sorbose by a membrane-bound D-sorbitol dehydrogenase of *G. oxydans* (Fig. 1.6). D-Sorbitol is obtained by electrolytic or catalytic reduction of D-glucose.

The overall practical yield of the Reichstein process, with the recent advances in chemical engineering, was thought to be approximately 50 % (Boudrant 1990). The effects of cross-linking agents, such as glutaraldehyde and polyethyleneimine on L-sorbose production by immobilized *G. oxydans* cells, were studied (Park et al. 1998). The theoretically maximal productivity of the biological oxidative step was achieved by using a *G. oxydans* mutant that was selected under conditions of substrate inhibition (De Wulf et al. 1996).

Several strategies including recombinant DNA technologies are being developed to shift the synthesis of ascorbic acid from chemical procedures to purely bioconversion routes, where the synthesis of 2-keto-L-gulonic acid is a key intermediate (Deppenmeier et al. 2002). Some *Gluconobacter* strains convert D-sorbitol to 2-keto-L-gulonic acid via L-sorbosone. The 2-keto-L-gulonic acid can be converted nonbiologically to L-ascorbic acid (Fig. 1.7; Hoshino et al. 1990; Saito et al. 1997).

Overexpression of the L-sorbose dehydrogenase and the L-sorbosone dehydrogenase genes in *G. oxydans* resulted in improved yields of 2-keto-L-gulonic acid (Saito et al. 1997). In this respect, the application of *Ketogulonigenium* strains belonging to the family *Rhodobacteraceae* may open new perspectives for the biological conversion of L-sorbose to 2-keto-L-gulonic acid (Urbance et al. 2001).

Production of Dihydroxyacetone and Acetylmethylcarbinol

Dihydroxyacetone is used as a cosmetic tanning agent and as an intermediate for the synthesis of various organic chemicals and surfactants. The oxidation of glycerol to dihydroxyacetone by *G. oxydans* strains is catalyzed by a membrane-bound PQQ-dependent glycerol dehydrogenase (Claret et al. 1994) and can be optimized by using immobilized *G. oxydans* cells (Tkac et al. 2001). Similarly *Gluconobacter* strains can be used to oxidize 2,3-butanediol to acetylmethylcarbinol.

Synthesis of 1-Deoxynojirimycin and Miglitol

1-Deoxynojirimycin and its *N*-substituted analog *N*-hydroxyethyl-1-deoxynojirimycin (miglitol) are strong inhibitors of α-glucosidases and are used for the treatment of

D-Glucos → 1-Amino-D-sorbitol → 6-Amino-L-sorbose → 1-Deoxynojirimycin

D-glucose → *N*-Hydroxyethyl-1-amino-D-sorbitol → *N*-Hydroxyethyl-6-amino-L-sorbose → *N*-Hydroxyethyl-1-deoxynojirimycin (miglitol)

Fig. 1.8

Synthesis of 1-deoxynojirimycin and miglitol from D-glucose. The conversions of 1-amino-D-sorbitol to 6-amino-L-sorbose and of *N*-hydroxyethyl-1-amino-D-sorbitol to *N*-hydroxyethyl-6-amino-L-sorbose are the regioselective oxidation by a *G. oxydans* strain. The other conversion are no biological steps. The first step is a chemical reductive amination of D-glucose and the last step is a chemical stereoselective ring closure

non-insulin-dependent diabetes (Campbell et al. 2000). The industrial production of these compounds follows a combined biotechnological-chemical synthesis, whereby a *G. oxydans* strain plays a key role in the oxidation of 1-aminosorbitol derivatives (Fig. 1.8; Deppenmeier et al. 2002). Because *Gluconobacter* strains cannot grow on aminopolyols, whole resting cells (grown on sorbitol) have to be used for this biotransformation process (Schledel 2000).

Gluconobacter as Biosensor

The cells and enzymes of *G. oxydans* find applications as biosensors for the estimation of the concentrations of various aldoses, polyalcohols, ethanol, glycerol, etc. (Lusta and Reshetilov 1998; Macauley et al. 2001; Tkac et al. 2000, 2001).

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2 The Family *Anaplasmataceae*

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Abstract
The family *Anaplasmataceae* is in the order *Rickettsiales* that includes the four genera *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia*, all of which are small Gram-negative pleomorphic cocci and obligatory intracellular bacteria. The *Rickettsiales* are most often cited as the alphaproteobacterial subgroup from which mitochondria arose.

Anaplasmataceae is a family in the order *Rickettsiales* that includes the four genera *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia*, all of which are small Gram-negative pleomorphic cocci and obligatory intracellular bacteria (Dumler et al. 2001; Williams et al. 2007). The *Rickettsiales* are most often cited as the alphaproteobacterial subgroup from which mitochondria arose (Fitzpatrick et al. 2006).

The genus *Wolbachia* is of particular interest due to its ubiquitous distribution and many different evolutionary interactions. It is one of the world's most common parasitic microbes and is possibly the most common reproductive parasite in the biosphere. As many as 70 % of all insect species are estimated to be potential hosts (Kozek and Rao 2007). Its interactions with its insect hosts are often complex and in some cases have evolved to be mutualistic rather than parasitic. Some host species cannot reproduce, or even survive, without *Wolbachia* infection (Zimmer 2001). The genomes of several *Wolbachia* strains show substantial differences at some rapidly evolving regions of the genome, primarily associated with prophage and repetitive elements (Duplouy et al. 2013). These genes include a number that have been recently horizontally transferred from phylogenetically distant bacterial taxa.

The ability of *Wolbachia* to spread through cytoplasmic incompatibility and render mosquitoes resistant to a variety of human pathogens (Moreira et al. 2009) has instigated the development of *Wolbachia*-based strategies for both suppression and replacement of disease vector populations (Hoffmann et al. 2011). Recently this effort has been extended to anopheles malaria vector mosquitoes, which are not naturally infected by *Wolbachia* spp. (Bian et al. 2013).

The genus *Anaplasma* comprises obligate intracellular rickettsial pathogens that are biologically transmitted by ticks (Telford et al. 1996). They target circulating blood cells of wild and domestic animals, as well as of humans, and are of global veterinary and human health importance. The type species, *Anaplasma marginale*, has been studied intensively (Kocan et al. 2000) and can be grown in cell lines derived from the tick *Ixodes scapularis* (Munderloh et al. 1994; Dyachenko et al. 2013). The complete genome sequence of *A. marginale*, the most prevalent tick-borne livestock pathogen worldwide, has been published (Brayton et al. 2005).

The obligate intracellular bacterium *Ehrlichia chaffeensis* that resides in mononuclear phagocytes is the etiologic agent of the emerging human disease monocytotropic ehrlichiosis (Thomas et al. 2010). *Ehrlichia* are round or ovoid Gram-negative bacteria and form a characteristic vacuole-contained microcolony (morula) in macrophages (Popov et al. 1998). *Ehrlichia* are transported between cells through the host cell filopodia during initial stages of infection, whereas in the final stages of infection, the pathogen ruptures the host cell membrane and spreads from cell to cell (Thomas et al. 2010). Complete genome sequencing revealed that the *Ehrlichia canis* genome consists of a single circular chromosome of 1,315,030 bp predicted to encode 925 proteins, 40 stable RNA species, 17 putative pseudogenes, and a substantial proportion of noncoding sequences (Mavromatis et al. 2006).

Discovered in 1984, *Neorickettsia* (formerly *Ehrlichia*) *risticii* is an obligate intracellular bacterium and the causative agent of Potomac horse fever (Rikihisa and Perry 1984). It was determined that *N. risticii* has similar genetic, antigenic, and morphologic characteristics to *Neorickettsia helminthoeca* (Pretzman et al. 1995), which were the major reasons it, as well as *Neorickettsia* (formerly *Rickettsia*, *Ehrlichia*) *sennetsu*, was regrouped into the genus *Neorickettsia* (Dumler et al. 2001).

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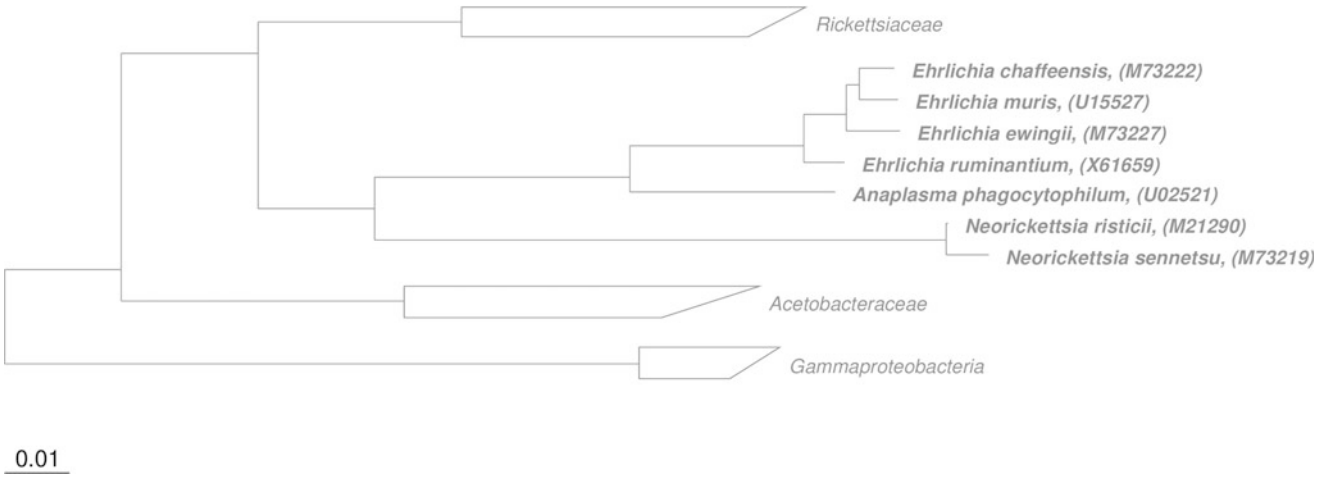

Fig. 2.1

Phylogenetic reconstruction of isolates belonging to the family *Anaplasmataceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (*LTP*) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. *Scale bar* indicates estimated sequence divergence

The identification of Neorickettsia proteins is now achievable with the availability of. The whole genome sequences of both the type strain (Miyayama) of *N. sennetsu* (Hotopp et al. 2006) and the type strain (Illinois) of *N. risticii* (Lin et al. 2009) are available (*Fig. 2.1*).

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3 The Family *Bartonellaceae*

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Abstract
Bartonellaceae is a family within the order *Rhizobiales* in the sub-class of *Alphaproteobacteria* which contains only the genus *Bartonella*. They are fastidious, facultative intracellular, slow-growing, gram-negative, slightly curved bacilli that cause a wide range of diseases in humans and animals, some species being zoonotic. Clinical manifestations in humans include Carrión's disease, trench fever, cat scratch disease, bacteremia with fever, bacillary angiomatosis and peliosis, endocarditis, and neuroretinitis. Colony morphology varies from clear to smooth white and rough tan. Several species embed in the agar. They are typically inert biochemically; the organisms are usually catalase and oxidase negative and aerobic, and they do not produce acid from carbohydrates. Some species are motile. The genus *Bartonella*, to date, contains 29 species and 3 subspecies. Most *Bartonella* species have successfully adapted to survival in specific domestic or wild mammals. Blood-sucking arthropod vectors are often involved. Clinical presentation generally depends on both the infecting *Bartonella* species and the immune status of the infected individual. *Bartonella* species are susceptible to many antibiotics in vitro; however, in vivo antibiotic susceptibilities do not correlate well for a number of antibiotics.

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Taxonomy, Historical and Current

Short Description of the Family

Bartonellaceae (*Bar.to.nel.la' ce.ae.* M.L. fem. n. *Bartonella*, type genus of the family; suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Bartonellaceae*, the *Bartonella* family; Gieszczykiewicz, 1939 emend. Brenner et al. 1993).

Type genus: *Bartonella* Strong, Tyzzer and Sellards 1915 emend. Birtles, Harrison, Saunders, and Molyneux 1995.

Phylogenetically, the family of *Bartonellaceae* is a member of the order *Rhizobiales*, class *Alphaproteobacteria* (subgroup 2) in the phylum Proteobacteria. The family contains only the genus *Bartonella* (type genus) (Birtles et al. 1995; Strong et al. 1915). The phylogenetic relationship of *Bartonella* to other *Alphaproteobacteria* indicates that they have a close evolutionary homology with the members of the genera *Brucella*, *Agrobacterium*, and *Rhizobium* (Brenner et al. 1993). These four genera have similar characteristic, such as the fact that they evolve toward a parasitic or mutualistic association, being *Bartonella* spp. and *Brucella* spp. intracellular parasites of mammalian cells, whereas *Agrobacterium* and *Rhizobium* species can parasitize or mutualistically associate with plant cells, respectively (Minnick and Anderson 2006). In addition, none of these bacteria are obligate parasites, and all can be cultivated in vitro.

The family *Bartonellaceae* suffered a major taxonomic reorganization in the 1990s. For decades, since the description of *Bartonella bacilliformis* in 1909 by Alberto L. Barton (1909), this species was the sole member of the genus *Bartonella*. However, in 1992, on the basis of DNA relatedness data, previous 16S rRNA sequence data, guanine-plus-cytosine contents, and phenotypic characteristics, Brenner and colleagues proposed that the genus *Rochalimaea* should be united with the genus *Bartonella* in the family *Bartonellaceae* (Brenner et al. 1993). The name *Bartonella* was retained as the genus name since it had nomenclatural priority over the name *Rochalimaea*. As a result, new combinations for the *Rochalimaea* species were created: *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb. nov., *Bartonella henselae* comb. nov., and *Bartonella elizabethae* comb. nov. (Brenner et al. 1993). This reclassification also resulted in the transfer of these organisms from the family *Rickettsiaceae* to the family *Bartonellaceae*, which included *Bartonella bacilliformis*. In addition, the family *Bartonellaceae* has been removed from the order *Rickettsiales*, a group that originally included *Anaplasmataceae*, *Rickettsiaceae*, and *Bartonellaceae*. Later, in 1995, based upon DNA relatedness data and phenotypic characteristics, it was proposed that the genera *Grahamella* and *Bartonella* should be unified and that the latter name should be retained (Birtles et al. 1995). *Bartonella talpae* comb. nov. and *Bartonella peromysci* comb. nov., new combinations for former *Grahamella* species, were created, and three new *Bartonella* species were described: *Bartonella grahamii* sp. nov., *Bartonella taylorii* sp. nov., and *Bartonella doshiae* sp. nov. In effect, these reclassifications eliminated the previous genera *Rochalimaea* and *Grahamella*. In 1996, *Bartonella vinsonii* subsp. *berkhoffii* subsp. nov. was designated a new subspecies, *Bartonella vinsonii* subsp. *vinsonii* subsp. nov. renamed (formerly *B. vinsonii*) (Kordick et al. 1996), and *Bartonella clarridgeiae* sp. nov. characterized as a new species (Lawson and Collins 1996). In 1998, *Bartonella tribocorum* sp. nov. was isolated from the blood of wild rats and characterized (Heller et al. 1998). On a sequence, *Bartonella koehlerae* sp. nov. (Droz et al. 2000), *Bartonella alsatica* sp. nov. (Heller et al. 1999), and *Bartonella vinsonii* subsp. *arupensis* subsp. nov. (Welch et al. 2000) were isolated and added to the growing list of *Bartonella* species and subspecies. In the year 2000, *Bartonella birtlessi* sp. nov. was isolated from small mammals (*Apodemus* spp.) and received the name after Richard J. Birtles, whose studies have contributed to an improved understanding of the taxonomy of the genus (Bermond et al. 2000). Also in the same decade, *Bartonella schoenbuchensis* sp. nov. (Dehio et al. 2001), *Bartonella bovis non* (formerly called *Bartonella weissi* or *Bartonella weissii*), *Bartonella capreoli* sp. nov. (Bermond et al. 2002), and *Bartonella chomelii* sp. nov. were isolated (Maillard et al. 2004). New species were later added with the identification of *Bartonella rattaustraliani* sp. nov. (Gundi et al. 2009), *Bartonella queenslandensis* sp. nov. (Gundi et al. 2009), *Bartonella coopersplainsensi* sp. nov. (Gundi et al. 2009), *Bartonella japonica* sp. nov. (Inoue et al. 2010), *Bartonella silvatica* sp. nov. (Inoue et al. 2010), and *Bartonella rochalimae* sp. nov. (Eremeeva et al. 2012). Recently, in 2013, *Bartonella jaculi* sp. nov., *Bartonella callosciuri* sp. nov., *Bartonella pachyuromydis* sp. nov., and *Bartonella acomydis* sp. nov. were isolated from wild Rodentia (Sato et al. 2013) and increased the list of species compromising the genus *Bartonella*. According to the *List of Prokaryotic Names with Standing in Nomenclature* (*LPSN*, 2014), the genus *Bartonella*, to date, contains 29 species and 3 subspecies. Further growth in the number of species and subspecies in the genus is thus expected to occur in the future.

It is noteworthy that some *Bartonella* organisms are considered *Candidatus*, a category used for describing prokaryotic entities for which more than a mere sequence is available but for which characteristics required for description according to the *Bacteriological Code* (1990 Revision) are lacking. These include "*Candidatus* Bartonella ancashi" (Blazes et al. 2013), "*Candidatus* Bartonella antechini" (Kaewmongkol et al. 2011a), "*Candidatus* Bartonella bandicootii" (Kaewmongkol et al. 2011b), "*Candidatus* Bartonella mayotimonensis" (Lin et al. 2010), "*Candidatus* Bartonella merieuxii" (Chomel et al. 2012), "*Candidatus* Bartonella thailandensis" (Saisongkorh et al. 2009), "*Candidatus* Bartonella washoensis subsp. cynomysii" (Bai et al. 2008), and "*Candidatus* Bartonella woyliei" (Kaewmongkol et al. 2011).

According to the Second Edition of *Bergey's Manual of Systematic Bacteriology*, the family *Bartonellaceae* is metabolically diverse and includes aerobic organisms. The Gram staining of these bacteria is negative and is not acid fast. The morphology is characterized by small coccobacilli that may be beaded or filamentous and <3 μm in their greatest diameter. Erythrocytic forms stain lightly with many aniline dyes but distinctly with

Giemsa stain after methanol fixation. Some species have polar flagella although most are nonmotile. Organisms are cultivable, but highly fastidious, in vitro, on blood-enriched bacteriological media (Welch 2005).

Clinical manifestations in humans and the vectors and reservoirs associated with each of the currently recognized species and subspecies of *Bartonella*, as well as discovery and first cultivation date are described in *Table 3.1*.

Molecular Analyses

Phylogenetic Structure of the Family and Its Genus

The *Bartonellaceae* family consists of closely related bacteria grouped into a single genus (*Fig. 3.1*). Molecular approaches are the most contemporary tools used to classify organisms and study their evolutionary relationships. These analyses are based on the variability of the sequences of certain genes, especially those encoding RNA molecules. The use of well-defined secondary structure in the identification of homologous residues (i.e., the alignment process) is an important advantage of the RNA sequences as compared with the genes encoding other proteins. To perform a phylogenetic study of prokaryotes, comparative analysis of *16S rDNA* (Woese 1987; Ludwig and Schleifer 1999) is considered the gold standard. Because of the high degree of *16S rDNA* sequence conservation, the phylogenetic differentiation at the species level is low. Thus, the use of this molecular marker in differentiating groups of closely related organisms, such as species of the genus *Bartonella*, can be a limiting factor (Fox et al. 1992; Suksawat et al. 2001; Houpikian and Raoult 2001a). This has led to problems in the identification of medically important bacteria of *Bartonella* genus when the molecular diagnosis was based on the 16S rDNA gene. Other genes have been successfully used to increase the sensitivity and specificity of analytical methods for the detection of these *Bartonella* species.

Alternative genes that may be used for phylogenetic purposes should be both highly conserved (i.e., housekeeping genes) and sufficiently variable to allow species identification (Olsen and Woese 1993). Useful alternative genes include a 17 kDa antigen gene (Sweger et al. 2000), *flaA* (Sander et al. 2000), *gltA* (Birtles and Raoult 1996; Roux et al. 2000; Houpikian et al. 2001), *htrA* (Anderson and Neuman 1997), *ftsZ* (Ehrenborg et al. 2000), and *groEL* (Marston et al. 1999), as well as the 16S and 23S rDNA internally transcribed spacer sequences (Jensen et al. 2000; Birtles et al. 2000). However, when used to infer the phylogenetic relationships within the genus *Bartonella*, these sequences suggested convicting relationships.

The *gltA* and 16S–23S genes are often used to classify bacterial species, especially those of the genus *Bartonella*. The trees generated from the *16S rDNA* gene have an improved resolution, but many of them lack statistical support (Birtles and Raoult 1996; Roux and Raoult 1995; Jensen et al. 2000). Phylogenetic trees derived from *ITS* gene for the *Bartonella* species showed reliable organizations, similar to derived trees of *groEL* gene (Houpikian and Raoult 2001a). Several studies have demonstrated the importance of the *groEL* gene as a molecular marker sensitive and valuable for determining phylogenetic relationships of bacteria species (Viale et al. 1994). Several bacteria species of *Bartonella* genus known to date have been classified by Zeaiter et al. (2002) using *groEL* gene sequences. In the same study, the *groEL* gene was used for subtyping of *B. henselae* and *B. quintana* isolates. Zeaiter et al. (2002) compared phylogenetic trees derived from 16S rDNA sequences; it was confirmed that this gene was unable to resolve the relationships within the genus *Bartonella* as most branches lacked statistical support, and only one reliable cluster was established by *B. elizabethae* and *B. tribocorum*. In contrast, trees generated using *groEL* sequences were much more informative. All three phylogenetic analysis methods provided similar and reliable topologies.

According Houpikian and Raoult (2001b), the sequences of protein-coding genes have been used for evolutionary inferences for being more conserved at the species level, but highly variable between species. Furthermore, these sequences may be analyzed by amino acid sequence as well as genomic level for improved reliability (Birtles and Raoult 1996; Marston et al. 1999; Birtles et al. 2000). In the literature, there is no consensus about the use of hypervariable region of the ITS gene as indicator of molecular evolution (Birtles et al. 2000; Roux and Raoult 1995). Nevertheless, the ability of a specific sequence to produce meaningful phylogenetic data can only be appreciated after comparison and reconstruction inferable, by assessing the statistical support of the resulting branching order. Thus, only the *16S rDNA*, *ITS*, *gltA*, and *groEL* are routinely used for phylogenetic studies. Use of other genes is hampered by the lack of *Bartonella* sequences.

Six clusters were consistently identified after genotyping studies based on phylogenetic information from the 16S rDNA, ITS, groEL, and gltA genes of *Bartonella* species (i.e., clusters which were statistically supported to at least two different genes). The cluster formed by *B. bacilliformis* (group A) is the only one that diverges from the others relatively well with 100 % bootstrap values for the 16S rDNA, ITS, groEL, and gltA genes. This divergence is also supported by differences in colony morphology, requirements for growth, fatty acid composition, protein, and antigenic structure (Anderson et al. 1995; Birtles and Raoult 1996; Marston et al. 1999; Birtles et al. 1991). Levels of similarity for amino acid sequences of ftsZ proteins, as well as for nucleotide sequences of *ribC* genes, are low between *B. bacilliformis* and other *Bartonella* species, supporting its divergent position (Bereswill et al. 1999; Kelly et al. 1998). *Bartonella clarridgeiae* may also be a divergent species (group B) according to *ITS* and *groEL* analyses. Strains of *Bartonella* sp. associated with cats in the Netherlands and the USA have a close phylogenetic relationship with *B. clarridgeiae* based on analyses of 16S rDNA gene. Studies have suggested that *B. clarridgeiae* and *B. bacilliformis* have a close phylogenetic relationship, since these species are flagellated, but statistical

Table 3.1

Current recognized species and subspecies of *Bartonella*, discovery and first cultivation date, reservoirs, vectors, and associated human disease

Bartonella species	Discovery (year)	First cultivation (year)	Reservoir	Vector	Disease in human	Reference
Human-specific species						
B. bacilliformis	1909	1919	Human	Sand fly	Carrión's disease: Oroya fever and verruga peruana	Guptill 2010; Mogollon-Pasapera et al. 2009; Jacomo et al. 2002
B. quintana	1916	1961	Human	Human body lice Cat flea (?)	Trench fever Endocarditis Bacillary angiomatosis–peliosis Chronic bacteremia Lytic bone lesions Central nervous system disorder	Maurin and Raoult 1996; Rolain et al. 2003; Mogollon-Pasapera et al. 2009
Zoonotic species						
B. alsatica	1999	1999	Rabbit	Rabbit flea (?)	Endocarditis Lymphadenitis	Heller et al. 1999; Raoult et al. 2006; Chomel and Kasten 2010; Kaiser et al. 2011
B. clarridgeiae	1995	1995	Cat	Cat flea (?)	Cat scratch disease (?)	Dehio et al. 2001; Mogollon-Pasapera et al. 2009; Lamas et al. 2008; Chomel and Kasten 2010
B. elizabethae	1993	1993	Rat	Oriental rat flea	Endocarditis Retinitis	Daly et al. 1993; Mogollon-Pasapera et al. 2009; Jacomo et al. 2002; Chomel and Kasten 2010
B. grahamii	1995	1995	Mouse Vole	Rodent flea Tick (?)	Neuroretinitis Cat scratch disease	Birtles et al. 1995; Dehio et al. 2001; Mogollon-Pasapera et al. 2009; Brown et al. 2004; Oksi et al. 2013
B. henselae	1992	1990	Cat Dog	Cat flea Tick (?)	Cat scratch disease Bacillary angiomatosis Parenchymal bacillary peliosis Septicemia Endocarditis	Regnery et al. 1992; Slater et al. 1990; Dehio et al. 2001; Mogollon-Pasapera et al. 2009; Kaiser et al. 2011
B. koehlerae	1999	1999	Cat	Cat flea	Cat scratch disease Endocarditis	Droz et al. 1999; Jacomo et al. 2002; Chomel and Kasten 2010
B. rochalimae	2007	2007	Foxes Raccoons Coyotes Dogs	Flea (?)	Septicemia Cutaneous lesions	Mogollon-Pasapera et al. 2009; Henn et al. 2009; Eremeeva et al. 2007; Chomel and Kasten 2010
B. vinsonii subsp. *arupensis*	1999	1999	White-footed mouse	Flea (?) Tick (?)	Bacteremia Arthralgia Neurologic disorders endocarditis	Welch et al. 1999; Mogollon-Pasapera et al. 2009; Jacomo et al. 2002; Kaiser et al. 2011; Chomel and Kasten 2010; Chomel et al. 2006
B. vinsonii subsp. *berkhoffii*	1996	1996	Coyote Dog	Tick (?)	Endocarditis Arthralgia Myalgia Septicemia	Kordick et al. 1996; Mogollon-Pasapera et al. 2009; Jacomo et al. 2002; Lamas et al. 2008

Table 3.1 (continued)

Bartonella species	Discovery (year)	First cultivation (year)	Reservoir	Vector	Disease in human	Reference
Animal-specific species						
B. acomydis	2013	2013	Unknown	Unknown	Not reported	Sato et al. 2013
B. birtlesii	2000	2000	Mouse	Tick	Not reported	Bermond et al. 2000; Reis et al. 2011; Kaiser et al. 2011
B. bovis	2002	2002	Deer Elk Beef Cattle	Biting fly (?) Tick (?)	Not reported	Bermond et al. 2002; Kaiser et al. 2011; Lamas et al. 2008; Chomel and Kasten 2010
B. callosciuri	2013	2013	Unknown	Unknown	Not reported	Sato et al. 2013
B. capreoli	2002	2002	Roe deer	Unknown	Not reported	Bermond et al. 2002; Kaiser et al. 2011
B. chomelii	2004	2004	Cattle	Unknown	Not reported	Maillard et al. 2004; Dehio 2005
B. coopersplainsensis	2009	2009	Unknown	Unknown	Not reported	Gundi et al. 2009
B. doshiae	1995	1995	Vole	Unknown	Not reported	Birtles et al. 1995; Jacomo et al. 2002; Kaiser et al. 2011
B. jaculi	2013	2013	Unknown	Unknown	Not reported	Sato et al. 2013
B. japonica	2010	2010	Unknown	Unknown	Not reported	Inoue et al. 2010
B. pachyuromydis	2013	2013	Unknown	Unknown	Not reported	Sato et al. 2013
B. peromysci	1942		Deer Mouse	Unknown	Not reported	Birtles et al. 1994; Rolain et al. 2004; Kaiser et al. 2011
B. queenslandensis	2009	2009	Unknown	Unknown	Not reported	Gundi et al. 2009
B. rattaustraliani	2009	2009	Unknown	Unknown	Not reported	Gundi et al. 2009
B. schoenbuchensis	2001	2001	Roe deer	Deer ked	Not reported	Dehio et al. 2001; Rolain et al. 2004; Kaiser et al. 2011
B. silvatica	2010	2010	Unknown	Unknown	Not reported	Inoue et al. 2010
B. talpae	1905		Mole	Unknown	Not reported	Birtles et al. 1994; Rolain et al. 2004
B. taylorii	1995	1995	Mouse Vole	Rodent flea	Not reported	Birtles et al. 1995; Brown et al. 2004; Kaiser et al. 2011
B. tribocorum	1998	1998	Rat	Unknown	Not reported	Heller et al. 1998; Jacomo et al. 2002
B. vinsonii subsp. *vinsonii*	1946	1946	Vole	Vole ear mite	Not reported	Baker 1946; Jacomo et al. 2002; Kaiser et al. 2011

support is lacking for such organization (Kosoy et al. 1997; Marston et al. 1999; Jensen et al. 2000). The third group (group C) is formed by *B. quintana* and *B. henselae*; the cluster is supported by bootstrap values >90 % with trees derived from *gltA* and *groEL* genes. Studies indicate that *B. henselae* and *B. quintana* are two species closely related; these studies were based on the high degree of conservation between *ftsZ* homologues, *ribC* proteins, and 17 kDa antigens (Bereswill et al. 1999; Kelly et al. 1998; Sweger et al. 2000). *Bartonella koehlerae* is a species more closely related to *B. henselae* based on a comparison of *16S rDNA* and *gltA* gene sequences.

Group D is formed by *Bartonella* species associated with rats and mice captured in Peru and the USA. This phylogenetic group was determined from the analysis of the *gltA* gene (Birtles et al. 1999; Kosoy et al. 1997; Ellis et al. 1999b; Birtles and Raoult 1996). Group D may be divided into two subgroups, one of which includes *Bartonella* species associated with mice, and the second subgroup includes species associated with rats. Phylogenetic studies have revealed a strong association between *B. vinsonii* subsp. *vinsonii* and *B. vinsonii* subsp. *berkhoffii* (group E) with sequences of *ITS*, *groEL*, and *gltA* genes, but not for the sequences of the *16S rDNA* gene (Kordick et al. 1996). The position of this *B. vinsonii* cluster relative to other *Bartonella* species remains unclear. The sixth group (F) is obtained with *16S rRNA*, *ITS*, *gltA*, *and groEL* gene sequence comparisons. Like group D, it contains rodent-associated organisms. *B. elizabethae*, *B. grahamii*, and *B. tribocorum* cluster into this clade, together with uncharacterized strains found in mice and rats from the USA, Peru, and Europe (Birtles et al. 1999; Ellis et al. 1999a; Birtles et al. 1994). Within group F, *B. elizabethae* clusters with isolates from Peruvian rats in a separate subclade supported by *gltA*

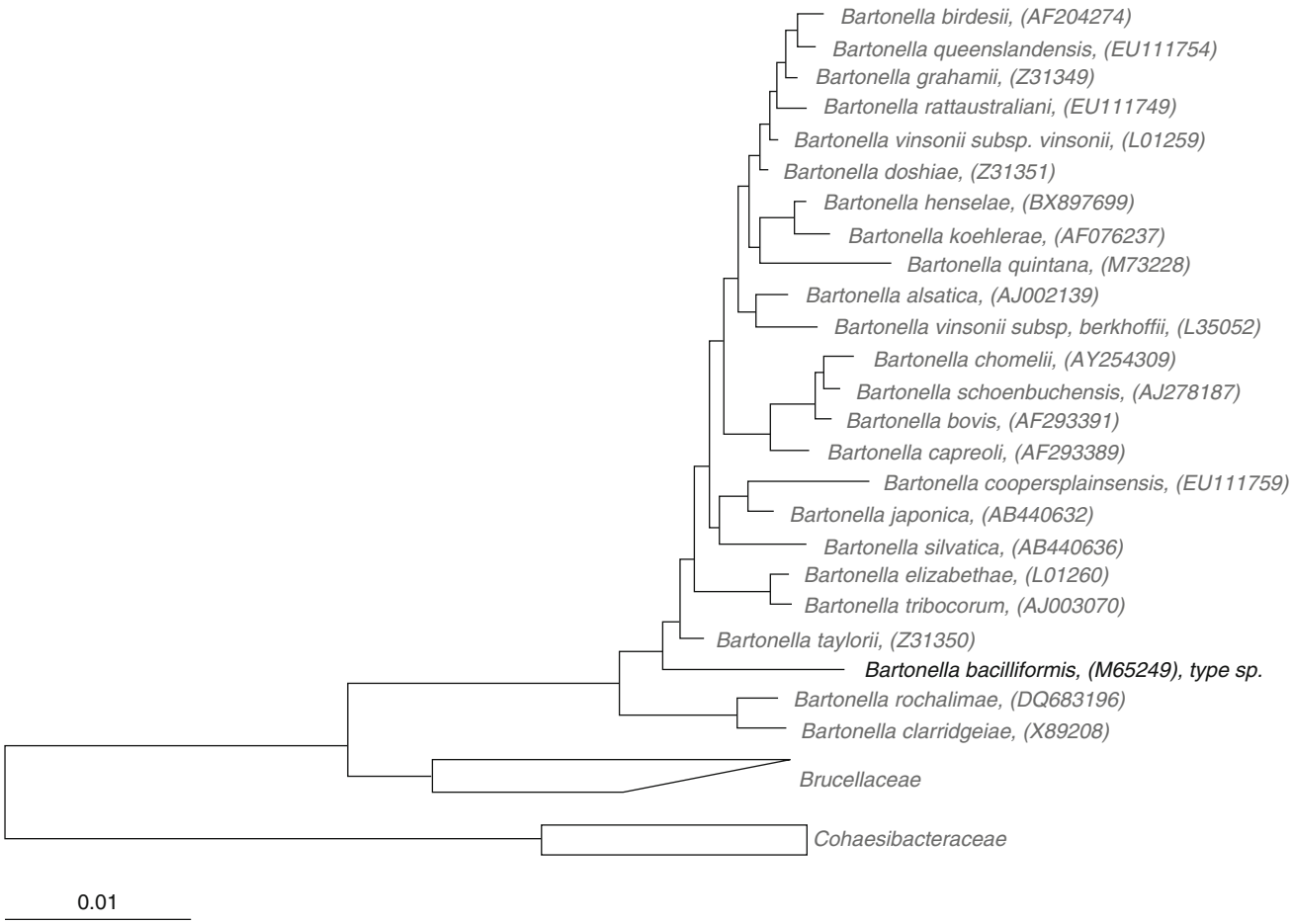

Fig. 3.1
Phylogenetic relationships of the organisms belong to *Bartonellaceae* family in relation of *Cohaesibacteraceae* and *Brucellaceae* families, based on the DNA sequences. The numbers of GenBank access are positioned right of the specie name

analysis. The taxonomic position of other species and strains, mainly *B. taylorii*, *B. doshiae*, and *B. alsatica*, could not be consistently determined on the basis of genetic sequence data available.

The phylogeny of *Bartonella* species remains confusing, and it is likely that this may be exacerbated as new isolates of *Bartonella* continue to be made in large numbers from mammal populations (Chang et al. 2000; Birtles and Raoult 1996; Birtles et al. 2000). Although 16S rDNA-based phylogenetic studies give little information on evolutionary processes occurring within the genus *Bartonella*, studies using comparisons of *ITS*, *gltA*, *and groEL* sequences have enabled six distinct evolutionary clades to be identified within the genus. As multiple gene data sets have been shown to be useful in determining the precise molecular relationships between genera and species (Marston et al. 1999), comparisons of the sequences of the ribC, the ftsZ, and the 17 kDa antigen genes might be used to improve the understanding of the evolutionary process. This will specifically help in the identification of lateral gene transfer, which occurs more commonly in intracellular pathogens (Doolittle 1999).

The phylogenetic data available for *Bartonella* species suggest that a phenomenon of coevolution occurred between the bacteria and their reservoir hosts. This host specificity may be partially offset by concomitant vector specificity. As a consequence of the restricted geographical distribution of their hosts and vectors, geographically distant *Bartonella* species may have evolved separately. It would be interesting to combine genetic classifications with phenotypic data sets such as duration of experimental bacteremia (Lucey et al. 1992) to better understand the natural history of *Bartonella* species and their association with specific mammal hosts and to confirm the apparent divisions identified within the genus.

Genome Analysis

According to the genome project database of the NCBI (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi), eight genomes of *Bartonella* species have been sequenced completely: *Bartonella quintana* RM-11, *Bartonella bacilliformis* KC583, *Bartonella henselae* str. Houston-1, *Bartonella vinsonii* subsp. *berkhoffii* str. Winnie, *Bartonella grahamii* as4aup, *Bartonella clarridgeiae* 73,

Table 3.2

***Bartonella* species with completely sequenced genome**

Bacteria	Genome	G + C %	Proteins	Genes	Reference/GenBank
Bartonella quintana RM-11	One chromosome (1,59 Mb)	38.8	1,203	1,251	NC_005955.1
Bartonella bacilliformis KC583	One chromosome (1,45 Mb)	38.2	1,283	1,374	NC_008783.1
Bartonella henselae str. Houston-1	One chromosome (1,93 Mb)	38.2	1,488	1,665	NC_005956.1
Bartonella vinsonii subsp. *berkhoffii* str. Winnie	One chromosome (1,80 Mb)	38.6	1,434	1,484	NC_020301.1
Bartonella grahamii as4aup	One chromosome (2,37 Mb) and one plasmid (pBGR3-28.19Kb)	38.1	1,768	2,053	NC_012846.1 NC_012847.1 (plasmid)
Bartonella clarridgeiae 73	One chromosome (1,52 Mb)	35.7	1,323	1,445	NC_014932.1
Bartonella tribocorum CIP 105,476	One chromosome (1,64 Mb) and one plasmid (pBT01-23.34Kb)	38.9	2,087	2,166	NC_010161.1 NC_010160.1 (plasmid)
Bartonella australis Aust/NH1	One chromosome (1,60 Mb)	41.8	1,264	1,313	NC_020300.1

Source: http://www.ncbi.nlm.nih.gov/genome/?term=Bartonella

Bartonella tribocorum CIP 105476, and *Bartonella australis* Aust/NH1 (Table 3.2). In other 14 species, the genome was partially sequenced: *Bartonella birtlesii* LL-WM9, *Bartonella elizabethae* ATCC 49927, *Bartonella bovis* 91–4, *Bartonella tamiae* Th239, *Bartonella doshiae* ATCC 700133, *Bartonella washoensis* 085–0475, *Bartonella schoenbuchensis* m07a, *Bartonella rochalimae* ATCCBAA-1498, *Bartonella queenslandensis* AUST/NH15, *Bartonella rattaustraliani* AUST/NH4, *Bartonella koehlerae* C-29, *Bartonella alsatica* IBS 382, *Bartonella taylorii* 8TBB, *Bartonella melophagi* K-2C, and *Bartonella rattimassiliensis* 15908. Only the *B. grahamii* and *B. tribocorum* species have plasmids.

Seubert et al. (2003a) was first to isolate and molecularly characterize the pBGR1, the first native plasmid isolated from the genus *Bartonella*. Cloning and sequencing revealed a 2725-bp cryptic plasmid comprising two open reading frames of considerable length, which were designated rep and mob. The regions containing rep and mob are separated by 140-bp inverted repeat sequences and display a difference in G+C content from one another. A 1435-bp SacI-BclI fragment containing the rep gene is sufficient to mediate replication in the species *B. henselae* and *B. tribocorum*.

Comparative genomics studies revealed that *Bartonella* species related to rodents have a higher of copy number of genes related with factors linked to adaptability to their host when compared with *Bartonella* species human-specific. These gene clusters are located in a highly dynamic region of 461 kb. A microarray designed for the *B. grahamii* genome was utilized to observe a massive, putatively phage-derived run-off replication of this region (Berglund et al. 2009). These authors identified a novel gene transfer agent, which packages the bacterial genome, with an overrepresentation of the amplified DNA, in 14 kb pieces. This is the first observation associating the products of run-off replication with a gene transfer agent. Because of the high concentration of gene clusters for host-adaptation proteins in the amplified region and since the genes encoding the gene transfer agent and the phage origin are well conserved in *Bartonella* (Berglund et al. 2009). There is the hypothesis that these systems are driven by selection. Berglund et al. (2009) proposed that the coupling of run-off replication with gene transfer agents promotes diversification and rapid spread of host-adaptability factors, facilitating host shifts in *Bartonella*.

The complete genome sequence of *B. tribocorum* was determined by shotgun sequencing. It was functionally identified 97 pathogenicity genes by signature-tagged mutagenesis (Saenz et al. 2007). In core genome (1,097 genes) 81 pathogenicity genes were identified which belong to the radiating lineage inferred from genome comparison of *B. tribocorum*, *B. henselae*, and *B. quintana*. Sixty-six pathogenicity genes are present in *B. bacilliformis*, and one has been lost by deletion. The 14 pathogenicity genes specific for the radiating lineage encode two laterally acquired type IV secretion systems, suggesting that these systems have a role in host adaptability.

Genomic comparison studies were performed between *B. quintana* and *B. henselae*, where a high degree of similarity was observed. The main differences were observed in genomic islands encoding filamentous hemagglutinin that are specific to *B. henselae*. Genomic reduction observed in *B. quintana* reminds that found in *Rickettsia prowazekii* (Alsmark et al. 2004). Both genomes are reduced versions of chromosome I from the highly related pathogen *Brucella melitensis*. Flanked by two rRNA operons is a segment with similarity to genes located on chromosome II of *B. melitensis*, suggesting that it was acquired by integration of megareplicon DNA in a common ancestor of the two *Bartonella* species. Comparisons of the vector–host ecology of these organisms suggest that the utilization of host-restricted vectors is associated with accelerated rates of genome degradation and may explain why human pathogens transmitted by specialist vectors are outnumbered by zoonotic agents, which use vectors of broad host ranges (Alsmark et al. 2004).

The experimentally characterized genes in *B. quintana*, *B. henselae*, and *B. tribocorum* are those coding for the type IV (VirB, Trw) and type V (adhesins, autotransporters) secretion systems. The VirB system translocates effector proteins into the cytoplasm of endothelial cells, thereby mediating antiapoptotic activity and angiogenic reprogramming (Schulein and Dehio 2002; Schulein et al. 2005; Schmid et al. 2006), whereas the Trw system is required for invasion of erythrocytes (Seubert et al. 2003b) and displays diversifying selection on the pilus proteins (Nystedt et al. 2008). The *Bartonella* adhesin (BadA) is involved in binding to endothelial cells, autoaggregation, and mediates a proangiogenic response in *B. henselae* (Kaiser et al. 2008; Riess et al. 2004). The homologous proteins in *B. quintana* have essentially the same function and are required for bloodstream infection but have been termed Vomps (variably expressed outer membrane proteins) since they have been shown to undergo sequence and expression variation in a monkey model system (Zhang et al. 2004; Schulte et al. 2006; Mackichan et al. 2008). Less is known about the autotransporters; however, they are known to be upregulated during infection of endothelial cells (Seubert et al. 2002) and may be involved in adhesion to host cells (Litwin et al. 2007).

Several of the genes for secretion systems are located in a dynamic region of the genome, up to several hundred kb in size, which is thought to have originated by the integration of an auxiliary replicon (Alsmark et al. 2004). DNA from this region was highly amplified in *B. henselae* upon prolonged growth (Lindroos et al. 2006). Microarray hybridizations suggested bidirectional replication starting from a region encoding a few phage genes (Lindroos et al. 2006). A similar phenomenon has also been observed in lambdoid phages of Salmonella (Frye et al. 2005) and has been termed escape replication or run-off replication. It has been suggested to arise from defects in prophage excision (Frye et al. 2005; Fukasawa et al. 1978). Despite the replication of large amounts of the bacterial chromosome, the prophage that induced escape replication in Salmonella produced phage particles that only contained phage DNA (Frye et al. 2005).

Bacteriophage particles have been identified in several species of *Bartonella* (Umemori et al. 1992; Anderson et al. 1994; Barbian and Minnick 2000; Chenoweth et al. 2004b; Maggi and Breitschwerdt 2005), and growth experiments have suggested phage induction and lysis of *B. henselae* cells as they enter stationary phase (Chenoweth et al. 2004a). While the morphology of the bacteriophage particles differed slightly—phages from *B. bacilliformis* and *B. vinsonii* ssp. *berkhoffii* were tailed, whereas those from *B. henselae* and *B. Quintana* lacked tails—they all contained 14 kb linear, double-stranded DNA, packaged in a round to icosahedral head of 40–80 nm in size (Anderson et al. 1994; Barbian and Minnick 2000; Maggi and Breitschwerdt 2005). No one has been able to fully characterize the 14 kb band; however, it has been suggested to consist of random or heterogeneous bacterial DNA (Anderson et al. 1994; Barbian and Minnick 2000; Maggi and Breitschwerdt 2005).

Alsmark et al. (2004) identified 128 pseudogenes and extensively degraded gene sequences in *B. henselae* (excluding 23 tandem duplication remnants), as compared with 175 fragmented genes in *B. quintana*. As many as 67 pseudogenes in *B. quintana* are represented by intact orthologs in *B. henselae*, where they code for proteins with basic metabolic functions. In contrast, only six *B. henselae* pseudogenes are present as intact genes in *B. quintana*, a few of which are found in the operon *fatBCD* that codes for proteins involved in iron transport. As many as 57 and 75 pseudogenes in *B. quintana* and *B. henselae* represent degraded fragments of genes present in the phage and the islands.

Phenotypic Analyses

The main features of members of genus *Bartonella* are listed in *Table 3.3*, as well as morphological, physiological and molecular characteristics differentiating species within the genus. As *Bartonella* are phenotypically similar, characterization and differentiation of species is extremely reliant on genotypic and phylogenetic methods.

Bartonella, Strong et al. 1915. Emend. Brenner et al. 1993 Emend. Birtles et al. 1995

Bar.to.nel'Ia. M.L. dim. ending *-ella;* M.L. fem. dim. n. *Bartonella* named after Alberto L. Barton, who described these organisms in 1909, after studying the agent of Carrion's disease.

Cells are gram-negative, slightly curved bacillus (0.5–0.6 × 1.0 μm). They are not acid fast and stain satisfactorily with Romanowsky or Giemsa stain. Warthin–Starry silver staining of fixed tissue sections reveals bacilli in clusters. They may be seen in stained blood films appearing as rounded or ellipsoidal forms or as slender, straight, curved, or bent rods, occurring singly or in groups. They characteristically occur in chains of several segmenting organisms, sometimes swollen at one or both ends and frequently beaded. In the tissues, they are situated within the cytoplasm of endothelial cells as isolated elements or are grouped in rounded masses. Intraerythrocytic forms occur in the blood of humans, felines, small rodents, birds, fish, and other animals. In cultures, the cells may be very autoadherent. Some species are motile since they possess unipolar flagella. Electron microscope examination showed multiple unipolar flagella in species such as *B. capreoli* and *B. rochalimae*. In *B. bacilliformis*, motility is achieved by means of 1–10 polar flagella, although a small number of cells appear to have subpolar or lateral flagella. The presence of pili is associated with the marked adherence. They are highly fastidious organisms and may be cultivated on media enriched with blood components in the presence of air or 5 % CO_2. The optimal incubation temperature varies from 20 °C to 37 °C after prolonged incubation (7–21 days). Optimum pH for growth is between pH 7.1 and 7.8. No growth on MacConkey agar nutrient agars. Succinate, pyruvate, and glutamine or glutamate, but not glucose, can be utilized as sources of energy for *B. quintana* and *B. bacilliformis*. Colony morphology varies from clear to smooth white and rough tan. Several species embed in the agar.

Table 3.3

Differential characteristics of *Bartonella* species and subspecies

Characteristic	*B.acomydis*	*B.alsatica*	*B.bacilliformis*	*B.birtlesii*	*B.bovis*	*B.callosciuri*	*B.capreoli*	*B.chomelii*	*B.clarridgeiae*	*B.coopersplainsensis*	*B.doshiae*	*B.elizabethae*	*B.grahamii*	*B.henselae*	*B.jaculi*	*B.japonica*	*B.koehlerae*	*B.pachyuromydis*	*B.peromysci*	*B.quintana*	*B.queenslandensis*	*B.rattaustraliani*	*B.rochalimae*	*B.schoenbuchii*	*B.silvatica*	*B.taylorii*	*B.tribocorum*	*B.vinsonii* subsp. *vinsonii*	*B.vinsonii* subsp. *arupensis*	*B.vinsonii* subsp. *berkhoffi*
Optimal temp, °C	35	35	25–30	35	35	35	35	35	35–37	37	35–37	35–37	35–37	35–37	35	35	35	35	20–28	35–37	37	37	35	37	35	35–37	35	35–37	35–37	35–37
Growth in nutrient broth		–	–						–		–	–	–	–			–		–	–						–	–	–	–	–
Growth on heart infusion agar with X factor	+					+						+		–	+		–	+		v								v	+	–
Hemolysis		–	–	–					–			v		–					–	–								–	–	–
Growth in < 10 days	–	–	+	–	–	–	–	–	+	–	v	+	+	v	–		–	–	+	+	+	+	–	+		+	–	+	+	+
Oxidase	–	–	–	–	–	–	–	–	–			–		–	–	–		–		v				–	–		–	v	–	–
Catalase	–	–	+	–	–	–	–	–	–			–		v	–	–		–		–				–	–		–	v	–	v
Nitrate reduction	–	–	–			–			–			–		–	–			–		–							–	–	–	–
Indole	–	–	–			–			–			–		–	–			–		–				–			–	–	–	–
Urease	–	–	–	–	–	–	–		–			–		–	–	–		–		–				–	–		–	–	–	–
Glucose oxidation or fermentation		–	–						–		–	–	–	–			–		–	–						–	–	–	–	–
Voges–Proskauer		–	–	–	–		–	–	–		+	–	+	–						–						+	–	–		
p-nitrophenyl-β-D-galactopyranosidase		–	–			–			–			–		–	–		–			–							–	–	–	–
p-nitrophenyl-α-D-galactopyranosidase		–	–			–			–		–	–	–	–	–		–			–						–	–	–	–	–
bis p-nitrophenyl-phosphatase	+	–	+	+	+	–	+					+		+	–	+	+	+		v					+		–	+	+	+
p-nitrophenyl-*N*-acetyl-β-D-glucosaminidase	–	–	–	–	+	–	+		–		–	–	–	–	–	–	–	–	–	–					–	–	–	–	–	–
p-nitrophenyl-α-D-glucopyranosidase	–	–	–			–			–		–	–	–	–	–		–	–		–						–	–	–	–	–
p-nitrophenyl-β-D-glucopyranosidase	–	–	–			–			–		–	–	–	–	–		–	–		–						–	–	–	–	–
p-nitrophenyl phosphatase	–	–				–					–	–	–	–	–		–	–	–	–				–		–	–	–	–	–

Table 3.3 (continued)

Characteristic	*B.acomydis*	*B.alsatica*	*B.bacilliformis*	*B.bittlesii*	*B.bovis*	*B.callosciuri*	*B.capreoli*	*B.chomelii*	*B.clarridgeiae*	*B.coopersplainsensis*	*B.doshiae*	*B.elizabethae*	*B.grahamii*	*B.henselae*	*B.jaculi*	*B.japonica*	*B.koehlerae*	*B.pachyuromydis*	*B.peromysci*	*B.quintana*	*B.queenslandensis*	*B.rattaustraliani*	*B.rochalimae*	*B.schoenbuchii*	*B.silvatica*	*B.taylorii*	*B.tribocorum*	*B.vinsonii* subsp. *vinsonii*	*B.vinsonii* subsp. *arupensis*	*B.vinsonii* subsp. *berkhoffii*
p-nitrophenyl-α-L-fucopyranosidase																														
p-nitrophenyl-α-D-mannopyranosidase																														
L-leucine-β-naphthyl-amidase	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+		+					+			+	+	+
DL-methionine-β-naphthyl-amidase	+	+	+	+	+	+	+					+		+	+	+	+	+		+								m		
L-lysine-β-naphthyl-amidase (alkaline)	+	+	+	+	+	+	+	+				+		+	+	+	+	+		+					+			+	+	+
L-lysine-β-naphthyl-amidase (acidic)	+	+	+	+	+	+	+	+				w		+	+	+		+												
Glycylglycine-β-naphthyl-amidase	+	+	+	+		+						+		+	+		+			+								+	+	+
Glycine-β-naphthyl-amidase	+	+	+	+	+	+	+	+	+			+		+	+	+	+	+		+				+	+			+	+	+
L-proline-β-naphthyl-amidase	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+		+					+			v		
L-arginine-β-naphthyl-amidase	+	+	+	+	+	+	+	+	+			+		+	+	+	+	+		+				+				+	+	
L-pyrrolidonyl-β-naphthyl-amidase																														

L-tryptophan-β-naphthyl-amidase	+	+	+	+	+	+	+					+		+	+	+	+	+		+					+		+	+	+	+
3-indoxyl phosphatase		–	–									–		–			–			–							–	–	–	–
Flagella	–	–	+	–	–	–	+	+	+	–		–		–	–	–	–	–	–	–	–	–	+	+	–		–	–	–	–
Twitching motility												–		+			–			+								–	–	–
Major cellular fatty acids:																														
$C_{16:0}$			+						+			+		+						+				+				+	+	+
$C_{16:1\ \omega\ 7c}$			+																											
$C_{17:0}$												+																	+	+	
$C_{18:0}$									+					+						+				+				+	+	+
$C_{18:1\ \omega\ 7c}$			+						+			+		+						+				+				+	+	+
$C_{19:0\ cyclo}$																														
$C_{Br\text{-}19:1}$																														
Reactivity with fluorescent antibody to																														
B. bacilliformis			+								–		–	–						–						–		–		
B. doshiae			–								+	–	w	–						w						w		w		
B. elizabethae			–								–	+	–	–						–						–		–		
B. grahamii			–								w	w	+	w						w						w		w		
B. henselae			–						–		–	–	–	+						v						–		v	–	
B. quintana			–						–		–	–	–	–						+						–		–	–	
B. taylorii			–								w	–	–	w						w						+		w		
B. vinsonii			–								–	–	–	–						w						–		+		

Symbols: + positive, – negative, *w* weakly positive, *v* variable (only some isolates have the characteristic)

Cocultivation with an endothelial cell line can also be performed. They are usually catalase and oxidase negative and aerobic. They are typically inert biochemically, with no acid or gas production from amygdalin, L-arabinose, dextrin, dukitol, fructose, D-galactose, D-glucose, inulin, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, salicin, sucrose, trehalose, or D-xylose. However, *B. bovis* and *B. capreoli* are able to hydrolyze trehalose and have trypsin-like activity and glycyl arylamidase activity, but no pyrrolidonyl arylamidase activity, as *B. birtlessi*, *B. japonica*, and *B. silvatica* also have glycyl arylamidase activity, but no pyrrolidonyl arylamidase activity. Additionally, *B. doshiae* and *B. grahamii* are able to hydrolyze butyrin (albeit weakly), and *B. doshiae* exhibits pyrazinamidase activity. *B. tribocorum* presents ability to hydrolyze trypsin but not proline and tributyrin. Gelatin is not liquefied. Esculin is not hydrolyzed. H_2S is not detected with lead acetate. Some species are Voges–Proskauer positive (*B. doshiae*, *B. grahamii*, *B. taylorii*). *B. henselae*, *B. coopersplainsensis*, *B. rattaustraliani*, and *B. queenslandensis* are readily stained with Gimenez stain. *Bartonella* species contain greater than 50 % $C_{18:1}$ isoacids, 16–25 % $C_{18:0}$, and 16–22 % $C_{16:0}$, with minor amounts of $C_{13:1}$ and $C_{17:0}$ fatty acids. *B. henselae* lack cellular fatty acids $C_{15:0}$ and $C_{12:0}$, which are present in isolates of *B. vinsonii* and *B. bacilliformis*, respectively. *B. quintana* can be differentiated from most *B. henselae* isolates by amounts of $C_{18:0}$. *B. elizabethae* has cellular fatty acid composition similar to *B. vinsonii* and includes $C_{15:0}$ (not found in *B. henselae* and *B. quintana*) and larger amounts of $C_{17:0}$ (21 %), than *B. henselae* (3 %), *B. quintana* (1 %), and *B. vinsonii* (9 %). *B. elizabethae* also contains smaller amounts of cellular fatty acid $C_{16:0}$ than other species. DNA G+C content varies from 37 to 41 mol%. *Bartonella* species have been distinguished by the concatenated sequence of its 16SrRNA, *ftsZ*, *gltA*, *groEL*, *ribC*, *rpoB* genes, and ITS region, by the total protein profiles in SDS-PAGE, by the banding patterns in the ERIC-PCR, as well as by whole DNA hybridization and some species by the unique mammalian host.

The type species is *Bartonella bacilliformis* (Strong et al. 1913, 1915). As mentioned before, the genus contains 29 species and 3 subspecies, in which etymology, total DNA G+C content, type strain, and *GenBank* accession number are outlined below.

Bartonella acomydis, Sato et al. 2013

a.co'my.dis. N.L. gen. n. *acomydis* of *Acomys*, isolated from *Acomys russatus*.

The mol % G+C of the DNA is 37.2 (based on the *ftsY* gene sequence).

Type strain: KS2-1^T (=JCM 17706^T = KCTC 23907^T), KS7-1 (=JCM 17707 = KCTC 23908)

GenBank accession number (16S rRNA): AB602533

Bartonella alsatica, Heller et al. 1999

al.sa'ti.ca. L. adj. *alsaticus* from Alsace, the region in the eastern part of France near the Rhine River where the wild rabbits were trapped and where the strains were isolated and identified.

The mol % G+C of the DNA is 37 (capillary electrophoresis method).

Type strain: IBS 382^T (CIP 105477^T).

GenBank accession number (16S rRNA): AJ002139

Bartonella bacilliformis, Strong et al. 1913, Strong et al. 1915

ba.cil.li.for'mis. L. dim. n. *bacillus*, a small staff, rodlet; L. adj. suffix *-formis -is -e* (from L. n. *forma*, figure, shape, appearance)-like, in the shape of; N.L. fem. adj. *bacilliformis*, rod shaped.

The mol % G+C of the DNA is 39 (T_m).

Type strain: KC583 (=Herrer 020/F12,63 = ATCC 35685)

GenBank accession number (16S rRNA): M65249

Bartonella birtlesii, Bermond et al. 2000

birt.les'i.i. M.L. gen. n. *birtlesii* of Richard J. Birtles, whose studies have contributed to an improved understanding of the taxonomy of the *Bartonella* genus.

The mol % G+C of the DNA is not determined.

Type strain: IBS 325^T (=CIP 106294^T = CCUG 44360^T)

GenBank accession number (16S rRNA): AF204274

Bartonella bovis, Bermond et al. 2002 Non Donatien and Lestoquard 1934

Bo'vis. L. gen. n. *bovis* of the ox.

OBS: *Bartonella weissi* or *Bartonella weissii* have no standing in nomenclature, and this species must be designated as *Bartonella bovis* Bermond et al. 2002.

The mol % G+C of the DNA is 38.

Type strain: 91-4^T (=CIP 106692^T = CCUG 43828^T)

GenBank accession number (16S rRNA): AF293391 and AF293394 (gltA)

Bartonella callosciuri, Sato et al. 2013

cal.lo.sci'u.ri. N.L. gen. n. *callosciuri* of *Callosciurus*, isolated from *Callosciurus notatus*.

The mol % G+C of the DNA is 35.5 (based on the *ftsY* gene sequence).

Type strain: BR11-1^T (=JCM 17709 T = KCTC 23909 T)

GenBank accession number (16S rRNA): AB602530

Bartonella capreoli, Bermond et al. 2002

ca.pre.o'li. L. gen. n. *capreoli* of the roe deer.

The mol % G+C of the DNA is 38.

Type strain: IBS 193^T (=CIP 106691^T = CCUG 43827^T)

GenBank accession number (16S rRNA): AF293389 and AF293392 (gltA)

Bartonella chomelii, Maillard et al. 2004

cho.mel'i.i. N.L. gen. n. *chomelii* in honor of Bruno B. Chomel, who was the first to experimentally demonstrate the transmission of *B. henselae* by fleas in cats and to isolate *B. bovis* from domestic cattle. His studies on *Bartonella* infection in animals contributed to an improved understanding of epidemiology and vectors of *Bartonella*-associated disease in animals.

The mol % G+C of the DNA is not determined.
Type strain: A828^T(CIP 107869^T)
GenBank accession number (16S rRNA): AY254309

Bartonella clarridgeiae, Lawson and Collins 1996

clar.ridge'i.a.e. M.L. fem. adj. *clarridgeiae* named in honor of Jill E. Clarridge III, the microbiologist who first isolated the organism in Houston, Texas.

The mol % G+C of the DNA is not determined.
Type strain: Houston-2 cat (ATCC 51734)
GenBank accession number (16S rRNA): X89208

Bartonella coopersplainsensis, Gundi et al. 2009

coo.pers.plain.sen'sis. N.L. fem. adj. *coopersplainsensis* pertaining to Coopers Plains, Queensland, the name of the area where the rat was trapped from which the type strain was isolated.

The mol % G+C of the DNA is not determined.
Type strain: AUST/NH20^T (=CIP 109064^T = CCUG 52174^T = CSUR B619^T)
GenBank accession number (16S rRNA): EU111759

Bartonella doshiae, Birtles et al. 1995

do'shi.ae. N. L. gen. n. *doshiae*, of Doshi, named after Nivedita Doshi, whose technical skills have been central to the work on *Legionella* and *Bartonella* at the Central Public Health Laboratory.

The mol % G+C of the DNA is 41(T_m).
Type strain: R18 (=ATCC 7000133 = NCTC 12862)
GenBank accession number (16S rRNA): Z31351

Bartonella elizabethae (Daly et al. 1993), Brenner et al. 1993

e.liz'a.beth.e.a. N.L. fem. adj. *elizabethae*, named after Saint Elizabeth's Hospital, Brighton, MA., where the organism was isolated.

The mol % G+C of the DNA is 41 (T_m).
Type strain: F9251 (=B91-002005 = ATCC 49927)
GenBank accession number (16S rRNA): LO1260

Bartonella grahamii, Birtles et al. 1995

gra.ham'i.i. N. L. gen. n. *grahamii*, of Graham, named after G. S. Graham-Smith, who observed similar organisms, subsequently named *Grahamella*, in the blood of moles in 1905.

The mol % G+C of the DNA is 40 (T_m).
Type strain: V2 (=ATCC 7000132 = NCTC 12860)
GenBank accession number (16S rRNA): Z31349

Bartonella henselae (Regnery et al. 1992), Brenner et al. 1993

hen'.sel.a.e. N. L. gen. n. *henselae* is named in honor of Diane M. Hensel, who isolated many of the original strains detected in bacteremic patients from Oklahoma.

The mol % G+C of the DNA is 41 (T_m).
Type strain: Houston 1 (=G5436 = ATCC 49882)
GenBank accession number (16S rRNA): M73229

Bartonella jaculi, Sato et al. 2013

ja'cu.li. N.L. gen. n. *jaculi* of *Jaculus*, isolated from *Jaculus orientalis*.

The mol % G+C of the DNA is 37.4 (based on the *ftsY* gene sequence).
Type strain: OY2-1^T (=JCM 17712^T = KCTC 23655^T) and OY5-1 (=JCM 17713 = KCTC 23656)
GenBank accession number (16S rRNA): AB602527

Bartonella japonica, Inoue et al. 2010

ja.po'ni.ca. N.L. fem. adj. *japonica* of Japan, where the host rodent, the small Japanese field mouse (*Apodemus argenteus*), from which the strain was isolated.

The mol % G+C of the DNA is 40.1.
Type strain: Fuji 18-1^T (=JCM 15567^T = CIP 109861^T)
GenBank accession number (16S rRNA): AB440632

Bartonella koehlerae, Droz et al. 2000

koeh'ler. ae. N. L. fem. adj. *koehlerae*, in honor of Jane E. Koehler, who was the first to isolate *Bartonella* species from bacillary angiomatosis lesions and whose studies of *B. quintana* and *B. henselae* isolates from human immunodeficiency virus-infected patients have contributed to an improved understanding of the molecular epidemiology, reservoirs, and vectors of *Bartonella*-associated disease in humans.

The mol % G+C of the DNA is not determined.
Type strain: C-29 (=ATCC 700693)
GenBank accession number (16S rRNA): AF076237

Bartonella pachyuromydis, Sato et al. 2013

pa.chy.u.ro'my.dis. N.L. gen. n. *pachyuromydis of Pachyuromys*, isolated from *Pachyuromys duprasi.*

The mol % G+C of the DNA is 35.7 (based on the *ftsY* gene sequence).
Type strain: FN15-2^T (=JCM 17714 T = KCTC 23657 T) and FN18-1 (=JCM 17715 = KCTC 23911)
GenBank accession number (16S rRNA): AB602531

Bartonella peromysci (Ristic and Kreier 1984), Birtles et al. 1995

pe.ro.mys' ci. M.L. gen. n. *peromysci*, of *Peromyscus* a genus of mice.

The mol % G+C of the DNA is unknown.
Type strain: No type strain available.
GenBank accession number (16S rRNA):

Bartonella queenslandensis, Gundi et al. 2009

queens.lan.den'sis. N.L. fem. adj. *queenslandensis* referring to Queensland, the Australian state where the rats were trapped from which the first strains were isolated.

The mol % G+C of the DNA is not determined.
Type strain: AUS^T/NH12^T (=CIP 109057^T = CCUG 52167^T = CSUR B617^T), AUST/NH5 (=CIP 109052 = CCUG 52162 = CSUR B614), AUST/NH8 (=CIP 109053 = CCUG 52163 = CSUR B615), AUST/NH11 (=CIP 109056 = CCUG 52166 = CSUR B616), and AUST/NH15 (=CIP 109060 = CCUG 52170 = CSUR B618).
GenBank accession number (16S rRNA): EU111754

Bartonella quintana (Schmincke 1917), Brenner et al. 1993

quin.ta' na. M.L. fem. adj. *quintana* fifth, referring to 5-day fever and the clinical disease produced by the species.

The mol % G+C of the DNA is 40 (T_m).
Type strain: ATCC VR-358 (=CIP 107027)
GenBank accession number (16S rRNA): M73228

Bartonella rattaustraliani, Gundi et al. 2009

ratt.aus.tra.li.a'ni. N.L. n. *Rattus* zoological name of a genus of rat; N.L. adj. *australianus* Australian; N.L. gen. n. *rattaustraliani* of an Australian rat, from which the first strains were isolated.

The mol % G+C of the DNA is not determined.
Type strain: AUST/NH4^T (=CIP 109051^T = CCUG 52161^T = SUR B609^T). Other isolates are AUST/NH9 (=CIP 109055 = CCUG 52164 = CSUR B610), AUST/NH10 (=CIP 109054 = CCUG 52165 = CSUR B611), AUST/NH14 (=CIP 109059 = CCUG 52169 = CSUR B612), and AUST/NH18 (=CIP 109063 = CCUG 52173 = CSUR B613).
GenBank accession number (16S rRNA): EU111749

Bartonella rochalimae, Eremeeva et al. 2012

ro.cha.li.ma'e. N.L. gen. masc. n. *rochalimae*, of Rochalima, named in honor of Henrique da Rocha Lima, an early Brazilian investigator of the etiology of rickettsial diseases.

The mol % G+C of the DNA is not determined.
Type strain: BMGH (=ATCC BAA-1498 = DSM 25050)
GenBank accession number (16S rRNA): DQ683196

Bartonella schoenbuchensis, Dehio et al. 2001

schoen.buch'i.i. N.L. gen. n. *schoenbuchii* from Schönbuch, a nature park near Tubingen in southwest Germany where most of the roe deer analyzed were shot.

The mol % G+C of the DNA is not determined.
Type strain: R1^T NCTC 13165^T and DSM 13525^T
GenBank accession number (16S rRNA): AJ278187

Bartonella silvatica, Inoue et al. 2010

sil.va'ti.ca. L. fem. adj. *silvatica* of the forest where the host rodent, the large Japanese field mouse (*Apodemus speciosus*), from which the strain was isolated, was captured.

The mol % G+C of the DNA is 40.4.
Type strain: Fuji 23-1^T (=JCM 15566^T = CIP 109862^T)
GenBank accession number (16S rRNA): AB440636

Bartonella talpae (Ristic and Kreier 1984), Birtles et al. 1995

tal' pae. M.L. gen. n. *talpae* of *Taipa*; M.L. fem. n. a genus of moles.

The mol % G+C of the DNA is unknown.
Type strain: No type strain available.
GenBank accession number (16S rRNA): Not available

Bartonella taylorii, Birtles et al. 1995

tay.lor'i.i. N. L. gen. n. *taylorii*, of Taylor, named after A. G. Taylor, whose foresight led various microbiologic studies at the Central Public Health Laboratory.

The mol % G+C of the DNA is 41 (T_m).

Type strain: M6 (=NCTC 12861)
GenBank accession number (16S rRNA): Z31350

Bartonella tribocorum, Heller et al. 1998

tri.bo.co'rum. L. n. gen. pl. *Triboci* named after the tribes mentioned by Caesar (51 BC) in his *Commentarii de Bello Gallico*, which were living in the region near the Rhine River in the eastern France. Wild rats, from which the two strains were isolated, were trapped there.

The mol % G+C of the DNA is 38 (capillary electrophoresis).
Type strain: IBS 506^T (=CIP 105476^T)
GenBank accession number (16S rRNA): AJ003070

Bartonella vinsonii, (Weiss and Dasch 1982), Brenner et al. 1993, Kordick et al. 1996

vin.so'ni.i. N.L. gen. n. *vinsonii*, of Vinson, named in honor of J. William Vinson who, with Henry S. Fuller, originally demonstrated that *Bartonella vinsonii* subsp. *vinsonii* (*Rochalimaea vinsonii*) could be grown on blood agar.

Bartonella vinsonii subsp. *vinsonii* Weiss and Dasch 1982; Kordick et al. 1996

The mol % G+C of the DNA is 41(T_m).
Type strain: ATCC VR-152 (strain Baker = CCUG 30453 = CIP 103738)
GenBank accession number (16S rRNA): L01259

Bartonella vinsonii subsp. *arupensis*, Welch et al. 2000

a.rup.en.'sis. N.L. fem. adj. *arupensis*, from Associated Regional and University Pathologists, Inc., in Salt Lake City, Utah, the laboratory where the type strain was initially characterized.

The mol % G+C of the DNA is not determined.
Type strain: OK 94–513 (5 = ATCC 700727)
GenBank accession number (16S rRNA): U71322

Bartonella vinsonii subsp. *berkhoffii*, Kordick et al. 1996

berk.hof'fi.i. N. L. gen. n. *berkhoffii*, of Berkhoff, in honor of Herman A. Berkhoff, a veterinary microbiologist whose research encompasses a wide spectrum of veterinary pathogens and who contributed to the study of *Bartonella* infection in domestic animals that led to the recognition of this subspecies.

The mol % G+C of the DNA is not determined.
Type strain: 93-CO1 (=ATCC 51672)
GenBank accession number (16S rRNA): L35052

Isolation, Enrichment, and Maintenance Procedures

The specimen sources of most isolates of *Bartonella* are blood and tissue (e.g., liver, spleen, lymph node, and skin). Specimens collected early in the clinical course are preferred, especially those obtained from lymph nodes biopsy from patients with suspected of cat scratch disease. Late-stage, suppurative lymph nodes may not yield organisms. Tissue specimens should be homogenized and plated on culture medium. Lysis-centrifugation methods may be used to enhance recovery of these bacteria from blood specimens (Koehler et al. 1992; Lucey et al. 1992; Larson et al. 1994). However, lysis-centrifugation is said to be prone to contamination due to extensive manipulation, including centrifugation and specimen separation. If storage of the specimens is necessary, they should be kept frozen. The blood collected in tubes containing EDTA can be plated after 26 days at –65 °C with no loss of sensitivity (Brenner et al. 1997). Freeze-thawing of a blood sample prior to direct plating has also been used (Varela et al. 1969; Regnery et al. 1992; Larson et al. 1994; Maggi et al. 2005a, b) and may enhance recovery, especially when blood is collected with heparin. Since *Bartonella* produce little or no CO_2 during growth, detection of these bacteria in automated blood culture systems (e.g., BACTEC; Bacti/Albert, etc.) may be problematic. However, species have been isolated using BACTEC 460 blood culture bottles (Lucey et al. 1992; Daly et al. 1993; Larson et al. 1994), BACTEC NR-660 infrared CO_2 detection system (Spach et al. 1993; Larson et al. 1994), and Septi-Chek biphasic system (Lucey et al. 1992; Larson et al. 1994).

Member of the genus *Bartonella* spp. has been isolated by plating onto an appropriate media (including agar, semisolid media, and broth) or by cultivation in cell culture. Cell culture methods are performed with shell vials containing a human endothelia cell monolayer (e.g., ECV 304 cell line) or other cell types (e.g., Vero cells, HeLa cells, L292 cells) (Drancourt et al. 1995; Koehler et al. 1992; Raoult et al. 1994; Zbinden et al. 1995). Vero E6 and L-929 (mouse fibroblasts) mammalian cell lines, for example, have been successfully utilized to culture *Bartonella* species (Chmielewski et al. 2007). Previous works have suggested that under mammalian cell culture-based conditions, cell coculture is important for *B. quintana* growth (Battisti and Minnick 2008); however, when the medium is modified to incorporate insect cell culture-based medium, coculturing with mammalian cells is no longer needed. Lynch et al. (2011) compared three culturing methodologies for *B. henselae*, *B. elizabethae*, *B. quintana*, *and B. tamiae*: showing that combination of mammalian and insect cell culture-based media (MS10 medium) are able to achieve peak growth comparable to that achieved with the insect cell culture-based medium alone (S10 medium) at 35 °C but that at 37 °C the MS10 medium performed significantly better for both *B. tamiae* and *B. quintana*. Data suggest that mammalian cell coculture is not necessary for growth of laboratory-adapted Bartonella strains; however, the combination medium is still able to support mammalian cell culture, which may remain an important variable during initial isolation of Bartonella from biological samples. Bovine endothelial cell line (i.e., CPA cells, ATCC cell line # 207) has also been used to isolate with success *B. henselae* and *B. quintana* from biopsies of bacillary angiomatosis lesions (Koehler et al. 1992). In addition, *B. quintana* has been isolated from the blood using ECV 304 cells (a continuous human endothelial cell line) (Drancourt et al. 1995).

Broth-based systems generally tend to have lower sensitivity for the isolation of *Bartonella* species (Spach et al. 1995). However, broth-enrichment recovery of *B. henselae* from human and cat blood has also been performed with a defined medium containing RPMI 1640 (a tissue culture liquid medium) supplemented with hemin, amino acids, and pyruvate (Wong et al. 1995). Combining the subculture of blood culture broth into shell vials has been reported to increase sensitivity for recovery (La Scola and Raoult 1999). The alphaproteobacteria growth medium (BAPGM) has also been used to isolate *Bartonella* species (Welch et al. 1992; Houpikian and Raoult 2002; Eremeeva et al. 2007; Kosoy et al. 2008). This is a modified formulation of liquid medium designed to support insect cells and requires sheep blood supplementation. It should be mentioned that BAPGM followed by PCR might provide a complimentary diagnostic approach for the isolation and identification of potentially fastidious bacteria from the blood and tissue samples. Another modified insect cell culture-based medium (Schneider's medium) capable of supporting *B. henselae*, *B. quintana*, and B. *vinsonii* was also described, without the requirement of whole-blood supplementation (Riess et al. 2008).

Agar-based culture methods include culture on heart infusion agar containing 5 % horse or rabbit blood. Blood-supplemented BHI, trypticase soy, and Columbia agars and enriched chocolate agar may also support growth (Lappin et al. 2008). For optimal recovery, the medium should be as freshly as possible prepared. Didi et al. (2013) compared strategies for the identification of *B. henselae* and outlined that growth can be obtained on biphasic medium, tryptic soy broth, trypticase soy agar with 5 % sheep blood, and brain–heart infusion agar, although colony morphology varied. Selective medium are not available, and media containing antimicrobial agents should not be used (Koneman et al. 2006). Growth is dependent on humid (>40 %), microaerophilic conditions (5–10 % CO_2) at temperatures ranging for most species between 35 °C and 37 °C (Maurin et al. 1997; La Scola et al. 2001; Rolain et al. 2004). The fastidious nature of these organisms requires that precaution be taken to minimize delayed attempts of isolation. *Bartonella* species grow slowly on blood agar, and primary isolates appear after 12–14 days on average (Jacomo et al. 2002), although it has been reported that primary isolation can take up to 45 days (Maurin et al. 1994). Subcultured colonies have been found to appear after only 3–5 days (Jacomo et al. 2002). Most *Bartonella* species do not grow under anaerobic conditions, at temperatures of 25 or 42 °C, or in the absence of hemin and CO_2. *B. bacilliformis*, however, is exceptional, in that this species favors a lower growth temperature (25–28 °C) and does not require supplemental CO_2 for growth (Koneman et al. 2006).

On primary isolation, *Bartonella* species appear usually initially as white, or gray-whitish, small (average 1–2 mm in diameter) adherent colonies that vary in shape and size. Some strains of *B. henselae*, *B. quintana*, and *B. elizabethae* may pit the agar surface during growth. Colonies of most of the species are smooth, although *B. birtlessi* colonies are rough. Characteristically, *B. henselae* colonies are white, dry, adherent, "cauliflower-like," embedded in the agar, and morphologically heterogenous. With multiple passages, the colonies become less dry, less adherent, and larger. Also, colonies of *B. schoenbuchensis* appear in primary culture within 4–6 days as firm adherent, deeply invaginated, "cauliflower-like" colonies which upon passaging may switch to a more rapidly growing, shiny, and smooth colony phenotype (Dehio et al. 2001). Colonies of *B. elizabethae* resemble *B. henselae* except that weak or partial hemolysis may be noted around colonies growing on heart infusion agar with 5 % rabbit blood (Daly et al. 1993).

According to the Second Edition of *Bergey's Manual of Systematic Bacteriology*, viability of cultures can usually be maintained by passage on blood or chocolate agar at biweekly intervals. Cultures are best maintained during storage at −70 °C in a blood- or hemoglobin-containing medium. Long-term storage is best accomplished by lyophilization. Retrieval of viable cultures that have been lyophilized may be enhanced by suspending dried cells in a blood–broth mixture followed by culture on fresh blood agar media (Welch 2005).

B. acomydis—isolated from the blood of golden spiny mouse (*Acomys russatus*). All the animals were imported to Japan as pets from Egypt, Thailand, and the Netherlands. Growth occurred on heart infusion agar plates (Difco™, Difco Laboratories, Inc., subsidiary of Becton, Dickinson and Company) containing 5 % defibrinated rabbit blood (HIA) at 35 °C in a moist atmosphere under 5 % CO_2 after 14 days of incubation. Also cultured on rabbit blood–chocolate HIA plates for 14 days at 35 °C in a moist atmosphere under 5 % CO_2 with H_2O_2 on a glass slide (Sato et al. 2013).

B. alsatica—the type strain (IBS 382^T) was isolated from the blood of wild rabbit (*Oryctolagus cuniculus*) caught in warrens in Alsace, France. Growth occurred on Columbia agar base containing 5 % defibrinated rabbit blood after 10-day postinoculation, and culture was detectable in Bactec Peds Plus liquid medium supplemented with Bacto Fildes enrichment (Difco Laboratories, Inc., subsidiary of Becton, Dickinson and Company) after 4 weeks. Incubation was performed at 35 °C in a moist atmosphere containing 5 % CO_2 (Heller et al. 1999).

B. bacilliformis—on isolation, type strain KC583 grew slowly (3–5 days), without hemolysis, on heart infusion agar with 5 % rabbit blood. Growth is best at 25 °C, weak at 35 °C, and absent at 42 °C (Brenner et al. 1991).

B. birtlesii—was isolated from the blood of small mammals belonging to the genus *Apodemus* in France and the UK. Growth occurred on Columbia base agar plates containing 5 % fresh, defibrinated rabbit blood at 35 °C in a moist atmosphere containing 5 % CO_2. Primary culture of type strain IBS325^T was obtained after 10-day incubation and subcultured after 6-day culture in the same conditions (Bermond et al. 2000).

B. bovis* Bermond et al. *non Donatien and Lestoquard 1934—was isolated from the blood samples collected from domestic cows belonging to a herd of dairy cattle located in Bissy, 50 km south of Paris, France. For isolation, the blood samples were thawed before plating and centrifuged at 1,800 g for 60 min. After centrifugation, the pellets were plated onto Columbia base agar plates (Difco Laboratories, Inc., subsidiary

of Becton, Dickinson and Company) containing 5 % fresh defibrinated rabbit blood. The plates were incubated at 35 °C in a moist atmosphere containing 5 % CO_2. Primary culture of type strain 91-4^T was obtained after 10-day incubation and subcultured after 4-day culture in the same conditions (Bermond et al. 2002).

B. callosciuri—isolated from the blood of plantain squirrel (*Callosciurus notatus*). Animals were imported to Japan as pets from Egypt, Thailand, and the Netherlands. Growth occurred on heart infusion agar plates (Difco Laboratories, Inc., subsidiary of Becton, Dickinson and Company) containing 5 % defibrinated rabbit blood (HIA) at 35 °C in a moist atmosphere under 5 % CO_2 after 14 days of incubation. Also cultured on rabbit blood–chocolate HIA plates for 14 days at 35 °C in a moist atmosphere under 5 % CO_2 with H_2O_2 on a glass slide (Sato et al. 2013).

B. capreoli—isolated from the blood of wild roe deer (*Capreolus capreolus*) captured in Chize forest conservation hunting area (western France). For isolation, blood samples were centrifuged at 1,800 g for 60 min and the pellets plated onto Columbia base agar plates (Difco Laboratories, Inc., subsidiary of Becton, Dickinson and Company) containing 5 % fresh defibrinated rabbit blood. The plates were incubated at 35 °C in a moist atmosphere containing 5 % CO_2. Primary culture of type strain (IBS 193^T) was obtained after 10 days of incubation, while subculture was obtained after 6 days, at 35 °C in a moist atmosphere containing 5 % CO_2 (Bermond et al. 2002).

B. chomelii—was isolated from blood domestic cows in two regions in France (Loire-Atlantique and Nord departments). For isolation, blood samples were thawed, centrifuged at 1,800 g for 75 min, and then plated onto agar plates containing 5 % fresh defibrinated rabbit blood. Incubation was held at 35 °C in a moist atmosphere containing 5 % CO_2. The type strain A828^T and A12.90 isolate grew slowly, in which primary culture were obtained after 10 days incubation and subculture after 6 days culture. Although it grows on rabbit blood and sheep blood agar plates, a higher efficiency was observed on rabbit blood agar plates (Maillard et al. 2004).

B. clarridgeiae—was first isolated from a kitten which belonged to a patient with cat scratch disease and from whom *B. henselae* had been isolated (Lawson and Collins 1996).

B. coopersplainsensis—isolated from the blood of wild rat of the species *Rattus leucopus*, which were trapped in Queensland, Australia. Good growth was observed on Columbia agar with 5 % sheep blood in a 5 % CO_2 atmosphere at 35 °C, after 20 days (Gundi et al. 2009).

B. doshiae—the type strain R18 was isolated from the blood of *Microtus agrestis*. It was cultivated on Columbia blood agar containing 10 % whole fresh horse blood. Plates were incubated at 37 °C in a moist atmosphere containing 5 % CO_2 (Birtles et al. 1995).

B. elizabethae—the type strain F9251 was isolated from a patient with endocarditis after the blood was drawn for culture into BACTEC 460 blood culture medium (Johnson Laboratories, Towson, Md.) and subcultured onto blood and chocolate agar and incubated for 14 days in an atmosphere of 5 % CO_2. Grows well on semisolid heart infusion agar supplemented with rabbit blood, on which it produced incomplete hemolysis.

B. grahamii—the type strain V2 was isolated from the blood of *Clethrionomys glareolus*. It was cultivated on Columbia blood agar containing 10 % whole fresh horse blood, with best growth at 37 °C in a moist atmosphere containing 5 % CO_2 (Birtles et al. 1995).

B. henselae—the type strain is Houston 1 (=G5436 = ATCC 49882) and was isolated from the blood of a febrile, 40-year-old human immunodeficiency virus-positive man. Optimal growth occurs on enriched medium with erythrocytes. Colonies were observed after 9–15 days of incubation at 35 °C in a 5 % CO_2, humidified atmosphere or at 30 °C in air; optimal growth does not occur anaerobically (Slater et al. 1990). Subsequent passages demonstrated more rapid growth (Regnery et al. 1992; Brenner et al. 1993). A second genotype among *B. henselae* isolates involved in cat scratch disease and endocarditis was identified in 1996 by Drancourt and colleagues. As so, *B. henselae* was divided into two geno-/serogroups: *B. henselae* Houston, 16S type I, and *B. henselae* Marseilles, 16S type II (Bergmans et al. 1996; Drancourt et al. 1996).

B. jaculi—isolated from the blood of wild-captured greater Egyptian jerboas (*Jaculus orientalis*) that were imported to Japan as pets from Egypt, Thailand, and the Netherlands. Growth occurred on heart infusion agar plates (Difco Laboratories, Inc., subsidiary of Becton, Dickinson and Company) containing 5 % defibrinated rabbit blood (HIA) at 35 °C in a moist atmosphere under 5 % CO_2 after 14 days of incubation. Also cultured on rabbit blood–chocolate HIA plates for 14 days at 35 °C in a moist atmosphere under 5 % CO_2 with H_2O_2 on a glass slide (Sato et al. 2013). The two strains, OY2-1^T (type strain) and strain OY5-1, were isolated from the same source (Sato et al. 2013).

B. japonica—the type strain, Fuji 18-1^T (=JCM 15567^T = CIP 109861^T), was isolated from the blood of the small field mice *Apodemus argenteus*, captured in the Mount Fuji forest in Japan. Growth was performed on heart infusion agar plates (Difco Laboratories, Inc., subsidiary of Becton, Dickinson and Company) containing 5 % (w/v) defibrinated rabbit blood at 35 °C with 5 % CO_2 (14 days) (Inoue et al. 2010).

B. koehlerae—two strains, C-29 (type strain) and C-30, were isolated from the blood of a healthy kitten during a prevalence study of *B. henselae* in domestic cats in the greater San Francisco Bay area of northern California. Optimal growth on chocolate agar, and primary colonies were observed after 14 days of incubation at 35 °C in a CO_2-enriched environment. Chocolate agar should be prepared with hemoglobin powder and GC agar base (Acumedia Manufacturers, Inc., Baltimore, Md.) and supplemented with 10 ml of IsoVitalex (Becton Dickinson). Usually fails to produce any growth on heart infusion agar supplemented with 5 % rabbit blood (Droz et al. 2000).

B. pachyuromydis—isolated from the blood of fat-tailed gerbil (*Pachyuromys duprasi*), imported to Japan as pets from Egypt, Thailand, and the Netherlands. Growth occurred on heart infusion agar plates (Difco Laboratories, Inc., subsidiary

of Becton, Dickinson and Company) containing 5 % defibrinated rabbit blood (HIA) at 35 °C in a moist atmosphere under 5 % CO_2 after 14 days of incubation. Also cultured on rabbit blood–chocolate HIA plates for 14 days at 35 °C in a moist atmosphere under 5 % CO_2 with H_2O_2 on a glass slide. The type strain (FN15-2^T) and strain FN18 were isolated from the same source (Sato et al. 2013).

B. queenslandensis—the type strain AUS^T/NH12^T was isolated from the blood of a wild rat belonging to the genus *Melomys* during mammal surveys carried out in Queensland, Australia. Other isolates belonging to the species, isolated from the blood of rats, are AUST/NH5 from *Rattus tunneyi*, AUST/NH8 from *Rattus fuscipes*, AUST/NH11 from *Rattus conatus*, and AUST/from *Rattus leucopus*. Good growth was observed on Columbia agar with 5 % sheep blood in a 5 % CO_2 atmosphere at 37 °C (Gundi et al. 2009).

B. quintana—in 1961, Vinson and Fuller reported the first successful cultivation of the agent of trench fever on cell-free media using blood-enriched agar. The authors went on to fulfill Koch's postulates by inducing trench fever in human volunteers following their inoculation with suspensions of the isolated organisms. The report also emphasized that different clinical manifestations of trench fever were observed in different volunteers inoculated with the same strain.

B. rattaustraliani—the type strain AUST/NH4^T was isolated from the blood of a wild rat of the species *Rattus tunneyi* which were trapped in Queensland, Australia. Other isolates are AUST/NH9 from *Uromys caudimaculatus*, AUST/NH10 from *Rattus leucopus*, AUST/from *Rattus conatus*, and AUST/NH18 from *Melomys* sp. Growth was observed on Columbia agar with 5 % sheep blood in a 5 % CO_2 atmosphere at 37 °C (Gundi et al. 2009).

B. rochalimae—the type strain BMGH was isolated from a 43-year-old woman with splenomegaly, fever, and anemia, which had recently traveled to Peru. Grows on fresh defibrinated rabbit blood agar at 35 °C in a candle extinction jar (Eremeeva et al. 2012).

B. schoenbuchensis—type strain R1^T was isolated from the blood of a 4-month-old female wild roe deer which was shot in a nature park near Tubingen in southwest Germany. For isolation, the blood which was preserved at −80 °C was thawed and streaked onto the blood agar plates made with a Columbia agar base supplemented with 5 % defibrinated sheep blood. Incubation of the plates was performed at 37 °C in a moist atmosphere containing 5 % CO_2. Primary cultured appeared within 4–6 days (Dehio et al. 2001).

B. silvatica—the type strain Fuji 23-1^T was isolated from the blood of *Apodemus speciosus* mice captured in the Mount Fuji forest in Japan. Growth on heart infusion agar plates (Difco Laboratories, Inc., subsidiary of Becton, Dickinson and Company) containing 5 % (w/v) defibrinated rabbit blood at 35 °C with 5 % CO_2 for 14 days (Inoue et al. 2010).

B. taylorii—the type strain M6 was isolated from the blood of small woodland mammals (*Apodemus* spp.) in the UK. Cultivated on Columbia blood agar containing 10 % whole fresh horse blood. Plates inoculated were incubated for up to 10 days at 30 °C in a moist atmosphere containing 5 % CO_2 (Birtles et al. 1995).

B. tribocorum—type strain IBS 506^T was isolated from wild rats (*Rattus norvegicus*) trapped in a wetland conservation area near the Rhine River, in the eastern part of France. For isolation, the blood was streaked without prior centrifugation onto the blood agar plates made with a Columbia agar base with 5 % defibrinated rabbit blood. Incubation of plates was performed at 35 °C in a moist atmosphere containing 5 % CO_2 (Heller et al. 1998).

Bartonella vinsonii* subsp. *arupensis—the type strain is OK 94–513 (5 = ATCC 700727) and was isolated from a 62-year-old bacteremic cattle rancher. Growth occurred on heart infusion agar in the presence of X factor and on Columbia agar supplemented with 5 % defibrinated sheep blood at 35 °C in 5–10 % CO_2, after 5–7 days (Welch et al. 2000).

Bartonella vinsonii* subsp. *berkhoffii—the type strain 93-CO1 was isolated from a dog with valvular endocarditis. The Isolate appeared following 8 days of incubation at 35 °C in the presence of 5 % CO_2, on trypticase soy agar supplemented with 5 % rabbit blood. Subcultures grew vigorously within 4 days. Did not grow on heart infusion agar supplemented with X factor (Kordick et al. 1996).

Bartonella vinsonii* subsp. *vinsonii—the type strain ATCC VR-152 was isolated from voles by Baker (1946). Grows on heart infusion agar supplemented with X factor (Kordick et al. 1996).

Ecology

Habitat

The species belonging to the genus *Bartonella* have been isolated and detected in a wild range of animal species. Potential animal reservoirs include cats, dogs, rodent, rabbits, ruminants, and sea mammals, as well as wild animals including wild cats, coyotes, deer, elk, and foxes. At least three species have been identified as major human pathogens, and their disease syndromes and treatment recommendation are described below. An additional seven species, including two other subspecies have been associated with disease in humans. Finally, currently nineteen species and one subspecies have been isolated from animals, and most remain of uncertain pathogenicity (*Table 3.1*). Additionally, some species have been associated with disease in animals, such as cats and dogs. The adaptation of *Bartonella* spp. to a broad range of mammalian hosts, as well as vectors which transmit these bacteria, is representative of the complexity of the ecological diversity of members of the *Bartonella* genus.

Considering host ecology, with the exception of *B. bacilliformis* and *B. quintana*, which are classified as "specialists" because humans are the only reservoirs, most *Bartonella* species are referred to as "generalists" because they are able to infect and persist in numerous animal hosts. Although *B. quintana* has been categorized as a "specialist," there is evidence to support the possibility that this species

may have adapted the means to utilize another mammalian species, the domestic cat, as a reservoir host (Breitschwerdt et al. 2007; Drancourt et al. 1996; La et al. 2005). In contrast to other vector-borne diseases which are typically limited to one host, many *Bartonella* species have the ability to be transmitted from a reservoir host to an accidental host. The establishment of a primary reservoir host for *Bartonella* spp. is key because it allows for persistent infection at a high level of bacteremia for a prolonged period of time in a host, permitting uptake by blood-feeding arthropods and subsequent transmission to other hosts. Accordingly, prolonged bacteremia in clinically normal reservoir hosts is considered common (Chomel et al. 2003, 2009; Jacomo et al. 2002). Cats appear to be the primary mammalian reservoir for the important zoonotic specie, *B. henselae*, and also for *B. clarridgeiae* and *B. koehlerae* (Chomel et al. 2004). Coyotes and dogs are considered mammalian reservoirs for *B. vinsonii* subsp. *berkhoffii* and foxes, coyotes, and dogs for *B. rochalimae* (Chomel et al. 2003; Henn et al. 2009a, b). Other examples of reservoir hosts include cattle, which appears to be the mammalian reservoir for *B. bovis* (Bermond et al. 2002). Infection of a single host animal with more than one *Bartonella* species has been reported in cats, dogs, cattle, and wild mammals (Cherry et al. 2009; Diniz et al. 2007; Gurfield et al. 1997; Telfer et al. 2007). Additionally, coinfection with multiple genetic types of *B. henselae* or *B. vinsonii* subspecies *berkhoffii* is reported for cats and dogs, respectively (Duncan et al. 2007; Gurfield et al. 1997).

Transmission of *Bartonella* to humans occurs through an insect vector for most *Bartonella* species. The list of vectors and potential vectors associated with transmission includes flies, flees, ticks, lice, and mites (*Table 3.1*). *B. quintana* is carried by human body lice (*Pediculus humanus*) and human hosts form the reservoir (Chang et al. 2001; Roux and Raoult 1995). *B. bacilliformis* has humans as the only reservoir host, and the sand fly, *Lutzomyia verrucarum*, is the transmitting vector. Transmission of *B. henselae* in cats has typically been associated with the cat flea (*Ctenocephalides felis*) via fleas acquiring *B. henselae* during a blood meal from an infected carrier and then later regurgitating infected saliva while feeding on an uninfected individual (Guptill 2003). Feline intraspecies transmission is regarded as vector-borne, and several studies have failed to demonstrate either seroconversion or bacteremia in uninfected cats living in prolonged association with infected cats but in an ectoparasite-free environment (Bradbury and Lappin 2010; Chomel et al. 1996; Guptill 2003; Kordick and Breitschwerdt 1998). Direct transmission between cats experimentally has also failed (Chang et al. 1999). In cats, the presence of arthropod vectors appears to be necessary for horizontal disease transmission (Abbott et al. 1997; Guptill 2003). Experimental studies have shown that fleas and intradermal inoculation of flea feces can spread the infection between cats. It is supported by the fact that transmission is reduced or absent when fleas are controlled. Domestic dogs are reported to be accidental hosts for *B. henselae*, and as in cats, intraspecies transmission is believed to rely on arthropod vectors (Chang et al. 1999; Guptill 2003). Transmission to humans is known to occur via dog bites, and oral swabs of dogs have confirmed at least four *Bartonella* species (*B. henselae*, *B. quintana*, B. *vinsonii* subsp. *berkhoffii*, and *B. bovis*) (Chomel et al. 2006; Duncan et al. 2007; Skerget et al. 2003). Humans have been assumed to acquire *B. henselae* infection when flea fecal material is introduced through the skin via a cat scratch or cat or dog bite (Skerget et al. 2003). Recent evidence suggests that iatrogenic transmission to humans via accidental needle stick may occur, as well as perinatal transmission (Breitschwerdt et al. 2010; Oliveira et al. 2010). It has also been suggested, but not proven, that fleas may be able to transmit *B. henselae* directly to humans (Center for Food Security and Public Health 2005).

Ticks have been proposed as vectors for transmission of *Bartonella* species among cats, humans, dogs, and other mammalian hosts (Bergmans 1996; Cotte et al. 2008; Pappalardo et al. 1997; Slater et al. 1990; Welch et al. 1999). *B. henselae* and other *Bartonella* spp. were detected by PCR in questing ticks (Chang et al. 2001, 2002; Sanogo et al. 2003). Transstadial transmission of *B. henselae* has been demonstrated in *Ixodes ricinusticks* (Cotte et al. 2008). However, the role of ticks is still unknown.

Pathogenicity, Clinical Relevance

Analysis of the species belonging to the genus *Bartonella* indicates the presence of human and animal pathogenic species/strains, although information about the pathogenicity for several species remains uncertain to date. Despite the large spectrum of antibiotic sensibility among the species, clinical use of antimicrobial agents in vivo are dependent on the infection (disease) caused by *Bartonella* species.

Human- and Animal-Related Pathogenicity

Bartonella spp. are facultative intracellular pathogens that employ a unique stealth infection strategy comprising immune evasion and modulation, intimate interaction with nucleated cells, and intraerythrocytic persistence. Infections with *Bartonella* are ubiquitous among mammals, and many species can infect humans either as their natural host or incidentally as zoonotic pathogens (*Table 3.1*). Upon inoculation into a naive host, the organism first colonizes a primary niche that is widely accepted to involve the manipulation of nucleated host cells in the microvasculature. Consistently, in vitro researches have demonstrated that *Bartonella* harbors an ample arsenal of virulence factors to modulate the response of such cells, gain entrance, and establish an intracellular niche (Dehio 2004; Harms and Dehio 2012). Subsequently, the bacteria are seeded into the bloodstream where they invade erythrocytes and give rise to a typically asymptomatic intraerythrocytic bacteremia. Residing intraerythrocytically allows the bacteria to evade the host's immune system and thus chronically infect the host (Greub and Raoult 2002). While this course of infection is characteristic for natural hosts, zoonotic infections or the

infection of immunocompromised patients may alter the path of *Bartonella* and result in considerable morbidity. The probability of infection is heightened in immunosuppressed transplant patients, chemotherapy patients, and patients who suffer from immunosuppressive disorders such as HIV/AIDS (Tappero et al. 1993).

Since *Bartonella* spp. resides within the red blood cells of the host, the transmissibility of the bacteria is greatly facilitated. Disease in the reservoir host, depending on the adaptation level to the host and *Bartonella* species, varies from an asymptomatic or subclinical to clinical manifestations with low morbidity and limited mortality, or even to life-threatening severe disease (Chomel et al. 2009). Besides erythrocytes, endothelial cells represent another major target cell type for *Bartonella*, and studies have shown that there is a phenomenon of host restriction. As so, when humans are the reservoir host, such as for *B. bacilliformis* and *B. quintana*, clinical manifestations result from both intraerythrocytic bacteremia and endothelial cell association (Chomel et al. 2003; Dehio 2001; Dehio and Sander 1999). On the other hand, other *Bartonella* species, for which humans are not the natural reservoir, tend to cause persistent bacteremia only in immunodeficient individuals, and in this case without evidence for intraerythrocytic localization, as typically observed in the reservoir host. For these zoonotic infections, the severity of the clinical presentation correlates well with the status of the host's immune system and often reflects a close association with proliferating endothelial cells (Dehio 2001; Dehio and Sander 1999; Resto-Ruiz et al. 2003).

Although, several studies have detected *Bartonella* sp., other than *B. bacilliformis* within erythrocytes (Mehock et al. 1998; Rolain et al. 2002), which ones may also localize and persist within vascular endothelial cells (Dehio 2001), the exact localization of *Bartonella* species in mammalian hosts has not been completely determined. *Bartonella* have a strong tendency to stick or clump together in tissues and in culture and also to stick to, and penetrate, red blood cells and endothelial cells. The ability to adhere to each other, as well as to the membranes of red blood cells and endothelial cells, leads to the wide and varied tissue pathogenesis observed in cats, dogs and people. Pili and a protein called deformin are probably responsible for the sticky properties (Xu et al. 1995). *Bartonella* proteins stimulate endothelial cells to proliferate causing neovascularization or angiogenesis, as well as an outpouring of inflammatory cytokines which recruit inflammatory cells such as lymphocytes, plasma cells, and macrophages. Studies upon virulence factors of *Bartonella* species suggest that they include adhesins, heme acquisition/utilization mechanisms, type IV secretion systems, outer membrane proteins, and a low-potency lipopolysaccharide, among others (Dehio 2004; Minnick and Battisti 2009; Harms and Dehio 2012). The T4SS Trw has been identified in *B. henselae* and *B. quintana* and is believed to be important in erythrocyte and endothelial cell invasion (Minnick and Battisti 2009; Dehio 2008). The VirB/VirD4T4SS, investigated in *B. henselae*, has important effects including activation of nuclear factor kappa B, leading to the release of proinflammatory or proangiogenic cytokines and inhibition of apoptosis of colonized endothelial cells (Dehio 2008; Minnick and Battisti 2009). It should be mentioned that the source of inoculum may substantially influence the pathogenicity of the disease. A synopsis of pathogenicity factors of *Bartonella* spp. and their function is highlighted below (*Table 3.4*), as reviewed by Dehio (2004) and Harms and Dehio (2012).

Several studies have indicated the lack of immune protection following *Bartonella* infection (Arvand et al. 2008; Kordick et al. 1995) and consequently relapsing bacteremia, such as 454 days for *B. henselae* or *B. clarridgeiae* (Kordick et al. 1999). Relapsing bacteremia may be facilitated by antigenic variation or by localization to the so-called "privileged" sites such as within erythrocytes or other host cells. In vitro studies indicate that *Bartonella* spp. can also infect professional macrophages, including dendritic cells, microglial cells, monocytes, and tissue macrophages (Chomel et al. 2009; Schulein et al. 2001; Muñana et al. 2001). In addition, experimental infection of dogs with *B. vinsonii* subsp. *berkhoffii* induced immunosuppression, characterized by sustained suppression of peripheral blood CD81 lymphocytes, accompanied by an altered cell surface phenotype and an increase in CD41 lymphocytes in the peripheral lymph nodes (Pappalardo et al. 2000, 2001). Studies have also demonstrated lack of or incomplete cross-protection among *Bartonella* isolates, likely due to genetic variation (Guptill et al. 2004). Experimentally infected cats became immune to challenge infection with homologous strains of *Bartonella*, but there is a lack of protection against reinfection with heterologous isolates (Greene et al. 1996; Yamamoto et al. 1997). Considering humoral immunity, the antibody response to acute experimental infection of *B. henselae* or *B. clarridgeiae* is similar to other diseases, with antibodies IgM being detected within 1 week followed by a rise in IgG 1–2 weeks later. Although some studies suggest that antibody titer may correlate with bacteremia, this association is unreliable for clinical use (Pressler 2006).

A wide spectrum of diseases has been described following *Bartonella* infection. Clinical disease ranges from painless lymphadenopathy to large cervical abscesses (Kabeya et al. 2002; Seubert et al. 2002; Ridder et al. 2005; Rie et al. 2003). Lymph nodes most commonly involved are those ones localized in the axilla, neck, and groin regions (Marakaki et al. 2003; Dyachenko et al. 2005). Although rare, serious diseases such as encephalopathy, pulmonary disease, pneumonia, neuroretinitis, endocarditis, aseptic meningitis, and osteomyelitis may complicate *Bartonella* infections, even in immunocompetent individuals (Ciervo et al. 2005; Ridder et al. 2005; Loa et al. 2006). In immunocompromised patients, disseminated and systemic infections such as fever, bacillary angiomatosis, and bacillary peliosis may occur (Harms and Dehio 2012; Johnson et al. 2003). In severe cases, HIV-positive patients develop cerebral lesions (Spach et al. 1992; Muñana et al. 2001) and dementia associated with relapsing *B. henselae* bacteremia (Lucey et al. 1992; Schwartzmann et al. 1995; Muñana et al. 2001). However, the pathogenesis and dysfunction of central nervous system is not fully understood (Muñana et al. 2001). Other diseases include meningoencephalitis, stellar retinitis, Parinaud's oculoglandular syndrome, erythema nodosum,

Table 3.4
Virulence factors of *Bartonella* spp.

Virulence factor(s)	Direct functions(s)	Contribution(s) to pathogenesis
Autotransporters	Unknown, cohemolysin activity (CAMP-like factor autotransporter)	Unknown
Deformin	Formation of invaginations in erythrocyte membranes	Enabling erythrocyte invasion
Filamentous hemagglutinins	Unknown	Unknown
Flagellation	Motility; mechanical force	Binding to and invasion of erythrocytes
Hemin-binding proteins (Hbps)	Hemin-binding, fibronectin binding (HbpA)	Host cell adhesion? Formation of a heme surface layer (antioxidant barrier? Nutritive reservoir?)
Invasion-associated locus (ial), encoding IalA and IalB	Unknown	Invasion of erythrocytes
Lipopolysaccharides	Invisibility to Toll-like receptor 4 (TLR4), antagonizing TLR4 signaling	Immune evasion and immunomodulation
OMP43 (outer membrane protein of *Bartonella*)	Fibronectin binding, host cell binding	Host cell adhesion?
Outer membrane proteins	Activation of NF-kB signaling, host cell adhesion	Secretion of monocyte chemoattractant protein 1 (MCP-1) and upregulation of E-selectin and ICAM-1 (as part of proangiogenic signaling?)
Secreted factor (GroEL heat shock protein)	Unknown, activation of Ca2+ signaling?	Inhibition of apoptosis and mitogenic stimulation of host cells
Trimeric autotransporter adhesins (i.e., *Bartonella* adhesin A of *B. henselae*, "variably expressed outer membrane of *B. quintana*)	Bacterial autoaggregation, attachment to extracellular matrix, host cell binding via β1-integrins, activation of hypoxia-inducible factor 1 (HIF-1) and NF-Kb, inhibition of macrophage phagocytosis	Stable integration with host cell as basis for further manipulation, secretion of proangiogenic cytokines
Trimeric autotransporters or the VirB/D4 type IV secretion system (T4SS), *Bartonella* effector proteins	Inhibition of apoptosis, proinflammatory activation, modulation of angiogenesis, invasome formation	Setup and control of intracellular niche
Trw type IV secretion system	Adhesion to erythrocyte surface	Enabling erythrocyte invasion
Unknown factor (probably surface protein)	Activation of cellular signaling via β1-integrins	Triggering host cell invasion in course of invasome formation

arthritis, pulmonary nodules, myelitis, granulomatous hepatitis, and lesions of almost every organ system (Anderson and Neuman 1997; Giladi et al. 2001; Greub and Raoult 2002).

Carrión's Disease and Verruga Peruana: The classical course of infection with *B. bacilliformis* is known as Carrion's disease and consists of Oroya fever as the acute phase with hemolytic anemia and a subsequent chronic phase hallmarked by multiple vasoproliferative lesions on the skin (verruga peruana for "Peruvian wart"). This infection is also known as Carrion's disease after Daniel Carrion, a Peruvian medical student in whom the disease developed after inoculating himself with material from an infectious lesion. The disease is considered endemic in South America, particularly in Peru (Maguina 1998), Colombia (Alexander 1995), Ecuador, Chile, and Guatemala (Sanchez et al. 2012), and it has afflicted travelers visiting the region. The first phase, Oroya fever, involves invasion of human erythrocytes and results in an often severe acute hemolytic anemia, and life-threatening septicemia usually develops (Chang et al. 2001; Houpikian and Raoult 2002). The second phase, verruga peruana, involves infection and prominent proliferation of endothelial cells, resulting in characteristic skin eruptions of papules and nodules (Anderson and Neuman 1997). These papules are often inflamed and bleed, and osteoarticular pain usually occurs in association with these vascular lesions (Rolain et al. 2004). However, atypical pathological angiogenesis during the verruga peruana cases involving other organs such as the spleen has been reported (Lydy et al. 2008). People are still the only known reservoirs of *B. bacilliformis*, and they serve as sources of infection for sand flies, which are the vectors of the disease. The devastating hemolytic anemia during Oroya fever is a critical difference between the pathologies of *B. bacilliformis* and the other *Bartonella* species, since severe

morbidity and severe hemolytic anemia are not usually observed with modern species. Mortality during Oroya fever ranges from close to zero in the case of hospitalized patients receiving antibiotic treatment to up to 88 % in untreated cases (Pachas 2000). Approximately 70 % of patients suffering from Oroya fever develop complications of the disease, of which approximately half are of an infectious cause and half of a noninfectious nature. Noninfectious complications such as fever, anorexia, or an altered mental status have been suggested to be primarily a consequence of systemic infection (Maguina et al. 2001). Secondary infections may occur during Oroya fever and contribute prominently to morbidity and mortality by provoking septicemia, most often with *Salmonella* (Pachas 2000; Maguina et al. 2001). However, it is known today that this classical course of Carrion's disease is not the only possible outcome of a *B. bacilliformis* infection, since natives in the areas of endemicity exhibit a significant baseline of asymptomatic bacteremia and suffer from Oroya fever relatively rarely, but they frequently develop verruga peruana without prior hemolytic anemia (Harms and Dehio 2012).

***B. quintana* Infections**: Trench fever, also known as 5-day fever, Wolhynia fever, or Quintan fever, is caused by *B. quintana* and is distributed throughout the world (Roux and Raoult 1999). Although symptoms vary between patients, the disease usually present mild to moderately severe fever (for up to 8 days, concurrent with the onset of erythrocyte infection), chills, malaise, myalgia, and leg pains and is especially prominent in the tibia (Maurin et al. 1997; Rolain et al. 2004). The acute signs usually resolve spontaneously, but recurrences of the disease may occur (Kostrzewski 1949). Occasionally, splenomegaly and a maculopapular rash resembling the rose spots of typhoid fever may develop (Strong 1918). Many of the infected individuals do not suffer from disease but develop a chronic asymptomatic, bacteremic carrier state (Raoult et al. 2001). Trench fever occurred in millions of troops in World War I, but with the introduction of louse control measures by armed forces, the disease was thought no longer to be a threat (Maurin and Raoult 1996). However, *B. quintana* has recently reemerged as a contemporary disease, in which manifestations include "urban" trench fever and several new disease manifestations including endocarditis and, specifically in HIV patients, a vasoproliferative disorder called bacillary angiomatosis. Major risk factors for acquiring *B. quintana* infections nowadays include poor living conditions, chronic alcoholism, and HIV-positive individuals (Jacomo et al. 2002). The vasoproliferative infection is usually progressive and fatal unless treated by antibiotic therapy (Bass et al. 1997).

Cat scratch Disease (CSD): Studies have shown that *B. henselae* Houston-1 strain constitutes the primary etiologic agent of CSD. However, *B. henselae* Marseille strain, *B. clarridgeiae*, *B. koehlerae*, and *B. grahamii* have also been identified as agents of the disease in limited cases (Kordick et al. 1997; Lamas et al. 2008; Margileth and Baehren 1998; Oksi et al. 2013; Windsor 2001). CSD has a worldwide distribution and is probably the most common zoonotic human infection caused by *Bartonella* spp. nowadays. Regional lymphadenopathy is the main clinical manifestation of CSD, associated with the nodes draining the site of the cat scratch and preceded by localized lesions with redness, swelling, and pustules also appearing at the site of initial inoculation. Lymph nodes usually regress in size over a period of weeks or months, but the lymphadenitis may become suppurative in 10 % of patients. However, atypical manifestations with involvement of various organs such as the eyes, liver, spleen, central nervous system, skin, and bones may occur including malaise, fatigue, myalgia and arthralgia, weight loss, splenomegaly, and Parinaud's oculoglandular syndrome (Dehio 2004). Encephalopathy and neuroretinitis are less common complications of CSD (Bass et al. 1997). Typically, the course of CSD is benign as it is observed in immunocompetent hosts, in whom the pathological response is granulomatous, suppurative, extracellular and intracellular, generally self-limited, and without bacteremia (Bass et al. 1997; Carithers 1985; Zangwill et al. 1993). However, severe immunosuppression, particularly secondary to HIV, as well as organ transplantation, is associated with disseminated and often tissue-invasive forms of CSD which have the potential to be life-threatening (Black et al. 1986). Manifestations in such patients include bacillary angiomatosis, hepatitis peliosis, endocarditis, and persistent or relapsing fever in association with bacteremia (Chomel et al. 1996; Higgins et al. 1996) Although CSD may occur in people of any age, most patients are under 18 years of age, perhaps because children are more likely to have close and rough contact with cats. The incidence of CSD is seasonal, with most cases occurring in August to October in northern temperate areas (Carithers 1985).

Endocarditis: *B. quintana*, *B. henselae*, *B. elizabethae*, *B. vinsonii* subsp. *berkhoffii*, *B. vinsonii* subsp. *arupensis*, *B. koehlerae*, and *B. alsatica* are known to cause infective endocarditis (Johnson et al. 2003; Lepidi et al. 2008). Several studies suggest that previous valve lesions predispose an individual to endocarditis with nonhuman *Bartonella* species, with *B. quintana* endocarditis being more frequently diagnosed in patients without previous heart diseases (Jacomo et al. 2002). *Bartonella*-caused endocarditis often requires valve replacement surgery, and since it is generally indolent and culture negative, delays in diagnosis contribute to a higher death rate than other forms of endocarditis (Rolain et al. 2004). Prescription of an aminoglycoside appears to be critical in the outcome of endocarditis (Jacomo et al. 2002). Endocarditis caused by *Bartonella* has been reported in both immunocompetent and immunosuppressed individuals (Minnick and Anderson 2006).

Bacillary Angiomatosis (BA) and Bacillary Peliosis (BP): BA and BP are vasoproliferative disorders that occur particularly in immunocompromised patients with *Bartonella* infections seen most commonly in patients with advanced HIV (Perkocha et al. 1990; Tappero et al. 1993; Dehio 2005). It is characterized by cutaneous manifestations of vascular tumors of the skin and subcutaneous tissues, including single or multiple discolored cutaneous nodules, hyperpigmented lichenoid plaques, and subcutaneous nodules with the potential to ulcerate (Conrad 2001; Klotz et al. 2011; Koehler and Tappero 1993). BA cutaneous

lesions may resemble pyogenic granulomas, Kaposi's sarcoma, lichenoid violaceous plaques, or verruga peruana (Koehler and Tappero 1993). A multicellular inflammatory infiltrate with polymorphonuclear lymphocytes that display leukocytoclastic characteristics is present in BA (Cockerell and LeBoit 1990). Both *B. quintana* and *B. henselae* are the species responsible for this manifestation (Chang et al. 2001; Houpikian and Raoult 2001b; Johnson et al. 2003; Stoler et al. 1983). BA lesions can also involve the bone marrow, liver, spleen, or lymph nodes. Risk factors for BA include exposure to infected cats or cat fleas (Koehler et al. 1994). The BP is caused by *B. henselae* and is considered by several researchers as the visceral manifestation of BA. It is characterized by vascular proliferation in single or multiple organs, including liver, spleen, and lymph nodes, which results in the formation of blood-filled cysts (Rolain et al. 2004; Slater et al. 1992; Tappero et al. 1993). Gastrointestinal distress, fever, and chills may accompany peliosis, and the syndrome may occur alone or in combination with BA or bacteremic syndrome (Minnick and Anderson 2006). BP can produce hepatomegaly and may result to an increase in the activity of liver enzymes and also death due to liver failure (Perkocha et al. 1990). Typically, patients with tuberculosis, advanced cancers, or those utilizing anabolic steroids are considered most prone to develop BP (Koehler et al. 1992, 1997).

***Bartonella* Species in Felines**: Natural infection of cats with five *Bartonella* species (*B. henselae*, *B. clarridgeiae*, *B. koehlerae*, *B. quintana*, and *B. bovis)* has been reported, although feline infection with species other than *B. henselae* and *B. clarridgeiae* is rarely reported (Guptill 2010). Nevertheless, pathogenesis of *Bartonella* infection, including the localization of the organism, in these hosts is not completely understood until nowadays (Guptill 2010; Jacomo et al. 2002; O'Reilly et al. 1999; Rolain et al. 2004). *B. henselae* and *B. clarridgeiae* bacteremia is usually asymptomatic, and the disease is strain dependent (Chenoweth et al. 2004a, b; Johnson et al. 2003; Koehler et al. 1994). *Bartonella* spp. are responsible for chronic bacteremia in kittens and adult cats, which may remain bacteremic for several months, or even years, without ever becoming symptomatic (Chomel et al. 1999). Most of the cats naturally infected with *B. henselae* do not show any clinical manifestation; however, some of them can demonstrate only mild clinical signs which are self-limiting (Stutzer and Hartmann 2012). Several studies have suggested a link between infection and clinical signs such as endocarditis, pyogranulomatous myocarditis and diaphragmatic myositis (Varanat et al. 2012), central nervous system diseases (Leibovitz et al. 2008), uveitis, conjunctivitis (Ketring et al. 2004) and possibly stomatitis, and various urinary tract disorders (Glaus et al. 1997). Additionally, clinical signs reported in cats inoculated with *B. henselae* include swelling at the site of inoculation, fever, lethargy, anorexia, myalgia, anemia, lymphadenopathy, and reproductive failure (Guptill et al. 1999; Kordick and Breitschwerdt 1997; O'Reilly et al. 1999). It should be mentioned, however, that establishing causal associations between clinical conditions and a pathogen like *Bartonella* that has a high prevalence in the reservoir host population is difficult. The likelihood that some clinical conditions have multiple causes must also be considered, particularly in cats with exposure to arthropod vectors (Guptill 2010).

***Bartonella* Species in Dogs**: Infection in dogs may be associated with *B. clarridgeiae*, *B. elizabethae*, *B. henselae*, *B. vinsonii subspecies berkhoffii*, *B. quintana*, and *B. rochalimae* (Boulouis et al. 2005; Breitschwerdt et al. 1995, 2002; Chomel et al. 2001, 2003; Henn et al. 2009a; Kelly et al. 2006; Kordick et al. 1996; Mexas et al. 2002). *Bartonella henselae* and *B. vinsonii* subspecies *berkhoffii* are currently believed to be the most common *Bartonella* infections in dogs (Skerget et al. 2003). A wide variety of clinical signs for *Bartonella* infection in dogs have been reported, including polyarthritis, cutaneous vasculitis, endocarditis, myocarditis, cardiac arrhythmias, epistaxis, peliosis hepatitis, and granulomatous inflammatory diseases in several organs (Breitschwerdt et al. 1995; Cadenas et al. 2008; Chomel et al. 2001, 2003; Cockwill et al. 2007; Henn et al. 2009a; Pasavento et al. 2005; Saunders and Monroe 2006). Endocarditis appears to be the clinical condition with the strongest association with *Bartonella* in dogs, and most of the cases are attributable to *B. vinsonii* subsp. *berkhoffii* (Guptill 2010). Thrombocytopenia, anemia, neutrophilic leukocytosis, and eosinophilia are the most commonly detected in dogs seropositive for *B. vinsonii* subsp. *berkhoffii* (Breitschwerdt et al. 2004). The role of dogs as reservoirs of *Bartonella* spp. is less clear than it is for cats because domestic dogs are more likely to be final, clinically affected hosts.

Other *Bartonella* Species: Numerous rodents are known to have intraerythrocytic *Bartonella* bacteremias, and in the past years, some have been associated with human disease, while others remain unknown. As mentioned before, *B. elizabethae*, which naturally infects rats (Ellis et al. 1999b), has been associated with endocarditis (Daly et al. 1993), as well as *B. vinsonii* spp. a*rupensis* (mice) which was isolated from a bacteremic patient with fever (Welch et al. 1999). Additionally, *B. grahamii* naturally infects rats and other rodents (Ellis et al. 1999; Koesling et al. 2001) and may cause neuroretinitis in humans (Kerkhoff et al. 1999). Other *Bartonella* species that infect small woodland mammals and herbivores and domesticated animals are of uncertain pathogenicity, including *B. birtlesii* (mouse), *B. bovis (ruminant)*, *B. capreoli* (roe deer), *B. doshiae* (rodent), *B. peromysci* (rodent), *B. schoenbuchensis* (roe deer), *B. talpae* (rodent), *B. taylorii* (rodent), *B. tribocorum* (rodent), *B. vinsonii* subsp. *vinsonii* (Canadian vole agent), *B. jaculi*, *B. callosciuri*, *B. pachyuromydis*, and *B. acomydis* (wild Rodentia).

Antibiotic Sensitivity

The antibiotic sensitivity is variable among the genus *Bartonella*, and the in vitro and in vivo antibiotic susceptibilities of *Bartonella* do not correlate well for a number of antibiotics; for instance, penicillin has no efficacy in vivo, despite the very low minimal inhibitory concentration (MIC) observed in vitro. Evaluation of susceptibilities to antibiotics has been performed either in the presence of eukaryotic cells or without cells, i.e., in axenic media. Determination of antibiotic susceptibility in

axenic media has been carried out either with solid media enriched with 5–10 % sheep or horse blood or with liquid media (Maurin et al. 1995; Rolain et al 2004).

Generally, *Bartonella* spp. display high levels of in vitro susceptibility to antibacterial agents. Agar dilution testing shows resistance only to fosfomycin, colistin, and vancomycin (Maurin et al. 1995). The highest sensitivity is observed to ketolides such as telithromycin, macrolides, particularly doxycycline, clarithromycin, and rifampin, as well as amoxicillin, cefotaxime, azithromycin, clarithromycin, gentamicin, tetracycline, minocycline, and deftriaxone (Bass et al. 1998; Dörbecker et al. 2006; Maurin and Raoult 1993). However, macrolide-resistant strains of *Bartonella* are reported, and studies suggest that these may arise as a result of animals being treated with macrolide antibiotics (Biswas et al. 2006). Additionally, resistance to azithromycin has been reported in vitro (Biswas et al. 2010). There is a considerable variability in the susceptibilities of isolates to the fluoroquinolones, and only the aminoglycosides (gentamicin, tobramycin, and amikacin) are found to be bactericidal (Jacomo et al. 2002). *B. bacilliformis* is resistant in vivo to neosalvarsan and to other arsenical compounds in general. It is sensitive to penicillin, streptomycin, chloramphenicol, and oxytetracycline. When grown with penicillin, the organism produces L forms (Sharp 1968). *B. henselae*, *B. quintana*, and *B. elizabethae* are susceptible to the macrolides azithromycin, clarithromycin, dirithromycin, erythromycin, and roxithromycin based on testing in Vero cell cultures (Ives et al. 1997). Results of a recent study showed good in vitro efficacy of pradofloxacin against *B henselae* (Biswas et al. 2010), although natural fluoroquinolone resistance in *Bartonella* isolates have been reported (Angelakis et al. 2008). Recently, specific antibiotic resistance mutations have been characterized in *B. henselae* (azithromycin, gentamicin, enrofloxacin, and ciprofloxacin), *B. quintana*, and B. *bacilliformis* (erythromycin, rifampicin, and ciprofloxacin) (Biswas et al. 2006, 2007, 2010; Biswas and Rolain 2010; Habib et al. 2009; Rolain et al. 2004; Meghari et al. 2006). Resistance in vitro to doxycycline or amoxicillin has not yet been induced (Guptill 2010). The results of susceptibility testing with *Bartonella* spp. were demonstrated in a review published by Rolain and colleagues (2004) (❯ *Table 3.5*).

Recommendations for the treatment of human *Bartonella* infections are summarized in ❯ *Table 3.6* according to the data available from several studies (Biswas and Rolain 2010; Rolain et al. 2004; Pérez-Martinez et al. 2010; Prutsky et al. 2013). Antibiotic susceptibilities are not routinely tested in clinical isolates as susceptibility studies often fail to predict response to therapy (Hammoud et al. 2008).

Penicillin G, chloramphenicol, tetracycline, and erythromycin have been used for the treatment of Oroya fever. Treatment with these drugs produces rapid defervescence, with disappearance of the organisms from the blood, usually within 24 h (Bass et al. 1997). However, because many patients suffer from secondary infections, especially salmonellosis and infections caused by other enteropathogenic bacteria, chloramphenicol has become the recommended antibiotic therapy (Rolain et al. 2004). Nevertheless, in some patients, simultaneous treatment with another antibiotic (especially a beta-lactam compound) is recommended (Maguina et al. 2001). Trimethoprim–sulfamethoxazole, macrolides (roxithromycin), and fluoroquinolones (norfloxacin and ciprofloxacin) have also been used successfully in some patients (Maguina 1998). Although, verruga peruana is caused by the same bacterium that causes Oroya fever, chloramphenicol is an ineffective treatment for this eruptive stage of infection with *B. bacilliformis* (Maguina 1998). The treatment used in this case has traditionally been streptomycin (15–20 mg/kg of body weight intramuscularly [i.m.] once daily) for 10 days, although rifampin may also be used (Maguina et al. 2001). Meta-analysis showed that there is no difference between them (Prutsky et al. 2013). However, failures of rifampin treatment have been reported, as well as resistance. Ciprofloxacin at 500 mg p.o. twice daily for 7–10 days has also been used with success for the treatment of adults with multiple eruptive-phase lesions, as has azithromycin (Maguina and Gotuzzo 2000).

Considering trench fever, recommendation for patients with the acute form of *B. quintana* bacteremia relies on gentamicin (3 mg/kg of body weight i.v. once daily for 14 days) in combination with doxycycline (200 mg p.o. daily) for 28 days. Rifampin resistance was readily induced in *B quintana*, and it is recommended that people with *Bartonella* infections should not be treated with rifampin alone (Biswas et al. 2008).

Agents that have been used in therapy of cat scratch disease include azithromycin and rifampin. A placebo-controlled clinical study showed that azithromycin caused an 80 % decrease in lymph node volume in 7 of 14 azithromycin-treated CSD patients during the first 30 days of observation (Bass et al. 1998). However, Rolain et al. (2004) and Prutsky et al. (2013) pointed out that it is not clear that antibiotic therapy is useful for the treatment of CSD in immunocompetent patients, and because antibiotic therapy adds the risk of adverse drug reactions and the generation of resistant flora, the current recommendation for the mild to moderately ill immunocompetent patient with CSD is not antibiotic treatment, being azithromycin used only in patient with extensive lymphadenopathy. Considering complicated CSD (retinitis, encephalopathy, and visceral forms), the combination of doxycycline with rifampin should be used (Reed et al. 1998). However, it remains unclear whether the antibiotic treatment of a localized disease reduces the risk of the development of a systemic disease.

Bartonella infections causing bacillary angiomatosis (BA), bacillary peliosis (BP), and endocarditis have been treated with a variety of antibiotics, including trimethoprim–sulfamethoxazole, doxycycline, erythromycin, ciprofloxacin, rifampin, gentamicin, clarithromycin, and azithromycin (Rolain et al. 2004). Nowadays, however, it is not recommended to use fluoroquinolones, trimethoprim–sulfamethoxazole, or narrow-spectrum cephalosporins for the treatment of BA or BP. Studies have demonstrated that treatment of BA and BP with oral doxycycline (100 mg twice daily) has been consistently successful (Koehler and Tappero 1993). The drug of choice for the treatment of BA is erythromycin, in which patients intolerant of erythromycin can be treated with doxycycline (Koehler 1995).

Table 3.5

Minimal inhibitory concentration (MIC) for *Bartonella* sp. strains determined by the agar dilution technique with Columbia agar supplemented with 5 % horse blood

Drug group and drug	MIC (μg/mL)				
	B. henselae	*B. quintana*	*B. bacilliformis*	*B. vinsonii*	*B. elizabethae*
Aminoglycosides					
Amikacin	2–4	4–8	2–8	4	1
Gentamicin	0.12–0.25	0.12–2	1–2	0.5	0.12
Streptomycin	ND	ND	4	ND	ND
Tobramycin	0.5–1	0.5–4	2–4	2	0.25
Cephalosporins					
Cefotaxime	0.12–0.25	0.12–0.25	0.03–0.12	0.12	0.06
Cefotetan	0.25–0.5	0.12–0.5	2	1	1
Ceftazidime	0.25–0.5	0.25–0.5	0.12–0.25	0.25	0.5
Ceftriaxone	0.12–0.25	0.06–0.25	0.003–0.006	0.06	0.12
Cephalothin	8–16	8–16	4–8	16	8
Macrolides					
Azithromycin	0.006–0.015	0.006–0.03	0.015	0.015	0.006
Clarithromycin	0.006–0.03	0.006–0.03	0.015–0.03	0.03	0.015
Erythromycin	0.06–0.25	0.06–0.12	0.06	0.25	0.12
Roxithromycin	0.015–0.03	0.015–0.06	0.03	0.12	0.06
Telithromycin	0.003	0.006	0.015	ND	ND
Penicillins					
Amoxicillin	0.6–0.12	0.03–0.06	0.03–0.06	0.06	0.03
Oxacillin	1–2	1–4	0.25–0.5	1	4
Penicillin G	0.03–0.06	0.03	0.015–0.03	0.03	0.015
Ticarcillin	0.25	0.06–0.25	0.06–0.12	0.25	0.12
Quinolones					
Ciprofloxacin	0.25–1	0.5–2	0.25–0.5	1	0.5
Pefloxacin	4–8	2–8	1–2	4	2
Sparfloxacin	0.06	0.06–0.12	0.25	0.06	0.06
Tetracyclines					
Doxycycline	0.12	0.06–0.25	0.03–0.06	0.25	0.06
Miscellaneous					
Clindamycin	2–4	4–16	32–64	8	8
Colistin	4–16	4–16	16	8	4
Fosfomycin	16–32	32–64	8–16	16	16
Imipenem	0.5	0.25–1	0.5–1	2	0.25
Rifampin	0.03–0.06	0.06–0.25	0.003	0.12	0.03
TMP-SMX	1/5	0.25/1.25–1/5	0.4/2–0.8/4	1/5	0.5/2.5
Vancomycin	2–8	8–16	4–8	8	8

Note that the in vitro MICs correlate poorly with the in vivo efficacies of antibiotics in patients with *Bartonella*-related infections, and thus, these MICs should not be used for the selection of antibiotics for patient treatment. MICs are the concentrations at which there is complete inhibition of growth. *Abbreviations*: *ND* not determined, *TMP-SMX* trimethoprim–sulfamethoxazole

No statistically significant difference was observed in treatment with erythromycin or doxycycline (Prutsky et al. 2013). BP responds more slowly than cutaneous BA to treatment. Considering *Bartonella* endocarditis, a retrospective study using aminoglycosides with either a beta-lactam or other antibiotics (vancomycin, doxycycline, rifampin, or co-trimoxazole) demonstrated that patients receiving an aminoglycoside were more likely to recover fully and, if they were treated for at least 14 days,

Table 3.6

Recommendations for the treatment of infections caused by *Bartonella* spp. for humans

Disease	Adults	Children
Carrion's disease Oroya fever	Chloramphenicol at 500 mg p.o. or i.v. QID for 14 days and another antibiotic (a beta-lactam is preferred) Or ciprofloxacin at 500 mg p.o. BID for 10 days	Chloramphenicol at 50–75 mg/kg/day p.o. or i.v. divided into four doses for 14 days and another antibiotic (a beta-lactam is preferred) Or ciprofloxacin in children 7–12 years 250 mg p.o. BID for 10 days Or doxycycline + gentamicin
Carrion's disease Verruga peruana	Doxycycline + gentamicin Or streptomycin at 15–20 mg/kg/day i.m. for 10 days Or rifampin at 10 mg/kg/day p.o. for 14–21 days 10 days	Rifampin at 10 mg/kg/day p.o. for 14 days (maximum total daily dose of 600 mg/day)
Trench fever or chronic bacteremia with *B. quintana*	Doxycycline at 200 mg p.o. QD for 4 weeks and gentamicin 3 mg/kg i.v. QD for 2 weeks	Unknown
Typical CSD	No recommendation	No recommendation
	For patients with extensive lymphadenopathy, consider azithromycin at 500 mg p.o. on the first day and 250 mg p.o. on days 2–5 as a single daily dose	For patients with extensive lymphadenopathy, consider azithromycin at 10 mg/kg p.o. on day 1 and 5 mg/kg p.o. on days 2–5 as a single daily dose
Retinitis	Doxycycline at 100 mg p.o. BID for 4–6 weeks and rifampin at 300 mg p.o. BID for 4–6 weeks	Unknown
Bacillary angiomatosis (BA)	Erythromycin at 500 mg p.o. QID for 3 months Or doxycycline at 100 mg p.o. BID for 3 months	Erythromycin 40 mg/kg/day p.o. divided in four divided doses (maximum total daily dose, 2 g/day) for 3 months
Bacillary peliosis (BP)	Erythromycin at 500 mg p.o. QID for 4 months Or doxycycline at 100 mg p.o. BID for 4 months	Erythromycin 40 mg/kg/day p.o. divided in four divided doses (maximum total daily dose, 2 g/day) for 4 months
Endocarditis	Suspected *Bartonella*, culture negative: Gentamicin at 3 mg/kg/day i.v. for 14 days and ceftriaxone at 2 g i.v. or i.m. QD for 6 weeks with or without doxycycline at 100 mg p.o. or i.v. BID for 6 weeks	Unknown
	Documented *Bartonella*, culture positive: Doxycycline at 100 mg p.o. BID for 6 weeks and gentamicin at 3 mg/kg/day i.v. for 14 days	

Abbreviations: BID, twice a day; QD, once a day; QID, four times a day

were more likely to survive (Raoult et al. 2003). These data strongly support the use of aminoglycoside therapy for at least 14 days for patients with suspect of *Bartonella* sp. endocarditis. Additionally, it is recommended that patients with suspected (but culture negative) *Bartonella* endocarditis receive treatment with gentamicin for the first 2 weeks and ceftriaxone with or without doxycycline (Habib et al. 2009; Raoult et al. 2003; Prutsky et al. 2013).

Considering feline bartonellosis, documenting clearance of *Bartonella* infections through antibiotic treatment is difficult because of the relapsing nature of the bacteremia. Treatment seems to require long-term (at least 4–6 weeks) antibiotic administration. No regimen of antibiotic treatment has been proved effective for definitively eliminating *Bartonella* infections in cats (Greene et al. 1996; Guptill 2010; Regnery et al. 1996). Enrofloxacin (3.5–11.4 mg/kg given by mouth every 12 h) treatment for 28 days appeared to be efficient for *B. henselae* or *B. clarridgeiae* (Kordick et al. 1997). However, enrofloxacin causes retinal degeneration and blindness in some cats when administered at more than 5 mg/kg/day, and use of a higher dose is contraindicated. In addition, doxycycline seems to clear *B. henselae* or *B. clarridgeiae* infection in cats, in which higher doses (e.g., 50 mg/cat by mouth every 12 h for 1 week) may be more likely to be effective (Greene et al. 1996; Kordick et al. 1997). Other antibiotics have also been tested (erythromycin, amoxicillin, amoxicillin/clavulanic acid, tetracycline hydrochloride) and proven to decrease the level of bacteremia in treated cats, although cats were not followed by a prolonged period of treatment (Guptill 2010). Rifampin should not be used alone because resistance develops quickly (Biswas et al. 2008); however, combination with doxycycline has been recommended. Azithromycin has been widely used to treat feline *Bartonella* infections, but there are no data from controlled studies to support this practice, and its efficacy may be limited (Guptill 2010). Additionally, azithromycin is not recommended as a first-line antibiotic for cats and dogs, due to the rapid development of resistance when compared to several other antibiotic classes. Considering that there are differences in antibiotic treatment protocols and that routine treatment of asymptomatic feline *Bartonella* infections may induce resistant strains, the treatment

is recommended only for cats showing clinical signs of disease, and doxycycline may be the antibiotic of choice (Guptill 2010). Experimental and clinical evidence in treatment of cats and dogs supports the lack of efficacy for doxycycline, particularly when given for periods of, at least, 4 weeks at 5 mg/kg every 12 h (i.e., the recommended dose and treatment period for canine ehrlichiosis). Thus, the combination of doxycycline and amikacin would be a reasonable initial antibiotic combination to treat canine or feline endocarditis cases. For long-term therapy, doxycycline and enrofloxacin should be considered, until other antibiotics or antibiotic combinations are proven to be effective. Ciprofloxacin has less favorable MICs compared to enrofloxacin and pradofloxacin, and because of variable intestinal absorption following oral administration, ciprofloxacin is not recommended for treatment of bartonellosis in dogs (Diniz et al. 2009). For canine cases with central nervous system involvement, doxycycline and rifampin in combination have been used successfully.

Application

There are very few evidence in the literature involving directly application of *Bartonella* species. However, some of them are used for serological diagnosis of species which cause important disease in humans. Several serodiagnostic methods, including Western blotting, enzyme-linked immunosorbent assay (ELISA), and indirect immunofluorescent antibody (IFA) assay, have been proposed (Chomel et al. 1995; Chamberlin et al. 2000; Matsuoka et al. 2013; Regnery et al. 1992; Tea et al. 2003). IFA assay is most commonly used for routine clinical diagnosis of Bartonella infections, using fixed bacterial cells cocultured with Vero cells on a slide as the antigen. Commercial kits are also available for the diagnosis of *B. henselae* (e.g., MRL Diagnostics) and *B. quintana* (e.g., Focus technologies, USA) (Seki et al. 2006; Zbinden et al. 1997). However, cross-reactivity with other species has been reported (Maurin et al. 1997; McGill et al. 1998; Regnery et al. 1992). Recombinant antigens, such as immunoreactive protein P26 of *B. henselae*, have also been used in ELISAs, resulting in a more sensitive test than *B. henselae* type I outer membrane proteins (Chenoweth et al. 2004b; Feng et al. 2009). Accordingly, use of a recombinant B. henselae 17-kDa antigen (the r17-kDa antigen) was used for the development of an IgG and an IgM capture-based ELISA for the detection of *B. henselae* infections, with good results of sensitivity and specificity (Anderson et al. 1995; Loa et al. 2006; Hoey et al. 2009). Recently, Matsuoka and colleagues (2013) reported that synthetic peptides HbpB2 (hemin-binding protein B) and HbpD3 (hemin-binding protein D) might be suitable for developing serological tools for differential diagnosis of *B. quintana* infections. Additionally, the potential application of affinity-purified recombinant Pap31 in ELISA for the diagnosis of *B. bacilliformis* infection has been demonstrated (Taye et al. 2005).

The development of a vaccine to prevent feline infection is warranted to reduce the prevalence of infection in the feline population and consequently to decrease the potential for zoonotic transmission. Several antigenic *B. henselae* proteins have been described, including the recombinant immunoreactive protein P26 of *B. henselae*, but to date, no effective protection has been achieved (Anderson et al. 1995; Feng et al. 2009; Kabeya et al. 2003; Werner et al. 2006).

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4 The Family *Beijerinckiaceae*

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Abstract
To date there are only 18 established species in the family *Beijerinckiaceae* and only 12 if outgroup members are not taken into account. This small number does not impede to consider them quite interesting from the metabolic and evolutionary point of view since it encompasses from obligate methanotrophs to chemoorganoheterotrophs, plus examples of the intermediate states: facultative methylotrophs and facultative methanotrophs. Nitrogen fixation is another remarkable trait of the family, enabling them to thrive in habitats in which other potential sources of nitrogen are scarce. Their global distribution is wide, with a preference for acidic soils of tropical regions (in the case of *Beijerinckia*) or of temperate and even polar regions in the case of *Methylocapsa*, *Methylocella*, *Methyloferula*, *Methylorosula*, and *Methylovirgula*.

Taxonomy, Historical and Current

The family *Beijerinckiaceae* belongs to the order *Rhizobiales* within the *Alphaproteobacteria* (Garrity et al. 2005). At the time of its proposal contained the genera *Beijerinckia*, *Chelatococcus*, *Methylocapsa*, and *Methylocella* and was described as a metabolically diverse family of aerobic bacteria capable of forming poly-β-hydroxybutyrate granules and fixing nitrogen (*Beijerinckia* and *Methylocella*). To date, four more genera have been formally described within this family: *Camelimonas*, *Methyloferula*, *Methylorosula*, and *Methylovirgula*. However, the phylogenetic analysis taking into account the newly described taxa (also within neighboring families) reveals that two of the eight genera, namely, *Camelimonas* and *Chelatococcus*, occupy an uncertain position close to *Bosea* spp. (family *Bradyrhizobiaceae*) and *Microvirga* (family *Methylobacteriaceae*) (◉ *Fig. 4.1*).

From the historical point of view, there is a clear separation in the taxonomic activity of the different genera. The type genus, *Beijerinckia*, named to honor the Dutch pioneering microbiologist Martinus Willem Beijerinck (1851–1931), has deep roots (Starkey and De 1939; Derx 1950a). There is account of intense research going on for decades concerning isolation of *Beijerinckia* sp. from different habitats and characterization by different methods (Becking 2006) and its beneficial role as nitrogen fixers. However, studies become infrequent after the nineties and there is only one species description of *Beijerinckia* in this century (Oggerin et al. 2009). For the other genera it is just the opposite since all descriptions appeared from 2000 on with the only exception of *Chelatococcus asaccharovorans* (Auling et al. 1993). The push of knowledge in recent times, and not only taxonomic, has been especially important in the case of the methanotrophic and methylotrophic aerobic bacteria of the genera *Methylocapsa*, *Methylocella*, *Methyloferula*, *Methylorosula*, and *Methylovirgula*, whose ecological importance and interest for understanding evolutionary processes go beyond the possibilities of this chapter.

Members of the family *Beijerinckiaceae* include chemoorganoheterotrophs, facultative methylotrophs, facultative methanotrophs, and obligate methanotrophs (◉ *Table 4.1*). The ability to utilize multicarbon compounds is variable but is generally broader in the chemoorganoheterotrophs, then facultative methylotrophs, and then facultative methanotrophs. Other important aspects related with C_1 compounds metabolism such as the carbon assimilation pathway or the type of methane monooxygenase, in those species affected, are additional sources of variability (◉ *Table 4.1*).

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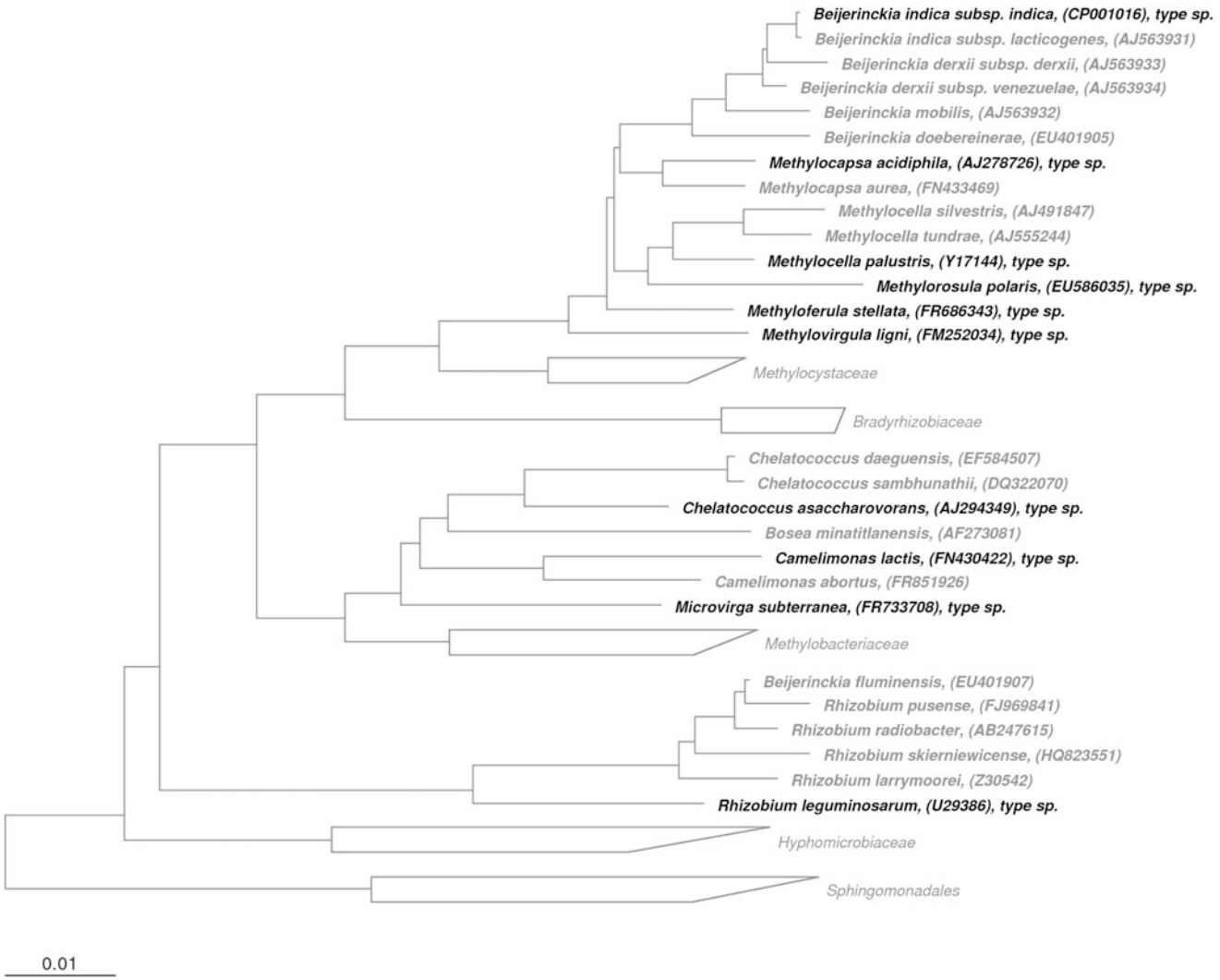

Fig. 4.1

Phylogenetic reconstruction of the family *Beijerinckiaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes–Cantor correction. The sequence data sets and alignments were used according to the All-Species Living Tree Project (*LTP*) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

Formation of poly-β-hydroxybutyrate granules is still a general trait, followed by exopolysaccharide production and nitrogen-fixing activity, with one and two exceptions known, respectively. Unfortunately, these three traits have not been determined in all species, and the main data gap occurs in the uncertain position genera *Camelimonas* and *Chelatococcus*, with no or only partial data, respectively (*Table 4.1*). In contrast, some of the characteristics determined in them (e.g., polyamine pattern or polar lipid composition) have not been explored in the other genera and therefore comparison is not possible. If we leave aside these two genera, the DNA G+C content is 55–63 mol %, a range that could well fit for a single genus.

All members of the family *Beijerinckiaceae* are aerobic but *C. daeguensis* and *C. sambhunathii* can also grow anaerobically with nitrate (Panday and Das 2010; Yoon et al. 2008). Growth factor requirements have been only reported in two species, *C. asaccharovorans* (Auling et al. 1993) and *M. ligni* (Vorob'ev et al. 2009).

During the course of a research project with free-living nitrogen-fixing bacteria, Oggerin et al. (2009) confirmed that strains *B. fluminensis* UQM 1685^T and *B. fluminensis* CIP 106281^T had the same origin but did not descend from the nomenclatural type of the species since they exhibit important phenotypic and genotypic differences

Table 4.1
Genera of the family *Beijerinckiaceae* and diagnostic traits

	1	2	3	4	5	6	7	8
Cell morphology	Rods	Rods	Cocci or rods	Curved coccoids or rods	Rods or ovoids	Rods	Rods	Rods
Metabolism[a]	COH/ FMlo	COH	COH	OMno/FMno	FMno	OMno	FMlo	FMlo
Multicarbon compounds used	Sugars and others	Sugars and others	Sugars and others	None or acetate	Acetate, ethanol, pyruvate, succinate, malate	None	Sugars and others	Ethanol, pyruvate, malate
C_1 assimilation pathway	RuBP	–	–	Serine	Serine	RuBP	RuBP	RuBP
Methane monooxygenase type	–	–	–	pMMO	sMMO	sMMO	–	–
PHB synthesis	+	ND	+[b]	+	+	+	ND	+
Exopolysaccharides	+	ND	+[b]	+	+	ND	+	–
Nitrogen fixation	+	ND	–[b]	+	+	+	–	+
G+C content (mol%)	54–61	65[c]	63–68	61–63	60–63	56–57	65	62–63

1, *Beijerinckia* (Derx 1950a, b; Döbereiner and Ruschel 1958; Oggerin et al. 2009, 2011; Starkey and De 1939; Tchan 1957; Thompson and Skerman 1979; Dedysh et al. 2005b); 2, *Camelimonas* (Kämpfer et al. 2010, 2012); 3, *Chelatococcus* (Auling et al. 1993; Panday and Das 2010; Yoon et al. 2008); 4, *Methylocapsa* (Dedysh et al. 2002; Dunfield et al. 2010); 5, *Methylocella* (Dedysh et al. 2000, 2004a; Dunfield et al. 2003); 6, *Methyloferula* (Vorobev et al. 2011); 7, *Methylorosula* (Berestovskaya et al. 2012); 8, *Methylovirgula* (Vorob'ev et al. 2009)
+ positive, *ND* not determined, – negative
[a]*COH* chemoorganoheterotrophs (non-methylotrophic), *FMno* facultative methanotroph, *FMlo* facultative methylotroph, *OMno* obligate methanotroph
[b]Reported for *Chelatococcus asaccharovorans* but not for *C. daeguensis* or *C. sambhunathii*
[c]Reported for *Camelimonas lactis* but not for *C. abortus*

when compared with the descriptions of *B. fluminensis* or any of the other species of the genus *Beijerinckia*. As a matter of fact, they were identified as *Rhizobium radiobacter* (Fig. 4.1). All other available equivalent strains (DSM 2327^T and CCUG 53676^T) were confirmed too as *R. radiobacter* (Oggerin et al. 2011). Subsequent attempts to find older deposits of the type strain, hopefully derived from the original isolate, or other existing strains of *B. fluminensis* that could be proposed as a neotype strain were fruitless, and the bibliographic search of the key publications (Döbereiner and Ruschel 1958; Hilger 1965; Skerman et al. 1980; Thompson and Skerman 1979) did not provide enough traceability or hints to locate such biological material. According to Rule 18c of the International Code of Nomenclature of Bacteria (Lapage et al. 1992), if a suitable replacement type strain or a neotype cannot be found or proposed, respectively, within 2 years of the publication of the request for an opinion (Oggerin et al. 2011), the Judicial Commission of the International Committee on Systematic of Prokaryotes is entitled to place the name *Beijerinckia fluminensis* on the list of rejected names. The next foreseeable meeting of the Judicial Commission is during the celebration of the 14th International Congress of Bacteriology and Applied Microbiology to be held in Montreal, Canada, from July 27 to August 1, 2014.

Subspeciation has only been proposed in the genus *Beijerinckia*, namely, in the species *B. derxii* and *B. indica* (Thompson and Skerman 1979).

Molecular Analyses

DDH

To the best of our knowledge, DDH methods have never been reported in the genus *Beijerinckia*, in spite of having the largest number of species, not in the monospecific genera *Methyloferula*, *Methylorosula*, and *Methylovirgula*. In the remaining four genera of the family *Beijerinckiaceae*, we find several examples.

Thus, the proposal of *Chelatococcus asaccharovorans* as novel genus and species (Auling et al. 1993) was based on two strains, TE1 and TE2^T, that could not be discriminated by DDH. However, much lower values (less than 30 %) were obtained against other nine nitrilotriacetate (NTA)-utilizing strains that were proposed in the same study as *Chelatobacter heintzii* gen. nov., sp. nov. For about 15 years, the genus contained one single species, but recently it was enlarged twice and in both cases DDH was considered necessary. The mean value obtained between

C. daeguensis K106^T and *C. asaccharovorans* DSM 6462^T was 13 % (Yoon et al. 2008), whereas *C. sambhunathii* HT4^T displayed 52 and 20 % to *C. daeguensis* CCUG 54519^T and *C. asaccharovorans* DSM 6462^T, respectively (Panday and Das 2010).

The description of *Methylocapsa acidiphila* (Dedysh et al. 2002) included the DDH value between the type strain B2^T and *Methylocella palustris* K^T (7 %). However, in the description of the second species of the genus, *M. aurea* (Dunfield et al. 2010), this information was absent.

In the case of the genus *Methylocella*, DDH was included in the proposal of the second and third species: *M. silvestris* BL2^T was confronted to *M. palustris* K^T resulting in 21–22 % values (Dunfield et al. 2003) and, later on, *M. tundrae* T4^T was hybridized to *M. silvestris* BL2^T and *M. palustris* K^T resulting in values of 36 and 27 %, respectively.

When proposing *Camelimonas lactis* as new genus and species based on strains M-2040^T, M 1973, and M 1878-SK2, Kämpfer et al. (2010) performed DDH experiments and obtained relatedness values among them ranging from 89 % to 100 %. The three strains where also confronted to *Chelatococcus asaccharovorans* DSM 6462^T and *Chelatococcus daeguensis* CCUG 54519^T resulting in values from 22.3 % to 49.2 % and 18.5 % to 49.6 %, respectively (reciprocal measurements included). Later, the proposal of the second species of the genus *Camelimonas* was supported by DNA–DNA relatedness values of 18.0 % and 13.6 % (reciprocal analysis) between *C. abortus* UK34/07-5^T and *C. lactis* M 2040^T (Kämpfer et al. 2012).

Molecular Phylogenetic Markers

An important metabolic ability in many *Beijerinckiaceae*, as has been already mentioned, is dinitrogen fixation that allows them to thrive in environments depleted in available nitrogen compounds. The sequences of *nifH* and *nifD* gene fragments from representatives of the genera *Methylocella*, *Methylocapsa*, *Beijerinckia*, together with some outgroup methanotrophs have been used to withdraw phylogenetic relationships (Dedysh et al. 2004b). The trees constructed for the inferred amino acid sequences of *nifH* and *nifD* were highly congruent. The phylogenetic relationships among methanotrophs in the NifH and NifD trees also agreed well with the corresponding 16S rRNA-based phylogeny, but two discrepancies were found. One suggested that an ancient event of lateral gene transfer was responsible for the aberrant branching of *Methylococcus capsulatus*. On the other hand, the identity values of NifH and NifD sequences between *Methylocapsa acidiphila* B2^T and representatives of *Beijerinckia* were clearly higher (98.5 % and 96.6 %, respectively) than would be expected from their 16S rRNA-based relationships. This can be taken as an indication that these two bacteria originated from a common acidophilic dinitrogen-fixing ancestor and were subject to similar evolutionary pressure with regard to nitrogen acquisition. This interpretation is corroborated by the observation that, in contrast to most other diazotrophs, *M. acidiphila* and *Beijerinckia* spp. are capable of active growth on nitrogen-free media under fully aerobic conditions.

In addition to these genes, the key genes of C_1 metabolism have also been widely used as phylogenetic and functional markers in a number of taxonomic and ecologic studies (Berestovskaya et al. 2012; Dedysh et al. 1998b, 2000, 2001b, 2002, 2004a; Dunfield et al. 2003, 2010; Lau et al. 2013; McDonald and Murrell 1997a, b; Vorob'ev et al. 2009; Vorobev et al. 2011).

The first step of bacterial methane oxidation is catalyzed by methane monooxygenase (MMO), which occurs in two forms: a membrane-bound or particulate (pMMO) and a cytoplasmic or soluble (sMMO). Virtually all known methanotrophs except perhaps, the *Methylocella* species, possess pMMO that consists of three membrane-associated polypeptides encoded by *pmoC*, *pmoA*, and *pmoB* (McDonald and Murrell 1997b; Murrell et al. 2000; Pacheco-Oliver et al. 2002). In addition to pMMO, several methanotrophs possess sMMO. The enzyme from strains belonging to *Methylosinus*, *Methylocystis*, and *Methylococcus* has been thoroughly studied, and the nucleotide sequence of the sMMO gene cluster *mmoX*, *mmoY*, *mmoB*, *mmoZ*, *mmoC*, and *mmoD* appears to be highly conserved (Trotsenko and Khmelenina 2005). The *pmoA* gene encoding a 26-kDa subunit that harbors the active site of the pMMO and *mmoX* gene coding for α-subunit of the sMMO hydroxylase component has been used as appropriate gene markers for the occurrence of the enzymes in various methanotrophs.

Methanol dehydrogenase (MDH), the second enzyme involved in methane oxidation, is present in all Gram-negative methylotrophs including methane and methanol utilizers, and *mxaF* is an appropriate indicator gene for their occurrence in the natural environment (McDonald and Murrell 1997a). Its potential as a functional and phylogenetic marker for methanotrophs has been recently addressed (Lau et al. 2013). This gene codes for the large α-subunit of methanol dehydrogenase and is highly conserved among distantly related methylotrophic species in the alpha-, beta-, and gammaproteobacteria. Topology tests revealed that *mxaF* and 16S rRNA genes of methanotrophs do not show congruent evolutionary histories. Moreover, the *mxaF* gene has poor resolving power for methanotrophs within the *Beijerinckiaceae* (genera *Methylocapsa*, *Methylocella*, and *Methyloferula* in this study), although it performed well for the families *Methylocystaceae* and *Methylococcaceae*. The polyphyletic nature seen for the *Beijerinckiaceae* and the fact that it includes obligate and facultative methylotrophs give rise to various hypothesis: (i) methanotrophy arose once in the group and was lost by some methylotrophic taxa, (b) methanotrophy arose separately in more than one taxon in the group, and/or (c) multiple occurrences of horizontal gene transfers have occurred in the common ancestor of methanotrophic *Beijerinckiaceae*. It therefore is difficult to ascertain whether microorganisms with *mxaF* gene sequences placed near the polyphyletic *Beijerinckiaceae* genera *Methylocapsa*, *Methylocella*, and *Methyloferula* are indeed methanotrophic.

Genomic Data

The first two complete genome sequences to be published in the family came in 2010, both obtained from type strains of separate species and genera.

On one hand, *Methylocella silvestris* BL2^T (Chen et al. 2010) was a judicious choice since it brought as well the first genome available for an alphaproteobacterial methanotroph. Moreover, the organism itself is interesting because it was the first fully authenticated example of facultative methanotroph growing not only on methane and other one-carbon (C_1) substrates but also on some compounds containing carbon–carbon bonds, such as acetate, pyruvate, propane, and succinate (Dedysh et al. 2005a). The genome is a circular chromosome of 4.31 Mbp (CP001280) and a G+C content of 63.1 mol%. In total, 3,917 candidate genes were predicted and 99 pseudogenes were found. Functionality was assigned to 67.9 % of the genes, while 30.9 % of the genes could not be assigned any known function. All tRNA-encoding regions were identified, and two identical rRNA operons were found (Chen et al. 2010). In agreement with previous knowledge of the metabolic capabilities of the organism, the genome analysis permitted to conclusively verify (i) the absence of any *pmoCAB* genes encoding a pMMO enzyme that is present in all other genera of methanotrophs and (ii) the presence of a complete operon encoding sMMO (*mmoXYBZDC*), a complete operon encoding methanol dehydrogenase (*mxaFJGIRSACKLDEH*), and all genes necessary for fixation of methane-derived carbon via the serine cycle. Genes encoding key enzymes in both the tetrahydrofolate and the tetrahydromethanopterin-mediated formaldehyde oxidation pathways were found. Acetate kinase- and phosphotransacetylase-encoding genes are present, allowing acetate to be fed into the tricarboxylic acid (TCA) cycle. Genes encoding glyoxylate bypass enzymes (i.e., isocitrate lyase and malate synthase), essential for bacteria growing on C_2 compounds, were also identified. A full gene set encoding enzymes of the TCA cycle is also present, including genes encoding α-ketoglutarate dehydrogenase, which are lacking in some methanotrophs. Interestingly, a gene cluster encoding di-iron-containing multicomponent propane monooxygenase is also present (Chen et al. 2010).

On the other hand, *B. indica* subsp. *indica* ATCC 9039^T was also a wise choice for its phylogenetic proximity yet different metabolism. Its genome consists of a chromosome of 4,170,153 bp and two plasmids of 181,736 and 66,727 bp (CP001016, CP001017, and CP001018, respectively). There are a total of 3,982 open reading frames predicted, of which 3,784 are predicted protein-coding genes and 2,695 (70 %) have been assigned a predicted function (Tamas et al. 2010). There are 134 pseudogenes, 52 tRNA genes, and three operons each containing 16S, 23S, and 5S rRNA genes. The G+C content is 57.0 % (56 % and 54 % in the plasmids). The initial analysis (Tamas et al. 2010) revealed that this strain lacks phosphofructokinase, the key enzyme of the Embden–Meyerhof pathway. Instead, it uses the Entner–Doudoroff or pentose phosphate pathway to catabolize sugars, which is typical of free-living *Rhizobiales*. The majority of the genes involved in N_2 fixation are clustered in two genomic islands (10 and 51 kb), with the notable exception of the *nifS* gene encoding cysteine desulfurase. *B. indica* is a metabolically versatile bacterium capable of growth on a variety of organic acids, sugars, and alcohols, while *M. silvestris* is a highly specialized methanotrophs capable of growth on very few substrates (Dedysh et al. 2005a). However, their genome sizes (4.17 vs. 4.30 Mbp, respectively) and the numbers of predicted protein-encoding genes (3,788 vs. 3,917, respectively) are remarkably similar. A BLAST analysis indicated that the 57 % of the genes in the genome of *B. indica* have homologues in *M. silvestris* (Tamas et al. 2010). Some key pathways of one-carbon metabolism (such as the tetrahydromethanopterin and serine pathways of formaldehyde metabolism) that are present in *M. silvestris* appear to be absent or incomplete in *B. indica*, which confirms previous experiments showing that the organism is incapable of methylotrophic growth (Dedysh et al. 2005b). However, an operon encoding a putative propane monooxygenase homologous to soluble propane/methane monooxygenases of *M. silvestris* BL2^T was identified. More in-depth comparison of these genomes will help elucidate what defines their distinct lifestyles.

Meanwhile three more genomic sequences (scaffolds or contigs) have become available. One corresponds to *Chelatococcus* sp. GW1, a crude-oil degrading strain that has 5.21 Mb and a G+C content of 68.7 mol%. The other two are from the types of two obligate methanotrophs, *Methylocapsa acidiphila* B2^T and *Methyloferula stellata* AR4^T, with sizes of 4.10 and 4.24 Mb and G+C contents of 61.8 and 59.5 mol%, respectively. This expands considerably the possibilities of performing comparative genome analyses for the study and understanding of methanotrophy.

Using a combination of proteomic, biochemical, and genetic approaches, the identification and characterization of trimethylamine (TMA) monooxygenase gene (*tmm*) from *M. silvestris* have been reported (Chen et al. 2011). This enzyme is an example of bacterial flavin-containing monooxygenase, a system much better studied in eukaryotes and with many important physiological functions. It contained the conserved sequence motif and typical flavin adenine dinucleotide and nicotinamide adenine dinucleotide phosphate-binding domains. It was highly expressed in TMA-grown *M. silvestris* and absent during growth on methanol. Indeed, the ability to use TMA as a sole carbon and/or nitrogen source is directly linked to the presence of *tmm* in the genomes as shown by recombination and mutagenesis experiments. Close homologues of *tmm* occur in many *Alphaproteobacteria*, in particular *Rhodobacteraceae* (marine *Roseobacter* clade) and the marine SAR11 clade (*Pelagibacter ubique*). It is highly abundant in the metagenomes of the Global Ocean Sampling expedition, and it has been estimated that 20 % of the bacteria in the surface ocean contain *tmm*, playing an important role in the global carbon and nitrogen cycles (Chen et al. 2011).

By comparing the genomes of the generalist organotroph *B. indica*, the facultative methanotroph *M. silvestris*, and the obligate methanotroph *M. acidiphila*, Tamas et al. (2014) obtained the first insights into the evolution of the family *Beijerinckiaceae*

Table 4.2

Differential traits between *Beijerinckia* species (Becking 2006; Dedysh et al. 2005b; Derx 1950a, b; Döbereiner and Ruschel 1958; Kennedy 2005; Oggerin et al. 2009, 2011; Starkey and De 1939; Tchan 1957; Thompson and Skerman 1979)

	B. indica	*B. derxii*	*B. doebereinerae*	*B. fluminensis*	*B. mobilis*
Cell size (μm)	0.5–1.2 × 1.6–3.0	1.5–2.0 × 3.5–4.5	1.0 × 3.25	1.0–1.5 × 3.0–3.5	0.6–1.0 × 1.6–3.0
Water-soluble, green fluorescent pigment	–	+	–	–	–
Colony color after aging	Pink	Beige	Cream	Amber brown	Brown
Motility	–/w[a]	–	–	–/w[a]	+
pH range for growth (optimal)	3.0–10.0 (4.0–10.0)	4.0–9.0 (6.0–7.0)	3.0–10.0 (6.5)	3.5–9.2 (ND)	3.0–10.0 (4.0–5.0)
Urease	+	+	–	–	+
Assimilation of					
Fructose	+	+	+	+	–
Maltose	–	+	+	v	–
Mannose	+	–	+	v	–
Sorbose	+	v	–	+	+
Xylose	–	–	–	+	–
Glycerol	+	–	–	+	+
Sorbitol	+	+	–	+	–
DNA G+C content (mol%)	54.7–58.8	57.5–60.7	57.1	54.4–58.0	57.3

[a]Negative or weak and only in young cultures

Fatty acid methyl ester profiles of *B. indica* LMG 2817^T, *B. doebereinerae* LMG 2819^T, and *B. mobilis* LMG 3912^T were obtained by Oggerin et al. (2009). The only major fatty acid was C18:1 ω7c accounting 86.0–92.4 %. C16:0 was the only minor fatty acid present in all three strains (at amounts between 2.8 and 6.9 %), the rest being present only in one or two strains and representing up to 7.0 % of the total composition: C17:0 iso, C18:0, C16:1 and/or C15:0 iso 2-OH, and C17:1 iso and/or anteiso

and into the trade-offs required for a specialist methanotrophic lifestyle compared with a generalist chemoorganotrophic lifestyle. Thus, highly resolved phylogenetic construction based on 29 universally conserved genes demonstrated that the *Beijerinckiaceae* forms a monophyletic cluster with the *Methylocystaceae* (each represented by three genomes in this study), the only other family of alphaproteobacterial methanotrophs. For methanotrophy and methylotrophy genes, a vertical inheritance pattern within these families could be seen. At the same time, many lateral gene transfer events were detected in the genome of *B. indica* for genes encoding carbohydrate transport and metabolism, energy production and conversion, and transcriptional regulation, suggesting that they have been acquired recently. *B. indica* also had a larger abundance of transporter elements, particularly periplasmic-binding proteins and major facilitator transporters. Thus, the authors conclude that the most parsimonious scenario for the evolution of methanotrophy in the *Alphaproteobacteria* is that it occurred only once, when a methylotroph acquired methane monooxygenases via lateral gene transfer. This was supported by a compositional analysis suggesting that all methane monooxygenases in *Alphaproteobacteria* methanotrophs are foreign in origin. Some members of the *Beijerinckiaceae* subsequently lost methanotrophic functions and regained the ability to grow on multicarbon energy substrates. According to that, *B. indica* is a recidivist multitroph, the only known example of a bacterium having completely abandoned an evolved lifestyle of specialized methanotrophy (Tamas et al. 2014).

Phenotypic Analyses

Beijerinckia

Formally, the description of the genus is that of Derx (1950a), although some traits have been studied later (Becking 2006; Kennedy 2005). *Beijerinckia* species are nonsymbiotic, aerobic, chemoheterotrophic bacteria with the ability to fix atmospheric dinitrogen. They utilize a wide range of multicarbon compounds, but sugars are the preferred growth substrates. Members of this genus are straight or slightly curved rods with rounded ends containing polar lipoid bodies (PHB). Cells occur singly or appear as dividing pairs. Individual cells measure 0.5–2.0 × 1.6–4.5 μm (*Table 4.2*), although larger misshapen cells can also be seen. Cysts (enclosing one cell) and capsules (enclosing several cells) may occur in some species. Gram negative. Motile by peritrichous flagella or nonmotile. Aerobic, having a strictly respiratory type of metabolism. N_2 is fixed under aerobic or microaerobic conditions. Growth occurs between 10°C and 35 °C (optimal 20–30 °C); no growth occurs at 37 °C. Growth occurs between pH 3.0 and 10.0. Liquid cultures can become a highly viscous, semitransparent mass; in

some species the whole medium becomes opalescent and turbid, and adhering slime is not produced. On agar media, especially under N_2-fixing conditions, copious slime is produced and giant colonies with a smooth, folded, or plicate surface develop; some strains form slime having a more granular consistency. Catalase positive. Glucose, fructose, and sucrose are utilized by all strains and oxidized to CO_2. No growth occurs on peptone medium. Glutamate is utilized poorly or not at all. Negative for indole production. The G+C of the DNA is 54.4–60.7 mol%.

The chemical structure of the water-soluble polysaccharide, CV-70, produced by one strain of *Beijerinckia* sp. was defined applying different analytical methods. Glucose, galactose, and fucose were identified as the components in the CV-70 polysaccharide, in a 3:1:3 ratio (Scamparini et al. 1997).

A recent study (Jendrossek et al. 2007) has shown that the location of poly-3-hydroxybutyrate granules in *B. indica* and other bacteria is nonrandom. The early stages of formation were examined by confocal laser scanning fluorescence microscopy of Nile red-stained cells and by transmission electron microscopy. Cells of this species harbored one PHB granule at each cell pole, often close to or even in physical contact with the cytoplasmic membrane.

As methanotrophy was being uncovered in the family *Beijerinckiaceae* and given the close phylogenetic distance between *Beijerinckia* and the new genera, and the fact that some of the new species were facultative and not obligate methanotrophs, it became more imperative to check for C_1 metabolism in the genus *Beijerinckia*. Dedysh et al. (2005b) tested five type strains of different *Beijerinckia* species/subspecies (*B. mobilis* DSM 2326^T, *B. indica* subsp. *indica* ATCC 9039^T, *B. indica* subsp. *lacticogenes* DSM 1719^T, *B. derxii* subsp. *derxii* DSM 2328^T, and *B. derxii* subsp. *venezuelae* DSM 2329^T) for their ability to grow on methanol as the sole carbon and energy source in either nitrogen-free medium or supplemented with KNO_3. The only species capable of growth on methanol was *B. mobilis*. Growth occurred under a wide range of methanol concentrations ranging from 0.01 % to 3 % (vol/vol) and was optimal between 0.05 % and 0.5 % (vol/vol) CH_3OH. Growth was obtained under both nitrogen-fixing and nitrogen-sufficient conditions and could be maintained continuously without loss of viability. The enzymatic activities in the metabolism of methanol and glucose have been measured (Smirnova et al. 2005) confirming that *B. mobilis* possesses all the enzymes necessary for oxidation of methanol to CO_2 via formaldehyde and formate coupled with the ribulose bisphosphate (RuBP) pathway of C_1 assimilation. Therefore, *B. mobilis* is a facultative chemoautotrophic methylotroph that in addition to growth on C_1-reduced compounds (methanol and formate) has the ability to use a wide range of multicarbon substrates. These studies also demonstrate that there is more metabolic versatility within representatives of the genus *Beijerinckia* than was previously thought. Although methylotrophic autotrophy could be attributed to only one species of this genus, future studies may extend the number of *Beijerinckia* species capable of C_1 metabolism. In this sense, it is important to mention that *B. doebereinerae* (Oggerin et al. 2009) was described after the publication of these studies and occupies a position that is intermediate between *B. mobilis* and *Methylocapsa* spp. (Fig. 4.1).

Table 4.3
Differential traits between *C. lactis* (Kämpfer et al. 2010) and *C. abortus* (Kämpfer et al. 2012)

	C. lactis	*C. abortus*
Growth on MacConkey agar	+	–
Utilization of		
Trehalose	–	+
4-Aminobutyrate	+	–
Citrate	+	–
Fumarate	+	–
Itaconate	+	–
DL-Lactate	+	–
Mesaconate	+	–
L-Alanine	+	–
Presence of		
Unidentified glycolipid	+	–
Unidentified phospholipid	–	+

Camelimonas

Cells of *Camelimonas* (Kämpfer et al. 2010) are Gram-negative, nonmotile, nonspore-forming rods, approximately 2 µm long and 1 µm wide. Oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, PYE agar, and nutrient agar at 25–30 °C. Beige, translucent, and shiny colonies with entire edges are formed within 24 h, with a diameter of about 2 mm. The characteristic peptidoglycan diamino acid is meso-diaminopimelic acid. The predominant compound in the polyamine pattern is spermidine, and sym-homospermidine is absent. The quinone system is ubiquinone Q-10. The polar lipid profile includes the major compounds phosphatidylcholine and diphosphatidylglycerol and moderate amounts of phosphatidylethanolamine, phosphatidylglycerol, an unidentified glycolipid, and two unidentified aminolipids. The major fatty acids are C19:0 cyclo ω8c, C18:1 ω7c, and C16:0, with C18:0 3-OH as the major hydroxylated fatty acid. The G+C content of the DNA of the type strain of the type species is 65 mol%.

The genus contains two species, *C. lactis* (type species) and *C. abortus*, that can be differentiated according to the traits in Table 4.3.

Chelatococcus

Description of the genus *Chelatococcus* is based on Auling et al. (1993) and the emendation by Yoon et al. (2008). Cells are cocci

Table 4.4
Differential traits between *C. asaccharovorans* (Auling et al. 1993), *C. daeguensis* (Yoon et al. 2008), and *C. sambhunathii* (Panday and Das 2010)

	C. asaccharovorans	*C. daeguensis*	*C. sambhunathii*
Cell morphology	Cocci	Rods	Rods
Motility	–	+	+
Hydrolysis of gelatin	+	–	+
NaCl tolerance range (w/v)	0.0–2.5	0.0–0.5	0.0–3.0
Utilization of			
Mannose, xylose, gluconate, D-arabinose, cysteine	+	+	–
Glycerol, salicin, inositol, adonitol, cellobiose, xylitol, aesculin, rhamnose, galactose, methionine, threonine, tyrosine, lysine	–	+	–
Glycine	+	–	+
Nitrilotriacetate	+	–	+
Acid production from			
Glucose, inositol, arabinose, rhamnose	–	+	–
pH range for growth	5.5–9.5	5.5–10.0	6.0–8.5
pH optimum for growth	7.0–8.0	7.0–7.5	7.5–8.0

or rods (Table 4.4). Gram negative. Nonmotile. Obligately aerobic. Optimal growth temperature is 35–37 °C and temperature range for growth is 4–41 °C. Poly-β-hydroxybutyrate is accumulated within the cells. Utilization of the metal-chelating nitrilotriacetic acid (NTA) as sole source of carbon, nitrogen, and energy is positive or negative. Ubiquinone Q-10 is present. The major fatty acids are C18:1 ω7c and C19:0 cyclo ω8c. Major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, two aminolipids, and two unidentified phospholipids. Major polyamines are sym-homospermidine, spermidine, and putrescine. The DNA G+C content is 63.3–68.3 mol%.

The type species, *C. asaccharovorans*, requires one or more vitamins (Auling et al. 1993), whereas growth factors are not needed by the two other established species of the genus. Besides the typical Gram-negative cell envelope (cytoplasmic membrane, murein layer, and outer membrane), *C. asaccharovorans* is surrounded by an additional proteinaceous S-layer approximately 15 nm wide.

There are some traits of the genus description, not formally emended though, that are discrepant in *C. daeguensis* and *C. sambhunathii*. These include temperature growth range (20–50 °C on both species), anaerobic growth with nitrate (both species), and motility (*C. sambhunathii* has one polar flagellum).

Methylocapsa

The description of *Methylocapsa* is that of Dedysh et al. (2002) later emended by Dunfield et al. (2010). Gram-negative, curved coccoids or thick curved rods (Table 4.5) that occur singly or in conglomerates, but do not form rosettes. Surrounded by an extracellular polysaccharide matrix (the etymologic meaning of the genus name is methyl-using cell covered by a capsule). Reproduce by normal cell division. Nonmotile. Produce intracellular poly-β-hydroxybutyrate granules. Resting cells are *Azotobacter* type cysts. Cells are not lysed by 2 % SDS. Cells possess a well-developed intracytoplasmic membrane system of type III which appears as stacks of membrane vesicles packed in parallel on only one side of the cell membrane. Strictly aerobic. Possess pMMO and do not express sMMO. Moderately acidophilic and mesophilic. Prefers dilute media of low salt content. Some members of the genus are obligate utilizers of C_1 compounds via the serine pathway, while others are also capable of growth on acetate, but sugars are not utilized. Does not contain the ribulose monophosphate and ribulose bisphosphate enzymes. Tricarboxylic acid cycle is complete. Capable of atmospheric nitrogen fixation. The major cellular fatty acid is C18:1 ω7c and the major phospholipids are phosphatidylglycerols. The G+C content is 61.4–63.1 mol%.

Methylocella

The isolation of acidophilic methane-oxidizing bacteria from northern peat wetlands (Dedysh et al. 1998a) paved the way for the description of the new genus and species *Methylocella palustris* (Dedysh et al. 2000). Two more species proposals followed, *M. silvestris* (Dunfield et al. 2003) and *M. tundrae* (Dedysh et al. 2004a), each time including an emendation of the genus description. Several characteristics of the genus make

Table 4.5
Differential traits between *M. acidiphila* (Dedysh et al. 2002) and *M. aurea* (Dunfield et al. 2010)

	M. acidiphila	*M. aurea*
Cell morphology	Curved coccoids	Thick curved rods
Cell size	0.5–0.8 × 0.8–1.2 μm	0.7–1.2 × 1.8–3.1 μm
Growth on solid media	Not achieved	Yellow colonies
Carbon sources	Methane and methanol (obligate methanotrophy)	Methane, methanol, formate, and acetate (facultative methanotrophy)
pH range for growth (optimal)	4.2–7.2 (5.0–5.5)	5.2–7.2 (6.0–6.2)
Temperature range for growth (optimal)	10–30 °C (20–24 °C)	2–33 °C (25–30 °C)
Growth in nitrogen-free medium under fully aerobic conditions	+	–
Growth inhibited by NaCl (w/v)	0.5 %	0.3 %

it unique compared with other methanotrophic bacteria. Representatives of this genus do not contain an intracytoplasmic membrane system like that in all other known methanotrophs and appear to possess only a soluble form of methane monooxygenase (sMMO).

Methylocella spp. are Gram-negative, polymorphic rods or ovoids that are 0.6–1.0 μm wide by 1.0–2.5 μm long with rounded ends. Produce large, highly refractile, intracellular poly-β-hydroxybutyrate granules. Reproduce by normal cell division. Cells occur singly or in irregularly shaped aggregates, but do not form rosettes. Nonmotile. Encapsulated. Cells are not lysed by 2 % SDS. Cells lack an extensive ICM system typical of both type I and type II methanotrophic bacteria but contain a vesicular membrane system composed of singular flattened or ovoid vesicles connected to the cytoplasmic membrane. Possess sMMO. The temperature range for growth is 4–30 °C with an optimum at 15–25 °C; no growth occurs at 37 °C. Growth occurs between pH 4.2 and 7.5. Highly sensitive to salt stress; prefer diluted media with a low salt content. Utilize C_1 compounds via the serine pathway. Do not contain the enzymes of the ribulose monophosphate and ribulose bisphosphate pathways. The tricarboxylic acid cycle is complete. Fix atmospheric nitrogen via an oxygen-sensitive nitrogenase. The major phospholipid fatty acids are 18:1 ω7c acids. The G+C content of the DNA is 60–63 mol%.

The intact phospholipid profiles of *M. palustris* using liquid chromatography/electrospray ionization/mass spectrometry (Fang et al. 2000) revealed it has a preponderance of phosphatidylmethylethanolamine phospholipids with C18:1 fatty acids. The relative abundance of C18:1 is one of the features that help to discriminate species of the genus (*Table 4.6*).

Methylocella species are considered the first fully authenticated examples of facultative methanotrophs (Dedysh et al. 2005a). In addition to methane and methanol, they can utilize as well multicarbon compounds, such as acetate, pyruvate, succinate, malate, and ethanol. This ability does not include other compounds tested, urea, glucose, fructose, sucrose, lactose, galactose, xylose, sorbose, maltose, raffinose, arabinose, ribose, lactate, oxalate, citrate, mannitol, or sorbitol, but clearly provides a competitive advantage in natural environments where methane production is temporally heterogeneous due to fluctuations in temperature, water content, and water table level (Segers 1998). The molecular regulation of methane oxidation in *M. silvestris* $BL2^T$ during growth on methane and acetate has been assessed (Theisen et al. 2005).

Methyloferula

Shortly after the description of *Methylovirgula* (Vorob'ev et al. 2009), another genus of rosette-forming bacteria was proposed in the family *Beijerinckiaceae*. *Methyloferula* (Vorobev et al. 2011), an obligate methanotroph, was described as Gram-negative, aerobic, colorless, nonmotile rods that occur singly or are arranged in rosettes and misshapen cell clusters. Reproduce by irregular fission. Colonies are small, circular, and smooth. The colony color varies from white to cream. Produce poly-β-hydroxybutyrate. Mesophilic and moderately acidophilic. Prefer dilute media of low salt content. Obligate utilizers of methane and methanol; the latter is the preferred growth substrate. Possess only a soluble MMO. Positive to RubisCO activity. Capable of atmospheric nitrogen fixation. The major fatty acid is 18:1 ω7c. The major quinone is Q-10. The G+C content of the DNA is 55.6–57.5 mol%.

The type species *M. stellata* is the only one of the genus to date. Its cells are 0.4–0.65 μm wide and 1.1–3.0 μm long. Carbon sources include methane and methanol. Grows optimally at methanol concentrations of 0.5–1 %. Nitrogen sources (0.05 %, w/v) include ammonia, nitrate, urea, and yeast extract. Capable of growth at pH 3.5–7.2 (optimum pH 4.8–5.2) and at 4–33 °C (optimum 20–23 °C). NaCl inhibits growth at concentrations above 0.7 % (w/v).

Methylorosula

Methylorosula (Berestovskaya et al. 2012) is described as Gram-negative rods that are not capable of nitrogen fixation. Aerobic, facultative methylotrophic bacteria.

Table 4.6
Differential traits between *M. palustris* (Dedysh et al. 2000), *M. silvestris* (Dunfield et al. 2003), and *M. tundrae* (Dedysh et al. 2004a). Substrate utilization from (Dedysh et al. 2005a)

	M. palustris	*M. silvestris*	*M. tundrae*
Cell morphology	Straight or curved short rods	Straight or curved short rods	Curved ovoids
Macrocapsule (slimy colonies)	+	+	–
pH range for growth (optimal)	4.5–7.0 (5.5)	4.5–7.0 (5.5)	4.2–7.5 (5.5–6.0)
Temperature range for growth (optimal)	10–28 °C (20 °C)	4–30 °C (15–25 °C)	5–30 °C (15 °C)
Growth inhibited by NaCl (w/v)	0.5 %	0.8 %	0.8 %
Concentration range of methanol (v/v) utilization	0.01–0.1 %	0.01–5 %	0.01–2 %
Substrate utilization			
Formate	–	–	w
Methylamine	w	+	+
Fatty acids (%)			
16:1 ω7t	5.8	0	0
17:0 cyclo	0	0	5.0–6.5
18:1 ω7c	78.6	82.2	59.2–61.7
19:0 ω8c cyclo	0	0	7.9–13.6

Chemoorganoheterotrophic. Use the RuBP pathway for carbon assimilation. The dominant cellular fatty acid is C18:1 ω7c.

M. polaris is the type species, and only species thus far, of the genus. Its cells are long, bipolar rods (0.5–0.6 × 1.3–4.5 μm) that are single or form rosettes. Reproduces by irregular fission. Young cells are motile by means of a subpolar flagellum. Forms pale, non-translucent slimy (mucoid) colonies with an uneven edge. Resting cells are not known. After 2 weeks of growth on methanol at 20 °C, colonies are 1.5–2.0 mm in size. Utilizes C_1 compounds, methanol and methylamines, sugars, polysaccharides, sugar alcohols, and amino acids as a carbon and energy source. The temperature and pH ranges for growth are 4–30 °C (optimum, 20–25 °C) and pH 4.0–7.8 (optimum, pH 5.5–6.0), respectively. NaCl is not required for growth and is tolerated up to a concentration of 2.0 % (w/v). The major fatty acid is C18:1 ω7c. The DNA G+C content of the type strain is 65.2 mol%.

Methylovirgula

The genus *Methylovirgula* (Vorob'ev et al. 2009) contains Gram-negative, aerobic, colorless, nonmotile, thin rods that occur singly or are arranged in rosettes and misshapen cell clusters. Reproduce by binary fission. Colonies are small, circular, smooth, and convex. The colony color varies from white to cream. Produce poly-β-hydroxybutyrate. Obligately acidophilic and mesophilic. Prefer dilute media of low salt content. Restricted facultative methylotrophs. Assimilate methanol-derived carbon via the RuBP pathway. Capable of atmospheric nitrogen fixation. The major phospholipid fatty acid is C18:1 ω7c, and the major phospholipids are phosphatidylethanolamine, phosphatidylcholine, and phosphatidylglycerols. The major quinone is Q-10. The G+C content of the DNA varies between 61.8 and 62.8 mol%.

The type and only species is *M. ligni*, whose cells are 0.3–0.65 μm wide and 1.2–2.5 μm long. Resting cell forms are absent. Carbon sources include methanol, ethanol, pyruvate, and malate. Grows optimally at methanol concentrations of 0.5–1 %. Nitrogen sources used (at 0.05 %, w/v) include ammonia, nitrate, and yeast extract. Growth factors are required. Capable of growth at pH 3.1–6.5 (optimum pH 4.5–5.0) and at 4–30 °C (optimum 20–24 °C). NaCl inhibits growth at concentrations above 0.7 % (w/v).

Enrichment, Isolation, and Maintenance Procedures

Strategies for enrichment and isolation are different depending on the metabolism and physiology considered.

Beijerinckia spp. are chemoheterotrophic diazotrophs, mesophilic, able to grow on a wide range of pH (including values as low as 3.0 units), and known to show a preference for sugars as carbon sources. Complex media such as peptone medium are even inhibitory and yield no growth. Thus, nitrogen-free media with low pH are considered selective for members this genus. A simple formulation used in early studies (Becking 1961; Derx 1950a, b) contains (g/l of distilled water) glucose, 20.0; KH_2PO_4, 1.0; and $MgSO_4 \cdot 7H_2O$, 0.5 (pH is adjusted to 5.0). Aeration is important to inhibit the development of nitrogen-fixing anaerobes. So, instead of using tubes, the medium can be poured as thin layers (2–3 mm) into Petri dishes. Approximately 0.5 g of soil per plate can be used as inoculum. This provides also the necessary trace elements. For water samples, for example,

irrigation water, larger inocula might be needed to compensate the much lower presence of *Beijerinckia* in this type of habitat. Enrichment can be then approached mixing the water sample with sterile double-strength medium before dispensing the dishes as before. Samples collected from the phyllosphere can be immersed in the enrichment medium for the same purpose. Recommended incubation is at 30 °C for 2 or more weeks. Positive enrichments cultures can be streaked on the following isolation nitrogen-free, mineral agar medium (Becking 2006) that contains (g/l of distilled water) glucose, 20.0; K_2HPO_4, 0.8; KH_2PO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.5; $FeCl_3 \cdot 6H_2O$, 0.025; $Na_2MoO_4 \cdot 2H_2O$, 0.005; $CaCl_2$, 0.05; and agar 15.0 (pH is adjusted to 6.9) that can also be used for routine maintenance. Development of exopolysaccharide, a common trait of *Beijerinckia*, can be easily recognized by the slimy appearance of the colonies or by the viscosity of liquid-aged cultures. An easy confirmation step is plating on peptone medium because *Beijerinckia* will not develop, whereas other free-living diazotrophs such as *Azotobacter* or *Azomonas* will grow readily.

Camelimonas have been isolated from two different farm animals in different countries but using very similar media. Strains *C. lactis* M 2040^T, M 1973, and M 1878-SK2 were initially cultivated on *Brucella* agar (Difco) from milk of different camels of a camel milk production farm in the United Arab Emirates during routine bacteriological testing for *Brucella* species in 2008 (Kämpfer et al. 2010). *C. abortus* UK34/07-5^T was initially cultured on Farrell's medium (Farrell 1974) from placental tissue following a case of abortion in a Holstein–Friesian cow in Derbyshire, UK, in 2007 (Kämpfer et al. 2012). Farrell's medium was manufactured using a *Brucella* medium base (CM0169; Oxoid) and a Brucella selective supplement (SR0209E; Oxoid). So, it seems that an adequate formulation is (g/l of distilled water) peptone, 10.0; "Lab-Lemco" powder, 5.0; glucose, 10.0; NaCl, 5.0; and agar, 15.0 (pH 7.5). Antibiotic combinations used as selective supplements for *Brucella* can also favor the growth of *Camelimonas* sp., as occurred with *C. abortus*. In both cases, the classical methods for identification of *Brucella* species failed because they are phenotypically very distinct. Subcultivation was done in tryptone soy agar (Kämpfer et al. 2010, 2012) and other common general media can be also employed.

The two strains that served for the description of ***Chelatococcus*** *assacharovorans* were isolated with a batch enrichment procedure using nitrilotriacetic acid (NTA) as the only source of carbon, energy, and nitrogen (Egli 1988). However, they were not as easily enriched as members of the genus *Chelatobacter*. The fact that the optimal growth temperature of *Chelatococcus* strains (35–37 °C) is considerably higher than that of *Chelatobacter* strains, together with their resistance to vancomycin, was proposed to enrich for *Chelatococcus* when using NTA as the only source of carbon and nitrogen for growth (Egli and Auling 2005). However, the next two species of the genus, each based on a single strain, were obtained following a more general approach. *C. daeguensis* K106^T (Yoon et al. 2008) was isolated from wastewater of a textile dye works in Korea, by means of the standard dilution-plating technique on trypticase soy agar (TSA; Difco) at 30 °C. *C. sambhunathii* HT4^T (Panday and Das 2010) was retrieved from sediment of an Indian hot spring that was inoculated into a 250 ml conical flask containing 50 ml nutrient broth (Difco) and incubated on a shaker at 200 r.p.m. and 45 °C. After 2 days incubation, the contents of the flasks were serially diluted and plated onto nutrient agar medium (Difco) and incubated at 37 °C for 4 days, after which colonies were picked and purified by repeated streaking on the same medium. Interestingly, *C. sambhunathii* HT4^T showed a nutritional pattern closer to *C. asaccharovorans* than to *C. daeguensis* (Table 4.4) and was also able to grow on NTA as single carbon, nitrogen, and energy source although it had not been used for the enrichment and isolation.

Methylocapsa, ***Methylocella***, and ***Methyloferula*** are methanotrophs, either facultative or obligately, that can be cultivated using liquid or solid mineral media with methane as growth substrate (Dedysh and Dunfield 2011). Facultative methanotrophic species can utilize multicarbon compounds, but its use for isolation or even maintenance is not recommended since these substrates give a selective advantage to chemoorganoheterotrophs that will contaminate and surpass the methanotrophic cultures. Two media have been successfully applied for the enrichment and isolation of most strains of these three genera:

- Nitrogen-free medium M2 (Dedysh et al. 1998b) containing (in grams per liter of distilled water) KH_2PO_4, 0.04; $MgSO_4 \cdot 7H_2O$, 0.02; and $CaCl_2 \cdot 2H_2O$, 0.004, with the addition of 0.1 % (by volume) of a trace elements stock solution containing (in grams per liter) EDTA, 5; $FeSO_4 \cdot 7H_2O$, 2; $ZnSO_4 \cdot 7H_2O$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.03; $CoCl_2 \cdot 6H_2O$, 0.2; $CuCl_2 \cdot 5H_2O$, 0.1; $NiCl_2 \cdot 6H_2O$, 0.02; and Na_2MoO_4, 0.03. The initial pH of the medium can be adjusted with concentrated phosphoric acid from 6 to 3.
- Dilute nitrate mineral salts (DNMS) medium (Dunfield et al. 2003) containing (in grams per liter of distilled water) KNO_3, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; $Na_2HPO_4 \cdot 12H_2O$, 0.143; KH_2PO_4, 0.054; and $CaCl_2 \cdot 6H_2O$, 0.04 and containing 1 mM NaH_2PO_4/Na_2HPO_4 buffer (pH 5.8).

For growth in liquid medium, screw cap serum bottles are used with a headspace/liquid space ratio of 4:1. After inoculation, the bottles are sealed with silicone rubber septa, and methane is added aseptically using a syringe equipped with a disposable filter (0.22 μm) to achieve a 15–20 % mixing ratio in the headspace. Bottles can be incubated on a rotary shaker (120 r.p.m.) at 20–25 °C. Solid media are prepared adding agar or gellan gum as solidifying agents (15 g per liter). These media are incubated in closed vessels or desiccators containing air supplemented with (v/v) CH_4 (10–30 %) and CO_2 (5 %) and can be used for maintenance.

Of course, other formulations are possible (Vorobev et al. 2011; Dedysh and Dunfield 2011) with variations in the presence or the amount of minerals and/or adjusted pH. Initial incubation times are long (up to 2 months). Methanotrophic cultures are frequently contaminated by heterotrophic bacteria that survive on metabolic by-products of methanotrophs. The presence of only a few satellite cells in a culture may lead to false

conclusions regarding substrate utilization, and several early reports of facultative methanotrophy are likely attributable to impure cultures. Another recurring mistake is the misidentification of non-methanotrophic facultative methylotrophs as facultative methanotrophs. Valuable advice on how to differentiate and identify facultative and obligate methanotrophs, not necessarily members of the family *Beijerinckiaceae*, is given by Dedysh and Dunfield (2011).

Media based on methane as carbon source are not suited for the facultative methylotrophic genera ***Methylorosula*** and ***Methylovirgula*** because they lack MMO. But actually, the three strains that conformed *Methylorosula polaris* (Berestovskaya et al. 2012) were isolated from acidophilic, methanotrophic enrichments obtained from *Sphagnum* peat of three tundra wetland sites of Northern Russia. Cell suspensions of these enrichments were spread onto medium 2A (Berestovskaya et al. 2002) agar plates, containing 2 % (w/v) agar (Difco) and amended with 0.5 % methanol as the sole carbon source. Mineral medium 2A had been specially developed to cultivate microorganisms from ultrafresh habitats due to its low mineralization (mg/l): NH_4SO, 500; $MgCl_2$, 40; KH_2PO_4, 70; and a solution of microelements, 1 ml/l (Berestovskaya et al. 2002). In the case of *Methylovirgula ligni*, each of the two strains that served for its description was recovered from a nutrient-rich, although diluted, medium. One of them, designated medium W5, had the following composition (per liter of demineralized water): NaCl, 1 g; yeast extract, 0.1 g; 2-[*N*-morpholino]ethane sulfonic acid (MES) as a buffering compound, 1.95 g; and agar, 20 g. Medium T5 contained (per liter) NaCl, 1 g; trypticase soy broth (Oxoid), 3 g; MES, 1.95 g; and agar, 20 g. Attempts to maintain both strains on their respective isolation media proved they were not suited for optimal growth and a slightly modified medium M2 (designated medium MM2) resulted in good development of the strains when methanol was provided as a growth substrate. Thus, further maintenance and cultivation experiments with these bacteria were performed using medium MM2 of the following composition (mg per liter distilled water): $(NH_4)_2SO_4$, 200; KH_2PO_4, 100; $MgSO_4$, 50; $CaCl_2 \cdot 2H_2O$, 20; yeast extract, 10; and methanol, 0.5 % (v/v) (pH 4.8–5.2). For growth in liquid media, 500 ml screw-capped serum bottles were used with a headspace/liquid space ratio of 4:1. After inoculation, methanol was added aseptically to the cultures, and the bottles were capped with silicone rubber septa to prevent loss of methanol by evaporation. Bottles were incubated on a rotary shaker (120 r.p.m.) at 24 °C.

In addition to periodic transfer using the maintenance media referred above, members of the family can be successfully maintained using the common long-term preservation protocols and techniques, that is, lyophilization and deep freezing of cell suspensions in the presence of lyo-/cryoprotectants. The possibility of preserving *Beijerinckia* cultures by encapsulation using a spray drier, for use in biotechnological processes in the production of biopolymers, has been addressed (Boza et al. 2003). Among different wall (coating) malt dextrin presented the greatest stability with respect to fermentative activity, although the glucose-encapsulated cells showed the highest percentage of viability during spray drying and during the storage period.

Ecology

Members of the type genus ***Beijerinckia*** are commonly found as free-living bacteria in acidic soils and also in plant rhizosphere and phyllosphere environments. The earliest recorded isolations (Becking 2006) are from a Malaysian quartzite soil (pH 4.5) by Altson (1936), from a Dacca, Bangladesh, soil (pH 4.9), and from a soil of Insein, Burma (pH 5.2), by Starkey and De (1939), although considered at that time *Azotobacter* spp. Later it was found to be widely distributed in the more acidic soils of mainly tropical regions, and it was also isolated from neutral and slightly alkaline soils and outside the tropics. In a large soil survey covering 392 soils of worldwide distribution, *Beijerinckia* was found in some temperate and subtropical soils of Europe (former Yugoslavia), South Africa, India, China, and Japan (Becking 1959, 1961). Other authors mention its isolation from other nontropical soils, i.e., from an acidic volcanic ash soil of Sendai, Tohoku District, Japan (lat. 38°N) (Suto 1954, 1957); from some Indian nontropical soils (up to lat. 27–30°N) (Barooah and Sen 1959); in some subtropical and nontropical soils in Australia (lat. 15–43°S) (Tchan 1953; Thompson 1968; Thompson and Skerman 1979); and in two soils of Egypt (Kharga Oasis, lat. 25°N) (Vančura et al. 1965). In addition, it was isolated from some Pacific Northwest soils in North America (Snake River Plain, Idaho, USA; lat. 44°N), from soil of the Squamish Bay Area, British Columbia, Canada (lat. 50°N) by Anderson (1966). Also, Jordan and McNicol (1978) reported the isolation of *Beijerinckia* from three sites of a permanently cold, high Arctic soil (Devon Island, Northwest Territories; lat. 75°N).

Distribution in the more tropical soils of the African continent was largely explored. In addition to the South African soils already mentioned (Becking 1959, 1961) *Beijerinckia* was observed to occur in soils of Zimbabwe (Meiklejohn 1968), Ivory Coast (Kauffmann and Toussaint 1951a, b), Sudan (Hegazi and Ayoub 1979), Sierra Leone, Nigeria, Ethiopia, Uganda, Kenya, Tanzania, and Congo/Zaire (Becking 1961). *Beijerinckia* was also found in many soil samples of South America: Argentina (Amor Asunción et al. 1980), Trinidad Island, Surinam, Venezuela, Bolivia, and Brazil (Becking 1961).

Decades of research on the biological nitrogen fixation in graminaceous plants in Brazil (Baldani and Baldani 2005) have provided many insights on ecology of *Beijerinckia*. It was demonstrated that the size of the populations was related to vegetation, physical, and chemical characteristics of the soil (Döbereiner and Castro 1955). Further studies on the occurrence of this genus in soil of several Brazilian States (Rio de Janeiro, São Paulo, Pernambuco and Paraná) led to the description of *B. fluminensis* (Döbereiner and Ruschel 1958). Analysis of 158 samples collected in different regions of Brazil showed that this species occurred predominantly in soils where sugarcane was cultivated (Döbereiner 1959a) and a direct influence of

the plant on the development of the bacteria was suggested (Döbereiner 1959b). Additional studies showed that roots as well as leaves and stems had a positive influence on *Beijerinckia* populations. This was influenced by the exudation of substances into the soil by the roots during rainfall (Döbereiner and Alvahydo 1959). The population of *Beijerinckia* was much more pronounced in the rhizoplane region (refers to the soil adherent to the root surface) than in the rhizosphere. In addition, it was shown that removal of the aerial part of the plant significantly reduced the population of bacteria in both the rhizoplane and rhizosphere regions (Döbereiner 1961). Rice plants grown in greenhouse and inoculated with *Beijerinckia* showed the establishment of the bacteria as well as an increase in the yield (Döbereiner and Ruschel 1961). The measurement of the nitrogenase activity in roots of sugarcane, thanks to the introduction of acetylene reduction methodology, showed that it was much higher than that observed in the rhizosphere and in soil between the plant rows. *B. indica* was the most abundant bacterial species in both roots and soil samples (Döbereiner et al. 1972). Quantification of nitrogen fixation in sugarcane based on the extrapolation of the nitrogenase activity data indicated a contribution of 50 kg N/ha/year to the soil/plant system (Döbereiner et al. 1973).

Concerning habitats other than soil, Ruinen (1956, 1961) found *Beijerinckia* as a regular component of the phyllosphere of tropical plants. Diem et al. (1978) have used the fluorescent antibody technique to study the behavior of a *Beijerinckia* isolate in the rhizosphere and seed region (spermosphere) of rice seedlings in Camargue (France). Also in rice, Karkhanis and Tikhe (1980) and Karkhanis (1987) reported not only its presence in the rhizosphere but also its intracortical occurrence within the rice root. Murty (1984) measured nitrogenase activities on the leaf surfaces of different varieties of cotton (*Gossypium hirsutum* L. and *G. herbaceum* L.) plants, and *Beijerinckia* sp. was observed to be the predominant nitrogen-fixing microorganism in the phyllosphere of these varieties.

Jackson and Denney (2011) studied the annual and seasonal variation in the phyllosphere bacterial community associated with leaves of the southern Magnolia (*Magnolia grandiflora*) using multivariate analysis of denaturing gradient gel electrophoresis profiles of 16S rRNA gene fragments. Temporal changes had a much greater variation than leaf to leaf. This was confirmed by sequencing and analysis of 16S rRNA gene clone libraries generated for each sample date. All phyllosphere communities were dominated by *Alphaproteobacteria*, with a reduction in the representation of *Beijerinckiaceae* related to *B. indica* and *M. silvestris* during the summer and a concurrent increase in the *Methylobacteriaceae* being the most significant seasonal change.

The knowledge about the ecology of ***Camelimonas*** is limited to the information of the habitat from where the two species described were isolated: milk of camels at a camel milk production farm in the United Arab Emirates for *C. lactis* (Kämpfer et al. 2010) and placental tissue following a case of abortion in a Holstein–Friesian cow in the UK for *C. abortus* (Kämpfer et al. 2012). This limitation does not occur with ***Chelatococcus***; the type species *C. asaccharovorans* was isolated from wastewater and soil in Switzerland (Auling et al. 1993), but its ubiquitous distribution in surface waters and sewage treatment plants was shown by immunofluorescence (Wilberg et al. 1993). *C. daeguensis* was indeed isolated from wastewater, in this case, from textile dye works in the Republic of Korea (Yoon et al. 2008), while *C. sambhunathii* originated from a hot spring sediment in India (Panday and Das 2010). In addition to water, soils can also be inhabited by *Chelatococcus*. In a recent study (Fredrickson et al. 2008) dealing with bacterial cultures obtained from irradiated soils in Washington (USA), 6 out of 47 were identified as *Chelatococcus* sp. For extremely ionizing radiation-resistant bacteria, survival has been attributed to protection of proteins from oxidative damage during irradiation, with the result that repair systems survive and function with far greater efficiency during recovery than in sensitive bacteria. The relationship between survival of dry climate soil bacteria and the level of cellular protein oxidation induced by desiccation was examined. Desiccation-resistant isolates accumulated high intracellular manganese and low iron concentrations compared to sensitive bacteria. In vivo, proteins of desiccation-resistant bacteria were protected from oxidative modifications that introduce carbonyl groups in sensitive bacteria during drying. Members of *Chelatococcus* have also been identified among other genera present in the headbox water of a paper machine in Canada (Prince et al. 2009), using an amplified ribosomal DNA restriction analysis.

Acidic wetlands, particularly *Sphagnum* peat bogs, from different sites of Russia and forest soils from Germany, Russia, and the Netherlands were the habitats from where the species of ***Methylocapsa***, ***Methylocella***, ***Methyloferula***, ***Methylorosula***, and ***Methylovirgula*** were originally isolated (Berestovskaya et al. 2012; Dedysh et al. 2000, 2002, 2004a; Dunfield et al. 2003, 2010; Vorob'ev et al. 2009; Vorobev et al. 2011).

Wetlands with peat-forming soils (peatlands) provide habitat for anaerobic archaeal methanogens that produce CH_4 under strictly anoxic conditions, usually below the water table. In contrast aerobic methane-oxidizing bacteria (methanotrophs) are active primarily above the water table in aerated portions of the soil and play an important role in wetlands by limiting CH_4 emissions to the atmosphere. Given the global scope of bogs, the number of molecular studies of methanotrophs in these sites is still small. Gupta et al. (2012) investigated the active methanotroph community in two contrasting North American peatlands, a nutrient-rich sedge fen and nutrient-poor *Sphagnum* bog using in vitro incubations and DNA stable-isotope probing (DNA-SIP) with ^{13}C–CH_4. This approach avoids cultivation biases or issues associated with facultative methanotrophs such as members of the genus *Methylocella* being active, but not necessarily using CH_4 as a substrate. Rates of CH_4 oxidation were slightly, but significantly, faster in the bog, and methanotrophs belonged to the class *Alphaproteobacteria* and were similar to other methanotrophs of the genera *Methylocystis*, *Methylosinus*, and *Methylocapsa* or *Methylocella* detected in, or isolated from, European bogs. The fen had a greater phylogenetic diversity of organisms that had

assimilated ^{13}C, including methanotrophs from both the *Alpha-* and *Gammaproteobacteria* classes and other potentially non-methanotrophic organisms that were similar to bacteria detected in a UK and Finnish fen. Based on similarities between bacteria in North American sites and those in Europe, including Russia, it was concluded that site physicochemical characteristics rather than biogeography controlled the phylogenetic diversity of active methanotrophs and that differences in phylogenetic diversity between the bog and fen did not relate to measured CH_4 oxidation rates (Gupta et al. 2012). Using a different approach, fluorescent in situ hybridization with 16S rRNA targeted oligonucleotide probes; Dedysh et al. (2003) detected *Methylocystis* spp. as the numerically largest methanotroph group in peat from Siberia and Germany, followed by *Methylocapsa acidiphila* and *Methylocella palustris*. *M. acidiphila* was enumerated in both samples at greater than 10^5 cells g^{-1} of peat. This accounted for 5 % and 2 % of total methanotroph cells detected by FISH in peat from Siberia and Germany, respectively. In contrast, the population sizes of *M. palustris* were significantly different in *Sphagnum* peat from West Siberia (10^6 cells g^{-1} of peat) and northern Germany (10^4 cells g^{-1} of peat). But, the possibility that some unknown species of acidophilic *Methylocella* and *Methylocapsa* escaped detection by FISH with the available set of oligonucleotide probes cannot be excluded. Besides, these values refer to single-time, single peat soil depth sample measurements. Significant variations in methanotroph community structure and abundance might occur in different subsites and depths as well as on a seasonal scale. In addition, some environmental factors (variation in temperature, aeration conditions, substrate availability) might also have a strong impact on population dynamics of different methanotrophs in acidic peatlands. Indeed, a previous study at Bakchar bog, Plotnikovo field station in west Siberia, 56°N, 82°E, revealed *M. palustris* as predominant methanotroph well above *Methylocystis* (Dedysh et al. 2001a).

Radajewski et al. (2002) applied DNA-SIP to characterize the active methylotroph populations in microcosms in an oak (*Quercus petraea*) forest soil (pH 3.5) collected from the Gisburn Forest Experiment, UK. The microcosms were exposed to $^{13}CH_3OH$ or $^{13}CH_4$ and distinct ^{13}C-labeled DNA fractions were resolved from total community DNA by CsCl density gradient centrifugation. Subsequent analysis of amplified 16S rRNA sequences from these ^{13}C-DNA revealed bacteria related to the genera *Methylocella*, *Methylocapsa*, *Methylocystis*, and *Rhodoblastus*. Enrichments targeted towards the active proteobacterial CH_3OH utilizers were successful, although none of these bacteria were isolated into pure culture. Chen et al. (2008) investigated the active methanotroph community in heather (*Calluna*)-covered moorlands and *Sphagnum-*/*Eriophorum*-covered UK peatlands. Direct extraction of mRNA from these soils facilitated detection of expression of methane monooxygenase genes, which revealed that particulate methane monooxygenase, and not soluble methane monooxygenase, was probably responsible for CH_4 oxidation in situ, because only *pmoA* transcripts (encoding a subunit of particulate methane monooxygenase) were readily detectable. Differences in methanotroph community structures were observed between both habitats. In *Calluna*-covered moorlands, in addition to *Methylocella* and *Methylocystis*, a unique group of peat-associated type I methanotrophs (*Gammaproteobacteria*) and a group of uncultivated type II methanotrophs (*Alphaproteobacteria*) were also found. The *pmoA* sequences of the latter were only distantly related to *Methylocapsa* and also to the RA-14 group of methanotrophs, which are believed to be involved in oxidation of atmospheric CH_4. Soil samples were also labeled with $^{13}CH_4$, and subsequent analysis of the ^{13}C-labeled phospholipid fatty acids showed that C16:1 ω7, C18:1 ω7, and C18:1 ω9 were the major labeled fatty acids. Since C18:1 ω9 is not a major fatty acid of any established species of methanotroph, it suggests the presence of novel species in this peatland. The *Sphagnum-*/*Eriophorum*-covered gullies were dominated by *Methylocystis*, but *Methylocella*- and *Methylocapsa*-related species were also present.

Corsican pine (*Pinus nigra*) roots colonized by the ectomycorrhizal (ECM) fungi, *Suillus variegatus* and *Tomentellopsis submollis*, were examined analyzing the diversity of bacterial nitrogenase genes (*nifH*) present and their mRNA transcription (Izumi et al. 2006). DNA-derived *nifH* PCR products were obtained from all samples, but only a few reverse transcription PCRs for *nifH* mRNA were successful, suggesting that nitrogenase genes were not always transcribed. Putative nitrogenase amino acid sequences revealed that more than half of the *nifH* products were derived from methylotrophic bacteria, such as *Methylocella* spp.

Methanotroph abundance was analyzed in control and long-term nitrogen-amended pine and hardwood soils in Massachusetts, USA (42°13′N, 72°11′W) using rRNA-targeted quantitative hybridization (Lau et al. 2007). Family-specific 16S rRNA and *pmoA*/*amoA* genes were analyzed via PCR-directed assays to elucidate methanotrophic bacteria inhabiting soils undergoing atmospheric methane consumption. Quantitative hybridizations suggested methanotrophs related to the family *Methylocystaceae* were one order of magnitude more abundant than *Methylococcaceae* and more sensitive to nitrogen addition in pine soils. 16S rRNA gene phylotypes related to known *Methylocystaceae* and acidophilic methanotrophs and *pmoA*/*amoA* gene sequences, including three related to the upland soil cluster *Alphaproteobacteria* (USCalpha) group, were detected across different treatments and soil depths. The result suggested that methanotrophic members of the *Methylocystaceae* and *Beijerinckiaceae* may be the candidates for soil atmospheric methane consumption (Lau et al. 2007).

Landfills are also a suitable habitat for methanotrophs with important ecological implications. The identity of active methanotrophs in Roscommon (Ireland) landfill cover soil, a slightly acidic peat soil, was assessed by DNA-SIP (Cébron et al. 2007). Landfill cover soil slurries were incubated with ^{13}C-labeled methane and under either nutrient-rich nitrate mineral salt medium or water. 16S rRNA gene analysis revealed that the cover soil was mainly dominated by Type II methanotrophs closely related to the genera *Methylocella* and *Methylocapsa* and to *Methylocystis* species. These results were supported by analysis

of *mmoX* genes in ^{13}C-DNA. Analysis of *pmoA* gene diversity indicated that a significant proportion of active bacteria were also closely related to the Type I methanotrophs, *Methylobacter*, and *Methylomonas* species. Phylogenetic analysis of mRNA recovered from the soil indicated that *Methylobacter*, *Methylosarcina*, *Methylomonas*, *Methylocystis*, and *Methylocella* were actively expressing genes involved in methane and methanol oxidation. Transcripts of *pmoA*, but not *mmoX*, were readily detected by reverse transcription polymerase chain reaction (RT-PCR), indicating that particulate methane monooxygenase may be largely responsible for methane oxidation in situ (Chen et al. 2007).

Another study (Han et al. 2009), involving 16S rRNA gene and functional gene clone libraries and microarray analyses using *pmoA*, investigated the methanotroph community structure in alkaline soil from a Chinese coal mine. This environment contained a high diversity of methanotrophs, including the type II *Methylosinus* and *Methylocystis*, type I *Methylobacter*, *Methylosoma*, *Methylococcus*, and a number of as yet uncultivated methanotrophs. In order to identify the metabolically active methane-oxidizing bacteria from this alkaline environment, DNA-SIP experiments using $^{13}CH_4$ were carried out. This showed that both type I and type II methanotrophs were active, together with methanotrophs related to *Methylocella*, which had previously been found only in acidic environments. This finding opened the question if *Methylocella* species are widely distributed in nature and not restricted to acidic environments. The affirmative answer came through another study (Rahman et al. 2011) employing a real-time quantitative PCR method and primers targeting *Methylocella mmoX*.

Application

Beijerinckia have received attention due to their plant growth-promoting properties (Miyasaka et al. 2003; Thuler et al. 2003) and for their abundant production of exopolysaccharide with potential biotechnological uses (Scamparini et al. 1997).

Nitrogen-fixing bacteria are probably among the most extensively studied soil microorganisms. Several genera of free-living, N_2-fixing bacteria occur in high numbers in the rhizosphere, rhizoplane, and phyllosphere, and *Beijerinckia* is one of the most notable genera. Inoculation of rice seeds with *Beijerinckia* showed that this genus is able to multiply in the soil, establishing itself in large numbers and reducing the number of other competing microorganisms (Döbereiner and Ruschel 1961). It has been observed (Miyasaka et al. 2003) that *B. derxii* maintains an increasing nitrogenase-specific activity during the stationary growth phase. To verify the destination of the nitrogen fixed during this phase, intra- and extracellular nitrogenated contents were analyzed. Organic nitrogen and amino acids were detected in the supernatant of the cultures. An increase in intracellular content of both nitrogen and protein occurred. Cytoplasmic granules indicated the presence of arginine. The ability of a non-diazotrophic bacterium (*Escherichia coli*) to use *B. derxii* proteins as a source of nitrogen was observed concomitantly with *E. coli* growth. This suggested that *B. derxii* contributes to the environment by both releasing nitrogenated substances and accumulating substances capable of being consumed after its death. Moreover, the ability of *B. derxii* to release certain plant growth regulators (indoleacetic acid, ethylene and polyamines) and amino acids into the growth medium has also been addressed (Thuler et al. 2003). The production of those substances was compared using cultures in which nitrogenase was active (N-free medium) and cultures in which nitrogenase was repressed (combined-N cultures) and with and without agitation. Total indoleacetic acid production was higher in agitated, N-free cultures, but specific production was greater in combined-N cultures under agitation. Putrescine and spermidine were detected under all conditions tested. Ethylene was produced in both N-free and combined-N cultures. Although a greatest diversity of amino acids was released in N-free cultures, it is important that production of growth regulators was not inhibited under conditions where nitrogenase was inactive, and so are independent activities. The fact that indoleacetic acid was released by *B. derxii* in three of the four tested conditions suggests that it is likely to be produced in the rhizosphere and therefore influence plant growth. The diversity of amino acids released by *B. derxii* was higher than that observed for *B. indica* (Pati et al. 1994) but in lower concentrations. The high concentration of glutamic acid detected under all conditions tested suggests that *B. derxii* actively excretes amino acids as the CFU number remained stable in N-free medium under agitation. The excretion of amino acids may be seen as a way for the bacteria to maintain a low intracellular N level, necessary for active N_2 fixation. However, this phenomenon may be particularly advantageous to the naturally surrounding environment where these amino acids may be directly assimilated and incorporated into the proteins of plants and a number of other living organisms. The growth-promoting effect of *B. mobilis* and *Clostridium* sp. cultures isolated from the pea rhizosphere on some agricultural crops was studied (Polianskaia et al. 2002). Seed soaking in bacterial suspensions followed by the soil application of the suspensions or their application by means of foliar spraying was found to be the most efficient method of bacterization. The application of both bacteria in combination with mineral fertilizers increased the crop production by 1.5–2.5 times. The study of the population dynamics of *B. mobilis* by genetic marking showed that it quickly colonized the rhizoplane of plants.

It is important to mention that a number of research articles that appeared between 1973 and 1996 dealing with *Beijerinckia* able to degrade polycyclic aromatic hydrocarbons such as anthracene, biphenyl, dibenzofuran, and phenanthrene, among others, correspond indeed to a different organism. The original strain, isolated from a polluted stream, is designated as B1 or Bwt and its mutant B8/36 (Gibson et al. 1973), but a polyphasic study concluded that it is a member of *Sphingomonas yanoikuyae* (Khan et al. 1996). It is interesting to note that two independent studies, employing apparently other isolates of *Beijerinckia*, do also claim polycyclic aromatic hydrocarbon degradative activity. One is strain *B. mobilis* 1f isolated from a soil contaminated

with creosote (Surovtseva et al. 1999), and the other one is a strain of *B. indica* proposed as a biomaterial for aerobic biofilm barriers for the removal of phenanthrene from groundwater (Lim et al. 2010).

As for the production of EPS, Lee et al. (2007) have characterized the water-soluble extracellular polysaccharide produced by *B. indica*, heteropolysaccharide-7 or PS-7, a gellan-like biopolymer. It is composed of β-D-glucose, α-L-rhamnose, and β-D-galacturonic acid in a molar ratio of approximately 5:1:1.3. The aqueous solution of PS-7 is highly viscous and shows high thermal stability over the temperature range from 4 °C to 93 °C. This PS-7 dispersion is stable in the wide pH range from 3.0 to 12.0 as well. The PS-7 dispersion shows distinct pseudoplastic behavior, which means the viscosity of PS-7 dispersion decreases as the shear rate increases. In the presence of cationic or polyvalent ions at high pH, PS-7 dispersion easily produced a gel matrix. Unique chemical structure and physical properties of PS-7 make them to be useful for a wide range of applications to the oil industries and potentially to dripless water-based latex well-joint cement adhesives and textile printing.

Chelatococcus has been considered for a number of applications mostly related with biodegradation and bioremediation. Aminopolycarboxylic acids are used in a broad range of household products and industrial applications to control the solubility and precipitation of metal ions. The two most widely used are ethylenediaminetetraacetate (EDTA) and nitrilotriacetate (NTA). Because their application is mostly water-based, they are disposed off in wastewater from where they reach the environment if not sufficiently well eliminated. A number of NTA- and EDTA-degrading bacterial strains have been isolated. The NTA-degrading genera *Chelatobacter* and *Chelatococcus* appear to be well represented in most aerobic environments, and their key enzymes involved in NTA and EDTA catabolism have been studied in considerable detail (Egli 2001).

Also related with environment protection but this time through waste treatment is the recent report of an effective candidate for simultaneous nitrification and denitrificaton at high temperature (Yang et al. 2013). Strain, *Chelatococcus daeguensis* TAD1, isolated from the biofilm of a biotrickling filter at a coal-fired power plant in China has the ability to denitrify at 50 °C under aerobic condition without accumulation of nitrite, nitric oxide, and nitrous oxide and with nitrogen gas as its terminal product. It can utilize not only nitrate and nitrite but also ammonium at this temperature. The denitrification genes were identified (*napA*, *nirK*, *cnorB*, and *nosZ*) and sequenced. Heterotrophic nitrification and aerobic denitrification by one single organism has attractive advantages over the two-step process involving anaerobic denitrification because of its easy operation, cost benefit, and relatively accelerated rate.

Another thermophilic member of the genus *Chelatococcus*, strain E1 isolated from a compost sample, is capable of low-molecular-weight polyethylene degradation, and this activity has been thoroughly assessed in a recent study (Jeon and Kim 2013).

In the field of energy production and diversification of strategies with less environmental concerns, the biotransformation of coal into a cleaner fuel is being explored to extend the utilization of coal-derived biofuels as an alternative source of energy. This requires isolation of microbes with the capability to degrade complex coal into simpler substrates to support methanogenesis in the coal beds. For that purpose aerobic bacteria able to solubilize and utilize coal as the sole source of carbon were searched in samples from an Indian coal bed (700 m depth). Two isolates fulfilling these requirements, strains BHU2 and BHU6, were characterized and identified as *Chelatococcus* spp. (Singh and Tripathi 2011).

Polyhydroxybutyrate (PHB) production by *Chelatococcus* has also been approached. Ibrahim and Steinbüchel (2010) examined different fermentation strategies that were employed for the cultivation of *Chelatococcus* sp. strain MW10, a new PHB-accumulating thermophilic bacterium, with the aim of achieving high-cell-density growth and high PHB productivity. Enhanced cultivation was achieved by a cyclic fed-batch fermentation technique (42 l scale), and maximal productivity (16.8 ± 4.2 g/l) was obtained during the second cycle. Strain MW10 has the advantage of accumulating PHB using cheap media made with renewable resources (Ibrahim et al. 2010), but also being a moderate thermophile can help to reduce the costs of bioreactor operation since cooling is not required during the exothermic fermentation process.

Motivated by the need to establish an economical and environmentally friendly methanol control technology for the pulp and paper industry, a bench-scale activated carbon biofiltration system was developed (Babbitt et al. 2009) that supported a diverse community of methanol-degrading bacteria, with high similarity to species in the genera *Methylophilus*, *Hyphomicrobium*, and ***Methylocella***. The system was evaluated for its performance in removing methanol from an artificially contaminated air stream and characterized for its bacterial diversity over time, under varied methanol loading rates, and in different spatial regions of the filter. The biofilter system, composed of a novel packing mixture, provided an excellent support for growth and activity of methanol-degrading bacteria, resulting in approximately 100 % methanol removal efficiency for loading rates of 1–17 g/m3 packing/h, when operated both with and without inoculum containing enriched methanol-degrading bacteria. Although bacterial diversity and abundance varied over the length of the biofilter, the populations present rapidly formed a stable community that was maintained over the entire 138-day operation of the system and through variable operating conditions, as observed by PCR–DGGE methods that targeted all bacteria as well as specific methanol-oxidizing microorganisms.

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5 The Family *Bradyrhizobiaceae*

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Abstract
Bradyrhizobiaceae is a family of *Rhizobiales* order into the *Alphaproteobacteria* class that presents 12 genera including the type genus *Bradyrhizobium*. Phylogenetic analyses based on 16S rRNA sequences evoke a versatile family presenting a broad taxonomic affiliation with organisms from different environments like soil, plant, or animal hosts. Bacteria are pleomorphic with predominance of rod-shaped form. There is a diversity of phenotypic, metabolic, and ecological properties associated with each genus, and their participation in biogeochemical cycles is of extreme importance. Biological nitrogen fixation particularly performed by *Bradyrhizobium* is one of the most important ecological properties with potential application in agriculture, besides other diazotrophic members. In addition, the genus *Afipia* is clinically relevant once *A. felis* is a human pathogen causing the cat scratch disease. A lot of members of *Bradyrhizobiaceae* already had their genome completely sequenced which in turn corroborates their taxonomic classification. Considering that, taxonomy into *Bradyrhizobiaceae* will become better illustrated with advances in genomic projects applied to other members of this family.

Taxonomy: Historical and Current

The traditional nomenclature of *Rhizobium* was first modified when the new genus *Bradyrhizobium* was described by Jordan (1982), which included all nitrogen-fixing bacteria (NFB) that have symbiotic associations with soybean plants, and the nomenclature is still based on physiological traits that are different from the other rhizobia. Advances in molecular methods for phylogenetic analysis, particularly those based on 16S rRNA gene sequences, have led to the creation of the family *Bradyrhizobiaceae*, which includes NFB genera of subclass α of *Proteobacteria* with different traits, but which have similar slow growth rates (5–6 days) in A1EG culture medium (Garrity et al. 2005; Turner and Young 2000). The family contains 12 genera, including the type genus *Bradyrhizobium* (derived from the Latin term *bradus*, meaning slow growing) (Garrity et al. 2005; Euzéby 2006).

Strains belonging to the rhizobial groups diverged even before the emergence of legumes. The symbiotic genes arose later in one or more strains and diversified themselves as they propagated by lateral gene transfer between different genera and strains (Kaneko et al. 2000; Hirsch et al. 2001; Galibert et al. 2001; Moulin et al. 2004). The divergence between bradyrhizobia and the fast-growing rhizobia (*Rhizobium*, *Sinorhizobium*, and *Mesorhizobium*) dates between 507 and 553 MYA (million years ago), as estimated by the divergence times of sequences using molecular clocks (Turner and Young 2000). Complex events during the evolution of genomes involved gene recruitment from paralogs, gene duplication, and lateral and vertical gene transfer, as shown by taxonomic studies based mainly on the diversity of 16S rRNA sequences, glutamine synthetases, nodulation genes (*nod*), and nitrogen fixation genes (*nif*) (Turner and Young 2000; Moulin et al. 2004; Parker 2012). Despite the high sequence diversity, *Bradyrhizobium* strains reveal a monophyletic character with few exceptions, particularly for photosynthetic Bradyrhizobia (Moulin et al. 2004).

In contrast to many NFB strains that interact with legumes, *Bradyrhizobium* does not group symbiotic genes in larger plasmids, but has a chromosomal symbiotic island (SI) that groups the genes related to nodulation and biological nitrogen fixation (BNF) (Kaneko et al. 2002). The high degree of genotypic plasticity in this genus associated with SI recombination may have contributed to the high degree of adaptation and selective advantage in this genus (Kaneko et al. 2002; Parker 2012).

Small genetic alterations can have strong pleiotropic effects on the phenotype. Thus, systematics based only on phenotype can lead to confused, divergent, or skewed results (Bécquer 2004). It is unfeasible to explain the genetic diversity of microorganisms according to one criterion alone. Modern polyphasic taxonomy (Colwell 1970; Zakhia and de Lajudie 2006) recommends combining genotypic and phenotypic information that

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integrates several cellular levels, including DNA, RNA, and other macromolecules and expressed traits, such as proteins and their activities, fatty acids, and chemotaxonomic markers. In this context, several techniques are employed and contribute to a more stable classification. Among them, sequencing the gene encoding the small ribosomal subunit 16S rRNA revolutionized bacterial taxonomy. Polyphasic taxonomy is therefore an example of maintaining traditional and modern, classical, and molecular techniques for microbial characterization because all genotypic, phenotypic, and phylogenetic information is included in its analysis (Bécquer 2004; Cohan and Perry 2007). Rhizobia are clearly polyphyletic, as there are no phylogenetic branches that contain only this group and no other bacteria (Young 1996; Bécquer 2004). When only considering *Bradyrhizobium*, polyphasic taxonomy already has consensus problems between the phenotypic and phylogenetic data (Young et al. 1991; Dupuy et al. 1994; Ladha and So 1994; Zakhia and de Lajudie 2001).

Currently, the family *Bradyrhizobiaceae* in particular demonstrates a marked effect of phylogenetic analysis based on 16S rRNA sequences (Garrity et al. 2005) (*Fig. 5.1*). In addition to the genus *Bradyrhizobium*, the following other genera comprise the family *Bradyrhizobiaceae*: *Afipia*, *Agromonas*, *Balneimonas*, *Blastobacter*, *Bosea*, *Nitrobacter*, *Oligotropha*, *Rhodoblastus*, *Rhodopseudomonas*, *Salinarimonas*, and *Tardiphaga*. The taxonomy in *Bradyrhizobiaceae* became quite confusing and makes this family one of the most distinct in terms of including genera that are quite phenotypically diverse and that exhibit extreme physiological and ecological characteristics.

Despite genetic, biochemical, physiological, and ecological characteristics being important to better elucidate the taxonomic classification in *Bradyrhizobiaceae*, particularly in this case, we still suggest that complete genome sequencing and comparative genomics seem to be the best alternatives in these cases. Given the advances in DNA sequencing techniques on a large scale, this trend can be seen as a common practice in bacteria that have small genomes and not complex genomes like those of eukaryotes.

Molecular Analyses

Currently, the family *Bradyrhizobiaceae* consists of 11 genera, as defined by 16S rRNA gene phylogenetic analysis (Garrity et al. 2005; Okubo et al. 2013), while considering that the inclusion of genus *Nitrobacter* is out of accordance with the Rules of the Bacteriological Code (1990 Revision) (Parte, 2014). However, these genera have high phenotypic, metabolic, and ecological diversity, including organisms that can fix nitrogen, photosynthetic organisms, organisms that employ aerobic and/or anaerobic respiration, organisms that are important in the sulfur cycle, and even organisms that are human pathogens.

The classification of the microorganisms in the family *Bradyrhizobiaceae*, with regard to genera, was recently altered, mainly due to the use of more effective molecular methods and comparison of genomic sequences or complete genomes. Sequencing of the *Agromonas oligothophica* genome (the only species in the genus *Agromonas*) showed that this species can be classified as a bacterium belonging to the genus *Bradyrhizobium* and oligotrophic *Bradyrhizobium* species (Okubo et al. 2013). Similarly, a new genus within the family *Bradyrhizobiaceae* was isolated and determined from this species, the genus *Salinarimonas* (Liu et al. 2010), whose species is *Salinarimonas rosea*, and more recently the genus *Tardiphaga*, whose species is *Tardiphaga robiniae* (De Meyer et al. 2012).

Currently, a large number of *Bradyrhizobiaceae* strains have had their complete and/or draft genome sequence deciphered according to the Genomes Online Database (Pagani et al. 2012). Indeed, genome sequencing is a powerful tool for investigating the biology of the bacterial world. In particular, for nitrogen fixation symbionts, genomic sequencing research during the last decades has yielded several insights regarding the molecular genetics and physiology of these organisms. For instance, it is well known that most of the genes related to nitrogen fixation and interaction with the plant host are located in specific genomic islands, known as symbiosis islands. In general, the genomic island forms syntenic groups of multiple accessory genes encoding a diverse set of functions and characteristics, most of which are associated with adaptation and virulence (Juhas et al. 2009). These genomic islands are acquired by lateral gene transfer (LGT) events, such as bacterial conjugation and are often inserted into various tRNA genes (Juhas et al. 2009). Conversely, to form functional nodules, some *Bradyrhizobiaceae*, such as *B. japonicum* USDA 110, use a type III secretion system to transport bacterial effector proteins into the cytoplasm of target eukaryotic cells (Tsukui et al. 2013). Indeed, some of the essential genes related to type III secretion systems are also found on symbiotic islands (Kaneko et al. 2002, 2011). Symbiosis islands have been very well explored in previous years by classical molecular analysis and more recently, due to the revolution generated by the genomics era, by sophisticated bioinformatics approaches, such as sequence comparison and analysis of the bias of nucleotide patterns and composition along the entire chromosome. However, the impact of symbiosis island transfer on symbiotic relationships is not well understood (Parker 2012). Recently, using Bayesian and comparative approaches, Parker (2012) concluded that symbiotic specificity is complex and difficult to characterize by only analyzing genomic data from the public databases. In this same study, Parker also observed that the host impact on symbiont population structure falls on different genomic regions and that the symbiosis islands are transferred across chromosomal lineages with few impacts to the bacterial genetic structure in other portions of the genome (Parker 2012). Therefore, we are just beginning to understand the evolutionary mechanisms related to these islands in the *Bradyrhizobiaceae* genera.

Moreover, other *Bradyrhizobiaceae* species do not carry these islands and are related to roles other than nitrogen-fixing activities. For instance, the *Afipia* genera is related to human diseases, *Rhodopseudomonas* and *Rhodoblastus* are related to phototropism in non-sulfur environments, *Bosea* is a commensal inhabitant of legume plants, and *Nitrobacter* and *Oligotropha* are chemolithoautotrophic microorganisms. The genetic traits

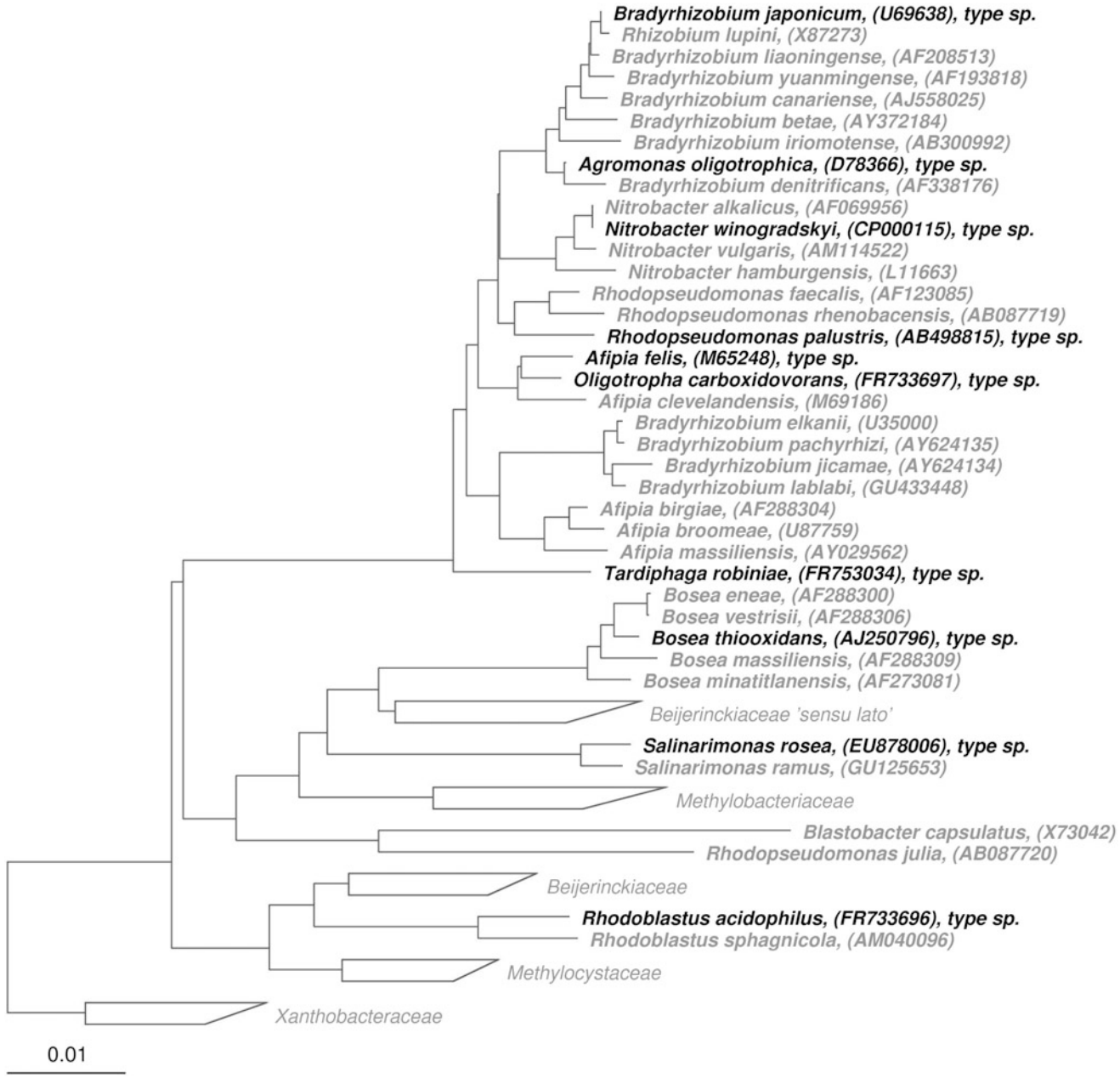

Fig. 5.1

Phylogenetic reconstruction of the family *Bradyrhizobiaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes–Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (*LTP*) database (Yarza et al. 2010, http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

related to each genus cited above are specific for their location on the chromosome and their genes. For most of these microorganisms, different research groups around the world in recent years have made and are indeed making efforts to generate the genome sequence for these organisms, with the main goal being to achieve these genetic traits and also to catalog the microbiological diversity of our planet. Currently, study and comparisons of the genome sequence using molecular and bioinformatics approaches, such as DNA–DNA hybridization and 16S rRNA and housekeeping gene phylogeny, indicate that the *Bradyrhizobiaceae* group is indeed genetically quite heterogeneous.

In this study, we will present and compile the major findings and features of the sequenced type strains of the

Table 5.1

Genome structure and architecture of the sequenced type strains of *Bradyrhizobiaceae*. The genomic information used to fill this table was collected from the GenBank and IMG databases and the literature. The absence of information regarding one of the selected features does not mean that these particular feature do not exist on the type strain considered in this table

Genus	*Bradyrhizobium*	*Afipia*	*Agromonas*	*Bosea*	*Salinarimonas*
Type strain considered	*B. japonicum* USDA 6	*A. felis* ATCC 53690	*A. oligotrophica* S58	*B. thiooxidans* DSM9653	*S. rosea* DSM 21201
Genome completeness	Complete	Permanent draft	Complete	Not sequenced yet	Permanent draft
GenBank accession	NC_017249.1	AGWZ01000000	NC_020453.1		AUBC01000000
Reference	Kaneko et al. 2011	Earl et al. 2012	Okubo et al. 2013		Kyrpides et al. 2013
Chromosomes and plasmids	1 chr	n/d	1 chr		n/d
Genome size (bp)	9,207,384 bp	4,203,290 bp	8,264,165 bp		5,245,321 bp
Number of coding genes	8,826	4,046	7,228		4,952
Pseudogenes	0	1	0		n/d
tRNAs coding genes	51	51	51		45
rRNA gene cluster	2	3	2		5
Number of genomic islands	15	n/d	n/d		n/d
Number of symbiosis island	3	n/d	n/d		n/d
Mobile genetic elements orfs	69	n/d	n/d		n/d

Genus	*Nitrobacter*	*Oligotropha*	*Rhodoblastus*	*Rhodopseudomonas*
Type strain considered	*N. winogradskyi* Nb-255	*O. carboxidovorans* strains OM4 and OM5	*R. acidophilus* DSM137T	*R. palustris* ATCC 17001
Genome completeness	Complete	Complete	Not sequenced yet	In progress (2013)
GenBank accession	NC_007406.1	PRJNA66839 and PRJNA41025		
Reference	Starkenburg et al. 2006	Volland et al. 2011		
Chromosomes	1 chr	1 chr and 2 pls		
Genome size (bp)	3,402,093 bp	3,839,771 and 3,896,074 bp		
Number of genes	3,143	3,574 and 3,629		
Pseudogenes	21	17 and 12		
tRNAs coding genes	49	48 and 48		
rRNA gene cluster	1	3 and 3		
Number of genomic islands	4	n/d		
Number of symbiosis island	n/d	n/d		
Mobile genetic elements orfs	276	n/d		

n/d not defined or determined

Bradyrhizobiaceae family. Each type strain described will be mainly focused on what advantages the genomic sequence has brought to the knowledge of biology and also implications for ecology and agriculture. In particular, we will focus on genome structure and architecture and symbiosis and genomic islands. An overview of the main strains considered in this review, summarizing the major genomic features, is presented in *Table 5.1*, and the following subtopics are explored based on the literature and the public genomic databases, such as GenBank, Integrated Microbial Genomes (IMG), and PATRIC (PathoSystem Resource Integration Center) (Schueller et al. 2007).

B. japonicum USDA 6. The soybean symbiont *B. japonicum* species exhibits a remarkable genetic diversity, and the strain USDA6 is an effective nitrogen-fixing bacterium and is considered the prototype strain of this species (Kaneko et al. 2011). In summary, the complete genome sequence was deciphered in 2011, having 9.2 Mb, and composed of a unique chromosome encoding 8,826 open reading frames (Kaneko et al. 2011) (*Table 5.1*). Comparative genomics against the closely related

strain, *B. japonicum* USDA 110, showed a high degree of identity and collinearity between both chromosomes and indicated that the major differences are due to three genomic rearrangements related to a large symbiosis island (Kaneko et al. 2011). Genomic analysis also indicates that the symbiosis island was split by large-scale genome rearrangements in three regions called Locus A, Locus B, and Locus C, accounting for a total of 860 kb of its genome (Kaneko et al. 2011). Indeed, the nitrogen fixation gene cluster (*nif*/*fix*) is located in the midst of this disrupted symbiosis island (Kaneko et al. 2011). Furthermore, at least 67 insertion sequences are located inside these loci of symbiosis islands (Kaneko et al. 2011), suggesting that the transposition mediated by insertion sequences may have contributed to the genomic rearrangements and thus to differences found in the collinearity between *B. japonicum* USDA 6 and *B. japonicum* USDA 110. Moreover along with the symbiosis island loci, an additional 15 varieties of typical GIs not associated with nitrogen fixation and with duplicated portions of tRNA genes at both ends were identified, which account for up to 592 laterally transferred genes (Kaneko et al. 2011). Therefore, a considerable proportion of laterally acquired elements carrying important genes related to soybean interaction, adaptation, and fitness are very common among *B. japonicum* species. It is noteworthy that *Bradyrhizobium* sp. S23321, which was isolated from paddy field soil, is unable to nodulate in siratro, a legume often used for testing the Nod factors, and interestingly shows a strong collinearity with *B. japonicum* USDA 6 and B. japonicum USDA 110 (Okubo et al. 2012). Genomic analysis strongly suggests that *Bradyrhizobium* sp. S23321 did not acquire the required symbiosis island present in both *B. japonicum* strains by horizontal gene transfer and instead carries a photosynthetic system, indicating the capacity to adapt to a wide range of environments (Okubo et al. 2012). These observations indicate that lateral gene transfer acts as a central player in the evolution of genome architecture in this group of important nitrogen-fixing bacteria.

A. felis ATCC 53690. The human intracellular, lymph node isolated, and putative etiologic agent of cat scratch disease, *A. felis* ATCC 53690, is an organism that is very difficult to isolate (Birkness et al. 1992). Although only a few reports indicate successful isolation and maintenance of the bacterium on artificial medium (Birkness et al. 1992), this organism has been studied since the early 1990s but only partially sequenced by the Broad Institute through the Human Microbiome Project (HMP) initiative in 2012. According to the Integrated Microbial Genomes Database (IMG), only 122 genes are putative products of LGT events, and only 1 gene and no genes code for viruses and transposase functions, respectively. This finding suggests a genome with no pressure for LGT events and with no extra chromosomal elements. This observation may be investigated in detail in future works. Indeed the HMP is underway, and insights into the role of *A. felis* should be revealed in the coming years.

A. oligotrophica S58. This bacterium, formally known as *Bradyrhizobium oligotrophicum*, is a soil-dwelling and nitrogen-fixing oligotroph that is capable of growing in extra-low-nutrient conditions (Okubo et al. 2013). Its complete genome sequence was recently deciphered and interestingly lacks the presence of important nitrogen-fixing landmarks, such as the symbiosis island and nodABC genes. Conversely, *A. oligotrophica* S58 carries nitrogen fixation and photosynthesis gene clusters and is able to nodulate *Aeschynomene indica*, an aquatic legume plant, in the absence of the Nod factor (Okubo et al. 2013). Inoculation experiments also suggested an endophytic lifestyle of *A. oligotrophica* S58 in rice plants (Okubo et al. 2013). Interestingly, both *A. oligotrophica* S58 and *Bradyrhizobium* sp. S23321 lack a typical symbiosis island (Okubo et al. 2013). Therefore, it is believed that key molecules for the Nod factors independent of symbiosis are also key components for maintaining basic cellular functions of *A. oligotrophica* S58, thus indicating that bradyrhizobial purine derivatives would be a key component for triggering the process of nodule formation (Okubo et al. 2013; Masson-Boivin et al. 2009).

B. thiooxidans DSM9653. The *Bosea thiooxidans* species was originally isolated from agricultural soils in India and also found as a commensal inhabitant of legume nodules in Tunisia, but it was not capable of inducing nodule formation (Das et al. 1996; Zakhia et al. 2006). The phenotypic traits of the genus *Bosea* are related to cellular fatty acid and substrate utilization. Unfortunately, there is no full-length or draft genome sequence determined yet for *B. thiooxidans* type strain DSM9653. However, different strains of *B. thiooxidans* have had nearly full-length portions of the 16S rRNA and nitrogenase genes, as well as other housekeeping genes such as *rpoB*, *gyrB*, *atpD*, *recA*, *dnaK*, and *nifH* genes, sequenced and added to the public databases for comparative studies. These sequenced genes have been used for phylogenetic studies in the last few years, which confirmed that the *Bosea* genus is near the *Salinarimonas* genus, according to the *Bradyrhizobiaceae* tree.

S. rosea DSM 21201. *S. rosea* DSM 21201 was isolated from Moxie, Yunnan, Southwest China. The genome sequence was deciphered as a permanent draft in 2013 by an initiative created by the DOE Joint Genome Institute, which has the aim to determine the genome sequence of 1,000 type strains from DSMZ and ATCC repositories and to create an encyclopedia of type strains in order to fill phylogenetic gaps and generate reference genomes. Genomic comparisons indicate that the closest relative is strain 69-29, which was isolated from sediments of the Guanting Reservoir in China and shares at least 98 % identity based on 16S rRNA sequence comparisons (Liu et al. 2010). Although a partial genome sequence is available, there is no published comparative genomic study available in the literature, and this species remains to be investigated in the very near future by the scientific community.

Nitrobacter winogradskyi Nb-255. The third member of the *Bradyrhizobiaceae* for which the genome has been fully sequenced is the nitrite-oxidizing bacteria, *N. winogradskyi* Nb-255, which is a facultative chemolithoautotrophic microorganism that fixes carbon dioxide as its source of carbon (Starkenburg et al. 2006). The genome sequence that was determined in 2006 indicated an important set of gene clusters related to nitrite oxidation and reduction to nitric oxide and to

autotrophy capabilities that are mostly mediated by type I ribulose-1,5-bisphosphate carboxylase–oxygenase (RuBisCO) via the Calvin–Benson–Bassham (CBB) cycle (Starkenburg et al. 2006). Previous studies based on DNA–DNA hybridization of *coxA*, *coxB*, and *coxC* genes revealed that cytochrome c oxidase genes are organized in a repeated gene cluster (Berben 1996). Cell motility is guaranteed by structural and regulatory genes necessary for flagellar synthesis and function in response to environmental changes (Starkenburg et al. 2006). Genomic comparisons based on 16S rRNA show that *N. winogradskyi* Nb-255 is 98 % and 95 % identical to *Bradyrhizobium japonicum* USDA110 and *Rhodopseudomonas palustris* CGA009, respectively (Starkenburg et al. 2006). Two-thirds of the predicted proteins in *N. winogradskyi* Nb-255 have the highest degree of sequence identity to homologs in *B. japonicum* and *R. palustris* (Starkenburg et al. 2006). For instance, at least 85 genes are closely related to *N. europaea* and encode transposases, a multicopper oxidase/cytochrome cluster, glycolate oxidase, sulfite reductase, and siderophore receptors (Starkenburg et al. 2006), thus suggesting an exchange of genetic material by lateral gene transfer events (Starkenburg et al. 2006). Moreover, several integration events mediated by the lateral gene transfer mechanism were identified and account for up to 276 of the coding sequences, whereas the insertion sequence and phage-like regions appear to be the most prevalent mobile genetic elements found in *N. winogradskyi* Nb-255, and they present a potential role for *Nitrobacter* evolution (Starkenburg et al. 2006).

Oligotropha carboxidovorans (Strains OM4 and OM5). Isolated from wastewater in Göttingen, Germany, the chemolithoautotrophic bacteria *O. carboxidovorans* is capable of using carbon monoxide and carbon dioxide hydrogen and also has the capability for heterotrophic growth under appropriate environmental conditions (Paul et al. 2008; Meyer et al. 1993). The genomes of strains OM4 and OM5 were sequenced in 2011 (Paul et al. 2008). These genome sequences revealed that the main differences between these strains were related to acquisition of an 8 kb prophage-like region and a 53 kb region encoding a dissimilatory nitrate reduction (*narGHJI*), nitrous oxide reduction (*nosRZDFYL*), transporters, and biosynthesis proteins in the OM5 strain (Paul et al. 2008). The corresponding 53 kb region in OM4 is formed of phage-related genes, transposases, pseudogenes, and only a single copy of the *narI* gene (Paul et al. 2008), suggesting that the lateral gene transfer events generate by mobile genetic elements, such as temperate phages, are the key agents of genome evolution and differentiation in these strains.

Rhodoblastus acidophilus DSM137T. The purple, non-sulfur bacteria *R. acidophilus* is adapted to acidic aquatic environments and has properties that are considered unique to the genus *Rhodoblastus* (Imhoff 2001). For instance, DNA–DNA hybridization against the closely related bacterium *Rhodopseudomonas palustris* indicates that there is less than 15 % similarity between these strains (Imhoff 2001). Moreover, 16S rRNA comparisons also support that *R. acidophilus* is quite different from the *Rhodopseudomonas* genera (Ivanova et al. 1988). In spite of the absence of full-length or draft genome sequences deposited in the public databases, several sequences of 16S rRNA and nitrogen-fixing genes from different strains of *R. acidophilus* are available in the public databases for evolutionary and functional studies.

R. palustris ATCC 17001. The phototrophic, purple, non-sulfur bacteria *R. palustris* has had several strains completely sequenced in the last few years according to the Genome Online Database (Pagani et al. 2012), whereas the genome sequence of the type strain ATCC 17001 is in progress by an initiative coordinated by the University of Washington. In general, the non-type and different strains of *R. palustris* harbor a unique replicon, ranging from 4.8 to 5.7 Mb and encoding from 4,500 to 5,200 proteins. The only exception is strain CGA009, which harbors a small plasmid of 8.4 kb. Therefore, molecular analyses based on 16S rRNA, internal transcribed spacer (ITS), *puf* genes, and DNA–DNA hybridization indicate that *R. palustris* is genetically quite heterogeneous within the genus. Only in the year 2009 was *R. palustris* considered a novel species of the genus *Rhodopseudomonas* (Okamura et al. 2009).

Phenotypic Analyses

Among the genera of this family, there are still many isolates that are not fully classified or characterized; some have phenotypic traits similar to species already described but without molecular support, and others have similar molecular traits but are biologically different. In addition, there are defined genera with species that have still not been obtained from pure cultures, for example, the type species of the genus *Blastobacter* (*B. henricii*).

The type species of each genus from family *Bradyrhizobiaceae* (Table 5.1) were used for comparative phenotypic analysis in this family. The general morphological, cultural, and metabolic characteristics of this large family are shown in Tables 5.2 and 5.3, which are exclusively based on the characteristics of the type species from each genus currently belonging to the family *Bradyrhizobiaceae* and were mostly obtained from texts described by Garrity et al. (2005) and updated as needed.

All species of the family *Bradyrhizobiaceae* are characterized as Gram-negative bacteria, rod shaped, and irregular or not, with sizes ranging from 0.5 to 1.3 μm wide by 1 to 5 μm long. Species of the genus *Rhodoblastus* stand out for having the largest size on average (Table 5.2). All exhibit mobility provided by the presence of one or two polar or subpolar flagella.

In the genus *Bradyrhizobium*, bacteria are small and rod shaped in young cultures, but these bacteria become pleomorphic (swollen and elongated) in older cultures or under adverse conditions. The different species of the genus *Bradyrhizobium* characteristically form symbiotic nodules on the roots of tropical legumes (Leguminosae), in which the bacteria live as nitrogen-fixing symbionts. *Bacteroides* are rod shaped and slightly swollen, but not branched or highly distorted. Both forms have poly-β-hydroxybutyrate granules, and *Bacteroides* also exhibit polyphosphate inclusions. Bacterial cells of this genus are characterized by a bifurcated division that results in Y-shaped cells in culture. In solid culture medium containing arabinose, yeast extract, or gluconate (Kuykendall 1987; Kuykendall et al. 1988),

Table 5.2

Morphological and molecular characteristics for type species of *Bradyrhizobiaceae*

Genera	*Bradyrhizobium*	*Afipia*	*Agromonas*	*Blastobacter*	*Bosea*	*Nitrobacter*	*Oligotropha*	*Rhodoblastus*	*Rhodopseudomonas*	*Salinarimonas*	*Tardiphaga*
Morphology	Rods (0.5–0.9 × 1.2–3.0 μm)	Rods (0.5 × 1.0 μm)	Irregular rod shape, bent, branched, and/or budding rods (0.6–1.0 × 2.0–7.0 μm)	Ovoid rods, wedge or club shaped (rosette formation), slightly curved, and occasionally branched (0.5–1.0 × 1.0–4,5 μm)	Rods (0.55 × 1.4–1.6 μm)	Pleomorphic rod or pear shaped (0.5–0.9 × 1.0–2.0 μm)	Rod shaped, slightly curved (0.4–0.7 × 1.0–3.0 μm). Star-shaped aggregates (rosettes) by polar pili and excreted slime	Rod shaped (1.0–1.3 × 2.0–5.0 μm)	Rod shaped (0.6–0.9 × 1.2–2.0 μm)	Rod shaped (0.4–1.0 × 1.2–1.45 μm)	Rod shaped, 0.4–0.6 μm by 1.2–2.2 μm
Cell division	*Bradyrhizobium* cells swell and branch (changing to a Y shape) in culture	Not determined	Cell division is by irregular budding and/or elongation pinching	Reproduce by budding at the poles or laterally; the cells are often attaching to form rosettes	Not determined	Cells reproduce by budding or binary fission	Reproduce by budding and sessile division	Polar ground, budding and asymmetric cell division, sessile	Polar ground, budding and asymmetric cell division, tube, or sessile. Formation of prosthecae and rosette-like cell aggregates	Not determined	Not determined
Capsule and special internal structures	Poly-β-hydroxybutyrate granules	None	Cytoplasm compartmentalization by invagination and growth of the cytoplasmic membrane	Capsule in some species. Reproduce by budding at the poles or laterally, form rosettes	None	Intracytoplasmatic membranes as a polar cap of flattened vesicle Inner side of wall and cytoplasmic and intracytoplasmic membranes are covered with a particles from 8 to 10 nm of the nitrite oxidoreductase enzyme	None	Internal photosynthetic membranes underlying and parallel to the cytoplasmatic membrane	Internal photosynthetic membranes underlying and parallel to the cytoplasmatic membrane	None	None

Table 5.2 (continued)

Genera	*Bradyrhizobium*	*Afipia*	*Agromonas*	*Blastobacter*	*Bosea*	*Nitrobacter*	*Oligotropha*	*Rhodoblastus*	*Rhodopseudomonas*	*Salinarimonas*	*Tardiphaga*
Motility	Motile (one polar or subpolar flagellum)	Motile (one or two polars, subpolar, or lateral flagella)	Motile (one polar flagella)	Motile (flagellated swarmer cells)	Motile (one polar flagella)	Motile (one polar or subpolar flagellum)	Motility variable; when present it is by one subpolar flagellum	Motile by polar flagellum	Motile by polar or subpolar flagellum	Motile by polar flagellum	Nonmotile
Storage and important compounds	Poly-β-hydroxybutyrate and water-soluble extracellular polysaccharide	None	Poly-β-hydroxybutyrate and fluorescent pigment	None	None	Poly-β-hydroxybutyrate, glycogen, and polyphosphates granules Carboxysomes in some species	None	Photosynthetic pigments (bacteriochlorophyll a and carotenoids)	Photosynthetic pigments (bacteriochlorophyll a and carotenoids of spirilloxanthin series)	None	None
DNA G+C%	61.0–65.0	61.5–69.0	65.1–66.0	59–60	65.0–69.0	59–62	62.5–63.1	62.2–66.8	64.8–66.3	67.7–71.8	60.8

Table 5.3

Cultural, biochemical, and biological characteristics associated with each genus

Genera	*Bradyrhizobium*	*Afipia*	*Agromonas*	*Blastobacter*	*Bosea*	*Nitrobacter*	*Oligotropha*	*Rhodoblastus*	*Rhodopseudomonas*	*Salinarimonas*	*Tardiphaga*
Colony size, appearance, and color	>1.0 mm (5–6 days incubation), circular, opaque (rarely translucent), white and convex	0.5–1.5 mm opaque, gray white, glistening, and convex with smooth edges	<0.5 mm, colorless/white, punctiform, pulvinate	*B. henricii* characteristic was observed only in an enrichment culture	Round, circular, smooth, mucoid, and cream colored	Growth on agar surface is limited	Colony is white or cream colored. Colony size is variable	Colony/culture is red to orange-red colored	Colony/culture is brown-red to red colored	Colonies are circular, entire, convex, and pink. with 2.5 mm diameter	Colonies are white, smooth, and round; have a diameter smaller than 0.3 mm; and are convex with entire margins
Optimal temperature	25–30 °C (maximum growth temperature 33–35 °C)	30 °C (not growth at/or above 42 °C)	25–27 °C	10–46 °C	30–32 °C (range 20–37 °C)	28–30 °C (range 5–37 °C)	30 °C	25–30 °C	30–37 °C	28–30 °C (range 15–37 °C)	28 °C. (range 15 °C–33 °C)
Optimal pH	pH 6–7 Acid tolerant	pH 6,8	Not determined	6.8–7.8	7.5–8.0 (range 6.0–9.0)	7.5–8.0	Not determined	5.5–6.0	6.9 (range 5.5–8.5)	7.0–8.0 (range 6.0–9.0)	Not determined
Metabolic and cultural characteristics	Aerobic	Aerobic	Aerobic and oligotrophic	Aerobic, heterotrophic	Aerobic	Aerobic but also capable of anaerobic respiration with nitrate	Aerobic and some strains can denitrify	Chemotrophic (microoxic and oxic conditions)	Chemotrophic (microoxic and oxic conditions)	Anaerobic facultative	Aerobic
	Chemoorganotrophic Does not grow in medium with 2 % NaCl	Has non-fermentative metabolism and grows in various common media	Grows under conditions of low organic carbon supply, fixes nitrogen under microaerobic conditions	Grows in low-nutrient medium	Chemolithoheterotrophic	Lithoautotrophic and chemoorganotrophic	Facultatively chemoorganotrophic	Photoheterotrophic (anoxic conditions and light)	Photoheterotrophic (anoxic conditions and light)	Halotolerant, grows in NaCl concentration 0–5 % (optimum, 3 %, w/v)	Very slow-growing bacteria showing visible growth after 7 days on aerobic incubated R2A and 10 times diluted
	Does not absorb Congo red from a yeast extract–mannitol mineral salts; the colonies are colorless or very faintly pink	Grows well in BCYE medium without NaCl and does not grow in medium with 6% NaCl		Some species can grow chemolithotrophically with hydrogen	Reduced sulfur in the presence of organic carbon	Grows in mineral liquid medium containing nitrite and in the dark or with organic C and N without nitrite	Colony formation is slower in solid medium (1–4 weeks) than in broth (generation time varies from 15 to 20 hs with 20 % CO)	Photoautotrophic growth (with hydrogen as electron donor; sulfide and thiosulfate can't be used) Sulfate is used as a sulfur source	Photoautotrophic growth (with hydrogen, sulfide, or thiosulfate as electron donor) Sulfate is used as a sulfur source		TSA

Table 5.3 (continued)

Genera	*Bradyrhizobium*	*Afipia*	*Agromonas*	*Blastobacter*	*Bosea*	*Nitrobacter*	*Oligotropha*	*Rhodoblastus*	*Rhodopseudomonas*	*Salinarimonas*	*Tardiphaga*
Main carbon source	Uses several carbohydrates and organic acids as a carbon source	Several – common agar and broth media (heart infusion with blood, trypticase soy with blood, tryptone glucose yeast extract and BCYE)	Ferulic, p-coumaric, and p-anisic acid with organic nutrients in the medium	Alcohol, sugars, organic acids, and some amino acids	Uses several monosaccharides, organic acids, and amino acids	Carbon dioxide (oxic conditions with nitrite)	CO (energy and carbon assimilation by Calvin cycle)	Carbon sources are several organic acids (but not sugars or sugar alcohol), methanol, and ethanol	Benzoate, pyruvate, formate acetate, fumarate, lactate, butyrate, and some sugar alcohol such as mannitol and glycerol	Sugar, amino acid, and organic acids	Don't use glucose for oxidation, utilization of citrate and gluconate
						Pyruvate, formate acetate (without nitrite)	Salts of pyruvate, formate, glyoxylate, and other organic acids; sugars and amino acids may be used by some strains				
Main nitrogen source	Ammonium salts, nitrate, and amino acids as nitrogen source	Not determined	Ammonium Sulfate in the presence of O_2 and N_2 in microaerophilic conditions	Ammonium, nitrate, urea, yeast extract casein hydrolyzed	Glutamate, glutamine, and aspartate	Peptone and yeast extract in heterotrophic medium without nitrite	Urea, ammonia, nitrite, and nitrate (exception when CO is the substrate)	Ammonia, dinitrogen, and amino acids	Several nitrogen sources (ammonia, guanine, xanthine amino acids, cytidine, citosyne, and others	Nitrate is reduced to nitrite	Not determined
					Neither ammonia nor nitrate or urea serves as nitrogen source			Complex nutrients (yeast extract) do not increase the growth			Positive for nitrate reduction
N_2 fixation	Yes, in symbiosis with root of leguminous plants or free-living state under special conditions	No	Yes, under microaerobic conditions	No, may reduce nitrate to nitrite	Denitrification occurs with gas production under microaerobic conditions with malate, succinate, glucose, and sucrose	No	No	Nitrogenase has been found in all species	Nitrogenase shows a "switch-off" effect by ammonia and some organic compounds	No	No
C assimilation	No	No	No	Some species when grown on methanol or methylated amines	No	Yes, under oxic conditions and in the presence of nitrite	Carbon monoxide oxidation can occur in some strains and it is assimilated in the Calvin cycle	Yes, under anoxic conditions	Yes, under anoxic conditions	No	No

Bradyrhizobium colonies are characterized by being opaque, rarely translucent, white, and convex; can have a granular aspect; and are never larger than 1 mm in diameter after incubation for 5–6 days. In liquid medium, turbidity only appears after 3–4 days, and the generation time of these species is calculated to be between 9 and 18 h. These traits were responsible for the name of this bacterial group, with slower growth than another group of symbiotic nitrogen-fixing bacteria, the genus *Rhizobium* from the family *Rhizobiaceae*.

Bacteria of the genus *Afipia* appear to be related to human infections, as *A. felis* was isolated or detected by PCR in the lymph nodes of patients with cat scratch disease (CSD), although the bacterium *Bartonella henselae* is considered a causative agent of CSD. The isolation and history of other species of the genus *Afipia* suggest that these bacteria may act as opportunistic pathogens in humans. Morphologically, bacteria belonging to the genus *Afipia* are straight rods and Gram-negative, do not form clusters or chains, and have one or two polars, subpolar, or lateral flagella easily visualized by the Ryu method (Ryu 1937; Weyant 1995). In BCYE medium, *Afipia* bacterial colonies have diameters ranging from 0.5 to 1.5 mm after 72 h of culture at 30 °C, have gray to white coloring, and are glistening, convex, and opaque with smooth edges.

The genus *Agromonas*, described by Ohta and Hattori (1983) and represented by a single species, *Agromonas oligotrophica*, is characterized by having irregular shapes of bent, branched, or budding rods and compartmentalization of the cytoplasm by invagination of the cytoplasmic membrane and growth. This bacterium shows poly-β-hydroxybutyrate granules and produces a fluorescent pigment. The colonies on solid media are small, colorless or white, punctiform, and pulvinate. However, in 2013, a comparison of 16S rRNA gene sequences and housekeeping gene sequence analysis, phenotypic characterization, and DNA–DNA hybridization led Ramírez-Bahena et al. (2013) to propose reclassification of *Agromonas oligotrophica* within the genus *Bradyrhizobium* as *Bradyrhizobium oligotrophicum*.

Rosette formation is a marked morphological trait of species from the genus *Blastobacter*. These bacterial species are ovoid rod, wedge, or club shaped; slightly curved; and occasionally branched. Cells generally reproduce by budding at the poles or laterally, with the cells attaching to form rosettes. A great difficulty in the study of the *Blastobacter* strains is the absence of a pure culture for the type species *B. henricii*, whose description was performed only by examination of an enrichment culture (Zavarzin 1961). Nevertheless, currently there are five species described for this genus, which have been isolated from water samples and activated sludge (Hirsch and Müller 1985; Doronina et al. 1983; Loginova and Trotsenko 1979).

The genus *Bosea* is characterized by cells with a bacillary shape and a single polar flagellum; the colonies are round, circular, smooth, mucoid, and cream colored in a medium containing an organic substrate supplemented with sodium thiosulfate. Various morphological, biochemical, and molecular traits (DNA–DNA hybridization analysis) determined the isolation of four more species in this genus, *B. eneae*, *B. vestrisii*, *B. massiliensis*, and *B. minatitlanensis* (La Scola et al. 2003; Ouattara et al. 2003), and the additional isolates *Bosea lupine*, *Bosea lathyri*, *and Bosea robiniae* were determined by multilocus analysis techniques (De Meyer and Willems 2012).

Nitrobacter is a genus described by Winslow et al. (1917) and amended by Watson (1971); this genus is morphologically described as being pleomorphic rod or pear shaped. The cells are characterized by the presence of intracytoplasmic membranes as a polar cap of flattened vesicles. The cell wall differs from that found in other Gram-negative bacteria. The inner side is more electron dense than the outer because it has densely packed particles of nitrite oxidoreductase enzyme (Spieck et al. 1996, 1998; Meincke et al. 2004). Poly-β-hydroxybutyrate, glycogen and polyphosphates granules, and (in some species) carboxysomes can be observed in the cytoplasm. Cells normally reproduce by budding (Holt et al. 1994), and growth on an agar surface is limited (Markowitz et al. 2012).

Bacteria belonging to the genus *Oligotropha* are rod shaped and slightly curved, form star-shaped aggregates by polar pili, and excrete slime, especially in the post-exponential and stationary growth phases. Reproduction is by budding and sessile division. Colonies are white or cream colored and are visible on agar plates after 1–4 weeks, depending on the medium. *Oligotropha carboxidovorans* is the sole species representative of the genus *Oligotropha* and has had its genome sequenced by Paul et al. (2010).

Rhodoblastus is a *Bradyrhizobiaceae* genus characterized by rod-shaped cells. Daughter cells originate from polar growth as sessile buds at the pole opposite the flagella; there are no tubes or filaments between the original and the new cell. When the daughter cell reaches the size of the mother cell, division occurs by constriction, and clusters can form. These cells form colonies that are red to orange-red colored, and this color also can be observed in broth culture. The bacteria have internal photosynthetic membranes underlining and parallel to the cytoplasmic membrane. They produce photosynthetic pigments, such as bacteriochlorophyll *a* and carotenoids. The type species from this genus is *Rhodoblastus acidophilus* (previously named *Rhodopseudomonas acidophila*), but another species was recently proposed, *Rhodoblastus sphagnicola* (Kulichevskaya et al. 2006). In contrast to *R. acidophilus*, this new species is not capable of aerobic growth in the dark, has no spirilloxanthin among the carotenoids, and differs in its pattern of substrate utilization. The value for DNA–DNA hybridization between the two strains is only 22 %.

Bacteria from the genus *Rhodopseudomonas* are very similar to *Rhodoblastus*, but in this genus the cells are smaller. The species are characterized by asymmetric cells during cell division and formation of prosthecae and rosette-like cell aggregates. The mother cell produces a slender prosthecae 1.5–2.0 times the length of the original cell at the opposite pole from the flagella. Rosette or cluster formation occurs mainly in older cultures. The colonies or culture suspensions are brown-red or red colored. Similar characteristics can be observed in all species from this genus. They produce photosynthetic pigments, such as bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series.

The newest genus from the *Bradyrhizobiaceae* family, the genus *Salinarimonas*, was proposed by Liu et al. (2010), and the genus *Tardiphaga* was proposed by De Meyer et al. (2012). The type species from *Salinarimonas* is *S. rosea* and another species, *S. ramus*, was defined (Cai et al. 2011). These bacteria have a rod shape, and the colonies formed after 5 days under optimal conditions are circular, entire, convex, and pink-colored. The type species from *Tardiphaga*, which has only one isolated species, *Tardiphaga robiniae*, is an aerobic, nonmotile, rod-shaped bacterium with a very slow growth rate that was isolated from *Robinia pseudoacacia* root nodules.

Guanine–cytosine content is an important attribute of bacterial genomes. It has been used to scan the basic makeup of the genome, as well as to understand coding sequence evolution. The variety in GC content is great in bacterial genomes. The percentage composition can range from 25 % to 75 % for GC richness among species (Table 5.2), but it is highly constrained and constant in a single species. However, this trait does not have a classificatory value for the levels of bacterial family and genus due to its large variation. The GC content in *B. henricii* is unknown, but this parameter ranges from 59 % to 69 % for other species of the genus. In contrast, the DNA GC content among all of the genera ranges from 59 % to 71 %, and the genus *Afipia* has the highest variation in this parameter (7.5 %). All of the species studied from the genera belonging to the family *Bradyrhizobiaceae* can be considered C+G-rich bacteria, which may relate to their mesophilic characteristics (Zheng and Wu 2010).

A wide diversity of behaviors can also be observed in the culture and biochemical characteristics of genera from the family *Bradyrhizobiaceae* (Table 5.3). The optimal pH for most species is between 6 and 7, with some acid tolerance occurring in *Bradyrhizobium*, and *Salinarimonas*, *Nitrobacter*, and *Rhodopseudomonas* can even grow in basic medium with a pH close to 8. The pH range for *Agromonas*, *Oligotropha*, and *Tardiphaga* has not yet been determined. The growth temperature for these bacteria ranges from 25 to 30 °C, where *Blastobacter* species exhibit greater extremes and can adapt to temperatures from 10 to 46 °C.

In in vitro culture, the size and shape of the colonies are also characteristic of each genus, and the colonies are usually circular and translucent but with different colorations: *Bradyrhizobium* colonies are characteristically white, *Afipia* have gray-white colonies, *Bosea* and *Oligotropha* have cream-colored colonies, and the colonies from *Rhodoblastus* and *Rhodopseudomonas* have orange-red-brown color and the color of colonies are pink. The type species *Nitrobacter* should be highlighted because it has limited growth in solid mineral medium. *N. winogradskyi* was described as a bacterium that forms small colonies that are yellowish white in color, although other species of the genus that were recently isolated have an orange color (Zare et al. 2012). In contrast within the genus *Blastobacter*, its type species *Blastobacter henricii* has been observed only in enrichment cultures, and thus the colony morphology cannot be determined. Other species from the genus *Blastobacter* have colorless to yellow colonies that become brownish when older.

Regarding the metabolic characteristics observed in genera from the family *Bradyrhizobiaceae*, this bacterial group comprises organisms with high diversity in the strategies for obtaining energy, carbon sources, and electrons needed for their development. The vast majority of species are aerobic, i.e., use oxygen as a final electron acceptor in the oxidative processes of obtaining energy (*Bradyrhizobium*, *Afipia*, *Agromonas*, *Blastobacter*, *Bosea*). Bacteria from the genera *Nitrobacter* and *Oligotrophica* are aerobic, but can survive anaerobically under specific conditions. In contrast, there are bacteria that preferentially develop in low-oxygen or anaerobic environments (*Rhodoblastus*, *Rhodopseudomonas*, and *Salinarimonas*).

Bacteria from the genus *Bradyrhizobium* are chemoorganotrophic, using a range of carbohydrates and salts from organic acids as carbon sources, but do not use cellulose or starch, and produce alkaline reactions on mineral salts medium with mannitol or other sugars. In this medium, the *Bradyrhizobium* strains lack the ability to absorb Congo red, and the colonies observed under this condition are colorless white or very faintly pink. This is a differential medium because most other soil bacteria produce red colonies under this condition. Ammonium salts (nitrates preferably) and some amino acids can be used as nitrogen sources. This organism may enter through the root hairs of tropical and temperate zone leguminous plants (Fabaceae) and induce nodule formation. In the nodule, the bacteria live as symbionts where they are able to fix nitrogen. Some strains exhibit hydrogenase activity with the soybean host and thus are more efficient in symbiotic nitrogen fixation (Maier 1981; Vauclare et al. 2013).

The main metabolic trait of *Agromonas oligotrophica*, currently classified as *Bradyrhizobium oligotrophicum* (Okubo et al. 2013), is its ability to grow in an environment that is poor in carbon. Important carbon sources in culture medium for this bacterium include the following organic acids: ferulic acid, p-coumaric acid, and p-anisic acid (Ohta 2000). The main nitrogen source in the presence of oxygen is ammonium sulfate, but this bacterium has the ability to fix nitrogen in microaerobic environments. Despite these data, metabolic knowledge about this bacterial species is still scarce, although it appears to be important for the degradation of organic matter and nutrient cycling in environments with low levels of this material considering its isolation site (rice paddy soils).

Afipia is a *Bradyrhizobiaceae* genus that is related to cat scratch disease, and it is a facultative intracellular bacterium. This bacterium has a non-fermentative and aerobic metabolism; it grows well on buffered charcoal yeast extract (BCYE) agar and nutrient broth without NaCl or less than 6 % of this salt. The bacteria from this genus grow well in several common medias, but generally these medias must be supplemented with heart infusion or blood. Some strains have the ability to reduce nitrate. *Afipia* bacteria do not produce acid with carbohydrate; they are positive for oxidase, urease, and litmus milk alkalinization.

Bacteria from the genus *Blastobacter* are aerobic and heterotrophic. They grow in low-nutrient medium, and they can use many alcohols, sugars, organic acids, or some amino

acids as carbon and energy sources. Ammonium, nitrate, urea, peptone, yeast extract, or casein hydrolysate may be utilized as nitrogen sources. These bacteria may reduce nitrate to nitrite or denitrify. Some species of these bacteria grow chemolithotrophically with hydrogen, and some species may fix CO_2 reductively when grown on methanol or methylated amines (Loginova and Trotsenko 1979). Because a culture of the type species *Blastobacter henricii* has never been isolated, the genus is characterized mainly by morphological traits, as budding cell division, which is not a phylogenetically useful characteristic and is observed in several genera from the *Alphaproteobacteria* class. Several authors have demonstrated a high degree of heterogeneity in species of the genus *Blastobacter* with respect to their phenotype and molecular phylogeny. Analysis of the 16SrRNA sequences of some species from the *Blastobacter* genus has determined that these species are in polyphyletic positions in the *Alphaproteobacteria* class (Sly and Hugenholtz 2005; Doronina et al. 1996); therefore, the taxonomy of the genus *Blastobacter* needs revision (Doronina and Trotsenko 2003).

Species from the genus *Bosea* are characterized as being aerobic and chemolithoheterotrophic bacteria, capable of obtaining energy from the oxidation of reduced sulfur compounds. Ammonia, urea, and nitrate cannot serve as nitrogen sources, and instead the type species of this genus, *B. thiooxidans*, uses glutamate, glutamine, and aspartate as sources of this element. Heterotrophic growth can be caused by organic compounds, such as glucose, fructose, sorbose, xylose, pyruvate, rhamnose, ribose, arabinose and galactose, citrate, gluconate, succinate, malate and acetate, glutamine, proline, aspartic acid, cysteine, serine, asparagine, alanine, and lysine. Under microaerobic conditions, denitrification occurs with gas production in GYM medium (glutamate–yeast extract–mineral salts medium) containing malate, succinate, glucose, or sucrose as a carbon source.

Nitrobacter grows lithoautotrophically or chemoorganotrophically, occurs in aerobic and microaerophilic atmospheres where organic matter is mineralized, and it is capable of anaerobic respiration. Under anaerobic conditions, nitrate is reduced to nitrite, nitric oxide, and nitrous oxide. Nitrobacter cells are facultative lithoautotrophs that obtain energy from the oxidation of nitrite to nitrate when the main carbon source is carbon dioxide. In the absence of nitrite, pyruvate, formate, and acetate can serve as energy and carbon sources. During mixotrophic growth, nitrite is oxidized first, followed by the oxidation of organic material. Under lithoautotrophic and mixotrophic conditions, bacteria have a generation time of 8–14 h, and under heterotrophic conditions, the generation time is 70–100 h.

The type species from the genus *Oligotropha*, *O. carboxidovorans*, is an obligately aerobic, facultative autotrophic bacterium. It is a chemolithoautotrophic bacterium that is capable of utilizing carbon monoxide, carbon dioxide, and hydrogen. It is also capable of heterotrophic growth under appropriate environmental conditions. It can utilize as its sole source of carbon and energy organic acids such as acetate, lactate, pyruvate, succinate, fumarate, I.-malate, formate, and crotonate. It cannot utilize sugars, amino acids, or citrate. Starch, tween, agar, gelatine, and polyhydroxybutyrate are not hydrolyzed. Nitrates are reduced to nitrites. It grows autotrophically at the expense of either carbon monoxide or hydrogen plus carbon dioxide. The carbon monoxide-oxidizing activity is bound to a small particle fraction, does not reduce NAD(P), and is only present in cells grown on $CO + O_2$. Hydrogenase is membrane bound, does not reduce NAD(P), and is present in cells grown on $H_2 + O_2 + CO_2$, $CO + O_2$, and pyruvate + O_2. *O. carboxidovorans* may have practical utility for bioenergy production. This species is capable of utilizing syngas, which is a mixture of CO, CO_2, and H_2 that results from gasification of organic wastes, for chemolithoautotrophic growth. Microbial fermentation of this gas mixture could serve as a source for biofuels.

The genus *Rhodoblastus* prefers photoheterotrophic growth with a number of organic carbon sources under anoxic conditions and light. Photoautotrophic growth is possible with hydrogen as an electron donor under anoxic conditions. In the dark and under microoxic or oxic conditions, organic carbon sources are acetate, propionate, butyrate, lactate, pyruvate, fumarate, malate, succinate, valerate, formate, methanol, and ethanol; these cells do not use sugars, sugar alcohols, glutamate, and other amino acids. As a sulfur source, it uses only sulfate, and for nitrogen ammonia, dinitrogen and some amino acids can be used. Recently, studies with new *Rhodoblastus acidophilus* isolates support the observation that few species of acidophilic purple bacteria appear to exist in nature. These isolates, in addition to methanol and ethanol, can grow on several other primary alcohols, results that were not reported in the original species description (Kempher and Madigan 2012).

Cells from the *Rhodopseudomonas* genus can live under aerobic, microaerobic, and anaerobic conditions. They exhibit photoautotrophic grow with hydrogen, sulfide, or thiosulfide as an electron donor. In the light and under anoxic conditions, the cells can grow photoheterotrophically, and under microoxic and oxic conditions, *Rhodopseudomonas* have chemotrophic growth. When necessary, the carbon source can be formate, acetate, pyruvate, lactate, malate, succinate, fumarate tartrate, gluconate, ethanol, or butyrate. Glucose, fructose, citrate, methanol, arginine, and glutamate are not used, and p-aminobenzoic acid and sometimes biotin are required as growth factors. Sulfate is the sole sulfur source. Nitrogenase shows no activity in medium with ammonia or some organic nitrogen compounds, and these cells can use several nitrogen compounds as a nitrogen source (amino acids, di- and trimethylamine, guanine, uric acid, and xanthine).

One of the newest genera from the *Bradyrhizobiaceae* family, *Salinarimonas*, is characterized as a facultative anaerobe; it was isolated under anaerobic conditions, but is able to grow well under aerobic conditions. *Salinarimonas* is halotolerant and must grow in medium supplemented with 0–5 % NaCl. These cells can use several organic compounds as carbon and nitrogen sources (Liu et al. 2010; Cai et al. 2011).

Another recently determined genus in this family is the genus *Tardiphaga* (de Meyer et al. 2012). The type species from this genus is *Tardiphaga robiniae*, which is characterized as an

aerobic bacterium that grows slowly on R2A at 28 °C. It can be distinguished from the other genera by positive nitrate reduction, positive β-glucosidase hydrolase, negative D-mannitol assimilation, and negative malate assimilation, as well as its use of capric acid. This species is negative for lysine and ornithine decarboxylase, tryptophan deaminase, and production of acetoin.

Isolation, Enrichment, and Maintenance Procedures

Bradyrhizobium species are common soil inhabitants, but the strains are difficult to isolate directly from this habitat. The isolation from symbiotic strains in the soil requires the use of trap hosts, a leguminous plant from which nodules are selected for isolation. *Bradyrhizobium* strains cause nodule production in *Glycine* (soybean), *Vigna* (cowpea), and *Macroptilium* (siratro), so these leguminous plants can be used as a trap hosts. The nodules with a small portion of root attached must first be surface sterilized (5–60 min in 3 % H_2O_2 or 5 % commercial hypochlorite) and washed in sterile water. The nodule is crushed in a small drop of sterile 0.05 % peptone or 0.1 M phosphate solution. A loopful of the suspension must be streaked onto surface plates with yeast extract–mannitol agar (YMA medium) (Vincent 1970), and the plate incubated at 28 °C for 3 or more days. The agar medium for initial isolation should contain 0.002 % actidione to avoid fungal contamination. Well-isolated, white, mucoid, glistening, hemispheric colonies are restreaked onto fresh plates for subsequent confirmation, which requires reinfection of the original host under aseptic conditions, and reisolation. The maintenance of *Bradyrhizobium* species consists of several procedures. These bacteria persist on YMA slants in tightly closed screw-capped tubes for more than 5 years at 4 °C. A suitable maintenance medium is A1EG (Kuykendall 1987) or arabinose-enriched (0.1 % yeast extract) gluconate (Kuykendall et al. 1988). Long-term storage at −20 °C or −80 °C is recommended using turbid suspensions from fresh broth cultures mixed with equal volumes of sterile 80 % glycerol in water. Lyophilized cultures stored at 4 °C remain viable for at least 25 years (Kuykendall 2005).

Agromonas oligothophica (the only species from the genus *Agromonas*) and the species *Bradyrhizobium oligotrophicum*, which was recently classified as belonging to the genus *Bradyrhizobium* (Okubo et al. 2013), are widely distributed in nature and share the ability to grow on low-nutrient media. Isolation of these strains is performed on plates with NB medium diluted 1/100. After 1 week to 1 month, the colonies must be tested for sensitivity to nutrients on NB medium without dilution. The cells can be maintained in screw-capped tubes with 1/100 NB medium at room temperature for 3–4 years. Another method for maintenance is the suspension of cells in 20 % glycerol and freezing at −80 °C.

Although no selective media have yet been described for the growth of *Afipia* strains, these strains can be grown at 30–32 °C under aerobic conditions on a variety of media, including nutrient broth, brain–heart infusion, heart infusion, chocolate, potato dextrose, trypticase soy, and BCYE agar. The isolation of *Afipia felis* strains was performed by grinding lymph nodes tissues in phosphate buffered and cultivation on brain–heart infusion or HeLa cell monolayers (English et al. 1988; Brenner et al. 1991). For long-term preservation of *Afipia* strains, it is necessary to mix freshly grown cells with 20 % glycerol or defibrinated rabbit blood and freeze at −70 °C. These strains can be maintained for the short term (less than 1 year) in a semisolid motility medium deep in tightly closed screw-capped tubes at room temperature (Weyant et al. 1995).

Low-nutrient medium containing glucose, peptone, and yeast extract (Stalwy 1981) is used for isolation or enrichment of *Blastobacter* strains from environmental samples or water. Enrichment may be performed by incubating samples at 18–23 °C for 1–3 weeks. Mineral salts medium with 1 % methanol at 30 °C for 7 days was used to isolate *Blastobacter viscosus* (Loginova and Trotsenko 1979). Cultures may be preserved in sucrose peptone broth containing 10 % glycerol by cryogenic storage or freeze-drying in glucose peptone medium with horse serum.

The *Bosea* genus is represented by *Bosea thiooxidans*. To isolate this strain, it is necessary to enrich the soil sample on medium containing sodium thiosulfate, sodium sulfide, and S° for 10 days. Then, the soil is incubated for 2 days in another mineral salt broth with sodium thiosulfate or sodium sulfide and yeast extract. The bacterial colonies can be obtained by culturing on salt–thiosulfate–yeast extract agar by incubation at 30 °C for 4 days. The cultures can be maintained on mixed substrate agar or Luria agar at 4 °C for 2 months or mixed in growth medium with 15 % glycerol and stored at −20 °C for approximately 1 year. For long-term preservation, lyophilization is the best method (Das 2005).

Mineral medium containing nitrite is used to isolate bacteria that oxidate nitrite. If these bacteria have lithotrophic, mixotrophic, or heterotrophic growth, the medium used needs to have different components (Watson and Waterbury 1971; Bock et al. 1983). An interesting summary of the composition of these media can be obtained from *Bergey's Manual of Systematic Bacteriology* (Garrity et al. 2005), where the composition of media for lithoautotrophic (terrestrial and marine), mixotrophic, and heterotrophic nitrite oxidizers is shown. For isolation, enrichment cultures must be incubated for one to several months in the dark. These cells are sensitive to oxygen, so growth on the agar surface is limited. *Nitrobacter* can survive in liquid medium at 17 °C for approximately 4 months. These cells can be stocked for one or more years by freezing in liquid nitrogen with a buffer containing sucrose or histidine or freeze-drying.

The enrichment and isolation of bacteria from the genus *Oligotropha* and other carbon monoxide-oxidizing microorganisms is performed in a mineral medium with CO as an energy source and CO_2 as the sole carbon source under aerobic conditions. Alternatively, enrichment may be carried out under anoxic conditions in the presence of an electron acceptor. In this case, the culture is performed in mineral medium under an

atmosphere composed of 5 % CO_2, 10 % O_2, and 85 % CO or 5 % CO_2, 45 % CO, and 50 % air. The culture is incubated for approximately 1 month (with or without shaking) at 30 °C in the dark. Every 2 weeks, subcultures must be done, and a sample must be streaked onto an agar plate with mineral medium supplemented with pyruvate. The plate has to be maintained in the same atmosphere. Slow-growing colonies (appear after 1 week) with a morphology consistent with these colonies and cells are evidence of axenic culture.

The media used for isolation, enrichment, and maintenance of *Rhodoblastus* and *Rhodopseudomonas* species are the same as that used to isolate other purple sulfur bacteria (Imhoff et al. 1994). A succinate mineral medium with pH 5.2 is selective for *Rhodoblastus acidophilus*. Benzoate is the carbon source that is generally used for enrichment of *Rhodopseudomonas palustris*. The *Rhodoblastus* and *Rhodopseudomonas* species can be preserved in liquid nitrogen, by lyophilization, or in a freezer at −80 °C (Imhoff 2005).

Bacteria from the genus *Salinarimonas* can be isolated on marine agar 2216 (MA; Difco) or seawater agar (ASW), as described by Cai et al. (2011) and Eguchi et al. (1996), respectively. The culture is cultivated at 28 to 30 °C under aerobic or anaerobic conditions. It can be maintained on MA slants at 4 °C and stored in a 20 % glycerol suspension at −80 °C (Liu et al. 2010).

The newest genus described from *Bradyrhizobiaceae*, *Tardiphaga*, was isolated and is maintained with a group of rhizobia associated with indigenous legumes. This bacterium was isolated in YMA medium from surface-sterilized root nodules. All YMA plates were incubated at 28 °C, and the isolates were stored in 15 % glycerol + YMA broth tubes at −20 °C (De Meyer et al. 2011).

Ecology

The ecological diversity observed in *Bradyrhizobiaceae* reflects the genetic, phenotypic, and metabolic diversity present in the family. The different bacteria isolated from soil, water, or hosts may or may not be able to perform fixation and/or other pathways of nitrogen assimilation, carbon assimilation, photosynthesis, aerobic or anaerobic respiration, and even pathogenicity in humans (Garrity et al. 2005).

The ability to use different nitrogen sources in their metabolism (*Table 5.3*) makes all genera of the family, except for *Afipia*, important components of the biogeochemical nitrogen cycle. Nitrogen is the fourth most common element in many biomolecules, and its cycle in soils is one of the most studied (Philippot and Germon 2005). Microorganisms are essential components in different processes that maintain the balance between reduced and oxidized nitrogen forms, ranging from ammonia (−3) to nitrate (+5). Nitrogen cycle fluxes are primarily driven by nitrogen fixation and mineralization through ammonification and nitrification (Robertson and Groffman 2007).

Nitrobacter participates in nitrogen mineralization and immobilization from organic matter, generating nitrate (NO_3^-), which is one of the main nitrogen forms used by most microorganisms and plants. Nitrification is performed via oxidation reactions to assimilate CO_2 and separates the bacteria into two groups based on their ability to oxidize ammonia into nitrite and oxidize nitrite into nitrate (De Boer and Kowalchuk 2001; Gubry-Rangin et al. 2010). *Nitrobacter* species ($NO_2^- \rightarrow NO_3^-$) are important obligatory chemooligotrophic nitrifiers (*Table 5.3*) in soils and allow for complete oxidation of NH_4^+ when combined with actions performed by members of the genus *Nitrosomonas* (Nitrosomonadaceae) ($NH_4^+ \rightarrow NO_2^-$) (Sundermeyer-Klinger et al. 1984; Zhu and Carreiro 1999; Philippot and Germon 2005).

In contrast, *Salinarimonas* and *Tardiphaga* exhibit nitrate reduction ability, generating nitrite under anaerobic conditions. This transformation is an important step in producing ammonia through a process called dissimilatory reduction of nitrate into ammonia. In the absence of oxygen, this process is important for maintaining functional respiratory activity and is an alternative mechanism for denitrification in the soil (Robertson and Groffman 2007).

Denitrification allows the nitrogen to return to the atmosphere, its main reservoir on the planet, by reducing nitrate to gasses, such as NO, N_2O, and N_2. Denitrifying bacteria have representatives from genera *Bosea* and *Blastobacter* (*Table 5.3*). This process occurs under anaerobic conditions because nitrate is a less efficient electron acceptor than oxygen. In some soils, denitrification occurs after the pores are saturated by flooding, which prevents oxygen diffusion and is the main point of the cycle in which the fixed nitrogen reenters the atmosphere as N_2 gas (Tiedje 1988; Čuhel et al. 2010).

Despite the enormous amount of nitrogen in the atmosphere, soil, and water, more than 99 % is present in the form of N_2 gas and unavailable for almost all living organisms on Earth (Herridge et al. 2008). Bacteria belonging to the genera *Bradyrhizobium*, *Agromonas*, *Rhodoblastus*, and *Rhodopseudomonas* are able to directly fix atmospheric nitrogen. These bacteria form a restricted group of microorganisms called biological nitrogen fixers and can perform this process via oxygen-sensitive nitrogenase enzyme complex activity. BNF may or may not occur exclusively in free-living bacteria in soil or water, under specific culture conditions in vitro, and/or in symbiosis with host plants (Garrity et al. 2005). In these processes, N_2 is reduced to ammonia (NH_3), which can be incorporated into the bacterial biomass or supplied to host plants when in symbiosis (Bottomley and Myrold 2007). BNF is important, as it reduces gaseous nitrogen by breaking its triple bond, converting it into inorganic reactive forms, and contributing to an input on the order of 100–175 million tons (Tg) of N per year on the planet (Bottomley and Myrold 2007). The anthropogenic action related to BNF adds another 50–70 million tons (Tg) per year of N used in agriculture (Herridge et al. 2008; Lindström et al. 2010).

BNF is only performed by prokaryotic species, and the NH_3 produced may be assimilated into inorganic nitrogen forms that comprise macromolecules or may be converted into NO_2^- and NO_3^- by nitrifying bacteria. NO_3^- can thus enter

Table 5.4
Examples of host plants associated with *Bradyrhizobium*

Bradyrhizobium species	Host plant
B. japonicum	*Glycine max, Glycine soja, Macroptilium atropurpureum, Lupinus* spp., *Arachis hypogaea, Aeschynomene americana, Rhynchosia minima, Crotalaria hyssopifolia, Bryaspis lupulina, Sesbania rostrata, Chamaecrista* sp., *Cassia absus, Acacia* spp.
B. elkanii	*Glycine max, Glycine soja, Parasponia, Cajanus cajan, Tephrosia purpúrea, Abrus stictosperma, Faidherbia albida, Aeschynomene uniflora, Aeschynomene afraspera, Astragalus canadensis, Faidherbia albida*
B. liaoningense	*Glycine max, Glycine soja, Phaseolus aureus*
B. yuanmingense	*Lespedeza* spp.
B. canariense	*Chamaecytisus proliferus, Teline* spp., *Lupinus* spp., *Adenocarpus* spp., *Spartocytisus supranubius, Ornithopus* spp.
B. diazoefficiens	*Glycine max*
B. betae	*Beta vulgaris*
B. cytisi	*Cytisus villosus*
B. daqingense	*Glycine max*
B. huanghuaihaiense	*Glycine max*
B. iriomotense	*Entrada koshunensis*
B. jicamae	*Pachyrhizus erosus*
B. labladi	*Lablad purpureus, Arachis hypogaea*
B. pachyrhizi	*Pachyrhizus erosus*
B. yuanmingense	*Lespedeza* spp.
Bradyrhizobium sp.	*Lupinus* spp., *Genista tinctoria, Faidherbia albida, Aeschynomene* spp., *Desmodium asperum, Crotalaria lathyroides, Sarothamnus* sp., *Astragalus mollissimus, Ornithopus compressus, Lotus* spp., *Vigna* spp., *Cicer* spp., *Sesbania* spp., *Leucaena* spp., *Mimosa* spp., *Acacia* spp., *Glycine* spp.

into organic metabolism through its reduction into NH_4^+ and assimilation into amino acids by bacteria, fungi, and plants, in addition to serving as an electron acceptor for denitrifying bacteria (Crawford et al. 2000). Thus, all biological processes associated with the nitrogen cycle together contribute to the balance and use of inorganic and organic nitrogen forms associated with different microorganisms, plants, and animals.

Pathogenicity: Clinical Relevance

In the family *Bradyrhizobiaceae*, pathogenicity is associated with the genus *Afipia*. *A. felis*, the cat scratch disease (CSD) bacillus, was first detected in the lymph nodes of human patients with the disease (English et al. 1988; Bergman et al. 1995; Brenner et al. 1991). CSD, also known as cat scratch fever, is a bacterial infection that affects the lymph nodes that drain the inoculation sites and usually develops in patients with a history of cat scratches or bites (Regnery and Tappero 1995). The disease develops as a chronic, localized lymphadenopathy in infected young people, while some immunocompetent patients develop severe systemic diseases or other atypical manifestations, which include oculoglandular syndrome, encephalitis, neuroretinitis, pneumonia, osteomyelitis, erythema nodosum, arthralgia, arthritis, and thrombocytopenic purpura (Carithers 1985; Abbasi and Chesney 1995; Marra 1995; de Kort et al. 2006).

Bartonella henselae is actually considered the main etiologic agent of CSD (Bergman et al. 1995; Regnery and Tappero 1995). Species of the genus *Afipia* can act as opportunistic pathogens capable of surviving in phagosomes by inhibiting the fusion of phagosomes with lysosomes (Schueller et al. 2007). *A. felis* strains are resistant to a wide variety of antimicrobial agents, which include many beta-lactams, ciprofloxacin, and tetracycline, and are susceptible to aminoglycosides and imipenem. *A. clevelandensis* and *A. broomeae* share a similar susceptibility pattern but are more resistant to aminoglycosides. *Afipia* genomospecies 1 and 2 are the most resistant, including all the antibiotics tested, whereas genomospecies 3 is the most sensitive, exhibiting susceptibility to all classes of antibiotics cited except for cefoperazone, cefotoxin, and ceftazidime (Garrity et al. 2005).

Application

Symbiotic nitrogen fixation with bacteria of genus *Bradyrhizobium* is one of the most important aspects of agroecosystem sustainability. In many parts of the world, soybean crops are one of the most relevant examples of the importance of symbiotic associations with bradyrhizobia, in which inoculants are applied to the soil (Lindström et al. 2010). BNF associated with cultivable plants is not only beneficial to them but also provides sustainability of crop development and is related to mitigation of greenhouse gas emissions because it can replace nitrogen fertilizers produced at a high cost of CO_2-generating fossil resources (Boddey et al. 2009).

Plants are considerably varied in acquiring and assimilating nitrogen compounds from the soil, according to their dependency and variation, where NO_3^- is the predominant form. In the case of plants hosting symbiotic N-fixers, this process occurs via endosymbionts hosted in root nodules (Reddy et al. 1997; Hirsch et al. 2001; Lindström et al. 2010). Inoculation of legumes with rhizobia is perhaps one of the oldest agri-biotechnological management strategies, and recognition of its importance dates back to 1888, when M. Beijerinck confirmed that a

microorganism, later identified as a bacterium, was responsible for the formation of root nodules in legumes (Crawford et al. 2000).

There are a variety of host plants associated with different *Bradyrhizobium* species strains (› *Table 5.4*). However, from an applied point of view, the following strains stand out for their ability to nodulate soybean (*Glycine max*): *B. japonicum* (Kaneko et al. 2011) and *B. elkanii* (de Souza et al. 2012). Well-established strains of these two species are available for formulation of commercial inoculants for soybean, and the search for more efficient strains and those adapted to each country's specific agrarian conditions is a constant concern.

Another important biotechnological aspect of bacteria belonging to the family *Bradyrhizobiaceae* is the ability that some species have to produce substances with great biotechnological applications, such as water-soluble extracellular polysaccharides (exopolysaccharides, EPSs). Many species of bacteria possess this ability, and biological EPSs provide protection from various environmental insults, such as desiccation, predation, and the effects of antibiotics (Donot et al. 2012). However, the interest in EPSs has increased considerably in recent years because these compounds are candidates for many commercial applications. Numerous types of exopolysaccharides have already been described (Castellane and Lemos 2007; Monteiro et al. 2012; Mota et al. 2013; Radchenkova et al. 2013; Silvi et al. 2013).

However, only three EPSs (i.e., dextran, xanthan, and gellan gums) have been successfully adopted for industrial purposes (Donot et al. 2012; Prajapati et al. 2013). Bacteria from the genera *Bradyrhizobium* and *Rhizobium* are ESP producers that exhibit important characteristics for use in diverse industrial sectors and bioremediation, although this is still not entirely applied on the commercial scale (Bomfeti et al. 2011). Similarly, these bacteria can also produce another polymer of biotechnological relevance, polyhydroxybutyrate (PHB), the most common representative of polyhydroxyalkanoates (PHA). PHAs are biopolymers that accumulate as carbon sources in many microorganisms, usually when essential nutrients, such as nitrogen or phosphorus, are available in limiting concentrations, as well as in the presence of excess carbon sources. The PHB microbial polyester has unique physicochemical properties, such as thermoplasticity, biodegradability, and biocompatibility (Kapritchkoff et al. 2006). Because of these properties, it could be used to make plastic packages, suture ties, bone prostheses, and medicine capsules. Rhizobia is not yet used for PHB industrial production, but Paganeli et al. (2011) demonstrated that some strains are potential candidates for this purpose because they can be produced in large quantities from inexpensive carbon sources.

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6 The Family *Brucellaceae*

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Abstract
The family *Brucellaceae* comprises the type genus *Brucella* and six further genera, namely, *Crabtreella, Daeguia*, *Mycoplana*, *Ochrobactrum*, *Paenochrobactrum*, and *Pseudochrobactrum*, phylogenetically members of the order *Rhizobiales* within the class *Alphaproteobacteria*. Organisms are Gram-negative and have a rod-shaped morphology with varying length, occasionally motile, do not produce spores, and have an aerobic respiratory type of metabolism. The majority of *Brucella* species have been isolated from animals and occasionally humans, whereas the species belonging to the other genera have been predominantly isolated from environmental sources such as sludge, soil, and water and only rarely from human or animal sources. *Brucella* species of the *B. melitensis* type (*B. melitensis, B. abortus*, *B. suis, B. ovis, B. neotomae*, *B. canis*, and B. *ceti*, considered by some taxonomists as one species, because of their high genetic relatedness) are the causal agents of Brucellosis, a severe disease of animals and man by comparative genetic analyses, it has been demonstrated that the family *Brucellaceae* especially the genus *Brucella* is genetically highly related to plant symbionts/pathogens, such as *Agrobacterium*, *Rhizobium*, and *Mesorhizobium*, and also to animal pathogens such as *Bartonella*.

Several *Ochrobactrum* species are opportunistic living organisms, which cycle from soil-rhizoplane to immunocompromised humans/animals; however, the species of the *Brucella melitensis* group live as intracellular primary parasites that do not require predisposing conditions and are capable to cycle directly from animal to animal.

Taxonomy, Historical, and Current

Short Description of the Family

Bru.cel.la'ce.ae. M.L. fem. n. *Brucella* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Brucellaceae* the *Brucella* family. The description is an emended version of the description given in *Bergey's Manual*, 2nd edition.

Phylogenetically a member of the order *Rhizobiales* within the class *Alphaproteobacteria*. The family contains the type genus *Brucella* (Meyer and Shaw 1920). In addition, six further genera, namely, *Crabtreella* (Crabtree and McCoy 1967; emended by Xie and Yokota 2006), *Daeguia* (Yoon et al. 2008), *Mycoplana* (Gray and Thornton 1928; emended by Urakami et al. 1990), *Ochrobactrum* (Holmes et al. 1988), *Paenochrobactrum* (Kämpfer et al. 2010), and *Pseudochrobactrum* (Kämpfer et al. 2006), are currently recognized. Organisms are Gram negative and have a rod-shaped

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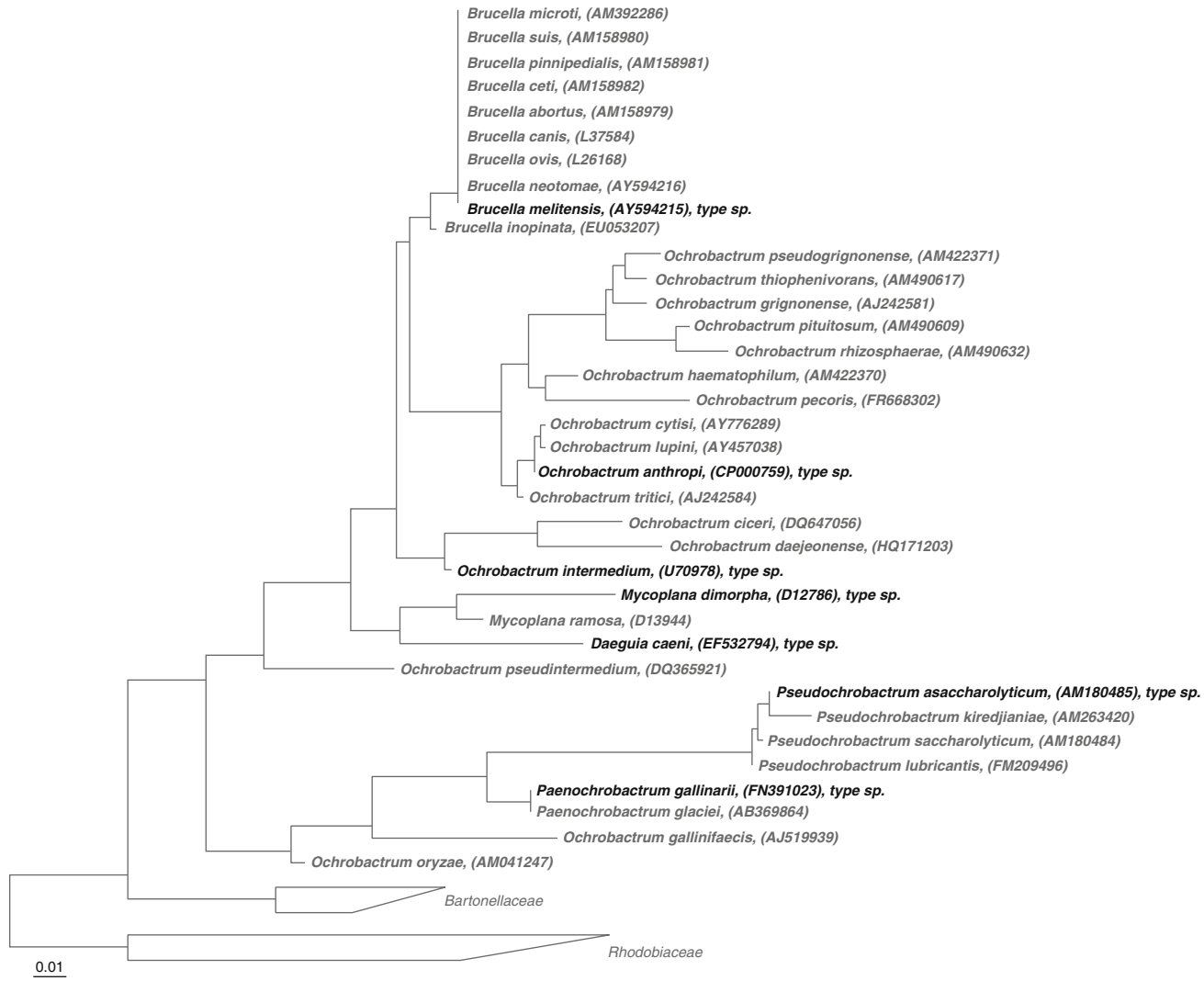

Fig. 6.1

Phylogenetic reconstruction of the family *Brucellaceae* based on 16S rRNA and created using the maximum likelihood algorithm PhyML (Guindon et al. 2010). The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as out-groups. Scale bar indicates estimated sequence divergence

morphology with varying length. Do not form spores. Cells are motile by means of one to several lateral or peritrichous flagella; nonmotile species occur among the genera *Brucella*, *Paenochrobactrum*, and *Pseudochrobactrum*. Aerobic, with a respiratory type of metabolism. Major fatty acids are $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:1}$ $\omega 7c$; other fatty acids such as $C_{14:0}$, $C_{14:0}$ 3-OH, $C_{16:0}$, $C_{16:0}$ 3-OH, and $C_{19:0}$ cyclo $\omega 8c$ may also be present. The predominant polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. The major ubiquinone system is Q-10. The mol % G+C content of the DNA lies between 49.7 and 65.3 %. Usually found in environmental sources such as sludge, soil, and water. Some species belonging to *Brucella* and *Ochrobactrum* have been predominantly isolated from human clinical samples.

Phylogenetic Structure of the Family and Its Genera

By comparative 16S rRNA gene analysis, DNA–DNA hybridization studies, and genome sequencing analysis, it has been demonstrated that the family *Brucellaceae* especially with the genus *Brucella* is genetically related to plant symbionts/pathogens such as *Agrobacterium*, *Rhizobium*, and *Mesorhizobium* (Paulsen et al. 2002) as well as to animal pathogens such as *Bartonella* (O'Connor et al. 1991; Brenner et al. 1993) within the class of *Alphaproteobacteria* and the order *Rhizobiales*.

A phylogenetic tree is shown in *Fig. 6.1*.

The genus *Brucella* consists of genetically highly related organisms. By means of DNA–DNA hybridization studies, it has been shown that all *Brucella* species share DNA–DNA

reassociation values >90 % (Hoyer and Mccullou 1968a, b; Verger et al. 1985; De Ley et al. 1987), which is in agreement with the single species concept (accepted limit for bacterial species delineation is 70 % DNA–DNA similarity). Also based on the information that *Brucella* species cannot be differentiated by 16S rRNA gene sequencing, it has been proposed to classify all *Brucella* species as biovars of a single species, *Brucella melitensis* (Verger et al. 1985). Nevertheless, recent molecular data are in support of the classical delineation into nomenspecies and biovars, which were defined earlier on the basis of phenotypic characteristics and host preference (Michaux-Charachon et al. 1997). The ten species that are currently recognized by the Subcommittee on Taxonomy of *Brucella* are *B. melitensis*, *B. abortus*, *B. suis, B. ovis, B. neotomae*, *B. canis*, B. *ceti*, B. *pinnipedialis, B. microti*, and B. *inopinata* (http://www.the-icsp.org/subcoms/Brucella.htm#taxa).

Investigation of the taxonomic neighborhood of *Brucella* by DNA–DNA hybridization led to the conclusion that *Brucella* and *Achromobacter* CDC Group Vd strains are closely related, belonging to the same rRNA superfamily IV (De Ley et al. 1987). Later, the genus *Ochrobactrum* was constituted on the basis that *Achromobacter* CDC Group Vd strains were distinct from the genus *Achromobacter* but also displayed less than 30 % DNA–DNA similarity with *Brucella* strains. *Ochrobactrum anthropi* was constituted as the single species of this novel genus (Holmes et al. 1988).

Additional analysis of *O. anthropi* strains by a polyphasic approach identified a subset of strains with a very high relatedness to *Brucella* with 16S rRNA gene sequence similarities of >98.5 %. However, these strains clearly differed in their protein patterns, Western blot profiles, and serology. Based on this finding, a new species *Ochrobactrum intermedium* was established, which name should reflect the intermediate position between *Ochrobactrum* and *Brucella* (Velasco et al. 1998). Even though these two genera share strong genetic similarities, they differ strongly in their biological characteristics (see *Table 6.1*). While *Ochrobactrum* are opportunistic organisms, which cycle from soil-rhizoplane to immunocompromised humans/animals, *Brucella* live as intracellular primary parasites that do not require predisposing conditions and are capable to cycle directly from animal to animal (Moreno and Moriyon 2006). In addition to *O. anthropi* and *O. intermedium*, 14 more species, such as *O. grignonense*, *O. tritici* (Lebhun et al. 2000), *O. cytisi* (Zurdo-Pineiro et al. 2007), and *O. oryzae* (Tripathi et al. 2006), have recently been described and included in the genus.

The genus *Mycoplan*a was first placed within the family *Mycobacteriaceae* in the order *Actinomycetales* on the basis of phenotypic characteristics (Gray and Thornton 1928). A detailed characterization of *Mycoplana bullata* and *Mycoplana dimorpha* was performed by Urakami et al. (1990) and two new species, *M. ramosa* and *M. segnis*, were proposed. More recent 16S rRNA similarity analyses revealed that the genus is paraphyletic. With the two species *M. dimorpha* and *M. ramosa*, *Mycoplana* represents a separate branch closely related to the genera *Brucella* and *Ochrobactrum* within the *Brucellaceae*. The two former *Mycoplan*a species *M. bullata* and *M. segnis* were placed in the emended genera of *Brevundimonas* and *Caulobacter*, respectively (Abraham et al. 1999).

Phylogenetic analysis and chemotaxonomic studies of *Zoogloea ramigera* strain ATCC 19623 (Crabtree and McCoy 1967) indicated a close relationship to the *Alphaproteobacteria* and distance to the genus *Zoogloea*. As a consequence, a new genus *Crabtreella* was constituted within the *Brucellaceae*, and *Z. ramigera* was reclassified as the type species *Crabtreella saccharophila* (Xie and Yokota 2006).

The genus *Pseudochrobactrum* was constituted on the basis of 16S rRNA sequence similarity and chemotaxonomic analysis of two newly isolated Gram-negative rod-shaped oxidase-positive bacteria (Kämpfer et al. 2006). 16S rRNA sequence analysis displayed that the two organisms belong to the *Alphaproteobacteria*, with similarities less than 95 % to established species within the genera *Bartonella*, *Brucella*, and *Ochrobactrum*. Even though both bacteria displayed highest 16S rRNA gene sequence similarity to members of *Brucella*, phenotypic characteristics (including chemotaxonomic features) were more similar to those of members of the genus *Ochrobactrum*. By *recA* sequence comparison, DNA–DNA hybridization, and biochemical analysis, it was possible to clearly differentiate the two strains from each other and all known *Ochrobactrum* species. As a result, the new genus *Pseudochrobactrum* with the two species *Pseudochrobactrum asaccharolyticum* (type species) and *Pseudochrobactrum saccharolyticum* was constituted. In addition, two more species, *P. lubricantis* and *P. kiredjianiae*, have been described (Kämpfer et al. 2007, 2009).

More recently, polyphasic analysis of a newly isolated bacterial strain K107^T has led to the constitution of the genus *Daeguia* with classification of *Daeguia caeni* K107^T as the single type species (Yoon et al. 2008). *D. caeni* K107^T displays 16S rRNA gene sequence similarity values of 94.7–97.1 % with respect to members of the genera *Brucella*, *Mycoplana*, *Ochrobactrum*, and *Pseudochrobactrum*. In addition, it could be differentiated from phylogenetically related genera by *recA* gene analysis, phenotypic properties, and fatty acid profiles.

The genus *Paenochrobactrum* was newly generated after analysis of a bacterial isolate Sa25^T, which could be clearly placed in the vicinity of the *Brucella–Ochrobactrum–Pseudochrobactrum* group by 16S rRNA gene and *recA* sequencing and with *Pseudochrobactrum glaciei* KMM 3858^T being the closest relative (Kämpfer et al. 2010). Due to the fact that strain Sa25^T and *P. glaciei* KMM 3858^T form a distinct lineage in the 16S rRNA gene sequence-based phylogenetic tree and that this separate position is supported by unique characteristics of their polar lipid profiles and polyamine patterns, the novel genus *Paenochrobactrum* was proposed with *Paenochrobactrum gallinarii* strain Sa25^T as the type species and reclassification of *Pseudochrobactrum glaciei* as *Paenochrobactrum glaciei* KMM 3858^T.

Phenotypic Analyses

The main characteristics of the members of the family *Brucellaceae* are listed in *Table 6.1*.

Table 6.1
Main characteristics of members of the family *Brucellaceae*

Characteristic	*Brucella*	*Crabtreella*	*Daeguia*	*Mycoplana*	*Ochrobactrum*	*Paenochrobactrum*	*Pseudochrobactrum*
Cell morphology	Cocci/short rods	Straight rods	Rods	Irregular rods	Rods	Rods	Rods
Size in μm	0.5–0.7 x 0.6–1.5	0.6–0.9 x 1.5–2.0	0.4–0.6 x 0.7–2.0	0.5–0.8 x 2.0–3.0	1.0 x 1.5–2.0	2.0	2.0
Gram-stain	–	–	–	–	–	–	–
Optimum growth temp. in °C	37	28–30	30–37	30	20–37	25–30	25–30
Optimum pH	6.6–7.4	7.0–7.5	7.0–8.0	6.0–8.0	ND	6.0–8.0 [d]	ND
Flagella	–	+	ND	+	+	–	–
Predominant fatty acids	$C_{14:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$	$C_{18:1}$	$C_{18:1}$ ω7c	$C_{18:1}$, $C_{16:0}$, $C_{16:1}$	$C_{18:1}$ ω7c, $C_{19:0}$ cyclo ω8c	$C_{18:1}$ ω7c, $C_{19:0}$ cyclo ω8c	$C_{18:1}$ ω7c
Ubiquinone	Q10	Q10	Q10	Q10	Q10	Q10	Q10
Urease	+[a]	–	–	+	v	–[d]	ND
Catalase	+	+	ND	+	+	+[d]	ND
Oxidase	+[b]	+	ND	+	+	+	+
Nitrate reduction	+[c]	–	+	–	+	–[d]	ND
O_2 requirement	+	+	+	+	+	+	+
CO_2 requirement	v	–	–	–	–	–	–
Utilization of sugars	+	+	v	+	+	v	v
Utilization of amino acids	v	+	v	+	+	v	v
Gas production	–	–	ND	–	–	ND	ND
Acid production from sugars	–	+	ND	+	+	–[d]	ND
Indole production	–	–	–	–	–	–[d]	ND
H_2S production	v	+	–	–	–	ND	ND
Mol % GC content of DNA	57.9–59.0	65.3	57.0	63.0–65.0	56.0–59.0	49.7 [e]	50.9 [f]

Data from Corbel and Banai (2005); Moreno and Moriyon (2006); Unz (1971); Xie and Yokota (2006); Yoon et al. (2008); Urakami et al. (1990); Holmes et al. (1988); Romanenko et al. (2008); Kämpfer et al. (2006, 2009, 2010, 2011)

ND not determined, + positive, – negative, *v* variable

[a] *B. ovis* is usually urease negative, but positive strains are also reported. Some urease negative strains of *B. abortus* have been reported

[b] *B. neotomae* and *B. ovis* are oxidase negative

[c] *B. ovis* and some *B. abortus* strains are negative for nitrate reduction

[d] Data for *P. glaciei* KMM 3858^T (Romanenko et al. 2008)

[e] Data for *P. gallinarii* Sa25^T (Kämpfer et al. 2010)

[f] Data for *P. assacharolyticum* CCUG 46016^T (Kämpfer et al. 2006)

Brucella (Meyer and Shaw 1920, 173[AL])

Bru.cel'la. L. dim. ending-*ella*; M.L. fem n. *Brucella* named after Sir David Bruce, who first recognized the organism causing undulant (Malta) fever.

Cells are Gram-negative nonspore-forming cocci, coccobacilli, or short rods with a size of 0.5–0.7 x 0.6–1.5 μm and occur singly and, less frequently, in pairs, short chains, or small groups. Nonmotile. True capsules are not formed. Do not usually display true bipolar staining. Colonies are transparent, raised, and convex, with an entire edge and a smooth, shiny surface when grown on serum-dextrose agar or other clear media. They appear in a pale honey color by transmitted light. Smooth strains produce a distinctive lipopolysaccharide (LPS) and perosamine synthetase. Nonsmooth variants of the smooth species exist, but stable nonsmooth nomenspecies with a characteristic host range do also occur. Growth occurs between 20 °C and 40 °C with an optimum growth temperature at 37 °C. Optimum pH for growth is between 6.6 and 7.4. Many strains require supplementary CO_2 for growth, especially for primary isolation. Cells are aerobic, having a respiratory type of metabolism and a cytochrome-based electron transport system with oxygen or nitrate as the terminal electron acceptor. Generally oxidase positive, but negative strains occur. Catalase and urease positive, except *B. ovis* which is usually urease negative. Nitrate reduction occurs. Voges–Proskauer test is usually negative, except for *B. inopinata* and *B. microti* which are positive for the production of acetyl methylcarbinol. Methyl red test is negative. Do not form indole. The major fatty acids are $C_{14:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$. Phosphatidylcholine is the predominant polar lipid; phosphatidylglycerol and diphosphatidylglycerol are also present as major phospholipids. Phosphatidyl ethanolamine, phosphatidyl serine, and cardiolipin are present in minor amounts. The ubiquinone system is Q-10. Chemoorganotrophic. For most strains, complex media containing several amino acids, nicotinamide, thiamine, iron, and magnesium ions are required; some strains may be induced to grow on minimal media containing an ammonium salt as the sole nitrogen source. Growth is improved by serum or blood, but hemin (X factor) and nicotinamide adenine dinucleotide (NAD: V factor) are not necessary. Acid production does not occur from carbohydrates in conventional media, except for *B. neotomae*. No liquefaction of inspissated serum or gelatine. No hemolysis. Organisms are intracellular parasites, contagious to a wide range of animal species including humans. The mol% G+C content of the DNA is 57.9–59.0. The type species is *Brucella melitensis* (Hughes 1893), Meyer and Shaw 1920, 179 (*Streptococcus melitensis* Hughes 1893, 235). Differential characteristics of the classical *Brucella* species are shown in *Table 6.2*. More detailed informations on the genus *Brucella*, including phenotypic characteristics, genetics, and pathology of Brucellosis, can be found in the comprehensive study of Moreno and Moriyon (2006).

Crabtreella Xie and Yokota 2006, 623[VP]

Crab.tre'el.la. N.L. fem. n. *Crabtreella* named after Dr K. Crabtree, the American microbiologist who isolated the type strain of the type species.

The type strain of the type species is *Crabtreella saccharophila* ATCC 19623[T] after reclassification of *Zoogloea ramigera* ATCC 19623[T], which was initially isolated from sludge (Crabtree and McCoy 1967). Currently the genus description is based on only one species. The description is based on the data compiled by Unz (1971) and Xie and Yokota (2006). Cells are Gram-negative, straight rods with rounded, blunt, or tapered ends. Cells have a size of 0.6–0.9 x 1.5–2.0 μm with one to several laterally attached flagella. Zoogloea formation is absent. Colonies are straw colored, glistening, circular, and raised when grown on CY agar. The optimum growth temperature is 28–30 °C, with slow growth at 10 °C and no growth at 45 °C. Optimum growth at pH 7.0–7.5 with no growth at pH 4.5 or 9.6. Growth occurs in the presence of 3 % (w/v) NaCl, but not at 6 % NaCl. PHB is accumulated. Catalase and oxidase positive, but urease negative. H_2S is generated in a peptone-cysteine-sulfated medium. Indole is not produced. Nitrate reduction is absent. The predominant fatty acid is $C_{18:1}$. The major hydroxy fatty acids produced are $C_{14:0}$ 3-OH, $C_{16:0}$ 3-OH, and $C_{18:0}$ 3-OH. The predominant ubiquinone is Q-10. Hydrolyses tyrosine agar with formation of brown pigment. Utilizes acetate, fumarate, malate, oxalacetate, pyruvate, arginine, asparagine, aspartate, citrulline, glutamate, histidine, ornithine, tyrosine, ammonia, *n*-propanol, ethanol, *n*-butanol, and ethanol, but not citrate, benzoate, or *m*-toluate. Arginine dihydrolase is present. Tributyrin is hydrolyzed. Acid is produced oxidatively from arabinose, fructose, ethanol, glucose, glycerol, mannitol, rhamnose, ribose, sucrose, and xylose, but no acid is formed from inulin or starch. The mol % G + C content of the DNA is 65.3 %.

Daeguia Yoon, Kang, Park and Oh 2008, 170[VP]

Dae.gu'i.a. N.L. fem. n. *Daeguia* pertaining to Daegu, the location of the textile dye works from which the type strain of the type species was isolated.

The type strain of the type species is *Daeguia caeni* K107[T] and was isolated from sludge collected from the wastewater treatment plant of textile dye works in Deagu, Korea (Yoon et al. 2008). Currently the genus description is based on only one species. Cells are aerobic, Gram-negative, nonspore-forming rods with a size of 0.4–0.6 x 0.7–2.0 μm. Colonies are grayish yellow, circular, convex, smooth, glistening, and 1.0–1.5 mm in diameter after incubation at 37 °C on TSA for 2 days. Growth occurs at 15–55 °C with an optimum at 30–37 °C and at pH 5.0–9.0 with an optimum at pH 7.0–8.0. Grows in the presence of 0–4 % (w/v) NaCl (optimum 0.5–1.0 %). The strain displays 16S rRNA gene sequence similarity values of

Table 6.2
Physiological characteristics of the classical *Brucella* species

Substrate	1	2	3	4	5	6	7	8	9	10
Oxidase	+	+	+	+	+	+	–	–	+	+
Urease	+	+[a]	+	+	+	+	+	–[b]	+	+
Nitrate reduction	+	+[a]	+	+	+	+	+	–	+	+
Voges–Proskauer	–	–	–	–	+	+	–	–	–	–
Esculin hydrolysis	–	–	v	–	–	–	–	–	–	v
Fumaric acid	–	–	–	–	+	+	–	–	–	–
Glutaric acid	–	–	–	–	+	+	–	–	–	–
Itaconic acid	–	–	–	–	+	+	–	–	–	–
Mesaconic acid	–	–	–	–	+	+	–	–	–	–
Succinic acid	–	–	–	–	+	+	–	–	–	–
α-Ketoglutaric acid	–	–	–	–	+	+	–	–	–	–
L-Citrulline	–	–	–	–	+	+	–	–	–	–
L-Glutamine	–	–	–	–	+	+	–	–	–	–
L-Histidine hydrochloride	–	–	–	–	+	+	–	–	–	–
D-Histidine	–	–	–	–	+	+	–	–	–	–
DAPI	–	–	–	–	+	+	–	–	–	–
Sarcosine hydrochloride	–	–	–	–	+	+	–	–	–	–
Arginine *p*-nitroanilide	–	–	–	–	+	+	–	–	–	–
L-Pyroglutamic acid β-naphthylamide	–	–	–	–	–	–	–	–	–	–
*p*NP *N*-Acetyl-β-D-galactosaminide (pH 7.5)	–	–	–	–	+	+	–	–	–	–
oNP *N*-Acetyl-β-D-galactosaminide (pH 7.5)	–	–	–	–	+	+	–	–	–	–
oNP *N*-Acetyl-β-D-galactosaminide (pH 7.5)	–	–	–	–	+	+	–	–	–	–
*p*NP *N*-Acetyl-β-D-glucosaminide (pH 7.5)	–	–	–	–	+	+	–	–	–	–
*p*NP *N*-Acetyl-1-thio-β-D-glucosaminide (pH 7.5)	–	–	–	–	+	v	–	–	–	–
4-Nitrophenyl-α-D-maltoheptaoside-4,6,O-ethylidene (pH 7.5)	–	–	–	–	+	+	–	–	–	–
*p*NP *N*-Acetyl-β-D-glucosamide (pH 5.5)	–	–	–	–	+	+	–	–	–	–
*p*NP *N*-α-L-Arabinopyranoside (pH 7.5)	–	–	–	–	+	+	–	–	–	–
oNP *N*-β-D-Xylopyranoside (pH 7.5)	–	–	–	–	+	+	–	–	–	–
*p*NP *N*-1-thio-β–D-Galactopyranoside (pH 7.5)	–	–	–	–	+	+	–	–	–	–
2-Methoxy-4-(2-nitrovinyl)-phenyl-β-D-galactopyranoside	–	–	–	–	+	+	–	–	–	–
*p*NP β-L-Fucopyranoside (pH 7.5)	–	–	–	–	+	+	–	–	–	–
*p*NP β-D-Thiofucopyranoside (pH 7.5)	–	–	–	–	+	+	–	–	–	–
*p*NP β-D-Maltoside (pH 7.5)	–	–	–	–	+	+	–	–	–	–

Table 6.2 (continued)

Substrate	1	2	3	4	5	6	7	8	9	10
*p*NP β-D-Lactopyranoside (pH 7.5)	–	–	–	–	+	v	–	–	–	–
*p*NP β-D-Glucuronide (pH 5.5)	–	–	–	–	+	+	–	–	–	–
oNP β-D-Galactopyranoside-6-phosphate	–	–	–	–	+	+	–	–	–	–
*p*NP Phosphate-di(2-amino-2-ethyl-1,3-propanediol) (pH 5.5)	–	–	–	–	+	+	–	–	–	–
Sensitivity to phage(s)	BK_2	BK2, Fi, TB, Wb,	R/C	Iz, Wb	Tb[c], F1[c], F25[c]	Tb[c], F1[c], F25[c]	BK2, Tb[c]	R/C	Iz, Tb[d], Wb,	Bk2, Tb[c], Wb
Preferred host	Sheep, goats	Cattle	Dogs	Cetaceans (whales, dolphins, porpoises)	Not known	Vole	Desert wood rats	Sheep	Seal	Pigs, hares, reindeer, rodents[e]

Data from Corbel and Banai (2005); Moreno and Moriyon (2006); Scholz et al. (2010); Taxa: 1, *B. melitensis* strains: 16M^T (= NCTC 10094^T) (bv. 1), 63/9 (= NCTC 10508) (bv. 2), Ether (= NCTC 10509) (bv. 3); 2, *B. abortus* strains: 544^T (= NCTC 10093^T) (bv. 1), 86/8/59 (= NCTC 10501) (bv. 2), Tulya (= NCTC 10502) (bv. 3), 292 (= NCTC 10503) (bv. 4), B3196 (= NCTC 10504) (bv. 5), 870 (= NCTC 10505) (bv. 6), 63175 (= NCTC 10506) (bv. 7), C68 (= NCTC 10507) (bv. 9); 3, *B. canis* RM6/66^T (= NCTC 10854^T); 4, *B. ceti* NCTC 12891^T; 5, *B. inopinata* BO1^T; 6, *B. microti* CCM 4915^T; 7, *B. neotomae* 5K33^T (= NCTC 10084^T); 8, *B. ovis* 63/290^T (= NCTC 10512^T); 9, *B. pinnipedialis* NCTC 12890^T; 10, *B . suis* strains: 1330^T (= NCTC 10316^T) (bv. 1), Thomsen (= NCTC 10510) (bv. 2), 686 (= NCTC 10511) (bv. 3), reference 40 (bv. 4), reference 513 (bv. 5)

+ positive, – negative, *v* variable, *pNP* para-nitrophenyl

[a]Some strains are negative in this test

[b]Some strains are positive in this test

[c]Lysis only at routine test dilution (RTD) x 10^4

[d]Some strains exhibit lysis with phage Tb

[e]Preferred host for *B. suis* biovar 1, 2 and 3: pigs, biovar 2: hares, biovar 4: reindeer, biovar 5: wild rodents

94.7–97.1 % with respect to members of the genera *Brucella*, *Mycoplana*, *Ochrobactrum*, and *Pseudochrobactrum*. The *recA* gene (550 nt) exhibits sequence similarity values of 77.6–84.5 % with respect to the genera *Brucella*, *Mycoplana*, *Ochrobactrum*, and *Pseudochrobactrum*. The predominant fatty acid is $C_{18:1}$ *ω7c*; a significant amount of $C_{18:1}$ 2-OH is produced. The major ubiquinone is Q-10. Urease negative. No nitrate reduction occurs. H_2S and indole are not produced. Assimilation of glucose, mannose, *N*-acetylglucosamine, inositol, adonitol, and phenylacetate. Adipate is weakly assimilated, but no assimilation of aesculin, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, caprate, cellobiose, citrate, dulcitol, erythritol, D-fucose, L-fucose, gentiobiose, gluconate, glycogen, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, malate, melezitose, melibiose, methyl α-D-mannoside, methyl α-D-glucoside, methyl β-D-xyloside, raffinose, salicin, starch, sorbose, D-tagatose, turanose, xylitol, and L-xylose. Hypoxanthine is hydrolyzed, but aesculin, casein, gelatin, starch, tyrosine, urea, xanthine, and Tween 20, 40, 60, and 80 are not. Alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, and naphthol-AS-BI-phosphohydrolase are present, but arginine dihydrolase, cystine arylamidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, valine arylamidase, lipase (C14), α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, trypsin, and α-chymotrypsin are absent. The organism is susceptible to carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, streptomycin, and tetracycline, but not to ampicillin, lincomycin, oleandomycin, penicillin G, or polymyxin B. The mol % G + C content of the DNA of the type strain of the type species is 57.0 %.

Mycoplana Gray and Thornton 1928, 82^{AL} emend. Urakami, Oyanagi, Araki, Suzuki and Komagata 1990, 439

My.co.pla'na. Gr. *Mykes* fungus; Gr. *plane* a wandering; M.L. fem. n. *Mycoplana* fungus wanderer.

Cells are Gram-negative and nonspore-forming curved or irregular rods with rounded ends and a size of 0.5–0.8 x 2.0–3.0 μm. The cells form branching filaments prior to fragmentation into irregular rods and occur singly or rarely in pairs. Motile by means of peritrichous flagella. Occur in soil. Colonies have a white to light yellow color and are not mucous. Good growth on standard culture media at 30 °C and between pH 6.0 and 8.0, but not at 42 °C and in the presence of 3 % (w/v) NaCl. Cells are aerobic, nonfermentative, with a strictly respiratory type of metabolism. Catalase, oxidase, and urease are produced. Voges–Proskauer is positive, but the methyl red test is negative. H_2S and indole are not formed. The predominant fatty acids are $C_{18:1}$, in large

amounts, $C_{16:0}$, and $C_{16:1}$. The major hydroxy fatty acid produced is $C_{14:0}$ 3-OH. The major ubiquinone is Q-10; small amounts of Q-9 and traces of Q-11 are also detected. Hydrolysis of gelatin and starch does not occur. Denitrification is negative, and litmus milk is not changed. Ammonia is produced. The cells accumulate granules of poly-β-hydroxybutyrate, and some have the ability to decompose aromatic compounds, such as phenol and *m*-cresol. A water-soluble fluorescent pigment is not formed on King A or King B media. Cells utilize many different compounds as sole as sole carbon sources. Currently the genus description is based on four species. In the original description of the genus (Gray and Thornton 1928), *Mycoplana* produced no acid from carbohydrates. This is inconsistent with the emended description (Urakami et al. 1990), where strains of *M. dimorpha* and *M. ramosa* have been shown to form acid oxidatively from L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, D-xylose, D-mannitol, D-sorbitol, and glycerol, but not from inositol, lactose, maltose, sucrose, trehalose, and soluble starch. The mol % G + C content of the DNA is between 63 and 65 %. The type species is *Mycoplana dimorpha* (Gray and Thornton 1928, 82 emend. Urakami et al. 1990, 440).

Ochrobactrum Holmes, Popoff, Kiredjian and Kersters 1988, 412[VP]

O.chro.bac'trum. Gr. adj. *ochros* pale, colorless; Gr. neut n. *baktron* a staff or stick; M.L. neut. n. *Ochrobactrum* a colorless rod.

Gram-negative rods with parallel sides and rounded ends with a size of 1.0 x 1.5–2.0 µm; shorter oval forms, 1.0–1.5 µm in length, may occur. Cells usually occur singly and are motile by means of peritrichous flagella. Colonies are < 0.5 mm in diameter, circular, smooth, low convex, moist, glistening, translucent, and butyrous and have an entire edge after incubation on nutrient agar for 24 h at 37 °C. Pigment is not produced. Growth occurs on MacConkey agar. Optimum growth temperature is between 20 °C and 37 °C. Few strains grow at 42 °C, and while clinical strains have not been reported to grow at 5 °C, environmental isolates will grow at 4 °C. Occur in human clinical specimens, also in soil samples and wheat roots. Cells are obligately aerobic, having a strictly respiratory type of metabolism. Catalase and oxidase positive. Indole is not produced. Nitrate reduction occurs. Urease usually positive, but ornithine decarboxylase (ODC) negative. Predominant fatty acids are $C_{18:1}$ ω7c, and $C_{19:0}$ cyclo ω8c (Kämpfer et al. 2011). The major ubiquinone is Q-10. Chemoorganotrophic, utilizing a variety of amino acids, organic acids, and carbohydrates as carbon sources. Acid is produced from arabinose, glucose, ethanol, fructose, rhamnose, and xylose. Substrate utilization patterns of various *Ochrobactrum* type strains are shown in Table 6.3. Clinical strains have been reported to be resistant to ampicillin, amoxicillin and clavulanic acid, aztreonam, cefamandole, cefonicid, cefoperazone, cefoxitin, cefsulodin, ceftazidime, cefuroxime, cephalothin, chloramphenicol, erythromycin, fosfomycin, kanamycin, mezlocillin, pipemidic acid, piperacillin, pristinamycin, streptomycin, and ticarcillin but have been shown to be susceptible to amikacin, cefoperazone, ceftriaxone, ciprofloxacin, gentamicin, imipenem, netilmicin, nalidixic acid, pefloxacin, rifampicin, tetracycline, and vancomycin; most are also susceptible to moxalactam. The mol % G+C content of the DNA is between 56 % and 59 %. The type species is *Ochrobactrum anthropi* (Holmes et al. 1988, 412).

Paenochrobactrum Kämpfer, Martin, Lodders, Jäckel, Huber, Schumann, Langer, Busse, Scholz 2010, 60[VP]

Pae'no.chro.bac'trum. L. adv. *Paene* nearly, almost; N.L. neut. n. *Ochrobactrum* a bacterial genus name; N.L. masc. n. *Paenochrobactrum* almost *Ochrobactrum.*

Cells are Gram-negative, nonmotile, nonspore-forming rods, approximately 2.0 µm long. On the basis of 16S rRNA gene sequence analysis, most closely related to the genera *Brucella*, *Ochrobactrum*, and *Pseudochrobactrum.* Currently the genus description is based on two species. Cells are oxidase positive, showing an oxidative metabolism. The predominant fatty acids are $C_{18:1}$ ω7c and $C_{19:0}$ cyclo *ω8c.* The major polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylmonomethylethanolamine, unknown aminolipid AL1, and moderate or minor amounts of unknown polar lipids L1 and L2. In addition, an aminolipid that stretches over a long range in the first chromatographic dimension (stretched aminolipid) is detectable. The predominant ubiquinone is Q-10. The polyamine pattern exhibits the major compound putrescine and moderate to large amounts of spermidine while sym-homospermidine is absent. *P. gallinarii* Sa25^T and *P. glaciei* KMM 3858^T are positive for the hydrolysis of L-alanine, *p*-nitroanilide (*p*NA), and L-proline *p*NA and weakly positive for hydrolysis of bis-*p*-nitrophenyl (*p*NP) phosphate, but do not hydrolyze aesculin, *p*NP-β-D-galactopyranoside, *p*NP-β-D-glucuronide, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, *p*NP-phosphorylcholine, *p*NP-phenylphosphonate, and 2-deoxythymidine-59-*p*NP phosphate. Both strains assimilate acetate and L-alanine, but no assimilation of adonitol, *p*-arbutin, cellobiose, D-gluconate, maltitol, maltose, *myo*-inositol, D-mannitol, α-melibiose, D-sorbitol, salicin, trehalose, putrescine, L-leucine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, *trans*-aconitate, adipate, citrate, itaconate, mesaconate, phenylacetate, suberate, azelate, and 4-hydroxybenzoate occurs. Table 6.4 shows assimilation of different substrates by *P. gallinarii* Sa25^T and *P. glaciei* KMM 3858^T. The type strain of the type species is *Paenochrobactrum gallinarii* Sa25^T and has a mol% G + C content of the DNA of 49.7 % (Kämpfer et al. 2010).

Table 6.3
Physiological characteristics of *Ochrobactrum* type strains

Test	1[a]	2[b]	3[b]	4[a]	5[a]	6[a]	7[a]	8[a]	9[c]	10[a]	11[d]	12[a]	13[a]	14[a]	15[a]
Hydrolysis of															
L-glutamate-γ-3-carboxy-*p*NA	(+)	ND	ND	–	–	–	+	+	ND	–	+	–	–	–	(+)
L-proline-*p*NA	+	ND	ND	+	+	+	(+)	+	ND	(+)	ND	+	+	+	(+)
*p*NP-phenyl-phosphonate	–	ND	ND	–	–	–	–	–	ND	+	+	(+)	+	(+)	(+)
Assimilation of															
D-fructose	+	ND	ND	–	+	+	+	+	+	+	ND	+	+	+	+
D-sorbitol	+	+	+	–	+	+	+	+	+	+	ND	+	+	+	+
i-inositol	+	+	+	–	+	+	+	+	+	(+)	ND	+	+	+	+
L-rhamnose	+	+	+	–	+	+	+	+	+	–	+	+	+	–	+
cis-aconitate	+			–	+	+	+	–	+	–	+	+	+	–	+
citrate	+	+	+	–	+	+	+	+	–	(+)	+	+	+	–	+
DL-3-hydroxybutyrate	+	ND	ND	–	+	+	+	+	+	(+)	ND	+	+	+	+
4-aminobutyrate, β-alanine	+	ND	ND	(+)	+	+	+	+	ND	+	ND	+	+	+	+
D-maltose,	+	ND	ND	–	–	+	+	+	+	+	+	–	+	–	+
D-gluconate	+	ND	ND	+	+	+	+	(+)	+	–	–	+	–	+	+
N-acetyl-D-glucosamine	+	ND	ND	–	–	+	+	+	+	+	+	–	(+)	(+)	+
adonitol,	+	+	+	–	–	+	+	+	+	+	+	+	+	–	+
sucrose	+	+	+	–	–	+	–	+	+	+	+	(+)	+	–	+
D-trehalose	+	–	+	–	–	+	–	+	+	+	+	(+)	+	–	+
maltitol	+	ND	ND	–	–	+	–	+	ND	–	+	–	+	–	–
trans-aconitate	–	ND	ND	–	–	+	(+)	–	ND	–	+	+	+	–	–
D-Cellobiose	+	+	+	–	–	–	+	–	–	+	+	–	+	–	–
N-acetyl-D-galactosamine	(+)	+	+	–	+	–	+	+	+	+	ND	+	+	+	+
Suberate	–	ND	ND	–	–	–	–	–	ND	–	ND	–	–	–	+
L-aspartate	+	ND	ND	+	+	+	+	+	+	+	ND	+	+	+	–
4-hydroxybenzoate	(+)	ND	ND	–	–	+	(+)	+	ND	–	ND	–	–	–	+
L-histidine	+	ND	ND	+	+	+	+	+	+	+	+	+	+	–	(+)
L-leucine	+	ND	ND	–	+	+	+	+	+	+	+	+	+	–	(+)

Taxa: 1, *O. anthropi* CIP 14970^T; 2, *O. ciceri* Ca-34^T; 3, *O. cytisi* DSM 19778^T; 4, *O. gallinifaecis* Iso 196^T; 5, *O. grignonense* DSM 13338^T; 6, *O. haematophilum* CCUG 38531^T; 7, *O. intermedium* LMG 3301^T; 8, *O. lupini*, LUP21^T; 9, *O. oryzae* DSM 17471^T; 10, *O. pecoris* 08RB2639^{T}and 08RB2781-1; 11, *O. pituitosum* CCUG 50899^T; 12, *O. pseudogrignonense* CCUG 30717^T and CCUG 43892; 13, *O. rhizosphaerae* PB17^T; 14, *O. thiophenivorans* 08RB2781-1^T; 15, *O. tritici* LMG 18957^T
+ positive, – negative, (+) weak positive, *ND* not determined, *pNP* para-nitrophenyl, *pNA* para-nitroanilide
[a]Data from Kämpfer et al. (2011)
[b]Data from Imran et al. (2010)
[c]Data from Tripathi et al. (2006)
[d]Data from Huber et al. (2010)

Pseudochrobactrum Kämpfer, Rosselló-Mora, Scholz, Welinder-Olsson, Falsen, Busse 2006, 1825[VP]

Pseud.och.ro.bac'trum. (Gr. adj. *pseudes* false; N.L. neut. n. *Ochrobactrum* a bacterial genus name; N.L. neut. n. *Pseudochrobactrum* false *Ochrobactrum*).

Cells are Gram-negative, nonmotile, nonspore-forming rods, approximately 2.0 μm long. The *recA* gene exhibits sequence similarity to species of *Ochrobactrum* (81 %), *Brucella* (80 %), and *Bartonella* (76 %). Currently the genus description is based on four species. Negative for a *Brucella abortus*- and *Brucella melitensis*-specific antigen (according to the assay of Baily et al. 1992). Cells are oxidase positive, showing an oxidative metabolism. Optimum growth temperature is between 25 °C and 30 °C. The major fatty acids produced are $C_{18:1}$ ω7*c* and moderate amounts of $C_{18:0}$ and $C_{19:0}$ cyclo ω8*c*. The predominant polar lipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. In addition,

Table 6.4
Physiological characteristics of *Paenochrobactrum* type strains

Assimilation of	*P. gallinarii* Sa25^T	*P. glaciei* KMM 3858^T
D-glucose	+	(+)
D-fructose	–	+
D-galactose	–	+
D-mannose	–	(+)
D-ribose	–	–
D-xylose	–	+
L-arabinose	–	–
L-rhamnose	–	–
DL-lactate	+	–
DL-3-hydroxybutyrate	–	–
Pyruvate	–	–
4-aminobutyrate	+	–
Propionate	+	–
Fumarate	–	–
Glutarate	–	–
L-aspartate	–	–
L-malate	–	–
cis-aconitate	–	–
β-alanine	+	–
L-histidine	–	+
Ornithine	–	–
N-acetyl-D-glucosamine	–	+

+ positive, – negative, (+) weak positive. Data from Kämpfer et al. (2010)

moderate amounts of phosphatidyldimethylethanolamine and unknown aminolipid AL1 and small amounts of unknown phospholipid PL1 and five unknown lipids (L1–L5) are detected. The major ubiquinone is Q-10. The polyamine pattern exhibits the major compounds putrescine and spermidine and small amounts of 1,3-diaminopropane and spermine. All *Pseudochrobactrum* strains are positive for hydrolysis of L-alanine-*p*NA and L-proline-*p*NA and weakly hydrolyze bis-*p*NP-phosphate, but do not hydrolyze *p*NP-β-D-galactopyranoside, *p*NP-β-D-glucuronide, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucopyranoside, *p*NP-phosphorylcholine, *p*NP-phenylphosphonate, 2-deoxythymidine-5′-*p*NP-phosphate, and aesculine. All strains are positive for assimilation of L-alanine, L-proline, L-serine, acetate, and oxoglutarate, but do not assimilate adonitol, *p*-arbutin, D-cellobiose, D-gluconate, i-inositol, D-maltose, D-maltitol, D-mannitol, α-D-melibiose, D-sorbitol, salicin, D-trehalose, putrescine, L-leucine L-phenylalanine, L-tryptophan, trans-aconitate, adipate, azelate, citrate, itaconate, mesaconate, phenylacetate, suberate, 3-hydroxybenzoate, and 4-hydroxybenzoate. *Table 6.5* shows assimilation of different substrates by *Pseudochrobactrum* type strains. The type strain of the type species is *Pseudochrobactrum asaccharolyticum* CCUG 46016^T and has a mol% G + C content of the DNA of 50.9 % (Kämpfer et al. 2006).

Molecular Analyses

All *Brucella* species are genetically highly related to each other, exhibiting sequence similarity values of 98–100 % in aligned genomic regions (core genomes). In fact, with the exception of *B. inopinata*, all *Brucella* species have identical 16S rRNA and *recA* gene sequences and show only few variations in most housekeeping genes. Thus, the population structure is clonal and *Brucella* represents a monophyletic genus. Because of this close genetic relatedness, DNA–DNA hybridization cannot be used for species differentiation. However, despite this close genetic relatedness, the various species show species-specific and to some extent even biovar-specific clustering in high-resolution molecular typing assays, such as multilocus sequence typing (MLST) or multilocus sequence analysis (MLSA). More recently, whole genome sequencing (WGS) and the resulting global genome-wide single-nucleotide polymorphism (SNP) analysis have become available to study phylogeny, evolution, host specificity, and pathogenicity of the genus *Brucella* at the highest level possible.

Species Delineation

Accurate species delineation can be achieved by conventional multiplex polymerase chain reaction (PCR) targeting species-specific genes, SNP analysis, MLST, or MLSA. The last approach is also suitable for phylogenetic reconstructions, owing to the highly clonal evolution of the different species and in particular to study *Brucella* at the strain level which is important for molecular epidemiologic studies, e.g., outbreak investigations.

PCR-Based Methods

One of the first PCR assays to differentiate among *Brucella* species was the so-called Abortus–Melitensis–Ovis–Suis (AMOS) PCR, developed by Bricker and Halling (1994). This PCR uses a single reverse primer, targeting the *Brucella*-specific insertion element IS*711*, and four different forward primers, each specific for a given species as estimated by testing representative isolates. Species are differentiated on the basis of different PCR fragment sizes. This assay distinguishes *B. melitensis*, *B. abortus*, *B. ovis*, and *B. suis*. The disadvantage of this PCR is that not all species can be identified (i.e., *B. canis* and *B. neotomae*) and that some biovars within a given species give negative results.

In 2006 a new conventional multiplex PCR (Bruce-ladder), using eight primer pairs in a single reaction, was developed by Garcia-Yoldi and colleagues (Garcia-Yoldi et al. 2006). Later, this PCR was enhanced, to cover novel species such as *B. microti* and *B. inopinata* and to differentiate between *B. ceti* and *B. pinnipedialis* (Mayer-Scholl et al. 2010). However, differentiation at the biovar level, or below, is not possible (*Fig. 6.2*). Consequently, PCR assays that allow discrimination among biovars within a given species were developed. The most recent

Table 6.5
Physiological characteristics of *Pseudochrobactrum* type strains

Assimilation of	*P. assacharolyticum* CCUG 46016^T	*P. kiredjianiae* CCUG 49584^T	*P. lubricantis* KSS 7.8^T	*P. saccharolyticum* CCUG 33853^T
D-glucose	–	+	+	+
D-fructose	–	–	+	+
D-galactose	–	(+)	+	+
D-mannose	+	–	+	+
D-ribose	–	+	+	+
D-xylose	–	–	–	+
L-arabinose	–	–	–	+
L-rhamnose	–	+	–	–
DL-lactate	–	+	+	+
DL-3-hydroxybutyrate	–	–	–	+
Pyruvate	–	–	+	+
4-aminobutyrate	–	+	+	+
Propionate	–	+	+	+
Fumarate	–	+	+	+
Glutarate	–	+	+	+
L-aspartate	–	(+)	+	+
L-malate	–	+	+	+
cis-aconitate	+	–	(+)	+
β-alanine	–	+	+	+
L-histidine	–	–	+	+
Ornithine	–	–	+	+
N-acetyl-D-glucosamine	–	(+)	+	+

+ positive, – negative, (+) weak positive. Data from Kämpfer et al. (2009)

multiplex PCR assay to differentiate among *B. suis* biovars 1 to 5 (Suis-ladder) was developed by López-Goñi et al. (2011).

To date, at least 400 reports have been published dealing with various PCR-based methods for *Brucella* detection and differentiation. For details on PCR-based assays, see Yu and Nielsen (2010).

Specific High-Resolution Assays

Multilocus Variable Number of Tandem Repeat Analysis

The MLVA assays take advantage of array-length variations in tandem repeats. Owing to the availability of whole genome sequences, tandem repeats can be readily identified and tested for polymorphism. Most tandem repeats have been evaluated, essentially by three groups (Bricker, Vergnaud, Whatmore, and colleagues), and tens of polymorphic loci have been identified. Different selections of such loci, used in MLVA assays, have been proposed to suit different purposes.

The first MLVA assay, named "HOOF-Prints" (hypervariable octameric oligonucleotide fingerprints), was developed by Bricker et al. in 2003 (Bricker et al. 2003). The *Brucella* genome contains a family of tandem repeats sharing the repeat unit "AGGGCAGT." Eight highly variable such loci, present in most *Brucella* species, were selected for use in the HOOF-Print assay. Variations of the repeat numbers at each locus can easily be investigated by amplifying the corresponding regions and subsequent gel electrophoresis or preferably capillary electrophoresis given the short repeat unit size. This selection of tandem repeats has a very high discriminatory power and can be useful for local outbreak investigations.

With the HOOF-Print assay, a reliable tool to study the relationship of human cases and outbreak dynamics became available for the first time (Valdezate et al. 2007). However, it cannot provide a species assignment owing to the high level of homoplasy (see below) at these loci.

Indeed, high-resolution markers allow the discrimination of individual strains and therefore can be used for trace-back analyses and epidemiological studies in outbreak scenarios. A high discriminatory power is desired when investigating an outbreak with very limited geographical and temporal distribution, and highly variable loci will then be preferred. However, rapidly evolving VNTR markers often suffer from homoplasy,

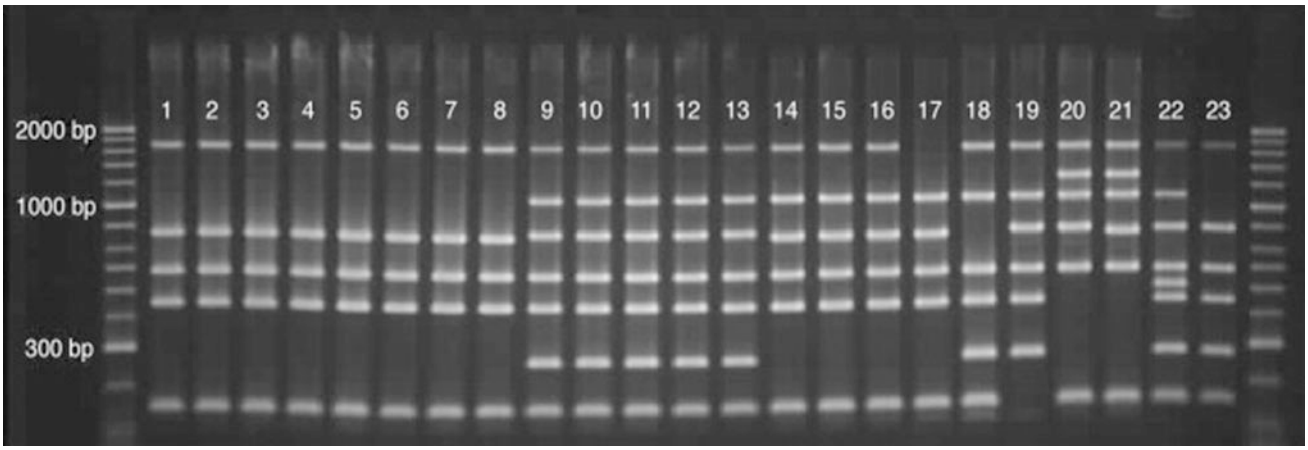

Fig. 6.2
Identification and differentiation of all known *Brucella* species and biotypes by modified multiplex PCR assay. Lanes *1–8*: *B. abortus* biotypes *1–7* and biotype *9*; lanes *9–13*: *B. suis* biotypes *1–5*; lanes *14–16*: *B. melitensis* biotypes *1–3*; lane *17*: *B. ovis*; lane *18*: *B. canis*; lane *19*: *B. neotomae*; lane *20*: *B. pinnipedialis*; lane *21*: *B. ceti*; lane *22*: *B. microti*; lane *23*: *B. inopinata*.; Mayer-Scholl et al. 2010

i.e., the appearance of the same genetic alteration in two or more branches of a phylogenetic tree. These phenomena can disrupt and confound the accurate phylogenetic placement of some isolates within an MLVA cluster and prevent accurate species-level designation.

A stronger phylogenetic signal and different selections of loci are needed when looking for species identification. Practical considerations (cost) also need to be taken into account. Although tens of VNTRs have been described in *Brucella* and are potentially useful, the number of loci in an MLVA assay should be kept to a minimum. Because the HOOF-Print MLVA assay could not be used for identification purposes at the species level, additional selections of tandem repeats were subsequently proposed (*Table 6.6*).

The MLVA15$_{Orsay}$ and MLVA16$_{Orsay}$ selection (Le Fleche et al. 2006) and/or their subpanels are currently the most commonly used, according to the literature, probably because of the existence of an accessible internet database which contains MLVA16 data from 1,492 isolates (see: http://mlva.u-psud.fr/brucella/ accessed 10 December 2012).

The MLVA16$_{Orsay}$ assay is divided in two different panels: one with a low discriminatory index that allows quick allocation to a major branch and species and a second panel with VNTR markers of high discriminatory power, suitable for outbreak investigations, some of which are used in the HOOF-Print assay (*Table 6.5*). A minimum spanning tree based on MLVA16$_{Orsay}$ data from 1,925 isolates is shown in *Fig. 6.3*.

Whereas MLVA is a highly efficient tool for clustering strains, it is not a phylogenetic tool because rapidly evolving VNTR markers often suffer from homoplasy. However, clusters can be easily connected upon a phylogenetic backbone, as soon as a subset of representative strains have been analyzed by both MLST (see below) and MLVA.

Given the clonal evolution of the main *Brucella* species, phylogeny makes sense, and phylogenetic investigations can conveniently be used to define lineages. In fact, MLST is probably the tool most commonly used in current phylogenetic investigations of *Brucella*.

Multilocus Sequence Typing of Single-Nucleotide Polymorphisms

The conventional MLST approach uses sequence divergence in housekeeping genes. About 7–9 housekeeping genes are commonly analyzed in order to obtain a reasonable balance between the acceptable identification power, time, and cost for the strain typing. From each housekeeping gene, approximately 450–500 base pairs (bp) are amplified by PCR, followed by DNA sequencing and subsequent comparative sequence analysis. Each unique sequence is assigned a specific allele number, and alleles are combined into an allelic profile and further assigned to a specific sequence type (ST). New alleles result in a new combination and therefore in a novel sequence type.

Because accumulated changes occur slowly and are regarded as selectively neutral, the MLST approach is a reliable tool for the overall characterization of microbial populations and the investigation of phylogenetic relationships. However, the slow molecular clock rate and the limited number of genes do not allow in-depth phylogenetic reconstructions and analysis of local epidemiological studies. Thus, unlike MLVA, MLST is of little value for outbreak investigations.

In 2006 Whatmore et al. developed an MLST assay based on nine different loci (Whatmore et al. 2006). Of the nine loci, seven correspond to classical housekeeping genes. The remaining two loci target the *omp25* gene and an intergenic region, respectively, resulting in more discriminatory power. Nine loci are sequenced, totalling almost 4,400 bp or 0.1 % of the genome. Owing to clonal evolution and congruence of the phylogeny of individual loci, these data are usually analyzed as MLSA in which the sequence

Table 6.6

List of VNTR markers used in different assays

locus_repeatunitsize	aliases[a]	Chr	HGDI	HGDI	Hoof-Prints	MLVA8Orsay	MLVA10Orsay	MLVA11Orsay	MLVA15Orsay	MLVA16Orsay	MLVA21Weybridge	MLVA15Flagstaff	MLVA10Ames
BRU28_13bp	Bruce54 VNTR21	**1**	**0.26**	**0.39**							X		X
BRU211_63bp	Bruce11	**1**	**0.84**			X	X	X	X	X			
BRU217_15bp	Bruce46 BruceVNTR25	**1**	**0.5**									X	
BRU233_18bp	Bruce45 VNTR7	**1**	**0.65**	**0.54**		X	X	X	X	X	X		
BRU379_12bp	Bruce43	**1**	**0.55**			X	X	X	X	X			
BRU424_125bp	Bruce42	**1**	**0.75**			X	X	X	X	X			
BRU588_8bp	Bruce09 HOOF8 BruceVNTR30	**1**	**0.72**	**0.80**	X				X	X	X	X	X
BRU1134_18bp	Bruce08 VNTR26	**1**	**0.53**	**0.44**		X	X	X	X	X	X		
BRU1234_15bp	Bruce36 VNTR27	**1**	**0.38**	**0.41**							X		
BRU1250a_8bp	Bruce07 VNTR5A BruceVNTR3[a]	**1**	**0.78**	**0.78**					X	X	X	X[a]	X
BRU1250b_8bp	VNTR5B BruceVNTR3[a]	**1**	**ND**	**0.88**							X	X[a]	X
BRU1322_134bp	Bruce06	**1**	**0.73**			X	X	X	X	X			
BRU1505_8bp	Bruce30 HOOF2	**1**	**0.7**	**0.77**	X				X	X	X		
BRU1543_8bp	Bruce04 HOOF6 BruceVNTR28	**1**	**0.87**	**0.86**	X				X	X	X	X	
BRU1915_8bp	Bruce25 HOOF3 BruceVNTR29	**1**	**0.9**	**0.74**	X						X	X	X
BRU1938_8bp	Bruce01 HOOF7 BruceVNTR1	**1**	**0.95**	**0.90**	X						X	X	
BRU1940_8bp	Bruce24 HOOF5 BruceVNTR2	**1**	**0.88**	**0.89**	X						X	X	
BRU2066_40bp	Bruce55 VNTR24	**1**	**0.69**	**0.75**		X	X	X	X	X	X		
BRU18_8bp	Bruce14 VNTR12B BruceVNTR33	**2**	**0.93**	**0.91**							X	X	
BRU19_8bp	Bruce13 VNTR12A	**2**	**0.85**	**0.85**							X		
BRU73_15bp	Bruce12	**2**	**0.82**			X	X	X	X	X			
BRU275_8bp	Bruce74 BruceVNTR7	**2**	**0.63**									X	
BRU285_28bp	Bruce73	**2**	**0.51**										
BRU322_8bp	Bruce22 HOOF1	**2**	**0.9**	**0.92**	X						X		X
BRU324_3bp	Bruce19[b]	**2**	**0.79**					X		X			
BRU329a_8bp	Bruce20 HOOF4	**2**	**ND**	**0.92**	X						X		X
BRU329b_8bp	Bruce21	**2**	**0.57**				X	X	X	X			

Table 6.6 (continued)

locus_repeatunitsize	aliases[a]	Chr	HGDI	HGDI	Hoof-Prints	MLVA8Orsay	MLVA10Orsay	MLVA11Orsay	MLVA15Orsay	MLVA16Orsay	MLVA21Weybridge	MLVA15Flagstaff	MLVA10Ames
BRU339_8bp	Bruce18 VNTR17 BruceVNTR16	**2**	**0.82**	**0.71**			X	X	X	X	X	X	X
BRU344_5bp	Bruce17 VNTR16 BruceVNTR31	**2**	**0.79**	**0.82**							X	X	X
BRU396_9bp	BruceVNTR14	**2**	**ND**	**0.00**								X	
BRU542_12bp	Bruce80 BruceVNTR20	**2**	**0.18**									X	
BRU548_8bp	Bruce16 VNTR2 BruceVNTR27	**2**	**0.85**	**0.75**					X	X	X	X	X
BRU564_18bp	Bruce68	**2**	**0.47**										
BRU574_6bp	BruceVNTR21	**2**	**ND**	**0.06**								X	
BRU609_31bp	Bruce67	**2**	**0.52**										
BRU652_17bp	Bruce66 VNTR14	**2**	**0.32**	**0.31**							X		

HOOF-designations are from Bricker et al. 2003; "Bruce" are from Le Flèche et al., 2006 (48), "VNTR" are from Whatmore et al. 2006 (49), "BruceVNTR" are from Tiller et al. 2009 (50); *HGDI* (Hunter–Gaston diversity index): discriminatory power

[a]The primers for BruceVNTR3 amplify simultaneously VNTR5A and VNTR5B

[b]Bruce19 was initially published by Le Flèche et al., 2006 as a 6 bp repeat unit VNTR. However due to a secondary internal deletion in the array, present in some lineages, it is considered as a 3 bp repeat unit for allele calling consistency purposes

from all nine loci is concatenated. Such a tree is shown in Fig. 6.4.

In a comprehensive study, Whatmore and colleagues investigated 160 *Brucella* isolates belonging to all known species and biovars (Whatmore et al. 2007). The assay grouped *B. abortus*, *B. melitensis*, *B. ovis*, and *B. neotomae* as well-separated clusters according to their species affiliation. *B. suis* biovars 1 to 4 also clustered together but demonstrated higher intraspecies heterogeneity when compared with the other species. *B. suis* biovar 5 formed a separated lineage. The ten *B. ovis* isolates, although from different geographical regions, were represented by a single sequence type. The *B. canis* strains grouped in proximity to *B. suis* biovars 3 and 4 but were distinguishable by a different sequence type. *Brucella* isolates from marine mammals also grouped as a separated cluster.

The results of that study confirmed the close proximity of *B. suis* biovars 3 and 4 with *B. canis* and the separate position of *B. suis* biovar 5, already suggested by phenotypic assays and molecular methods. On the basis of these results, the authors concluded that this assay can be used to provide a strong phylogenetic backbone for the *Brucella* genus.

Real-Time Polymerase Chain Reaction Assays of Single-Nucleotide Polymorphisms

The MLST technique requires amplification of the corresponding target genes by PCR, followed by double-strand DNA sequencing of the products and subsequent sequence analysis. The overall procedure is expensive and time consuming. Consequently, a new platform for the rapid detection of SNPs was developed.

Based on the results from MLST, Gopaul et al. developed a rapid assay to distinguish the major *Brucella* clades using the minor groove binder real-time PCR technology (Gopaul et al. 2008). For each allele, a highly specific 5′ labeled, 3′ minor groove binding (MGB) TaqMan probe was designed. The assay was evaluated by testing over 300 isolates of *Brucella*. Almost at the same time, a similar approach to distinguish among the various *Brucella* species was developed by Foster et al. (2008). However, it is important to keep in mind that assays based on typing selected SNPs are highly biased. They collapse tree branches along linear trees.

Whole Genome Sequencing and Global Analysis of Single-Nucleotide Polymorphisms

The first two genome sequences available were from *B. melitensis* 16 M and *B. suis* 1330 (DelVecchio et al. 2002; Paulsen et al. 2002). From genome sequencing analysis, in particular the genome of *B. suis* 1330, it became evident that *Brucella* shares extensive genetic similarity with some plant pathogens and microbes that live symbiotically with plants, such as *Agrobacterium, Rhizobium*, and *Mesorhizobium* (Paulsen et al. 2002). Paulsen et al. found that a total of 1,902 *B. suis* ORFs were conserved in all three genomes of *M. loti*, *S. meliloti*, and *A. tumefaciens*, and 2,408 *B. suis* 1330 ORFs were conserved in at least one of these three genomes. In addition to gene sequence conservation, *B. suis* 1330 chromosome 1 shares extensive regions of gene synteny (gene order) with the *M. loti* chromosome, whereas chromosome 2 does not. Instead, chromosome 2 possesses a cluster of plasmid-like replication genes similar to plasmid replication genes from *Agrobacterium* Ti plasmids and plasmids from other organisms including *Rhizobium* spp. (Paulsen et al. 2002).

Detailed sequence analysis revealed the existence of an intact beta-ketoadipate pathway in *B. suis* 1330 (Paulsen et al. 2002) and also in *B. microti* (Audic et al. 2009). This pathway, which enables the bacterium to break down plant-derived aromatic compounds and lignin monomers, is widely distributed and highly conserved in soil bacteria and fungi, including *Pseudomonas putida*, *Acinetobacter calcoaceticus*, *Agrobacterium tumefaciens*, and *Rhodococcus erythropolis* (Harwood and Parales 1996). Therefore, it seems likely that some *B. suis* strains can persist and even might multiply in the environment. Various studies have been performed to confirm this hypothesis. In a study, carried out by the US Environmental Protection Agency (EPA), the persistence of *B. suis* biotype 1 strain Battelle BRU163 on various outdoor materials was evaluated. *B. suis* persisted as long as 28 days (longest period tested) on various matrices (aluminum, wood, glass, soil) under low and moderate temperatures without UV light (report see: EPA/600/R-10/026; April 2010; www.epa.gov/ord). Thus, it seems likely that *Brucella* has evolved from a soil/plant-associated ancestral bacterium, and speculatively, at least some strains of *B. suis* and *B. microti* may be have a replicative life cycle outside of a mammalian host. Sequencing of the *B. abortus* field isolate 9–941 in 2005 (Halling et al. 2005) and comparison to the genome sequences of *B. melitensis* 16 M and *B. suis 1330* revealed a striking similarity, with nearly identical genetic content and gene organization.

In 2009 the genome sequence of *B. microti*, originally isolated from the common vole in the Czech Republic, was published (Audic et al. 2009). Although *B. microti* is metabolically very active displaying numerous metabolic reactions that are absent from all classical *Brucella* species (Scholz et al. 2008; Al Dahouk et al. 2012), the genome sequence of *B. microti* is almost identical to that of *Brucella suis* 1330 with an overall sequence identity of 99.84 % in aligned regions (Audic et al. 2009). Hence, the authors concluded that the different phenotype is rather a matter of differential gene regulation than based on the presence of additional genes in the chromosome of *B. microti*.

In 2011 the first genome sequence of a *Brucella* strain isolated from a marine mammal, *Brucella pinnipedialis* B2/94, was published (Audic et al. 2011).

To date, a total of 40 *Brucella* genomes have been sequenced, with an average size of 3.3 MB ranging from

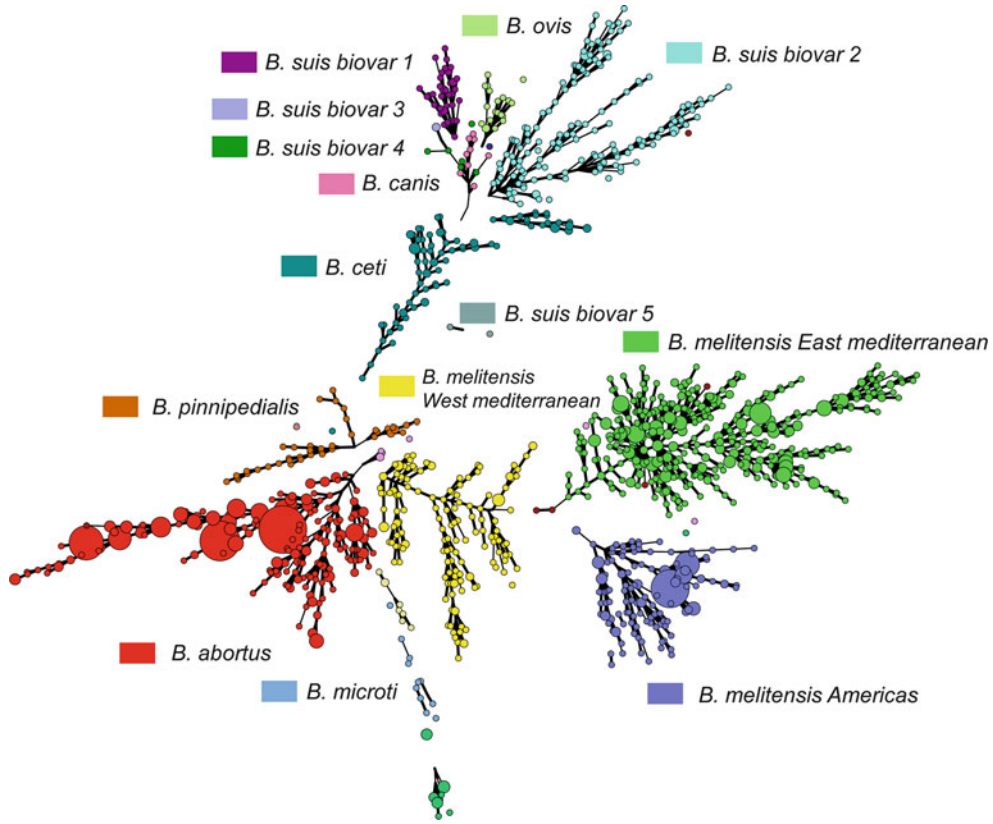

Fig. 6.3

Minimum spanning tree based on MLVA15Orsay data from 1925 isolates, the majority being compiled from the literature. Branch length up to 3 are shown. The figure illustrates how MLVA can be used to draw the image of a population at low cost and then pick the rare isolates (shown with different *colors*) with long branches for whole genome draft sequencing (Figure provided by G.Vergnaud)

3.11 MB (*Brucella* sp. NF2653) to 3.4 MB (*B. pinnipedialis* B2/94). The average number of annotated genes per genome is 3,524, ranging from 3,325 (*Brucella* sp. 83/13) to 3,709 (*B. ceti* Cudo) (Sobral and Wattam 2011). With the exception of *B. suis* biovar 3, which consists of a single 3.15 MB chromosome, all other so far sequenced *Brucella* genomes are composed of two chromosomes. Plasmids are absent in all *Brucella* sequenced so far. To date, there has been no comparative analysis including all 40 genomes; however, two comparative studies have recently been published covering 10 and 37 *Brucella* genomes, respectively (Wattam et al. 2009; Bohlin et al. 2010).

Wattam et al. (2009) analyzed the genomes of *Brucella* strains that have recently been described as atypical *Brucella* because of their different phenotype compared with the classical *Brucella* species. These analyses included stain BO1 (*B. inopinata*), isolated from a breast implant infection of a 71-year-old woman (De et al. 2008); strain BO2, isolated from a patient with obstructive pneumonia in Australia (Tiller et al. 2010a); and two strains, 83–13 and NF2653, isolated from Australian rodents (Tiller et al. 2010b). They found that the atypical strains grouped separately from the classical *Brucella* species but markedly closer to them as *Ochrobactrum*, the genetically closest genus in the family *Brucellaceae*, suggesting the atypical strains as true members of the genus *Brucella* – at least from a genetically point of view. Furthermore, all so far sequenced atypical *Brucella* strains, including *B. inopinata* and *B. microti*, harbor the *Brucella*-specific genetic marker *bcps*31 (31 KDa surface protein) and the insertion element (IS) *IS711*, confirming their affiliation to the genus *Brucella*. Wattam et al. (2009) also found a set of genes specific for each group, respectively, or genes which are absent in one of the groups. In fact, the atypical *Brucella* strains harbor unique regions not found in the classic *Brucella* genomes and vice versa. A recent genome study, carried out by Bohlin and colleagues (Bohlin et al. 2010), compared a set of *Brucella* species with closely related bacteria, such as *Ochrobactrum* and *Agrobacterium*, using base compositional- and proteome-based methods. Both methods were in good agreement with the current *Brucella* taxonomy but demonstrated that *Brucella* species are characterized by extremely high levels of nucleotide similarity and yet vary in microbial and disease phenotypes as well as in pathogenicity and host preference.

Draft whole genome sequencing is increasingly being used in replacement of MLST and large-scale SNP typing because it is an unbiased approach and provides an incomparable wealth of data, at a cost which is now getting closer to that

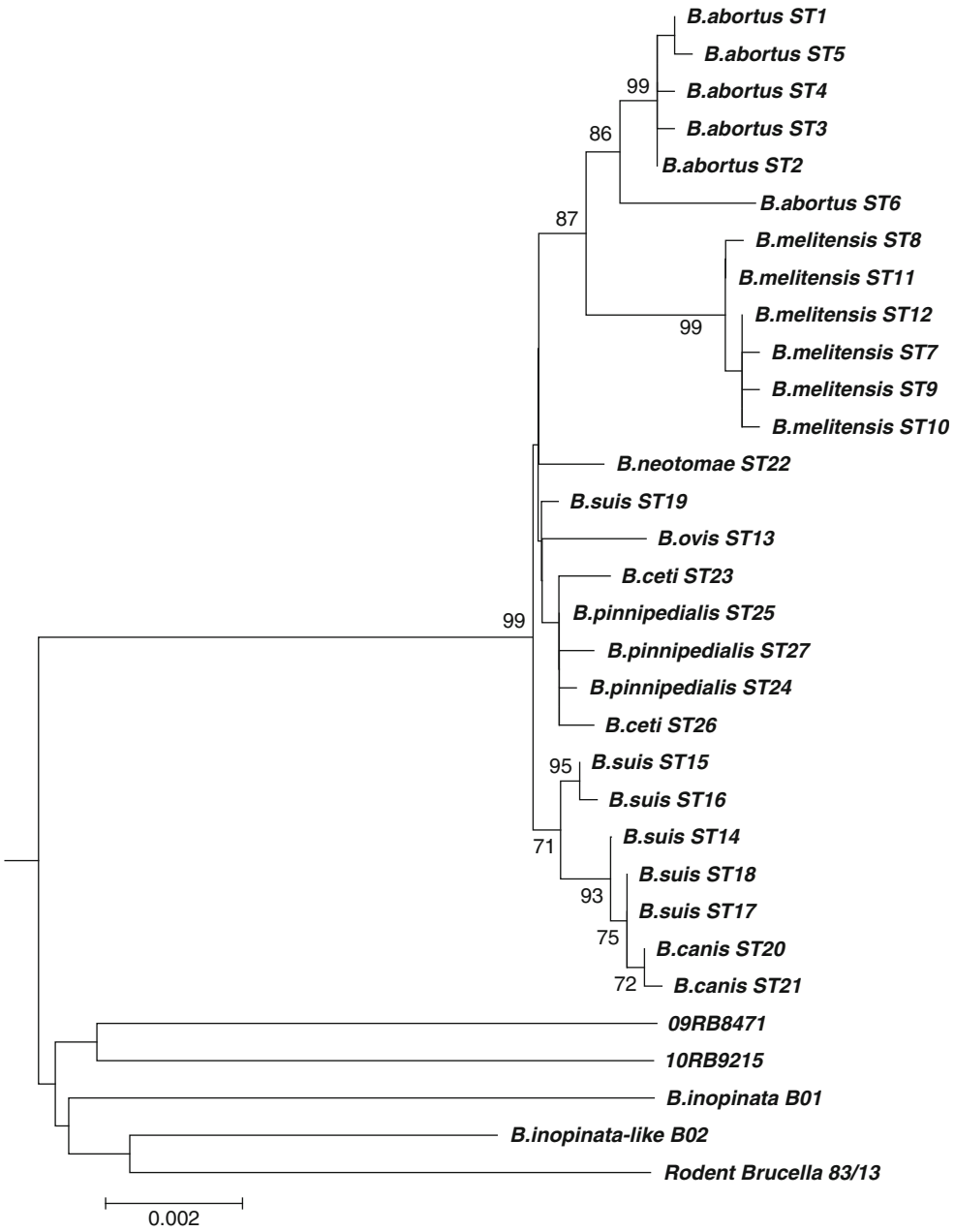

Fig. 6.4

Relationships among the *Brucella* complex based on eight loci MLSA. The extended *Brucella* group, including *B. inopinata*, the rodent isolate 83/13, strain BO2, and the two isolates (09RB8471 and 09RB9215) from African bullfrogs cluster separately from the core *Brucella*. Sequences were concatenated and the phylogenetic tree constructed using the neighbor-joining approach. Numbers at nodes correspond to proportions of 100 resamplings that support the topology shown with only values >70 % indicated. Bar = number of substitutions per nucleotide position (Figure provided by AM Whatmore)

of the previously described assays. Hundreds of isolates are currently being sequenced and analyzed at draft level for whole genome SNP discovery (O'Callaghan and Whatmore 2011). Compared with MLSA, this will provide much higher resolution. From the resulting information, it will be possible to devise specific SNP assays that efficiently complement MLVA typing and clustering analysis when necessary (Foster et al. 2009).

Phages

A major criterion for species definition of *Brucella* is their susceptibility to phages. Over twenty *Brucella* phages have been isolated from various sources such as *Brucella*-infected animals, blood, milk, fetuses and from the bacteria themselves (Moreno and Moriyon 2006). Based on their host specificity, *Brucella* phages may be classified into seven

distinct groups with numbers that reflect the order they were isolated or developed (Corbel and Banai 2005). The characteristics of the main *Brucella* phages are shown in Table 6.7. Due to their narrow host range, phages are valuable for the identification of *Brucella* at the genus and, combined with other test, even at the species level. Although phage typing mainly provides a precise result, some *Brucella* strains may display variation from the standard lysis pattern. This is predominantly the case for *B. melitensis* strains, which can be classified into subtypes according to their susceptibility pattern for Bk_2, Iz_1, and Wb phages (Corbel 1987, 1989). More detailed information on *Brucella* phages can be found at Corbel and Banai (2005) and Moreno and Moriyon (2006).

MALDI-TOF Analysis

Despite the close genetic relatedness, it is possible to differentiate the various *Brucella* species using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In a recent study, Lista et al. (2011) investigated 170 *Brucella* strains covering all classical *Brucella* species. Identification was possible at the species level.

Isolation, Enrichment, and Maintenance Procedures

Detailed information on isolation and culture of *Brucella* can be found at Corbel and Banai (2005) and Moreno and Moriyon (2006). *Brucella* has been classified as a risk group 3 microorganism and should be handled with the appropriate precautions. *Brucella* may be isolated from clinical specimens such as any tissue or secretion. The material which will most likely yield positive cultures includes abortion material (amniotic fluid, fetal gastric contents, fetal lung, fetal liver, placenta cotyledon, and vaginal discharge), accessory glands, bone marrow, lymph nodes, mammary gland, uterus, seminal vesicles, testes and epididymides, or other organs with local lesions, as well as body fluids like blood, colostrum, milk, and semen (Corbel and Banai 2005).

To isolate *Brucella* from samples where contaminating bacteria are expected, a selective isolation medium is advised. Most smooth strains of *Brucella* can be isolated with SDA supplemented with the antibiotic formulation of Farrell (1974), yet this medium might inhibit growth of *B. canis* and *B. ovis*. These two species may be isolated from contaminated material by using SDA supplemented with 10 % (v/v) heated horse serum and VCN-F inhibitor (BBL) or modified Thayer–Martin medium (Brown et al. 1971). These media have shown to be less selective compared to Farrell's SDA; therefore, it is recommended to dilute heavily contaminated material, such as semen, in 5–10 volumes of sterile isotonic solution and subsequently filter it through a membrane filter (0.8 µm pore size) before plating. Inoculated plates should be incubated at 37 °C under an air atmosphere supplemented with 5–10 % (v/v) CO_2. Incubation with CO_2 supplementation can be omitted when isolation of a CO_2-independent *Brucella* species is being attempted. Direct inoculation of heavily infected materials onto selective media generally leads to a satisfactory isolation of *Brucella*, even if contaminating bacteria are present. Uncontaminated sample materials may be plated directly on to SDA plates.

When the concentration of *Brucella* in the sample is expected to be low, as it is the case for body fluids such as blood, milk, or semen, enrichment procedures should be employed. To enrich *Brucella* in milk, samples should be incubated at 4 °C, leading to concentration of *Brucella* cells in the cream layer, which can then be cultured. Enrichment of *B. canis* in blood of tissue material may be achieved by inoculation of the yolk sacs of 6–8-day-old chicken embryos. Yolk from eggs with dead embryos is plated onto SDA or other suitable media. Cell wall-defective forms of *Brucella* may be enriched by culturing blood, solid tissue, and synovial fluid samples on SDA supplemented with 20 % (v/v) horse serum and with the antibiotic formulation of Farrell (1974). The inoculated plates should be incubated for at least 2 weeks and be inspected for microcolonies under a stereomicroscope.

Another way to enrich for *Brucella* is either by growth in a liquid enrichment medium or by intramuscular injection of the sample material into guinea pigs followed by subsequent culturing of the spleen tissue past 4 weeks. However, enrichment in guinea pigs is only presumably being successful with virulent strains of *B. abortus*, *B. melitensis*, and *B. suis*. Enrichment cultures can be carried out using a two-phase culturing system established by Castañeda (1947). The system contains a liquid phase, which consists of SDB supplemented with the antibiotic formulation of Farrell (1974) and 25 % (w/v) trisodium citrate, and a solid phase containing SDA with 2.5 % (w/v) agar. At first, the liquid phase is inoculated with the sample, and bottles are then incubated at 37 °C under air atmosphere (supplemented with CO_2, if required). Every 2–3 days, an aliquot of the liquid phase is poured over the solid phase. Bottles are re-incubated for up to 6 weeks or until colonies start to grow on the solid phase, whichever is sooner. For enrichment cultures of *B. canis* and *B. ovis*, the antibiotic formulation can be substituted by VCN-F inhibitor.

Short time maintenance of *Brucella* cultures can be achieved by streaking them onto SDA slants. Following incubation at 37 °C for 72 h under air atmosphere (supplemented with 10 % CO_2 (v/v), if required), the slants are sealed and stored at 4 °C. Subculturing has to be repeated at intervals of 6–8 weeks. However, for long-term preservation of *Brucella* strains, this procedure is insufficient, as these tend to alter their characteristics when subcultered repeatedly.

Cultures of *Brucella* may be maintained appropriately for long-term periods by vacuum drying. The strains are incubated on SDA slants at 37 °C for 72 h under air (+ 10 % CO_2 (v/v), if required). The culture is then washed off the slants and suspended in sterile rabbit serum to reach a final concentration of about 10^{10} bacteria/ml. Aliquots of 0.1 ml are filled into sterile

tubes sealed with cotton-wool plugs and are stored over phosphorus pentoxide in a desiccator kept at 4 °C. The desiccator has to be evacuated on a daily basis until a stable pressure of 0.05-mmHg is reached. Finally, the tubes are sealed under vacuum in glass ampoules including a small quantity of silica gel. Preserved this way, *Brucella* cultures may stay viable for several years.

In addition, *Brucella* cultures may be preserved adequately by freeze-drying. General instructions for this procedure have been provided by Lapage et al. (1970), and further details about the technique are given by Boyce and Edgar (1966).

Storage of *Brucella* cultures in liquid nitrogen is another option for preservation, yielding to a higher degree of viable cells compared to vacuum drying or freeze-drying (Davies et al. 1973). *Brucella* are incubated on SDA slants at 37 °C for 72 h under air (+10 % CO_2 (v/v), if required). The culture is then suspended in single-strength Bacto-glutamate medium to give a dense suspension. The bacterial suspension is left alone at 4 °C for 1 week, and then aliquots of 1 ml are filled into sterile glass screw-capped vials. Finally, the vials are placed in the vapor phase or are directly immersed in the liquid nitrogen.

Isolation of *Crabtreella saccharophila* (*Zoogloea ramigera* ATCC 19623^T) from sludge was initially described by Crabtree and McCoy (1967): flocs obtained from activated sludge samples are gently washed several times in sterile phosphate buffer (pH 7.2), then collected by sedimentation and decantation to free them from floating cells and debris. The washed flocs are further homogenized and subsequently used to inoculate arginine broth (arginine 0.05 %, K_2HPO_4 0.2 %, KH_2PO_4 0.1 %, $MgSO_4$ x 7 H_2O 0.02 %, biotin and vitamin B_{12} 2 µg/ml each). Penicillin G (2–5 units/ml) is added to the medium to inhibit contaminating Gram-positive bacteria. Cultures are incubated under gentle agitation at 30 °C for 12–24 h. Following incubation, cells in the enrichment culture are homogenized once more, and suitable dilutions are plated on trypticase soy (0. 5 %) agar. Colonies of selected isolates are re-inoculated in arginine broth and in flocculation test medium (arginine broth + 5 % glucose). Flocculation in the glucose medium but not in the plain broth indicates selection of the right isolates, which may be further purified. Isolates can be maintained on trypticase soy (0. 5 %) agar slants with transfer every 6 months.

Daeguia caeni was isolated from sludge of a wastewater treatment plant by using standard dilution plating techniques (Yoon et al. 2008). Diluted samples are plated on trypticase soy agar and incubated aerobically at 30 °C. Isolates can be further cultured in trypticase soy broth. Special maintenance procedures have not been reported. Organisms may be maintained for short time periods on trypticase soy agar slants.

Isolation of *Mycoplana* strains has been originally described by Gray and Thornton (1928): inoculate 0.5–1 g of soil sample in 100 ml of a mineral salt solution containing 0.05–0.1 % phenol or 0.05 % *m*-cresol. If growth is observed, transfer the culture into a new flask of the same medium. *Mycoplana* strains can be isolated by further plating and purification on agar medium.

It is possible to maintain *Mycoplana* cultures on most common media. Good growth occurs in nutrient broth, PYG broth and peptone water, and on soil media, when cultures are incubated aerobically at 30 °C. Cultures may be kept for short periods at room temperature or at 4 °C. Subculturing should be repeated every week, although longer storage of cultures is possible when stored at 4 °C. Long-term maintenance can be achieved by freezing at −80 °C, storage in liquid nitrogen, or lyophilization. If organisms are frozen, cultures with a high cell density should be used and cryoprotective agents such as DMSO or 10 % glycerol should be added.

For the isolation of *Ochrobactrum*, no special procedures are necessary. Cultures may be maintained for several months on Dorset egg medium slants (Barrow and Feltham 1993) in a screw-capped bottle stored at 4 °C. Longtime preservation may be achieved by lyophilization or by suspension of an 18-h-old agar slant culture in a small tube containing defibrinated rabbit blood, which is then frozen in a mixture of alcohol and dry ice prior to storage at −50 °C.

Paenochrobactrum gallinarii was isolated from air collected in a duck barn (Kämpfer et al. 2010). Bioaerosol samples are collected on gelatine filters. Filters are dissolved and decimally diluted in isotonic NaCl. Subsequently, tryptone soy agar plates are inoculated with an aliquot of each dilution and incubated at 25 °C. Isolates may be picked from the plates inoculated with liquid of the first decimal dilution. Further subcultivation may be performed aerobically on TSA at 28 °C for 48 h.

Paenochrobactrum glaciei has been recovered from sea ice (Romanenko et al. 2008). The sea ice sample is collected in a sterile flask together with a small quantity of sterile sea water. The sample is stored for 2 days at 15 °C and melted carefully. An aliquot of melting sea ice is inoculated onto a marine 2216 agar plate and incubated for 1 week at 15 °C. Subcultivation of isolates may be performed on marine 2216 agar or in marine broth, trypticase soy agar or trypticase soy broth, and R2A agar aerobically at 28 °C. Long-term maintenance may be achieved by storage of cells at −80 °C in liquid marine broth supplemented with 30 % (v/v) glycerol.

Isolation of various *Pseudochrobactrum* strains has been described by Kämpfer et al. (2006, 2007, and 2009). Most strains have been isolated from industrial samples by inoculation on blood agar, nutrient agar, or standard plate count agar, following aerobically incubation at 30–37 °C. Subculturing may be performed on tryptone soy agar at 28 °C for 48 h. Growth occurs also on MacConkey and R2A agar when incubated at 30 °C. Longtime maintenance procedures have not been reported so far but may be achieved by freezing of the culture together with a cryoprotective agent at −80 °C.

Ecology

Habitat

The main habitat of organisms belonging to the *Brucellaceae* seems to be environmental habitats such as soil and water.

Table 6.7
Characteristics of the main *Brucella* phages

Group	Phage-type strain	Origin	Propagating strain	Host lysis	Other features
1	Tb	Isolated from liquid manure of a cowshed in Tbilisi (Russia)	*B. abortus* 544 and S19	*B. abortus*, *B. inopinata*, *B. neotomae*, *B. microti*, *B. suis*, some *B. pinnipedialis* strains	Does not lyse rough mutants
2	Fi 75/13	Obtained from *B. abortus* culture in Firenze (Italy)	*B. abortus* S19	*B. abortus*, *B. neotomae*, *B. suis*	Does not lyse rough mutants. Plating efficiency is to some extend higher on *B. abortus* strains
3	Wb	Obtained from *B. suis* culture in Weybridge (England)	*B. abortus* S19, *B. suis* 1330	*B. abortus*, *B. ceti*, *B. neotomae*, *B. suis, B. pinnipedialis*, some *B. melitensis* strains	Does not lyse rough mutants
4	Bk_2	Obtained by plating phage Bk_0 on *B. melitensis* 16 M in Berkley (USA)	*B. melitensis* Isfahan and 16 M	*B. abortus*, *B. neotomae*, *B. suis, B. melitensis*	Relative of phage Bk_1 Does not lyse rough mutants
5	R/C	Obtained from phage R/O by serial propagation on *B. canis* RM 6/66	*B. canis* RM 6/66 and Mex.51	*B. canis*, *B. ovis*, rough *B. abortus*, and some rough *B. melitensis* mutant strains	Does not lyse smooth *Brucella* strains. Relative of phage R/M, which lyses some *B. melitensis* strains
	R/M	Obtained from phage R/C by serial propagation on *B. melitensis* 115	*B. melitensis* 115	Some rough *B. melitensis* strains	Genetically unstable. Reported to be more consistently lytic for rough *B. melitensis*
6	Iz_1	Isolated from goat and sheep feces in Izatnagar (India)	*B. abortus* S19, *B. melitensis* 16M and 115	*B. abortus*, *B. ceti*, *B. neotomae*, *B. suis, B. melitensis*, *B. pinnipedialis*	Lysis of all smooth *Brucella*. Also lysis of rough *B. abortus*, *B. suis, B. melitensis*
7	Np	Obtained from atypical B. abortus strains in Nepean (Canada)	*B. abortus* S19	*B. abortus*, *B. neotomae*	Does not lyse rough mutants

Adapted from Moreno and Moriyon (2006)

However, mammalian animals including humans present a reservoir for various *Brucella* species, whereas they may also be present in the environment. Several species especially of the genus *Pseudochrobactrum* have been isolated from industrial environments, while some species belonging to the genera *Brucella* and *Ochrobactrum* have been predominantly found in human clinical samples.

Even though *Brucella* species are cultivated in vitro without problems, under natural conditions, they behave as obligate intracellular pathogens and do not seek for an existence apart from their animal hosts. Nevertheless, they are capable to persist for longer times in the environment, such as in soil or surface water, under the appropriate humidity, low temperature conditions (between 2 and 8 °C), and when protected from sun light. *Brucella* species are distributed worldwide and can infect various hosts, whereas the host range of a specific *Brucella* species may be enormous. The common natural hosts for *B. melitensis* are goats and sheep, but other mammals like cattle, humans, and pigs may also be infected (Corbel and Banai 2005). For *B. abortus*, cattle has been described as the natural host. Yet, *B. abortus* has been found to persist in various other hosts, such as camels, dogs, elk, goats, horses, pigs, sheep, yaks (Crawford et al. 1990), and humans (Arimi et al. 2005; Swai and Schoonman 2009). Biovars 1, 2, and 3 of *B. suis* naturally infect pig. In addition, all biovars of *B. suis*, except for biovar 2, are pathogenic for humans. Other species, such as dogs, horses, and rodents, may also serve as hosts (Corbel and Banai 2005). Under certain conditions, *Brucella* is also capable to replicate within lice, fleas, ticks, and worms, which may serve as vectors for transmission to mammalian hosts (Moreno and Moriyon 2006). Another group of *Brucella* species has been isolated from marine mammals initially designated as *B. maris*. These marine mammalian *Brucella* isolates have a host preference for either the order *Cetacea* (whales, dolphins, and porpoises) or *Pinnipedia* (seals, sea lions, and walruses) (Thakur et al. 2012). Instead of the designation *B. maris*, 7 novel species have recently been described. These include *B. delphini*, *B. phoecoenae*, *B. ceti*, *B. pinnipedialis*, and *B. phocae*, with dolphins, porpoises, cetaceans, and seals as their preferred hosts, respectively (Corbel and Banai 2005; Foster et al. 2007).

The two *Mycoplana* species *M. dimorpha* and *M. ramosa* were both isolated from soil (Gray and Thornton 1928). The same applies to several species of the genus *Ochrobactrum*: *O. grignonense* has been isolated from a French soil, and *O. tritici* has been isolated from wheat root (Lebhun et al. 2000); *O. oryzae* originated from surface-sterilized seeds and plant tissue from

deep-water rice cultivated in Suraha Tal Lake in northern India (Tripathi et al. 2006); another species *O. cytisi* was recovered from nodules of common broom growing in a Spanish soil (Zurdo-Pineiro et al. 2007). Strains of *O. intermedium* have been isolated from French soil (Holmes et al. 1988) and chromium-contaminated soil from India (Kavita and Keharia 2012); other *O. intermedium* strains are from human blood (Velasco et al. 1998). *Ochrobactrum pseudintermedium* has been isolated from human clinical samples (Teyssier et al. 2007). The most predominant isolates from human clinical samples are strains of *O. anthropi*, which have been recovered from human blood, wounds, urogenital tracts or urine, respiratory tracts, and feces; a few strains have also been reported from environmental sources like activated sludge, biofilm in water supply lines, soil, and the termite gut (Holmes et al. 1988; Laura et al. 1996; Kuhnigk and Konig 1997).

Paenochrobactrum have so far only been isolated occasionally from environmental samples. *P. gallinarii* was isolated from air of a duck barn (Kämpfer et al. 2010), and *P. glaciei* was isolated from a sea ice sample from the Sea of Japan (Romanenko et al. 2008).

Pseudochrobactrum species have been isolated more often especially from various industrial environments. *P. lubricantis* was isolated from a water-mixed metal-working fluid (Kämpfer et al. 2009), and *P. kiredjianiae* has been recovered from a seafood-processing plant sample in New Zealand (Kämpfer et al. 2007). *P. saccharolyticum* was isolated from industrial glue, whereas *P. asaccharolyticum* originates from a knee aspirate of a 66-year-old man (Kämpfer et al. 2006).

The habitat of members of the monospecific genera and of those species for which only a single strain, the type strain, is available must be considered tentative.

Pathogenicity and Clinical Relevance

Brucella species are pathogens that cause infections in a wide range of animals and in humans. Generalized infections with an initial bacteremic phase are often followed by localization in the reproductive organs and reticuloendothelial system. In vivo, organisms grow intracellular and may survive within immune cells such as monocytes and granulocytes. Infection may involve any organ or body system, causing a variety of lesions. Infections in the natural host are generally mild and seldom lethal. Clinical manifestations occur mainly in the pregnant animal, were abortion is often a result of placental and fetal infection. *Brucella* may also be excreted in the milk after localization in the mammary tissue. In general, all main species of meat- and milk-producing domesticated animals are susceptible to *Brucella* infections and act therefore as possible sources of human infection (Corbel and Banai 2005).

Human infections are caused by direct or indirect contact with infected animals or by ingestion of contaminated milk or meat products. A transmission from person-to-person hardly ever takes places and does not play a role in the natural history of the disease. *B. melitensis* is responsible for the majority of severe infections, followed by *B. suis*. Milder infections are generally associated with *B. abortus* and *B. canis*. Organisms may enter the body via the gastrointestinal or respiratory mucosa or via percutaneous penetration. The pathogenesis is thought to be analogous to the disease progression in experimental animals, with an initial bacteremic phase of variable duration causing proliferation of the organisms in lymphoid tissue, which is in a few cases followed by localization in specific organs. The infection can have a complete subclinical progression, or it may cause a subacute or acute febrile sickness. If not treated appropriate with adequate antibiotic therapy, the infection may persist for several months and can be accompanied by drastic complications like arthritis, endocarditis, meningoencephalitis, orchitis, and spondylitis. *Brucella* infection stimulates both antibody production and cell-mediated immunity. The prevailing antigen in the serological response is LPS. However, immunological response to a number of proteins can also develop. More detailed information is given by Young and Corbel (1989) and Madkour (2000).

O. anthropi has been isolated from a variety of human clinical specimens, predominantly from cases of bacteremia (Gransden and Eykyn 1992; Kern et al. 1993) but also of pancreatic abscess (Appelbaum and Campbell 1980), necrotizing fasciitis (Brivet et al. 1993), and puncture wound osteochondritis of the foot (Barson et al. 1987). In most cases, it seems that *O. anthropi* is community acquired; moreover catheters have been suggested as a pathogenic niche for this organism (Gransden and Eykyn 1992). It may be pathogenic in immunocompromised or critically ill patients, with or without indwelling catheters. Even though it can cause serious clinically infections, it appears to have a relatively low virulent potential (Corbel and Banai 2005). Most strains display a multiple resistance to a variety of antibiotics, being only susceptible to aminoglycosides, fluoroquinolones, imipenem, and trimethoprim–sulfamethoxazole. Monotherapy with an appropriate aminoglycoside has yielded a good clinical response in the treatment of bacteremia. However, despite determining in vitro susceptibility to imipenem in initial isolates, treatment of patients with this agent failed to eradicate the organism. Due to the unpredictable susceptibility pattern, choice of antibiotics should be based on susceptibility tests for each isolate. In addition, multidrug therapy may be advised (Kern et al. 1993).

O. intermedium may also cause infections in immunocompromised patients; however, case reports are limited. The organism has so far been isolated from two cases of bacteremia, from a liver transplanted patient (Moller et al. 1999) as well as from a patient with bladder cancer (Apisarnthanarak et al. 2005). It may also be possible that certain infections believed to be caused by *O. anthropi* were actually caused by *O. intermedium*, since both organisms cannot be easily differentiated by biochemical tests. Nevertheless, both organisms may be discriminated by genotypic methods, and their antibiotic resistance to colistin and polymyxin B, with susceptibility of *O. anthropi* and resistance of *O. intermedium*, isolates to both antibiotics (Velasco et al. 1998; Moller et al. 1999).

So far there is no comprehensive data on the pathogenic potential of other organisms belonging to the *Brucellaceae*.

Acknowledgments

The excellent and comprehensive treatise of Moreno and Moriyon from the 2nd edition of *The Prokaryotes* is still highly recommended for a more deep study on classical approaches in the biology of *Brucella*.

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7 The Family *Caulobacteraceae*

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Abstract
The family *Caulobacteraceae* comprises Gram-negative, aerobic, or facultatively anaerobic, rod-shaped, and motile bacteria which do not form spores. Although *Caulobacteraceae* have first been recognized because of their unusual cell morphology and cell cycle, their genera harbor both prosthecate or non-prosthecate species. Prosthecate cells often attach to surfaces with the prostheca at one end and forming polarly flagellated swarming cells at the other end. Prostheca of several cells may form rosettes. The species are chemo-organotrophic, some species require peptone or B vitamins and amino acids. Some species denitrify. All species have alkaline phosphatase activity, but none of α-glucuronidase, α-mannosidase, or α-fucosidase. In most species, the major isoprenoid quinone is Q-10. Main polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranuronosyl diacylglycerol, 6-phosphatidyl-α-D-glucopyranosyl diacylglycerol (but lacking in *Asticcacaulis*), and phosphatidylglycerol. Members of the family have been isolated from freshwater, soil, sea water, plants, animals, and humans. The family is a member of the *Alphaproteobacteria* Garrity et al. 2006 and comprises the type genus *Caulobacter* Henrici et Johnson 1935, emend. Abraham et al. 1999 and the genera *Brevundimonas* Segers et al. 1994, emend. Abraham et al., 1999, *Asticcacaulis* Poindexter 1964 and *Phenylobacterium* Lingens et al. 1985, emend. Oh et Roh, 2012. The species live in very diverse habitats, mostly aquatic, but also in soil, at the surfaces of eukaryotes. The temperature range of these habitats is moderate, but some species also thrive at low temperatures. Few species are pathogens. Because of their life cycle, most species are associated with surfaces where they form biofilms.

Taxonomy: Historical and Current

The first report on the isolation of a species of the *Caulobacteraceae* appeared more than a century ago (Loeffler 1890). The strain could not be assigned to a known taxon and was named "*Vibrio* (*?*) *spermatozoides*," but even then, its unusually low nutrient requirements were noticed. Almost half a century later, Henrici and Johnson (1935) described the genus *Caulobacter* with *Caulobacter vibrioides* as the type species. Henrici and Johnson did not isolate *Caulobacter vibrioides*, and

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therefore, a type strain was not named. Poindexter (1964) enlarged the monotypic genus considerably by describing nine species and two subspecies, thereafter adding *C. variabilis* (Poindexter 1989). Strain CB51 was proposed as the neotype strain of *C. vibrioides* (Poindexter and Lewis 1966). At that time, the taxonomy of the species of the genus was based almost exclusively on the characteristic morphology and mode of reproduction (Poindexter 1981b). They are dimorphic, prosthecate bacteria in which reproduction occurs by binary fission and results in the separation of two cells that are morphologically distinct from each other. The prosthecate cell is sessile, being nonmotile, and, possessing at least one elongated, cylindrical appendage, a prosthecum (Staley 1968). The other cell is motile by one polar flagellum. The mode of reproduction of the dimorphic, prosthecate bacteria is unique and helps to disperse the population at each generation, thereby minimizing competition among siblings for resources. This enables these bacteria to exist in oligotrophic habitats (Poindexter 1981a) and to tolerate prolonged starvation. In 1964, Poindexter described the genus *Asticcacaulis* harboring species with prostheca but attaching to substrate with holdfasts at the cell poles and not at the prosthecum (Poindexter 1964).

In 1973 Krassilnikov and Belyaev described eight additional species (*Caulobacter robiginosus, C. rutilus, C. glutinosus, C. kusnezovii, C. rossi, C. flexibilis, C. metschnikovii*, and *C. fulvus*) and five subspecies (*C. vibrioides ssp. luridus, C. bacteroides ssp. modicus, C. bacteroides ssp. podsolicum, C. bacteroides ssp. creteus*, and *C. variabilis ssp. albus*) (Krassilnikov and Belyaev 1973). These taxa, however, were not validly described because no type strains were given. Consequently, the species described by Krassilnikov and Belyaev in 1973 were all not accepted in the Approved Lists. They were discussed by Poindexter (1981a) but otherwise largely ignored probably also due to the fact that the publication of Krassilnikov and Belyaev was in Russian.

The phylogenetic position of *Caulobacter crescentus* within the *Alphaproteobacteria* was first deduced from 5S rRNA sequences (Stackebrandt et al. 1988). First hints that not all prosthecate bacteria may belong to a single genus came from analyses of a large set of strains which revealed differences between freshwater and marine caulobacteria (Anast and Smit 1988). The differences between these two groups could be substantiated by a phylogenetic analysis using 16S rRNA gene sequences of several *Caulobacter* isolates (Stahl et al. 1992). This study revealed, for the first time, that *Caulobacter* actually forms two different lineages, one comprising mainly the freshwater and brackish water *Caulobacter* spp. and the other the true marine species. Surprisingly, the study also showed that the non-prosthecate *Brevundimonas diminuta* (Segers et al. 1994) clusters with the group of freshwater *Caulobacter* spp. The 16S rDNA sequences of *Mycoplana segnis* and *Mycoplana bullata* also fell in the freshwater *Caulobacter-Brevundimonas* cluster, thus confirming that the presence of a prosthecum is not a sufficient criterion for taxonomy (Yanagi and Yamasato 1993). The diversity of more than 70 strains of *Caulobacter*, *Brevundimonas*, and *Mycoplana* was evaluated using lipid analysis, 16S rRNA gene sequences, and profiling of the 16S-23S rRNA gene interspacer (ITS) regions and comparison of these results with physiological data. As a consequence, the descriptions of the genera *Caulobacter* and *Brevundimonas* were emended and a number of *Caulobacter* species were transferred to the genus *Brevundimonas* (Abraham et al. 1999). *Mycoplana bullata* was found to belong to the genus *Brevundimonas, Caulobacter crescentus* to be conspecific with *C. vibrioides*, and *Mycoplana segnis* was transferred to *Caulobacter* as *C. segnis*.

Phenylobacterium, for many years a monospecific genus consisting of a degrader of xenobiotics, was found to be closely related with *Caulobacter* and the prosthecate species *Phenylobacterium conjunctum* was described (Abraham et al. 2008). Currently, the family *Caulobacteraceae* comprises the genera *Caulobacter*, *Asticcacaulis*, *Brevundimonas*, and *Phenylobacterium*. Within the family only the genus *Asticcacaulis* exclusively contains prosthecate species; the other genera habor both prosthecate and non-prosthecate species.

Description of *Caulobacteraceae*

Caulobacteraceae (Cau.lo.bac.te.ra′ceae. N.L. fem. n. *Caulobacter* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Caulobacteraceae* the *Caulobacter* family).

Gram-negative, rod-shaped bacteria. Motile. Do not form spores. Cells are either prosthecate or non-prosthecate. Prosthecate cells often attach to surfaces with the prostheca at one end and form polarly flagellated swarming cells at the other end. Prostheca of several cells may form rosettes. Chemoorganotrophic. Some species require peptone or B vitamins and amino acids. Aerobic or facultatively anaerobic. Some species denitrify. All species have alkaline phosphatase activity but none of α-glucuronidase, α-mannosidase, or α-fucosidase. In most species, the major isoprenoid quinone is Q-10. Main polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranuronosyl diacylglycerol, 6-phosphatidyl-α-D-glucopyranosyl diacylglycerol (but lacking in *Asticcacaulis*), and phosphatidylglycerol. Members of the family have been isolated from freshwater, soil, sea water, plants animals, and humans. The family is a member of the *Alphaproteobacteria* Garrity et al. 2006 and comprises the type genus *Caulobacter* Henrici et Johnson 1935, emend. Abraham et al. 1999 and the genera *Brevundimonas* Segers et al. 1994, emend. Abraham et al., 1999, *Asticcacaulis* Poindexter 1964 and *Phenylobacterium* Lingens et al. 1985, emend. Oh et Roh, 2012.

Molecular Analyses

DNA-DNA hybridizations between strains of *Caulobacteraceae* (Moore et al. 1978) revealed a diversity to their genomes which is high compared to the diversity of their 16S rRNA gene

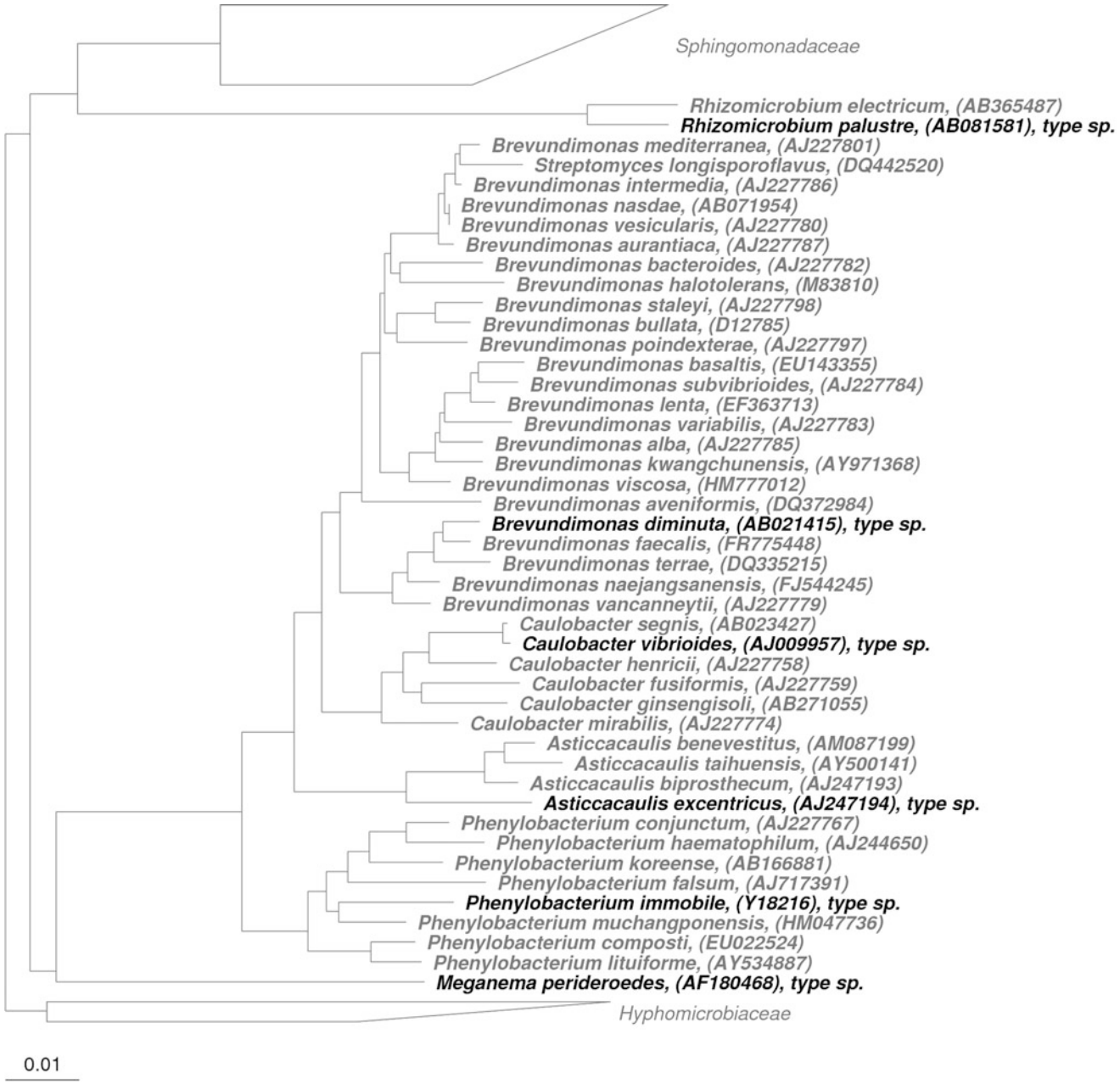

Fig. 7.1

Phylogeny of *Caulobacteraceae* based on their 16S rRNA gene sequences. For comparison, the closest related families are also shown

sequences. This results in DNA-DNA hybridizations below 70 % similarity for strains with otherwise almost identical 16S rRNA gene sequences, as has been reported, e.g., for *Caulobacter vibrioides* and *C. segnis* (Abraham et al. 1999). A similar tendency has been found in such an analysis for *Brevundimonas bacteroides* isolates (Ariskina 1995). *Caulobacteraceae* comprises four different genera, *Caulobacter*, *Brevundimonas*, *Asticcacaulis*, and *Phenylobacterium*, which are well resolved in phylogenetic trees based on the 16S rRNA sequences of their type strains (*Fig. 7.1*).

Whole-Genome Analyses

The completion of the first complete genome of a *Caulobacter* strain was carried out with *Caulobacter crescentus* (= *C. vibrioides*) CB15 in 2001 (Nierman et al. 2001). At that time, the genome of *C. vibrioides* CB15 was the first publicly available from a free-living *Alphaproteobacteria*. The final version of the genome was deposited in the GenBank database (accession no. AE005673 or NC_002696). The complete genome sequence of *C. vibrioides* CB15 was determined to be 4,016,947

base pairs in a single circular chromosome, encoding 3,767 genes. By means of this accomplishment, the genome of the probably most prevalent organism specially adapted to survive in nutrient-poor aquatic and marine habitats was determined and annotated. Consequently, the complete and detailed description of the genetic circuitry at the genome level of the *Caulobacter* cell cycle; which controls the processes of differentiation, growth, and progression of their cycle, established the basis for exploring and understanding the biology of this group of bacteria, at the molecular level. The detailed genome analysis showed that the *C. vibrioides* genome encoded for up to 105 of two-component signaling proteins, a number significantly high. It is well known that these systems, together with DNA methylation, are of interest from the point of view of the regulatory mechanisms implied in cell cycle.

Moreover, through the comprehensive analysis of the annotated genome sequence, numerous gene clusters were pointed out coding for proteins needed for the subsistence of the microorganism in oligotrophic habitats. The repertoire for this environmental survival includes genes for chemotaxis, outer membrane channel function, biodegradation of aromatic compounds, the use of plant-derived carbon sources by microorganisms as growing substrates, and several bacteria sigma factors involved in the regulation of extracytoplasmic functions (termed as ECF or extracytoplasmic functions). This heterogeneous subfamily group of alternative sigma factors is assumed to be involved in the regulation of the gene expression in response to a variety of conditions, including stress. Thus, as a result of the data obtained from the annotated genome analysis, it could be concluded that *C. vibrioides* is well provided with the ability to respond to a wide range of environmental fluctuations.

More recently, the genome of *C. vibrioides* has been used to point out evidences for close relationships between two dimorphic prosthecate bacteria, comparing *C. vibrioides* with *Hyphomonas neptunium* (Badger et al. 2006); and for the establishment of the genetic basis of laboratory adaptation in *C. vibrioides* (Marks et al. 2010). Interestingly, the comparison between the genomes of *H. neptunium* ATCC 15444^{T} and *C. vibrioides* CB15 revealed some unexpected results and found that both strains share more proteins than does *H. neptunium* with the on the basis of 16S rRNA gene phylogeny more closely related species, *Silicibacter pomeroyi*. This fact may perhaps be a demonstration that genome comparison could be an important approach to confirm or reclassify the phylogeny of microorganisms, like the genus *Hyphomonas*, although we have shown that the observed dissimilarity between phylogenies based on the 16S and the 23S rRNA genes was simply caused by the lack of available 23S rRNA gene sequences (see the chapter 12: The Family *Hyphomonadaceae* in this edition). Phylogenetic trees using the currently available sequences of these two rRNAs are consistent and do not show the disparity observed by Badgers et al.

Combining whole-genome sequencing with classical molecular approaches, the genetic basis underlying the multiple derived phenotypes between two closely related *C. vibrioides* strains, CB15 and NA1000, was characterized through genome comparison. Both strains had a common ancestor but have evolved marked phenotypic differences while having been cultured over more than 50 years in the laboratory environment (Marks et al. 2010). *Caulobacter vibrioides* NA1000 was described as a spontaneous mutant of *C. vibrioides* CB15, and is routinely used worldwide because it can easily be synchronized, allowing for studies related to the bacterial cell cycle. The changes detected in that study aiming to determine the mechanisms underlying adaptation to laboratory are related to growth rate, mucosity, adhesion, sedimentation, phage susceptibility, and stationary-phase survival. The work demonstrated that the rapid adaptation of *C. vibrioides* to the laboratory environment occurred in both strains and was characterized by few genetic changes. These changes encompassed non-synonymous mutations, non-coding regulatory changes, the acquisition of new genes, and inactivation of already existing genes, each with a large phenotypic effect (Marks et al. 2010). This study underlines once more the reasons why *C. vibrioides* is an excellent system to study the nature of selection and phenotypic microevolution in prokaryotes during colonization of new environments.

In addition to the whole-genome sequencing projects described above, pointing toward characterization and annotation of the different protein-coding sequences, a remarkable study focusing on the characterization of the essential genome of *C. vibrioides* was performed (Christen et al. 2011). The essential genome of *C. vibrioides* includes not only protein-coding sequences but also structural elements, non-coding RNAs, and regulatory sequences. The study was done basically by applying hypersaturated transposon mutagenesis followed by high-throughput DNA sequencing to determine the transposon insertion sites. The obtained essential genome for *C. vibrioides* was of 492,941 bp in size, covering 12.19 % of its entire genome. This proposed essential genome for *Caulobacter vibrioides* or even for most *Caulobacter* species comprised up to 1,012 features, including 480 open reading frames (ORFs), 402 regulatory elements, and 130 non-coding sequences. Remarkably, 38 % of the essential protein-coding sequences were absent in most species not belonging to the *Alphaproteobacteria* evaluated and 10 % were unique to *Caulobacter*. The results reported in this study included also the identification of those promoter elements essential for the cell-cycle–regulated genes. An interesting conclusion refers to the location of the essential elements, which seems to be preferentially positioned near both origin and terminus of the *Caulobacter* chromosome.

Besides the leading coverage in the literature or in the databases genomic projects of *C. vibrioides* had, other *Caulobacteraceae* genome projects are either completed or running. *Table 7.1* summarizes the information related to genome projects for *Caulobacteraceae* publicly available in databases. For example, the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) is trying to gain further insights into the biological and environmental activities of oligotrophs in general, but *Caulobacter* isolates in particular, through the comparison of genomes of species from each of the major *Caulobacter* habitats. For this reason, and in addition to the genomic DNA sequence already mentioned about *C. vibrioides* CB15, the genomic DNA

Table 7.1
Summary of *Caulobacteraceae* complete genomes publicly available

Organism	Size (Mb)	GC %	Gene	Protein	rRNA	tRNA	RefSeq (NCBI)	Notes
Asticcacaulis biprosthecium C19	5.3	61.1	4,767	4,712	3	41	NZ_ADUH00000000.1	Chromosome
Asticcacaulis excentricus CB 48	2.59	59.2	2,409	2,330	3	22	NC_014816.1	Chromosome 1
	1.32	60.4	1,177	1,121	6	27	NC_014817.1	Chromosome 2
	0.244	59.8	180	172			NC_014818.1	Plasmid 1
							NC_014819.1	Plasmid 2
Brevundimonas sp. BAL3	3.64	67.4	3,520	3,470		50	NZ_ABRU00000000.1	Chromosome
Brevundimonas diminuta ATCC 11568	3.24	67.1	3,056	3,002	3	47	NZ_ADUI00000000.1	Chromosome
Brevundimonas subvibrioides ATCC 15264 (formerly *Caulobacter subvibrioides*)	3.45	68.4	3,393	3,327	6	47	NC_014375.1	Chromosome
Caulobacter sp. K31	5.48	67.5	5,133	5,061	7	49	NC_010338.1	Chromosome 1
	0.234	67.0	212	212			NC_010335.1	Plasmid 1
	0.178	64.3	170	165			NC_010333.1	Plasmid 2
Caulobacter crescentus CB15 (formerly known as *Caulobacter vibrioides*)	4.02	67.2	3,819	3,737	6	51	NC_002696.2	Chromosome
Caulobacter crescentus NA1000	4.04	67.2	3,971	3,877	6	51	NC_011916.1	Chromosome
Caulobacter segnis ATCC 21756^T (Formerly *Mycoplana segnis*)	4.66	67.7	4,325	4,139	6	51	NC_014100.1	Chromosome
Phenylobacterium zucineum HLK1	4.00	71.3	3,579	3,529	3	42	NC_011144.1	Chromosome
	0.383	68.5	327	325			NC_011143.1	Plasmid

sequences of *Maricaulis maris* MCS10 (GenBank accession nº CP000449), a stalked, biofilm-forming marine bacterium of the *Hyphomonadaceae* family, and *Caulobacter* sp. strain K31, a groundwater isolate, were both generated at JGI. Through comparative genome analyses of these microorganisms, it is expected to obtain information related to the specific functions of each, i.e., the pangenome adaptations may be revealed for each natural niche.

The complete list of *Caulobacteraceae* microorganisms for which complete genome sequences currently exist includes *Phenylobacterium zucineum* HLKI which was isolated from the human leukemia cell line K562 (Luo et al. 2008). This facultative intracellular bacterium maintains, unlike other known intracellular pathogens, a stable association with the host cell without affecting the growth and morphology of the latter. The detailed genome analysis revealed the fundamental basis for this strain to invade and persistently survive in human cells. The relationship between *P. zucineum* and *C. vibrioides* was determined on the basis of comparative genome analysis (Luo et al. 2008). Although the genome size and protein number of *P. zucineum* (4.37 Mb, 3,529 proteins respectively) were similar to those of *C. vibrioides* (4.02 Mb, 3,737 proteins), no significant synteny was found between both genomes. The largest synteny block was about 30 kb, coding for 24 proteins. The conservation region with the largest number of proteins was an operon coding for 27 ribosomal proteins. Moreover, both species share only 57.8 % of the orthologous proteins. Probably related to functional elements involved in the efficient response to environmental changes, the genome of *P. zucineum* contains abundant two-component signal transduction proteins, transcriptional regulators, and the largest number of heat shock response proteins when compared with 5 *Alphaproteobacteria* and 93 bacteria (Luo et al. 2008). Interestingly, the *P. zucineum* genome contains also information on features involved in the adaptation of *P. zucineum* to the intracellular lifestyle, like genes crucial for the defense against oxidative stress or the use of protein-bound iron, e.g., siderophore transporter systems, to cope with the low intracellular levels of iron not appropriate to support bacterial life. Finally, the comparative analysis of cell-cycle genes between *C. vibrioides* and *P. zucineum* also indicated that the CtrA regulon (which controls the transcription of 95 genes involved in the cycle) is well conserved, with 93.07 % and 89.88 % amino acid and nucleotide sequence identities respectively.

Other strains of *Caulobacteraceae* currently used for comparative genome analysis include *Brevundimonas diminuta* 470-4, an isolate obtained from the human oral cavity and proposed as reference genome for the Human Microbiome (NCBI BioProject PRJNA67195). The data generated from the whole-genome shotgun assembly of *B. diminuta* 470-4 consists of 168 contigs (GenBank accession nº AMFA00000000.1), comprising 3,506 protein sequences. Similarly, the genomes of a group of uncharacterized isolates from a population of *Caulobacter*, which are clearly distinct from currently recognized species, are sequenced. One of these uncharacterized isolates is *Caulobacter* sp. AP07 (BioProjects at NCBI PRJNA171682 and PRJNA83031); for which a genome assembly of 326 contigs is

available at the GenBank (accession nº AKKF00000000.1). The assembly comprises a genome size of 5.62 Mb, from which 5,311 genes were detected (5,260 protein sequences). The *Caulobacter* sp. AP07 strain has been proposed for Plant-Microbe Interfaces (PMI) projects focusing on the study of the dynamic interface that exists between plants, microbes, and the common environment that they do occupy. Furthermore, *Caulobacter* sp. JGI 0001010-J14 (BioProject PRJNA169806), JGI 0001013-D04 (BioProject PRJNA169803), and JGI 0001013-O16 (BioProject PRJNA169704) have been proposed as grand challenge to perform single cell sequencing at the rhizosphere.

Comparative analyses of the actually existing and the new upcoming genome sequences will not only provide a multitude of insights into the genomic and physiological diversities in the *Caulobacteraceae*, but will also aid researchers in exploring and advancing through new trends on traditional subjects such as the prokaryotic cell cycle, morphogenetic regulatory genes, and systems. Continuing with the acquisition and deeper understanding of the information obtained from the genomes of these organisms is expected to shed light on the potential for practical applications of the *Caulobacteraceae*.

Phenotypic Analyses

All species are chemo-organotrophic and have a respiratory, never a fermentative metabolism. Cells usually possess prostheca, but a number of species do not. The prosthecate cells undergo a remarkable and complex cell cycle. The motile swarmer cells become sessile forming prostheca and attach to surfaces by a holdfast. The cell cycle is completed by binary fission of the prosthecate cell forming again a new swarmer cell. The morphogenesis of *Caulobacteraceae*, especially of *Caulobacter crescentus*, now *Caulobacter vibrioides* (Abraham et al. 1999), has been studied in detail and the main features of the regulation process have been revealed; however, many details are still unclear (Kirkpatrick and Viollier 2012).

The Prosthecum

Most species of the family *Caulobacteraceae* produce cells with filiform extensions of the cell, devoid of reproductive function, the prosthecum (Gr. n. *prosthece*, appendage). The prosthecum does not contain DNA, ribosomes, and cytoplasmic material (Poindexter and Cohen-Bazire 1964). The length of the prosthecum depends on the availability of nutrients but also on phosphate concentration. By growing cells under phosphate limitation, it has been demonstrated that the prosthecum is synthesized at the junction of the prosthecum with the cell (Schmidt and Stanier 1966). The extended prosthecum under phosphate limitation points to a role of the prosthecum as nutrient scavenger (Poindexter 1984). The nutrient transport into the prosthecum has been studied by several groups, and it was found that free glucose accumulated within the prosthecum at a concentration 60–200 times higher than the surrounding concentration. Glucose transport into prostheca was stereospecific for D-glucose and neither methyl α-D-glucopyranoside nor 2-deoxyglucose was transported (Larson and Pate 1976). Amino acids are also actively transported into the prosthecum, and three different transport systems could be identified. The G system transported all 20 amino acids tested, while the P system transported seven amino acids, including proline, and the A system only glutamate and aspartate (Tam and Pate 1985). Two-dimensional gel (2D gel) electrophoresis of the proteome of the prosthecum showed that the prosthecum is mostly free of cytoplasmic proteins and has a protein composition very similar to that of the cell membrane. Several TonB-dependent receptors, two OmpA family proteins, two alkaline phosphatases, 3-phytase and a putative ToIC protein were identified, corroborating the view that the prosthecum plays a role in nutrient uptake (Ireland et al. 2002).

In many *Caulobacter* and *Asticcacaulis* species, crossbands have been observed in the prosthecum. In the case of *Asticcacaulis*, these crossbands consist of a series of concentric rings and can be digested by lysozyme (Schmidt and Swafford 1975). These crossbands differ from the ones found in *Caulobacter*. While the *Brevundimonas bacteroides* and *Caulobacter vibrioides* crossbands are restrictions, undivided septation was detected in *Asticcacaulis excentricus* and *A. biprosthecum* (Poindexter and Hagenzieker 1981). A band it added to the *Caulobacter* prosthecum during each cell cycle allowed the determination of cell age (Poindexter and Staley 1996) and in situ reproductive rate of *Caulobacter* (Poindexter et al. 2000). The cell division protein FtsZ is needed for the formation of these crossbands, and it is also needed for proper prosthecum formation (Divakaruni et al. 2007).

The Holdfast

Many *Caulobacteraceae* species stick to surfaces with the aid of a holdfast. The holdfast sits at the tip of the prosthecum in the prosthecate *Caulobacter*, *Brevundimonas*, and *Phenylobacterium* species, but in *Asticcacaulis* species, the holdfast is located at the cell pole. In *Caulobacter vibrioides*, the holdfast is composed both of protein and polysaccharide. It can be labeled by wheat germ agglutinin lectin, indicating the presence of *N*-acetylglucosamine. It also reacts with chitinase which cleaves β-1 → 4 linkages; therefore, the polysaccharide of the holdfast has some β-1 → 4-linkages (Merker and Smit 1988). Biophysically the holdfast can be seen as a gel-like substance possessing an impressive adhesiveness (Tsang et al. 2006). Such an immense stickiness is important for the ecology of *Caulobacter* and *Brevundimonas* species living, e.g., in fast running creeks (Zhang et al. 2010).

The holdfasts of other *Caulobacteraceae* have been characterized, and they contain a variety of polysaccharides (MacRae and Smit 1991), differing from those of *Maricaulis* species

(Merker and Smit 1988). The holdfasts of *Caulobacter henricii* and *C. vibroides* have α-linked *N*-acetylgalactosamine and *N*-acetylglucosamine while the *Brevundimonas subvibroides* holdfast contains α-linked mannose, α-linked fucose, and *N*-acetylgalactosamine. The holdfasts of all prosthecate *Caulobacteraceae* species examined bind cationic gold, suggesting that the polysaccharides of the holdfast are negatively charged (Merker and Smit 1988). The variation in sugar composition and holdfast structure may cause also differences in the stickiness to various substrata allowing *Caulobacteraceae* to attach to surfaces with different physicochemical properties depending on the specific environment they colonize and minimizing competition. An intriguing phenomenon concerning the selectivity of *Caulobacter* attachment was observed: While the holdfast sticks to a wide variety of materials and surfaces, it never sticks to *Caulobacter* cells. Assumptions that the presence of an S-layer is responsible for this selectivity proved to be wrong because also in S-layer-lacking mutants, no holdfasts were found attached to the cells of such mutants.

The S-layer

Paracrystalline protein surface arrays, S-layers, were identified on many Bacteria and Archaea. Their functions are diverse, e.g., they are virulence factors, protective against predators, a depository for surface-exposed enzymes, shape-determining agents, and crystallization points for mineral formation (Beveridge et al. 1997). More than 30 years ago, proteins arranged in a precisely ordered crystalline array forming an S-layer were found on *C. vibrioides* cells (Smit et al. 1981). They are formed by the RsaA protein with the length of 1,026 amino acids in the case of *C. vibrioides* CB15A which is not cleaved during export (Gilchrist et al. 1992). The extreme N-terminus of the protein directs its export but is not essential for secretion (Bingle et al. 1996). The RsaA protein is secreted by a type I secretion mechanism involving an ABC transporter protein (Awram and Smit 1998) and two outer membrane proteins (Toporowski et al. 2004). For all *Caulobacteraceae* strains forming S-layers, such a type I excretion system has been detected (Iuga et al. 2004). Staining of the S-layer proteins revealed distinct *de novo* biosynthesis at the region of cell division and at the prosthecum during the cell cycle of *C. vibrioides* (Smit and Agabian 1982).

When the S-layer proteins of several cauliform freshwater isolates were extracted by treatment with HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer at pH 2, for most strains, S-layer proteins were found. This method likely disrupted calcium-mediated bonds which were re-established after addition of calcium but not magnesium or strontium, allowing recrystallization of the proteins. From 35 strains out of 42 strains producing an S-layer, a single characteristic protein could be isolated, ranging in size from 100 to 193 kDa. A specific S-layer-associated oligosaccharide was detected in all S-layer-producing strains (Walker et al. 1992; Awram and Smit 2001). However, not in all examined *Caulobacteraceae* isolates S-layer proteins were found, and they were absent in *Brevundimonas poindexterae* FWC40^T, *Brevundimonas staleyi* FWC43^T, (Abraham et al. 2010), *Caulobacter mirabilis* FWC 38^T and *Phenylobacterium conjunctum* FWC21^T (Abraham et al. 2008).

The Shape of the Cell

Cell shape seems to be an important advantage for the survival of *Caulobacteraceae*, also highlighted by a dramatic change in cell shapes at the late stationary phase. Cell shape is controlled in *Caulobacteraceae* as in most rod-shaped bacteria by the murein cluster *mre* (Ingerson-Mahar and Gitai 2012). In *Caulobacter vibrioides*, MreB is an actin-like protein, essential for cell shape during growth (Gitai et al. 2004) and prosthecum elongation (Wagner et al. 2005). Transcript and protein levels of MreB remain constant throughout the cell cycle, but MreB is localized during the cell cycle. MreB forms a cytoplasmic spiral which condenses into a ring at the mid-cell during cell division similar to FtsZ (Lam et al. 2006). MreB maintains its position in the cell by polymerizing new monomers at one end of the polymer and cleaving old monomers from the other end (Kim et al. 2006). Other proteins from the *mre* operon also contribute to cell shape and two of them, MreC and PBP2, interact and form also spirals of polymers which are offset from the MreB spirals. They also play a role in cell wall synthesis (Divakaruni et al. 2005), and MreB and MreD polymers are responsible for the organization of murein biosynthetic enzymes (White et al. 2010). Two different cytoskeletal proteins of the group of bactofilins are also present in *Caulobacter vibrioides*. They form a sheet-like structure covering the membrane around the prosthecate cell pole and mediate polar localization of a peptidoglycan synthase involved in prosthecum morphogenesis again connecting cell shape and cell wall synthesis in *Caulobacter* (Kühn et al. 2010). A model developed from electron cryotomography data on sacculi of *Caulobacter vibrioides* showed that a single layer of linked glycan strands encircles the cell in a disorganized hoop-like fashion. From this model follows that peptidoglycan cannot determine cell shape but requires factors like the cytoskeletal filaments to guide peptidoglycan synthesis and control cell shape (Gan et al. 2008).

Responsible for the curved form of *Caulobacter vibrioides* cells is the protein crescentin, as has been demonstrated in deletion mutants (Ausmees et al. 2003). Crescentin is a remarkable protein because it resembles intermediate filaments only known from metazoa. It is a coiled coil-rich protein with essential domains similar to the tripartite architecture of intermediate filament proteins (Cabeen et al. 2011), it assembles into 10-nm wide filaments without the requirement of cofactors and it forms a higher-order helical structure localized along the concave side of the cell (Ausmees 2006). With the aid of modern superresolution microscopy, the three-dimensional superstructures of the polymers could recently be visualized and confirmed (Lew et al. 2011).

The Life Cycle of *Caulobacteraceae*

The characteristic form of *Caulobacter* cells and their dimorphism led to the recognition of a distinct cell cycle (review by Curtis and Brun 2010). The swarmer cell carries a flagellum and pili at a single pole. This cell is incapable of DNA replication, and its role is dispersal of the strain for which it is motile and possesses chemotaxis. After some time, the flagellar pole of the swarmer cells undergoes differentiation and secretes a polysaccharide adhesin. This adhesin, also called the holdfast, mediates the surface attachment of the cell. The next step in the cell cycle is the loss of the pili and the flagellum and the formation of a prosthecum, a thin extension of the cell envelope. Only this prosthecate cell is able to reproduce, forming swarmer cells which closes the cell cycle (Brown et al. 2009). Their cell cycle, especially their asymmetric cell division, made *Caulobacter* species a model system for bacterial development. Synchronization of the cell cycle to study morphogenesis of *Caulobacteraceae* can easily be achieved by separating swarmer cells from prosthecate cells by centrifugation in a density gradient (Evinger and Agabian 1977). Almost all studies were done with *Caulobacter crescentus* which is synonym to *C. vibrioides* (Abraham et al. 1999).

How is this life cycle coordinated? One key element is the strict control of DNA replication which occurs only once per cell cycle (Marczynski 1999) and that is during the transition of the swarmer cell to the prosthecate cell (Marczynski and Shapiro 1992). Responsible for the control of DNA replication is the transcription factor CtrA. Global transcription analyses of *Caulobacter vibrioides* revealed that the transcription of 553 genes, i.e., 19 % of the genome, varied with the cell cycle. A single regulatory factor, CtrA, is involved in the control of 26 % of these genes; hence, CtrA has been dubbed the master regulator (Laub et al. 2000). CtrA is a response regulator and binds in the phosphorylated form CtrA∼P to 5 sites at the chromosomal origin silencing the origin in swarmer cells (Quon et al. 1998). Interestingly, this function of CtrA is required for the cell cycle in rich media but is not important in minimal media (Bastedo and Marczynski 2009). Nevertheless, CtrA is essential for the cell because it binds to about 95 promoters on the chromosome, regulating their activities (Laub et al. 2002).

A rather complex network is active during the cell cycle to ensure that CtrA is only active when it is needed. The histidine kinases, PlecC and DivJ, are localized in the predivisional cell at opposite cell poles. While DivJ phosphorylates the response regulators DivK and PleD, PleC is a phosphatase for DivK∼P and inhibits the formation of PleD∼P. In the swarmer cell, PleC is localized at the flagellated pole and cleaves DivK∼P which stabilizes CckA and CtrA∼P. When the swarmer cell differentiates to the prosthecate cell, PleC delocalizes and DivJ is synthesized and bound to the prosthecate pole. Here Div J phosphorylates DivK and the formed DivK∼P migrates to the flagellar pole of the predivisional cell where it delocalized CckA, stopping the phosphorylation of CtrA, allowing the initiation of chromosome replication (Lam et al. 2003).

In order to initiate DNA replication, CtrA, resp. CtrA∼P, has to be deactivated. Probably because of the essential role of CtrA, regulation is achieved at several levels, including dephosphorylation, hydrolysis, and repression of transcription. Phosphorylation of CtrA is controlled by CckA, a hybrid histidine kinase/response regulator (Jacobs et al. 2003). CckA is present throughout the entire cell cycle but is not evenly distributed in the cell. It becomes localized to the swarmer cell pole before cell division (Jacobs et al. 1999), a characteristic essential for proper temporal activation of CtrA. Depletion of CckA leads to a decrease of CtrA∼P but also to destabilization of CtrA. To achieve this, CckA uses a phospho-relay by transferring the phosphate to ChpT (Biondi et al. 2006) which transfers it further either to CtrA or CpdR. CdpR is a response regulator and localizes the ATP-dependent protease ClpXP to the prosthecate pole, where phosphorylated CpdR cannot target ClpXP (Iniesta et al. 2006). Here regulation by dephosphorylation and proteolysis of CtrA are connected because ClpXP degrades CtrA (Jenal and Fuchs 1998). The consequence is that when CckA is active, it activates CtrA by phosphorylation and prevents its degradation by inactivating CpdR again by phosphorylation. For DNA replication, initiation CckA is inactivated which leads to a lack of phosphorylation, both of CtrA and of CpdR, and this results in CtrA inactivation, both due to a lack of phosphorylation and proteolysis by CpdR.

It underlines the importance of CtrA that *Caulobacter vibrioides* has a third mechanism of controlling CtrA. This mechanism is based on epigenetics by controlling DNA expression via methylation. In *C. vibrioides* and probably other *Caulobacteraceae*, DNA methylation is performed by the CcrM methylase which is essential for the viability of the cells (Zweiger et al. 1994). CcrM is active in the monomeric form but dimerizes in solution, probably to resist proteolysis by the Lon protease (Shier et al. 2001). CcrM is active only shortly before cytokinesis in the predivisional cell and was found preferentially in the prosthecate cell (Reuter and Shapiro 1987). This is achieved by a spike of *ccrM* transcription which overcomes temporarily the degradation rate of CcrM by Lon and this is also under the control of CtrA (Reisenauer et al. 1999). The *ctrA* gene has the weak P_1 and the strong P_2 promoter (Domian et al. 1999). The promoter P_1 is only active in the hemimethylated form and is regulated by GcrA (Holtzendorff et al. 2004). Therefore, when GcrA is active and the chromosome has been duplicated past the *ctrA* gene, *ctra* expression increases steadily. The formed CtrA∼P represses promoter P_1 and activates the stronger P_2 promoter, leading to a strong burst of CtrA. The high concentration of CtrA is then able to bring CcrM synthesis to a level where it overcomes degradation of CcrM by Lon. The now abundant CcrM methylates the newly synthesized DNA only when CtrA is present which also binds to the replication origin, blocking reinitiation. The outcome of this regulation cascade is that due to the dependence of CcrM on the presence of CtrA, the chromosome is only replicated once per cell cycle.

Before DNA replication is initiated the chromosomal origin is localized at the site where the prosthecate pole of the *C. vibrioides* cell will be located. One copy of a newly replicated locus is immediately segregated to its new location at the future swarmer cell while the other remains in the prosthecum cell section (Viollier et al. 2004). To start replication, the initiator protein DnaA is required. DnaA is an essential replication factor, and 40 genes were shown to be DnaA-dependent (Hottes et al. 2005). DnaA binds to two different types of DnaA boxes, the two G-boxes with moderate affinity and five W-boxes with weak affinities for DnaA. DnaA and CtrA compete for binding at the chromosomal origin and activate or repress replication at different stages of the cell cycle (Taylor et al. 2011). This is achieved by a tight control of DnaA level, being the highest in prosthecate cells (Cheng and Keiler 2009). DnaA activity is dependent on ATP-binding, and as a consequence, hydrolysis of ATP deactivates DnaA. DnaA activates HdaA expression, shutting itself down by promoting the formation of its own inhibitor (Grünenfelder et al. 2001). The replisome-associated protein HdaA stimulates ATPase activity and acts therefore as a DnaA inhibitor (Collier and Shapiro 2009).

Chromosome replication is also regulated at the level of RNA. Here the hybrid tRNA-mRNA, called tmRNA, encoded by the *ssrA* gene, plays a key role. The main function of tmRNA is to release ribosomes that are blocked by mRNAs without a stop codon by adding the SsrA degradation tag at the C-terminal end of these incomplete proteins. One domain of tmRNA is folded into an alanyl-tRNA-like structure and is loaded by alanyl-tRNA synthetase, and its 3′ end does not contain an anticodon but encodes the degradation tag that is added to the C-terminal end of incomplete proteins (Keiler 2007). The small protein SmpB interacts with tmRNA by increasing its stability and interaction with the ribosome. This mechanism is used in *Caulobacter vibrioides* to regulate protein biosynthesis and changes of tmRNA concentrations over the cell cycle (Keiler and Shapiro 2003). It is produced in swarmer and predivisional cells. A burst in transcription of the ssrA promoter leads to high levels of tmRNA during the swarmer to prosthecate cell transition while tmRNA is rapidly degraded in prosthecate cells. The cell-cycle regulation of tmRNA stability is controlled by the degradation by the 3–5′ exoribonuclease RNase R on the one hand and the stabilization by the SmpB protein on the other hand (Hong et al. 2005). RNase R degrades tmRNA, but its level is relatively constant during the cell cycle, indicating that the cell-cycle variation in tmRNA is not caused by the regulation of RNase R expression but by the level of SmpB. SmpB stabilizes and selectively protects tmRNA from degradation by RNase R. SmpB abundance dramatically increases together with tMRNA during the swarmer to prosthecate cell differentiation, and SmpB is rapidly degraded again together with tmRNA in prosthecate cells (Keiler and Shapiro 2003). Therefore, the cell-cycle-dependent fluctuation of tmRNA is mediated by the protective action of SmpB against RNase R degradation. This model has been confirmed by fluorescent in situ hybridization (FISH) of tmRNA over the time course of the cell cycle (Russell and Keiler 2009). A proteomic identification of tmRNA substrates identified 73 proteins that are tagged by tmRNA (Hong et al. 2007).

The formation of the flagellum is also a tightly regulated process. First, the cell has to know where to assemble the flagellum, and for this, it uses the protein TipN. TipN is also called the birth scar protein and is only found at the new poles of both the swarmer and the prosthecate cells (Lam et al. 2006). TipF, a c-di-GMP phosphodiesterase, is then localized to TipN, a process essential for flagellum assembly (Huitema et al. 2006). The synthesis of the flagellum apparatus can be divided into four classes, and the expression of one class of genes requires confirmation of the successful action of proteins from the previous class. Class I genes are those that lead to expression of class II genes dependent on the cell cycle. Class I genes are regulated by CtrA (Dingwall et al. 1992). Class II genes encode the MS ring, switch complex, protein export apparatus, and regulatory proteins sigma 54, FliX, and FlbD. FliX and FlbD form the next important checkpoint in flagellum synthesis. FliX inactivates FlbD by complexation, but after completion of the MS ring, FliX repression of FlbD is relieved by an unknown mechanism (Muir et al. 2005). FlbD activates then transcription of the genes of both class III, comprising P ring, L ring, rod, and hook, and class IV which are the flagellin proteins. At this stage, translation of class IV genes is inhibited by the mRNA-binding protein FlbT (Anderson and Gober 2000), but the mechanism sensing the completion of class III proteins and transducing this information is unknown.

The attachment of *Caulobacter vibrioides* is regulated by a photosensory two-component system, LovK and LovR. LOV stands for light, oxygen, or voltage, because these proteins contain a domain that regulates blue-light-dependent processes. LovK is a histidine kinase and LovR a single-domain response regulator. Maximal transcription of *lovK* and *lovR* occurs during swarmer cell differentiation, when holdfast synthesis is active and loss of pili and flagella occur (Purcell et al. 2007).

Lipids

For decades, the cellular fatty acid composition of bacterial cells has been used as chemotaxonomic marker to compare species. Today a standardized protocol, easy handling, and a large database allow a reliable identification of isolates at genus level and often even at species level. Only fatty acids $C_{16:0}$ and $C_{18:1}\omega7c$ were found in all strains of the *Caulobacteraceae*. *Caulobacter* species contained in addition significant amounts of the unidentified fatty acid ECL 11.798 and fatty acids $C_{14:0}$, $C_{15:0}$, $C_{16:1}\omega7c$, $C_{17:0}$, $C_{17:1}\omega6c$, $C_{17:1}\omega8c$, $C_{18:1}\omega7c$ and 11-methyl-$C_{18:1}\omega5t$. *Brevundimonas* species differed from all other genera of *Caulobacteraceae* by the presence of an unidentified fatty acid at ECL 17.897. Fatty acids present in all *Brevundimonas* strains were $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}\omega7c$, $C_{17:0}$, $C_{17:1}\omega6c$, $C_{17:1}\omega8c$, and $C_{18:1}\omega7c$. *Brevundimonas* strains are differentiated from *Caulobacter* strains by the absence of ECL 11.789 and by the presence of at least traces of ECL 17.897 and by a higher content

of the dominant fatty acid $C_{18:1}\omega7c$ (Abraham et al. 1999). *Phenylobacterium* differed from those of the genus *Caulobacter* by the presence of only trace amounts of $C_{14:0}$ and from the genus *Brevundimonas* by the occurrence of only trace amounts of $C_{14:0}$ and the presence of $C_{12:0}$. 11-Methyl-$C_{18:1}\omega5t$ is present in *Caulobacter* and *Phenylobacterium* species but absent in *Brevundimonas* and *Asticcacaulis* strains. A number of fatty acids, e.g., $C_{14:0}$, $C_{17:0}$, or $C_{17:1}\omega8c$, usually found in species of the other genera are found only infrequently in *Asticcacaulis* species (Vasilyeva et al. 2006).

Especially hydroxy-fatty acids have been found to be valuable biomarkers for the identification of genera. *Caulobacter* and *Asticcacaulis* species (Vasilyeva et al. 2006) contained significant amounts of the fatty acids $C_{12:1}$ 3-OH, which is probably $C_{12:1}\omega6$ 3-OH (Bellmann and Lingens 1985), while the main hydroxy-fatty acid of *Brevundimonas* strains is $C_{12:0}$ 3-OH. From this scheme *Asticcacaulis taihuensis* deviated by considerable amounts of $C_{16:0}$ 2-OH (Liu et al. 2005). Species of the genus *Phenylobacterium* differ from those of the genus *Caulobacter* by a higher amount of $C_{12:0}$ 3-OH and from the genus *Brevundimonas* by the presence of $C_{12:1}\omega6$ 3-OH. In *P. composti*, *P. lituiforme*, and *P. koreense*, $C_{18:1}$ 2-OH has also been detected, a fatty acid not found in any of the other three genera (Weon et al. 2008).

The glycolipids of *Brevundimonas diminuta* have been identified as 1-0-α-D-glucopyranosyl-D-2,3-diacylglycerol, 1-*O*-α-D-glucopyranuronosyl-D-2,3-diacylglycerol, and 1-[0-β-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranuronosyl]-D-2,3-diacylglycerol. One of the phospholipids was phosphatidylglycerol; the other appeared to be a new phosphoglucolipid (Wilkinson 1969). The structure of this novel phosphoglucolipid has then been elucidated, revealing 3-α-D-[6′-(1″,2″-diacyl-sn-glycero-3-phospho)] glucopyranosyl-sn-diacylglycerol (Wilkinson and Bell 1971). From *Brevundimonas bacteroides* NP-105, the same five polar lipids together with 1,2-diacyl-3-α-D-(6′-sulfo)quinovopyranosyl-sn-glycerol have been isolated (Batrakov et al. 1997). This finding of sulfoquinovosyl-diacylglycerols was surprising, because this lipid is widely known to occur in photosynthesizing microbes but is extremely rare in non-photosynthesizing bacteria. Analysis of the polar lipids of *Brevundimonas vesicularis* revealed the same lipids obtained from *B. diminuta* but also a novel lipid identified as 1,2-diacyl-3-*O*-α-D-glycero-D-glucoheptopyranosylglycerol and bearing mainly $C_{18:1}$ and $C_{16:0}$ as major fatty acids (Wilkinson and Galbraith 1979).

These studies revealed often surprisingly high concentrations of glycolipids. Some of these glycolipids are strongly acidic, and it has been speculated that these lipids could be able to function instead of the usual phospholipids. Such a concept has been substantiated by the finding that low concentrations of phosphate lead to a reduction of phospholipids in *Brevundimonas diminuta* (and in many *Hypomonadaceae*) species. This can even lead to a reduction of phospholipids as low as 0.3 % of the polar lipids (Minnikin et al. 1974).

For the described comparison of fatty acid composition, the cellular fatty acids need to be saponified from their respective lipids, proteins or saccharides. This destruction of more complex structures causes a loss of information; therefore, it was suggested that intact polar lipids could be used as biomarkers. By ionization of the polar lipids in a mass spectrometer, isolation of individual molecular ions, and analysis of their fragments, the structure of individual lipids can be elucidated. The fragmentation of the fatty acid at sn-1 of glycerol is different from the one at sn-2, allowing unambiguous assignment of their position at the glycerol moiety. For a number of isolates of the *Caulobacteraceae* and *Hyphomonadaceae*, comparison between cellular fatty acids and polar lipids profiles revealed a better chemotaxonomic resolution of the intact lipids. More than 40 different lipids belonging to the groups of phosphatidyl-, sulfoquinovosyl-, glucoronotaurine amide-, and phosphatidyl-glucopyranosyl-diacylglycerols could be identified. All *Caulobacteraceae* cells contained phosphatidylglycerol and 18:1-16:0-PG was usually the most abundant one. A huge number of different phosphatidyl-glucopyranosyl-diacylglycerols could be identified. This lipid type was present in all *Caulobacter*, *Brevundimonas*, and *Phenylobacterium* species but absent in the strains of the genus *Asticcacaulis* (Abraham et al. 2001). *Caulobacter* strains are characterized by the presence of tetraacyl-3-0-[6′-(sn-glycero-3″-phosphoryl)α-D-glucopyranosyl]-sn-glycerols of the mass 1,411 Da. *Brevundimonas* strains produce the same phosphoglycolipids, but they are characterized by the main lipid of the mass 1,413 Da and homologous compounds of 1,427, 1,439, and 1,453 Da. Both genera showed the phosphoglucolipid with the molecular mass of 1,439 Da (Abraham et al. 1997). Most *Phenylobacterium* species have the phosphoglucolipid of 1,413 Da, characteristic for *Brevundimonas* species, only *P. conjunctum* has the lighter one of 1,411 Da. *Phenylobacterium* strains also have sulfoquinovosyl-diacylglycerols which can also be found in *Brevundimonas* species but not in those of the genus *Caulobacter*. The sulfoquinovosyl-diacylglycerols from *Phenylobacterium* have, however, a fatty acid composition different to the one found in *Brevundimonas* strains (Abraham et al. 2008).

The same analysis applied to the glycolipid fractions of 70 strains of *Caulobacter*, *Brevundimonas* and *Phenylobacterium* revealed as for the phospholipids a high diversity of glycolipids. The four glucolipid types already reported from *Brevundimonas* species were confirmed, and 60 different compounds could be elucidated, 59 of them were novel. It was found that the occurrence of glucosyl-glucuronosyl-lipids was restricted to *Brevundimonas* species but 1,2-diacyl-3-*O*-α-D-glycero-D-glucoheptopyranosylglycerol was often found in *Caulobacter*, *Brevundimonas*, and *Phenylobacterium* strains but absent in *Asticcacaulis* species (Abraham et al. unpublished).

It is also interesting to have a look at the structural variations observed in the phosphoglucolipids because the individual polar lipids are not produced by chance as little variation of these lipids has been observed after *Brevundimonas diminuta* had been grown in five different media (Abraham et al. 1997). All phosphoglucolipids have C18:1ω7*c* at sn-1 of the glycerol of the phosphatidyl part, indicating that this fatty acid is important or even essential for the function of these polar lipids. Saturated

fatty acids, $C_{16:1}\omega7c$ and $C_{17:1}\omega8c$, are exclusively found at sn-2 of the glycerol. The unusual fatty acid 11-methyl-$C_{18:1}\omega5t$ (Abraham et al. 2008) is only found at the glucosidic glycerol. So the observed diversity of these phosphoglucolipids is caused by the variation of the fatty acids at sn-2, indicating that the esters at sn-2 are not as important as those at sn-1 for the still unknown function of these phosphoglucolipids (Abraham et al. 1997).

A number of species have been reported to store energy in poly-hydroxyalkonate droplets, but hydroxy-fatty acids are usually found at the lipid A core of lipopolysaccharides. Structure elucidation of lipid A from *Caulobacter vibrioides* revealed a novel structure comprising the tetrasaccharide backbone alpha-D-galactopyranuronic acid-(1 → 4)-beta-D-2,3-diamino-2,3-dideoxyglucopyranose-(1 → 6)-alpha-D-2,3-diamino-2,3-dideoxyglucopyranose-(1 → 1)-alpha-D-galactopyranuronic acid (alpha-D-GalpA-(1 → 4)-beta-D-DAG-(1 → 6)-alpha-D-DAG-(1 → 1)-alpha-D-GalpA), substituted with six fatty acids. The major lipid A component had 12:0[3-*O*-[3-OH-12:1ω6]] and 12:0[3-*O*-12:1ω6] fatty acids at either the 3′- or the 2′-positions of the distal subunit 2,3-diamino-2,3-dideoxyglucopyranose, and 3-OH-12:0 and 3,6-diOH-12:0 fatty acyl chains at 3- and 2-positions of the reducing 2,3-diamino-2,3-dideoxyglucopyranose, respectively (Smit et al. 2008). The unusual fatty acid 3-OH-12:1ω6 has also been reported from Lipid A of *Phenylobacterium immobile* (Lodowska et al. 2007).

Polyamines

The distribution of polyamines as a chemotaxonomic marker was introduced by Busse and Auling after comparing 50 species of the *Proteobacteria* (Busse and Auling 1988). This analysis has been applied to a number of *Brevundimonas* and "*Brevundimonas*-like" strains which had significant amount of homospermidine or spermidine or both. While *B. diminuta* had spermidine as the main polyamine, *B. vesicularis* had homospermidine and *Phenylobacterium haematophilum* LMG 11050^T had higher amounts of homospermidine than the *B. vesicularis* strains analyzed (Segers et al. 1994). Polyamine analyses of *Caulobacter fusiformis*, *C. vibrioides*, and five *Brevundimonas* species revealed homospermidine as the main compound. In the extracts of *Brevundimonas bullata* and *B. vesicularis*, spermidine was also found (Hamana et al. 2001). The occurrence of two different polyamines as main compounds in *Brevundimonas* is rather exceptional (Hamana et al. 2003), but the polyamine distribution does not allow any differentiation at the species level.

Polysaccharides

Caulobacteraceae are usually members of biofilm communities and contribute to their mechanical stability by the production of exopolysaccharides. From two *Caulobacter vibrioides* strains, CB2A and CB15A, the chemical composition of the exopolysaccharides has been analyzed. Analysis of the monomers revealed that both strains produce unique exopolysaccharides. While one strain CB2A contained D-glucose, D-gulose, and D-fucose in a ratio of 3:1:1, the CB15A polymer contained D-galactose, D-glucose, D-mannose, and D-fucose in a 1:1:1:1 ratio. Further analyses of the CB2A polymer showed the presence of terminal glucose and gulose groups, 3-linked fucosyl, and two 3,4-linked glucosyl units, resulting in a pentasaccharide repeating unit. Contrarily, for strain CB15A, a tetrasaccharide repeating unit consisting of terminal galactose, 4-linked fucosyl, 3-linked glucosyl, and 3,4-linked mannosyl residues was characterized (Ravenscroft et al. 1991). From *Brevundimonas vesicularis* isolated from a paper mill, the exopolysaccharide has been purified and analyzed. Size exclusion chromatography showed a mean molecular weight above 2 MDa. The exopolysaccharide consisted of rhamnose, glucose, galacturonic, and glucuronic acid. After mild hydrolysis of the polymer, a repeating unit could be identified by mass spectrometry and nuclear magnetic resonance. The structure of the repeating unit was novel and elucidated to be → 4)-α-L-glucopyranosyluronate-(1 → 4)-α-D-galactopyranosyluronate-(1 → 4)-β-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl(1 → (= → 4)-α-L-Glc*p*A-(1 → 4)-α-D-Gal*p*A-(1 → 4)-β-L-Rha*p*-(1 → 4)-β-D-Glc*p*(1→). It has been speculated that the variation in sugars, conformation, and linkage type present would limit enzymatic degradation, adding another element of stability to biofilms of *Brevundimonas vesicularis* and probably also other species of *Caulobacteraceae* (Verhoef et al. 2002).

Peptidoglycan

The peptidoglycan of *Caulobacter vibrioides* CB13 has been found to contain only the amino acids alanine, diaminopimelic acid, and glutamic acid in the ratio 2:1:1 (Fujiki et al. 1976). For *Caulobacter vibrioides*, *C. fusiformis* CB27, *Brevundimonas bacteroides* CB11a, and *Asticcacaulis biprosthecum*, a ratio of alanine: glutamic acid: α,ω-diaminopimelic acid: muramic acid: glucosamine was determined to be 2:1:1:1.2:0.8. Such a peptidoglycan is richer in muramic acid than the peptidoglycan of most Gram-negative bacteria. While the ratio of these amino acids does not vary between prosthecate and swarmer cells, the composition of the sugar components does. The prosthecum of *C. vibrioides* was found to be enriched in glucosamine compared to the cell (Poindexter and Hagenzieker 1982). The lysozyme-degradation product of the murein of *Phenylobacterium immobile* was identical to the C6 muropeptide of *E. coli* (Lingens et al. 1985).

Caulobacter Henrici and Johnson 1935, emend. Abraham et al. 1999

The description of the genus *Caulobacter* follows the one given by Abraham et al. 1999. Gram-negative cells, rod-shaped, fusiform, or vibroid, 0.4–0.5 by 1.2 μm. Cells usually possess

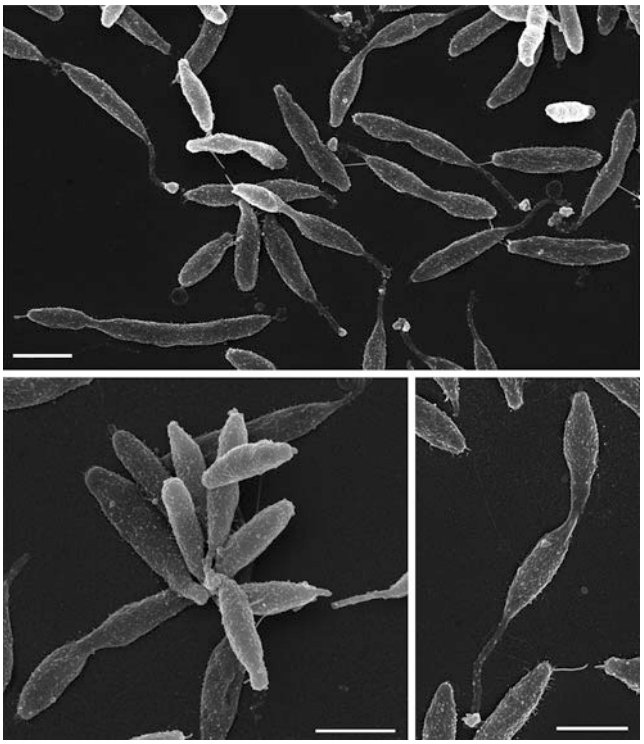

Fig. 7.2
Scanning electron micrographs of *Caulobacter fusiformis* showing the characteristic morphology found in many *Caulobacter*, *Brevundimonas* and *Phenylobacterium* species

Table 7.2
Characteristics useful to discern the following *Caulobacter* species: *1 Caulobacter fusiformis*, *2 C. ginsengisoli*, *3 C. henricii*, *4 C. mirabilis*, *5 C. segnis*, *6 C. vibrioides*

	1	2[a]	3[b]	4[b]	5[b]	6[b]
prosthecum	+	+	+	+	–	+
Utilization of:						
N-Acetyl-glucosamine	–	–	–	+	–	–
L-Aspartate	–	ND	–	+	–	–
D-Glucose	–	–	–	+	–	–
L-Glutamate	–	ND	–	+	–	–
β-Hydroxybutyrate	–	+	–	+	–	–
Lactate	–	–	–	+	–	–
Phenylalanine	–	ND	–	+	–	–
L-Proline	–	–	–	+	–	–
α-L-Rhamnose	–	+	–	+	–	–
Succinate	–	ND	–	+	–	–
G + C %		65.5	ND	68.0	67.5	ND

ND not determined
Data [a]from Liu et al. (2010)
From [b]Abraham et al. (2008)

Table 7.3
Characteristics useful to discern the following *Asticcacaulis* species: *1 Asticcacaulis benevestidus*, *2 A. biprosthecum*, *3 A. excentricus*, *4 A. taihuensis*

	1[a]	2[a]	3[a]	4[b]
No of prostheca	1	(1)-2	1	1
Arabinose	+	–	v	+
Cellobiose	–	ND	ND	+
Mannose	–	–	+	+
D-melibiose	–	ND	ND	+
Sucrose	+	–	+	+
Pyruvate	–	+	+	ND
Optimal temperature [°C]	15–20	30	30	ND
NaCl requirement	–	+	+	ND
G + C [%]	60.4	61	55	59

+ positive, – negative, *ND* not determined
Data from [a]Vasilyeva et al. (2006)
[b]Liu et al. (Liu et al. 2005)

a prostheca, but some species do not (Fig. 7.2). They tolerate only small amounts of NaCl, grow without it, but some grow optimally with 5 g NaCl l^{-1}. No growth with salt concentrations above 20 g l^{-1} was observed. All strains contain significant amounts of the fatty acids 3-OH $C_{12:1}$, $C_{14:0}$, $C_{16:0}$, sum4 (see Table 7.3), sum7 (see Table 7.3), and 11-Me $C_{18:1}\omega5$. All strains, except *C. segnis*, additionally contain ECL 11.798, $C_{15:0}$, $C_{17:0}$, $C_{17:1}\omega6c$, and $C_{17:1}\omega8c$. Polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranuronosyl diacylglycerol, 6-phosphatidyl-α-D-glucopyranosyl diacylglycerol (main mass number 1,411 Da), and phosphatidylglycerol. The GC content is 65.5–68.0 mol%. Type species is *Caulobacter vibrioides*.

The genus comprises currently the species *Caulobacter fusiformis* Poindexter 1964, *C. ginsengisoli* Liu et al. 2010, *C. henricii* Poindexter 1964, *C. mirabilis* Abraham et al. 2008, *C. segnis* (Urakami et al. 1990; Abraham et al. 1999), and *C. vibrioides* Henrici and Johnson 1935. Their distinct characteristics are compared in Table 7.2.

Asticcacaulis Poindexter 1964

The description of the genus is the emended version of the one given by Poindexter (1964) and based on our own studies (Abraham et al. 2001). Unicellular prosthecate bacteria. The prosthecum arises from a site on the cell which is not coincidental with the center of the pole of the cell (Figs. 7.3 and 7.4). The prosthecum does not possess adhesive material. Multiplication occurs by division of the prosthecate cell, giving

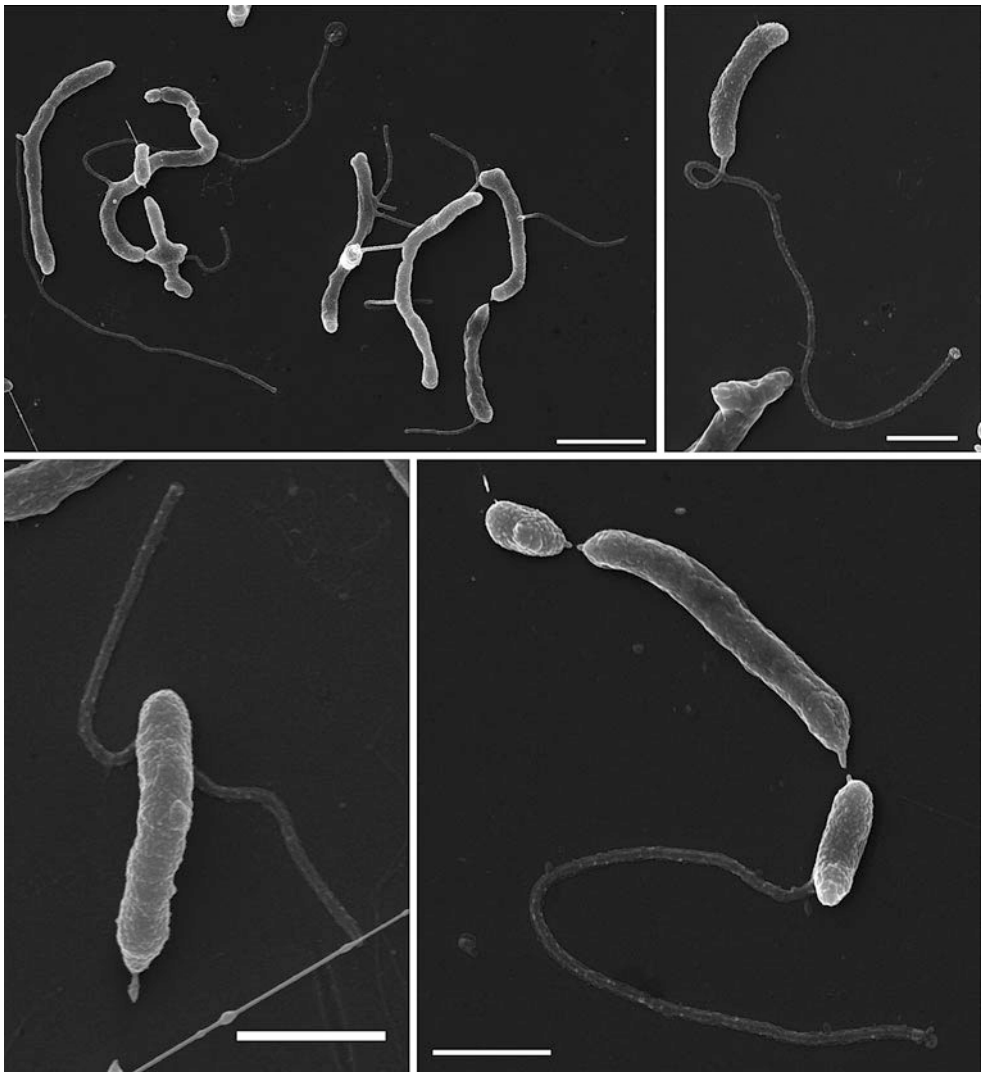

Fig. 7.3
Morphology of *Asticcacaulis biprosthecum* (SEM). Under certain growth conditions, the cells tend to be pleomorphic

rise to a non-prosthecate sibling which is smaller than the prosthecate sibling and is motile by means of a single flagellum which arises in an eccentric position on the pole of the cell. This cell develops a prosthecum and enters the immotile vegetative phase. Adhesive material is secreted by cells in both phases at or near the pole of the cell at a site different from that at which the prosthecum develops. The adhesive material allows cells to attach to a variety of solid substrates. The cells of known types are rod-shaped and colorless. Main hydroxy-fatty acid is 3-OH $C_{12:1}$. Species of *Asticcacaulis* differ from species of *Caulobacter* and *Brevundimonas* by the lack of 1,2-diacyl-3-*O*-[6′-phosphatidyl-*a*-D-glucopyranosyl]glycerol. They also do not contain 1,2-di-*O*-acyl-3-*O*-[D-glucopyranosyl-(1 → 4)-*a*-D-glucuronopyranosyl]-glycerol, which is found in most *Brevundimonas* species but not in strains of the genus *Caulobacter*. The GC content is 55–61 mol%. Type species of the genus is *Asticcacaulis excentricus*.

The genus comprises currently the species *Asticcacaulis benevestitus* (Vasilyeva et al. 2006), *A. biprosthecum* (Pate et al. 1973), *A. excentricus* (Poindexter 1964) and *A. taihuensis* (Liu et al. 2005). Their distinct characteristics are compared in *Table 7.3*.

Phenylobacterium Lingens et al. 1985 emend. Kanso and Patel 2004 emend. Tiago et al. 2005 emend. Zhang et al. 2007 emend. Abraham et al. 2008 emend. Oh and Roh 2012

The description follows the one emended Oh and Roh 2012: Cells stain Gram-negative, are non-spore-forming straight to slightly curved rods, coccobacilli or cocci measuring 0.7–1.0 × 1.0–2.0 μm and occur singly, in pairs, or short chains. Cells of some species have prosthecae. Cells may form rosettes.

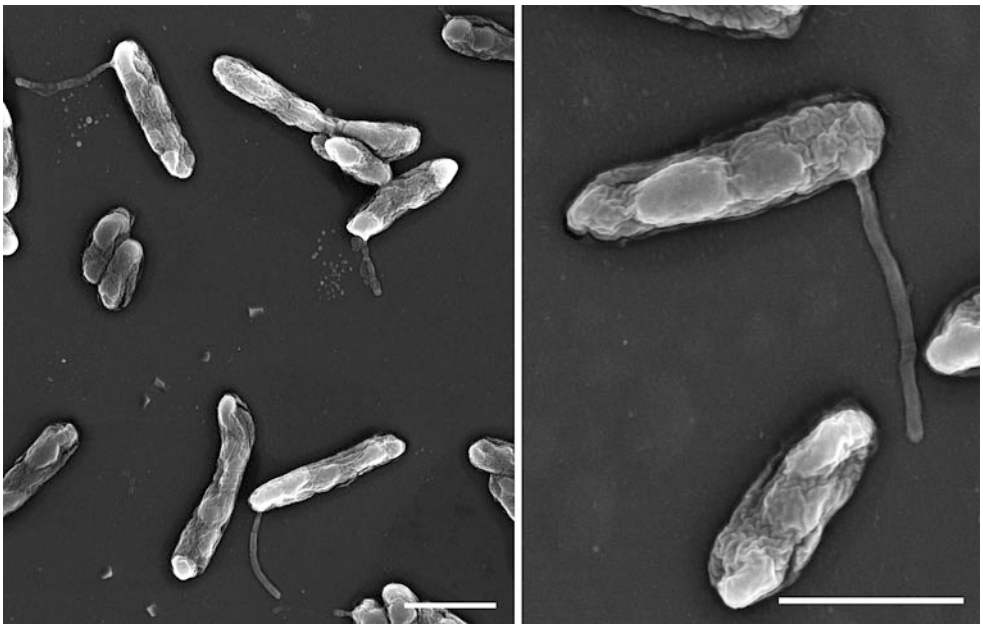

Fig. 7.4
Micrographs of *Asticcacaulis excentricus* showing the eccentric location of the prosthecum

Filamentous cells tend to form in old cultures. Species may be strict aerobes or facultative anaerobes and may be motile or nonmotile. Cells do not form sheaths and are not acid-fast. Species are negative for lipase (C14), α-galactosidase, α-mannosidase, β-glucosidase, and α-fucosidase. Utilization of antipyrine or chloridazon as sole carbon source, reduction of nitrate to nitrite, oxidase activity, and hydrolysis of casein are species dependent. All species of the genus *Phenylobacterium* have 122-G in their 16S rRNA gene sequences, while the other genera of the order *Caulobacterales* genera have 122-A. Furthermore, species of the genus *Phenylobacterium* have a 178-T which is either 178-G or 178-A in the genera *Caulobacter*, *Brevundimonas*, and *Asticcacaulis*. These three genera have a 359-G which is 359-A in all species of the genus *Phenylobacterium*. Main cellular fatty acids are $C_{16:0}$ and $C_{18:1}\omega7$. In contrast with species of the genera *Brevundimonas* and *Caulobacter*, $C_{14:0}$ is present only in traces. Hydroxy-fatty acids are 3-OH $C_{12:0}$ and 3-OH $C_{12:1}$. All species of the genus *Phenylobacterium* have the phospholipids phosphatidylglycerol and 3-O-[6′-(*sn*-glycero-3″-phosphoryl)-α-D-glucopyranosyl]-sn-glycerols. Some species also possess the sulfolipids sulfoquinovosyl-diacylglycerols. The DNA G + C ratio is between 66.5 and 72.3 mol%. Isolated from soil, water, and blood. The type species is *Phenylobacterium immobile*.

The genus comprises currently the species *Phenylobacterium composti* (Weon et al. 2008), *P. conjunctum* (Abraham et al. 2008), *P. falsum* (Tiago et al 2005), *P. haematophilum* (Abraham et al. 2008), *P. immobile* (Lingens et al. 1985), *P. koreense* (Aslam et al. 2005), *P. lituiforme* (Kanso and Patel 2004), and *P. muchangponense* (Oh and Roh 2012). Another species, "*P. zucineum*," has been invalidly described (Zhang et al. 2007), but it is discussed in this chapter because its genome has been sequenced. Characteristics of the species are shown in *Table 7.4*.

Brevundimonas Segers et al. 1994, emend. Abraham et al. 1999

Gram-negative cells, rod-shaped, fusiform, bacteroid, or vibroid, 0.4–0.5 by 1–2 μm. Cells do not usually possess a prostheca (*Fig. 7.5*), but some species do. They grow without NaCl, grow optimally with 5–20 g NaCl l^{-1}, growth is reduced at 30–80 g NaCl l^{-1} depending on the species, and they do not grow with NaCl concentrations above 80 g l^{-1}. Dominant fatty acids for all *Brevundimonas* strains are 3-OH $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, sum4, $C_{17:0}$, $C_{17:1}\omega6c$, $C_{17:1}\omega8c$, and sum7. They are differentiated from *Caulobacter* strains by the absence of ECL 11.789 and significant amounts of 3-OH $C_{12:1}$, by the presence of at least traces of ECL 17.897 and higher amounts of 3-OH $C_{12:0}$, and by a higher content of the dominant fatty acid $C_{18:1}\omega7$. Polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranuronosyl diacylglycerol, 1,2-di-*O*-acyl-3-*O*-[D-glucopyranosyl-(1 → 4)-α-D-glucopyranuronosyl]glycerol, 6-phosphatidyl-α-D-glucopyranosyl diacylglycerol (main mass numbers 1,413 and 1,439 Da), and phosphatidylglycerol. Most strains contain sulfoquinovosyl diacylglycerol. The GC content is 61.0–68.7 mol%. Type species is *Brevundimonas diminuta*; type strain is LMG 2089^T.

The genus *Brevundimonas* is currently the largest genus of the *Caulobacteraceae* comprising the species *Brevundimonas*

Table 7.4

Characteristics useful to discern the following *Phenylobacterium* species: *1 Phenylobacterium composti, 2 P. conjunctum, 3 P. falsum, 4 P. haematophilum, 5 P. immobile, 6 P. koreense, 7 P. lituiforme, 8 P. muchangponense, 9 "P. zucineum"*

	1[c]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]	8[b]	9[a]
Prosthecum	–	+	–	–	–	–	–	–	–
NO_3 reduction	–	–	–	–	–	+	+	–	+
Utilization of:									
Acetate	+	–	ND	–	–	+	+	–	–
N-acetyl-glucosamine	–	–	ND	–	–	–	–	+	–
L-alanine	–	–	+	–	–	–	–	–	–
L-aspartate	ND	–	–	–	–	ND	–	ND	ND
Citrate	–	+	–	+	–	–	+	–	–
Fumarate	ND	ND	ND	ND	+	–	+	ND	ND
D-glucose	–	+	–	+	–	–	+	–	+
L-glutamate	ND	–	+	–	–	ND	–	ND	ND
Glycerol	ND	–	–	–	–	–	+	ND	ND
3-hydroxybutyrate	+	–	–	–	–	+	–	+	+
Lactate	+	–	ND	–	–	–	–	–	–
Malonate	ND	+	ND	–	–	–	–	–	–
Phenylalanine	+	–	–	–	+	+	+	+	+
L-proline	+	–	+	–	–	+	–	+	+
α-L-rhamnose	–	–	–	–	–	–	–	–	–
Succinate	ND	–	+	–	+	–	+	–	ND
Sucrose	–	–	–	–	–	–	+	–	–
Xylose	ND	ND	–	–	–	–	+	–	ND
Dominant OH-FAME	3-OH $C_{12:0}$	3-OH $C_{12:1}$	3-OH $C_{12:1}$	3-OH $C_{12:0}$	3-OH $C_{12:1}$	2-OH $C_{18:1}$, 3-OH $C_{12:1}$	3-OH $C_{12:1}$	2-OH $C_{18:1}$	ND
G + C %	67.5	67	66.9	67.8	67.8	68.1	66.5	72.3	71.2

ND not determined
Data [a]from Abraham et al. (2008)
[b]From Oh and Roh (2012)
[c]From Weon et al. (2008)

alba (Poindexter 1964; Abraham et al 1999), *B. aurantiaca* (Poindexter 1964; Abraham et al. 1999), *B. aveniformis* (Ryu et al. 2007), *B. bacteroides* (Poindexter 1964; Abraham et al. 1999), *B. basaltis* (Choi et al. 2010), *B. bullata* (Gray and Thornton 1928; Kang et al. 2009), *B. diminuta* (Leifson and Hugh 1954; Segers et al. 1994), *B. faecalis* (Scotta et al. 2011), *B. halotolerans* (Abraham et al. 2010), *B. intermedia* (Poindexter 1964; Abraham et al. 1999), *B. kwangchunensis* (Yoon et al. 2006a), *B. lenta* (Yoon et al. 2007), *B. mediterranea* (Fritz et al. 2005), *B. naejangsanensis* (Kang et al. 2009), *B. nasdae* (Li et al. 2004), *B. poindexterae* (Abraham et al. 2010), *B. staleyi* (Abraham et al. 2010), *B. subvibrioides* (Poindexter 1964; Abraham et al. 1999), *B. terrae* (Yoon et al. 2006b), *B. vancanneytii* (Estrela and Abraham 2010), *B. variabilis* (Poindexter 1964; Abraham et al. 1999), *B. vesicularis* (Büsing et al. 1953; Segers et al. 1994), and *B. viscosa* (Wang et al. 2012). The distinct characteristics of these species have been listed in *Table 7.5*.

The 16S rRNA gene sequence DQ442520 submitted for *Streptomyces longisporoflavus* falls in the middle of the *Brevundimonas* phylogenetic tree. It is highly unlikely that spore-forming cells producing such a wealth of antibiotics belong to the genus *Brevundimonas*. Instead it must be assumed that a *Brevundimonas* species contaminating the *Streptomyces longisporoflavus* culture has erroneously been analyzed, and its 16S rRNA gene sequence has been deposited instead of that of the type strain of *Streptomyces longisporoflavus*. A *S. longisporoflavus* cultures properly identified by morphological and biochemical criteria should be analyzed to clarify this contradiction.

Isolation, Enrichment, and Maintenance Procedures

When isolating *Caulobacteraceae*, one has to be aware that cell densities of *Caulobacteraceae* in water and soil samples can be low because of their preferences for oligotrophic habitats, but a number of *Brevundimonas* species have also been isolated from carbon-rich sites. Many *Caulobacteraceae* stick

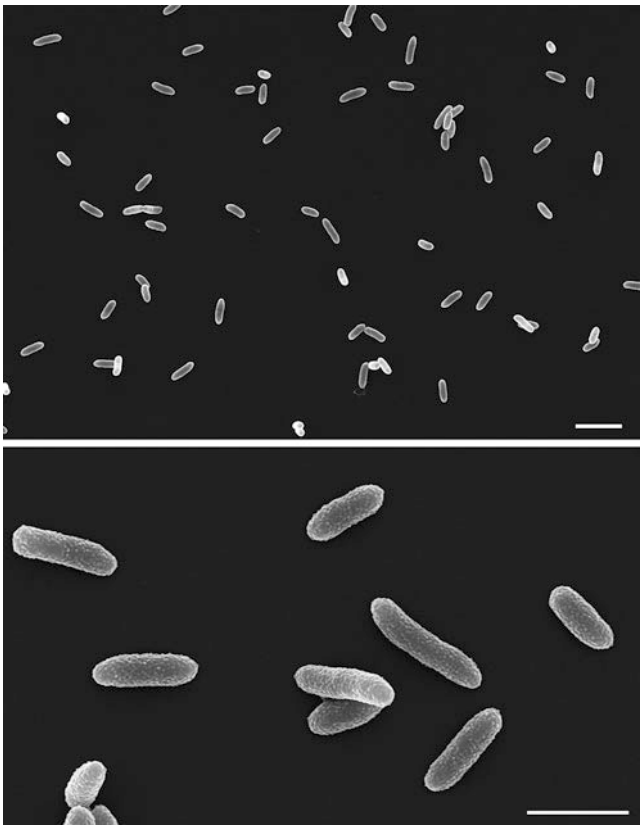

Fig. 7.5
Cells of *Brevundimonas diminuta* do not develop any prostheca. They form one example of prosthecum-less cells found in the family, other examples are *Caulobacter segnis* or *Phenylobacterium immobil*

with their holdfasts to surfaces and these surfaces can be other cells or any other substratum. Therefore, filtering the water samples before plating would lead to a loss of bacteria of many prosthecate *Caulobacteraceae*. The adaptation of the cells to starvation can be used for their isolation. Strains of *Caulobacteraceae* can be isolated from water using a standard dilution-plating method on an oligotrophic medium, e.g., R2A agar, diluted in the ratio 1:10 in tap water (1/10R2A). Media with more than 1 g l^{-1} soluble organic substrates should be avoided for bacteria from oligotrophic sites because it can cause pleomorphic cells. Cells growth is usually slow, and colonies are visible often only after one or even more weeks. Another isolation method is keeping the water sample after addition of 10–30 mg l^{-1} peptone or other carbon source at room temperature for 1–2 months and plate the formed biofilm on diluted agar. One should keep in mind that prosthecate species form clumps, and, hence, these isolates have to be checked carefully for any mixed cultures.

Most strains can be grown in

(i) PYE medium containing 200-mg peptone, 100-mg yeast extract, and 20-mg $MgSO_4 \times 7H_2O$ in 1 l autoclaved tap or deionized water (Poindexter 1964) or

(ii) PCa medium: 200-mg peptone, 20-mg $MgSO_4 \times 7H_2O$, 15-mg $CaCl_2 \times 2\ H_2O$ in 1 l autoclaved tap or deionized water (Poindexter 2006).

The great majority of isolates of the family *Caulobacteraceae* require one or more organic growth factors. For a few such strains, growth in defined media is supported by one or more B vitamins (Larson and Pate 1975; Poindexter 1964), but for most isolates, the requirements have not been determined. Significant stalk elongation of *Caulobacter vibrioides* and *Asticcacaulis excentricus* relative to cell elongation has been observed when growth is phosphate-limited (Poindexter 1981). One consequence of the lack of defined media for the majority of *Caulobacteraceae* isolates is that most studies focus on *Caulobacter vibrioides* where defined media for growth are known.

Most *Caulobacter vibrioides* isolates grow in the defined HiGg medium containing 5–10 mM glucose, 5–10 mM monosodium glutamate (filter sterilized), 0.1–1 mM KH_2PO_4, 5–10 mM NH_4Cl, and 5 mM imidazole and Hutner's mineral base (Cohen-Bazire et al. 1957; Poindexter 1978). Another minimal medium is the M2 medium: 20 mM sodium phosphate and potassium phosphate, 9.3 mM NH_4Cl, 0.5 mM $MgSO_4$, 0.5 mM $CaCl_2$, 0.01 mM $FeSO_4$, 0.008 mM ethylenediaminetetraacetic acid (Johnson and Ely 1977).

A definite medium, MS-B-AA medium, for the cultivation of *Asticcacaulis biprosthecum*, has been described: 0.1 % glucose, alanine, glutamate, serine, proline and aspartate 100 mg L-1 each, 0.075 mM $(NH_4)_2HPO_4$, 0.5 mM $NaH_2PO_4 \times H_2O$, 0.5 mM KH_2PO_4, 0.01 % $MgSO_4 \times 7H_2O$, 0.01 % sodium citrate, 4 µg l-1 D-biotin and 10-mg $CaCl_2 \times 2H_2O$, 10 mg $FeSO_4 \times 7H_2O$, 10 mg $MnSO_4 \times H_2O$, 10-mg $ZnSO_4 \times 7H_2O$, 1-mg $CuSO_4$, 1-mg $CoCl_2 \times 6H_2O$, 1-mg $K_2B_4O_7 \times 4H_2O$, and 1-mg MoO_3, per liter trace minerals (Larson and Pate 1975).

Caulobacteraceae are generally cultivated aerobically, but *Asticcacaulis biprosthecum* seems to be here an exception. Its growth was delayed and yield was reduced when vigorously aerated, but it grew when incubated under air without agitation. In fermenter cultures, it grew in the presence of 6.0–6.7 mg of 0_2 per liter, but not at levels greater than 6.7 mg l^{-1} (90 % saturation). Thus, *A. biprosthecum* appears to be an O_2-sensitive oxybiontic organism (Larson and Pate 1975; Poindexter 1981).

Caulobacteraceae are usually grown at 27 °C or 30 °C. Most species can grow well between 20 °C and 40 °C. Some species show reasonable growth at 10 °C, very few grow slowly at 4 °C (especially *Brevundimonas* isolates from cold habitats) and none at 50 °C (Abraham unpublished). The pH range for growth is around neutrality. Most strains of *Caulobacter* and *Brevundimonas* differ by their salt tolerances. While all isolates can grow with no sodium chloride, 0.5 g l^{-1} usually leads to better growth. While most *Caulobacter* species can tolerate 10 g l^{-1} of NaCl, most *Brevundimonas* species can grow well at 35 g l^{-1} and some even with 60 g l^{-1} (usually isolates from brackish or sea water). No growth with 80 g l^{-1} sodium chloride was observed (Abraham et al. 1999).

Table 7.5

Characteristics useful to discern the following *Brevundimonas* species: *1 Brevundimonas alba, 2 B. aurantiaca, 3 B. aveniformis, 4 B. bacteroides, 5 B. basaltis, 6 B. bullata, 7 B. diminuta, 8 B. faecalis, 9 B. halotolerans. 10 B. intermedia, 11 B. kwangchunensis. 12 B. lenta, 13 B. mediterranea, 14 B. naejangsanensis, 15 B. nasdae, 16 B. poindexterae, 17 B. staleyi, 18 B. subvibrioides, 19 B. terrae, 20 B. vancanneytii, 21 B. variabilis, 22 B. vesicularis, 23 B. viscosa*

	1[a]	2[a]	3b	4[a]	5c	6[a]	7[a]	8d	9e	10[a]	11[a]	12f	13[a]	14 g	15[a]	16e	17e	18[a]	19[a]	20 h	21[a]	22[a]	23 k
Prosthecum	+	+	+	+	–	–	–	–	+	+	–	–	–	–	–	+	+	+	–	–	+	–	–
Beta-galactosidase	ND	–	–	ND	–	ND	–	–	–	+	–	ND	–	–	+	–	+	ND	–	–	ND	–	–
NO_3 reduction	–	–	+	v	ND	–	–	–	+	–	–	–	–	ND	–	–	–	–	–	–	–	–	–
Hydrolysis																							
Aesculin	ND	+	–	ND	+	–	–	ND	+	+	+	ND	+	–	+	+	+	ND	–	–	ND	+	+
Starch	–	+	–	+	ND	–	–	ND	–	+	–	ND	ND	–	–	–	–	+	–	ND	v	–	ND
Biology																							
Cellobiose	ND	+	–	+	–	+	–	–	–	+	+	–	+	–	+	+	+	+	–	–	ND	+	–
D-galactose	+	–	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–	+	+	+	–
D-glucose	+	+	–	+	–	+	–	–	–	+	+	–	+	–	+	+	+	+	–	–	+	+	+
Maltose	+	+	–	+	–	+	–	–	–	+	+	–	+	–	+	+	–	–	–	+	+	+	–
Acetate	+	–	–	–	–	+	+	–	–	+	–	–	+	+	+	–	–	–	–	+	+	+	–
Lactate	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	+	–	–	+	–	+	–
Propionate	ND	–	ND	–	–	+	–	+	–	–	–	–	–	+	+	–	–	–	+	+	ND	–	–
Succinate	+	–	+	–	–	+	–	–	–	+	v	–	+	ND	+	+	+	–	–	+	+	+	–
L-aspartate	+	–	+	–	+	+	+	+	–	–	v	–	–	ND	+	–	–	–	+	+	–	–	–
L-glutamate	+	+	ND	+	–	+	+	+	–	+	v	+	+	+	+	+	+	+	+	+	+	+	–
L-leucine	v	–	–	–	–	+	–	+	–	–	–	–	–	+	+	–	–	–	+	–	+	–	–
L-proline	v	–	+	–	–	+	+	+	–	–	v	+	–	+	+	+	+	–	+	–	+	+	+
L-threonine	ND	–	ND	–	–	+	–	+	–	–	+	–	+	+	+	–	–	–	+	+	ND	+	–
OH-FAME	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $iC_{17:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$	3-OH $C_{12:0}$
G + C %	nd	65.6	64.1	66	66.3	67	67	65.1	64.6	66.1	68.5	68.7	67.3	67.0	66.5	67.0	66.5	67	61.8	66.3	61.0	65.5	66.7

ND not determined, *v* variable
[a]From Yoon et al. (2006b)
[b]From Ryu et al. (2007)
[c]From Choi et al. (2010)
[d]From Scotta et al. (2012)
[e]From Abraham et al. (2010)
[f]From Yoon et al. (2007)
[g]From Kang et al. (2009)
[h]From Estrela and Abraham (2010)
[k]From Wang et al. (2012)

For long time storage, most strains can be preserved in glycerol suspension (10 %, v/v) at −75 °C. Vegetative stocks should be maintained on 1 % 1/10 R2A agar slants at 4 °C and transferred every 2 or 3 months, incubated 2 or 3 days at room temperature then kept at 4 °C. For most strains, lyophilization is another option.

Ecology

The physiology and cell cycle of *Caulobacteraceae* demonstrate their excellent adaptation to oligotrophic habitats. This also includes motility, and it has been found that *Caulobacter vibrioides* swims about 10 times more efficiently than *E. coli* cells (Li and Tang 2006). Consequently, members of the genera *Caulobacter* and *Brevundimonas* have been reported from a wide variety of aquatic habitats (Poindexter 1981b; Ariskina et al. 2011). These habitats are mainly freshwater habitats and only few species of *Brevundimonas* have been found to thrive in brackish or even sea water (Abraham et al. 2010). Furthermore, *Brevundimonas*, *Asticcacaulis*, and *Phenylobacterium* species have also been isolated from other habitats, e.g., soil, polluted sites, ice, or even from humans. *Brevundimonas* sp. CCM 4020 comes from necrotic lesions of the epidermis of the plant *Cyprinus carpio*. Due to their excellent adaptation to oligotrophic habitats, the formation of biofilms and their ability to withstand long periods of fame *Caulobacter* species have also been found in drinking water reservoirs and even in bottled mineral drinking water (González et al. 1987). *Brevundimonas* species have been isolated from Antarctic ice cores, but it remained open whether these were indigenous bacteria or cells which have been transported by wind-blown dust into the ice (Antony et al. 2012). A *Brevundimonas* species, closely resembling *B. diminuta* but being able to grow at 5 °C, has been isolated from the water outlet of the Mont Cook Glacier, New Zealand (Abraham, unpublished). Psychrotolerance, however, is not limited to species of the genus *Brevundimonas* because a psychrotolerant *Caulobacter* strain Z-0024 has been isolated and characterized from tundra soil. It uses a wider range of organic substrates and can tolerate sodium chloride concentrations of up to 15 g l^{-1}. DNA-DNA hybridizations between strain Z-0024 and *C. vibrioides* ATCC 15252 gave only 24 % similarity; therefore, strain Z-0024 has been described as *C. tundrae*. However, the description remained invalid (Berestovskaia et al. 2006).

Brevundimonas species have also been found associated with the crude oil but not in the water at the Enermark oil field, Medicine Hat, Canada (Kryachko et al. 2012). A study on the metabolic functions of lake snow aggregates identified *Brevundimonas* species as one of the main players in the degradation of the aggregates and the release of amino acids to be consumed by other microbes (Schweitzer et al. 2001). When dry river sediments of a river in Italy was rewetted which may happen after heavy rain, *Caulobacter* and *Brevundimonas* species were among the first colonizers of the river water, demonstrating again their ability to form biofilms and to survive harsh times (Fazi et al. 2008). The ability of *Caulobacteraceae* to grow in high carbon environment and to tolerate several toxic compounds is also reflected in their occurrence in wastewater treatment systems. Here mainly *Brevundimonas* species but also some *Caulobacter* species have been isolated and characterized (MacRae and Smit 1991). In a polluted river in U.S.A., *Caulobacter* and *Asticcacaulis* species have been detected by both direct and viable counting. No significant correlation between the number of prosthecate bacteria and total coliforms in the stream could be established. During the coldest period, when the total viable counts decreased, the caulobacters increased in numbers (Staley 1971).

Prosthecate members of the *Caulobacteraceae* have mainly a sessile lifestyle sticking with their holdfasts to interfaces. Here they form monolayers where the cell densities are controlled by the availability of nutrients at the respective habitat. In flow chamber experiments, *Caulobacter vibrioides* CB15 formed also large mushroom-shaped structures. For the formation of these mushroom-like aggregates, pili are essential as pili-less mutants were not able to form these aggregates (Entcheva-Dimitrov and Spormann 2004). The ecological consequences of this finding remain to be seen, and one has to keep in mind that flow-chambers are model systems and do not always mirror conditions usually found in the environment.

Forming a biofilm, however, is not a one-way process and cells should also be able under deteriorating growth conditions to escape the biofilm community, revert to the planktonic state, and move to more favorable habitats, forming there new biofilm communities. We only just started to uncover the diverse mechanisms of bacteria for their reconversion to planktonic cells. Here *Caulobacter vibrioides* seems to have a novel and rather unique way: How to force its offsprings to alternative habitats. In many biofilms, considerable amounts of extracellular DNA (eDNA) have been detected. It seems to be produced by the autolysis of cells in the biofilm. Mechanisms by which eDNA is released are diverse and comprise, e.g., lysis of a bacterial subpopulation under the control of quorum sensing systems in *Pseudomonas aeruginosa* or autolysis by programmed cell death similar to apoptosis of eukaryotic cells in some form of an altruistic suicide in *Staphylococcus aureus* (Montanaro et al. 2011). The role of eDNA in the biofilm is not yet very clear, but it seems to contribute to its stability because biofilms can be inhibited or even dispersed by the addition of DNase I. eDNA does not seem to have such a stabilizing effect in *Caulobacter* biofilms because addition of DNase I has no effect on biofilm formation. For *Caulobacter vibrioides*, eDNA, however, has been shown to prevent the settling of swarmer cells on these biofilm communities. This is achieved by the binding of eDNA to the holdfast of swarmer cells during their transition to become sessile. The binding is very specific and only DNA from *Caulobacter* strains was able to block completely the adhesive material of the holdfast while eDNA from the closely related *Brevundimonas diminuta* was much less efficient. The mechanism for the species-specificity of the eDNA for blocking the

attachment of swarmer cells is still unknown, and several hypotheses have been discussed. While eDNA blocks holdfasts of swarmer cells, it had no effect on those of prosthecate cells, thus acting specifically on new born cells. By targeting this specific cell type, *Caulobacter* species in biofilm communities promote dispersal of their own kin, leaving the existing biofilm undisturbed and undissolved (Berne et al. 2010). This minimization of intraspecies competition is in line with the oligotrophic lifestyle of *C. vibrioides*, and it remains to be determined whether a similar mechanism is also working in other species of the *Caulobacteraceae*.

Stress Response

As most *Caulobacteraceae* thrive in oligotrophic habitats, not only their unusual cell cycle is optimized for living in such environment, they also bear a number of mechanisms allowing them to deal with the shortage of nutrient. One important goal under carbon starvation is to preserve energy, and this means reducing growth and replication. A complex network of regulons has been unraveled from *Caulobacter vibrioides*, leading to the uncoupling of the transition from the swarmer to the prosthecate cell under carbon starvation (Britos et al. 2011). Several small non-coding regulatory RNAs (sRNAs) play a key role in the posttranscriptional regulation of many genes under substrate limitation. One sRNA, specifically induced upon carbon starvation, is CrfA which strongly activates 27 genes, one-third of them are TonB-dependent receptors (Landt et al. 2010). TonB-dependent receptors are used for the uptake of compounds by active transport which are too large for uptake by simple passive diffusion. These receptors are anchored in the outer membrane and are also energy-dependent using a proton gradient. The transport process requires energy and a complex of three inner membrane proteins, TonB-ExbB-ExbD, to transduce this energy to the outer membrane. The transported substrates range in complexity from simple small molecules such as citrate to large proteins; however, the transport mechanism is still unclear (Noinaj et al. 2010). According to their oligotrophic habitats, the known genomes of *Caulobacteraceae* contain a large number of TonB-dependent receptors, e.g., *Caulobacter vibrioides* has 63 (Nierman et al. 2001).

The oligotrophic habitats of *Caulobacteraceae* may have also other limitations, e.g., that of phosphate or iron. *Caulobacter vibrioides* was shown to deal with such shortage of phosphate by the production of the outer membrane–bound lipoprotein, ElpS, which is further cleaved and released in the medium in a T2SS-dependent manner. Together with ElpS, an alkaline phosphatase is exported to enable the cells to gather inorganic phosphates (Le Blastier et al. 2010). In *Caulobacter vibrioides*, as in most other bacteria, the ferric uptake regulator, Fur, controls iron homeostasis. Genes involved in iron uptake are repressed by iron-Fur but induced under conditions of iron limitation, and genes of iron-using proteins are activated by Fur under conditions of iron availability. Contrary to a number of other bacteria, several genes are directly regulated by Fur in *C. vibrioides* and not via small RNAs. In *C. vibrioides*, Fur integrates iron metabolism and oxidative stress response (da Silva Neto et al. 2009).

When *Caulobacter vibrioides* is exposed to oxygen stress but also to cadmium, rpoE is expressed which is otherwise repressed by ChrR. The transcription of rpoE is achieved by the aid of sigma(E), a member of the ECF (extracytoplasmic function) subfamily of RNA polymerase sigma factors, which regulate gene expression in response to environmental stresses. Mutagenesis revealed that the amino acid C188 in ChrR is critical for the cadmium response, while amino acids H140 and H142 are required for oxidative stress (Lourenço and Gomes 2009). This, however, is only one aspect of the reactions of the cells to metal stress. High concentrations of uranium are simply reduced by the formation of extracellular calcium-uranium-phosphate precipitates. Analysis of whole-genome expression activities under metal stress showed that intracellular metal concentrations are reduced by several efflux pumps, shutdown of a sulfate transporter to reduce chromium uptake, and upregulation of genes involved in oxidative stress, e.g., superoxide dismutase SodA, DNA repair enzymes, etc. (Hu et al. 2005).

Predators

With the intensified study of *Caulobacteraceae*, bacteriophages were discovered infecting this group of bacteria. From sewage, the *Caulobacter vibrioides* CB13B1a bacteriophage Cd1 was isolated and found to infect both prosthecate and swarmer cells. ΦCd1 is an icosahedral DNA-phage with a short noncontractile tail (West et al. 1976). However, the DNA-containing *Caulobacter* phage phi 6 revealed different infection behaviors, attaching to pili more frequently than to flagella. As a consequence pilus-less mutants of *C. vibrioides* were resistant to its infection while flagellum-less mutants with pili were susceptible (Scholl and Jollick 1980).

Bacteriophages are not only known for *Caulobacter* species, and three phages have been isolated and characterized which were specific for *Asticcacaulis biprosthecium*. These phages with elongated cylindrical heads and noncontractile tails showed different ways of attachment to their host. Phage ΦAcM4 attached to the flagellum with the region where phage head and the tail joins which leaves the distal end of the tail free for attachment to the *Asticcacaulis* cell. Phage ΦAcM2 had a different attachment site which was at the pili and the pole of the cell by the phage tail. The third phage showed both characteristics of attachment (Pate et al. 1979). Flagellotropic phages have also been isolated from *Caulobacter* cultures (Schmidt 1966). Closer inspection of the mechanism using cryo-electron microscopy revealed that the phage attached through a filament on the phage head first with the bacterial flagellum. This results in concentration of phage particles around the pilus portals of the bacterial cell. Such a mechanism increases the likelihood of infection (Guerrero-Ferreira et al. 2011).

From nine *Brevundimonas vesicularis* isolates, seven phages were isolated and genetically characterized. All phages contained linear double-stranded DNA of a size of about 37 kb. Three phages belonged to the family *Siphoviridae* and four to the *Podoviridae* (Beilstein and Dreiseikelmann 2006). Bacteriophages of the phiCbK type, infecting *Caulobacter* species, are much larger than the ones described from *Brevundimonas*. They have 205–280 kb encoding for 318–448 proteins. Interestingly, 5 of the 6 sequenced phages possessed homologues of the *C. vibrioides* cell-cycle regulator GcrA (Gill et al. 2012). Such a homolog was also reported from another genome of a phiCbk phage (Panis et al. 2012). Such homologues may allow the phages to influence the replication status of the host cell, optimizing the condition for infection.

The paracrystalline surface (S) layer is assumed to function in the protection of the cell against predators, and it has been demonstrated that the presence of an S-layer protected *Caulobacter vibrioides* against lysis by *Bdellovibrio bacteriovorus* (Koval and Hynes 1991). However, this seems not to be the case for the predator *Bdellovibrio exovorus* (Koval et al. 2013). Pathogens however have learned to deal with such an obstacle and the bacteriophage ΦCr30 even became dependent on the presence of such an S-layer to achieve infections of *Caulobacter* cells. When *rsaA*, the gene for the S-layer protein, is deleted or mutated, *Caulobacter vibrioides* cells became resistant against this phage and susceptible again when *rsaA* was introduced via a plasmid (Edwards and Smit 1991).

Pathogenicity: Clinical Relevance

In the clinics, the ordinary microbiological analyses focus on the identification of known pathogens. Only in the last decade, it was noticed that beside these well known pathogens, a number of other bacterial species live in biofilms in the human body. Assessment both by cultivation and culture-independent methods of the phylogenetic diversity of bacteria colonizing prosthetic hip joints revealed *Brevundimonas* species both on hip joints, revealing clinical infections and asymptomatic ones (Dempsey et al. 2007). Deep-sequencing of the microbiota of the ocular surfaces of healthy volunteers revealed also highly diverse microbial communities with high intersubject variability. However, in all samples, species of *Brevundimonas* could be detected as they are members of the "core" communities in this habitat (Dong et al. 2011). Assessment of a very different habitat of the human body, aortic aneurysms, by molecular techniques detected *Brevundimonas diminuta* as members of the biofilm communities (Marques da Silva et al. 2006). The specific roles of the individual members in these biofilm communities are largely unknown, and the mere detection of *Brevundimonas* species does not implicate their role in infection processes.

Caulobacteraceae, however, play a role in infections. There was only one report of a *Caulobacter* infection in humans (Justesen et al. 2007), but a number of infections are known caused by *Brevundimonas* species. A case study of bacteremia caused by *Brevundimonas* in mostly immunocompromised patients in a hospital in Taiwan identified *Brevundimonas vesicularis* as the main pathogen (63 %), followed by *B. nasdae* and *B. diminuta*. The 30-day mortality rate was found to be 17 % and the pathogens were resistant against ciprofloxacin and colistin but susceptible to piperacillin-tazobactam and amikacin (Lee et al. 2011). *B. vesicularis* infections were also reported from neonates and could be treated with the same antibiotics (Karadag et al. 2012). These infections seem to be not limited to immunocompromised patients but were also found in immunocompetent persons (Chi et al. 2004). *Brevundimonas vesicularis* infections have also been observed in infective endocarditis (Yang et al. 2006), septic arthritis (Sofer et al. 2007), CAPD peritonitis (Choi et al. 2006), keratitis (Pelletier et al. 2010; Pandit 2012), nosocomial meningitis (Mondello et al. 2006), cutaneous infection (Panasiti et al. 2008), cystic fibrosis patient (Menuet et al. 2008), and liver abscess (Yoo et al. 2012). *Brevundimonas vancanneytii* has been isolated from blood of a patient with endocarditis and may also function as human pathogen (Estrela and Abraham 2010).

All bacteria in the human body are subject to occasional antibiotic exposure during treatment of infections. As a consequence, antibiotic resistances have also been noticed in *Brevundimonas* species. A study of clinical *B. vesicularis* isolates collected over 20 years in a hospital in Taiwan showed that 90.9 % of the isolates were susceptible to cefpirome, imipenem, and piperacillin-tazobactam; 86.4 % to gentamicin, amikacin, and ciprofloxacin; 63.6 % to ceftazidime, but only 59.1 % were susceptible to trimethoprim-sulfamethoxazole (TMP-SMX) (Zhang et al. 2012). *Brevundimonas diminuta* isolates carrying metallo-β-lactamase genes have been reported including a clinical isolate carrying the VIM-2 metallo-β-lactamase gene and protecting the isolate against carbapenem (Almuzara et al. 2012). *B. diminuta* from cancer patients were all resistant against multiple fluoroquinolones, and further examination of these isolates led to the conclusion that *B. diminuta* is intrinsically resistant to fluoroquinolones (Han and Andrade 2005). From the sewage of a hospital in Palma de Mallorca, three *Brevundimonas* strains were isolated which were resistant against the antibiotic ceftazidime. Two isolates belonged to *B. diminuta* and one to a novel species, described as *Brevundimonas faecalis*. *B. faecalis* was not shown to be a human pathogen, but it may act as a reservoir for antibiotic resistance in hospitals (Scotta et al. 2011).

Phenylobacterium species, besides species of the genera *Caulobacter* and *Brevundimonas*, were also connected to infections in humans. *Phenylobacterium haematophilum* was isolated from human blood (Abraham et al. 2008) and *P. zucineum* from a human erythroleukemia cell (Zhang et al. 2007) which may indicate their role as human commensals or pathogens. From granulomatous lesions of a patient with myelodysplastic syndrome, a *Phenylobacterium* species has been isolated as causative agent for inflammation, proving that some *Phenylobacterium* species can cause disease (Zhu et al. 2010). These reports connect three of the four *Caulobacteraceae* genera with human infections, leaving *Asticcacaulis* as the sole genus of this family with no clinical reports.

Application

Biodegradation and Biotransformation

The type species of *Phenylobacterium*, *P. immobile*, was isolated as a degrader of the herbicide chloridazon (Lingens et al. 1985). This gave species of the genus *Phenylobacterium* the character of degraders of xenobiotics. This notion has been confirmed by more reports on the involvement of *Phenylobacterium* species in degradation processes. A degrader of linear alkylbenzene sulfonate has been isolated which is mobile and can also use the xenobiotic compounds chloridazon and antipyrine as sole carbon sources. The 16S rRNA gene sequence similarity to its nearest neighbor *Phenylobacterium immobile* was 97.49 %, but the DNA-DNA relatedness was only 40 %; therefore, the species has been identified as a new member of the genus and invalidly named *Phenylobacterium mobile* GZ6T (Ke et al. 2003). Using stable isotope labeling, *Phenylobacterium* species have also been identified as one of the main degraders of the wood preservative Cu-HDO, containing the chelation product of copper and *N*-cyclohexyldiazenium dioxide (HDO) (Jakobs-Schönwandt et al. 2010).

More important for biotechnological application than *Phenylobacterium* species were *Brevundimonas* species. An isolate most closely related to *Brevundimonas mediterranea* was found to degrade fenamiphos (ethyl 4-methylthio-m-tolyl isopropylphosphoramidate), a systemic organophosphorus nematicide (Cabrera et al. 2010). Enzymes capable of cleaving organic phosphates are very interesting because of their potential application in the detoxification of organic phosphates. An isolate identified as *Brevundimonas diminuta* could saponify the phosphate ester of parathion (Serdar et al. 1982). Responsible for this metabolic capability is a plasmid which was also found in *Flavobacterium* sp. ATCC 27551 (Harper et al. 1988) and which is self-transmissible (Pandeeti et al. 2011). Fusing the phosphatase to the green fluorescent protein (gfp) revealed that the phophatase is targeted to the periplasmic face of the inner membrane in the *Brevundimonas* cell (Gorla et al. 2009). Another *Brevundimonas* strain, LY-2, was isolated from agricultural soil and proved to be a good degrader of the herbicide lactofen. Inactivation of this toxin was achieved by cleavage of the ester side chain by an esterase (Liang et al. 2010).

The urinary metabolite D-4-hydroxy-3-methoxymandelic acid is a major metabolite in humans, and the determination of this compound is used in the diagnosis of life-threatening catecholamine-secreting tumors (Kinoshita et al. 1988). A bacterial strain, tentatively identified as *Brevundimonas diminuta*, could use this substrate as sole source of carbon and energy. The degradation pathway of this strain converts the substrate to vanillylglyoxylate, which is then decarboxylated, dehydrogenated, and demethylated to protocatechuate, which is then cleaved by a dioxygenase to 2-hydroxy-4-carboxymuconic semialdehyde. The dehydrogenase converting the substrate to vanillylglyoxylate has been further characterized, and a rather narrow substrate spectrum has been revealed, making this enzyme as a potential candidate for the detection and monitoring of catecholamine-secreting tumors (Turner et al. 1996).

From a marine sediment, *Brevundimonas diminuta* MTCC 8486 has been isolated. The strains could withstand high nitrate concentrations, up to 10 g l^{-1}, and could reduce nitrate. In order to remove nitrate from ground water, the culture conditions of this strain were optimized. In a pilot treatment process, nitrate concentrations could be reduced by the application of this strain to levels below the level acceptable for drinking water (Kavitha et al. 2009). *Brevundimonas* species were also found to be good strains for the biosorption of heavy metals. From a hot spring with high background radiation, *Brevundimonas* sp. ZF12 has been isolated and shown to adsorb cadmium in high yields under acidic conditions (Masoudzadeh et al. 2011).

Brevundimonas species were also reported to be valuable strains for biotransformations. *Brevundimonas* sp. SGJ could hydroxylate under resting cell conditions L-tyrosine to give L-DOPA (3,4-dihydroxyphenyl-L-alanine), the most widely used drug for the treatment of Parkinson's disease (Surwase et al. 2012). Vitamin C, L-ascorbic acid, is widely added to food products but is not very stable in solution, especially when exposed to high temperatures and oxygen concentrations. Here, more stable derivatives of vitamin C would be of advantage, ascorbic acid-2-phosphate being one of them. From soil samples, nine isolates were obtained which were able to produce ascorbic acid-2-phosphate from L-ascorbic acid. Most isolates were identified as *Brevundimonas* species by 16S phylogenetic analysis, and *B. diminuta* KACC 10306 gave the highest yield under resting cell conditions (Shin et al. 2007).

Metabolites

Metabolites of *Brevundimonas* species were also studied, and some of them may have potential in biotechnology. Polyhydroxyalkanoates have an immense potential as biodegradable polymers, and many attempts have been made for their cheap production by microbes. When *Brevundimonas vesicularis* LMG P-23615 grows on acid-hydrolyzed sawdust as carbon source, it stores some of the carbon as poly-beta-hydroxyalkanoate granules. Such a process may offer an attractive way of converting waste into valuable products (Silva et al. 2007).

A number of *Brevundimonas* species can produce carotenoid dyes. *Brevundimonas vesicularis* DC263, isolated from surface soil, produced carotenoids, identified as hydroxylated astaxanthin. A study of the carotenoid synthesis genes identified *crtW* encoding the 4,4′-beta-ionone ring ketolase, *crtZ* encoding the 3,3′-beta-ionone ring hydroxylase, and *crtG* encoding the 2,2′-beta-ionone ring hydroxylase which can hydroxylate astaxanthin. The genes of strain DC263 had the highest sequence similarities to the corresponding genes in *Brevundimonas* sp. strain SD212, a marine isolate that also produced hydroxylated astaxanthin. The genetic organization of the carotenoid synthesis gene clusters in the two *Brevundimonas* strains was identical, suggesting a close relationship (Tao et al. 2006). When the *crtG* gene of *Brevundimonas* sp. DC263 was introduced into an *E. coli*

strain which synthesizes zeaxanthin and canthaxanthin, these carotenoids were hydroxylated to the 2-hydroxy- and 2,2-dihydroxy-products. For the rare hydroxylated canthaxanthins, a strong inhibitory effect on lipid peroxidation was demonstrated (Nishida et al. 2005).

Enzymes and Proteins

Brevundimonas species were also the source of unusual enzymes with potentials in biotechnological applications. The mononuclear molybdoenzyme isoquinoline 1-oxidoreductase from *Brevundimonas diminuta* belongs to the xanthine-dehydrogenase family of proteins. It is a molybdenum hydroxylase containing a molybdopterin cytosine dinucleotide molybdenum cofactor and two distinct iron-sulfur clusters (Israel et al. 2002). It catalyzes the conversion of isoquinoline to isoquinoline-1-one, the first step in the degradation of isoquinolines. The primary sequence and behavior, specifically its substrate specificity and lipophilicity, of the *Brevundimonas diminuta* enzyme differs from other members of the family and has been studied by crystallography (Boer et al. 2005). Another strain, *Brevundimonas diminuta* TPU 5720, produces an amidase which hydrolyzes phenylalaninamide stereoselectively to L-phenylalanine. The enzyme is Co-dependent and contains 491 amino acids with significant similarity to an aminopeptidase found in the genome of *Caulobacter vibrioides* (Komeda et al. 2006). A comparison of the N-terminal sequences of malate dehydrogenases from ten strains belonging to seven different genera of *Proteobacteria* demonstrated the enzyme sequences of *Brevundimonas* species are structurally distinct from others in the study. They clearly resembled those malate dehydrogenases with greatest similarity to lactate dehydrogenases. Further analyses of subunit molecular weights, peptide mapping, and enzyme mobilities confirmed the distinct position of the malate dehydrogenases from *Brevundimonas* species (Charnock 1997). *Asticcacaulis* strain SA7 was isolated from plants roots and could grow on agar using it as sole carbon source. It produces an extracellular agarose which may have some biotechnological applications (Hosoda and Sakai 2006).

Efficient control of mosquitoes is gaining importance for the control of several severe diseases distributed by these vectors. While chemical insecticides endanger the environment, bacterial control of mosquito larvae with *Bacillus* species producing protein toxins became attractive. However, applications using these bacteria have several drawbacks. An alternative could be genetically engineered aquatic bacteria producing the *Bacillus* toxins. *Asticcacaulis excentricus* were transformed with optimized plasmids carrying the binary toxin of *B. sphaericus*. In laboratory studies, constructs based on *A. excentricus* exhibited toxicity to *Culex* and *Anopheles* mosquito larvae, similar or even better than *B. sphaericus* strains used to control these larvae but *Caulobacter vibrioides* constructs were not viable (Liu et al. 1996).

The S-layer of *Caulobacter vibrioides* has been used for the display of proteins by genetic engineering techniques opening a broad spectrum of applications in nanobiotechnology (Ilk et al. 2011). For optimal performance, the production of the S-layer protein RsaA of *C. vibrioides* has been optimized based on an unusually stable *rsaA* mRNA with a half-life of 36 min. Using multiple copies of *rsaA*, the best constructs produced 51 % of their cell proteins as RsaA (Lau et al. 2010). Such an optimized strain has been used for the generation of anti-HIV antibodies. To achieve this goal, either domain 1 of CD4 or MIP1alpha, components needed for virus entry, were heterologous presented at the S-layer of *C. vibrioides*. The combination of both constructs was able to inhibit virus entry of many HIV-1 isolates (Nomellini et al. 2010). The same approach has been used to express the immunoglobulin G (IgG)-binding streptococcal protein G at the S-layer to be used, e.g., for immunoprecipitation or immunoadsorption-based assays (Nomellini et al. 2007), a *Pseudomonas aeruginosa* vaccine candidate protein (Umelo-Njaka et al. 2001) and a recombinant vaccine against infectious hematopoietic necrosis virus (Simon et al. 2001). Not only vaccines but also enzymes can be produced in this way as has been shown for beta-1,4-glycanase, which showed however a much reduced activity at the S-layer, probably due to misfolding (Duncan et al. 2005).

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8 The Family *Cohaesibacteraceae*: The Genera *Cohaesibacter* and *Breoghania*

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Abstract
This chapter reviews the recently described family *Cohaesibacteraceae*, their two genera (*Cohaesibacter* and *Breoghania*) and three species (*Cohaesibacter gelatinilyticus* Hwang and Cho 2008, *Cohaesibacter marisflavi* Qu et al. 2011, and *Breoghania corrubedonensis* Gallego et al. 2010). The type species for each genus are *Cohaesibacter gelatinilyticus* and *Breoghania corrubedonensis*, respectively. *Cohaesibacteraceae* was created to accommodate strains that were not genetically similar enough to be classified into existing families of the order *Rhizobiales*. Besides the genetic dissimilarity, the sole major respiratory quinone (Q-10) and the polar lipid composition differentiate *Cohaesibacteraceae* from the other families of this order.

The members of *Cohaesibacteraceae* are Gram-negative, facultative anaerobic (*Cohaesibacter*) or aerobic (*Breoghania*) rods and are motile by polar flagella. *Cohaesibacter* comprises strains isolated from coastal waters of the east coast of Korea and China, whereas *Breoghania* has its type strain isolated from coastal waters of northwest Spain. Nothing is known about their applications or ecological importance, so this chapter only provides phenotypic and genetic characterization. Additionally, the phylogenetic relationship with other families of the order *Rhizobiales* is presented. Finally, the formal taxonomical descriptions of both genera and their respective species are given.

Introduction and General Characteristics

The family *Cohaesibacteraceae* is in the order *Rhizobiales*, which is one of the seven orders of Alphaproteobacteria. *Cohaesibacteraceae* is distinguished from the other 12 families of Rhizobiales (*Rhizobiaceae*, *Bartonellaceae*, *Brucellaceae*, *Phyllobacteriaceae*, *Methylocystaceae*, *Beijerinckiaceae*, *Bradyrhizobiaceae*, *Hyphomicrobiaceae*, *Methylobacteriaceae*, *Rhodobiaceae*, *Aurantimonadaceae*, and *Xanthobacteraceae*) by phylogenetic analysis of ribosomal DNA (16S). This recently proposed family includes two genera: *Cohaesibacter* and *Breoghania*. They are distinct lineages formed by strains from coastal marine environments that did not cluster with any other families in 16S phylogenetic analysis. In addition, the members of *Cohaesibacteraceae* have other genetic and phenotypic characteristics, which are discussed in detail in this chapter. The family and the type species of the genus *Cohaesibacter*, *Cohaesibacter gelatinilyticus*, had their emended description approved by the International Committee on Systematics of Prokaryotes and its judicial commission (Euzéby 2011).

Phylogeny

Phylogenetic analyses, including secondary structure information of 16S rRNA, from 189 species of the order *Rhizobiales* determined that strains from coastal seawaters of the east coast of Korea (CL-GR15 and CL-GR35) belong to that order but do not represent any previously described family. Thus, the family *Cohaesibacteraceae* was characterized. Later, another seawater strain (DQHS21) from the east coast of China showed greater similarity with 16S of *Cohaesibacter gelatinilyticus* Hwang and Cho 2008 than with the other Alphaproteobacteria genera. Hwang and Cho (2008) detected three signature nucleotides in the 16S gene that characterize the genus *Cohaesibacter* in a study describing the type species *Cohaesibacter gelatinilyticus*. In addition, a strain (UBF-B1) isolated from a beach in the northwest of Spain after an oil spill was phylogenetic compared with *Rhizobiales* sequences (including *Cohaesibacteraceae* strains) using the 16S fragment and other genes (atpD, pyrG, rpo and fusA). Results showed UBF-B1 clustering together with *Cohaesibacter gelatinilyticus*; however, they had only 92 % similarity. These data resulted in a new genus description: *Breoghania*.

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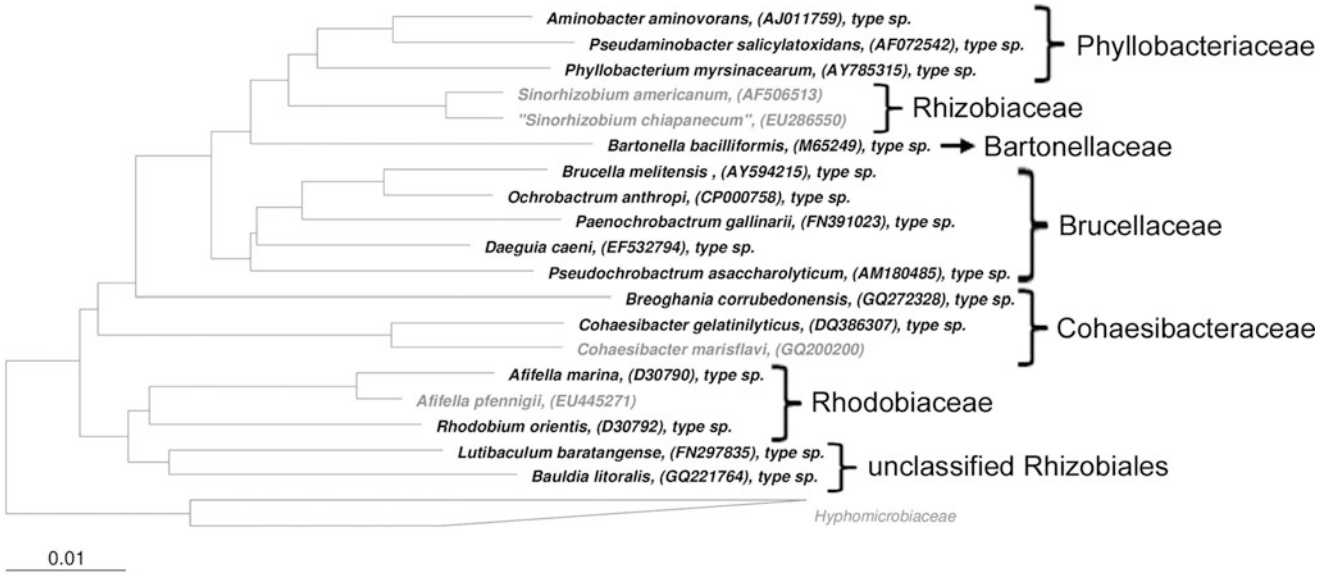

Fig. 8.1

Phylogenetic position of the family *Cohaesibacteraceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with a representative set of nearly 750 high-quality type-strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

Taxonomy

Family *Cohaesibacteraceae*, Hwang and Cho (2008) emend. Gallego et al. (2010)

Cohaesibacteraceae (Co.hae.si.bact.er.a'ce.ae. N.L. masc. n. *Cohaesibacter*, type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Cohaesibacteraceae*, the Cohaesibacter family) (Hwang and Cho 2008).

Cohaesibacteraceae is a family phylogenetically positioned in the order *Rhizobiales* in the class *Alphaproteobacteria*. It is most closely related with the families *Brucellaceae* (genera *Ochrobactrum* and *Brucella*: 90.9–92.5 % similarity); *Rhizobiaceae* (genus *Sinorhizobium*: 90.9–91.5 %); *Bartonellaceae* (genus *Bartonella*: 90.6–91.9 %); *Rhodobiaceae* (genus *Rhodobium*: 89.3–91.2 %), and *Phyllobacteriaceae* (genus *Phyllobacterium*: 89.3–91.2 %) (◉ *Fig. 8.1*).

The members of the family have varying capabilities for nitrate reduction and the DNA composition ranges from 52 to 64 mol% G+C. The sole major respiratory quinone (Q-10) differentiates *Cohaesibacteraceae* from the families *Aurantimonadaceae*, *Phyllobacteriaceae*, *Rhodobiaceae*, *Bradyrhizobiaceae*, *Methylocystaceae* and *Hyphomicrobiaceae*. *Cohaesibacteraceae* is also differentiated from 10 families in the order *Rhizobiales* (except the families *Brucellaceae* and *Phyllobacteriaceae*) by polar lipid composition (◉ *Table 8.1*).

Genus *Cohaesibacter*, Hwang and Cho (2008) emend. Gallego et al. (2010)

Cohaesibacter [Co.hae.s'i.bac.ter. L. part. adj. *cohaesus* (from L. v. *cohaereo*) pressed together, clung together; N.L. masc. n. bacter a rod; N.L. masc. n. *Cohaesibacter* rods that appear cohesive with each other].

The members of this genus are Gram-negative, facultative anaerobic rods. They have oxidase activity and variable catalase activity. The predominant fatty acids are $C_{18:1}\omega7c$ and $C_{15:0}$ iso 2-OH and/or $C_{16:1}\omega7c$ and $C_{18:0}$. The respiratory quinone is ubiquinone 10 (Q-10). The DNA G+C content ranges from 53.0 to 55.2 mol%. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, an unidentified aminolipid (AL1) and an unidentified glycolipid. The species of the genus have genetic signatures that are two compensatory transversion mutations (positions 678: A and 712: T) and a single transversion mutation (position 194: T). The type species is *Cohaesibacter gelatinilyticus*.

Description of *Cohaesibacter gelatinilyticus*, Hwang and Cho (2008)

Cohaesibacter gelatinilyticus (ge.la.ti.ni.ly'ti.cus. N.L. n. *gelatinum* gelatin; Gr. adj. *lutikos* able to dissolve; N.L. adj. *lyticus* dissolving; N.L. masc. adj. *gelatinilyticus* gelatin-dissolving).

Table 8.1

Families: *1 Cohaesibacteraceae* fam. nov., *2 Brucellaceae*, *3 Bartonellaceae*, *4 Aurantimonadaceae*, *5 Rhodobiaceae*, *6 Phyllobacteriaceae*, *7 Rhizobiaceae*, *8 Xanthobacteraceae*, *9*, *Bradyrhizobiaceae*, *10 Methylobacteriaceae*, *11 Beijerinckiaceae*, *12 Methylocystaceae*, and *13 Hyphomicrobiaceae*. Table from Hwang and Cho (2008) updated, with permission

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Nitrate reduction	V	V	–	–	V	V	+	V	V	V	NA	+	V
Gelatin hydrolysis	V	–	–	–	–	V	–	V	–	V	NA	V	V
Arginine dihydrolase	V	–	+	+	NA	–	–	V	–	NA	NA	NA	–
Urease	V	V	–	+	NA	V	+	V	+	V	V	V	+
Major quinone[a]	Q-10	Q-10	Q-10	Q-10, Q-9	Q-10, MK-10	Q-10, Q-11	Q-10	Q-10,	Q-10, MK-10, RQ-10	Q-10	Q-10	Q-10, Q-8	Q-10, Q-9, MK-9, RQ-10
Polar lipids ϕ	PC, PG, PE, DPG, PME, GL, ALs, Ls	PC, PG, PE, DPG PME, AL (PS, PN, PLs)	NA	PC, PG, PE, DPG, PME, PDE, Ls	NA	PC, PG, PE, DPG (PME, PDE, ALs, PL)	PC, PG, PE, DPG, PME, PDE	PE, PDE (PC, PG, PA)	PC, PG, PE, DPG, ALs	PC, PG, PE, DPG	PME (PG, PE)	PC, PG, PE, PDE (PME, PS, PL)	PC, PG, PE, DPG, PDE (PA, BPG)
DNA G+C content (mol%)	52–64 %	54.5–59	37–41	57.6–66.3	65.2–65.7	53.1–65.1	57–67.4	65–70	59–69	63.5–72.4	54.7–63.1	61–70	59–71.4

+ Positive, – negative, *V* variable, *NA* data not available

[a]Q Ubiquinone, MK Menaquinone, RQ Rhodoquinone

ϕ Polar lipids in parentheses were observed in <50 % of genera for which data are available in each family. *PC* Phosphatidylcholine, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *BPG* bisphosphatidylglycerol, *PE* phosphatidylethanolamine, *PME* phosphatidylmonomethylethanolamine, *PDE* phosphatidyldimethylethanolamine, *PA* phosphatidic acid, *PS* phosphatidylserine, *PN* aminophospholipid, *PL* unidentified phospholipid, *GL* unidentified glycolipid, *AL* an unidentified aminolipid, *L* an unidentified lipid

The cells are approximately 0.2–0.4 μm wide and 1.0–3.0 μm long. They are weakly motile by a polar flagellum. Reproduction occurs by bussing, binary fission, or asymmetric division. Rosette formation occurs. No growth occurs on trypticase soy agar (TSA), fivefold-diluted TSA, Czapek–Dox agar, MacConkey agar, blood agar, or the above media supplemented with either 3 % (w/v) NaCl or 3 % (w/v) sea salts. Circular, entire, convex and creamy white colonies appear on marine agar 2216 or R2A agar supplemented with 3 % (w/v) NaCl. At optimal growth conditions, colonies are approximately 2 mm in diameter after incubation for 1 week. Intracellular granules of poly-β-hydroxybutyrate are formed. Growth occurs on acetate, α-ketobutyric acid, citrate, D-fructose, D-glucose, mannitol, D-mannose, ribose, sorbitol, glutamic acid, glycerol, glycogen, inositol, inulin, L-arginine, L-asparagine, L-lysine, L-ornithine, *N*-acetylglucosamine, polyethylene glycol, L-pyruvate, sodium succinate, sucrose, thiamine, casamino acids, L-proline, peptone, tryptone and yeast extract. No growth occurs on acetamide, benzoate, DL-cysteine, cellobiose, D-galactose, raffinose, salicin, trehalose, L-xylose, ethanol, formic acid, glycine, 2-propanol, D-lactose, L-arabinose, ascorbate, L-rhamnose, maleic acid, oxalic acid, salicylate, tartrate or urea.

Growth occurs in temperatures between 15 °C and 31 °C (optimum, 25–30 °C), at pH 6–9 (optimum pH 8) and NaCl concentrations of 2–5 % (w/v, optimum 3 %). Decomposition of casein, cellulose, xanthine, hypoxanthine, and hydrolyses of gelatin and aesculin occur. Negative results are observed for nitrate reduction, indole production, arginine dihydrolase and urease. Alkaline phosphatase and trypsin are present. Esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-phosphohydrolase, and *N*-acetyl-β-glucosaminidase activities are weakly present. Lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α- and β-glucosidase, α- and β-galactosidase, α-mannosidase and α-fucosidase activities are absent.

Acid production from glycerol, DL-arabinose, D-ribose, DL-xylose, D-glucose, D-fructose, D-mannose, inositol, D-mannitol, D-sorbitol, *N*-acetylglucosamine, D-lyxose, L-fucose, potassium gluconate, and potassium 5-ketogluconate were observed. Acid production was not observed from erythritol, D-adonitol, methyl β-D-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, aesculin, salicin, D-cellobiose, maltose, D-lactose, melibiose, sucrose, trehalose, inulin,

melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-tagatose, D-fucose, DL-arabitol, or potassium 2-ketogluconate. The cells are sensitive to (μg per disc) gentamicin (6), cephalexin (20), vancomycin (20), mitomycin C (0.6), kanamycin (20), penicillin (6), erythromycin (10), chloramphenicol (20), ciprofloxacin (3), and ampicillin (6). Cells are resistant to tetracycline (20 μg per disc), nalidixic acid (20 μg per disc) and streptomycin (6 μg per disc).

The major fatty acids are $C_{18:1}\omega7c$ (54.3–55.1 %) and $C_{15:0}$ iso 2-OH and/or $C_{16:1}\omega7c$ (summed feature 3; 19.2–20.4 %), $C_{20:1}\omega7c$ (9.6–11.1 %), $C_{18:0}$ (3.1–3.3 %), $C_{14:0}$ 3-OH and/or $C_{16:1}$ iso I (3.0 %), $C_{18:0}$ 3-OH (1.9–2.0 %), $C_{17:1}\omega8c$ (1.1–1.5 %) and $C_{16:0}$ (1.1 %). Other minor fatty acids (<1 %) are $C_{9:0}$, $C_{14:0}$, $C_{17:0}$, $C_{19:0}$, $C_{20:0}$, $C_{14:1}\omega5c$, $C_{19:0}$ cyclo ω8*c*, $C_{16:0}$ 3-OH, $C_{17:0}$ iso 3-OH and $C_{19:0}$ 10-methyl. Besides the polar lipids of the genus, *C. gelatinilyticus* has also minor amounts of an unidentified aminolipid (AL2) and unidentified lipids (L1–4). The type strain, CL-GR15^T (= KCCM 42319^T = DSM 18289^T), was isolated from coastal waters of the east coast of Korea.

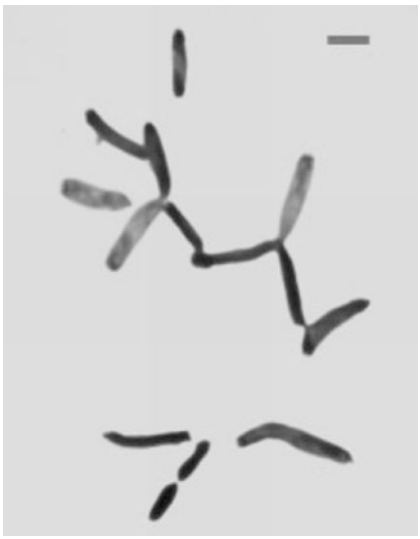

Fig. 8.2
Transmission electron micrograph of negatively stained cells of strain CL-GR15T. Bar, 1 μm (From Hwang and Cho (2008), with permission)

Description of *Cohaesibacter marisflavi*, Qu et al. (2011)

Cohaesibacter marisflavi (ma.ris.fla'vi. L. neut. n. *mare –is* the sea; L. adj. *flavus -a -um* yellow; N.L. gen. n. *marisflavi* of the Yellow Sea, referring to the isolation of the type strain).

The cells are Gram-negative, catalase-negative, oxidase-positive, facultative anaerobic rods that are 0.6–0.7 mm wide and 1.8–2.0 mm long (Fig. 8.2). Cells are motile by a single polar flagella. They reproduce by binary fission or asymmetrical division. Colonies, which grow on 2216E agar medium, are white, smooth, circular, lightly transparent, and 1.0–2.0 μm in diameter after 3–5 days of cultivation at 30 °C. Intracellular poly-b-hydroxybutyrate granules are accumulated. Positive results were obtained in API 20NE tests for indole production, utilization of D-glucose, D-mannose, malic acid, *N*-acetylglucosamine, and trisodium citrate and weakly positive for utilization of β-galactosidase, maltose and L-arabinose; results were negative for gelatin hydrolysis, D-glucose fermentation, arginine dihydrolase, and utilization of D-mannitol, potassium gluconate, capric acid, adipic acid, and phenylacetic acid. In the Biolog GN2 system, the tests were positive for utilization of dextrin, D-fructose, raffinose, glycerol, L-arabinose, *N*-acetyl-D-glucosamine and sucrose. Growth occurs in temperatures between 10 °C and 38 °C (optimum 25–30 °C) at pH 4–9 (optimum pH 7–8) and in 0.5–15 % (w/v) NaCl (optimum 3 %).

In the API ZYM test strip, the results were positive for esterase (C4), naphthol-AS-BIphosphohydrolase, and trypsin; weakly positive for acid phosphatase, alkaline phosphatase, esterase lipase (C8), and leucine aminopeptidase; and negative for cystine aminopeptidase, lipase (C14), *N*-acetyl-β-glucosaminidase, valine aminopeptidase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase, and β-glucuronidase. They also have urease and β-glucosidase (aesculin hydrolysis) activities and nitrate reduction. Cells are sensitive to (μg per disc, unless noted) ampicillin (10), carbenicillin (100), cefalexin (30), chloramphenicol (30), ciprofloxacin (5), doxycycline (30), erythromycin (15), kanamycin (30), nalidixic acid (30), neomycin (30), novobiocin (30), penicillin G (10 IU), rifampicin (5), streptomycin (10), and tetracycline (30). Cells are resistant to (μg per disc, unless noted) clindamycin (2), lincomycin (2), oxacillin (1), polymyxin B (300 IU), and vancomycin (30).

The major fatty acids are $C_{18:1}\omega7c$, $C_{18:0}$, $C_{16:0}$ and $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH. Ubiquinone 10 is the major quinone. The DNA G+C content is 55.2 mol%. The type strain, DQHS21^T (=CGMCC 1.9157^T = NCCB 100300^T) was isolated from sediment sampled from a seawater pond used for sea cucumber culture at Jimo, Qingdao province, China, on the west coast of the Yellow Sea.

Genus *Breoghania*, Gallego et al. (2010)

Breoghania (Bre.o.gha'ni.a. N.L. fem. n. *Breoghania*, named after *Breoghan*, according to Celtic mythology (*Leabhar Ghabhala*, XII century), the first Celtic king of *Gallaecia* (actual Galicia), founder of the city of *Brigantia* (probably A. Coruña) who built a tower on the coast from where Eire (Ireland) could be seen.

The members of this genus are Gram-negative, aerobic rods that are motile by polar flagella. Cellular division occurs by binary fission or asymmetric division. They present oxidase and catalase activity. The DNA G+C content of the type strain is 63.9 mol%. The respiratory quinone is ubiquinone 10 (Q-10). The predominant fatty acid is $C_{18:1}\omega7c$ (75.3 %); and other fatty acids found in smaller amounts are $C_{19:0}$ cycloω8c and $C_{16:0}$. The major polar

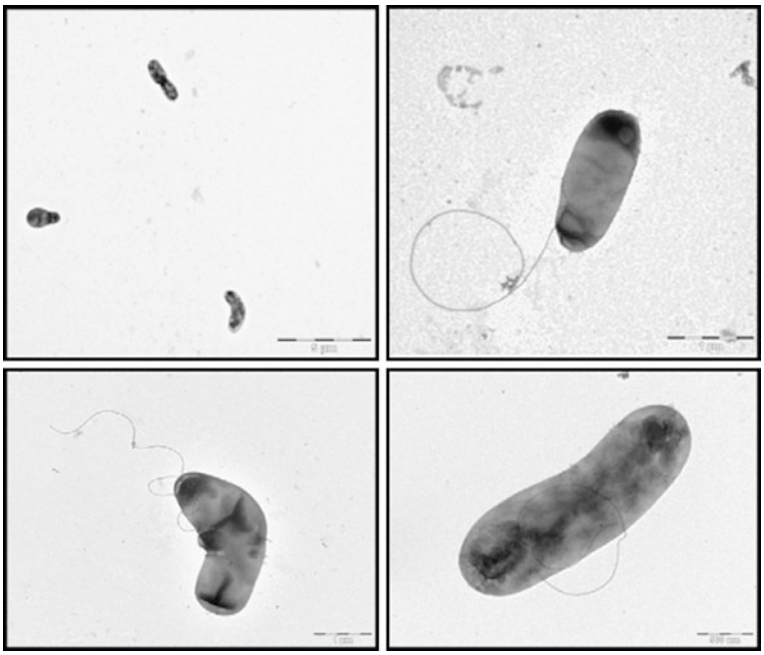

Fig. 8.3
Transmission electron micrographs of negatively stained cells of strain UBFP1T (From Gallego et al. (2010), with permission)

lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, and phosphatidylcholine. The type species is *Breoghania corrubedonensis*.

Description of *Breoghania corrubedonensis*, Gallego et al. (2010)

Breoghania corrubedonensis (Co.ru.be.do.nen'sis N.L. fem. adj. corrubedonensis, of or belonging to Corrubedo, northwest Spain, isolated from the beach of Corrubedo, the location of the sand sample that was used to inoculate the enrichment cultures from which strain UBF-P1^T was isolated).

Cells are regular, irregular, or bulbous rods that are approximately 0.6–0.7 μm wide and 2–3.5 μm long (*Fig. 8.3*). Cells are motile by one or two subpolar flagella. Reproduction occurs by binary fission or asymmetric division. Circular, entire, convex, mucoid, and creamy white colonies appear on LB ASW, LB 3 % NaCl, or marine agar. After incubation for 5 days at optimal growth conditions, colonies are approximately 1 mm in diameter. Growth occurs on glucose, arabinose, mannitol, D-sorbitol, adipate, gluconate (w), phenyl acetate, acetate, succinate, malate, pyruvate, Casamino acids (Difco), acetone, and Tween 20. No growth occurs on D-xylose, mannose, maltose, starch, caprate, *N*-acetyl-glucosamine, citrate, lactate, oxalacetate, propionate, or methanol. Hydrolysis of gelatine or aesculin does not occur. Nitrate is reduced to nitrite, but nitrite reduction does not occur, nor does indole production or H2S formation. β-Glucosidase and β-galactosidase are not produced. Starch, DNA, and Tween 80 are not degraded. Growth occurs in temperatures between 15 °C and 40 °C (optimum 30 °C), at pH 5–8.5 (optimum 7.5) and with NaCl concentrations of 1–10 %. Aminopeptidase, arginine dihydrolase, ornithine decarboxylase, urease, catalase, oxidase, and nitrate reduction are observed. Acid production was weak produced from L-arabinose.

The major fatty acids are $C_{18:1}\omega7c$ (75.3 %), $C_{19:0}$ cyclo $\omega8c$ (6.4 %), $C_{16:0}$ (4.4 %), $C_{16:1}$ $\omega7c$ and/or $C_{15:0}$ iso 2-OH (summed feature 3, 2.5 %), $C_{18:0}$ 3-OH (2.4 %), $C_{18:0}$ (2.2 %), and $C_{20:1}$ $\omega9c$ (1.3 %). Other minor (<1 %) fatty acids are $C_{14:0}$ 3-OH or $C_{16:1}$ iso I (summed feature 2), $C_{17:1}$ $\omega8c$, $C_{17:1}$ $\omega6c$, and $C_{17:0}$. The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PME), and phosphatidylcholine (PC). The DNA G+C content is 63.9 mol%. The type strain was isolated from the beaches of Corrubedo in northwest Spain. It has been deposited in three culture collections under the numbers CECT 7622, LMG 25482, and DSM 23382.

Remarks

The whole genome sequencing of the type strain *Cohaesibacter gelatinilyticus* CL-GR15 is in progress. The South Korean project is sequencing the genome using Illumina Hiseq 2000, 454-GS-FLX (http://www.genomesonline.org/cgi-bin/GOLD/GOLDCards.cgi?goldstamp=Gi0034926).

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9 The Family *Erythrobacteraceae*

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Abstract
Erythrobacteraceae is one of the two families that belong to the order *Sphingomonadales*, and it is affiliated with the class *Alphaproteobacteria*. The family *Erythrobacteraceae* includes five genera – *Altererythrobacter*, *Croceicoccus*, *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter* – for a total of 33 species, including 29 with validly published names and 4 new species. Members of the family are Gram-negative, aerobic, rod-shaped or pleomorphic coccoid bacteria; they are motile or nonmotile, chemo-organotrophic, produce pigments (yellow, orange, red or pink), and do not form spores. Some species require biotin. Most members contain bacteriochlorophyll a (*BChl a*), several types of carotenoids, and monosaccharide-type glycosphingolipid. Representatives of the clade have been isolated from diverse environments: wild rice, cold-seep sediment, desert sand, tepid water, seawater, tidal flats, marine sediment, and marine invertebrates. Whole-genome sequencing has been reported in only two genera of the *Erythrobacteraceae* family, including *Erythrobacter* (three strains) and *Porphyrobacter* (one strain). Family members offer a valuable source of information for further studies focused on aerobic anoxygenic phototrophic (AAP) metabolism, physiological nature, and high potential for biotechnological purposes by the presence of important hydrolases.

Taxonomy: Historical and Current

Erythrobacteraceae (E.ry.thro.bac.te.ra'ceae. N.L. masc. n. *Erythrobacter* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Erythrobacteraceae* the *Erythrobacter* family) (Lee et al. 2005 emend. Xu et al. 2009).

Erythrobacteraceae DNA mol% G+C content varies between 54.5 and 71.5. The type genus is *Erythrobacter*. *Erythrobacteraceae* is phylogenetically closest related to the family *Sphingomonadaceae*. These two families integrate the order *Sphingomonadales*, and they are affiliated with the class *Alphaproteobacteria*.

Physiologically, the group is very similar. Members are Gram-negative, rod-shaped, or pleomorphic coccoid bacteria and aerobic chemoorganotrophs. Most species contain carotenoids and require NaCl for growth (although it is not essential in many cases), some species contain *BChl a*. Autotrophic growth has not been reported, but one genus may be facultative photoheterotrophs. Members of this taxon have been isolated

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mainly from aquatic environments, but there are also isolation reports from sediment, sand, and rice.

Erythrobacter, the type genus of the *Erythrobacteraceae* family, was originally described by Shiba and Simidu in 1982. Members of this genus are orange- and pink-pigmented Gram-negative bacteria. They are aerobic chemoorganotrophs that are motile by subpolar flagella. *Erythrobacter* are halophilic carotenoids that are able to use glucose, pyruvate, acetate, butyrate, and glutamate as sole organic carbon sources, but not methanol. Strains require biotin and are able to hydrolyze gelatin, tween 80, and alginate. Although these organisms do not grow phototrophically, the presence of bacteriochlorophyll *a* indicates that this taxon is most closely related to the *Rhodospirillaceae* family (*Rhodospirillales* order), so it was maintained as a new genus inside this family. Later, the nonphotosynthetic *Sphingomonas* group was included in the α-4 subclass of *Proteobacteria* (Yabuuchi et al. 1990), as well as the *Erythrobacter-Porphyrobacter-Erythromicrobium* cluster, which was defined to be most closely related to members of the genus *Sphingomonas* (Yurkov et al. 1994). A new order was proposed to accommodate this clade. The *Sphingomonadales* order was created based on 16S rRNA sequence comparisons, as well as phenotypic and morphological characteristics (Yabuuchi and Kosako 2005).

Strain representatives of the *Porphyrobacter* genus proposed by Fuerst et al. (1993) shared ultrastructural similarities with members of the *Planctomycetales*. However, 16S rRNA sequence data indicated that these bacteria shared a position within the Alfa subclass of the *Proteobacteria* with *Erythrobacter longus*, but they were distinguishable in their sequences and in other characteristics, such as their requirement of vitamins for growth; acetate, glutamate, and butyrate utilization; gelatin hydrolysis; and quantitative differences in detectable cellular fatty acids.

The photosynthetic apparatus, biochemical, morphological, and 16S rRNA sequence data for strain E5T (= DSM 8510^T) also supported the proposal that a new genus and a new species should be described. The *Erythromicrobium* genus was introduced by Yurkov et al. (1992) as a genus of freshwater, obligatory aerobic, facultative, photoheterotrophic bacteria that included five species tentatively identified on the basis of DNA–DNA hybridization and phenotypic data (Yurkov et al. 1991): *Erythromicrobium sibiricum*, *Erythromicrobium ursincola*, *Erythromicrobium ezovicum*, *Erythromicrobium hydrolyticum*, and *Erythromicrobium ramosum*. However, the genus was validly published in 1994 with *Erythromicrobium ramosum* as the type strain (Yurkov et al. 1994) and the unique valid named species in the genus. In 1997, the genus *Erythromicrobium* was taxonomically reorganized, resulting in the exclusion of *E. sibiricum* and *E. ursincola* from the genus, being transferred to new genera, *Sandaracinobacter* and *Erythromonas*, respectively (Yurkov et al. 1997). Subsequently, phylogenetic analysis of the class *Alphaproteobacteria* based on 16S rRNA sequence comparisons through phylogenetic tree led to the reclassification of the genera *Erythrobacter* (Shiba and Simidu 1982), *Porphyrobacter* (Fuerst et al. 1993), and *Erythromicrobium* (Yurkov et al. 1994) into a new family named *Erythrobacteraceae* by Lee and colleagues (2005).

Further, the *Altererythrobacter* genus was suggested by Kwon et al. (2007) to accommodate new species in the clade. *Altererythrobacter* showed high similarity to the genus *Erythrobacter* but did not share its phyletic line. Another taxonomic rearrangement was related to reclassification of *Erythrobacter luteolus* (Yoon et al. 2005a) as *Altererythrobacter luteolus* comb. nov. (Kwon et al. 2007).

The last genus introduced in *Erythrobacteraceae* family was *Croceicoccus* (Xu et al. 2009), represented by aerobic and chemoheterotrophic bacteria containing carotenoids but not *BChl a*. The emended description of the family *Erythrobacteraceae* was also included in the study of Xu et al. (2009).

At the time of writing (September 2012), the family *Erythrobacteraceae* consisted of five genera (*Table 9.1*) with 33 species (*Tables 9.2*, *9.3*, *9.4*, and *9.5*): *Erythrobacter* (type genus) (13 species, 2 of which were only effectively published), *Altererythrobacter* (12 species, 2 of which were only effectively published) *Croceicoccus* (1 species), *Erythromicrobium* (1 species), and *Porphyrobacter* (6 species).

Phylogenetic Structure of the Family and Its Genera

The phylogenetic reconstruction of the 33 type species of the family *Erythrobacteraceae* based on 16S rRNA gene sequence is represented in *Fig. 9.1*. *Erythrobacteraceae* is most closely related to *Sphingomonadaceae*. These families belong to the order *Sphingomonadales*, and it is affiliated with the class *Alphaproteobacteria*.

The five genera (*Erythrobacter*, *Altererythrobacter*, *Croceicoccus*, *Erythromicrobium* and *Porphyrobacter*) currently recognized within the *Erythrobacteraceae* family are separated in the tree based on the topology created using the randomized axelerated maximum likelihood algorithm (RAxML; Stamatakis 2006). Representative sequences from *Rhodospirillaceae* and *Rodobiaceae*, which are closely related taxa, were used as outgroups. In addition, a 10 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment of the sequences. The scale bar in *Fig. 9.1* represents 0.02-bp substitutions per nucleotide position.

Molecular Analyses

DNA–DNA Hybridization

DNA-DNA hybridization (DDH) studies have been performed on several *Erythrobacteraceae* family members including *Altererythrobacter*, *Erythrobacter*, and *Porphyrobacter* genera, confirming the taxonomic positions of the species proposed in each taxon. *Tables 9.6*, *9.7*, and *9.8* summarize the DNA–DNA similarities among the type strains of the *Erythrobacteraceae* family.

Levels of DNA–DNA relatedness between *A. marensis* MSW-14^T and its closest relatives, *A. epoxidivorans* KCCM

Table 9.1

The genera classified within the family *Erythrobacteraceae*, as on September 2012

Genus	Number of species[a]	Type species	General properties
Erythrobacter (type genus)	13	*Erythrobacter longus*	Gram-negative aerobic chemoorganotrophs non-spore-forming containing ubiquinone-10 (Q-10) as the predominant respiratory lipoquinone, most species contain carotenoids, require NaCl for growth, utilize glucose, but do not hydrolyze starch, gelatin and do not reduce nitrate. Autotrophic growth has not been reported. Two species contain bacteriochlorophyll *a* (*BChl a*)
Altererythrobacter	12	*Altererythrobacter epoxidivorans*	The members share many phenotypic and chemotaxonomic characteristics such as the absence of motility, rod-shaped cells. C18:1 ω7*c* and ubiquinone-10 are the dominant fatty acid and respiratory quinone, respectively. Members can be characterized by the yellow to orange-red colony color on agar plates, lack of *BChl a*. DNA G+C content ranging between 54.5 and 67.2 mol%, and the temperature range for optimal growth occurs at 15–35 °C. All species can grow in the presence of NaCl, though not essential in many cases
Croceicoccus	1	*Croceicoccus marinus*	Gram-negative and non-spore-forming cocci. Divide by binary division. Capable of producing multifibrillar stalk-like fascicle structures on the cell surface. Contains carotenoids, but not *BChl a*. Aerobic and chemoheterotrophic. No growth occurs anaerobically in the light. Ubiquinone-10 is the major respiratory quinone. The polar lipid profiles comprise phosphatidylglycerol, two unidentified glycolipids, phosphatidylcholine and an unidentified phospholipid
Erythromicrobium	1	*Erythromicrobium ramosum*	Gram-negative, rod-shaped, and usually motile by means of flagella. Branching may occur. The cells are orange, contain *Bchl a* and carotenoids, and multiply by binary division. Aerobic chemoorganotrophs and facultative photoheterotrophs. No growth occurs anaerobically in the light. Ribulose diphosphate carboxylase is not detected. No fermentation and no denitrification activities occur
Porphyrobacter	6	*Porphyrobacter neustonensis*	Gram-negative, pleomorphic motile rods or cocci, non-sporulating, reproducing by polar growth or budding. Capable of producing multifibrillar stalk-like fascicle structures and crateriform structures on the cell surface. Aerobic. Chemoheterotrophic Synthesizes *BChl a* on low-nutrient media under aerobic and semiaerobic conditions. DNA base composition is 63.8–66.8 mol% G+C. Positive for calatase, presence of BChl a, and utilization of D-glucose, negative for utilization of citrate and L-Arabinose

[a]Including species whose names have been effectively but not yet validly published

42314^T and *A. luteolus* KCTC 12311^T were determined to be 26.0–27.3 % and 9.8–15.2 %, respectively (Seo and Lee 2010). The value between *A. xinjiangensis* S3-63^T and the type strain of the phylogenetically most closely related species *A. marinus* H32^T was 54.5 ± 2 % (Xue et al. 2012). DNA–DNA similarity between *A. troitsensis* KMM 6042^T and *A. dongtanensis* was 34.4 % ± 7.6 % (Nedashkovskaya et al. 2013).

DNA–DNA relatedness between *E. citreus* strains RE35F/1^T and RE10F/45 was (79 % ± 6 %). However, values between *E. citreus* strains and *E. litoralis* DSM 8509^T were 25 % ± 2 % and 34 % ± 2 %, respectively, and between *E. citreus* and *Erythromicrobium ramosum* DSM 8510^T were 20 % ± 3 % and 34 % ± 4 %, respectively (Denner et al. 2002). *E. flavus* strains SW-46^T and SW-52 exhibited DNA–DNA relatedness levels of 94.0 % and 94.7 % against each other, although *E. flavus* strains and the type strains of *E. longus* DSM 6997^T, *E. litoralis* DSM 8509^T, and *E. citreus* DSM 14432^T were 4.2 % and 3.6 %; 6.0 % and 7.4 %; and 13.1 % and 14.7 %, respectively (Yoon et al. 2003).

Levels of DNA relatedness observed between *E. aquimaris* strains SW-110^T, SW-116, and SW-140 were 88.5–102.1 %, but DNA relatedness of *E. aquimaris* strains against *E. longus*, *E. litoralis*, *E. citreus*, and *E. flavus* were between 5.3 % and 12.7 % (Yoon et al. 2004a). DNA similarity between *E. seohaensis* SW-135^T and *E. gaetbuli* SW-161^T was 12.3 %. Moreover, *E. seohaensis* SW-135^T and *E. gaetbuli* SW-161^T exhibited DNA similarity levels of 9.7–20.2 % and 8.5–18.9 % against *E. longus*, *E. litoralis*, *E. citreus*, *E. flavus*, and *E. aquimaris* (Yoon et al. 2005b).

DNA–DNA hybridization similarity among four *E. vulgaris* strains (i.e., 022-2-10^T, 022-2-9, 022-2-12 and 022-4-7) was 94–98 %. DNA relatedness between *E. vulgaris* and *E. flavus* KCCM 41642^T, *E. citreus* DSM 14432^T, *E. litoralis* DSM 8509^T, *E. aquimaris* KCCM 41818^T, and *E. longus* ATCC 33941^T were 39 %, 37 %, 33 %, 33 %, and 30 %, respectively (Ivanova et al. 2006). DNA–DNA relatedness values between *E. nanhaisediminis* T30^T and *E. aquimaris* JCM 12189^T, *E. seohaensis* JCM 21815^T, *E. citreus* JCM 21816^T, *E. vulgaris* DSM 17792^T, and *E. longus* JCM 6170^T were 56.9 %, 40.3 %, 19.7 %, 14.7 %, and 14.5 %, respectively (Xu et al. 2010). DNA–DNA relatedness between *E. pelagi* UST081027-248^T and *E. citreus* DSM14432^T,

Table 9.2
Comparison of selected characteristics of the members of the genus *Erythrobacter*

Character	*E. aquimaris*	*E. citreus*	*E. flavus*	*E. gaetbuli*	*E. gangjinensis*	*"E. jejuensis"*	*E. litoralis*
Type strain	KCCM 41818^T = JCM 12189^T	CIP 107092^T = DSM 14432^T	KCCM 41642^T = JCM 11808^T	DSM 16225^T = KCTC 12227^T	JCM 15420^T = KCTC 22330^T	KCTC 23090^T = JCM 16677^T	DSM 8509^T
Acession no 16S rRNA gene	AY461441	AF118020	AF500004	AY562220	EU428782	DQ453142	AF465836
Description year	2004	2002	2003	2005	2010	2012	1994
Number of strain in the clade	3	2	2	1	1	1	1
Colony color	Orange	Yellow	Yellow	Orange-yellow	Orange	Yellow	Red-orange
Cell size (μm)	0.6–0.9 × 2.0–4.0	0.3–0.7 × 1.0–1.5	0.7–0.9 × 1.5–2.5	0.6–0.8 × 2.0–4.0	0.3–0.4 × 0.6–0.8	0.2–0.3 × 0.6–0.9	0.2–0.3 × 1.0–1.3
Motility	−	−	+, polar flagellum	−	−	−	+
Optimal pH	6.5–7.5	NR	6.5–7.5	7.0–8.0	7.0–8.0	7.0–8.0	NR
Optimal temperature (°C)	30–37	25–30	30–37	30–37	30	30	25–30
Tolerance to NaCl (%)	10	10[a]	14	9	5	5	NR
Growth requirement	NaCl	−	NaCl	NaCl	NaCl	NR	−
Nitrate reduction	−	+	−	−	−	−	−
Utilization of:							
Glucose	+	(+)	−	+	−	−	+
Acetate	+	+	+	+	NR	NR	+
Pyruvate	+	−	+	+	NR	NR	+
Glutamate	−	+[b]	−	−	NR	NR	+
Butyrate	+	NR	+	+	NR	NR	+
Methanol	−	NR	−	−	NR	NR	−
Susceptibility to:							
Chloramphenicol	+ (100 pg)	+ (30 ug)	+ (100 ug)	− (100 pg)	+ (30 ug)	+ (30 ug)	+ (100 ug)
Penicillin	− (20 U)	− (10 U)	− (20 U)	− (20 U)	+ (10 U)	+ (10 U)	− (20 U)
Tetracycline	− (30 ug)[c]	− (30 ug)	− (30 ug)[c]	− (30 ug)[d]	+ (30 ug)	+ (30 ug)	+
Hydrolysis of:							
Gelatin	−	−	−	−	−	−	−
Starch	(+)	−	+	−	+	−	−
Tween 80	+	−[b]	+	+	+	+	+
Voges-Proskauer reaction	−[c]	−[c]	+[c]	NR	−	−	NR

H_2S production	–	NR	–	–	–	–	NR
Indole production	NR	NR	NR	NR	–	–	NR
Bacteriochlorophyll a	–	–	–	–	–	–	+
Major quinone	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	NR
G+C content of DNA (mol%)	62.2–62.9 (62.2)	62.0–62.4 (62)	64.0–64.1 (64.0)	64.5	61.6	58.9	67
Sample source and site	Seawater, Korea	Seawater, France	Seawater, Korea	Tidal flat, Korea	Seawater, Korea	Seawater, Korea	Cyanobacterial mat, The Netherlands

Character	*E. longus*	*"E. marinus"*	*E. nanhaisediminis*	*E. pelagi*	*E. seohaensis*	*E. vulgaris*
Type strain	IF0 14126^T	KCTC 23554^T = CCUG 43 60528^T	CGMCC 1.7715^T = JCM 16125^T	JCM 17468^T = NRRL 59511^T	KCTC 12228^T = DSM 16221^T	CIP 108956^T = KMM 3465^T
Acession no 16S rRNA gene	AF465835	HQ117934	FJ654473	HQ203045	AY562219	AY706935
Description year	1982	2011	2010	2012	2005	2006
Number of strain in the clade	11	1	1	1	1	4
Colony color	Orange	Yellowish-orange	Orange	NR	Orange-yellow	Yellow
Cell size (μm)	0.3–0.4 × 2.0–5.0	0.3–0.5 × 0.5–1.0	0.3–0.5 × 0.5–1.7	2.5–3.0 × 0.5–0.6	0.6–0.8 × 1.5–4.0	0.6–0.8 × 1.2–1.8
Motility	+, subpolar flagella	–	+	–	–	–
Optimal pH	7.0–8.0	7.0–8.0	7.0–7.5	8.0–9.0	7.0–8.0	7.5–8.5
Optimal temperature (°C)	25–30	25	30	28–36	30–35	20–30
Tolerance to NaCl (%)	7	5	10	8	9	8
Growth requirement	Biotin, NaCl	NaCl	NaCl	NaCl	NaCl	NaCl
Nitrate reduction	+	–	–	+	–	–
Utilization of:						
Glucose	+	+	+	+	+	–
Acetate	+	–	+	+	+	–[b]
Pyruvate	+	+	+	+	+	+[a]
Glutamate	+	+	+	+	–	–
Butyrate	+	NR	+	NR	+	NR
Methanol	–	NR	–	NR	–	NR
Susceptibility to:						
Chloramphenicol	+ (100 pg)	– (100 ug)	+ (30 ug)	+ (30 ug)	– (100 pg)	NR
Penicillin	+ (20 U)	+ (20 U)	– (10 U)	+ (20 U)	– (20 U)	– (10 U)[a]
Tetracycline	– (50 pg)	+ (30 ug)	– (30 ug)	+ (30 ug)	– (30 ug)[c]	– (30 ug)

Table 9.2 (continued)

Character	*E. longus*	*"E. marinus"*	*E. nanhaisediminis*	*E. pelagi*	*E. seohaensis*	*E. vulgaris*
Hydrolysis of:						
Gelatin	+	–	–	–	–	–
Starch	–	–	–	–	–	–
Tween 80	(+)	+	+	–	+	+
Voges-Proskauer reaction	–	NR	NR	NR	NR	–
H_2S production	–	–	–	–	–	–
Indole production	+	NR	NR	+	NR	–
Bacteriochlorophyll a	+	–	–	–	–	–
Major quinone	NR	Q-10	Q-10	Q-10	Q-10	NR
G+C content of DNA (mol%)	60–64 (60.7)	66.1	59.5	60.4	62.2	60–62 (60.5)
Sample source and site	Seaweeds, Japan	Seawater, Korea	Sea sediment, China	Seawater, Israel	Tidal flat, Korea	Marine invertebrate, China

Data taken from:
[a]Xu et al. (2010)
[b]Wu et al. (2012)
[c]Lee et al. (2010)
[d]Yoon et al. (2013)
NR not reported
Additional data on growth substrates are given in the original species descriptions

Table 9.3

Comparison of selected characteristics of the members of the genus *Altererythrobacter*

Character	*A. aestuarii*	*A. dongtanensis*	*A. epoxidivorans*	*"A. gangjinensis"*	*A. indicus*	*A. ishigakiensis*	*A. luteolus*
Earlier name							*"Erythrobacter luteolus"*
Type strain	KCTC 22735^T = JCM 16339^T	KCTC 22672^T = CCTCC AB 209199^T	KCCM 42314^T = JCM 13815^T	KACC 16190^T = JCM 17802^T	LMG 23789^T = DSM 18604^T	NITE-AP48T = ATCC BAA-2084^T	KCTC 12311^T = JCM 12599^T
Acession no 16S rRNA gene	FJ997597	GU166344	DQ304436	JF751048	DQ399262	AB363004	AY739662
Description year	2011	2011	2007	2012	2008	2011	2007
Number of strain in the clade	1	1	1	1	1	1	1
Colony color	Yellow	Yellow	Yellow	Ochre	Yellow	Orange-red	Yellow
Cell shape	Rod	Rod	Ovoid-rod	Rod	Rod	Rod	Rod
Motility	–	–	–	–	–	–	–
Optimal pH	6–8	7–9	6.5	6.5–7.0	7	7.5	7–8
Temperature range (°C)	10–40	10–37	20–40	7–35	4–42	25–40	4–36
NaCl concentration range (%)	0–6	0–1	0–6	0–9	0–12	1.5–2	0.5–9
Nitrate reduction	–	–	–	–	–	+	–
Indole production	–	–	–[a]	–	–	–	–[a]
H_2S production	–	–	–	–	–[b]	NR	–
Utilization of							
Glucose	–	+	–	–	+	–	+
Mannose	–	–	–[c]	–	–[c]	–	–
Malate	–	+	–	–	+	–	–
Acetate	–	+	–	NR	+	NR	–
Hydrolysis of							
Gelatin	–	–	–	–	–[b]	–	–
Aesculin	+	–	–	+	+	+	+
Urea	–		+[d]	–	–[d]	–	–
Enzyme activity of							
Esterase	–	+	+	–	+[d]	NR	+[a]
Trypsin	+	–	+	+	–[d]	NR	+[a]
B-galactosidase	–	+	+	+	+[d]	+	+
Bacteriochlorophyll a	–	NR	–	–	–	–	–
G+C content of DNA (mol%)	67.2	66.4	54.5	60.2	66.8	59.1	60.3
Sample source and site	Seawater, Korea	Tidal flat, China	Sediments, Japan	Tidal flat, Korea	Mangrove- rice, India	Sediment, Japan	Tidal flat, Korea

Table 9.3 (continued)

Character	*A. marensis*	*A. marinus*	*A. namhicola*	*"A. troitsensis"*	*A. xinjiangensis*
Earlier name					
Type strain	KCTC 22370^T = DSM 21428^T	CCTCC AB 208229^T = LMG 24629^T	KCTC 22736^T = JCM 16345^T	KCTC 12303^T = JCM 17037^T	CCTCC AB 207166^T = CIP 110125^T
Acession no 16S rRNA gene	FM177586	EU726272	FJ935793	AY676115	HM028673
Description year	2010	2009	2011	2012	2012
Number of strain in the clade	1	1	1	1	1
Colony color	Yellow	Yellow	Orange	Yellow	Yellow
Cell shape	Rod	Rod	Rod	Ovoid-rod	Rod
Motility	+	–	–	+	–
Optimal pH	7.1	7–8	6–7	7.2–7.6	8
Temperature range (°C)	4–42	10–42	15–37	4–39	20–37
NaCl concentration range (%)	0–9	0.5–5	1–2	0–4	0–3
Nitrate reduction	–	–	–	+	–
Indole production	–	–	–	–	–
H_2S production	NR	–	–	NR	NR
Utilization of					
Glucose	–	–	–	–	+
Mannose	–	–	–	–	–
Malate	+	–	+	–	+[e]
Acetate	NR	+	–	+	NR
Hydrolysis of					
Gelatin	–	–	–	–	–
Aesculin	+	+	+	+	+[e]
Urea	–	–	–	–	–
Enzyme activity of					
Esterase	w	w	–	w	+
Trypsin	–	+	+	–	+
B-galactosidase	–	–	–	–	+
Bacteriochlorophyll a	–	–	–	–	–
G+C content of DNA (mol%)	63.1	66.5	63.8	65	64.6
Sample source and site	Seawater, Korea	Seawater, Indian Ocean	Seawater, Korea	Sea urchin, East sea	Desert, China

Data taken from:

[a]Seo and Lee (2010)

[b]Lai et al. (2009)

[c]Matsumoto et al. (2011)

[d]Park et al. (2011)

[e]Nedashkovskaya et al. (2013)

NR not reported

Additional data on growth substrates are given in the original species descriptions

Table 9.4
Comparison of selected characteristics of the members of the genera *Croceicoccus* and *Erythromicrobium*

Character	*Croceicoccus marinus* (2009)	*Erythromicrobium ramosum* (1994)
Type strain	CGMCC 1.6776^T = JCM 14846^T	DSM 8510^T
Acession no 16S rRNA gene	EF623998	AF465837
Cell shape	Cocci	Rod
Color of colony	Yellow	Red-orange
Motility	+	+
Presence of BChl a	–	+
Growth in NaCl (%):	0–10 (0–1)	NR
Growth pH:	6.0–9.0 (7.0)	(7.0–8.5)
Growth temperature (°C):	4–42 (25)	(25–30)
H_2S production	–	NR
Hydrolysis of:		
Aesculin	+	NR
Casein	+	+
Gelatin	+	–
Starch	+	–
Tween 80	+	–
Acid production from:		
Glucose	+	NR
Maltose	+	NR
Sucrose	+	NR
Utilization of:		
Acetate	+	+
Cellobiose	–	NR
Citrate	–	+
Ethanol	+	+
Glutamate	+	+
Glycerol	+	–
Lactate	+	+
Malate	+	+
Maltose	+	+
Mannose	+	NR
Pyruvate	+	+
Succinate	+	+
Sucrose	+	+
Xylose	–	
Major cellular fatty acids	Anteiso-$C_{15:0}$	$C_{17:1}$ ω6*c*, $C_{18:1}$ ω7*c*
DNA G+C content (mol%)	71.5	64.2
Sample source	Deep-sea sediment, East Pacific Ocean	Freshwater cyanobacterial mat from alkaline spring

Data taken from Yurkov et al. (1994), Rainey et al. (2003), Xu et al. (2009). *NR* not reported
Additional data on growth substrates are given in the original species descriptions

E. seohaensis DSM 16221^T, *E. flavus* DSM16421^T, *E. vulgaris* DSM 17792^T, and *E. nanhaisediminis* DSM 16125^T were 62 %, 48 %, 24 %, 14 %, and 14 %, respectively (Wu et al. 2012).

P. sanguineus strains IAM 12620^T and ATCC 25661 showed DNA–DNA relatedness of 88 % against each other. In addition, the DNA relatedness between *P. sanguineus* IAM 12620^T and its phylogenetic neighbors *Erytromicrobium ramosum* DSM 8510^T, *P. tepidarius* DSM 10594^T, *P. neustonensis* DSM9434^T, *Erytrobacter litoralis* DSM8509^T, *Erytrobacter longus* IFO 14126^T, *Blastomonas natatoria* DSM 3183^T, and *Sphingomonas paucimobilis* IFO 13935^T were 38 %, 36 %, 31 %, 18 %, 14 %, 2 %, and <1 %, respectively (Hiraishi et al. 2002). DNA–DNA relatedness between *P. neustonensis* DSM 9434^T and *P. tepidarius* DSM 10594^T, *P. sanguineus* ATCC 25661 and IAM 12620^T, *Erytromicrobium ramosum* DSM 8510^T, *Erytrobacter litoralis* DSM8509^T, *Erytrobacter longus* IFO 14126^T, *Blastomonas natatoria* DSM 3183^T, and *Sphingomonas paucimobilis* IFO 13935^T were 38 %, 32 %, 29 %, 29 %, 14 %, 11 %, 4 %, and <1 %, respectively (Hiraishi et al. 2002).

DNA–DNA relatedness of *P. cryptus* ALC-2^T and ALC-3 strains against *P. neustonensis*, *P. tepidarius*, *Erythrobacter longus*, and *Erythromicrobium ramosum* were 25 %, 31 %, 35 %, 40 %, 19 %, 20 %, 22 %, and 21 %, respectively. DDH relatedness of *P. neustonensis* against *P. tepidarius*, *E. longus*, and *E. ramosum* were 24 %, 21 %, and 24 %, respectively. Values for *P. tepidarius* against *E. longus* and *E. ramosum* were 22 % and 24 %, respectively. DNA similarity between *E. longus* and *E. ramosum* was 25 % (Rainey et al. 2003). *P. donghaensis* strains SW-132^T and SW-158 exhibited a mean level of DNA–DNA relatedness of approximately 86 %. Values between *P. donghaensis* strains and the type strains of *Porphyrobacter* species (i.e., *P. neustonensis*, *P. tepidarius*, *P. sanguineus* and *P. cryptus*) were in the range of 9.4–20.3 % (Yoon et al. 2004b). Levels of DNA–DNA relatedness between *P. dokdonensis* DSW-74^T and the type strains *P. neustonensis*, *P. tepidarius*, *P. sanguineus*, *P. cryptus*, and *P. donghaensis* were in the range of 9–25 % (Yoon et al. 2006).

Genome Analysis

As of May 2013, no type strains genome sequence is available in the *Erythrobacteraceae* family. However, three genomes belonging to the *Erythrobacter* genus have been reported, but only one is completely closed. Moreover, a draft genome has also stated for the *Porphyrobacter* genus. The main features of these genomes are summarized in *Table 9.9*.

The *E. litoralis* genome, strain HTCC2594, was isolated from a depth of 10 m in the Sargasso Sea, Atlantic Ocean, and cultured in a low-nutrient heterotrophic medium. It contains one circular chromosome of 3,052,398 bp, 3.011 protein coding genes, one copy of 16S-23S-5S rRNA, 45 tRNA genes encoding 19 aminoacyl-tRNA sequences, and 63.07 % G+C content (Oh et al. 2009). No plasmids are present. Moreover, the HTCC2594 genome lacks reaction center genes for phototrophic metabolism, genes for CO_2 fixation such as ribulose-1,5-bisphosphate carboxylase/oxygenase, and components of the reverse trichloroacetic acid cycle.

Table 9.5

Comparison of selected characteristics of the members of the genus *Porphyrobacter*

Character	*P. cryptus*	*P. dokdonensis*	*P. donghaensis*	*P. neustonensis*	*P. sanguineus*	*P. tepidarius*
Type strain	DSM 12079^T = ATCC BAA-386^T	KCTC 12395^T = DSM 17193^T	KCTC 12229^T = DSM 16220^T	ACM 2844^T	ATCC 25659 ^{T} = IAM 12620^T	DSM 10595^T
Accession no 16S rRNA gene	AF465834	DQ011529	AY559428	AB033327	AB021493	AB033328
Description year	2003	2006	2004	1993	2002	1997
Strain number in the clade	2	1	2	4	2	1
Cell morphology	Short rods	Pleomorphic	Pleomorphic	Pleomorphic	Pleomorphic	Ovoid or short rods
Presence of BChl a	+	+	+	+	+	+
Oxidase	+	+	+	−	+	−
Growth in NaCl (%):	0 – NR	0–7 (2)	0–7 (2)	0 – NR	(1)	0–1.3
Growth pH:	6–9 (7.5–8)	5.5–8 (7–8)	5–8 (7–8)	(7.2)	(7–7.5)	NR
Growth temperature (°C):	<60 (50–55)	10–43 (35–37)	10–45 (30–37)	10–37 (30)	20–37 (30)	<52 (40–48)
Motility	+	−	−	+	+	−
Hydrolysis of:						
Aesculin	+	+	+	V (+)	+	+
Casein	−	−	−	V (−)	−	−
Gelatin	+	−	−	−	−	−
Starch	+	+	+	−	−	+
Tween 80	+	+	+	+	−	+
Utilization of:						
D-Fructose	−	−	−	V (−)	−	−
D-Galactose	−	−	−	+	−	−
D-Cellobiose	+	+	+	V (−)	+	+
D-Mannose	−	−	−	+	−	−
D-Trehalose	−	−	−	V (+)	+	−
D-Xylose	+	−	V (+)	+	−	−
L-Arabinose	−	−	−	−	−	−
Sucrose	+	−	V (−)	+	+	−
Acetate	−	−	V (−)	−	+	+
Succinate	−	+	+	V (+)	−	−
L-Malate	−	+	+	−	−	−
Pyruvate	+	+	+	V (+)	+	−
L-Glutamate	+	−	−	−	+	+
DNA G+C content (mol%)	66.2	65.8	65.9−66.8 (66.8)	65.7−66.4 (66.4)	63.8−64	65
Sample source and site	Hot spring, Portugal	Seawater, Korea	Seawater, Korea	Freshwater, Australian	Brackish and marine environments, Baltic sea	Brackish hot spring

Data taken from Fuerst et al. (1993), Hanada et al. (1997), Hiraishi et al. (2002), Rainey et al. (2003), Yoon et al. (2004b). *V* variable reaction; data in parentheses are for the type strain. Optimal growth conditions are in parentheses. *NR* not reported. Additional data on growth substrates are given in the original species descriptions

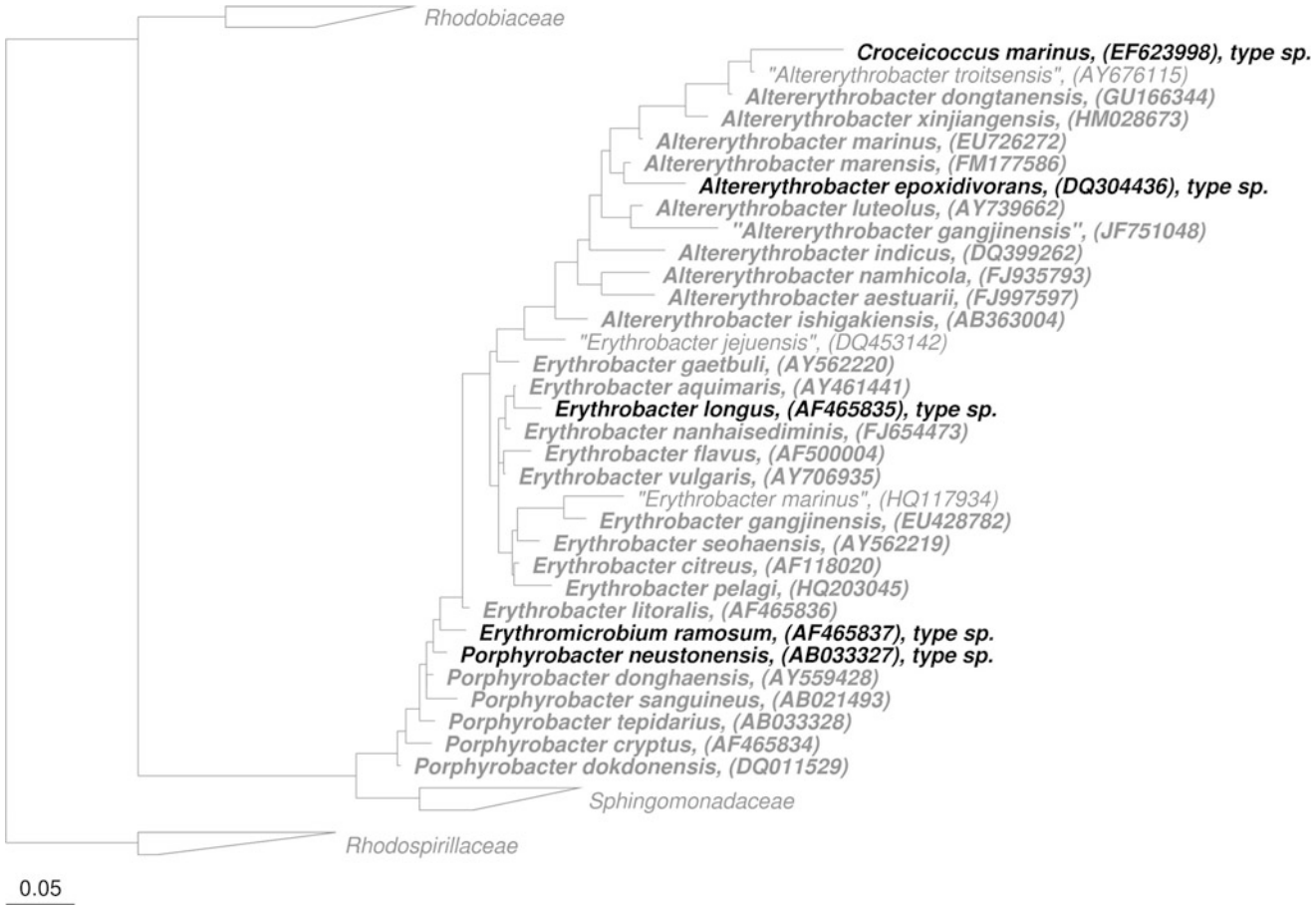

Fig. 9.1

Phylogenetic reconstruction of the family *Erythrobacteraceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, a 10 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar 2 % indicates estimated sequence divergence

The HTCC2594 strain encodes three distinct epoxide hydrolases with enantioselectivity towards styrene oxides, which may be exploited for biotechnological purposes (Woo et al. 2007; Oh et al. 2009).

So far, two other *Erythrobacter* draft genomes have been documented: *Erythrobacter* sp. strain NAP1, available as 2 scaffolds, and *Erythrobacter* sp. strain SD-21, available as 19 scaffolds. The NAP1 strain, which is an aerobic, anoxygenic, phototrophic bacterium, was isolated by plating on minimal medium from a water sample collected in the Northwest Atlantic in 2000 (Kolber et al. 2001; Koblízek et al. 2003). The genome of *Erythrobacter* sp. NAP1 consists of a single circular chromosome of 3,264,238 bp with 3.177 putative genes that cover 92 % of the genome, one copy of each of the rRNA genes, 45 genes for tRNAs, G+C content of 61 %, and a complete set of genes for bacteriochlorophyll biosynthesis and reaction center proteins (Koblízek et al. 2011). The continuous 38.9-kb-long photosynthetic gene cluster is organized as follows: *bchIDO-crtCDF-bchCXYZ-pufBALM-tspO-bch*PORF-*bchG-ppsR-ppaA-bchFNBHLM-lhaA-puhABC-acsF-puhEhemA-cycA*. Homologs of regulatory genes *ppaA* (cobalamin binding) and *ppsR* (*crtJ* homologue) are also present in the gene cluster, but most of the carotenoid biosynthesis genes are located outside the cluster (Koblízek et al. 2011). The NAP1 genome contains a full set of genes for heme biosynthesis; γ-aminolevulinic acid is synthesized through the Shemin (C4) pathway, besides a complete set of genes for the siroheme biosynthesis pathway, although final enzymes of cobalamin (vitamin B12) biosynthesis are missing.

Although the NAP1 strain lacks the genes of any autotrophic CO_2 fixation pathway (Koblízek et al. 2011), this strain may augment heterotrophic growth with light harvesting encoded by the *puf* operon and ribulose-1,5-bisphosphate carboxylase/oxygenase independent CO_2 fixation (Koblízek et al. 2003). Genes for nitrogenase or nitrate reductase are also absent in this genome, which is consistent with its inability to grow on nitrate. The NAP1 genome offers a valuable source of information for further studies focused on AAP metabolism and physiological nature (Koblízek et al. 2011).

Table 9.6
DNA–DNA hybridization similarities (%) of *Altererythrobacter* genus members

Taxon	Species name	1	2	3	4	5	6	7
1	*A. marensis* MSW-14^T	**100**						
2	*A. epoxidivorans* KCCM 42314^T	26.0–27.3[a]	**100**					
3	*A. luteolus* KCTC 12311^T	9.8–15.2[a]		**100**				
4	*A. xinjiangensis* S3-63^T				**100**			
5	*A. marinus* H32^T				54.5 ± 2[b]	**100**		
6	*A. troitsensis* KMM 6042^T						**100**	
7	*A. dongtanensis*						34.4 ± 7.6[c]	**100**

Original data:
[a]Seo and Lee (2010)
[b]Xue et al. (2012)
[c]Nedashkovskaya et al. (2013)

Table 9.7
DNA-DNA hybridization similarities (%) among type strains of the *Erythrobacter* genus

Taxon	Species name	1	2	3	4	5	6	7	8	9	10	11
1	*E. citreus* RE35F/1^T = DSM14432^T JCM 21816^T	**100**										
2	*E. litoralis* DSM 8509^T	25 ± 2[a]	**100**									
3	*E. flavus* SW-46^T = KCCM 41642^T = DSM16421^T	13.1[b]	6.0[b]	**100**								
4	*E. longus* DSM 6997^T = JCM 6170^T = ATCC 33941^T			4.2[b]	**100**							
5	*E. aquimaris* SW-110^T = KCCM 41818^T = JCM 12189^T	Range 5.3–12.7[c]				**100**						
6	*E. seohaensis* SW-135^T = JCM 21815^T = DSM 16221^T	Range 9.7–20.2[d]					**100**					
7	*E. gaetbuli* SW-161^T	Range 8.5–18.9[d]					12.3[d]	**100**				
8	***E. vulgaris*** 022-2-10^T = DSM 17792^T	37[e]	33[e]	39[e]	30[e]	33[e]			**100**			
9	*E. nanhaisediminis* T30^T = DSM 16125^T	19.7[f]			14.5[f]	56.9[f]	40.3[f]		14.7[f]	**100**		
10	*E. pelagi* UST081027-248^T	62[g]		24[g]			48[g]		14[g]	14[g]	**100**	
11	*Erythromicrobium ramosum* DSM 8510^T	20 ± 3[a]										**100**

Original data:
[a]Denner et al. (2002)
[b]Yoon et al. (2003)
[c]Yoon et al. (2004a)
[d]Yoon et al. (2005b)
[e]Ivanova et al. (2006)
[f]Xu et al. (2010)
[g]Wu et al. (2012)

Erythrobacter sp. strain SD-21 was isolated from surface sediment from the San Diego Bay, based on its ability to oxidize soluble Mn(II) to insoluble Mn(III, IV) oxides. The genome of SD-21 strain contains 2,970,874 bp, 2.941 protein coding genes that cover 85 % of the genome, one copy of the rRNA genes, 41 genes for tRNAs, and 62.9 % G+C content (http://www.ncbi.nlm.nih.gov/genome/browse/; https://moore.jcvi.org/moore/) (*Table 9.9*). The SD-21 strain was reported as the first bacterium within the alpha-4 Proteobacteria that is able to oxidize Mn(II) as well as the first marine Gram-negative bacterium containing Mn(II)-oxidizing proteins (Francis et al. 2001). In addition to being a robust Mn(II) oxidizer, SD-21 clusters with the aerobic anoxygenic phototrophs, which may comprise as much as 11 % of the microbial community in the upper ocean and exert a significant impact on global carbon cycling (Kolber et al. 2001). This nonphotosynthetic, manganese(II)-oxidizing bacterium (Francis et al. 2001; Oh et al. 2009) has a new type of manganese-oxidizing enzyme (Anderson et al. 2009) and may serve as a useful model for studying the mechanism of Mn(II) oxidation within the α-*Proteobacteria* and the biological function of bacterial Mn(II) oxidation.

Porphyrobacter sp. strain AAP82 was isolated from the Huguangyan Maar Lake in southern China. The genome of the AAP82 strain has a BioProject number in the Genebank

Table 9.8

DNA–DNA hybridization similarities (%) among type strains of the *Porphyrobacter* genus

Taxon	Species name	1	2	3	4	5	6	7	8	9	10	11
1	*P. sanguineus* IAM 12620^T = DSM11032^T	**100**		29[a]								
2	*P. tepidarius* DSM10594^T	36[a]	**100**	24[b]								
3	*P. neustonensis* DSM9434^T	31[a]	38[a]	**100**					21[b]			24[b]
4	*P. cryptus* ALC-2^T = DSM 12079^T		31[b]	25[b]	**100**							
5	*P. donghaensis* SW132^T	Range 9.4–20.3[c]				**100**						
6	*P. dokdonensis* DSW74^T	Range 9–25[d]					**100**					
7	*Erythrobacter litoralis* DSM8509^T	18[a]		14[a]				**100**				
8	*Erythrobacter longus* IFO14126^T = DSM6997^T	14[a]	22[b]	11[a]	35[b]				**100**			
9	*Blastomonas natatoria* DSM3183^T	2[a]		4[a]						**100**		
10	*Sphingomonas paucimobilis* IFO13935^T	<1[a]		<1[a]							**100**	
11	*Erythromicrobium ramosum* DSM8510^T	38[a]	24[b]	29[a]	40[b]				25[b]			**100**

Original data:
[a]Hiraishi et al. (2002)
[b]Rainey et al. (2003)
[c]Yoon et al. (2004b)
[d]Yoon et al. (2006)

Table 9.9

Properties of the sequenced genomes of members of the *Erythrobacteraceae* (as of May 2013)

	Erythrobacter litoralis HTCC2594[a]	*Erythrobacter* sp. strain NAP1[b]	*Erythrobacter* sp. strain SD-21[c,d]	*Porphyrobacter* sp. strain AAP82[d,e]
Accession number	CP000157.1	AAMW00000000	ABCG00000000	ANFX00000000
RefSeq	NC_007722.1	NZ_AAMW00000000	NZ_ABCG00000000	NZ_ANFX00000000
Genome length (bp)	3,052,398	3,264,238	2,970,874	2,899,072
G+C content	63.1	61	62.9	67.3
Protein sequence	3.011	3.177	2.941	NR
rRNA	3	3	1	3
tRNA	45	45	41	44
Gene	3.059	3223	2986	2789
Chromosomes	1	NR	NR	NR
Scaffolds	–	2	19	52

Data taken from:
[a]Oh et al. (2009)
[b]Koblízek et al. (2011)
[c]J. Craig Venter Institute (https://moore.jcvi.org/moore/)
[d]GeneBank (http://www.ncbi.nlm.nih.gov/genome/browse/)
[e]Rapid Annotation Subsystem Technology (RAST)
NR not reported

database (http://www.ncbi.nlm.nih.gov/genome/browse/), but no valid publication has been reported yet. This AAP bacterium represents an important microbial component in the upper layers of the water column in various freshwater lakes. The AAP82 strain genome is available in 52 scaffolds and contains 2,899,072 bp, 2789 coding sequences, one copy of each of the rRNA genes, 44 genes for tRNAs, and G+C content of 67.3 % (RAST server; Aziz et al. 2008).

The comparative analyses among these four genomes of the *Erythrobacteraceae* family has been represented by Genome Atlas (*Fig. 9.2*) based on the GeneWiz browser 0.94 server. *Sphingomonas wittichii* RW1 was used as the reference genome.

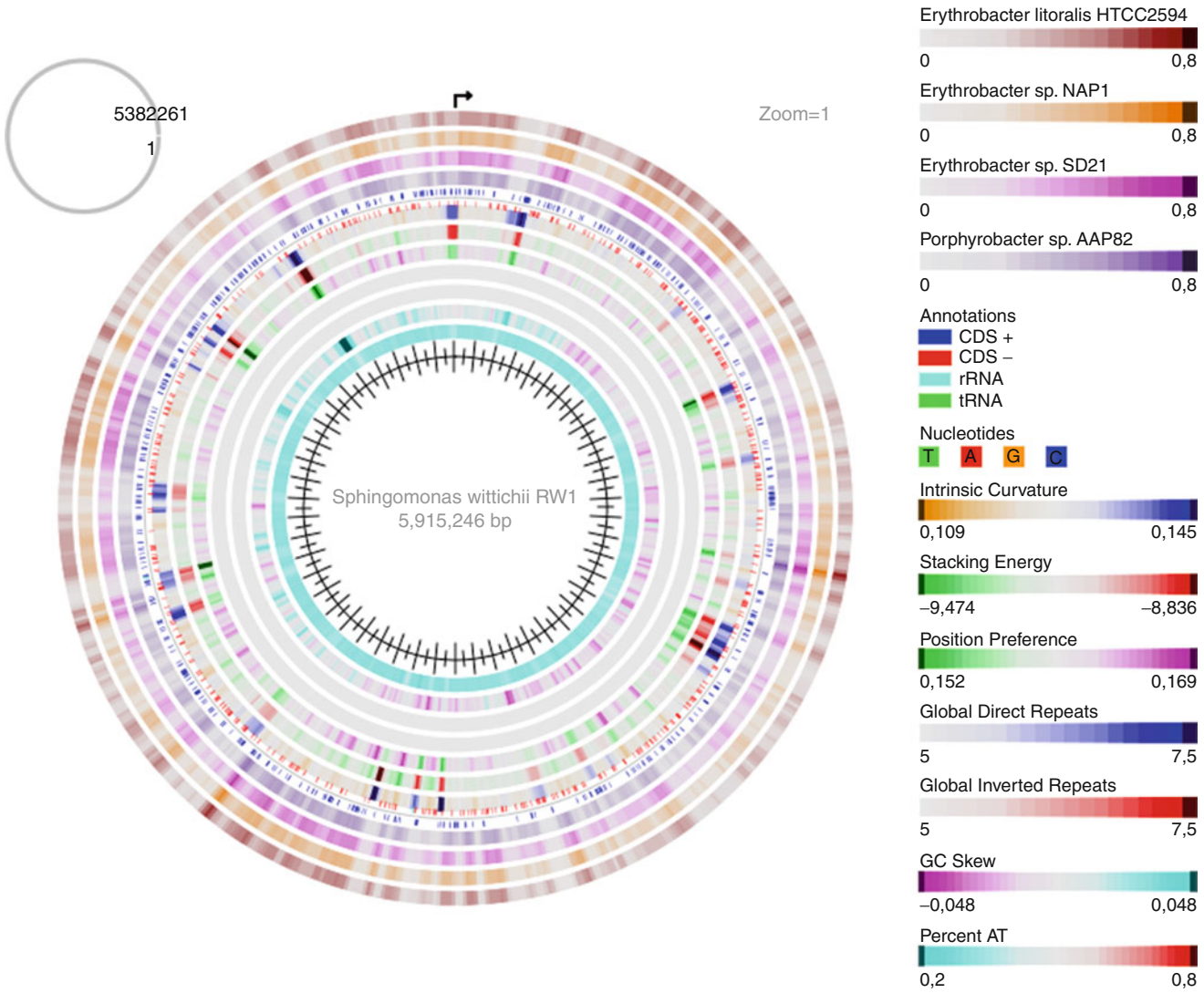

Fig. 9.2
Genome Atlas. The Atlas shows the genome comparison among *Erythrobacter litoralis* HTCC2594, *Erythrobacter* sp. strain NAP1, *Erythrobacter* sp. strain SD-21 and *Porphyrobacter* sp. strain AAP82 chromosomes. *Sphingomonas wittichii* RW1 was used as reference genome

Phages

Two prophage sequences are reported in the genome of *Erythrobacter litoralis* strain HTCC2594: a large prophage region (157,555–185,998 bp) and a medium prophage region (2,812,472–2,826,784 bp) (Oh et al. 2009). Based on the Rast Server annotation (Aziz et al. 2008), which uses the subsystem feature counts, three sequences have been allocated in the phages: a prophages category from *Erythrobacter* sp. NAP1 and two from *Erythrobacter* sp. SD-21 genomes. The main functions of the proteins in this category were phage packaging machinery and phage capsid proteins. The comparative chromosomal region for the phage genes among the three *Eryhtrobacter* strains is represented in *Fig. 9.3*. No sequence has been reported for *Porphyrobacter* sp. strain AAP82 in phages, prophages, transposable elements, or plasmids categories.

Phenotypic Analyses

Family *Erythrobacteraceae* Lee, Liu, Anzai, Kim, Aono and Oyaizu (2005). Emend Xu, Wu, Wang, Wang, Oren and Wu (2009)

Erythrobacteraceae (E.ry.thro.bac.te.ra'ceae. N.L. masc. n. *Erythrobacter* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Erythrobacteraceae* the *Erythrobacter* family.

The main features of *Erythrobacteraceae* family members are summarized below and listed in *Tables 9.1*, *9.2*, *9.3*, *9.4*, and *9.5*. The family *Erythrobacteraceae* was circumscribed on the basis of the phylogenetic analysis of 16S rRNA gene sequences, hybridizations based on similarities between DNA–DNA (DDH), and phenotypic characteristics. The family is

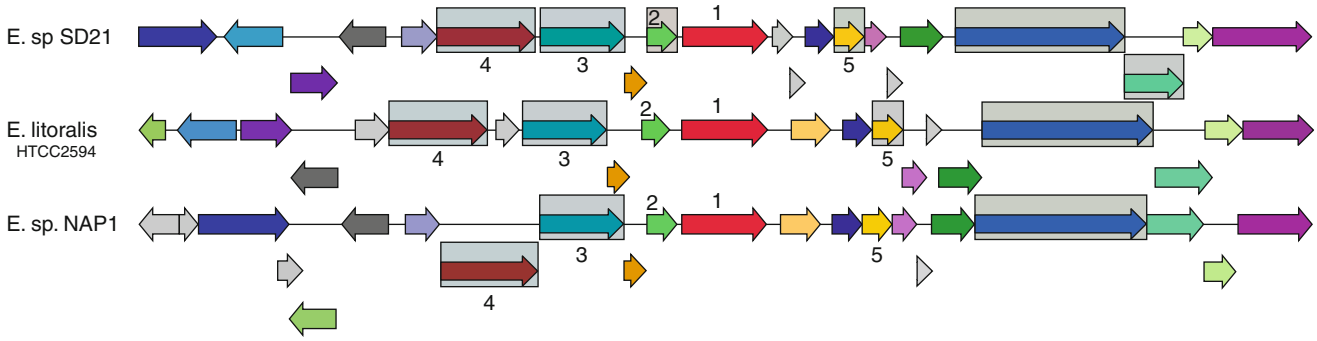

Fig. 9.3

Comparative chromosome region for phage genes among three *Erythrobacter* strains. The function predicted is represented by number: *1* Phage major capsid protein, *2* Gene transfer prohead protease, *3* Phage portal protein, *4* Gene transfer agent terminase protein, *5* Gene transfer tail protein. Sets of genes with similar sequence are grouped with the same number and color. Genes whose relative position is conserved are functionally coupled and share gray background boxes

phenotypically, metabolically, and ecologically similar. It basically includes aerobic chemoorganotrophs, but one genus may be facultative photoheterotrophs.

Type genus: *Erythrobacter.*

Genus *Erythrobacter* Shiba and Simidu (1982)

Erythrobacter (Gr. adj. *eiythrus* red; M. L. masc. n. *bacter* rod or staff; M. L. mas. n. *Erythrobacter* red rod)

Cells are yellow, orange, orange-yellow, or red-orange; ovoid to rod-shaped; and 0.3–3.0 by 0.5–5.0 um. The cells contain ubiquinone-10 (Q-10) as the predominant respiratory lipoquinone. Optimal growth occurs at temperatures between 25 °C and 37 °C and at pH values between 7.0 and 8.0. They are Gram-negative, non-spore-forming, and multiply by binary fission. Cells are motile by means of polar or subpolar flagella, or they are nonmotile. Most species contain carotenoids, require NaCl for growth, utilize glucose but do not hydrolyze starch or gelatin, and do not reduce nitrate. The Voges-Proskauer test is mostly negative. They are aerobic chemoorganotrophs; only two species present bacteriochlorophyll *a*. No growth is reported anaerobically in the light and no autotrophic growth occurs with H_2. Although small amounts of acid are produced from a wide range of carbohydrates under microaerobic conditions, metabolism is predominantly respiratory. Most species are mainly susceptible to chloramphenicol. Methanol is not utilized. Oxidase and catalase can be produced. The G+C of the DNA is 58.9–67 mol%.

Type species: *Erythrobacter longus.*

The genus currently contains 13 species, although only 2 of which were effectively published: *E. longus* (Shiba and Simidu 1982); *E. litoralis* (Yurkov et al. 1994); *E. citreus* (Denner et al. 2002); *E. flavus* (Yoon et al. 2003); *E. aquimaris* (Yoon et al. 2004a); *E. gaetbuli* and *E. seohaensis* (Yoon et al. 2005b); *E. vulgaris* (Ivanova et al. 2006); *E. gangjinensis* (Lee et al. 2010); *E. nanhaisediminis* (Xu et al. 2010); *E. pelagi* (Wu et al. 2012); "*E. jejuensis*" (Yoon et al. 2013); and "*E. marinus*" (Jung et al. 2012). *Erythrobacter luteolus* was isolated from a tidal flat of the Yellow Sea in Korea (Yoon et al. 2005a) has been transferred to *Altererythrobacter luteolus* comb. nov. (Kwon et al. 2007). *Tables 9.1* and *9.2* summarize the properties of the *Erythrobacter* genus.

Genus *Altererythrobacter* Kwon, Woo, Yang, Kang, Kang, Kim, Sato and Kato (2007). Emend Xue, Zhang, Cai, Dai, Wang, Rahman, Peng and Fang (2012)

Altererythrobacter (Al.ter.e.ryth'ro.bac'ter. L. adj. *alius*, *alterius* another, other, different; N.L. masc. n. *Erythrobacter* a genus name; N.L. masc. n. *Altererythrobacter* another or different *Erythrobacter*, because the genus shows high similarity to the genus *Erythrobacter* but does not share its phyletic line).

Cells are Gram-negative and motile or nonmotile; the color of cell suspensions and colonies can be yellow, orange-red, or ochre. Strains produce catalase and can be positive or negative for oxidase. Methanol-soluble pigments are species-dependent and are characterized by absorption maxima at 447 and 473 nm. They do not contain *BChl a* as a photosynthetic pigment. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and sphingoglycolipid. Anaerobic growth does not occur on marine agar (MA) or on MA supplemented with nitrate. The dominant fatty acid is C18:1 ω7*c*. They do not produce H_2S or indole, do not utilize manose, and do not hydrolyze gelatin. The dominant respiratory quinone is Q10. The optimal growth temperature occurs in mesophilic conditions. All species can grow in the presence of NaCl, although it is not essential in many cases. The DNA G+C content is 54.5–67.2 mol%.

Type species: *Altererythrobacter epoxidivorans.*

The genus currently contains 12 species, although only 2 of which were effectively published: *A. luteolus* (Yoon et al. 2005a; Kwon et al. 2007); *A. epoxidivorans* (Kwon et al. 2007);

A. indicus (Kumar et al. 2008); *A. marinus* (Lai et al. 2009); *A. marensis* (Seo and Lee 2010); *A. aestuarii* (Park et al. 2011); *A. dongtanensis* (Fan et al. 2011); *A. ishigakiensis* (Matsumoto et al. 2011); *A. namhicola* (Park et al. 2011); *A. xinjiangensis* (Xue et al. 2012); "*A. gangjinensis*" (Jeong et al. 2013); "*A. troitsensis*" (Nedashkovskaya et al. 2013).

Tables 9.1 and *9.3* summarize the properties of *Altererythrobacter*.

Genus *Croceicoccus* Xu, Wu, Wang, Wang, Oren and Wu (2009)

Croceicoccus [Cro.ce.i.coc'cus. L. adj. *croceus* yellow, golden; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos*) grain or berry; N.L. masc. n. *Croceicoccus* referring to a yellow coccoid-shaped bacterium].

Cells are Gram-negative and non-spore-forming cocci. Cells are motile and occur in pairs. Young cultures show pleomorphic coccoid cells (0.8–1.0 mm). Colonies on complex agar containing peptone, casamino acids, and yeast extract are 1–2 mm in diameter, circular, smooth, elevated, opaque, and yellow pigmented. They are oxidase negative, catalase positive, divide by binary division, and are capable of producing multifibrillar stalk-like fascicle structures on the cell surface. Cells are aerobic and chemoheterotrophic. They contain carotenoids but not *BChl a*. No growth occurs anaerobically in the light.

Ubiquinone-10 is the major respiratory quinone. The polar lipid profiles comprise phosphatidylglycerol, two unidentified glycolipids, phosphatidylcholine, and an unidentified phospholipid. The major fatty acids are anteiso-C_{15}: 0, iso-C_{14} : 0 and iso-C_{15} : 0. Cells are susceptible to ampicillin (10 mg), bacitracin (0.04 U), cefalexin (30 mg), ceftriaxone (30 mg), chloramphenicol (30 mg), erythromycin (15 mg), gentamicin (10 mg), minocycline (30 mg), neomycin (30 mg), novobiocin (30 mg), penicillin (10 mg), streptomycin (10 mg), and tetracycline (30 mg). The phylogenetic position is in the α-4 subgroup of the class *Alphaproteobacteria*. The G+C of the DNA of the type strain of the single species is 71.5 mol%.

Type species: *Croceicoccus marinus*.

Tables 9.1 and *9.4* summarizes the properties of *Croceicoccus marinus* and *Erythromicrobium ramosum*.

Genus *Erythromicrobium* Yurkov, Stackebrandt, Holmes, Fuerst, Hugenholtz, Golecki, Gad'on, Gorlenko, Kompantseva and Drews (1994)

Erythromicrobium (E.ry.thro.mi.cro'bi.um. Gr. adj. *erythrus*, red; Gr. adj. *micros*, small; Gr. n. *bios*, life; N.L. n. *Erythromicrobium*, red microbe).

The description of *Erythromicrobium* is supported by biochemical, morphological, and 16S rDNA sequence data. Cells are Gram-negative, rod-shaped cells, 0.6–1.0 by 1.3–2.5 μm, and usually motile by means of flagella. Branching may occur. The cells are orange and contain *BChl a* and carotenoids. The major carotenoids are the very polar compound erythroxanthin sulfate and bacteriorubixanthinal. Optimal growth occurs at temperatures between 25 °C and 30 °C and at pH values between 7.0 and 8.5. Multiplication occurs by binary or ternary fission. Cells are aerobic chemoorganotrophs and facultative photoheterotrophs. No growth occurs anaerobically in the light. Ribulose diphosphate carboxylase is not detected. No fermentation or denitrification activities occur. Methanol is not utilized. Cells are phylogenetically related to members of the *Proteobacteria* α-4 subclass. They are frequently found in freshwater habitats and are not halophilic. The G+C of the DNA of the type strain of the single species is 64.2 mol%.

Type species: *Erythromicrobium ramosum*.

Erythromicrobium ramosum is the only valid species published in this genus (Yurkov et al. 1994). "*Erythromicrobium sibiricum*," "*Erythromicrobium ursincola*", "*Erythromicrobium ezovicum*," and "*Erythromicrobium hydrolyticum*" were tentatively allocated in this genus by Yurkov et al. (1991). However, later "*E. sibiricum*" and "*E. ursincola*" were taxonomic transferred from this genus to new genera, *Sandaracinobacter* and *Erythromonas*, respectively, and validly published by Yurkov et al. in 1997. *Tables 9.1* and *9.4* summarize the properties of *Erythromicrobium ramosum* and *Croceicoccus marinus*.

Genus *Porphyrobacter* Fuerst, Hawkins, Holmes, Sly, Moore and Stackebrandt (1993)

Porphyrobacter (Por.phy.ro.bac.ter. Gr. adj. *porphyreos*, purple; Gr. n. *bacter*, rod; M.L. masc. n. *Porphyrobacter*, porphyrin-producing rod, referring to bacteriochlorophyll production).

Cells are members of the α-subclass of *Proteobacteria*. They are Gram-negative, ovoid-to-short rods, pleomorphic, 0.5–1.0 × 0.5–2.8 μm, and reproduce by polar growth, budding, or asymmetric cell division. Colonies on complex media containing peptone are circular, smooth, opaque, and orange or red. They are neutrophilic freshwater bacteria, mesophilic to moderately thermophilic. Growth occurs at 10–60 °C, with an optimum temperature between 30 °C and 37 °C for most species. Cells are motile by peritrichous flagella or nonmotile. They are capable of producing structures that are multifibrillar stalk-like fascicles and crateriforms on the cell surface. Cells do not form any type of internal membranes. Spores and capsules are not formed. They are aerobic and chemoheterotrophic.

Cells synthesize *BChl a* on low-nutrient media under aerobic and semiaerobic conditions. Carotenoids are present. They are positive for calatase, D-glucose utilization, and hydrolyze of aesculine, but negative for citrate, L-arabinose, and D-fructose utilization. Cells do not grow phototrophically under anoxic conditions in the light. Simple organic compounds, peptone, and yeast extract are used as electron donors and carbon sources. Straight-chain octadecenoic acid ($C_{18:1}$) is the major cellular fatty acid. 2-Hydroxy fatty acids and sphingoglycolipids are present, but 3-hydroxy fatty acids are absent. Ubiquinone-10 is the major quinine. Some strains may require vitamins for growth. The G+C of the DNA is 66.8–63.8 mol%.

Type species: *Porphyrobacter neustonensis.*

The genus currently contains six species with validly published names: *P. neustonensis* (Fuerst et al. 1993); *P. tepidarius* (Hanada et al. 1997); *P. sanguineus* (Ahrens 1968; Ahrens and Rheinheimer 1967; Hiraishi et al. 2002); *P. cryptus* (Rainey et al. 2003); *P. donghaensis* (Yoon et al. 2004b); *P. dokdonensis* (Yoon et al. 2006).

Tables 9.1 and *9.5* summarize the properties of the *Porphyrobacter* genus.

Isolation, Enrichment, and Maintenance Procedures

Members of this taxon have been isolated mainly from aquatic environments. However, there are also isolation reports from desert sand and rice in the *Altererythrobacter* genus. Growth media and culture conditions commonly used for the isolation of freshwater aerobic bacteria can be used for obtainment of *Erythrobacteraceae* members. Marine surface water, sediment, invertebrates, and freshwater environments are possible isolation sources for these organisms. MA and marine broth (MB) are the main culture media used to isolate and cultivate isolates. However, other media have been reported to be useful for isolation of *Erythrobacteraceae* members.

R_2A (Difco) plates supplemented with 1 % NaCl and solidified with 1.5 % gellan have been used to isolate *Altererythrobacter dongtanensis* (Fan et al. 2011). Tryptone soy agar was used to isolate *Altererythrobacter indicus* (Kumar et al. 2008) and *Altererythrobacter xinjiangensis* (Xue et al. 2012).

R_3A media solidified with agar (2 %, w/v) (Reasoner and Geldreich 1985; Williams and da Costa 1992) was used to isolate *Porphyrobacter cryptus* (Rainey et al. 2003). PE medium containing sodium glutamate, sodium succinate, sodium acetate, yeast extract, casamino acids, and a vitamin mixture (Hanada et al. 1995, 1997) supplemented with 1.5 % agar was used for isolation of *Porphyrobacter tepidarius.* Lake water agar (Franzmann and Skerman 1981) was used to isolate *Polphyrobacter neustonensis* (Fuerst et al. 1993). *P. sanguineum* strains were grown in MP medium, which consisted of marine broth and 0.5 % peptone water (1:1, vol/vol) (Hiraishi et al. 2002).

Erythromicrobium ramosum was isolated in a medium containing (per liter) 1.0 g of yeast extract, 1.0 g of bacto peptone, 1.0 g of sodium acetate, 0.3 g of KCl, 0.5 g of $MgS0_4.7H_20$, 0.05 g of $CaC1_2.2H_20$, 0.3 g of NH_4Cl, 0.3 g of K_2HP0_4, 20 pg of vitamin B12, and 1 ml of a trace element solution (Drews 1983; Yurkov et al. 1994). *Croceicoccus marinus* (Xu et al. 2009) was isolated by using ZoBell marine-casamino acids medium, which contained (per liter distilled water): NaCl, 19.45 g; $MgCl_2$, 8.8 g; Na_2SO_4, 3.24 g; $CaCl_2$, 1.8 g; KCl, 0.55 g; $NaHCO_3$, 0.16 g; $C_6H_5FeO_7$. $5H_2O$, 0.1 g; KBr, 0.08 g; $CsCl_2$, 34 mg; H_3BO_3, 22 mg; Na_2SiO_3, 4.0 mg; NaF, 2.4 mg; NH_4NO_3, 1.6 mg; Na_3PO_4, 8.0 mg; peptone, 0.5 g; yeast extract, 0.1 g; and casamino acids 0.1 g at a pH of 7.2 (ZoBell 1941).

Maintenance

It has been found that liquid cultures (taken from the late logarithmic growth phase) and agar surface cultures remained viable after storage at 4 °C for at least 2 months (Yurkov and Beatty 1998). Members of this family do not require special procedures for maintenance in medium and long-term storage. Strains may be preserved in screw-capped vials by freezing cell suspensions of liquid cultures or supplemented with MB (cultures mid-logarithmic growth phase) in 20 % (v/v) glycerol as a protective agent at −20 °C or at −70 °C. Long-term preservation is by lyophilization or by freezing cell suspensions in liquid nitrogen or at −80 °C in the presence of a cryoprotectant (Yurkov and Beatty 1998).

Physiological and Biochemical Features

Ecology

The habitat range of the family *Erythrobacteraceae* includes mainly aquatic environments. The original cultures were isolated from high-tidal seaweeds (Shiba and Simidu 1982), freshwater (Fuerst et al 1993; Yurkov et al 1994), cold-seep sediment (Kwon et al. 2007), and deep-sea sediment (Xu et al. 2009). This applies to most species described or reclassified recently. For instance, *Erythrobacter marinus*, *E. pelagi*, and *Porphyrobacter dokdonensis* were isolated from seawater (Yoon et al. 2006; Jung et al. 2012; Wu et al. 2012); *Altererythrobacter dongtanensis* and *A. aestuarii* were isolated from a tidal flat (Fan et al. 2011; Park et al. 2011), and *A. ishigakiensis* was isolated from marine sediment (Matsumoto et al. 2011). To date, all *Erythrobacter* species were retrieved from seawater environments, including marine invertebrates (*E. vulgaris*; Ivanova et al. 2006), as well as the monospecific *Croceicoccus* (*C. marinus*; Xu et al. 2009). The representative monospecific *Erythromicrobium* was isolated from freshwater (*E. ramosum*, Yurkov et al. 1994). *Porphyrobacter* genus includes members isolated from freshwater, brackish, and marine environments. To this genus belong isolates found in hot springs: *P. tepidarius* was isolated from a brackish hot spring (Hanada et al. 1997) and *P. cryptus* from a hot spring at Alcafache in central Portugal (Rainey et al. 2003). Both are moderately thermophilic. *Altererythrobacter* genus includes exceptions in addition to aquatic habitats: *A. indicus*, isolated from wild rice rhizosphere (Kumar et al. 2008) and *A. xinjiangensis*, isolated from desert sand (Xue et al. 2012).

The NCBI taxonomy browser *Erythrobacter* list includes isolates from a broader source than those of the type strains: marine aerosols (HQ188568); bacterioneuston (AY576736); coastal subseafloor (AB094461); soda pond (FN395246); biofilm on a copper-based antifouling paint (JN594622); *Scrippsiella* sp. laboratory culture (DQ486511); Dunhuang Mogao Grottoes 245# (JN244985); ancient salt deposits of the Yipinglang Salt Mine (EF177676); magnetite mine drainage (HQ652571); and chemocline of the hypersaline deep-sea Urania basin (AF321064).

The same search for *Porphyrobacter* resulted the following unusual sources: mine tailings ore and sand (JQ429465); an air sample collected 25 m above sea level (GQ484916); ozone-biological activated carbon filters for drinking water treatment (DQ884358); industrial site soil containing high amounts of heavy oil and heavy metals (HQ588835); Hamelin Pool stromatolites in Shark Bay, Australia (EF150743); aerobic fermentation of a mixture of bovine dung and urine, cow's milk, and yogurt (GQ246725); commercial nitrifying inoculum (AM236300); aquatic microbial mat from Antarctica (FR772128); arsenate resistant culture from estuary (AY788979); HAA degrading bacteria from drinking water (JN547328); oak (*Quercus alba*) leaf infusion (EF685171); ancient salt deposits of the Yipinglang Salt Mine (EF177679); and endophytic from a pine tree with Pine Wilt Disease (FJ784659). The NCBI taxonomy browser also includes *P. meromictius* isolated from a $Na_2SO_4^-$ dominated meromictic lake (Mahoney Lake in the Okanagan Valley of British Columbia, Canada) but the name is not valid (Rathgeber et al. 2007).

Erythromicrobium was found in mud volcano soil in Baratang Island, India (FN397674) and in arsenic-contaminated soil (AFI42455). The NCBI taxonomy browser also includes *E. ezovicum* and *E. hydrolyticum*, which are not valid names. These strains were reported to pertain to Vladimir Yurkov's personal collection and were related to tellurite resistance and reduction (Yurkov et al 1996). *Altererythrobacter* has also been recovered from Polycyclic Aromatic Hydrocarbons (PAHs) and crude oil degrading consortia enriched from marine sediments (GQ505272); mud volcano soil in Baratang Island, India (FN397680); aquatic microbial mat from Antarctica (FR772131); root interior of *Cymbidium goeringii* (GQ476825); and farming field soil (JN848799).

The genera *Erythrobacter*, *Porphyrobacter* and *Erythromicrobium* include AAP members. AAPs contain *BChl a* as a main light-harvesting pigment; however, in contrast to purple nonsulfur photosynthetic bacteria, these are obligate aerobes (Yurkov and Beatty 1998). They are facultative photoheterotrophs, metabolizing organic carbon when available, but are capable of photosynthetic light utilization when organic carbon is scarce. The first AAP bacteria discovered was *Erythrobacter longus* (Shiba et al. 1979; Shiba and Simidu 1982). *Porphyrobacter* and *Erythromicrobium* members present *BChl a* (Yoon et al. 2006; Yurkov et al. 1994; Yurkov and Gorlenko 1993), whereas the *Erythrobacter* genus includes 2 species (out of 13) that synthesize *BChl a*: the former isolated *E. longus* retrieved from high-tidal seaweeds of Tokyo Bay (Shiba and Simidu 1982) and *E. litoralis* found in marine cyanobacterial mat in a supralitoral zone (Yurkov et al. 1994). It had been suggested that cultivated *Erythrobacter* species represent the predominant AAPs in the upper ocean and should be main players in carbon cycling in the ocean, mainly in the euphotic zone (Shiba et al. 1991; Fenchel 2001; Kolber et al. 2001; Karl 2002). Later, culture-independent genomic analyses targeting photosynthetic operon genes (i.e., *puf*M) showed dissimilar results. No sequences recovered in Monterey Bay waters or in the central North Pacific Ocean (Karl and Lukas 1996) were similar to those of *Erythrobacter* species (Béjà et al. 2002).

An extensive study included samples from the Pacific, Atlantic, and Indian Oceans and West Pacific marginal seas, including the East and South China Seas – covering tropical, subtropical, and temperate zones, as well as coastal, shelf, and oceanic waters; it was able to account *Erythrobacter*- and *Roseobacter*-like sequences for approximately a quarter of the totality sequences obtained in the study (Jiao et al. 2007). For nearshore/offshore samples from a Pacific Ocean transect, only one station (out of three) presented sequences (32 %) most similar to those from cultured *Erythrobacter*, but altogether with *Rhodobacter* and *Roseobacter* genera (Ritchie and Johnson 2012). It is now clear that *Erythrobacter* species are not the oceanic-dominating AAP population and the counterparts include a wide variety of bacterial types. Therefore, the relative contribution of *Erythrobacter* spp. to oceanic carbon cycling may not be as large as previously suggested. The studies quantify *Bchl a*, and it is widespread among a phylogenetically diverse group – the AAP bacteria – in which oceanic representation of *Erythrobacter* spp. is not a consensus and may be variable (Béjà et al. 2002; Rathgeber et al. 2004; Venter et al. 2004; Jiao et al. 2007; Yutin et al. 2007; Ritchie and Johnson 2012).

Pathogenicity: Clinical Relevance

No information on pathogenicity for members of *Erythrobacteriaceae* is available heretofore (as of February 2013). The detection of naturally occurring β-lactamases in *Erythrobacter* spp. gave rise to the idea that this group, as many other marine bacteria, plays an important role as reservoir of β-lactam resistance genes, namely the oceanic resistome. A beta-lactamase II from *Erythrobacter litoralis* HTCC 2594 (ELbla2) showing *in silico* similarity with New Delhi metallo-beta-lactamase (MBL) was expressed in *E. coli* cells. These two mature proteins were purified from cultures and both presented as a monomer of 25 kDa. Antimicrobial susceptibility assay revealed that both shared similar substrate specificities, being sensitive to aztreonam and tigecycline (Zheng et al. 2011). Further, several chromosomally located MBLs have been identified from *Erythrobacter* spp. by bioinformatic analyses followed by cloning and expressing in *E. coli* cells. These MBLs showed a large diversity and belonged to subclasses B1 or B3. None of them could be considered a progenitor of the plasmid-mediated carbapenemases disseminated worldwide.

Clones expressing the MBL from *E. flavus* and *E. longus* presented decreased sensitivity to carbapenems (Girlich et al. 2012). It was hypothesized that resistance genes are transferred to the human gut microbiota by means of the consumption of nonsterilized seafood (e.g., seaweeds) (Hehemann et al. 2010; Zheng et al. 2011), given that the virus-like gene transfer system identified, likely Gene Transfer Agent (GTA) systems, are common in marine bacteria (e.g., *E. litoralis*) (Biers et al., 2008), and

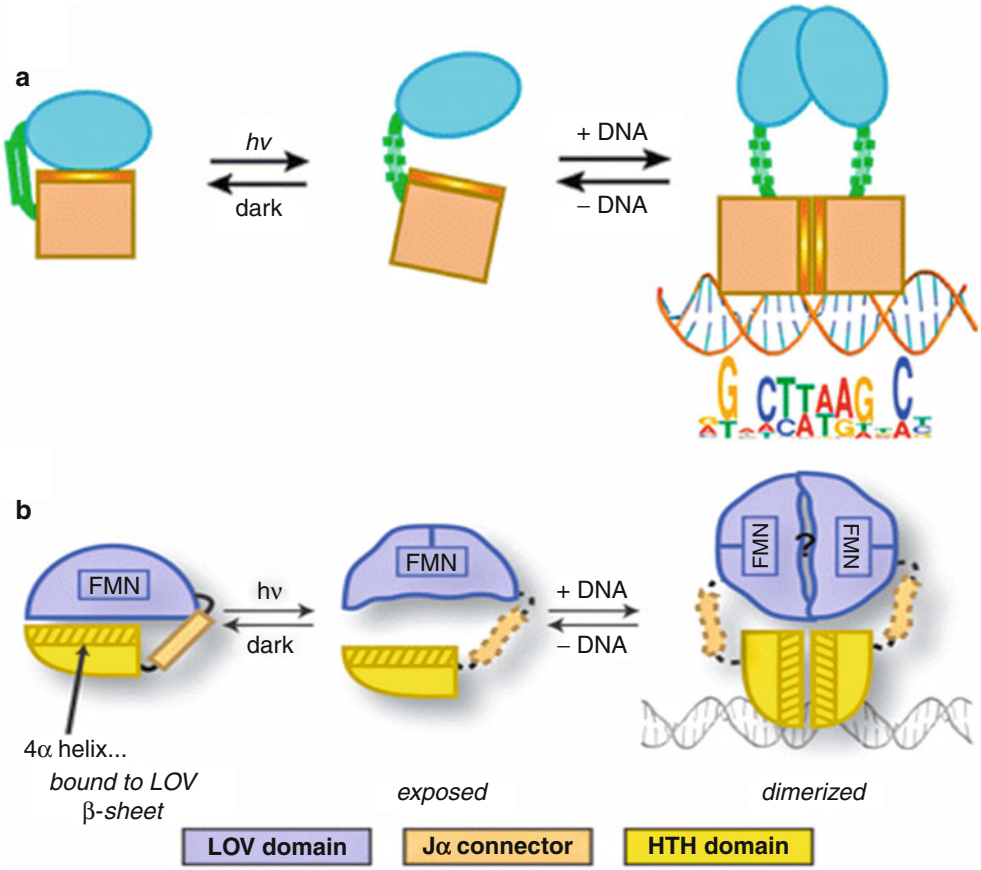

Fig. 9.4

(a) Light-oxygen-voltage (LOV) domains activation schematic model. LOV module inactive in the dark (*left*) and after blue light. activation (*center*): direct DNA binding (*right*) to regulate diverse functions (Adapted from Rivera-Cancel et al. (2012)). (b) Model for *E. litoralis* HTCC2594 LOV domain (EL222) activation. In the dark EL222 is incapable of binding DNA as the LOV domain interacts with the helix-turn-helix (HTH) module. Photo activation induces the formation of a cystein/flavin adduct in the LOV domain resulting in conformational changes that release LOV/HTH interactions. The 4α-helix is free to participate in HTH homodimerization upon binding DNA substrates (Adapted from Nash et al. (2011))

that GTAs account for an horizontal gene transfer frequency that is a million times the frequencies of transformation and transduction (McDaniel et al. 2010).

Application

A summary of the main application properties for the *Erythrobacteraceae* members is presented in *Table 9.10*.

Bioremediation

There are field and experimental evidences of the potential of *Erythrobacteraceae* members for bioremediation of heavy metals and a variety of xenobiotics, including hydrocarbons, aromatics and halogenates. For instance, *Erythrobacter* members were detected as part of the population related to alkane degradation from shoreline environments (Costa da Morte, Northwestern Spain) affected by the Prestige oil spill (Alonso-Gutiérrez et al. 2009). *Erythrobacter longus* and *E. citreus* were the closest phylogenetic neighbors related to the dominant clones identified after a bioremediated microcosm (nitrogen supplemented) experiment (Röling et al. 2002). A petroleum-aromatic-degrading active *Altererythrobacter* isolate is suggested to be a possible agent of bioremediation in and around nutrient-rich tropical marine environments (Teramoto et al. 2010). A *Porphyrobacter* strain, Oxy6 (according to 16S rRNA–based molecular phylogenetic analysis), isolated from subtropical Western Atlantic seawater, showed an ability to methyl halides degradation. This strain was even able to co-oxidize CH_3Br while growing on toluene. A variety of toluene pathway intermediates, such as benzyl alcohol, benzylaldehyde, benzoate, and catechol, could also be oxidized. Furthermore, the bacterium also oxidized *o*-xylene and the xylene monooxygenase pathway intermediate 3-methylcatechol. Considering the widespread

Table 9.10
Summary of the main application properties for the *Erythrobacteraceae* members

Bacteria name	Application	Function
Erythrobacter longus	Bioremediation	Alkane degradation[a, b]
Erythrobacter citreus		
Altererythrobacter		Petroleum-aromatic-degrading active[c]
Porphyrobacter strain – Oxy6		Methyl halides degradation[d], co-oxidize CH_3Br[d], oxidize *o*-xylene[d] and xylene monooxygenase (XMO)[d]
Erythrobacter litoralis		Resistance to tellurite and accumulation of metallic tellurium crystals[e]
Erythromicrobium ramosum		
Erythrobacter longus	Carotenoids	Twenty different carotenoids such as β-carotene and monocyclic carotenoids (e.g., rubixanthan)[f]
Erythrobacter JPCC M sp. strain 1436		Astaxanthin productivity[g]
Erythrobacter sp. strain SNB-035	Cytotoxic Compounds	Benzothiazoles Erythrazoles A-B, Erythrolic acid D (a meroterpenoid)[h]
E. litoralis strain HTCC 2594	LOV Domains	LOV-histidine kinase (LOV-HK) domain (which mediates light-activated histidine phosphorylation)[i,] EL222 DNA-binding protein (LOV domain binds and inhibits a helix-turn-helix (HTH) DNA binding domain in the dark)[j,k]

[a]Röling et al. (2002)
[b]Alonso-Gutiérrez et al. (2009)
[c]Teramoto et al. (2010)
[d]Goodwin et al. (2005)
[e]Yurkov et al. (1996), Yurkov and Beatty (1998)
[f]Takaichi et al. (1990)
[g]European Patent Register Publication Number EP 2157169A1 (2010)
[h]Hu and MacMillan (2011), Hu et al. (2012)
[i]Swartz et al. (2007)
[j]Nash et al. (2011)
[k]Rivera-Cancel et al. (2012)

inhibitory effect of toluene on seawater samples and the substrate oxidation pattern of this *Porphyrobacter* sp., the authors suggested a possible link between aromatic hydrocarbon utilization and the biogeochemical cycle of methyl halides (Goodwin et al. 2005).

Erythrobacter and *Erythromicrobium* isolates demonstrated high-level resistance to tellurite and accumulation of metallic tellurium crystals. In *Erythrobacter litoralis* and *Erythromicrobium ramosum* isolates, crystals occupied 20–30 % of the cell volume. This reduction of tellurite to the relatively inert metallic tellurium [Te(IV) to Te(0)] with accumulation as intracellular deposits is suited to the development of microbiological methods for environmental remediation. Tellurite compounds are toxic to other bacteria and other organisms, including humans. Furthermore, bacterial cells can be harvested for pure tellurium metal in the mineral ore (Yurkov et al. 1996; Yurkov and Beatty 1998).

Carotenoids

A remarkable characteristic of many members of Erythrobacteriaceae is the pigmentation (pink/red/orange/yellow) due to the production of carotenoids (Takaichi et al. 1988, 1990, 1991; Hanada et al. 1997; Yurkov et al. 1994; Denner et al. 2002; Xu et al. 2009). *Erythrobacter longus* produces approximately 20 different carotenoids, such as β-carotene and monocyclic carotenoids (e.g., rubixanthan) (Takaichi et al. 1990). The carotenoid biosynthesis genes lycopene cyclase (*crtY*) and phytoene desaturase (*crtI*), from *E. longus* strain Och101, were cloned, sequenced, and expressed in *Escherichia coli*. The *E. coli* HB101 employed contained the other required biosynthetic genes from *Erwinia herbicola*. Zeaxanthin and lycopene were produced and accumulated in the transformed cells, but transformants containing *crtY* and *crtI* genes from *E. herbicola* gave a higher pigment yield. The weak expression of *E. longus crt* genes in *E. coli* was attributed to codon usage bias (Matsumura et al. 1997).

Erythrobacter JPCC M sp. strain 1436 was included in a patent process for its astaxanthin productivity, reported to be 35 % or more by mass of the all produced pigments. According to 16S rDNA sequence similarity, the closest neighbor was *Erythrobacter luteolus* SW-109^T (GenBank AY739662), with a low nucleotide identity of 95.7 %. Based on 16S rDNA phylogeny, the authors considered *Erythrobacter* JPCCM sp. strain 1436 (accession number NITE BP-340), a novel species belonging to the genus *Erythrobacter*, at the time of priority application (April 2007). The process was entitled "Astaxanthin-producing

bacterium, bacterium culture product, astaxanthin-containing composition, and method for production of astaxanthin"; the European Patent Register Publication Number is EP 2157169 A1 (February 2010). The invention is claimed to be useful for the production of supplement foods and feedstuffs (color-enhancing feedstuffs).

Cytotoxic Compounds

Erythrobacter sp. strain SNB-035, isolated from mangrove sediments in Trinity Bay (Galveston, TX), yielded cytotoxic compounds: the benzothiazoles erythrazoles A-B and erythrolic acid D, a meroterpenoid. Cytotoxicity was observed against non-small cell lung cancer cell lines. Erythrazole B was cytotoxic, with IC_{50} values of 1.5, 2.5, and 6.8 μM against H1325, H2122, and HCC366, respectively. Erythrolic acid D was cytotoxic, with a modest IC_{50} value of 2.5 μM against HCC44. Strain SNB-035 shares 98 % 16S rRNA identity with *E. citreus*. (Hu and MacMillan 2011; Hu et al. 2012).

Light-Oxygen-Voltage Domains

Light-oxygen-voltage (LOV) domains are photosensory-signaling modules acting as blue light–dependent regulators of numerous activities, such as enzymes and DNA binding. LOV domains are widespread and highly conserved in eukaryotes, prokaryotes (including cyanobacteria), and archaeans; thus, they are suited for model-based studies. They can be readily produced in *E. coli* in amounts sufficient for biochemical and spectroscopic analysis. LOV domains can also be engineered into a variety of exogenous targets, allowing similar regulation for new protein-based reagents (Briggs et al. 2007; Möglich and Moffat 2010; Strickland et al. 2012). *Erythrobacter litoralis* presents the LOV-histidine kinase (LOV-HK) domain, which mediates light-activated histidine phosphorylation. *E. litoralis* strain HTCC 2594 has had full-length modules expressed in *E. coli* (EL346-LOV-HK and EL368-LOV-HK). The same was performed with modules from *Brucella melitensis* (animal pathogen) and *Pseudomonas syringae* (plant pathogen), allowing descriptive and comparative analysis (Swartz et al. 2007; Briggs et al. 2007; Tseng et al. 2010). EL222, a DNA-binding protein from the same E. *litoralis* strain, is also a model system. Despite its compactness (222 amino acids), it includes both sensor and effector domains. Nash et al. (2011) demonstrated that this LOV domain binds and inhibits a helix-turn-helix (HTH) DNA binding domain in the dark, releasing these interactions upon illumination. Furthermore, the same research group identified optimal DNA binding sites for EL222 and demonstrated the light-dependent activation of transcription (*Fig. 9.4*). EL222 LOV-HTH native targets are yet to be determined; nevertheless, the portability of the system, the direct regulation of DNA binding with light, together with the fact that the DNA binding sites are identified, make it attractive for engineering in heterologous systems (Rivera-Cancel et al. 2012).

Enzymes

Three epoxide hydrolases (EHases) genes (eeh1, eeh2, and eeh3) of *Erythrobacter litoralis* HTCC2594 were cloned in *E. coli*, and the recombinant proteins (rEEH1, rEEH2, and rEEH3) were purified. The functionality of purified proteins was proved by hydrolytic activities towards styrene oxide (SO). EEH1 preferentially hydrolyzed (R)-styrene oxide, whereas EEH3 preferred to hydrolyze (S)-SO, representing enantioselective hydrolysis of styrene oxide. EEH2 could hydrolyze (R)- and (S)-SO at an equal rate. The hydrolysis rate of EEH1 towards various epoxide substrates was superior to those of EEH2 or EEH3 (Woo et al. 2007). *Erythrobacter* sp. JCS358 also presents enantioselective epoxide hydrolyzing activity. Its performance was outstanding among marine strains isolated by the capability of living on SO and further screened for retaining enantioselective EHase activities towards SO (Hwang et al. 2008). *Altererythrobacter epoxidivorans* JCS350^T was described as presenting EHase activity, but it was not reported if this activity was enantioselective (Kwon et al. 2007). Pharmaceutical industries are eager for enantioselective EHases for the production of enantiopure epoxides.

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10 The Family *Holosporaceae*

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Abstract
The *Holosporaceae* family (Görtz H-D, Schmidt HJ (2005) Genus *Holospora*. In: Garrity et al. (eds) Bergey's manual of systematic of bacteriology, vol 2, part C, 2nd edn. Springer, New York, pp 149–151) in the *Rickettsiales* order (Gieszczykiewicz 1939) includes a group of gram-negative bacteria without motility and obligatory symbionts. Routinely, these bacteria occupy specific compartments in their host cells, which are generally rich in metabolites (Heckmann, K. Görtz H-D (1991) Procaryotic symbionts of ciliates. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds) The prokaryotes, 2nd edn. Springer, Berlin/Heidelberg/New York, pp 3865–3890; Fokin and Karpov, Endocyt Cell Res 11:81–94, 1995). Individuals of this family are mostly endosymbionts of ciliated protozoa of the genus *Paramecium* (Müller 1772) that parasitize, according to the species, the macronucleus and micronucleus. The family has only one consolidated genus, *Holospora*, and others seven genera considered to be *incertae sedis* in this family, including *Caedibacter, Lyticum, Odyssella, Paracedibacter, Pseudocaedibacter, Pseudolyticum*, and *Tectibacter* (Fujishima and Fujita, J Cell Sci 76:179–187, 1985). However, *Lyticum* was recently moved to the *Midichloriaceae* family (Boscaro et al., Microb Ecol 65:255–267, 2013). Among those cited, *Holospora* and *Caedibacter* are commonly studied. Thus, *H. obtusa*, *H. caryophila*, and *H. curviuscula* are parasitize the macronucleus of *P. caudatum* and *P. biaurelia*; *H. elegans*, *H. undulate*, and *H. accuminata* can be found in micronucleus of *P. caudatum* and *P. bursaria*. The specificity for the host does not occur only by the success of cell penetration, which demonstrates that the specificity for a habitat is influenced by nuclear genes (Fujishima and Fujita, J Cell Sci 76:179–187, 1985). Its distribution is still not clearly defined, but it is believed to be associated with the distribution of its host (Fokin et al., Eur J Protistol 32:19–24, 1996).

Taxonomy, Historical and Current

Short Description of the Family and Their Genera

The taxonomy was described by Garrity et al. (2004):

Domain Bacteria (Haeckel 1894) C.R. Woese et al. 1990
Phylum Proteobacteria Garrity et al. 2005
Class *Alfaproteobacteria* Garrity et al. 2006
Order *Rickettsiales* (Gieszczykiewicz 1939) Dumler et al. 2001
Family *Holosporaceae* Görtz and Schmidt 2005.

Holosporaceae Hans-Dieter Görtz and Helmut J. Schmidt 2005, 351[VP]

Ho.lo'spo.ra.ce.ae. Gr. Fem. Adj. *holos* whole, complete; Gr. N. *sporus* seed; M.L. n. *spora* spore; M.L. fem. N. *Holospora* whole spore; *-ceae* ending to denote family; *Holosporaceae* family of *Holospora* bacteria.

Type genus: *Holospora* (ex Hafkine 1890) Gromov and Ossipov 1981, 351[VP].

Genus *Holospora (ex* Hafkine 1890) nom. rev., emend. (Ho.lo'spci.ra. Gr. adj. *holos* whole, complete; Gr. noun *sporus* seed; M.L. noun *spora* spore; M.L. fem. noun *Holospora* whole spore.). This genus is consolidated within the *Holosporaceae* family, with nine *Holospora* species described (Fokin et al. 1996; Garrity et al. 2005; Rautian and Wackerow-Kouzova 2013): *Holospora caryophila, Holospora bacillata, Holospora curvata, Holospora obtusa, Holospora undulata, Holospora elegans, Holospora recta, Holospora acuminate*, and *Holospora curviuscula*.

The *Holosporaceae* family has been undergoing constant changes based on studies being conducted. However,

E. Rosenberg et al. (eds.), *The Prokaryotes – Alphaproteobacteria and Betaproteobacteria*, DOI 10.1007/978-3-642-30197-1_264,

Table 10.1

Main characteristics of *Holosporaceae* in relation to other principal families of the *Rickettsiales* order: *Rickettsiaceae* and *Anaplasmataceae*

Character	*Holosporaceae*	*Rickettsiaceae*	*Anaplasmataceae*
Morphology[a]	Two forms:	Diplococcus-shaped, rod shaped, coccoid	Two forms: dense-core and reticulate bodies
	Reproductive form (short rod, 1.0–3.0 × 0.5 μm)		The mulberry-like appearance of the inclusions has led to the use of the term "morulae"
	Long infectious form (5.0–20.0 μm)		
Gram type	Gram negative[a]	Gram negative[a]	Gram negative[a]
Motility	Nonmotile[a]	Only *Candidatus* Trichorickettsia mobilis and *Candidatus* Gigarickettsia flagellate (these organisms possess flagella and swimming behavior inside the host cell)[b]	Nonmotile[a]
		The other specimens of this family are not motile[a]	
Acting	Obligate symbiont of protist[a]; invade host nuclei or cytoplasm[c]	Obligately intracellular (residing free in the cytoplasm or infecting the nucleus of eukaryotic host cell, depending on the species)[a]	Obligately intracellular[a] (the organism grows within cytoplasmic vacuole, infects many types of cells, as erythrocytes, granulocytes, platelets, mononuclear phagocytes, trematodes cells[d], arthropods ovaries[e])
G + C content of DNA (Mol%)	Not determined[a]	29–33[a]	30–56[a]

[a]Garrity et al. 2005
[b]Vannini et al. 2014
[c]Vakkerov-Kouzova and Rautian 2011
[d]Occur in *Neorickettsia* genus
[e]Occur in *Wolbachia* genus

independent of these suggestions and assumptions, the previous classification has been maintained. This family is included in the order *Rickettsiales*; its particular characteristics are compared with neighboring families of this order, as described in *Table 10.1*.

Regarding the context of the evolution of the whole order *Rickettsiales* (Weinert et al. 2009), a molecular clock to date the divergence of different groups showed that the common ancestor was presumably free-living. According to Weinert et al. (2009), approximately 525–775 million years ago there was a transition to living within cells, followed by a split into agent groups that originated in the *Rickettsiales* order, endosymbionts of protists (*Holospora*) and a clade that primarily infects arthropods.

Phylogenetic Structure of the Family and Its Genera

Holosporaceae is a family that belongs to *Rickettsiales* order, which has two more families, *Anaplasmataceae* and *Rickettsiaceae*, that have being known for a long time. Two new families were proposed recently: *Pelagibacteraceae* (Thrash et al. 2011) and *Midichloriaceae* (Montagna et al. 2013) (*Fig. 10.1*).

Although Garrity et al. (2004) infer that, with the exception of *Holospora*, all should be listed as "genus *incertae sedis*", many studies (Birtles et al. 2000; Baker et al. 2003; Garrity et al. 2005; Eschbach et al. 2009; Georgiades et al. 2011; Nunan et al. 2013; Boscaro et al. 2013) try to prove that other genera may be included, such as *Candidatus* Captivus, *Candidatus* Gortzia, *Candidatus* Hepatobacter, *Candidatus* Paraholospora, *Candidatus* Paracaedibacter, *Caedibacter*, *Odyssella*, and others unclassified *Holosporaceae*.

According to Lee et al. (2005), it is ambiguous to classify endosymbionts as a taxon based on 16S rRNA gene sequence analysis because of the inconsistency in defining the boundaries of a taxon with low bootstrap values, such as those found in this study (69 %). They suggest that more extensive studies for endosymbionts should be carried out to allow definite taxonomic conclusions to be made.

Ferla et al. (2013) studied phylogenies with a new rRNA gene based on the *Alphaproteobacteria* to provide perspective on major groups, mitochondrial ancestry, and phylogenetic instability. They proposed three subclasses within the *Alphaproteobacteria*: the *Caulobacteridae*, the *Rickettsidae*, and the *Magnetococcidae*. They concluded that the *Holosporaceae* did not belong more to the *Rickettsiales*, so a new order should be created, named *Holosporales* and belonging to Caulobacteridae. This would insert the *Holosporaceae* family into this classification (*Fig. 10.2*). However, this study is still very recent and additional proposals may emerge with new studies on phylogeny.

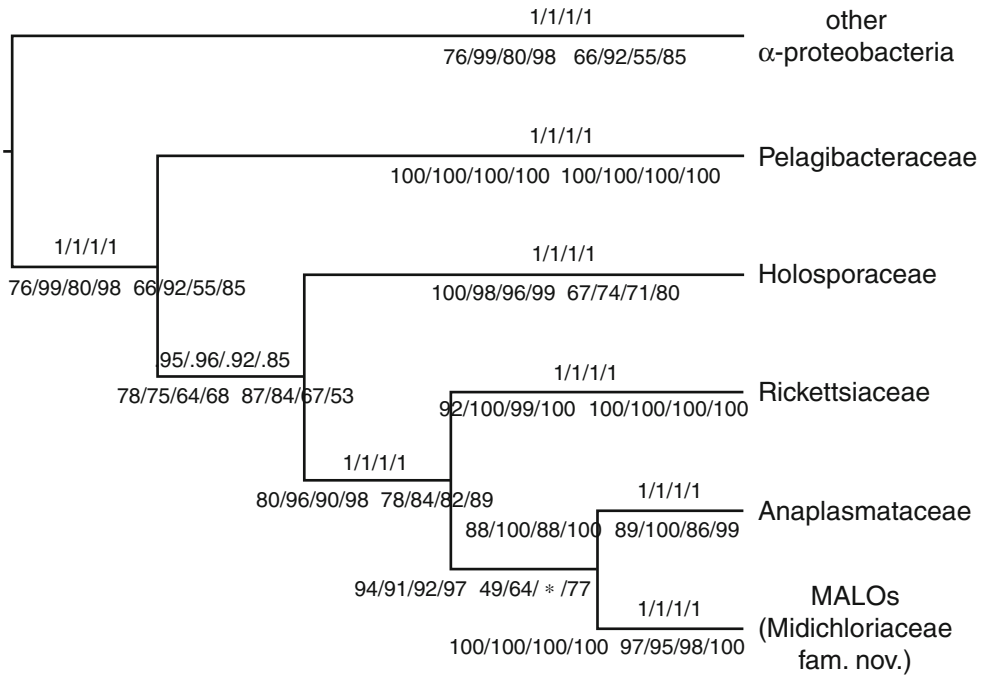

Fig. 10.1
Cladogram of the four *Rickettsiales* families, including *Holosporaceae* and *Midichloriaceae*, based on four 16S rRNA gene sequencing. The *asterisk* represents the sole branch that does not agree with the majority-rule consensus cladogram (Montagna et al. 2013)

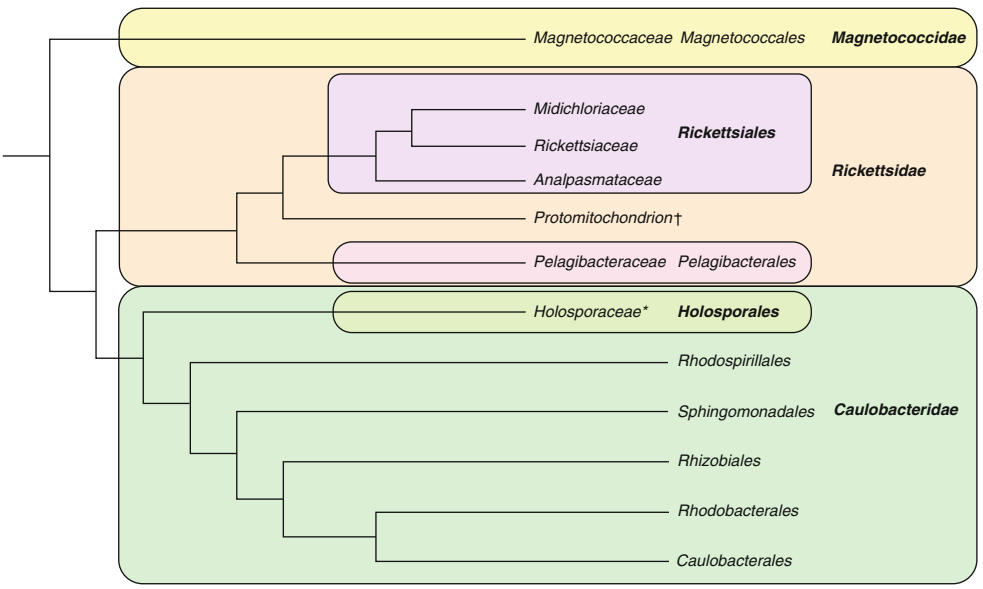

Fig. 10.2
Proposed subclasses of the *Alphaproteobacteria* (Ferla et al. 2013)

Molecular Analyses

The main studies on alphaproteocbacteria focus on the possible association with mitochondrial origin (Ferla et al. 2013) and the relationship of the symbiosis of species belonging to the *Holosporaceae* family with free-living protists. A study carried out by Lang et al. (2005), which analyzed the *H. obtusa* genome, showed that α-proteobacterial endosymbionts represent an unpredicted extensive diversity, both phylogenetically and metabolically. According to these authors, the complete genome sequence of *H. obtusa* will be the key to better understanding eukaryotic host–pathogen interactions. These results plus new investigations may also be able to provide insight into the early history of mitochondrial endosymbiosis.

Concerning *Holosporaceae*-like endosymbionts, many studies use microorganisms belonging to the *Holospora* genus to understand the mechanism of infection of Paramecia (Nunan et al. 2013) and investigate the effects on different aspects of parasite life history (Nidelet et al. 2009), for example. In a study with *Rickttsiales*-like endosymbionts, Baker et al. (2003) detected intervening sequences (IVSs) in their 16S rRNA genes. They concluded that IVSs are uncommon in rRNA genes and seem to be restricted to bacteria that live in close association with eukaryotes. In this study, the secondary structure predictions of the IVSs were made with DNA mfold analysis, a web server that is used for nucleic acid folding and hybridization prediction (Baker et al. 2003). The function of IVSs remains unknown (Pronk and Sanderson 2001), but their formation may be part of the overall process of adapting to a close working relationship with a eukaryotic host, which in bacterial endosymbionts includes genome reduction and lateral gene transfer to hosts (Andersson et al. 1998). Perhaps the IVSs specifically contribute to the likely increased communication that is required between the host and endosymbiont. In support of this hypothesis, IVSs investigated at the RNA level are all excised from the transcribed rRNA and have pronounced secondary structure suggestive of a functional RNA independent of the ribosome, but transcriptionally linked to protein production in the endosymbiont and, in some cases, the host.

Analysis of molecular variance (AMOVA) is a technique of estimating population differentiation directly from molecular information and testing suppositions about such diversity. A multiplicity of molecular data, including molecular marker data (e.g., Restriction fragment length polymorphism (RFLP) or Amplified fragment length polymorphism (AFLP)), direct sequence data, and phylogenetic trees based on such molecular information, may be analyzed using this method (Excoffier et al. 1992). A study based on the analysis of all 16S rRNA gene sequences for available *Rickettsiales* taxonomically plus 16S rRNA gene sequences available for a new family description (Montagna et al. 2013) used the method mentioned above, which was used to perform this analysis for the four *Rickttsiales* families.

Different methods are used for molecular analysis, such as AMOVA. The generalized mixed yule coalescent (GMYC) method is a likelihood method for delimiting species by fitting within- and between-species branching models to reconstructed gene trees (Fujisawa and Barraclough 2013). Principal-coordinate analysis (PCoA) (Gower 1966) is another common ordination method for data organized into distance matrices (Sneath and Sokal 1973); it is used to place the analyzed species/operational taxonomic units in a new coordinate system.

An analysis by Montagna et al. (2013) aimed to detect evolutionarily significant units within the *Rickettsiales* and the *Midichloria* and like organisms clades through the GMYC method. These authors developed for the detection of evolutionary significant units within a given data set based on a maximum-likelihood approach, which has also been applied to a 16S rRNA gene sequence data set from prokaryotes. The results obtained by different approaches (i.e., phylogeny, GMYC, PCoA, and AMOVA) indicate that current evidence places the family of ciliate-infecting bacteria *Holosporaceae* as the sister group of the lineage leading to *Rickettsiaceae*, *Anaplasmataceae*, and "*Candidatus* Midichloriaceae" Montagna et al. (2013). Although the method has been widely used, it has not previously been described in detail or evaluated fully against simulations of alternative scenarios of true patterns of population variation and divergence between species. Thus, the findings of Fujisawa and Barraclough (2013) support the robustness of GMYC as a tool for delimiting species when only single-locus information is available.

According to Reid and Carstens (2012), the GMYC model can be useful under a wide variety of circumstances, particularly in cases where divergences are deeper or taxon sampling is incomplete, as in many studies of ecological communities. However, in accordance with expectations from coalescent theory, rapid recent radiations may yield inaccurate results.

Fluorescence in situ hybridization (FISH) is a powerful technique used in the detection of chromosomal abnormalities. The high sensitivity and specificity of FISH and the speed with which the assays can be performed have made this technique a pivotal cytogenetic technique that has provided significant advances in biomedical research (Bishop 2010).

In a study by Boscaro et al. (2013) of the *Rickettsiales* order, the FISH technique was used with transmission electron microscopy, 16S rRNA gene sequencing, and phylogenetic analysis to reassess the de *Lyticum* genus. Hybridizations were performed with the specific oligonucleotide probes and Paramecium strains containing different alphaproteobacterial symbionts were employed as negative controls. This genus was framed as "*incertae sedis*" within the *Holosporacea* family (Garrity et al 2005). This research work using different techniques, including FISH, defined this genus as belonging to a new *Midichloriaceae* family (*Fig. 10.3*) (Boscaro et al. 2013).

Other molecular research was undertaken for the *Holosporaceae* family. Sequences of 1,312 and 1,293 bp were obtained for two main species, *H. obtusa* and *H. undulata*, respectively. The similarity values calculated by BLASTN with the sequence of *H. obtusa* X58198 were 99.5 % and 98.5 % (Boscaro et al. 2013). No data were found about the number of chromosomes, although *H. obtusa* and *H. undulata* were included in GenBank (NCBI 2014).

Genome

Dorah and collaborators (2013) presented the draft genome sequence of *H. undulata* strain HU1, which provides insight into the symbiotic strategy of this organism. This genome was sequenced and found to contain 1,420 protein-coding sequences, of which 650 (46 %) are hypothetical proteins without any known functions. Among the 1,420 proteins, 857 proteins (60 %) were assigned to different functional categories of National Center for Biotechnology Information (NCBI) Clusters of Orthologous Groups (COG). Eighty-six

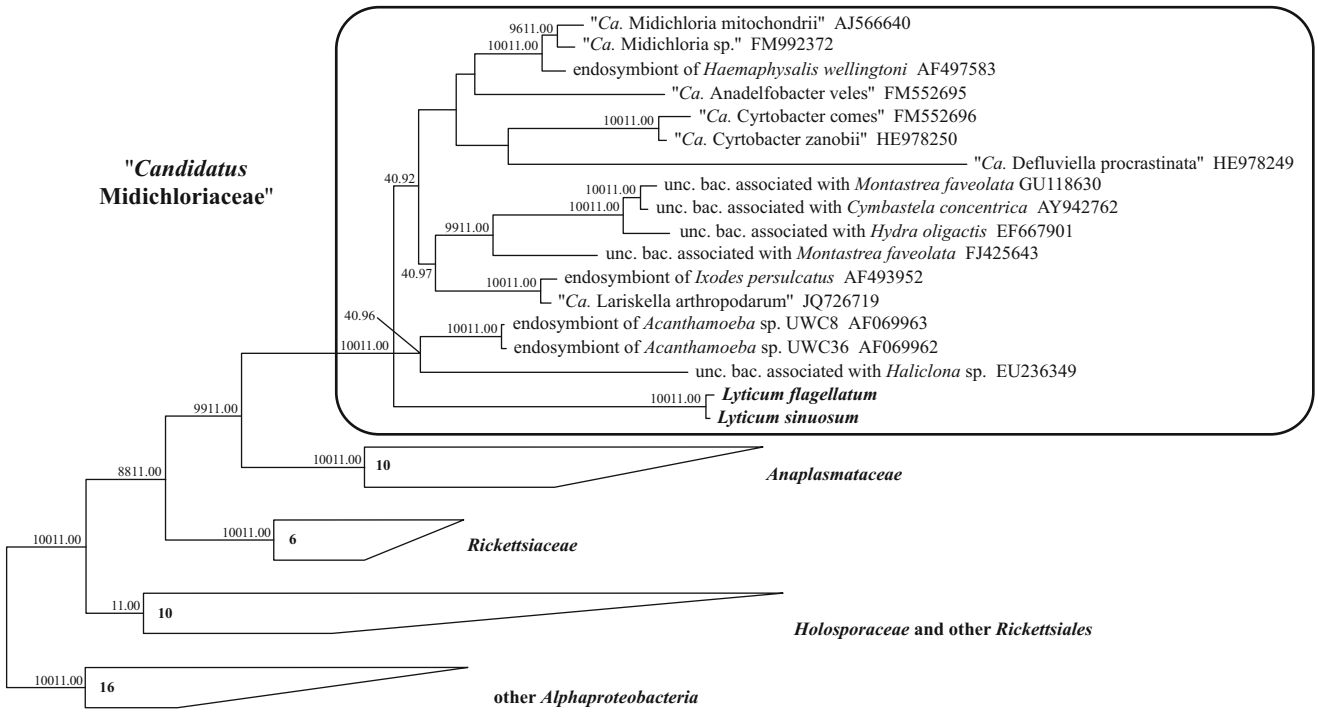

Fig. 10.3
Phylogenetic position of *Lyticum* species (Boscaro et al. 2013)

proteins were allocated to multiple COG categories. The most plentiful COG category was "Replication, recombination, and repair" (186 proteins) followed by "Translation, ribosomal structure, and biogenesis" (125 proteins), and "Cell wall/membrane/envelope biogenesis" (86 proteins). This genome represents a valuable resource for future genomic studies. The sequencing results are archived in the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (DRA) database with accession number DRA001008. The *H. undulata* strain HU1 draft genome sequence has been included in GenBank with the accession number ARPM00000000 (Dorah et al. 2013).

In the NCBI database, currently the only species described with the *Holosporaceae* genome were *H. obtusa* and *H. undulate*, and both were deposited by Yamaguchi University. For the *H. obtusa* F1 strain, the total sequence length is nearly 1,334, the number of contigs is 91, and the Contig N50 has a total of 24,415. The accession number is GCA_000469665 (NCBI 2014c). The *H. undulate* HU1 strain, after actualizations, was deposited in GenBank with accession number GCA_000388175, with a total sequence length nearly of 1,402; the number of contigs represents 208, and Contig N50 had a total of 10,714 (NCBI 2014c).

Phages

It is not clear if phages exist in the *Holosporaceae* family. However, the presence of phage particles has been found in infections initiated by *Rickettsiales*-like organisms in marine bivalves (Darriba et al. 2012).

No descriptions for the *Holospora* species were found. However, endosymbiotic bacteria belong to *Caedibacter* genus, which is a "*incertae sedis*" genus of *Rickettsiales*; they were observed to inhabit the cytoplasm of the freshwater ciliate *Paramecium novaurelia*. The bacteria conferred a killer trait to their host. The production of a proteinaceous inclusion body ("R-body") in the bacterial cell makes them toxic to other paramecia after they become enclosed in food vacuoles. "R-bodies" of *Caedibacter* spp. were associated with phages, which are known in most other *Caedibacter* species to code for the R-body proteins (Kusch et al. 2000). Knowledge of the evolutionary history of *Caedibacter* will increase understanding of its species. In this unique symbiotic system, plasmids or phages induce bacterial hosts to produce "R bodies" and associated toxins, which provide their eukaryotic host cells with a selective advantage against closely related ciliates (Beier et al. 2002).

Phenotypic Analyses

A mutualistic relationship between bacteria and ciliate protozoa is very common (Görtz 1983; Fokin 2004). The bacterial symbionts have a short reproductive form (1.5–2 μm in length) and a long infectious form (5–20 μm in length) in their lifecycle. The short infectious form is observed in the nuclei of host cells,

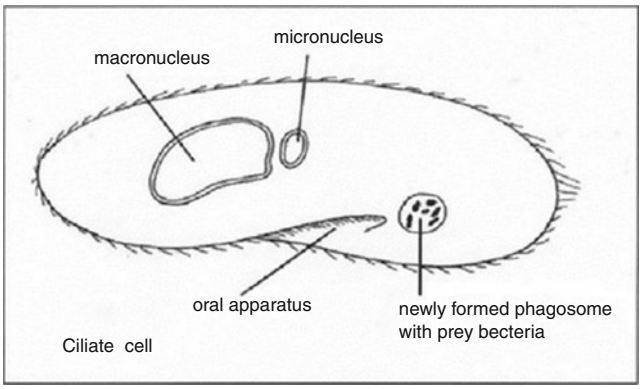

Fig. 10.4
The ciliate protozoa. Macronucleus and micronucleus are encircled by the nuclear envelope. The perinuclear space, between the nuclear envelope and the nucleus, can be colonized by bacterial symbionts. The oral apparatus (or cytostome) is where phagocytosis takes place (digestive vacuole) (Görtz 2006)

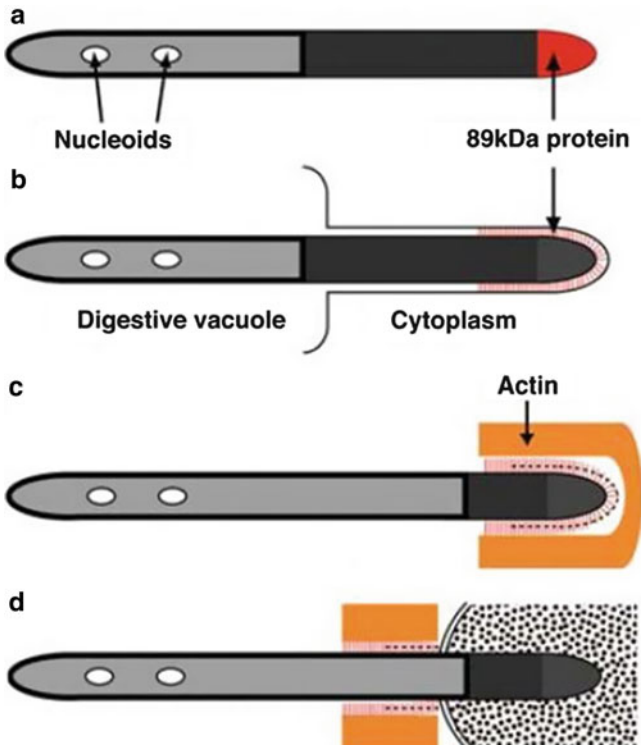

Fig. 10.5
The Infectious Form (IF) cell of *Holospora obtusa* model shows the 89-kDa-protein translocation and actin-based movement. (a) The 89-kDa-protein in the invasion tip of *H. obtusa* IF cell. (b) The vacuole membrane surrounds the bacteria starting from the invasion tip, forming the digestive vacuole in the host cell. A fibrous structure is formed between the bacteria and digestive vacuole membrane. (c) The host actin accumulates around the invasion tip in the cytoplasm. (d) In the macronucleus, the 89-kDa-protein, the actin, and the fibrous products are left behind (Masahiro 2009)

mainly in vegetative growth. The long infectious form is observed in the nuclei of starved cells; this can only infect the specific nucleus through the food vacuole, where a homogenate of bacteria-bearing cells is added to homogenized bacteria-free cells (Fujishima and Gortz 1983).

Holospora obtusa, an extensively studied obligate macronuclear endobiont of the ciliate *Paramecium caudatum*, has an infectious stage of rod-like cells (10–20 μm long) and a vegetative stage (1–3 μm long) of short cells. Morphologically, three compartments can be distinguished: the nucleoplasm, the periplasm, and the invasion tip (Görtz and Wiemann 1989).

The long infectious form of *H. obtusa* and *Holospora elegans* always enters the nuclear membrane with one of its ends. This special end of the bacterium, responsible for the nucleus entrance, can be distinguished from the other end by optical and electron microscopy as non-refractive and electron lucent. This special end is never observed in reproductive short forms (Görtz 1983). This end of *Holospora* appears to have a role in nucleus infectivity and specificity in the flagellates' protozoa. The process of entering the macronucleus or micronucleus takes about 1 h to occur (Görtz 1986), depending on the bacteria species.

Members of the spotted fever group in *Rickettsiaceae* induce polymerization of the host actin in entering hosts' cytoplasm (Heinzen et al. 1993, 1999; Gouin et al. 1999). A similar mechanism is used by *Holospora*, which forms a comet-tail-like structure around the periplasm when it is migrating through the host cytoplasm (Görtz and Wiemann 1989); the 89-kDa-protein in the special end has two actin-binding motifs. In addition of the 89-kDa-protein, this special end is called the invasion tip; it forms a fibrous structure between the bacteria and the digestive vacuole (*Fig. 10.4*). This is important for polymerization of the host's actin, the escape from the host's digestive vacuole, and the invasion of host's macronucleus (*Fig. 10.5*).

On the second day after infection of the ciliate protozoa by bacteria, almost all bacteria are still in long shapes. If these cells are transferred to a culture medium at this stage, the distribution of bacteria occurs to daughter cells without multiplication. On the third day after infection, the bacteria are in long shapes of 15 μm in length, producing about 10 reproductive short forms of 1–5 μm in length. This short form can multiply by binary fission in the host macronucleus (Fujishima and Fujita 1985).

After infection of the *Paramecium multimicronucleatum* macronucleus, bacteria multiply for 2–5 days, forming aggregates. These aggregates disappear from the host cell macronucleus around 6–12 h after the development of the first aggregate; this phenomenon occurs synchronously in all infected cells. It is still not clear if the bacteria disappear within the macronucleus or after migrating to the cytoplasm (Fujishima and Fujita 1985). Fokin et al. (2005) indicated that *P. multimicronucleatum* cells have some kind of defensive reaction against bacterial infection.

Isolation, Enrichment and Maintenance Procedures

Isolation and Enrichment

Ecology

About the biologic cycle, bacteria from this family reproduce into protozoans and possess two forms: reproductive (short cells) and infecting (long cells). In experiments with *P. multimicronucleatum*, Fok and Alen (1988) observed that the digestive vacuole (DV) of *Paramecium* can be classified into four stages, according to the penetration of infective forms of *Holospora*. When the long cells penetrate in the first form of DV (DVI), the fusion of acidisome (the organelle function of producing medium acidification) occurs. This acidification causes the bacteria to be expelled from the DVI and migrate into the macro- and micronuclei. On reaching the macronucleus or micronucleus (*H. obtusa* and *H. undulata*, respectively), the short form is multiplied by binary fission, subsequently to suffer differentiation and elongation to give rise to the long (infective) form, with the latter being able to parasite a new cell. This multiplication follows the growth of the protozoan and ceases when *Paramecium* is ingested by a host. The inhibition of protein synthesis occurs (interrupting the multiplication process) and begins the maintenance phase, distributing short forms of the nucleus of the protozoan until saturation of the medium occurs, thus promoting the death of the parasite. This allows new infectious forms to fall into the extracellular environment, which are able to infect a new cell (Dohra and Fujishima 1999; Fujishima et al. 2012). Still in the reproduction process, the infecting forms are expelled from the DVI and possibly penetrate other phases of DV, but they are not able to multiply inside the *Paramecium* and they are excreted through the cytoproct (*Fig. 10.6*).

The *Holospora* genus is composed mainly of two species, *H. undulata* and *H. obtusa*. The major differences between the species are related to the cellular type that is parasitic and to the size of its evolutive forms. *H. undulata* develops in the micronucleus of *P. caudatum*; the vegetative cells are slender and are up to 3 μm in length, whereas the infecting forms are sinuous filaments about 16 μm in length. *H. obtusa* develops in the macronucleus of *P. caudatum*; the vegetative portion is rounded and is about 3 μm in length, whereas infecting cells are lines and long, approximately 18 μm in length (Gromov and Ossipov 1981).

Caedibacter, as cited above, is am "incertae sedis" genus. It is the only one capable of producing refrateis inclusion bodies. These bodies are extended approximately 10 μm in length and are coils within the bacterial cells. As a cell is phagocytized, its bodies are unwound. These bodies are structures that possess the function of producing a major infection in the parasitized protozoan. When the *Paramecium* is parasitized by *Caedibacter*, it

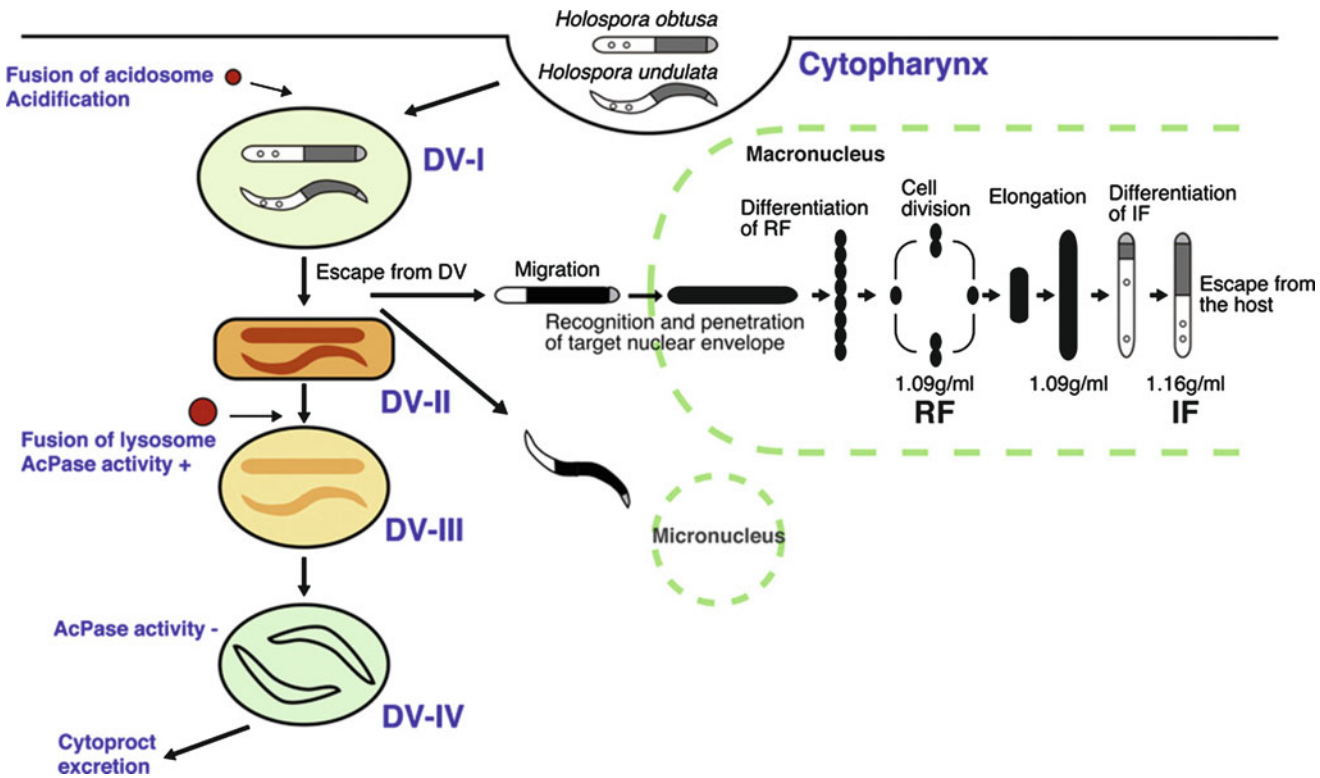

Fig. 10.6
Infection process and lifecycle of the *Holospora* species (Fujishima and Kodama 2012)

gets an ecological advantage in the niche it inhabits. These bacteria are eliminated permanently through a structure called the cytopyge. In *Paramecium* environments, when non-parasitized agents ingest bacteria, a change occurs in the pH of unfolding inclusion bodies, promoting a pathogenic action to *Paramecium* to be free of *Caedibacter*. This mechanism gives the parasitized *Paramecium* a selective advantage over competitors (Beier et al. 2002; Schrallhammer 2010). Morphologically, *Caedibacter taeniospiralis* presents rods of 0.4–0.7 × 1.0–2.5 μm, which are found at cytoplasm of *P. tetraurelia. C. caryophilus* is found in the macronucleus of *P. caudatum*, with refractory bodies measuring 1–1.5 × 0.4 μm. *C. paraconjugatus* show small rods and less than 1 % of cell have refractory bodies, having little infectivity to other *Paramecium*; they are found mainly in *P. biaurelia* (Schrallhammer 2010).

Members of this family have difficulty growing in a cultivated medium (Schmidt et al. 1987; Amann et al. 1991; Springer et al. 1993). When these bacteria are cryopreserved, good viability is observed without loss of infectivity. *H. obtusa* remained viable at −85 °C and −20 °C for up to 24 weeks (Fujishima et al. 1991). Fujishima et al. (1991) observed that the infectivity of *Holospora* is influenced by variations in its physical-chemical conditions because of verified major changes when subjected to high temperatures and large variations in pH. Thus, the bacteria lose their infective power when the temperature reaches around 30 °C and when pH undergoes major changes. However, the same study found that enzymes and other chemicals do not exert significant effects on *Holospora* infection in protozoa.

Pathogenicity and Clinical Significance

Members of this family are parasitic protozoan ciliates. Thus, they do not having pathogenicity or clinical relevance to vertebrates. However, they do act as causative agents in a poly-infection of necrotizing Hepatopancreatite of shrimp, which is a disease caused by *H. obtusa* and *C. caryophilus* that causes losses in shrimp production in Texas (Loy et al. 1996).

Application

The *Holosporaceae* family is highlighted in studies involving ecological relationships. It is a suitable model for research involving endosymbiosis because the relationship between *Paramecium* and *Holospora* is one of the most successfully studied to elucidate issues related to the origin of mitochondria. It is known that this organelle evolved from endosymbiosis between a unicellular organism and α-proteobacteria. This theory was enhanced when it was determined that the mitochondrial DNA (mtDNA) has a bacterial genome. Knowledge about α-proteobacteria, which was originally the mitochondria, is still a mystery despite persistent research (Burger and Lang 2003; Lang et al. 2005). Hori and Fujishima (2003) demonstrated that these bacteria promote the expression of heat shock protein hsp70, which is involved in stress-related physiological responses caused by variations in temperature and salinity. This increases the survival of these protozoa, which are extremely sensitive to such variations (Wichterman 1986).

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11 The Family *Hyphomicrobiaceae*

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Abstract
The family *Hyphomicrobiaceae*, first proposed in 1950, is affiliated with the *Alphaproteobacteria*. Currently (June 2012) it

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encompasses 18 genera: *Hyphomicrobium* [type genus], *Ancalomicrobium*, *Angulomicrobium*, *Aquabacter*, *Blastochloris*, *Cucumibacter*, *Devosia*, *Dichotomicrobium*, *Filomicrobium*, *Maritalea*, *Methylorhabdus*, *Pedomicrobium*, *Pelagibacterium*, *Prosthecomicrobium*, *Rhodomicrobium*, *Rhodoplanes*, *Seliberia*, and *Vasilyevaea*, with a total of 54 species. Morphologically and physiologically the family is highly diverse. Many representatives are characterized by the presence of prosthecae, and many species divide by budding. Many prosthecate species are oligocarbophilic, thriving only in the presence of low concentrations of suitable carbon sources and unable to grow in rich media. Most are aerobic chemoheterotrophs. A few representatives can grow anaerobically by denitrification or mixed-acid fermentation. *Blastochloris*, *Rhodomicrobium*, and *Rhodoplanes* are genera of facultative photoheterotrophs. Facultative chemolithotrophy with hydrogen and/or reduced sulfur compounds is also encountered. Representatives of the family can be found worldwide in soils, freshwater lakes and streams, and also in the marine environment.

Taxonomy, Historical and Current

Family *Hyphomicrobiaceae* (Babudieri 1950 589[AL])

Hy.pho.mi.cro.bi.a.ce'ae. N.L. neut. n. *Hyphomicrobium*, type genus of the family; *-aceae* ending to denote a family; N.L. fem. n. *Hyphomicrobiaceae*, the *Hyphomicrobium* family.

The family *Hyphomicrobiaceae*, consisting of Gram-negative bacteria, was circumscribed on the basis of phylogenetic analysis of 16S rRNA sequences. The family is affiliated with the *Alphaproteobacteria*, order *Hyphomicrobiales*. The family is phenotypically, metabolically, and ecologically diverse. Many members reproduce by budding. The family includes organisms that are chemoheterotrophic, facultatively methylotrophic, facultatively chemolithoautotrophic, and photosynthetic (Garrity et al. 2005).

Type genus: *Hyphomicrobium*.

The mol% G+C of the DNA varies between 49 and 72.

At the time of writing (June 2012), the family contained 18 genera with 54 species whose names have standing in the nomenclature (*Tables 11.1–11.9*): *Hyphomicrobium* [type genus] (ten species, including one for which the type is no longer available), *Ancalomicrobium* (one species), *Angulomicrobium* (two species), *Aquabacter* (one species), *Blastochloris* (three species), *Cucumibacter* (one species), *Devosia* (13 species), *Dichotomicrobium* (one species), *Filomicrobium* (two species), *Maritalea* (three species), *Methylorhabdus* (one species), *Pedomicrobium* (species, including one for which the type is no longer available), *Pelagibacterium* (two species), *Prosthecomicrobium* (two species), *Rhodomicrobium* (one species), *Rhodoplanes* (four species), *Seliberia* (one species), and *Vasilyevaea* (two species).

Based on 16S rRNA sequence comparisons, a number of taxonomic rearrangements have been made in the past:

1. Reclassification of organisms formerly classified within the *Hyphomicrobiaceae* into other families:
   - The transfer of *Hyphomicrobium neptunium* (Leifson 1964) to the genus *Hyphomonas* (family *Hyphomonadaceae*, order *Caulobacterales* within the *Alphaproteobacteria*) as *Hyphomonas neptunium* (Moore et al. 1984) comb. nov.
   - The reclassification of *Hyphomicrobium indicum* (Johnson and Weisrock, 1969) as *Photobacterium indicum* (family *Vibrionaceae* in the *Gammaproteobacteria*) (Ivanova et al. 2004; Xie and Yokota 2004).
   - The transfer of *Prosthecomicrobium litoralum* (Bauld et al. 1983) and *Prosthecomicrobium consociatum* (Vasil'eva et al. 1991; Vasil'eva et al. in Validation List no. 127, 2009) to the newly established genus *Bauldia* (yet to be assigned to a family) as *Bauldia litoralis* comb. nov. and *Bauldia consociata* comb. nov. (Yee et al. 2010).
2. Placement of organisms earlier attributed to other groups as new members of the family:
   - The transfer of "*Pseudomonas riboflavina*" (Foster 1944) to become the type species of the new genus *Devosia* as *Devosia riboflavina* (Nakagawa et al. 1996).
   - The reclassification of *Rhodopseudomonas rosea* (Janssen and Harfoot 1991) to become the type species of the new genus *Rhodoplanes* as *Rhodoplanes roseus* comb. nov. (Hiraishi and Ueda 1994).
   - The reclassification of "*Rhodopseudomonas cryptolactis*" (Stadtwald-Demchick et al. 1990), a name that was effectively published but not validated, as *Rhodoplanes cryptolactis* (Okamura et al. 2007).
   - The transfer of *Rhodopseudomonas viridis* (Drews and Giesbrecht 1966) and *Rhodopseudomonas sulfoviridis* (Keppen and Gorlenko 1975) to the newly established genus *Blastochloris* as *Blastochloris viridis* and *Blastochloris sulfoviridis*, respectively (Hiraishi 1997).
3. Rearrangements within the *Hyphomicrobiaceae*, which include the following:
   - The transfer of *Prosthecomicrobium enhydrum* (Staley 1968) and *Prosthecomicrobium mishustinii* (Vasil'eva et al. 1991; Vasil'eva et al. in Validation List no. 127, 2009) to the new genus *Vasilyevaea* as *Vasilyevaea enhydra* comb. nov. and *Vasilyevaea mishustinii* comb. nov. (Yee et al. 2010).
   - The proposal to consider the genus *Maritalea* (Hwang et al. 2009) as an earlier heterotypic synonym of *Zhangella* (Xu et al. 2009), thereby renaming *Zhangella mobilis* as *Maritalea mobilis* (Fukui et al. 2012).

Some sources, including the 2nd edition of *Bergey's Manual of Systematic Bacteriology*, classify the obligately anaerobic fermentative genus *Gemmiger* within the *Hyphomicrobiaceae*. Similar to many representatives of the *Hyphomicrobiaceae*, *Gemmiger* multiplies by budding. However, phylogenetically it belongs to the order *Clostridiales*, family *Ruminococcaceae*. Therefore it is not discussed in the framework of this chapter.

Table 11.1

The genera classified within the family *Hyphomicrobiaceae*, as of June 2012

Genus	Number of species	Type species	General properties
Hyphomicrobium [type genus]	10	*Hyphomicrobium vulgare*	Cells rod-shaped with pointed ends, or oval egg, or bean-shaped, producing monopolar or bipolar hyphae or prosthecae of varying length. Hyphae may be truly branched; secondary branches are rare. Multiply by budding of a daughter cell at one hyphal tip at a time. Mature buds become motile swarmers that break off and may attach to surfaces of other cells to form clumps or rosettes. Motility is lost soon after swarmer liberation and/or attachment. Aerobic, chemoorganotrophic. Growth can occur on mineral salts media without added carbon source, possibly resulting from the presence of volatile carbon and energy sources. Good growth with C1 compounds such as methanol and methylamine, Widely distributed in soils and aquatic habitats.
Ancalomicrobium	1	*Ancalomicrobium adetum*	Non-motile bacteria with conical cells, possessing 2–8 or more prosthecae 2–5 μm in length. Prosthecae are cylindrical and taper gradually. Prosthecae may be bifurcated. Cells multiply by budding. Buds are formed directly from the mother cell, never from prosthecae. Cells contain gas vesicles. Facultatively anaerobic chemoheterotrophs that use sugars aerobically and anaerobically. Sugars are fermented by mixed acid fermentation. Some organic acids are used aerobically but not fermentatively.
Angulomicrobium	2	*Angulomicrobium tetraedrale*	Obligately aerobic, chemoorganotrohpic non-motile bacteria, having polygonal (tetrahedral or mushroom-like) cells with radial symmetry. Division is by budding. Prosthecae and gas vesicles are absent. A variety of organic acids, monosaccharides and amino acids are used. Methanol and formate can serve as energy sources only in the presence of yeast extract.
Aquabacter	1	*Aquabacter spiritensis*	Unicellular encapsulated rod-shaped cells that may become motile by flagella. Aerobic and chemoorganotrophic, using organic acids and some amino acids as carbon source. Ammonium ions can serve as sole nitrogen source. Gas vesicles may be present.
Blastochloris	3	*Blastochloris viridis*	Rod-shaped to ovoid cells, motile by means of subpolar flagella. They exhibit polar growth, budding, and asymmetric cell division and form rosette-like aggregates. Internal photosynthetic membranes are present as lamellae underlying and parallel to the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll *b* and carotenoids. Ubiquinones and menaquinones are present. Preferentially grow photoheterotrophically under anoxic conditions in the light. Anaerobic photoautotrophic growth may occur with thiosulfate or sulfide as electron donor. Chemotrophic growth is possible under microoxic conditions in the dark.
Cucumibacter	1	*Cucumibacter marinus*	Rod-shaped cells, motile by means of monopolar flagella. Short prosthecae are present on the cell surface. Strictly aerobic and slight halophilic. Chemoheterotrophic, using a range of sugars and some amino acids as growth substrates. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and a number of unidentified lipids. The predominant cellular fatty acids are $C_{18:1\ \omega7c}$, 11 methyl $C_{18:1\ \omega7c}$ and $C_{18:0}$. The predominant quinone is Q-10.
Devosia	13	*Devosia riboflavina*	Rod-shaped, sometimes oval cells, motile by one or several polar flagella; rarely nonmotile. Aerobic, chemoheterotrophic; sugars are used aerobically as carbon source. The predominant quinone is Q-10 or Q-11. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and unknown glycolipids. The major fatty acids are $C_{18:1\ \omega7c}$, 11-methyl $C_{18:1\ \omega7c}$ and $C_{16:0}$. 3-Hydroxy fatty acids such as $C_{10:0\ 3\text{-OH}}$, $C_{18:0\ 3\text{-OH}}$, $C_{24:1\ 3\text{-OH}}$ and $C_{26:1\ 3\text{-OH}}$ are present. A relatively high content of iso-$C_{15:0}$ or $C_{18:0}$ (> 10 %) occurs in several species.
Dichotomicrobium	1	*Dichotomicrobium thermohalophilum*	Tetrahedral to spherical cells with up to four prosthecae of 0.2–0.3 μm width and varying length. Cells and hyphae may be covered with short, rigid, bent pili. Hyphae and mother cells may branch dichotomously. Cells multiply by terminal bud formation on hyphae or by intercalary budding. Propagation cells are initially spherical or pear-shaped, later tetrahedral, and never motile. Obligately aerobic, moderately thermophilic and halophilic. Inorganic nitrogen compounds and urea

Table 11.1 (continued)

Genus	Number of species	Type species	General properties
			do not serve as nitrogen sources. Carbon sources are acetate, malate, succinate, and α-ketoglutarate.
Filomicrobium	2	*Filomicrobium fusiforme*	Non-motile fusiform or bean-shaped cells with several polar prosthecae, which are approximately 0.2 μm in diameter and up to 40 μm in length. Buds are formed at the tips of the prosthecae. Aerobic and chemoheterotrophic. Acetate is used as carbon source. Glucose is not fermented. Major cellular fatty acids are $C_{18:1\ \omega7c}$ and $C_{19:0\ \omega8c\ cyclo}$. $C_{14:0\ 3\text{-}OH}$, $C_{16:0}$ and $C_{18:2\ \omega7,13}$ are present in some species. The major respiratory quinone is Q-9.
Maritalea	3	*Maritalea myrionectae*	Rod-shaped cells, motile by means of a single polar flagellum or peritrichous flagella. Strictly aerobic and slightly halophilic, found in marine environments. Chemoheterotrophic; glucose can be used as sole carbon source. Major polar lipids are phosphatidylglycerol and several unidentified lipids. The content of diphosphatidylglycerol varies between species. Minor amounts of phosphatidylethanolamine can be detected. Major cellular fatty acids are $C_{18:1\ \omega7c}$ and 11-methyl $C_{18:1\ \omega7c}$. The predominant quinone is Q-10.
Methylorhabdus	1	*Methylorhabdus multivorans*	Nonmotile, non-pigmented rods that multiply by fission, with constriction. Nitrate is reduced to nitrite. Aerobic, chemoorganotrophic and. facultatively methylotrophic. C1 compounds (dichloromethane, methanol, methylamine) are assimilated via a variant of the serine pathway. The major quinone is Q-10. Major fatty acids include $C_{18:1\ \omega7}$, $C_{19:0\ cyclo}$ and $C_{16:0}$. Major phospholipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine and cardiolipin.
Pedomicrobium	4	*Pedomicrobium ferrugineum*	Oval, spherical or rod-shaped encapsulated cells with 1–5 hyphae. At least one hypha originates laterally; others may appear at the cell poles. Buds are formed at the tips of the hyphae. Mature buds either separate from the hyphae as uniflagellated swarmers or remain attached. Oxidized iron or manganese compounds are deposited on mother cells and later on hyphae. Swarmer cells are motile by a single subpolar or polar flagellum. Other stages of the cell cycle are nonmotile. Chemoorganotrophic. Acetate is utilized as a carbon source; most strains also grow on caproate or pyruvate. Organic nitrogen sources are generally required. The major respiratory quinone is Q-9. Found in podzolic and other soils, freshwater, iron springs and seawater.
Pelagibacterium	2	*Pelagibacterium halotolerans*	Motile aerobic heterotrophic bacteria that divide by binary division. Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids. Major fatty acids include $C_{19:0\ cyclo}$, $C_{18:0}$, $C_{18:0\ 3\text{-}OH}$, $C_{18:1}$ and $C_{16:0}$. The main respiratory quinone is Q-10, with Q-9 as a minor component.
Prosthecobacterium	2	*Prosthecomicrobium pneumaticum*	Unicellular motile or non-motile coccobacilli or rod-shaped cells containing numerous prosthecae extending from all locations on the cell surface. Prosthecae (10 to >30 per cell) are typically <1.0 μm in length; some species also produce longer prosthecae (>2.0 μm). Cells divide by budding. Buds are produced directly from the mother cell, never from the tips of prosthecae. Motile organisms produce single polar or subpolar flagella. One species forms gas vacuoles but no flagella. Obligately aerobic, nonfermentative heterotrophs, using a variety of sugars and organic acids.
Rhodomicrobium	1	*Rhodomicrobium vannielii*	Ovoid to elongate-ovoid bacteria showing polar growth with formation of peritrichously flagellated swarmer cells and non-motile ''mother cells'', which form long prosthecae. Daughter cells originate as spherical buds at the end of the prosthecae. Internal photosynthetic membranes are of the lamellar type. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. The predominant cellular fatty acid is $C_{18:1}$. Q-10 and RQ-10 are present. Cells grow preferentially photoheterotrophically under anoxic conditions in the light. Photoautotrophic growth may be possible with hydrogen and sulfide as electron sources. Various organic substrates, molecular hydrogen, ferrous ion, and sulfide at low concentrations may be used as photosynthetic electron donors. Cells can also grow under microoxic to oxic conditions in the dark. Freshwater bacteria with a preference for acidic pH between 5.2 and 6.5.

Table 11.1 (continued)

Genus	Number of species	Type species	General properties
Rhodoplanes	5[a]	*Rhodoplanes roseus*	Motile rod-shaped cells that multiply by budding and asymmetric cell division. Internal photosynthetic membranes are present as lamellar stacks parallel to the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. $C_{18:1}$ and $C_{18:0}$ are the predominant components of the cellular fatty acids. Quinones are Q-10 and RQ-10. Growth is preferentially photoheterotrophic under anoxic conditions in the light, with simple organic substrates as carbon and energy sources. Photoautotrophic growth with sulfide as the electron donor does not occur. Chemotrophic growth is possible under oxic conditions in the dark at full atmospheric oxygen tension and by denitrification under anoxic conditions in the presence of nitrate. Mesophilic freshwater bacteria with preference for neutral pH. Some representatives are thermotolerant.
Seliberia	1	*Seliberia stellata*	Chemoorganotrophic, strictly aerobic rods with a helically sculptured or furrowed topography and blunt or rounded ends. Stellate aggregates (rosettes) of sessile rods joined at one pole, and individual, shorter motile rods (swarmers) occur in the same culture. An adhesive holdfast, secreted at one pole, mediates attachment into rosettes. Growth on appropriate soil extract media may permit formation of round to ovoid "generative cells". Following unidirectional polar cell growth, a shorter motile swarmer cell and a longer sessile cell are produced by asymmetric transverse fission. A single subpolar ensheathed flagellum is characteristically present on the swarmer; several lateral flagella may also occur.
Vasilyevaea	2	*Vasilyevaea mishustinii*	Budding, prosthecate bacteria with numerous short appendages, <1 μm in length, that cover the cell surface. Aerobic and heterotrophic, using a variety of carbon sources including sugars and sugar alcohols. Cells may be pigmented.

[a]Including *Rhodoplanes cryptolactis*, a name effectively but not yet validly published

Phylogenetic Structure of the Family and Its Genera

Phylogenetically the family *Hyphomicrobiaceae* belongs to the class *Alphaproteobacteria*, order *Hyphomicrobiales* (Douglas 1957). The proposal by Kuykendall (2005) to classify the *Hyphomicrobiaceae* within the newly proposed order *Rhizobiales* cannot be accepted because of matters of priority: *Hyphomicrobium* is the type genus of the order *Hyphomicrobiales*, and therefore the name *Rhizobiales* for the order is illegitimate.

Figure 11.1 shows a neighbor-joining tree of the type strains of 52 species of the family *Hyphomicrobiaceae*. The tree clearly shows that the family *Hyphomicrobiaceae* is polyphyletic, as the genera now classified within the family are interspersed between lineages of other families: *Xanthobacteraceae*, *Beijerinckiaceae*, *Methylocystaceae*, *Methylobacteriaceae*, *Bartonellaceae*, *Cohaesibacteraceae*, and *Rhodobiaceae*.

A number of separate clusters can be observed: (1) a group that contains the prosthecate genera *Hyphomicrobium*, *Pedomicrobium*, and *Filomicrobium*, the genus *Hyphomicrobium* being split into two separate branches as shown earlier (Rainey et al. 1998; Lee et al. 2005); (2) a cluster with two other genera of prosthecate heterotrophs, *Ancalomicrobium* and *Prosthecomicrobium*, confirming the close relationship of these genera demonstrated by Schlesner et al. (1989); (3) the thermophilic and halophilic prosthecate genus *Dichotomicrobium*, phylogenetically only distantly related to the mesophilic prosthecate bacteria within the family; (4) a branch that contains two out of the three genera of phototrophs—*Rhodoplanes* and *Blastochloris*; (5) the third phototrophic genus *Rhodomicrobium* on a deep branch; (6) a large cluster that contains genera without clear prosthecae, *Devosia*, *Vasilyevaea*, *Pelagibacterium*, *Cucumibacter*, and *Maritalea*; based on the tree topology, the genus *Vasilyevaea* is not well separated from *Devosia*; (7) a branch that contains the disparate genera *Angulomicrobium* and *Methylorhabdus* (see also Fritz et al. 2004); (8) *Aquabacter*, which clusters with the *Xanthobacteraceae* as noted earlier (Rainey and Wiegel 1996); (9) *Seliberia*, forming a separate deep lineage. A maximum likelihood tree constructed based on the same dataset (not shown) differs in some details of topology, but the overall picture obtained is similar.

Based on this tree, it is clear that a thorough reevaluation of the classification of the genera of the *Hyphomicrobiaceae* and related families within the alphaprotebacterial order *Hyphomicrobiales* ("*Rhizobiales*") is long overdue.

Genome Analysis

At the time of writing (June 2012), 5 genomes of members of the *Hyphomicrobiaceae* had been sequenced and annotated, and 4 of them were published (*Table 11.10*): the type strains of

Table 11.2

Comparison of selected characteristics of the members of the genus *Hyphomicrobium*

Character	*H. vulgare*[a]	*H. aestuarii*[a]	*H. chlorometha-nicum*[b]	*H. coagulans*[c]	*H. denitrificans*[d]	*H. facile* subsp. *facile*[a]	*H. hollandicum*[a]	*H. methylovorum*[e]	*H. sulfonivorans*[f]	*H. zavarzinii*[a]
Type strain	ATCC 33404	ATCC 27483	NCIMB 13687	Not available	DSM 1869	DSM 1565	ATCC 27498	ATCC 35216	ATCC BAA-113	ATCC 27496
Cell size of mother cell (μm)	1–3	1.6	1.3–1.8	1.2–2.0	1.0–3.0	2.0	1.7	0.5–1.2	0.5–1.3	1.8
Flagella	Subpolar	Subpolar	Arrangement NR	Polar	NR	Subpolar	Subpolar	Lateral	NR	Subpolar
Rosette formation	–	–	+	–	+	–	–	–	+	+
Pellicle formed on liquids	v	+	NR	+	–	–	v	–	+	v
Temperature range/optimum (°C)	15–37	5–45	Opt. 28–30	Opt. 35	Opt. 30	5–37	25–37	14–33 (Opt. 28)	15–37	15–37
pH optimum	6.5–7.5	6.5–7.5	6.5–7.5	6.0	6.0–8.0	6.5–7.0	>7.5	7.0	7.3–7.6	6.5–7.5
Nitrate reduction	+[g]	+	–	–	+	–	–	–	–	+
Oligocarbophilic	+	+	+	NR	NR	+	+	–	NR	+
Growth on methanol	+	+	+	+	+	+	+	+	+	+
Methylamine	+	+	+	+	+	+	+	+	+	+
Acetate	+	+	NR	–	+	w	–	–	+	w
n-butyrate	+	w	NR	NR	–	–	–	NR	NR	+
Lactate	+	+	NR	–	–	+	+	–	–	–
Succinate	+	+	NR	NR	–	–	+	–	+	–
Chloromethane	NR	–	+	–	NR	–	–	NR	NR	–
Peptones	–	+	NR	+	–	–	–	NR	NR	+
Formamide	NR	+	NR	NR	NR	–	–	+	NR	–
Respiratory quinone	Q-9	NR	Q-9	NR	Q-9	Q-9	Q-9	NR	NR	NR
G+C content of DNA (mol%)	61.1–61.4	64.1–66.1	60.0	NR	60–61	59.5–61.2	62.4–62.9	58.5–60.6	62	61.8–64.8
Sample source	Soil	Seawater and brackish water	Polluted soil	Rice field soil	Soil	Soil	Sewage	Soil	Garden soil	Peaty soil

Characteristics that differentiate the three subspecies of *H. facile* (subsp. *facile*, subsp. *tolerans*, subsp. *ureiphilum*):

subsp. *facile* grows on lactate and does not grow on butyrate

subsp. *facile* has a lower temperature minimum and maximum than the other subspecies

subsp. *tolerans*, named for its tolerance of high CO concentrations, is the only subspecies that grows on peptone

subsp. *ureiphilum* grows well on urea and shows α-hemolysis on sheep blood

w weakly positive, *v* variable, depending on growth conditions, *NR* not reported

Data taken from:

[a]Gliesche et al. (2005)

[b]McDonald et al. (2001)

[c]Takada (1975)

[d]Urakami et al. (1995)

[e]Izumi et al. (1982)

[f]Borodina et al. (2002). Additional data on growth substrates are given by Gliesche et al. (2005) and in the original species descriptions

[g]Gliesche et al. (2005) noted that *H. vulgare* (Stutzer and Hartleb 1899) could grow under anaerobic conditions with KNO_3, but nitrate reduction was considered to be negative

Table 11.3

Comparison of selected characteristics of the members of the genus *Pedomicrobium*

Character	*P. ferrugineum*[a,b,c]	*P. americanum*[a,b,c]	*P. australicum*[b,c]	*P. manganicum*[b,c]
Type strain	ATCC 33119	ATCC 43612	ATCC 43611	ATCC 33121
Prosthecae	1-3 hyphae	1-3 hyphae	1-3 hyphae	1-5 hyphae
Bud formation	+	+	+	+
Cell shape	Polymorphic, including oval, spherical, rod-shaped, tetrahedral, or bean-shaped	Polymorphic, including oval, tetrahedral, short rods, bean- or spindle-shaped	Polymorphic, including oval, tetrahedral, short rods, bean- or spindle-shaped	Polymorphic, including spherical, oval or short rods
Cell size (μm)	0.6–2.0 × 0.6–2.5	1.3 × 1.8	1.2 × 1.8	0.4–0.9 × 0.4–1.5
Motility	Single polar or subpolar flagellum	Single subpolar flagellum	Single subpolar flagellum	Single subpolar flagellum
NaCl range for growth and optimum (%)	0–0.1	NR	NR	NR
pH range for growth and optimum	3.5–10 (9)	(7.6–8.4)[d]	(7.3–7.6)[d]	NR
Temperature range for growth and optimum (°C)	10–43 (29–30)	15–41 (32–38)	15–36 (29–32)	(30)
Deposition of Fe^{3+}	+	+	+	v
Deposition of Mn oxide	−	+	+	+
Poly-β-hydroxybutyrate production	+	+	+	+
Nitrate reduction	+	+	+	NR
Assimilation of:				
L-Arginine, L-leucine, L-lysine, L-isoleucine	+	+	w	NR
L-Aspartate, L-glutamate, glycine, L-histidine, phenylalanine, L-serine, L-threonine, D-valine	+	+	+	NR
Formate	−	W	+	w
Glucose	−	−	+	w
Lactose	−	−	+	NR
Malate, D-mannitol	w	W	+	NR
L-proline	+	−	w	NR
Pyruvate	+	+	−	+
L-tyrosine	+	+	NR	NR
Main respiratory quinone	Q-10	Q-10	Q-10	Q-10
G+C content of DNA (mol%)	64.5–66.8	64–65	63–65	65
Sample source and site	Podzolic soil, Germany	Bog water and freshwater puddles, USA	Freshwater reservoir, Australia	Quartzite rock pool in France

+ positive, − negative, *NR* not reported, *w* weak

Data taken from:

[a]Gebers (1981)

[b]Hirsch and Gebers (2005)

[c]Gebers and Beese (1988)

[d]The values in brackets are the optimal pH of buffered media, whereas those of unbuffered media of *P. americanum* and *P. australicum* are 6.6–7.5 and 6.9–7.5, respectively

Table 11.4

Comparison of selected characteristics of the members of the genera *Ancalomicrobium*, *Angulomicrobium*, and *Seliberia*

Character	*Ancalomicrobium adetum*[a]	*Angulomicrobium tetraedrale*[b]	*Angulomicrobium amanitiforme*[c]	*Seliberia stellata*[d]
Type strain	ATCC 23632	DSM 5895	NCIMB 1785	VKM B-1340
Cell size (μm)	~1.0	1.1–1.5	1.0–1.5	0.5–0.8 × 1–12
Cell shape	2-8 prosthecae of 0.2 × 2–5 μm	Tetrahedral cells with radial symmetry, mushroom-like or flat	Tetrahedral cells with radial symmetry	Helically sculptured or furrowed rods with an adhesive holdfast
Motility	−	−	−	+ (swarmer cells)
Gas vesicles	+	−	−	−
Mode of cell division	Budding	Budding	Budding	Symmetric transverse fission
pH range for growth and optimum	6.3–7.5; Opt. 7.0	Opt. 6.8–7.0	5.2–8.0; Opt. 6.8–7.0	4.5–9.0
Temperature range for growth and optimum (°C)	9–39	15–35; Opt. 28–30	15–40; Opt. 28–30	15–37; Opt. 25–30
Oxidase	+	+	+	+
Catalase	+	+	+	+
Aerobic metabolism	Chemoheterotrophic, respiring a range of sugars and a few other compounds (pyruvate, malate, glycerol)	Chemoheterotrophic on a variety of organic acids, monosaccharides and amino acids; citrate, D-ribose and L-serine are not used; D-malate, D-mannose, D-melibiose, methylamine and tartrate are used	Chemoheterotrophic on a variety of organic acids, monosaccharides and amino acids; citrate, D-ribose and L-serine are used; D-malate, D-mannose, D-melibiose, methylamine and tartrate are not used	Chemoheterotrophic, growing in dilute complex media
Anaerobic metabolism	Mixed acid fermentation; glucose is fermented to acetate, lactate, formate, succinate, H_2 and CO_2	–	–	Some strains grow anaerobically by denitrification
Requirement for growth factors	Pantothenic acid required, others are stimulatory	No, but stimulatory	No, but stimulatory	–
Major fatty acids	NR	$C_{18:1\ \omega7}$, $C_{19:0\ \Delta8,9}$, $C_{20:1\ \omega9}$, $C_{18:0}$[e]	$C_{18:1\ \omega7}$, $C_{19:0\ \Delta8,9}$, $C_{14:0}$	NR
Main respiratory quinone	NR	NR	NR	NR
G+C content of DNA (mol%)	70.4	64.3–68.2	67.7	63–66
Sample source and site	Freshwater	Swamps, freshwater, pulp mill aeration lagoon	Freshwater	Soil and freshwater

+ positive, − negative, *NR* not reported

Data taken from:

[a]Staley (1968); Jenkins et al. (2005b)

[b]Vasil'eva et al. (1979); Fritz et al. (2004); Vasilyeva (2005)

[c]Fritz et al. (2004)

[d]Aristovskaya and Parinkina (1963); Schmidt and Kelly (2005)

[e]Fatty acids content of phospholipids

Hyphomicrobium denitrificans (Brown et al. 2011), *Pelagibacterium halotolerans* (Huo et al. 2012), *Rhodomicrobium vannielii* (Brown et al. 2011), a second strain assigned to *H. denitrificans*, and a chloromethane-degrading *Hyphomicrobium* isolate designated strain MC1 and isolated from industrial sewage (Vuilleumier et al. 2011). The chromosomes are 3.64–4.76 Mbp in length and contain 3,600–4,952 predicted genes. *P. halotolerans* in addition contains a 4-kb plasmid.

Notable features detected in the genome of the marine *P. halotolerans* are genes for the biosynthesis and uptake of the organic osmotic solutes ectoine and glycine betaine (Huo et al. 2012). *Hyphomicrobium* MC1 possesses the genes encoding enzymes for the synthesis of the cofactor pyrroloquinoline quinone (PQQ) and for both tetrahydrofolate- and tetrahydromethanopterin-linked pathways of C_1 metabolism (Vuilleumier et al. 2011). *P. halotolerans* has proteins for the biosynthesis of PQQ, as well as PQQ-dependent alcohol and glycerol dehydrogenases. Genes for the complete serine and ethylmalonyl-CoA pathways for carbon assimilation are present, as well as nitrogen fixation genes, which are absent in the type strain of *H. denitrificans* (Vuilleumier et al. 2011).

From the "pre-genomics era" there is a report about the genome sizes of a number of members of the *Hyphomicrobiaceae*, calculated from DNA renaturation kinetics. Estimated genome sizes (in Da) for the type strains of *Hyphomicrobium vulgare*, *H. aestuarii*, *H. hollandicum*, *H. facile* subsp. *facile*, *Pedomicrobium ferrugineum*, *P. manganicum*, *Filomicrobium fusiforme* and *Dichotomicrobium thermohalophilum* were, respectively, 2.13×10^9, 2.62×10^9, 2.43×10^9, 2.35×10^9, 3.43×10^9, 2.83×10^9, 2.03×10^9, and 1.73×10^9 (Kölbel-Boelke et al. 1985). Based on a molecular mass of 607.4 Da per base pair, these values translate to 3.5, 4.3, 4.0, 5.6, 4.7, 3.3, and 2.8 Mbp. Using a the same technique, Potts et al. (1980) estimated the genome size of the type strain of *R. vannielii* at 2.1×10^9 Da = 3.45 Mbp, a value now known to be too low compared to the 4.01 Mbp of the complete genome sequence (Brown et al. 2011).

Phages

Thus far, phages have only been reported for the genus *Hyphomicrobium*. Phage Hy*Φ*30 has a molecular mass of 55.4 $\times 10^6$ Da and contains linear double-stranded DNA of 29.7 $\times 10^6$ Da (Kaplan et al. 1976). It adsorbs only to the tip of the growing stalk and the evolving daughter cell until a constriction in the stalk is formed and the daughter cell becomes flagellated. Phage maturation only occurs within the daughter cell and the stalk (Voelz et al. 1971). Specific adsorption to the developing daughter cell was found in the *Hyphomicrobium* double-stranded DNA phages Hy*Φ*22a, Hy*Φ*1a and Hy*Φ*32a. Hy*Φ*22a and Hy*Φ*1a both have a polyhedral head and a short noncontractile tail, but differ in host range and sizes of protein components. Hy*Φ*32a had a polyhedral head and a long flexible non-contractile tail with fibers attached to its distal end (Yelton et al. 1979). Preissner et al. (1988) isolated a bacteriophage from raw sewage that, out of the 21 *Hyphomicrobium* strains and 22 other bacteria tested, was only active on *Hyphomicrobium* WI-926 isolated from a German forest pond. This representative of the *Podoviridae* has an isometric head 67 nm in diameter and short 12-nm tail. Adsorption is most efficient on the daughter cells, and phage multiplication was limited to the daughter cells. Fifty-five lytic phages of different morphologies specific for *Hyphomicrobium* strains were recovered from water and soil samples. These belonged to the *Myoviridae* (5), *Styloviridae* (41) with flexible or rigid tails, and the *Podoviridae* (9). They preferentially attached to the proximal pole of the mother cell, the hyphal tip, the distal pole of the bud, and the distal pole of the swarmer cell (Gliesche et al. 1988).

Phenotypic Analyses

The Properties of the Genera and Species of *Hyphomicrobiaceae*

Phenotypically the members of the family *Hyphomicrobiaceae* are quite diverse. Most species prefer an aerobic lifestyle. Some can also grow anaerobically using nitrate as an electron acceptor. Fermentative growth is rarely encountered, with the notable exception of *Ancalomicrobium* which, in addition to aerobic respiration, can grow by fermenting sugars to acetate, ethanol, lactate, formate, succinate, H_2, and CO_2 ("mixed-acid fermentation"). The facultatively photoheterotrophic genera *Rhodomicrobium*, *Rhodoplanes*, and *Blastochloris* lead a phototrophic life under anaerobic conditions only. In the presence of oxygen, no bacteriochlorophyll is produced, and the cells live by aerobic respiration. *Blastochloris* spp. can also grow photoautotrophically. Chemolithoautotrophic growth is seldom found, but at least one *Hyphomicrobium* strain can grow autotrophically using hydrogen as an electron donor.

As expected for *Alphaproteobacteria*, the *Hyphomicrobiaceae* all possess a Gram-negative type of cell wall. The presence of prosthecae is a characteristic property of many genera of the family (Poindexter 2006), but is by no means restricted to the group. Prosthecae can be defined as "semirigid appendages extending from a prokaryotic cell with a diameter which is always smaller than that of the mature cell, and which is bounded by the cell wall" (Staley 1968). Such prosthecate species typically multiply by asymmetric division ("budding") rather than by binary fission. Endospore formation was never observed. ❯ *Figure 11.2* shows a selection of prosthecate representatives of the family with diverse types of cell morphology.

Chemotaxonomically the members of the *Hyphomicrobiaceae* resemble most other groups of *Alphaproteobacteria*. In most species, the most abundant fatty acids are $C_{18:1\omega7c}$, 11-methyl $C_{18:1\omega7c}$ and $C_{16:0}$ and $C_{18:0}$. Hydroxy fatty acids are present as well. More detailed information on the fatty acids and the types of polar lipids present can be found in ❯ *Tables 11.1*– *11.9* and in the original species descriptions.

Table 11.5
Comparison of selected characteristics of the members of the genera *Prosthecomicrobium*, *Vasilyevaea*, *Dichotomicrobium*, and *Filomicrobium*

Character	*Prosthecomicrobium pneumaticum* [a]	*Prosthecomicrobium hirschii*[b]	*Vasilyevaea enhydra*[c]	*Vasilyevaea mishustinii*[d]	*Dichotomicrobium thermohalophilum*[e]	*Filomicrobium fusiforme*[f]	*Filomicrobium insigne*[g]
Basonym			*Prosthecomicrobium enhydrum*	*Prosthecomicrobium mishustinii*			
Type strain	ATCC 23633	ATCC 27832	ATCC 23634	VKM B-2499	DSM 5002	IFAM 1315	CGMCC 1.6497
Cell size (μm)	~1.0	0.8–1.2 × 1.0–2.0	0.4–0.5 × 0.5–0.8	0.6–1.2 × 0.8–1.5	0.8–1.8 × 0.8 x 2.0	0.5–0.7 × 1.0–4.0	0.5–1.0 × 1.0–2.5
Cell shape	Prosthecae generally <1.0 μm long, occasionally longer	Both short <1.0 μm and long, >2.0 μm prosthecae	Irregular cells with ~0.5 μm long prosthecae; colonies white, yellow or red	Short rods with 0.2–0.65 μm long prosthecae; colonies yellowish-orange	Tetrahedral to spherical with up to 4 prosthecae of varying length; cells and hyphae may be covered with short rigid bent pili. Hyphae and mother cells may branch dichotomously	Spindle-shaped with 2 (rarely 3) polar hyphae up to 40 μm and longer	Prostheca or bipolar prosthecae and buds on the prosthecal tips
Motility	−	+	+	−	−	−	+
Gas vesicles	+	−	−	−	−	−	−
Mode of cell division	Budding[f]	Budding	Budding[f]	Budding	Budding	Budding	Budding
pH range for growth and optimum	Opt. 6.0–6.5, good growth between 6.0–7.2	NR	>6.6; Opt. 7.0	Neutral	5.8–9.5; Opt. 8.0–8.5	NR	6.0–9.0; Opt. 7.0–7.5
Temperature range for growth and optimum (°C)	9–42	9–40	9–37	Opt. 28–30	20–65; Opt. 44–50	Up to 33–35; Opt. 20–38	4–45; Opt. 28–30
Salinity range (% NaCl)	Freshwarter	Freshwater	Freshwater	Freshwater	0.8–22.2; Opt. 8–14.2	0.4–3.5; Opt. ~1.7	0–7; Opt. 1–2
Oxidase	NR	+	NR	+	+	+	+
Catalase	NR	+	+	+	+	+	+

Substrates for aerobic metabolism	Acetate, arabinose, cellobiose, fructose, fucose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, sorbitol, trehalose, xylose	Acetate, arabinose, caproate, ethanol, formate, fructose, fucose, galactose, glucose, glycerol, malate, mannose, methanol, propionate, pyruvate, ribose, valerate, xylose	Glucose, lactose, malate, maltose, pyruvate	Glucose, lactose, malate, maltose, mannitol	Organic acids such as acetate, malate, succinate	Acetate, fumarate, glutamate, malate, propionate, succinate	Cellobiose, ethanol, formate, fructose, methanol, methylamine, trehalose
			No growth on mannitol	Pyruvate poorly used	Use of sugars variable	No growth on ethanol, fructose, glucose, glycerol, lactate, lactose, mannitol, methanol, methlylamine, pyruvate	
		No growth on cellobiose, glucosamine, inositol, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, sorbitol, trehalose				Urease positive	No growth on glycerol, lactate, maltose, mannitol, pyruvate, succinate, sucrose
	No growth on caproate, ethanol, formate, glucosamine, malate, methanol, propionate, pyruvate, raffinose, valerate						Urease-negative
Requirement for growth factors	Biotin, thiamine, vitamin B_{12}	Biotin, nicotinic acid, pantothenate, thiamine	Thiamine	Yeast extract or B-vitamins	Yeast extract	Vitamin B_{12}	NR
Major fatty acids	NR	NR	$C_{18:1\ \omega7c}$, 11-methyl $C_{18:1\ \omega6}$, $C_{16:0}$.	NR	$C_{18:1\ \omega7}$, $C_{19:0\ cyclo\ \omega7c}$, $C_{18:0}$	$C_{18:1\ \omega7}$,$C_{19:0\ cyclo\ \omega7c}$, $C_{18:2\ \omega7,13}$	$C_{18:1\ \omega7c}$, $C_{16:0}$, $C_{19:0\ cyclo\ \omega8c}$
Main respiratory quinone	NR	NR	NR	NR	Q-10	Q-9	Q-9
G+C content of DNA (mol%)	69–70	67.9–69.9	65.8	63.7–65.2	62–64	61.9	59.5
Sample source and site	Freshwater	Rivers, freshwater ponds and creeks	Freshwater	Soil, manure	Hypersaline Solar Lake, Sinai	Brackish water, Baltic Sea	Coastal oil field, China

+ Positive, − negative, *NR* not reported

Data taken from:

[a]Staley (1968), (1984); Jenkins et al. (2005a)

[b]Staley (1984); Jenkins et al. (2005a)

[c]Staley (1968); Jenkins et al. (2005b); Yee et al. (2010)

[d]Vasil'eva et al., (1991); Jenkins et al. (2005b); Yee et al. (2010)

[e]Hirsch and Hoffmann (1989); Hirsch (2005)

[f]Schlesner (1987), (2005); Wu et al. (2009)

[g]Wu et al. (2009)

Further information is given by Nakagawa et al. (2005)

[f]Originally, *P. pneumaticum* and *V. enhydra* were described as dividing by binary transverse fission (Staley 1968). However, a careful re-examination of slide cultures revealed that they divide by budding (Staley 1984)

Table 11.6

Comparison of selected characteristics of the members of the genus *Devosia*

Character	*D. riboflavina*[a,b,k]	*D. albogilva*[b]	*D. chinhatensis*[c,b,k]	*D. crocina*[b]	*D. geojensis*[d,k]
Basonym	*Pseudomonas riboflavina*				
Type strain	ATCC 9526	CCM 7427	CCM 7426	CCM 7425	KCTC 22082
Cell shape	Rod	Oval or rod	Rod	Oval	Rod
Cell size (μm)	0.4–0.8 x 2.0–8.0	NR	NR	NR	0.5–0.8 x 1.0–2.0
Motility	Several polar flagella	Single polar flagellum	Single polar flagellum	Single polar flagellum	Single polar flagellum
Colony color	Cream	Cream	Cream	Orange	White
NaCl range for growth and optimum (%)	NR	0–3	0–3	0–3	NR
pH range for growth and optimum	NR	5–9	5–9	5–9	6–9.5 (Opt. 7.5–9)
Temperature range for growth and optimum (°C)	NR	5–45	5–40	5–32	15–45 (Opt. 30–35)
Oxidase	+	+	+	+	+
Urease	+	+	+	+	+
Nitrate reduction	w	−	−	−	−
Hydrolysis of					
Casein	−	−	−	−	+
Starch	−	−	−	−	−
Assimilation of					
N-Acetylglucosamine	+	NR	w	NR	+
Glucose	+	+	+	−	+
L-arabinose	+	+	+	+	−
D-mannitol	+	+	+	+	+
Mannose	+	+	+	+	+
Maltose	+	+	+	+	+
Sucrose	−	+	+	−	NR
Enzyme activities (API ZYM, bioMérieux)					
Acid phosphatase	+	NR	+	NR	w
Alkaline phosphatase	NR	NR	NR	NR	w
α-Chymotrypsin	NR	NR	NR	NR	−
Cystine arylamidase	−	NR	w	NR	−
Esterase (C4)	w	NR	+	NR	+
Esterase lipase (C8)	+	NR	+	NR	w
α-fucosidase	+	NR	−	NR	−
α-galactosidase	+	NR	+	NR	v
β-galactosidase	+	NR	+	NR	w
α-glucosidase	+	NR	+	NR	v
β-glucosidase	+	NR	+	NR	w
β-glucuronidase	NR	NR	NR	NR	−
Leucine arylamidase	NR	NR	NR	NR	+
Lipase (C14)	−	NR	−	NR	−
α-mannosidase	+	NR	−	NR	−
Naphthol-AS-BI-phosphohydrolase	−	NR	−	NR	w
Trypsin	+	NR	+	NR	w
Valine arylamidase	−	NR	+	NR	w
Predominant ubiquinone	Q-10	NR	NR	NR	Q-10
G+C content of DNA (mol%)	61.4	NR	63.5	NR	60.8
Sample source	Riboflav*in*-rich soil	Hexachlorocyclohexane dump site	Hexachlorocyclohexane dump site	Hexachlorocyclohexane dump site	Diesel-contaminated soil

+ Positive, − negative, *NR* not reported, *w* weak, *v* variable with growth conditions among different references

Data taken from:

[a]Nakagawa et al. (1996); [b]Verma et al. (2009); [c]Kumar et al. (2008); [d]Ryu et al. (2008); [e]Zhang et al. (2012); [f]Yoon et al. (2007); [g]Vanparys et al. (2005); [h]Rivas et al. (2003); [i]Yoo et al. (2006); [j]Lee (2007); [k]Bautista et al. (2010)

D. glacialis[e]	*D. insulae*[f,k]	*D. limi*[g,k]	*D. neptuniae*[b,k]	*D. psychrophila*[e]	*D. soli*[i,b,k]	*D. subaequoris*[j,k]	*D. yakushimensis*[k]
CGMCC 1.10691	KCTC 12821	LMG 22951	LMG 21357	CGMCC 1.10210	KACC 11509	KCTC 12772	KCTC 22147
Rod	Oval or rod	Rod	Rod	Rod	Rod	Rod	Rod
0.8–1.0 x 1.5–2.0	0.3–0.5 x 0.5–2.0	0.5–1.0 x 1.0–3.0	0.7–0.9 x 1.1–1.4	0.4–0.6 x 4.0–5.0	0.4–0.6 x 1.5–3.5	0.7 x 1.2	0.4–0.6 x 1.2–2.0
Polar flagellation	Single flagellum	–	Single subpolar flagellum	Polar flagellation	NR	Single polar flagellum	Single polar flagellum
Light pink	Ivory	Light yellow to light brown	Pearl white	White	Light beige	Light yellow to light brown	Beige
0–2	0.5–2 (Opt. 0.5)	NR	NR	0–1	0–5	0–3	0–2.5
7–8	Opt. 6.5–7.5	NR	NR	7–8	4–8	5.1–12.1	5.5–7 (Opt. 7)
1–20	10–31 (Opt. 25)	NR	NR	1–25	10–37	20–42	Opt. 28
+	–	+	+	+	–	+	+
+	+	–	+	–	+	–	+
+	–	–	+	–	–	–	NR
NR	–	NR	+[b]	NR	–	–	NR
–	–	NR	–[b]	–	+	–	NR
–	–	–	–	+	–	–	–
–	–	v	+	+	–	–	+
–	–	v	+	+	–	–	+
–	–	–	+	+	–	w	+
–	–	v	+	+	–	w	+
–	–	v	+	+	–	w	+
NR	NR	NR	–	NR	–	NR	NR
+	+	+	+	+	+	–	+
+	–	+	NR	+	+	+	+
NR	–	–	NR	NR	–	–	–
NR	–	–	–	NR	–	–	–
+	v	w	–	+	+	w	w
+	+	+	+	+	+	+	w
–	+	–	+	–	–	–	–
NR	–	–	+	NR	v	–	+
+	+	v	+	+	+	v	+
+	+	v	–	NR	+	–	+
+	+	+	+	+	+	v	+
–	–	–	NR	–	–	–	–
+	+	+	NR	+	+	+	+
NR	–	w	–	–	–	–	–
NR	–	–	+	NR	+	–	+
+	v	v	w	+	+	v	w
NR	–	–	–	NR	–	w	+
+	–	+	–	+	+	–	–
Q-10	Q-11	NR	NR	Q-10	Q-10	Q-10	Q-10
63.6	66.2	61.9	62.0–62.4	61.4	59.5	59.1	65.3
Glacier cryoconite	Soil	Commercial nitrifying inoculum	Nodules of aquatic legume	Glacier cryoconite	Green-house soil	Beach sediment	Root nodules of leguminous plant

Table 11.7

Comparison of selected characteristics of the members of the genera *Cucumibacter*, *Maritalea* and *Pelagibacterium*

Character	*Cucumibacter marinus*[a]	*Maritalea mobilis*[b]	*Maritalea myrionectae*[c]	*Maritalea porphyrae*[d]	*Pelagibacterium halotolerans*[e]	*Pelagibacterium luteolum*[e]
Basonym		*Zhangella mobilis*				
Type strain	KCCM 90027	CGMCC 1.7002	KCCM 90060	LMG 25872	CGMCC 1.7692	CGMCC 1.10267
Prosthecae	+	NR	NR	NR	–	–
Bud formation	NR	NR	+	–	NR	NR
Cell shape	Rod	Rod	Rod	Rod	Slightly curved rod	Short rod
Cell size (μm)	0.4–0.6 x 1.5–2.0	0.4–0.7 x 1.8–3.3	0.4–0.5 x 1.0–2.0	0.42 x 1.58	0.4–0.6 x 2–3	0.5–0.9 x 1.5–2.5
Motility	Monopolar flagella	Single polar flagellum	Peritrichous flagella	Single polar flagellum	Monopolar flagella	Single polar flagellum
Colony color	Cream	Pale yellow	Cream	Cream	Light yellow	Yellow
NaCl range for growth and optimum (%)	1–3 (Opt. 2)	0.5–7.5 (Opt. 2)	1–10 (Opt. 2–5)[g]	1–5 (Opt. 2)	0–13 (3–4)	0–5 (0.5)
pH range for growth and optimum	6–9 (Opt. 7)	7–10 (Opt. 8.2)	6.3–9.8 (Opt. 7.2–8.0)	5–8 (Opt. 7)	6.0–9.5 (7)	6.0–9.5 (7.5)
Temperature range for growth and optimum (°C)	15–40 (Opt. 30–35)	4–42 (Opt. 36)	10–40 (Opt. 30–35)	10–40 (Opt. 30)	10–42 (30)	4–37 (30)
Nitrate reduction	–	+	NR	+	–	–
Urease	–[b,e]	–	NR	NR	+	+
Hydrolysis of:						
Casein	–[e]	–	NR	NR	+	+
Gelatin	+	–	+	+	–	–
Starch	+	–	+	–	–	–
Tween 80	NR	–	+ (but –[d])	+	–	–
Assimilation of:						
Gluconate	–[e]	–[e]	NR	NR	+	+
L-ornithine	+[e]	+[e]	NR	NR	+	–
Salicin	+[e]	+[e]	NR	+	+	–
Sorbitol	–[e]	–	–[d]	+	–	+
Succinate	–	+[e]	–	NR	+	+
Sucrose	+	+	+	–	+	+
Enzyme activities (API ZYM, bioMérieux):						
N-Acetyl-β-glucosaminidase	–	+	–	–	+	+
Acid phosphatase	+	+	–	+	+	+
Alkaline phosphatase	+	+	+	+	+	–
α-chymotrypsin	–	+	+	+	–	–
Cystine arylamidase	–	+	+	+	–	–
β-galactosidase	+	+	+	–	–	–
α-glucosidase	–	+	+	–	+	–
β-glucosidase	+	–	+	–	+	+

Table 11.7 (continued)

Character	*Cucumibacter marinus*[a]	*Maritalea mobilis*[b]	*Maritalea myrionectae*[c]	*Maritalea porphyrae*[d]	*Pelagibacterium halotolerans*[e]	*Pelagibacterium luteolum*[e]
Lipase (C14)	+	–	–	+	–	–
Trypsin	–	+	+	+	+	–
Valine arylamidase	–	+	+	+	–	–
Major fatty acids	$C_{18:1\ \omega7c}$, 11 methyl $C_{18:1\ \omega7c}$, $C_{18:0}$	$C_{18:1\ \omega7c}$, 11 methyl $C_{18:1\ \omega7c}$, $C_{18:0}$[d]	$C_{18:1\ \omega7c}$, 11 methyl $C_{18:1\ \omega7c}$, $C_{18:0}$[d]	$C_{18:1\ \omega7c}$, 11 methyl $C_{18:1\ \omega7c}$, $C_{16:1\ \omega7c}$, $C_{18:0}$	$C_{18:1\ \omega7c}$, 11-methyl $C_{18:1\ \omega7c}$, $C_{19:0\ \omega8c\ cyclo}$, $C_{18:0}$, $C_{16:0}$	$C_{18:1\ \omega7c}$, 11-methyl $C_{18:1\ \omega7c}$, $C_{19:0\ \omega8c\ cyclo}$, $C_{18:0}$
Major polar lipids[f]	PG, DPG, two GLs, an PL and an unidentified lipid	PG, DPG, two GLs, an APL and an unidentified lipid[d]	PG, DPG, two GLs, an PL and an unidentified lipid	PG, two GLs, an AL and an unidentified lipid	PG, DPG and three GLs	PG, DPG and two GLs
Minor polar lipids[f]	PC, PE, an PL, an APL and three unidentified lipids	PE, an PL and three unidentified lipids[d]	PE and an APL	PE, DPG, an PL, five unidentified lipids and an APL	Nine unidentified lipids	Ten unidentified lipids
G+C content of DNA (mol%)	62.9	53.1	52.7	49.6	59.3	58.1
Sample source	Seawater	Seawater	Culture of a marine phototrophic ciliate	Red alga	Seawater	Semi-coke

All taxa are positive for oxidase and catalase activity and able to utilization of glucose. In the API ZYM system, positive for esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase; and negative for α-fucosidase, α-galactosidase, β-glucuronidase and α-mannosidase. The predominant ubiquinone is Q-10.

+ Positive, – negative, *NR* not reported

Data taken from:

[a]Hwang and Cho (2008)

[b]Xu et al. (2009)

[c]Hwang et al. (2009)

[d]Fukui et al. (2012)

[e]Xu et al. (2011)

[f]*PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *GL* unidentified glycolipid, *PL* unidentified phospholipid, *APL* unidentified aminophospholipid, *AL* unidentified amino lipid

[g]The NaCl range for growth was determined using by sea salts (Sigma) in synthetic ZoBell broth

The most common lipiquinones are ubiquinone Q-10 or Q-9 and rarely Q-11 (Sittig and Hirsch 1992; Sittig and Schlessner, 1993). Menaquinones and rhodoquinones are found in addition in the photoheterotrophic genera.

The Prosthecate and Budding, Non-phototrophic Genera *Hyphomicrobium*, *Pedomicrobium*, *Ancalomicrobium*, *Angulomicrobium*, *Seliberia*, *Prosthecomicrobium*, *Vasilyevaea*, *Dichotomicrobium* and *Filomicrobium*

Genus *Hyphomicrobium* (Stutzer and Hartleb 1899[AL])

Hy.pho.mi.cro'bi.um. Gr. n. *huphê*, a web, thread; Gr. adj. *mikros*, small; Gr. masc. n. *bios*, life; N.L. neut. n. *Hyphomicrobium*, thread-producing microbe.

Cells are 0.3–1.2 × 1.0–3.0 μm in size; rod shaped with pointed ends or oval; they produce monopolar or bipolar filamentous hyphae or prosthecae, 0.2–0.3 μm in diameter and of varying length. Hyphae may be branched; secondary branches are rare. Cells multiply by budding of a daughter cell at one hyphal tip at a time. The mature buds break off to become motile swarmer cells. These may attach to surfaces, lose their motility, and form clumps or rosettes. Poly-β-hydroxybutyrate can be stored, usually at a distinct cell pole. Colonies on solid media are small, even after prolonged incubation. Older colonies often display concentric rings and change color to darker brown or bright yellow orange. Aerobic, chemoorganotrophic, and oligocarbophilic: growth is possible on mineral salts media without added carbon source, the cells growing on volatile carbon and energy sources. Good growth is possible with low concentrations of C_1 compounds (methanol, methylamine, and others). Ammonium is a good nitrogen source, but some amino acids are also used. Some species grow by denitrification when supplied with nitrate. Widely distributed in soils and aquatic habitats.

Table 11.8
Selected characteristics of the members of the genera *Aquabacter* and *Methylorhabdus*

Character	*Aquabacter spiritensis*[a]	*Methylorhabdus multivorans*[b]
Type strain	ATCC 43981	VKM B-2030
Cell shape	Encapsulated rod	Rod
Cell size (μm)	0.5–1.0 × 1.5–3.0	0.4–0.6 × 1.2–2.0
Prosthecae	–	–
Gas vesicles	+	–
Mode of division	Binary fission	Fission with formation of a constriction
Motility	+ (under certain conditions)	–
NaCl range for growth and optimum (%)	Freshwater	< 3%
pH range for growth and optimum	Opt. 7.0–9.0	6,0–8.0 (Opt. 6.8–7.4)
Temperature range for growth and optimum (°C)	25–37 (Opt. 35)	10–45 (Opt. 28–34)
Oxidase	+	–
Catalase	+	+
Nitrate reduction	+	+
C_1 compounds used for growth	–	Dichloromethane, methanol, methylamine
C_1 compounds not used	Methanol	Formaldehyde, dimethylamine, trimethylamine, dimethylsulfoxide
Organic compounds used for growth	Acetate, alanine, butyrate, glutamate, glutarate, glycerate, β-hydroxybutyrate, α-ketoglutarate, lactate, malate, maleate, propionate, pyruvate, succinate, triburytin	Acetate, adonitol, alanine, arabinose, dulcitol, ethanol, fructose, fumarate, glucose, glycerol, glutamate, α-ketoglutarate, maltose, mannitol, mannose, oxaloacetate, iso-propanol, propionate, pyruvate, sarcosine, sorbitol, succinate, sucrose, xylose
Organic compounds not used	Arabinose, aspartate, arginine, citrate, ethanol, formate, fructose, fumarate, gluconate, glucose, glycerol, lactose, lysine, malonate, maltose, mannitol, proline, rhamnose, sorbitol, sucrose, meso-tartrate and numerous carbohydrates	Acetamide, inulin, rhamnose, raffinose
Major fatty acids	NR	$C_{18:1\ \omega7}$, $C_{19:0\ cyclo}$, $C_{16:0}$
Main respiratory quinone	NR	Q-10
G+C content of DNA (mol%)	67.3	66–67
Sample source and site	Spirit Lake, WA, USA	Groundwater contaminated with dichloromethane

+ Positive, – negative, *NR* not reported
Data taken from:
[a]Irgens et al. (1991)
[b]Doronina et al. (1995); Doronina and Trotsenko (2005)

The mol% G+C of the DNA is 58–66.

Type species: *Hyphomicrobium vulgare*.

The genus *Hyphomicrobium* currently contains 10 species: *H. aestuarii*, *H. chloromethanicum*, *H. coagulans*, *H. denitrificans*, *H. facile*, *H. hollandicum*, *H. methylovorum*, *H. sulfonivorans*, *H. vulgare*, and *H. zavarzinii*. Three subspecies have been described for *H. facile*: *H. facile* subsp. *facile*, *H. facile* subsp. *tolerans*, and *H. facile* subsp. *ureaphilum*. The main features of the members of the genus *Hyphomicrobium* are summarized in *Table 11.2*.

Additional comments:

- The type strain of *Hyphomicrobium coagulans* (strain Takada 10–2) does not appear to be available from culture collections. Although the species name was validly published in 1989, the organism is currently unavailable for study.
- Attachment of *Hyphomicrobium* swarmer cells to surfaces may be inhibited by light (Gliesche et al. 2005).
- Up to 12 consecutive buds can be formed on one hyphal tip, and the size of the swarmer cells produced increases with the age of the mother cell (Gliesche et al. 2005).

- Dimethylsulfone – $(CH_3)_2SO_2$ – a major product of chemical oxidation of dimethylsulfide in the atmosphere, is a growth substrate for *H. sulfonivorans* (Borodina et al. 2000).
- *Hyphomicrobium* spp. EG and VS oxidize sulfide to thiosulfate (Suylen and Kuenen 1986; Pol et al. 1994).
- *Hyphomicrobium* sp. 53–49 can grow autotrophically with $H_2/CO_2/O_2$ (Uebayasi et al. 1981).
- Small amounts of squalene and sterols (Hop-22(29)-ene) were detected in *Hyphomicrobium* strains (Urakami and Komagata 1986).

Genus *Pedomicrobium* (Aristovskaya 1961, 957[AL]) (Emend. Gebers and Beese 1988, 305)

Pe.do.mi.cro'bi.um. Gr. n. *pedon*, soil; N.L. neut. n. *microbium*, microbe; N.L. neut. n. *Pedomicrobium*, soil microbe.

Cells are oval, spherical or rod-shaped, 0.4–2.0 × 0.4–2.5 μm, with 1–5 hyphae of 0.15–0.3 μm in diameter. At least one hypha originates laterally; other hyphae may appear at the cell poles. Buds are formed at the tips of the hyphae. Mature buds either separate from the hyphae as swarmer cells or remain attached. Extracellular polymers form thick capsules around mother cells. Oxidized iron or manganese compounds are deposited around mother cells and hyphae. Swarmer cells are motile by a single subpolar or polar flagellum. Colonies are yellowish or reddish brown to dark brown, due to accumulated iron or manganese oxides. Acetate is utilized as a carbon source; most strains also grow on pyruvate or caproate. Organic nitrogen sources utilized by most strains are glutamate, aspartate, glycine, serine, threonine, and valine. Inorganic nitrogen compounds allow poor growth of some isolates; nitrate is reduced by most strains. Vitamin mixtures stimulate growth; lack of vitamins results in pleomorphic cells, which produce large granules of poly-β-hydroxybutyric acid. The major respiratory quinone is Q-10. Found in soils, freshwater, iron springs, and seawater.

The mol% G+C of the DNA is 63–67.

The type species: *Pedomicrobium ferrugineum.*

The genus currently contains four species (June 2012): *P. ferrugineum*, *P. americanum*, *P. australicum*, and *P. manganicum* (Table 11.3).

Additional comment:

- The type strain of *P. australicum* (IFAM ST-1306 = ATCC 43611) is no longer available. Phylogenetic analysis was based on DNA recovered from nonviable lyophilized cells. A second isolate of the species, strain IFAM WD-1355, studied by Gerbers and Beese (1988) was lost as well. The prospect of designating a neotype strain is remote in the absence of suitable differentiating phenotypic characteristics. Therefore Cox and Sly (1997) proposed that the species *Pedomicrobium australicum* Gebers and Beese 1988 should not be retained.

Genus *Ancalomicrobium* (Staley 1968, 1940[AL])

An.ca.lo.mi.cro'bi.um. Gr. masc. n. *ankalis*, arm; N.L. neut. n. *microbium*, microbe; N.L. neut. n. *Ancalomicrobium*, arm (−producing) microbe.

Cells are nonmotile, conical, ~1 μm in diameter, possessing 2–8 or more prosthecae. Prosthecae are cylindrical and taper gradually to a distal diameter of ~0.2 μm and a length of 2–5 μm, and may be bifurcated. Cells multiply by budding. Buds are formed from the mother cell, never from prosthecae. Gas vesicles may be present. Chemoheterotrophic, sugars can aerobically be respired or anaerobically fermented by mixed-acid fermentation. Some organic acids are used aerobically, but not fermentatively. Ammonium can be used as sole nitrogen source. Vitamins are required. Occur in freshwaters and in pulp mill oxidation lagoons.

The mol% G+C of the DNA is 70.

Type species (and currently single species): *Ancalomicrobium adetum.*

The main features of the members of the genus *Ancalomicrobium* are summarized in Table 11.4.

Additional comments:

- Buds first develop as small protuberances on a non-appendaged area of the mother cell and then enlarge and differentiate so that the daughter cell is a pseudo-mirror image of the mother cell at the time of cell separation (Jenkins et al. 2005b).
- *Ancalomicrobium* is the only member of the *Alphaproteobacteria* known to perform mixed-acid fermentation. Products of sugar fermentation are acetate, ethanol, lactate, formate, succinate, H_2, and CO_2 (Oertli et al. 2006).
- Production of gas vesicles may be especially pronounced under anaerobic conditions and at low temperature (<18 °C) (Jenkins et al. 2005b).

Genus *Angulomicrobium* (Vasil'eva, Lafitskaya, and Namsaraev 1986, 354[VP]—Validation List no. 20) (Effective Publication: Vasil'eva, Lafitskaya, and Namsaraev 1979, 1037)

An.gu.lo.mi.cro'bi.um. L. adj. *angularis*, having corners or angles, angular; N.L. neut. n. *microbium*, microbe; N.L. neut. n. *Angulomicrobium*, angular microbe.

Cells are nonmotile, polygonal (tetrahedral or mushroomlike in shape during some stages of the life cycle) with radial symmetry, 1.1–1.5 μm in diameter. Division is by budding; buds are produced on the conical point of elongation of the mother cell with a short tube connecting two cells. Prosthecae and gas vesicles are not formed. A variety of organic acids, monosaccharides, and amino acids are used. Methanol and formate can serve as energy sources only in the presence of yeast extract.

The mol% G+C of the DNA is 64–69.

Type species: *Angulomicrobium tetraedrale.*

Table 11.9

Comparison of selected characteristics of the members of the genera *Rhodomicrobium*, *Rhodoplanes*, and *Blastochloris*

Character	*Rhodomicrobium vannielii*[a]	*Rhodoplanes roseus*[b]	*Rhodoplanes cryptolactis*[c]	*Rhodoplanes elegans*[d]	*Rhodoplanes piscinae*[e]
Basonym		*Rhodopseudomonas rosea*	Earlier described as 'Rhodopseudomonas cryptolactis'		
Type strain	ATCC 17100	DSM 5909	DSM 9987	JCM 9224	JCM14934
Cell size (μm)	1.0–1.2 x 2.0–2.8	0.8–1.0 x 1.8–2.5	1.0 x 2.5–4.0	0.8–1.0 x 2.0–3.5	0.7–1.0 x 1.5–3.5
Motility	+	+	+	+	+
Rosette formation	Complex aggregates	–	+	+	+
Photosynthetic membranes	Lamellae	Lamellae	Lamellae	Lamellae	Lamellae
Photosynthetic pigments	Bchl *a*, carotenoids (spirilloxanthin series)	Bchl *a*, carotenoids (spirilloxanthin series)	Bchl *a*, carotenoids (spirilloxanthin series)	Bchl *a*, carotenoids (spirilloxanthin series)	Bchl *a*, carotenoids (spirilloxanthin series)
Color of photo-trophically grown culture	Pale orange-brown	Pink	Pink-red	Pink	Pink
Optimal pH	6.0	7.0–7.5	7.0	7.0	6.5–8.0
Optimal temperature (°C)	30	30	40	30–35	30
Denitrification	NR	+	+	+	+
Sulfate assimilation	+	NR	–	NR	+
Aerobic dark growth	+	+	+	+	+
Photoautotrophic growth with	H_2, Sulfide	Thiosulfate	H_2	Thiosulfate	Thiosulfate
Growth factors	None	Niacin	B_{12}, niacin, *p*-amino-benzoic acid	Thiamine, *p*-amino-benzoic acid	Niacin
Utilization of: Benzoate	–	–	–	–	–
Citrate	–	+	–	+	+
Formate	+/–	–	NR	–	–
Glucose	–	–	–	–	–
Tartrate	–	+	–	+	w
Major quinones	Q-10, RQ-10	Q-10, RQ-10	Q-10, RQ-10	Q-10, RQ-10	Q-10, RQ-10
G+C content of DNA (mol%)	61.8–63.8	66–66.8	69.9	69.6–69.7	69.9–71.3
Sample source and site	Ponds, lakes, wastewater	Freshwater, lake sediment	Geothermal hot spring	Activated sludge	Wastewater treatment pond

NR not reported, +/– variable, *w* weak

Data taken from:

[a]Duchow and Douglas (1949); Imhoff (2005b)

[b]Janssen and Hartfoot (1991); Hiraishi and Ueda (1994)

[c]Stadtwald-Demchick et al. (1990); Okamura et al. (2007); Okamura et al. (2009)

[d]Hiraishi and Ueda (1994)

[e]Chakravarthy et al. (2010)

[f]Lakshmi et al. (2009)

[g]Okamura et al. (2009)

[h]Drews and Giesbrecht (1966); Hiraishi (1997)

[i]Venkata Ramana et al. (2011)

[j]Keppen and Gorlenko (1975); Hiraishi (1997). Additional data on growth substrates are given by Hiraishi and Imhoff (2005), Imhoff (2005a, b), and in the original species descriptions

[k]As stated in the protologue, *Rhodoplanes pokkaliisoli* does not use citrate (Lakshmi et al. 2009). However, according to *Table 11.1* in the same paper, the type strain can use citrate

Rhodoplanes pokkaliisoli[f]	*Rhodoplanes serenus*[g]	*Blastochloris viridis*[h]	*Blastochloris gulmargensis*[i]	*Blastochloris sulfoviridis*[j]
		Rhodopseudomonas viridis		*Rhodopseudomonas sulfoviridis*
KCTC 5711	DSM 18633	ATCC 19567	DSM 19786	DSM 729
1.0–1.2 x 3.0–5.0	0.8–1.0 x 2.0–3.0	0.6–0.9 x 1.2–2.0	1.0–1.5 x 3.0–5.0	0.5–0.9 x 1.0–1.8
+	+	+	+	+
+	+	+	NR	NR
Lamellae	Lamellae	Lamellae	Lamellae	Lamellae
Bchl *a*, carotenoids (spirilloxanthin series)	Bchl *a*, carotenoids (spirilloxanthin series)	Bchl *b*, carotenoids (spirilloxanthin series)	Bchl *b*, carotenoids (spirilloxanthin series)	Bchl *b*, carotenoids (spirilloxanthin series)
Yellowish-brown	Pink-red	Green to olive-green	Yellow-green	Olive-green
7.0	8.0	6.7–7.0	6.5–7.5	7.0
30	35	25–30	25	28–30
+	+	–	NR	–
+	+	+	+	–
+	+	w	+	w
-	Thiosulfate	-	-	Thiosulfate, Sulfide
Niacin, pantothenate, *p*-aminobenzoic acid	-	Biotin, *p*-amino-benzoic acid	Biotin, *p*-amino-benzoic acid	Biotin, *p*-amino-benzoic acid, pyridoxine
–	–	–	–	–
–[k]	+	–	–	–
–	–	–	–	–
–	–	w	–	+
–	+	–	–	–
Q-10, RQ-10	Q-10, RQ-10	Q-9, MK-9	Q-8, MK-8	Q-8/10, MK-7/8
67.2	69.5	66.3–71.4	63.8	67.8–68.4
Rice field, India	Pond water	Freshwater	Cold sulfur spring biofilm	Sulfur spring, activated sludge

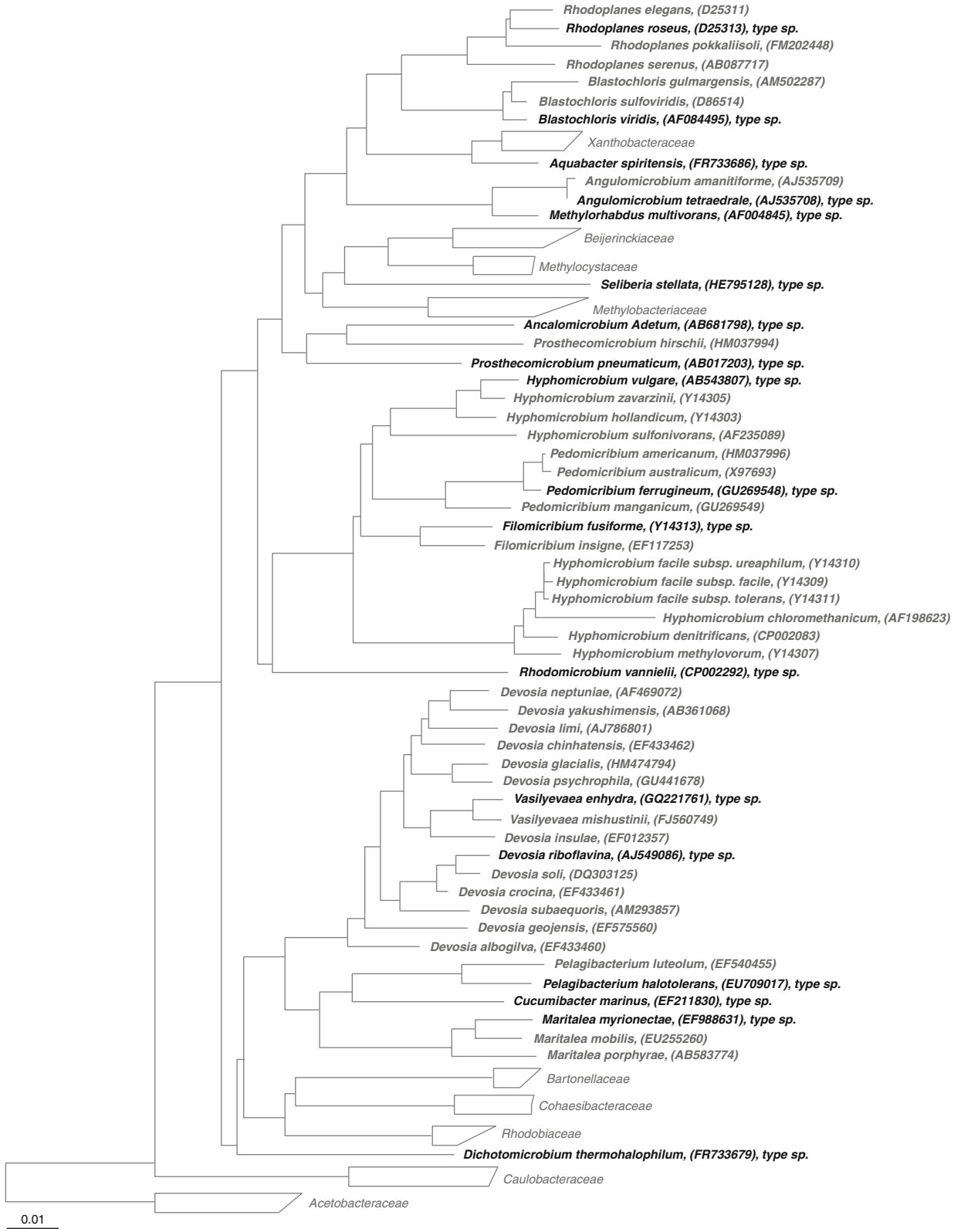

Fig. 11.1

Phylogenetic reconstruction of the family *Hyphomicrobiaceae* based on the neighbor-joining algorithm with the Jukes-Cantor correction. Sequence dataset and alignments according to the All-Species Living Tree Project, release LTPs108 (Yarza et al. 2010). The tree topology was stabilized with the use of a representative set of 767 high quality type strain sequences proportionally distributed among the different bacterial and archeal phyla. In addition, a 40 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence

Table 11.10
Properties of the sequenced genomes of members of the *Hyphomicrobiaceae* (as of June 2012)

Property	*Hyphomicrobium denitrificans* ATCC 51888[Ta]	*Hyphomicrobium denitriticans* 1NES1	*Hyphomicrobium* sp. MC1[b]	*Pelagibacterium halotolerans* B2[Tc]	*Rhodomicrobium vannielii* ATCC 17100[Ta]
Accession number	CP002083	AGIS00000000	FQ859181	CP003075 (chromosome) CP003076 (plasmid)	CP002292
Genome length (bp)	3,638,969	3,808,687	4,757,528	3,944,837	4,014,469
G+C content	60.8	59.7	59.2	61.4	62.2
Extrachromosomal elements	–	NR	–	1 (4,050 bp; 56.1% G+C)	–
Number of predicted genes	3,600	3,815	4,952	3,940	3,739
Predicted protein-coding genes	3,512	3,762	4,893	3,878 (chromosome) + 3 (plasmid)	3,565
Number of 16S rRNA genes	1	1	1	2	2

NR not reported
[a]Brown et al. (2011)
[b]Vuilleumier et al. (2011)
[c]Huo et al. (2012)

The genus *Angulomicrobium* currently contains two species: *A. amanitiforme* and *A. tetraedrale*. The main features of the members of the genus *Angulomicrobium* are summarized in Table 11.4.

Additional comment:

– Both *Angulomicrobium* species contain phosphatidylglycerol, phosphatidyl-*N*,*N*'-dimethylethylamine, phosphatidylcholine, and unknown phospholipids. Phosphatidylglycerol was found in *A. tetraedrale* but not in *A. amanitiforme* (Fritz et al. 2004).

Genus *Seliberia* (Aristovskaya and Parinkina 1963, 56[AL])

Se.li.be'ri.a. N.L. fem. n. *Seliberia*, named for the Russian microbiologist, Professor G. L. Seliber.

Cells are rod shaped, 0.5–0.8 × 1–12 μm, with a characteristic screw-like twisting of the rod surface. The ends of the cell may be either blunt or rounded. Cultures may contain stellate aggregates (rosettes) of sessile rods joined at one pole and shorter motile rods (swarmers). Cells produce an adhesive holdfast, secreted at one pole. Capsules are not produced, but a thin glycocalyx is produced later in the growth cycle. Unidirectional polar growth followed by asymmetric transverse fission produces a shorter motile cell (a swarmer) and a longer sessile cell. Swarmer cells have a single subpolar ensheathed flagellum; several non-ensheathed lateral flagella may also be present. In liquid medium, only the sheathed type of flagellum is produced. Growth is strictly aerobic, chemoorganotrophic. Nitrate is reduced to nitrite either aerobic or anaerobic conditions. Found in soil and freshwater environments.

The mol% G+C of the DNA is 63–66.

The type species: *Seliberia stellata*.

Type species (and currently single species): *Seliberia stellata*. The main features of the members of the genus *Seliberia* are summarized in Table 11.4.

Additional comments:

– In the literature, names such as *Seliberia carboxyhydrogena* and *Seliberia hydrogenophila* can be found, referring to chemoautotrophic bacteria that grow on H_2 + CO_2 and/or on carbon monoxide (Fedorova and Volova 1988; Kryukov et al. 1990). These names have not been validly published, and the affiliation of these isolates with *S. stellata* is not clear. Therefore these isolates are not further discussed here.
– Because of problems encountered in obtaining cell lysis, it is difficult to obtain good DNA preparations of *S. stellata* (Schmidt and Kelly 2005).

Genus *Prosthecomicrobium* (Staley 1968, 1940[AL] emend. Staley 1984, 304)

Pros.the.co.mi.cro'bi.um. Gr. fem. n. *prosthêkê*, appendage; N.L. neut. n. *microbium*, microbe; N.L. neut. n. *Prosthecomicrobium*, appendage (−bearing) microbe.

Cells are coccobacilli or rod shaped, ranging in diameter from 0.8 μm to 1.2 μm, and contain numerous prosthecae

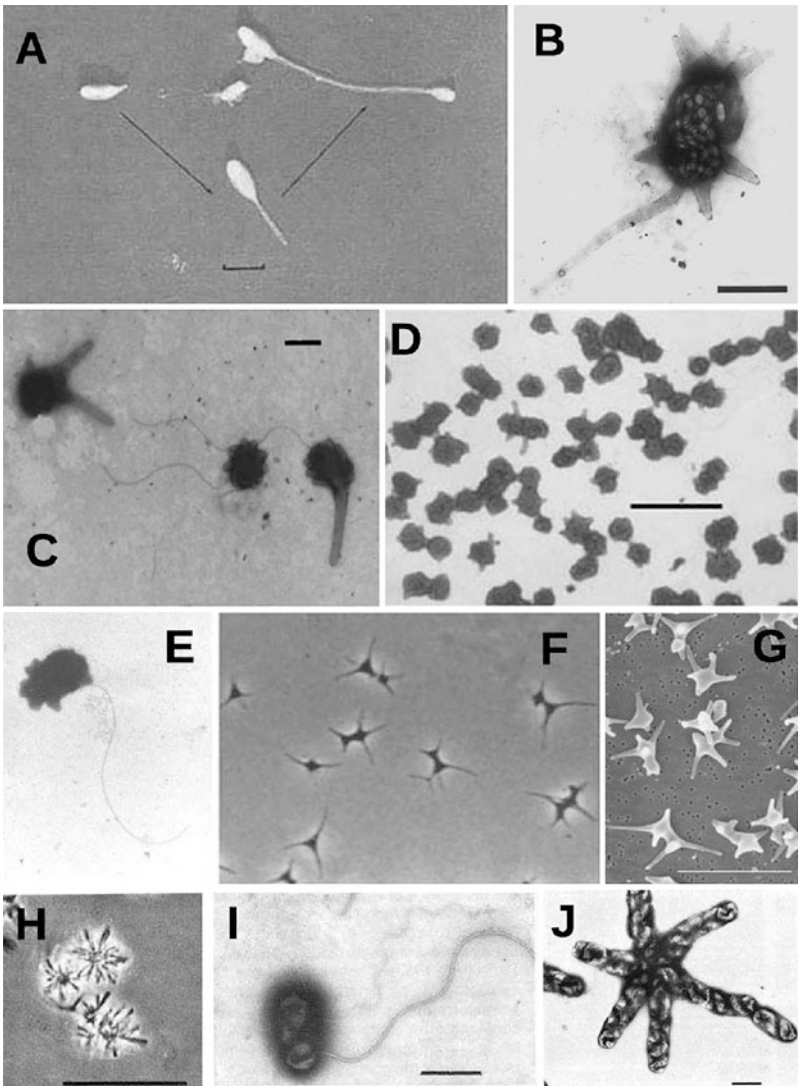

Fig. 11.2
The morphology of selected members of the *Hyphomicrobiaceae*: (a) *Hyphomicrobium* sp. Left to right in the transmission electron micrograph: swarmer, prosthecate cell, and reproducing cell. Bar = 1 μm; (b) *Prosthecomicrobium pneumaticum* ATCC 23633. Transmission electron micrograph showing gas vesicles within the cell, many short prosthecae, and a single long prostheca. Bar = 1.0 μm; (c) *Prosthecomicrobium hirschii*. Electron micrograph showing the two typical morphologies of *P. hirschii* cells. Nonmotile cells lacking flagella with long appendages, and motile cells with a single polar flagellum and shorter appendages. Bar = 1.0 μm; (d) *Prosthecomicrobium enhydrum*. Electron micrograph of a field of *P. enhydrum* cells showing the variability in prosthecae length. Bar = 5.0 μm; (e) *Prosthecomicrobium enhydrum*. Electron micrograph showing a single cell with a single subpolar flagellum. (f) *Ancalomicrobium adetum* ATCC 23632. Phase contrast image of *A. adetum* cells showing long appendages and including one with a branch; (g) *Ancalomicrobium adetum* ATCC 23632. Scanning electron micrograph. Bar = 5.0 μm; (h) *Seliberia stellata* aggregates, phase contrast microscopy. Bar = 10 μm; (i) Negative contrasted (0.5 % Na tungstate) *S. stellata* cell, having two structurally different flagella, grown on peptone-yeast extract solid medium. Bar = 1.0 μm; (j) Star-shaped rosettes of *S. stellata*, showing surface sculpturing. Bar = 1.0 μm (From Poindexter 2006 (panel a), Oertli et al. 2006 (panels b–g), and Schmidt and Swafford 2006 (panels h–i))

extending from all locations on the cell surface. Prosthecae (from 10 to more than 30/cell) are typically <1.0 μm in length; some species also produce longer prosthecae. Cells divide by budding. Buds are produced directly from the mother cell, not from the tips of prosthecae. Nonmotile or motile by means of a single polar or subpolar flagellum. One species forms gas vesicles but no flagella. A variety of sugars and organic acids are used as energy sources for growth. All strains tested require one or more B vitamins for growth. Found in freshwaters.

The mol% G+C of the DNA is 68–70.

Type species: *Prosthecomicrobium pneumaticum*.

The genus *Prosthecomicrobium* currently contains two species: *P. pneumaticum* and *P. hirschii*. The main features of the members of the genus *Prosthecomicrobium* are summarized in Table 11.5.

Additional comment:

– *P. hirschii* has a dimorphic lifestyle with a long-appendaged nonmotile stage and a short-appendaged motile stage (Semenov and Staley 1992). It is unable to use any disaccharides for growth but uses methanol and ethanol (Staley 1984).

Genus *Vasilyevaea* (Yee, Oertli, Fuerst and Staley, 2010, 2962^{VP})

Va.si.ly.e.vae'a. N.L. fem. n. *Vasilyevaea*, of Vasilyeva, named in honor of Lina Vasilyeva, a Russian microbiologist who has dedicated her career to the investigation of prosthecate bacteria and has named several new genera within this group.

Cells are less than 1 μm in length, budding, prosthecate with numerous short appendages that cover the cell surface. They use a variety of carbon sources including sugars and sugar alcohols. Thiamine, yeast extract, or B vitamins are required for growth. Cells may be pigmented.

The mol% G+C of the DNA is 63–66.

Type species: *Vasilyevaea mishustinii*.

The genus *Vasilyevaea* currently contains two species: *V. enhydra* and *V. mishustinii*. The main features of the members of the genus *Vasilyevaea* are summarized in Table 11.5.

Additional comment:

– *V. enhydra* contains two types of polar lipids only: phosphatidylglycerol and bisphosphatidylglycerol (Yee et al. 2010).

Genus *Dichotomicrobium* (Hirsch and Hoffmann 1989, 495^{VP}—Validation List no. 31) (Effective Publication: Hirsch and Hoffmann 1989, 300)

Di.cho.to.mi.cro'bi.um. Gr. adj. *dichotomos*, cutting in two, divided equally; N.L. neut. n. *microbium*, microbe; N.L. neut. n. *Dichotomicrobium*, a forked microbe.

Cells are tetrahedral to spherical, 0.8–1.8 × 0.8–2.0 μm, and have up to four prosthecae of 0.2–0.3 μm width and varying length. Cells and hyphae may be covered with short, rigid, bent pili. Hyphae and mother cells may branch dichotomously. Cells are pigmented red by carotenoids. Cells multiply by the formation of terminal buds on hyphae or by intercalary budding. Propagation cells are initially spherical or pear-shaped, later tetrahedral, and nonmotile. Poly-β-hydroxybutyrate granules may be produced, even in the hyphae. Cells are obligately aerobic, moderately halophilic, and thermophilic (maximum temperature 52–65 °C). Yeast extract is required for growth. Carbon sources are acetate, malate, succinate, and α-ketoglutarate. Inorganic nitrogen compounds and urea do not serve as nitrogen sources. The major respiratory quinone is Q-10.

The mol% G+C of the DNA is 62–64.

Type species (and currently single species): *Dichotomicrobium thermohalophilum*.

The main features of the members of the genus *Dichotomicrobium* are summarized in Table 11.5.

Genus *Filomicrobium* (Schlesner 1988, 220^{VP}—Validation List no. 25) (Effective Publication: Schlesner 1987, 65)

Fi.lo.mi.cro'bi.um. L. n. *filum*, thread; N.L. neut. n. *microbium*, microbe; N.L. neut. n. *Filomicrobium*, thread-like microbe.

Cells are fusiform or bean-shaped, 0.5–1.0 × 1.0–4.0 μm, with several polar prosthecae which are ~0.2 μm in diameter and up to 40 μm in length. Buds are formed at the tips of the prosthecae. Acetate is used as carbon source. Glucose is not fermented. Cells produce storage granules of poly-β-hydroxybutyrate. The major respiratory quinone is Q-9. Found in brackish water and oil-polluted soil.

The mol% G+C of the DNA is 59–62.

The type species: *Filomicrobium fusiforme*.

The genus currently contains two species (June 2012): *F. fusiforme* and *F. insigne* (Table 11.5).

Additional comments:

– *F. fusiforme* is pigmented red by carotenoids (canthaxanthin, echinenone) (Schlesner 1987, 2005).
– *F. fusiforme* does not use C_1 compounds, but *F. insigne* can grow on methanol and on methylamine (Wu et al. 2009).

The Non-photosynthetic Genera *Devosia*, *Cucumibacter*, *Maritalea*, *Pelagibacterium*, *Aquabacter* and *Methylorhabdus*

Genus *Devosia* (Nakagawa, Sakane and Yokota 1996, 20^{VP}) (Emend. Rivas, Willems, Subba-Rao, Mateos, Dazzo, Kroppenstedt, Martínez-Molina, Gillis and Velázquez 2003, 52, Emend. Yoo, Weon, Kim, Hong, Kwon, Cho, Go and Stackebrandt 2006, 2691, Emend. Yoon, Kang, Park and Oh 2007, 1314, Emend. Zhang, Redzic, Liu, Zhou, Schinner and Margesin 2012, 713)

De.vos'i.a.. N.L. fem. n. *Devosia*, honoring Paul De Vos, a Belgian microbiologist, for his basic contribution to the taxonomy of pseudomonads.

Cells are rod shaped, sometimes oval, 0.3–1.0 × 1.0–8.0 μm. Most species are motile by one or several polar flagella. Usually growth occurs at NaCl concentrations of 0–3 % (w/v) and

occasionally up to 5 %. Sugars are used aerobically as carbon source. Most species are negative for lipase (C14) and β-glucuronidase, and nearly all possess esterase lipase (C8) and β-galactosidase. The predominant quinone is Q-10 or Q-11. The major components in the polar lipid profile are phosphatidylglycerol, diphosphatidylglycerol, and unknown glycolipids. Found in soil and freshwater environments, including contaminated soil, glacier cryoconite, beach sediment, and plant root nodules.

The mol% G+C of the DNA is 59–67.

Type species: *Devosia riboflavina*.

The genus currently contains 13 species (June 2012): *D. albogilva*, *D. chinhatensis*, *D. crocina*, *D. geojensis*, *D. glacialis*, *D. insulae*, *D. limi*, *D. neptuniae*, *D. psychrophila*, *D. riboflavina*, *D. soli*, *D. subaequoris*, and *D. yakushimensis*.

Additional comments:

- While all other members of the genus *Devosia*, as well as most of the other genera of *Hyphomicrobiaceae*, are oxidase positive, *D. soli* was reported oxidase negative (Yoo et al. 2006). *D. psychrophila* is the only species of *Hyphomicrobiaceae* reported to be catalase negative (Zhang et al. 2012).
- All tested species have Q-10 as their major respiratory quinone, with the exception of *D. insulae* which has Q-11.
- *D. riboflavina*, a soil bacterium that oxidizes riboflavin, has unusually large amounts of the long hydroxy fatty acids 3-OH $C_{24:1}$ and 3-OH $C_{26:1}$. Hydroxy fatty acids shorter than 3-OH $C_{18:0}$ are absent (Nakagawa et al. 1996). The presence of such long-chain hydroxy fatty acids is not a general characteristic of the genus (Rivas et al. 2003).
- *D. chinhatensis*, isolated from a hexachlorocyclohexane dump site in India, contains the branched fatty acid iso-H $C_{18:1}$, a compound not present in other *Devosia* species (Kumar et al. 2008).
- *D. subaequoris*, isolated from beach sediment, was reported to grow within an unusually wide pH range between 5.1 and 12.1 (Lee 2007). However, no controlled experiments in buffered media were reported to confirm that indeed sustained growth above pH 12 is possible.
- *D. albogilva* and *D. crocina* were isolated from a hexachlorocyclohexane dump site. They tolerate high concentrations of hexachlorocyclohexane, but do not degrade the compound (Verma et al. 2009).
- *D. neptuniae* strains $J1^T$ and J2 contain two plasmids, one of which (1,600 kb) carries nitrogen-fixing (*nifH*) and nodulating (*nodD*) symbiotic genes (Rivas et al. 2003). Attempts to amplify *nifH* and *nodD* gene fragments from *D. yakushimensis* (a species isolated from root nodules of *Pueraria lobata*, a leguminous plant in Japan) or from *D. riboflavina*, *D. soli*, and *D. limi* were not successful (Bautista et al. 2010; Yoo et al. 2006).
- The marine ciliated protozoon *Euplotes magnicirratus* contains endosymbiotic bacteria in their cytoplasm. Based on 16S rRNA gene analysis and fluorescence in situ hybridization, these bacteria are affiliated with the genus *Devosia*. The organism has not yet been cultured, and the provisional name "*Candidatus* Devosia euplotis" was proposed (Vannini et al. 2004).

Genus *Cucumibacter* (Hwang and Cho 2008, 1594^{VP})

Cu.cu.mi.bac'ter L. n. *cucumis*, cucumber; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Cucumibacter*, a cucumber-like rod.

Gram negative and rod shaped, 0.4–0.6 × 1.5–2.0 μm. Short prosthecae are present on the cell surface. Cells are motile by means of monopolar flagella. Slightly halophilic. Growth occurs at NaCl concentrations of 1–3 % (w/v) (optimum 2 %) or sea salts concentrations of 1–10 % (optimum 3–4 %). The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, two unidentified glycolipids, an unidentified phospholipid and an unidentified lipid. The predominant quinone is Q-10. Found in seawater.

The mol% G+C of the DNA is 62–63.

Type species (and currently single species): *Cucumibacter marinus* (Hwang and Cho 2008). The main features of the members of the genus *Cucumibacter* are summarized in Table 11.7.

Genus *Maritalea* (Hwang, Cho, Yih and Cho 2009, 613^{VP})

Ma.ri.ta'le.a. L. neut. n. *mare*, the sea; L. fem. n. *talea*, a staff, rod; N.L. fem. n. *Maritalea*, rod inhabiting marine environments.

Cells are rod-shaped cells, 0.4–0.7 × 1.0–3.3 μm, and motile by means of a single polar flagellum or peritrichous flagella. Slight halophilic: growth occurs at NaCl concentrations of 0.5–7.5 % (optimum 2 %) or sea salts concentrations of 1–10 % (w/v) (optimum 2–5 %). Glucose can be used as sole carbon source. The major polar lipids are phosphatidylglycerol, two unidentified glycolipids, an unidentified phospholipid, and another unidentified lipid. The predominant quinone is Q-10. Found in marine environments, seawater, and associated with marine organisms.

The mol% G+C of the DNA is 49–54.

The type species: *Maritalea myrionectae*.

The genus was proposed in 2009 (Hwang et al. 2009) and currently (June 2012) contains three species: *M. mobilis*, *M. myrionectae* and *M. porphyrae* (Table 11.7).

Additional comments:

- Bud formation has been observed in the species *M. myrionectae* (Hwang et al. 2009), but not in *M. porphyrae* (Fukui et al. 2012).
- *Maritalea myrionectae* was recovered from a culture of the marine ciliate *Myrionecta rubra* (Hwang et al. 2009), and *M. porphyrae* was isolated from the red alga *Porphyra yezoensis* (Fukui et al. 2012).

Genus *Pelagibacterium* (Xu, Huo, Wang, Oren, Cui, Vedler and Wu 2011, 1820^{VP})

Pe.la.gi.bac.te'ri.um. L. n. *pelagus*, the sea; N.L. n. *bacterium*, from Gr. n. *bakterion*, a small rod; N.L. neut. n. *Pelagibacterium*, a rod isolated from the sea.

Cells are motile short rod-shaped bacteria, 0.4–0.9 × 1.5–2.5 µm, which divide by binary division. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and two unidentified glycolipids. The main respiratory quinone is Q-10, with Q-9 as a minor component. Found in marine environments and in semi-coke samples.

The mol% G+C of the DNA is 58–60.

The type species: *Pelagibacterium halotolerans*.

The genus currently contains two species (June 2012): *P. halotolerans* and *P. luteolum* (Table 11.7).

Genus *Aquabacter* (Irgens, Kersters, Gillis and Staley 1993, 864^{VP}—Validation List no. 47) (Effective Publication: Irgens et al. 1991, 141)

A.qua.bac'ter. L. n. *aqua*, water; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Aquabacter*, aquatic rod.

Cells are encapsulated rods, 0.5–1.0 × 1.5–3.0 µm. Under certain conditions cells become motile by flagella. Organic acids and some amino acids are used as carbon source. Growth occurs on minimal medium with acetate, succinate, or pyruvate as sole carbon source and ammonium ions as sole nitrogen source, along with B vitamins. Gas vesicles may be present.

The mol% G+C of the DNA is 67.

Type species (and currently single species): *Aquabacter spiritensis*.

The main features of the members of the genus *Aquabacter* are summarized in Table 11.8.

Additional comment:

– Flagella are produced when the cells are grown in semisolid media; no motility is seen when grown in typical broth media (Staley 2005).

Genus *Methylorhabdus* (Doronina, Braus-Stromeyer, Leisinger and Trotsenko 1996, 362^{VP}—Validation List no. 56) (Effective Publication: Doronina, Braus-Stromeyer, Leisinger and Trotsenko 1995, 97)

Me.thy.lo.rhab'dus. N.L. n. *methylum* (from Fr. *méthyle*, coined from Gr. n. *methu*, wine and Gr. n. *hulê*, wood), methyl radical; N.L. pref. *methylo-*, pertaining to the methyl radical; G. fem. n. *rhabdos*, rod; N.L. fem. n. *Methylorhabdus*, methyl rod.

Cells are nonmotile nonpigmented rods, 0.4–0.6 × 1.2–2.5 µm, that multiply by fission with constriction. Oxidase negative and catalase and urease positive. Some sugars and organic acids are used as carbon and energy sources. Facultatively methylotrophic. Assimilate C_1 compounds (methanol, methylamine, dichloromethane) via the isocitrate lyase-negative variant of the serine pathway. Nitrate is reduced to nitrite. Ammonium, nitrate, methylated amines, and some amino acids can be used as nitrogen sources. The major ubiquinone is Q-10. Major phospholipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, and cardiolipins. Found in groundwater.

The mol% G+C of the DNA is 66–67.

Type species (and currently single species): *Methylorhabdus multivorans* (Doronina et al. 1995). The main features of the members of the genus *Methylorhabdus* are summarized in Table 11.8.

Additional comment:

– After division the cells remain connected by a constriction apparently formed by the outer membrane (Doronina et al. 1995).

The Facultative Anoxygenic Phototrophs: Genera *Rhodomicrobium*, *Rhodoplanes* and *Blastochloris*

Genus *Rhodomicrobium* (Duchow and Douglas 1949, 415^{AL}, Emend. Imhoff, Trüper and Pfennig 194, 341)

Rho.do.mi.cro'bi.um. Gr. n. *rhodon*, the rose; N.L. neut. n. *microbium*, microbe; N.L. neut. n. *Rhodomicrobium*, red microbe.

Cells are ovoid to elongate-ovoid, showing polar growth and performing a characteristic vegetative growth cycle, involving formation of peritrichously flagellated swarmer cells and nonmotile mother cells that form long prosthecae. Daughter cells originate as spherical buds at the end of the prosthecae. Internal photosynthetic membranes are of the lamellar type. Contains photosynthetic pigments bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. $C_{18:1}$ comprises >80 % of the membrane-bound fatty acids. Ubiquinone and rhodoquinone with ten isoprene units are present, and the lipopolysaccharides are characterized by a glucosamine-containing, phosphate-free lipid A with amide-bound $C_{16:0}$ 3OH. Cells grow preferentially photoheterotrophically under anoxic conditions in the light. Photoautotrophic growth may be possible with hydrogen and sulfide as electron sources. Various organic substrates, molecular hydrogen, ferrous ion, and sulfide at low concentrations may be used as photosynthetic electron donors. Cells are also able to grow under microoxic to oxic conditions in the dark. Mesophilic freshwater bacteria with a preference for acidic pH between 5.2 and 6.5.

The mol% G+C of the DNA is 62–64.

Type species (and currently single species): *Rhodomicrobium vannielii*.

The main features of the members of the genus *Rhodomicrobium* are summarized in Table 11.9.

Additional comments:

- A well-illustrated overview of the morphogenesis and differentiation in *R. vannielii* was given by Whittenbury and Dow (1977).
- A number of aromatic compounds by can be photocatabolized by *R. vannielii*. Benzoate was photocatabolized by all five strains tested, and benzylalcohol by 4 out of 5. Other substrates that yielded good growth were vanillate (4-hydroxy-3-methoxybenzoate) and syringate (4-hydroxy-3,5-dimethoxybenzoate). However, catabolism of vanillate and syringate led to a significant inhibition of bacteriochlorophyll synthesis, eventually causing cultures to cease growing (Wright and Madigan 1991).

Genus *Rhodoplanes* (Hiraishi and Ueda 1994, 671^{VP})

Rho.do.pla'nes. Gr. n. *rhodon*, the rose; Gr. masc. n. *planos*, a vagabond, a wanderer; N.L. masc. n. *Rhodoplanes*, a red wanderer.

Cells are rod shaped; motile by means of polar, subpolar, or lateral flagella; and multiply by budding and asymmetric cell division. Internal photosynthetic membranes are present as lamellar stacks parallel to the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. Quinones are Q-10 and RQ-10. Growth is preferentially photoheterotrophic under anoxic conditions in the light, with simple organic substrates as carbon and energy sources. Photoautotrophic growth with sulfide as the electron donor does not occur. Chemotrophic growth is possible under oxic conditions in the dark at full atmospheric oxygen tension and by denitrification under anoxic conditions in the presence of nitrate. Growth factors may be required. Found in freshwater with preference for neutral pH. Some representatives may be thermotolerant.

The mol% G+C of the DNA is 66–71.

Type species: *Rhodoplanes roseus*.

The genus *Rhodoplanes* currently (June 2012) contains five species: *R. elegans*, *R. piscinae*, *R. pokkalisoli*, *R. roseus*, and *R. serenus*. The name *R. cryptolactis* was effectively but not yet validly published (Okamura et al. 2007). The main features of the members of the genus *Rhodoplanes* are summarized in Table 11.9.

Additional comments:

- *Rhodoplanes* cells are often motile in young cultures, but motile cells become extremely rare at the late-exponential phase of growth (Hiraishi and Imhoff 2005).
- *R. roseus* can also grow anaerobically or microaerophilically on pyruvate in the dark (Janssen and Harfoot 1991).
- "*Rhodoplanes cryptolactis*" (Okamura et al. 2007), originally described as "*Rhodopseudomonas cryptolactis*" (Stadtwald-Demchick et al. 1990), is a thermotolerant species, isolated from the Thermopolis Hot Springs, Wyoming.

Genus *Blastochloris* (Hiraishi 1997, 218^{VP})

Blas.to.chlo'ris. Gr. n. *blastos*, bud shoot; Gr. adj. *chlôros*, green; N.L. fem. n. *Blastochloris*, green bud shoot.

Cells are rod shaped to ovoid and motile by means of subpolar flagella. They exhibit polar growth, budding, and asymmetric cell division and form rosette-like aggregates. Internal photosynthetic membranes are present as lamellae underlying and parallel to the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll *b* and carotenoids. Ubiquinones and menaquinones are present, and the lipopolysaccharides are characterized by 2,3-diamino-2,3-deoxy-D-glucose-containing phosphate-free lipid A with amide-bound $C_{14:0}$ 3OH. They preferentially grow photoheterotrophically, using organic acids and other carbon sources under anoxic conditions in the light. Photoautotrophic growth may occur under anoxic conditions with thiosulfate or sulfide as electron donor. Chemotrophic growth is possible under microoxic conditions in the dark. Growth factors may be required. Found in aquatic environments of neutral pH.

The mol% G+C of the DNA is 63–72.

Type species: *Blastochloris viridis*.

The genus *Blastochloris* currently (June 2012) contains 3 species: *B. gulmargensis*, *B. sulfoviridis*, and *B. viridis*. The main features of the members of the genus *Blastochloris* are summarized in Table 11.9.

Additional comment:

- Additional comparative taxonomic and chemotaxonomic information on *Blastochloris* spp. was given by Kompantseva et al. (1998, 2007).

Isolation, Enrichment and Maintenance Procedures

Hyphomicrobium spp. can be selectively enriched and isolated using anaerobic media with methanol (0.5 %) as carbon source and nitrate (0.5 % KNO_3) as the electron acceptor (Attwood and Harder 1972; Poindexter 2006). For the other genera of the *Hyphomicrobiaceae*, no reliable enrichment and isolation procedures have been developed. One common property of many representatives of the group is their "oligocarbophilic" nature, i.e., their ability to thrive at extremely low concentrations of organic nutrients or even without any organic carbon sources added to the medium (Hirsch and Conti 1964; Gliesche et al. 2005). Growth is then based on the diffusion of volatile

carbon and energy sources from the air. Under these conditions, their growth may be slow, but they may outcompete most other groups of aerobic chemoheterotrophs. Thus, the development of *Hyphomicrobium* colonies on agar plates can be accelerated by placing a bottle of methanol in the incubator (Poindexter 2006). A compilation of suitable growth media for the enrichment and cultivation of *Hyphomicrobium* spp. was given by Gliesche et al. (2005).

No selective medium exists for the isolation of *Pedomicrobium* spp. For enrichment and isolation of aquatic strains, a dilute peptone medium (0.1 g/l peptone + mineral salts and vitamins) was recommended. Stationary cultures are incubated at 20–30 °C until a surface pellicle develops in the highest positive dilution tubes (Hirsch and Gebers 2005). Generally suitable complex media for *Pedomicrobium* spp. contain an organic acid (e.g., acetate, malate, pyruvate, succinate, gluconate) at 10 mM, a complex organic mixture (peptone, casamino acids, yeast extract), and a mixture of vitamins (Poindexter 2006). An example is medium PYVM (Gebers and Beese 1988), which contains 10 mM DL-malate, 0.25 g/l peptone, 0.25 g/l yeast extract, and vitamins and minerals solutions, pH 7.5. Gebers (1981) recommended a "*Pedomicrobium* standard medium" (PSM) with 10 mM Na-acetate, 0.5 g/l yeast extract, minerals and vitamins, pH 9.0. For the isolation of slowly growing manganese-oxidizing *Pedomicrobium* from water sources, a micromanipulation method was proposed. Colonies of Mn-oxidizers on PC medium (0.05 g/l yeast extract, 0.02 g/l $MnSO_4.4H_2O$, 2 % agar, with 50 µg/ml cycloheximide to prevent fungal growth) can be detected at an early stage of development because of the accumulation of black manganese oxide. Micromanipulation then allows selection of cells from the microcolonies before these are overgrown by faster growing bacteria (Sly and Arunpairojana 1987).

No selective enrichment protocols are known for the genera *Angulomicrobium*, *Prosthecomicrobium*, and *Ancalomicrobium*. Strains of *Angulomicrobium* have been isolated on mineral base medium supplemented with low concentrations of organic substances such as glucose (Vasilyeva 2005). Most enrichments for *Prosthecomicrobium* and *Ancalomicrobium* used low-nutrient media, e.g., 100 mg/l peptone and/or 0.5 g/l glucose, supplemented with minerals and vitamins solutions (Schlesner et al. 1989; Oertli et al. 2006). The morphological properties of *Ancalomicrobium adetum* strongly depend on the nutrient levels in the medium: when grown at >200 mg/l peptone the cells lost their prosthecae, growth became slower and finally ceased; in >0.2 g/l glucose the prosthecae became shorter and the cells lost their motility (Semenov and Staley 1992). *Prosthecomicrobium hirschii* was enriched in 0.1 g/l peptone, then isolated by streaking on MMB medium (0.15 g/l peptone, 0.15 g/l yeast extract, 1 g/l glucose, salts and vitamins) (Staley 1984).

It has been observed that cells with multiple appendages (*Prosthecomicrobium*, *Ancalomicrobium*) have less tendency to adhere to glass surfaces than non-appendaged cells. A strategy was developed on the basis of this phenomenon to enrich the appendaged forms. A culture is loaded on a column filled with 0.2 mm diameter glass beads, and the column is washed with sterile medium. Alternate drops of the effluent are added to liquid medium and spread on plates of the same medium. If growth in liquid medium after 3 weeks incubation at room temperature shows prosthecate forms, then the colonies on the plates are examined. The first drops containing cells tend to be enriched in appendaged types (Staley 1968).

To enrich (nonspecifically) for *Seliberia*-like bacteria of aquatic origin, water samples (250–800 ml) in glass beakers, either without added nutrients or with 0.01–0.05 g/l peptone, are covered with aluminum foil or plastic film and incubated at 24–26 °C for a few weeks to several months. *Seliberia* often floats at the air-water interface. Inserting glass slides or cover slips near the surface may improve the results (Schmidt and Swafford 2006).

For enrichment of *Dichotomicrobium*, clean sterile glass slides were inserted in hypersaline water from Solar Lake, Sinai. After 8–10 days the slides with attached bacteria were transferred to Solar Lake water with 0.25 g/l yeast extract. Cells can also be directly isolated by streaking water samples on plates of oligotrophic medium PYGV prepared in 2.5× concentrated artificial seawater (Hirsch and Hoffmann 1989; Hirsch 2005).

A number of *Devosia* species were first obtained as colonies on the low-nutrient R2A agar medium, commonly used for the isolation and enumeration of soil bacteria. These include *D. soli* isolated from greenhouse soil in Korea (Yoo et al. 2006), *D. geojensis* isolated from diesel-contaminated soil in Korea (Ryu et al. 2008), and *D. psychrophila*, and *D. glacialis* isolated from alpine glacier cryoconite (Zhang et al. 2012). *D. insulae* was found as a colony on a plate containing 10× diluted nutrient agar (Yoon et al. 2007).

A low-nutrient medium (0.1 g/l peptone and mineral salts) was also used for the isolation of *Aquabacter spiritensis*. The appearance of a chalky-white colony led to the discovery of this gas-vacuolate species (Irgens et al. 1991). *Maritella mobilis* was isolated by serial dilutions of a seawater sample onto a solidified seawater medium low in organic nutrients (Xu et al. 2009).

For the enrichment and cultivation of *Pedomicrobium* and *Seliberia* spp., special media were proposed based on soil extract (Aristovskaya and Parinkina 1963) or "humic gel agar." This medium contains liter 5 g (wet weight) of "humic gel" (i.e., fulvic acid iron sesquioxide complexes extracted from podzolic soils) and 18–20 g Bacto-agar, pH 5.7 (for further information, including detailed protocols for the preparation of the "humic gel" see Gebers 1981; Gebers and Beese 1988; Poindexter 2006; Schmidt and Swafford 2006). The properties of such media strongly depend on the type of soil used, and reproducibility may be poor. Hirsch and Gebers (2005) therefore dismissed routine testing of *Pedomicrobium* spp. for iron and manganese oxide accumulation from fulvic acid sesquioxide complexes as impractical because these humic substances are available solely from podzolic soils, which only occur in certain regions on earth. Instead of fuvic acid complexes embedded in agar media they recommended use of elemental iron, FeS, or $MnSO_4$ in conventional media.

Not all species of *Hyphomicrobiaceae* were isolated on special, more or less selective low-nutrient media. Quite a few were discovered during the screening of colonies on normal, high-nutrient media. *Devosia albogilva* and *D. crocina* were recovered from colonies on Luria-Bertani (LB) agar inoculated with material from a hexachlorocyclohexane dump site (Verma et al. 2009); *D. yakushimensis* was isolated by serial dilution of material from a root nodules of *Pueraria lobata* on yeast extract—mannitol agar (Bautista et al. 2010). *Cucumibacter marinus* and *Pelagibacterium halotolerans* were obtained by plating seawater directly on marine agar (Hwang and Cho 2008. Xu et al. 2011).

Some members of the family were retrieved on the basis of special substrates degraded by them: *Methylorhabdus multivorans* was isolated by plating of dichloromethane-contaminated groundwater on dichloromethane containing agar (Doronina et al. 1995). *Devosia riboflavina* was enriched in medium containing 1 g/l riboflavin and 1 g/l glucose and in addition phosphate and magnesium sulfate (Foster 1944). *Filomicrobium insigne* was enriched in a mineral medium amended with crude oil (Wu et al. 2009).

The facultatively anaerobic photoheterotrophs classified within the *Hyphomicrobiaceae* were all obtained by anaerobic enrichment in the light using different carbon sources. *Rhodomicrobium vannielii* can be enriched with succinate or ethanol in mineral media with as initial pH of 5.2–5.5 (Duchow and Douglas 1949; Imhoff 2005b). *Rhodoplanes roseus* was obtained from a Winogradsky column enrichment prepared with mud from a duck pond in a park in New Zealand (Janssen and Harfoot 1991). *Rhodoplanes pokkaliisoli* and *R. serenus* were both enriched using pyruvate as carbon source (Lakshmi et al. 2009; Okamura et al. 2009). The bacteriochlorophyll *b*-containing members of the genus *Blastochloris* were isolated on pyruvate (*B. gulmargensis*; Ramana et al. 2011), malate + yeast extract (*B. viridis*; Drews and Giesbrecht 1966) or toluene (another strain of *B. sulfoviridis*; Zengler et al. 1999). Use of appropriate light filters that allow only long wavelength radiation to penetrate increases the selectivity of the enrichment procedure (Zengler et al. 1999; Imhoff 2005a).

Maintenance

Standard techniques for maintenance of cultures can be used for the *Hyphomicrobiaceae*. For short-time maintenance (up to a number of weeks), cultures can be kept refrigerated at 4 °C. Freezing is possible as well. Gliesche et al. (2005) recommended for *Hyphomicrobium* spp. suspension in phosphate buffer and sterile glycerol, followed by immediate mixing and cooling down to −25 °C. Cells can then be kept frozen at −20 to −25 °C for several years. For *Prosthecomicrobium* and *Ancalomicrobium* spp. frozen cultures made using 15 % (w/v) glycerol and stored at −70 °C have proven to remain viable for at least 4 years. Storage of glycerol stocks at −20 °C is not recommended (Oertli et al. 2006). *Devosia* spp. can be preserved by freezing cell suspensions in nutrient both + 10 % glycerol + 7 % dimethyl sulfoxide as cryoprotective agents at −80 °C or −196 °C (Nakagawa et al. 2005).

For long-term preservation, lyophilization is recommended. Lyophilization in skim milk is the optimal method for maintenance of *Hyphomicrobium* cultures (Gliesche et al. 2005). For *Dichotomicrobium* lyophilization in growth medium gave better results than in skim milk (Hirsch 2005).

Physiological and Biochemical Features

A few special biochemical properties of a number of *Hyphomicrobiaceae* deserve to be highlighted: C_1 metabolism and metabolism of dimethyl sulfone, dichloromethane, and methamidophos by *Hyphomicrobium* spp.; C_1 metabolism in *Angulomicrobium tetraedrale*; and toluene metabolism in a strain of *Blastochloris sulfoviridis*.

Methanol is oxidized in *Hyphomicrobium* spp. by a periplasmic methanol dehydrogenase which contains PQQ (pyrroloquinoline quinone) as cofactor. Hyphomicrobia contain both tetrahydrofolate- and tetrahydromethanopterin-linked C_1 metabolites (Gliesche et al. 2005). Carbon is assimilated by the serine pathway (Izumi et al. 1982). *Methylorhabdus multivorans* uses the isocitrate lyase-negative variant of the serine pathway (Doronina et al. 1995). On the other hand, *Angulomicrobium tetraedrale* uses ribulose 1,5-bisphosphate carboxylase/oxidase (RuBisCO) when grown on C_1 substrates. It can grow on methanol and on formate, and possesses all enzymes needed for the oxidation of methanol via formaldehyde and formate to CO_2. It also can grow chemolithoautotrophically in an atmosphere of $CO_2 + H_2 + O_2$ (Doronina and Trotsenko 2006).

Dimethyl sulfone metabolism in *Hyphomicrobium sulfonivorans* strain S1 is based on constitutive separate membrane-associated dimethyl sulfone and dimethyl sulfoxide reductases. Cells growing on dimethylsulfone also oxidize DMSO and DMS. DMSO is reduced to DMS in an NADH-dependent reaction, and DMS is converted by a monooxygenase to methanethiol and formaldehyde. Methanethiol is then oxidized to formaldehyde and to sulfide, which is further oxidized to sulfate (Borodina et al. 2002).

Hyphomicrobium sp. strain DM2 uses dichloromethane as the sole carbon source for under denitrification conditions. A dichloromethane dehalogenase is induced both aerobically and anaerobically with nitrate (Kohler-Staub et al. 1995). *Hyphomicrobium chloromethanicum* strain $CM2^T$ has an inducible enzyme system for the utilization of chloromethane. The degradation is initiated by two methyltransferases: CmuA (chloromethane + a cobalamin → HCl + methyl-bound cobalamin) and CmuB (transferring the methyl group to tetrahydrofolate, McAnnula et al. 2001).

Hyphomicrobium species MAP-1 degrades the organophosphorus insecticide methamidophos and can use the compound as its sole carbon, nitrogen and phosphorus source. It is first cleaved at the P-N bond to form *O,S*-dimethyl thiophosphate and NH_3. *O,S*-dimethyl thiophosphate is hydrolyzed at the P-O

bond to release $-OCH_3$ and with the formation of *S*-methyl dihydrogen thiophosphate (Wang et al. 2010).

The type strain of *Blastochloris sulfoviridis* is unable to use toluene or benzoate as its carbon source. A new isolate was obtained that also grows on toluene under anoxic conditions in the light. Following incubation of cell extracts with toluene and fumarate benzylsuccinate was detected, showing that the anaerobic activation of the aromatic ring uses the same mechanism reported for denitrifying and sulfate reducing bacteria (Zengler et al. 1999).

Ecology

Members of the family *Hyphomicrobiaceae* can be found virtually anywhere—in freshwater, in soils, in seawater and brackish waters, and even in moderately hypersaline and hot environments. Thus, *Hyphomicrobium* spp. could be isolated from all soil samples and from nearly all water samples tested (Hirsch and Conti 1964). In freshwater habitats they are especially prevalent in the neuston layer, on submerged surfaces, and in the upper sediment layer (Gliesche et al. 2005). As discussed above, many types may have a selective advantage in oligotrophic environments. This is particularly true for the prosthecate genera, as the prosthecae increase the cells' surface area to increase uptake of nutrients (Semenov and Staley 1992). Thus, *Seliberia*-like organisms have been observed in stored samples of laboratory distilled water (Schmidt and Swafford 2006). It should be noted that prosthecae development may be aberrant, cell shape can be irregular, lysis is frequent, and cell viability in the colonies is low. Therefore the prosthecate forms are more often sighted in the environment than isolated (Poindexter 2006).

Prosthecate bacteria such as *Hyphomicrobium*, *Prosthecomicrobium*, and *Ancalomicrobium* spp. generally occur there in relatively low numbers, typically forming a small percentage of the viable colony-forming cells of heterotrophs. They have been found in freshwater (Staley 1968), brackish and marine water (Bauld et al. 1983; Schlesner et al. 1989), in groundwater, and in soil. They have been sighted in unusually large numbers in two specialized environments. A survey of 11 pulp mills waste aeration lagoons located in 6 states throughout the USA showed that 0.6–10.5 % of the prokaryotes observed ($>10^6$ per ml) could be recognized and assigned to genera of prosthecate bacteria on the basis of their morphology (Stanley et al. 1979). An unusual bloom of starlike prosthecate bacteria and filaments, with $>2 \times 10^6$ cells/ml with *Ancalomicrobium* morphology, was found in seawater used for shrimp aquaculture in Polynesia. It is possible that these prosthecate cells are not grazed by microflagellates but are available for mesoplankton larvae (Bianchi 1989).

There have been relatively few molecular ecology studies specifically targeting the *Hyphomicrobiaceae*. A specific probe for fluorescence in situ hybridization (FISH) studies of iron-depositing *Pedomicrobium* species was developed (probe "Pedo 1250", 5′-CGCUGGGUGGCUGCCCAC-3′, being complementary to the hypervariable region 8, position 1250–1268). It was successfully applied in a study of natural biofilms from the Odertal National Park, Germany (Braun et al. 2009). Another molecular study dealt with the characterization of a bacterial endosymbionts of the marine ciliate *Euplotes magnicirratus* (*Ciliophora*, *Hypotrichia*). Based on 16S rRNA sequence comparisons, this symbiont is affiliated with the genus *Devosia*, and it was provisionally named '*Candidatus* Devosia euplotis'. Oligonucleotide probe DevEup_993, 5′-AAGTCGTCCTGGTATGTC-3′, was developed for the specific visualization of the endosymbiont in FISH studies (Vannini et al. 2004).

Devosia neptuniae was isolated from the aquatic leguminous plant *Neptunia natans* indigenous to tropical and subtropical areas. It possesses genes for both nitrogen fixing and nodulation (Rivas et al. 2002, 2003). To what extent this bacterium is indeed responsible for *Neptunia*-associated nitrogen fixation remains to be ascertained. Another plant-associated *Devosia* species is *D. yakushimensis*, isolated from root nodules of *Pueraria lobata* (Bautista et al. 2010). Also here the possible nature of its interactions with the plant has not yet been reported.

Some representatives of the *Hyphomicrobiaceae* have been isolated from polluted environments: *Hyphomicrobium chloromethanicum* from soil at a petrochemical factory in Russia (McDonald et al. 2001), *Devosia albogilva* and *D. crocina* from a hexachlorocyclohexane dump site in India (Verma et al. 2009), and *Pelagibacterium luteolum* from a semi-coke sample in Estonia (Xu et al. 2011).

Pedomicrobium spp. and *Seliberia* are known to deposit iron and manganese on their cells (Aristovskaya 1961; Ghiorse and Hirsch 1979; Verkhovtseva et al. 1988; Larsen et al. 1999). Treatment with glutaraldehyde, $HgCl_2$ or heat abolished the accumulation of iron oxides by *Pedomicrobium* sp., showing that the process is biologically mediated, but did not affect manganese deposition (Ghiorse and Hirsch 1979). A later study with *Pedomicrobium* sp. ACM 3067 showed that Mn(II) adsorption to whole cells is enhanced by heat treatment but Mn(II) oxidation was abolished (Larsen et al. 1999).

The photoheterotroph *Rhodomicrobium vannielii* is commonly found in mud and water of ponds and lakes, as well as in wastewater. It prefers slightly acidic waters (Imhoff 2005b). Some *Rhodomicrobium* strains can also deposit iron hydroxides on the cell surface. Strain BS-1, enriched from a ditch in Germany, oxidizes Fe(II) in the light. This reaction appears to be a side activity of the cells, as Fe(II) oxidation does not support growth in the light over prolonged periods of time (Heising and Schink 1998).

Pathogenicity and Clinical Relevance

Recently there has been a report of an infection of a human patient in China by a bacterium claimed to be a *Rhodoplanes* sp. The bacterium first caused skin abscesses and ulcer, and later a systemic infection occurred.

Table 11.11

Sensitivity of species of the family *Hyphomicrobiaceae* to selected antibiotics

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Ampicillin	+	+	+	+	+	+	+	+/−	+/−	+	NR	+	+	NR	NR	NR	NR	NR	NR	NR	+	NR	+	+	+	+	+	−	+	+
Carbenicillin	NR	NR	NR	NR	+	+	NR	NR	NR	NR	NR	+	NR	NR	+	NR	NR	NR	NR	NR	+	NR	+	NR	NR	NR	NR	NR	NR	NR
Cephalothin	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	v	+	+	−	NR	NR
Chloramphenicol	+	+	+	+	−	+	+/−	+/−	+/−	+	+	+	NR	+	+	NR	NR	NR	NR	NR	+	+	+	NR	+	+	+	−	+	+
Ciprofloxacin	NR	+	+	+	NR	NR	−	+/−	−	+	NR	−	NR	NR	NR	NR	NR	NR	NR	NR	+	NR	+	NR	NR	NR	NR	NR	NR	NR
Erythromycin	+	NR	NR	NR	NR	NR	−	−	−	NR	NR	NR	NR	NR	+	+	+	NR	NR	NR	+	+	+	−	NR	NR	NR	NR	+	+
Gentamycin	−	+	+/−	+	−	−	+/−	+/−	+/−	+/−	−	−	NR	NR	NR	NR	NR	NR	NR	NR	+	+	+	−	NR	NR	+/−	+	NR	NR
Kanamycin	+	−	−	−	−	−	−	−	−	−	−	−	NR	NR	+	+	+	+	+	+	+	+	−	−	NR	NR	NR	NR	−	+
Nalidixic acid	−	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	−	NR	NR	NR	NR	NR	NR	+	−	−	NR	NR	−	−	NR	NR
Neomycin	+	NR	−	NR	−/+	−	+	+	+	+	−	+	NR	NR	NR	+	+	+	+	+	+	NR	+	+	+	+	+	+	+	+
Novobiocin	+	NR	NR	NR	−	+	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	NR	+	NR	+	+	NR	NR	NR	NR	+	+
Penicillin	+	NR	NR	NR	+	+	NR	−	−	NR	NR	NR	+	+	NR	NR	NR	NR	NR	NR	+	+	+	NR	+	+	+	NR	+	+
Polymyxin B	NR	−	−	−	−	+	−	w	−	−	NR	−	NR	NR	+	NR	NR	NR	NR	NR	+	NR	−	NR	+	+	−	+	−	−
Rifampicin	NR	+	+	+	NR	NR	−	−	−	+	NR	+	NR	NR	+	NR	NR	NR	NR	NR	+	NR	+	NR	NR	NR	+	−	+	+
Streptomycin	−	NR	NR	NR	−	−	NR	NR	NR	NR	NR	NR	+	+	+	+	+	NR	+	+	−	−	−	−	+	+	+	NR	−	−
Tetracycline	NR	+	+	+	+	+/−	+/w	+/−	+	+	+	+	NR	+	NR	+	+	+	NR	+	+	NR	+	NR	NR	NR	+	−	+	+
Vancomycin	+	+	+	+	+	−	+	+	+	+	+	+	NR	NR	NR	NR	NR	NR	NR	NR	+	+	+	NR	NR	NR	NR	NR	NR	NR

Species: (1), *Cucumibacter marinus* (Hwang et al. 2009; Xu et al. 2011); (2), *Devosia albogilva* (Verma et al. 2009); (3), *D. chinhatensis* (Kumar et al. 2008; Bautista et al. 2010); (4), *D. crocina* (Verma et al. 2009); (5), *D. geojensis* (Ryu et al. 2008; Bautista et al. 2010); (6), *D. insulae* (Yoon et al. 2007; Bautista et al. 2010); (7), *D. limi* (Vanparys et al. 2005; Bautista et al. 2010); (8), *D. neptunia* (Rivas et al. 2003; Vanparys et al. 2005; Bautista et al. 2010); (9), *D. riboflavina* (Rivas et al. 2003; Vanparys et al. 2005; Bautista et al. 2010); (10), *D. soli* (Yoo et al. 2006; Bautista et al. 2010); (11), *D. subaequoris* (Bautista et al. 2010); (12), *D. yakushimensis* (Bautista et al. 2010); (13), *Dichotomicrobium thermohalophilum* (Hirsch and Hoffmann 1989); (14), *Filomicrobium fusiforme* (Schlesner 1987); (15), *F. insigne* (Wu et al. 2009); (16), *Hyphomicrobium aestuarii* (Gliesche et al. 2005); (17), *H. facile* subsp. *facile* (Gliesche et al. 2005); (18), *H. hollandicum* (Gliesche et al. 2005); (19), *H. vulgare* (Gliesche et al. 2005); (20) *H. zavarzinii* (Gliesche et al. 2005); (21), *Maritalea mobilis (*Xu et al. 2009); (22) *M. myrionectae* (Hwang et al. 2009); (23) *M. prophyrae* (Fukui et al. 2012); (24), *Methylorhabdus multivorans* (Doronina et al. 1995); (25), *Pedomicrobium americanum* (Gebers and Beese 1988),; (26) *P. australicum* (Gebers and Beese 1988); (27), *P. ferrugineum* (Gebers 1981; Gebers and Beese 1988; Hirsch and Gebers 2005); (28), *P. manganicum* (Gebers 1981); (29), *Pelagibacterium halotolerans* (Xu et al. 2011); (29), *P. luteolum* (Xu et al. 2011)

+ sensitive,− resistant, +/− different reports by different authors, *NR* not reported, *w* weak, *v* variable among strains

The organism was isolated and was assigned to the genus *Rhodoplanes* solely on account of its rRNA gene sequence. No information was provided that could have confirmed the identification such as the formation of bacteriochlorophyll *a* and carotenoid pigments under anaerobic conditions in the light (Zhang et al. 2011). Beyond this single poorly documented report none of the members of the *Hyphomicrobiaceae* is known to be pathogenic to humans, animals or plants.

Table 11.11 summarizes data on the sensitivity of members of the *Hyphomicrobiaceae* to different antibiotics. Such data are not available for all members of the family, and the information present does not show any clear patterns, except for a general sensitivity to ampicillin, tetracycline, and vancomycin and for the resistance of *Devosia* spp. to kanamycin and polymyxin B.

Application

The current applications of the *Hyphomicrobiaceae* are of three main types: (1) biodegradation and bioremediation, (2) biosensors and enzymes for analytical purposes, and (3) biosynthesis of valuable chemicals. In nearly all cases members of the genus *Hyphomicrobium* are involved (Gliesche et al. 2005), but the applicative potential of *Devosia riboflavina* has also been proposed (Kizaki et al. 2008).

Diverse *Hyphomicrobium* populations are associated with sewage and water treatment plants (Holm et al. 1996). The ability of *Hyphomicrobium* spp. to remove nitrate by denitrification while using methanol as the oxidant is especially valuable, as methanol is a cheap, non-fermentable substrate that is oxidized to CO_2 (Liessens et al. 1993). Hyphomicrobia have been used for the removal of odorous volatile sulfur compounds such as H_2S, methane thiol and dimethylsulfide from air and gasses. *Hyphomicrobium* sp. strain VS, isolated from activated sludge using a medium with a low concentration of dimethyl sulfide, can use dimethylsulfide, dimethylsulfoxide, methanol, formaldehyde, formate, and methylated amines as substrates. Dimethylsulfide-grown cells respired sulfide, thiosulfate, methanethiol, dimethyldisulfide, and dimethyltrisulfide. A laboratory-scale trickling biofilter filled with this bacterium, using polyurethane and lava stone as carrier material, efficiently and rapidly removed high concentrations (1–2 μM) of dimethylsulfide from air (Pol et al. 1994). Dimethyl sulfoxide was degraded by cells of *Hyphomicrobium denitrificans* WU-K217 immobilized in calcium-alginate gel. The system may be applicable to wastewater treatment systems for removal of dimethyl sulfoxide (Murakami-Nitta et al. 2003). Specialized *Hyphomicrobium* strains can be applied to the biodegradation and bioremediation of dichloromethane in industrial wastes and in polluted groundwater (Gälli and Leisinger 1985; Diks et al. 1994; Kohler-Staub et al. 1995).

Specific strains of *Hyphomicrobiaceae* and enzymes obtained from them have been applied in biosensors for the detection of methanol, methylsulfates, methylated amines, dihalomethanes, L-serine, and glyoxylate. These applications and potential applications are based on the advantage of *Hyphomicrobium*-derived dehydrogenases such as formaldehyde dehydrogenase and methylamine dehydrogenase use the covalently bound cofactor pyrroloquinoline quinone and not the water-soluble and costly NAD or NADP that need to be supplied continuously (Jérôme et al. 2007; Hilbrig et al. 2009). Thus, a trehalose-stabilized methanol dehydrogenase from *Hyphomicrobium* X can be used for the detection of methanol (Argall and Smith 1993). A *Hyphomicrobium*-containing electrode was developed for the quantitative determination of monomethylsulfate (Schär and Ghisalba 1985). *Hyphomicrobium*-derived enzymes can be used for the assay of trimethylamine, e.g., to assess the quality of fish (Large and McDougall 1975; Wong and Gill 1987). Use of enzymes involved in the serine pathway for carbon assimilation of hyphomicrobia enabled the development of enzymatic assays for L-serine and glyoxylate (Yoshida et al. 1993).

The enzyme systems used in the above mentioned analytical applications can also be applied to large-scale stereospecific synthesis of valuable chemicals. Thus *H. methylovorum* can be used for the production of L-serine from glycine and methanol by action of methanol dehydrogenase and serine hydroxymethyltransferase. With resting cells of the wild type of *H. methylovorum* 24 mg/l of L-serine was produced from 100 mg/ml glycine and 48 mg/ml of methanol in 3 days. Use of a glycine-resistant mutant enabled improved serine production (32–34 mg/ml) (Yamada et al. 1987; Izumi et al. 1993). A novel NADH-dependent alcohol dehydrogenase of *Devosia riboflavina* can be applied in the stereoselective reduction of 3-pyrrolidinone derivatives. Optically active *N*-benzyl-3-pyrrolidinols are versatile chiral building blocks for diverse chemical syntheses (Kizaki et al. 2008).

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12 The Family *Hyphomonadaceae*

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Abstract
The family *Hyphomonadaceae* comprises strict aerobic and stalked and non-stalked species. Cells in this family can have two differing cell cycles. Species of the genera *Algimonas*, *Maricaulis*, *Oceanicaulis*, *Ponticaulis*, *Robiginitomaculum*, and *Woodsholea* divide by binary fission of the cell leading to flagellated offsprings. Species of the genera *Hellea*, *Hirschia*, *Hyphomonas*, and *Litorimonas* divide by budding, forming a flagellated cell at the tip of the prosthecum. All flagellated cells are mobile but later develop a stalk, shed the flagella, and become sessile. These two-cell different cycles are both optimal for living in oligotrophic habitats where the competition for food is fierce and should be minimized between siblings. The genus *Henriciella* is currently the only genus with species lacking any stalks. With the possible exception of *Hyphomonas polymorpha*, all species are marine isolates and require sodium chloride for optimal growth. Species of *Hyphomonadaceae* occupy various habitats in the oceans where they are usually found in oligotrophic niches from the Antarctic to deep sea vents. Only few isolates can grow on minimal media with defined carbon sources; however, most species require complex growth factors for growth. Polar lipids for most species are monoglycosyl and monoglucuronosyl diacylglycerols. Common phospholipids are phosphatidylglycerols and many species possess sulfoquinovosyl diacylglycerols and glucuronopyranosyl diacylglycerol taurine amides as well. Most species reduce nitrate and are alkaline phosphatase and leucine arylamidase positive.

Taxonomy, Historical and Current

For many decades, dimorphic, prosthecate bacteria which reproduce by the separation of two cells that are morphologically and behaviorally distinct from each other were called *Caulobacter*. One of these cells is sessile and nonmotile and prosthecate, possessing at least one elongated, cylindrical appendage, a prostheca (Staley 1968). The other cell is flagellated, bearing one polar flagellum, by which it is motile. The motile cell later develops a stalk, losses it flagellum, and becomes sessile. The mode of reproduction of the dimorphic, prosthecate bacteria is unique as a regular feature of a prokaryotic reproductive cycle. It helps to disperse the population at each generation, minimizing competition among siblings for resources. Loeffler was the first to report on the isolation of these bacteria (Loeffler 1890), but only in 1935, Henrici and Johnson described the genus *Caulobacter*, with *Caulobacter vibrioides* as the type species (Henrici and Johnson 1935). Three decades later, Poindexter (1964) described *Caulobacter maris* and *Caulobacter halobacteroides*, the first two caulobacteria from seawater. At that time, the taxonomy of the members of the genus was based almost exclusively on the characteristic morphology and mode of reproduction (Poindexter 1981). Stahl et al. (1992) sequenced the 16S rRNA of several isolates belonging to *Caulobacter*, revealing that members of *Caulobacter* actually formed three distinct lineages, one comprising the freshwater and brackish water *Caulobacter* strains and the other comprising the marine *Caulobacter* strains. Bacteria of the third lineage belong to the genus *Sphingomonas* with *S. leidyi* (Poindexter) as the only prosthecate *Sphingomonas* species so far described (Chen et al. 2012a). In 1999 Abraham et al. transferred *Caulobacter maris* to the new genus *Maricaulis* as the type

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species *M. maris*. For prosthecate species also proliferating by fission but being in several aspect differing from species belonging to *Maricaulis*, the novel genera *Oceanicaulis* (Strömpl et al. 2003) and *Woodsholea* (Abraham et al. 2004) were described.

In 1957, Pongratz reported the isolation of a prosthecate, but budding bacterium from the nasal mucous of a patient with sinusitis (Pongratz 1957). Because this organism grew on complex media, such as blood agar, and was unable to grow on simple mineral salt media containing only one-carbon compounds as the sole sources of carbon and energy, a new genus was proposed to accommodate this organism, and the organism was named *Hyphomonas polymorpha*. Another budding species, *Hirschia baltica*, was described in 1990, which is still the only known species of the *Hyphomonadaceae* coming from brackish water (Schlesner et al. 1990). Only with the advent of gene sequencing it became apparent that *Maricaulis*, *Oceanicaulis*, and *Woodsholea*, reproducing by fission, and *Hyphomonas* and *Hirschia*, reproducing by budding, belong to the same family which was described in 2005 as *Hyphomonadaceae* (Lee et al. 2005). This family grew considerable in size when the genera *Robiginitomaculum* (Lee et al. 2007), *Hellea* (Alain et al. 2008), *Henriciella* (Quan et al. 2009; emend. Lee et al. 2011), *Ponticaulis* (Kang and Lee 2009a), *Litorimonas* (Jung et al. 2011), and *Algimonas* (Fukui et al. 2012) were established.

Hy.pho.mo.na.da'ceae. N.L. fem. n. *Hyphomonas* type genus of the family; -aceae ending to denote a family (N.L. fem. pl. n. *Hyphomonadaceae* the *Hyphomonas* family).

The description of the family is an emended version of the description given by Lee et al. (2005): Phylogenetically together with the *Caulobacteraceae* (Henrici and Johnson 1935) and the *Rhodobacteraceae* nom. ill. (Garrity et al. 2005), a member of the order *Caulobacterales* (Henrici and Johnson 1935), phylum Proteobacteria, class *Alphaproteobacteria* (Garrity et al. 2005). Gram-negative, rod-shaped bacteria, motile. Cells do not form spores. Chemoorganotrophic. Some species require peptone or B vitamins and amino acids. Species are aerobic or facultatively anaerobic. Some species denitrify. In most species, the major isoprenoid quinone is Q-10. Most species contain the polar lipids α-D-glucopyranosyl diacylglycerol, α-D-glucuronopyranosyl diacylglycerol, and sulfoquinovosyl diacylglycerol; in some genera α-D-glucuronopyranosyl diacylglycerol taurine amide and phosphatidyl diacylglycerol are also present. Many species show a reduction of the relative amount of phospholipids, sometimes down to <1 %, in favor of glyco- and sulfolipids (Abraham et al. 1997; Batrakov et al. 1996a). Members of the family have been isolated from seawater. The family comprises the type genus *Hyphomonas* (Pongratz 1957; emend. by Moore et al. 1984) and the genera *Hirschia* (Schlesner et al. 1990), *Maricaulis* (Abraham et al. 1999), *Oceanicaulis* (Strömpl et al. 2003), *Woodsholea* (Abraham et al. 2004), *Robiginitomaculum* (Lee et al. 2007), *Hellea* (Alain et al. 2008), *Henriciella* (Quan et al. 2009; emend. Lee et al. 2011), *Ponticaulis* (Kang and Lee 2009a), *Litorimonas* (Jung et al. 2011), and *Algimonas* (Fukui et al. 2012) (*Table 12.1*).

Molecular Analyses

The four genomes of *Hyphomonadaceae* sequenced and annotated so far contain between 3.17 and 3.71 megabases. They are therefore somewhat smaller than most genomes of *Caulobacteraceae*. Usually one chromosome is present but cells of *Hirschia baltica* also harbor a plasmid of 84.492 bp. These genomes contain between 3.077 and 3.568 genes coding 3.029–3.505 proteins. Most genomes have two identical copies of ribosomal RNA operons; only *Hyphomonas neptunium* ATCC 15444^T has only one. The number of tRNA genes is also pretty uniform, and 43–45 tRNA genes were predicted from the genome sequences (*Table 12.2*).

To thrive in oligotrophic environment, highly efficient mechanisms for nutrient uptake are essential in *Hyphomonadaceae*. For the uptake of compounds which are too large for uptake by simple passive diffusion, active transports by TonB-dependent receptors are used which are both anchored in the outer membrane and energy dependent. The known genomes of *Caulobacterales* and *Hyphomonadaceae* contain a large number of TonB-dependent receptors, e.g., *Caulobacter crescentus* has 63 and *Hyphomonas neptunium* and *Hirschia baltica* 46 of these genes (Chertkov et al. 2011). However, only 24 TonB-genes were identified in the *Oceanicaulis* sp. HTCC2633 genome (Oh et al. 2011), but far too little is known to draw any conclusions from these differences.

All species of *Hyphomonadaceae* have a very characteristic cell cycle similar to most species of *Caulobacterales*. Bioinformatic analysis of genes controlling the dimorphic cell cycle revealed the conservation of 14 key proteins that function in the regulation of the cell cycle (Brilli et al. 2010). There is, however, one notable exception: DivJ, a histidine kinase, is absent in the *Hyphomonas neptunium* and *Hirschia baltica* genomes but present in the *Oceanicaulis* sp. HTCC2633 and *Maricaulis maris* MCS 10 genomes. Bacteria belonging to the *Caulobacterales* and *Hyphomonadaceae* are also known for the ability to produce a holdfast, a polar polysaccharide, required for strong adhesion to surfaces. The genes required for the synthesis (Smith et al. 2003) and anchoring (Hardy et al. 2010) of the holdfast have been identified and characterized both in *Caulobacter crescentus* and *Hirschia baltica* but were largely absent from the genome of *Hyphomonas neptunium*, which does not produce a polar holdfast. The observation that most developmental regulators and holdfast genes are conserved in the budding bacteria belonging to the *Hyphomonadaceae* as well as the non-budding bacteria belonging both to the *Caulobacterales* and the *Hyphomonadaceae* can be interpreted that this regulation of the cell cycle evolved prior to the separation of the *Hyphomonadaceae* and *Caulobacterales*.

Quite different results came from studies of the control of chromosome replication. The dimorphic growth of *Hyphomonadaceae* requires cell-type-specific controls of chromosome replication preventing replication in swarmer cells but enabling the progeny stalked cell to perform chromosome replication. This is achieved by the CtrA protein which

Table 12.1
Characteristics of genera of *Hyphomonadaceae*

Genus	Replication	Polar lipids	Major fatty acids	G+C content	Ubiquinone
Algimonas[a]	Binary fission	MGDG[a], GUDG, PG	C18:1ω7c	58.5	
			2-OH C18:1		
Hellea	Budding	MGDG, GUDG, PG[b]	C18:1ω7c, C19:18c	47.9	
			2-OH iC19:1		
Hirschia	Budding		C18:1ω7c, C16:0	43.7–44.5	Q-10
			3-OH C12:1		
Henriciella	Binary fission	MGDG, GUDG, Tau, PG	C18:1ω7c, C16:0	55.2–61.0	Q-10
			3-OH C12:0		
Hyphomonas	Budding	MGDG, GUDG, Tau, PG	C18:1w7,	57–64	Q-11 (Q-10)
			3-OH C12:1		
Litorimonas	Budding	MGDG, GUDG, PG[b]	C18:1ω7c, C17:0	63.6	Q-10
			2-OH C18:1	47.1[a]	
Maricaulis	Binary fission	MGDG, GUDG, Tau, SQDG, PG	C18:1ω7		
			3-OH C12:0		
Oceanicaulis	Binary fission	MGDG, GUDG, SQDG, PG	C18:1ω7, C18:0	61–62	Q-10, Q-9
			3-OH C12:0		
Ponticaulis	Binary fission		C18:1ω7c, C16:0	53.3	Q-10
Robiginitomaculum	Budding		C18:1ω7, C17:1ω8	60.3	Q-10
			3-OH C11:0		
Woodsholea	Binary fission	MGDG, GUDG, Tau, SQDG	C18:1ω7	65	
			3-OH C12:0		

[a]*MGDG* monoglucosyl diacylglycerol, *GUDG* α-D-glucopyranuronosyl diacylglycerol, *PG* phosphatidyl glycerol, *Tau* 1,2-diacyl-3-α-D-glucuronopyranosyl-sn-glycerol taurine amide, *SQDG* 1,2-diacyl-3-O-sulfoquinovosylglycerol
[b]Fukui et al. (2012)

Table 12.2
Characteristics of annotated genomes of *Hyphomonadaceae*

Strain	Size (Mb)		rRNA operons	tRNAs	Genes	Proteins
Hirschia baltica ATCC 49814^T (Chertkov et al. 2011)	3.54	1 chromosome	2	44	3.269	3.187
	0.08	1 plasmid				
Hyphomonas neptunium ATCC 15444^T (Badger et al. 2006)	3.71	1 chromosome	1	43	3.568	3.505
Maricaulis maris MCS 10 (Copeland et al. 2011)	3.37	1 chromosome	2	45	3.133	3.063
Oceanicaulis sp. HTCC 2633 (Oh et al. 2011)	3.17	1 chromosome	2	44	3.077	3.029

binds to the chromosome origin and prevents its replication. As a consequence the CtrA protein is present in swarmer cells but absent in stalked cells (Bowers et al. 2008). A study directed to the replication origins of *Caulobacter*, *Oceanicaulis*, and *Maricaulis* strains revealed that the genera of *Caulobacteraceae* and *Hyphomonadaceae* developed differently. While all these replication origins use CtrA to repress replication, the study showed that CtrA usage evolved separately among these two families confirming the phylogeny derived from their 16S rRNA gene sequences (Shaheen et al. 2009).

Further differences between *Caulobacter* and *Hyphomonas* were found in their selenoproteins. While *Hyphomonas neptunium* encodes an L-seryl-tRNA selenium transferase, a selenocysteine-specific translation elongation factor, and a selenoprotein of unknown function, all these selenoproteins are absent in *C. crescentus*. Another argument for a clear distinction between *Caulobacteraceae* and *Hyphomonadaceae* comes from the biosyntheses of holdfasts. While *H. neptunium* and *C. crescentus* both synthesize holdfasts, none of the genes required for its synthesis have orthologs encoded in the

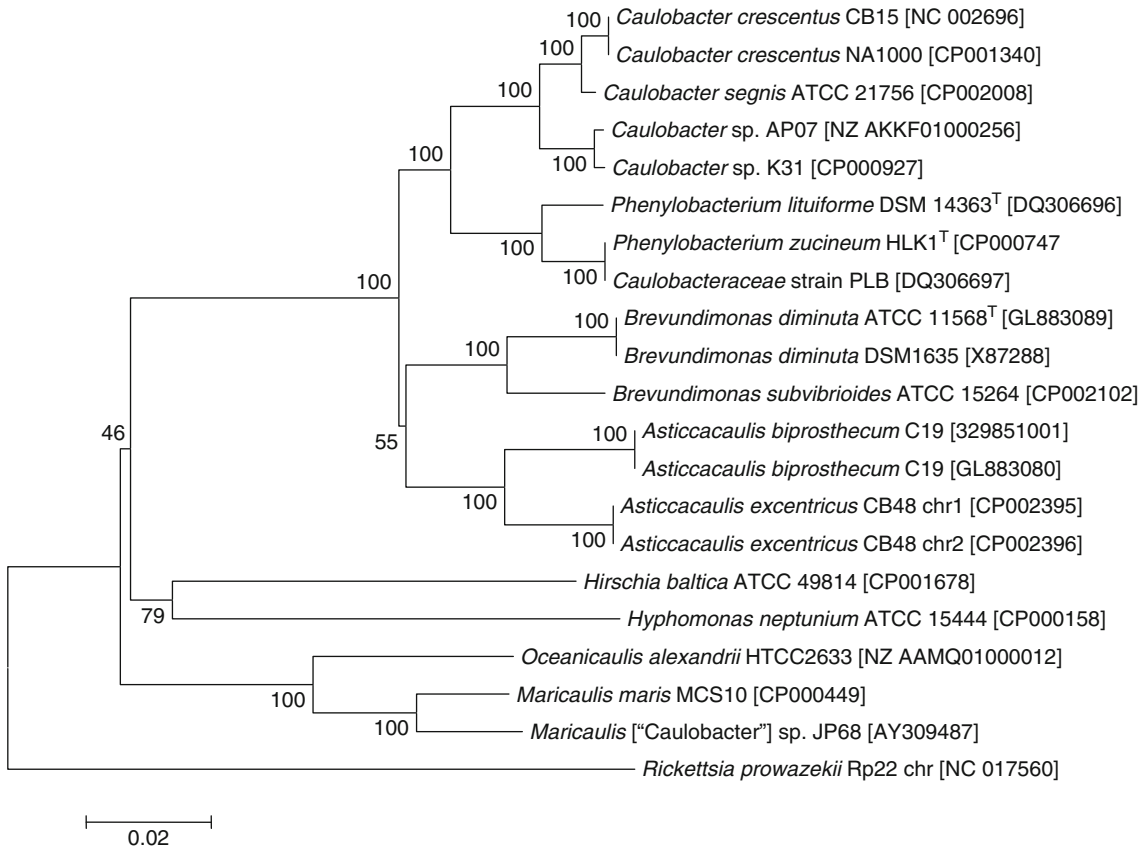

Fig. 12.1
Phylogenetic tree based on LSU rRNA gene sequences (neighbor joining, 1,000 bootstrap replication, *Rickettsia prowazekii* served as outgroup)

corresponding other genome (Badger et al. 2006). Therefore, it seems that holdfast synthesis and attachment must be achieved differently in both species. In line with these observation is also the gene organization for flagella formation in both species. While in *H. neptunium* most of the genes required for the flagellum and its synthesis are organized in one region comprising 35 contiguous open reading frames, the corresponding genes are scattered over the entire genome of *C. crescentus*. Furthermore, transcription of many flagellar genes in *C. crescentus* requires a σ^{54}-RNA polymerase holoenzyme which seems not to be involved in the corresponding transcriptions in *H. neptunium*.

Badger et al. (2005) reported that phylogenetic trees based on the 23S rRNA gene sequences differ remarkably from those calculated using the 16S rRNA gene sequences. However, using a much larger set of data, we could not find this discrepancy but a clear distinction between *Caulobacteraceae* and *Hyphomonadaceae* in phylogenetic trees based either on the 16S or the 23S rRNA gene sequences (*Figs. 12.1* and *12.2*).

DNA–DNA hybridizations between *Hyphomonas* isolates (Weiner et al. 2000), between *Maricaulis* isolates (Abraham et al. 2002), and between Henriciella isolates (Lee et al. 2011) have been reported. Applying the definition of species being distinct if DNA–DNA hybridization values are 70 % or less (Rosselló-Mora and Amann 2001) and comparing these results with the phylogenetic tree based on the 16S rRNA gene sequences, it can be concluded that only a few base pairs difference between two isolates of these genera is usually sufficient to assume two distinct species. This situation is similar to the one found in the *Caulobacteraceae* family (Abraham et al. 1999) (*Tables 12.3* and *12.4*).

This statement is corroborated by the DNA–DNA relatednesses reported between *Henriciella litoralis* SD10^T and *H. marina* Iso4^T of 12.0 % and between *H. marina* Iso4^T and *H. aquamarina* LMG 24711^T of 22.9 %.

Phenotypic Analyses

All species are oligotrophic organisms and the cells can be deformed, damaged, or even killed by higher substrate concentrations. Most strains have complex nutrient and growth factor requirements which cannot be fulfilled by simple addition of vitamins or amino acids. As a consequence cultivation on complex media is usually the only way to grow them. Most strains form biofilms and the formation of rosettes by attachment of the holdfast is a characteristic feature easily seen under the microscope.

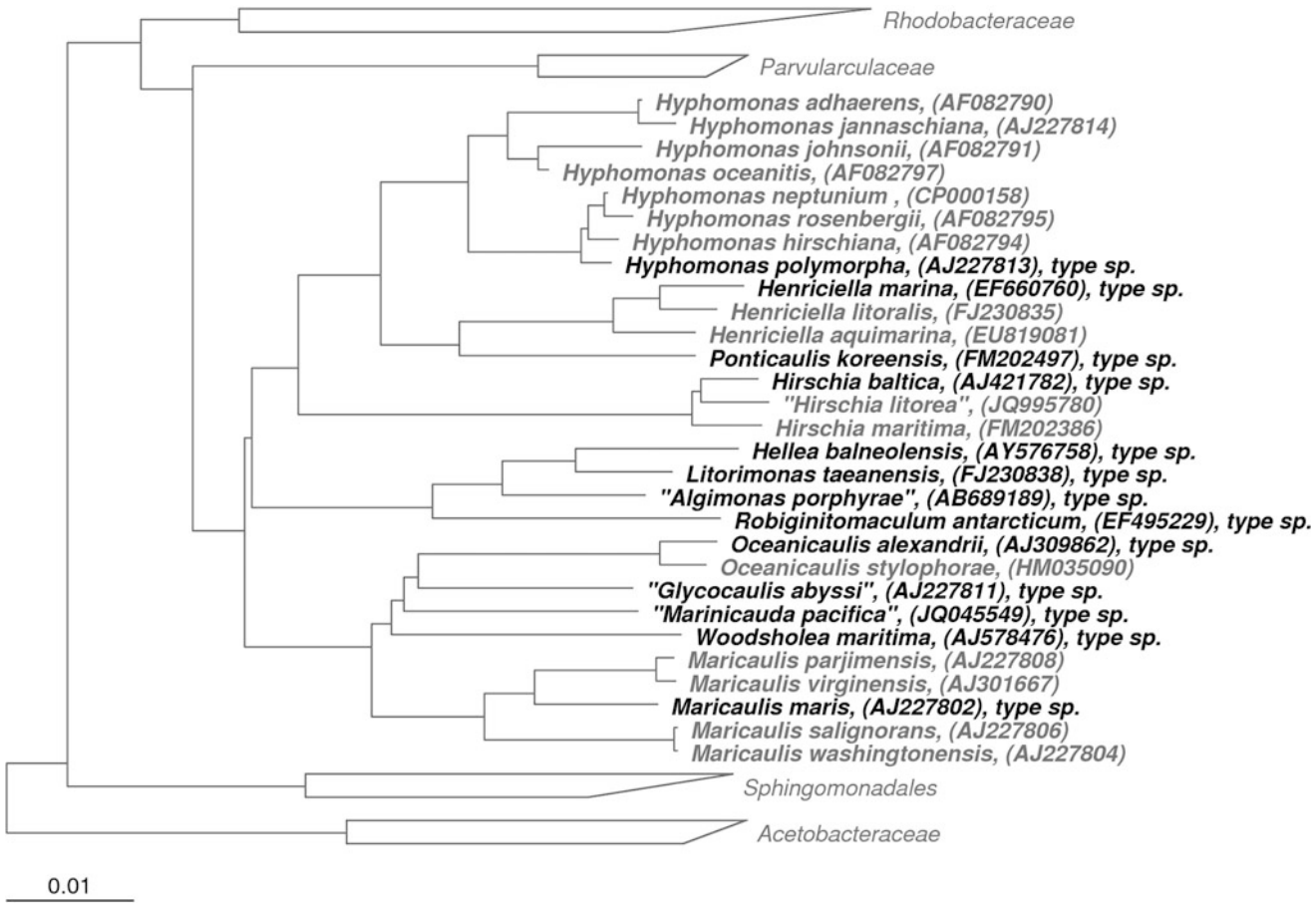

Fig. 12.2

Phylogenetic reconstruction of the family *Hyphomonadaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. Scale bar indicates estimated sequence divergence

Table 12.3

DNA–DNA hybridization between the *Hyphomonas* type strains (Weiner et al. 2000)

	PS-728^T	MHS-3^T	LE-670^T	MHS-2^T	VP-5^T	Sch89^T	VP-1^T	VP-6^T
H. polymorpha PS-728^T	100							
H. adhaerens MHS-3^T	32	100						
H. neptunium LE-670^T	27	66	100					
H. johnsonii MHS-2^T	13	25	25	100				
H. hirschiana VP-5^T	34	19	54	12	100			
H. oceanitis Sch89^T	28	11	12	23	20	100		
H. jannaschiana VP-1^T	8	41	32	18	23	26	100	
H. rosenbergii VP-6^T	5	30	11	21	19	2	4	100

Information on the antibiotic sensitivity of *Hyphomonadaceae* is scarce because only a minority of strains has been tested for their susceptibility against antibiotics. Most strains were susceptible against novobiocin, penicillin, and ampicillin, but *Hyphomonas oceanitis* SCH89^T and *Litorimonas taeanensis* G5^T were resistant against all three. *Litorimonas taeanensis* G5^T and *Robiginitomaculum antarcticum* IMCC3195^T showed also resistance against streptomycin and kanamycin. In the genome of *Oceanicaulis* sp. HTCC 2633, 15 genes for beta-lactamases and a bicyclomycin resistance protein were detected (Oh et al. 2011).

All *Hyphomonadaceae* have high activities in alkaline phosphatase. Esterase (C_4) and esterase/lipase (C_8) activities are

Table 12.4
DNA–DNA hybridization between the *Maricaulis* type strains (Abraham et al. 2002)

	ATCC 15268^T	MCS 25^T	VKM B-1513^T	MCS 6^T	MCS 18^T
M. maris ATCC 15268^T	100				
M. maris ATCC 15269	82				
M. parjimensis MCS 25^T	23	100			
M. virginensis VKM B-1513^T	n.d.	32	100		
M. virginensis VKM B-1514	33	28	83		
M. washingtonensis MCS 6^T	13	21	25	100	
M. salignorans MCS 18^T	32	18	23	59	100

n.d. not determined

present in all species; however, the activity is medium in *Woodsholea*, *Henricella*, and *Ponticaulis* species. Lipase (C_{14}) activity is absent or weak in all species. Leucine arylamidase is also present in all strains and usually high, while the valine and cystine arylamidases activities are always lower and in some species weak. Trypsin activities vary considerably between strains and have not been found in some species, e.g., *Hyphomonas polymorpha* DSM 2665^T, *Algimonas* porphyrae OC-2-2^T, or *Hellea balneolensis* DSM 19091^T. Similarly α-chymotrypsin varies being strong in *Henriciella* spp., *Oceanicaulis* spp., *Maricaulis parjimensis* MCS 25^T, and *M. maris* ATCC 15268^T but weak in *M. salignorans* MCS 18^T and *M. washingtonensis* MCS 6^T and even absent in *Hirschia* spp., *Algominas porphyrae* OC-2-2^T, and *Hyphomonas polymorpha* DSM 2665^T. The activity of acid phosphatase is rather abundant but even more variable being very active in *Maricaulis parjimensis* MCS 25^T, but absent in *M. maris* ATCC 15268^T, *M. washingtonensis* MCS 6^T, *Hellea balneolensis* DSM 19091^T, and *Robiginitomaculum antarcticum* IMCC3195^T. The same behavior was found for naphthol-AS-BI-phosphohydrolase. From the group of sugar-cleaving enzymes, α-glucosidase is the most frequent enzyme. Its activity has been reported from all *Henriciella* species, *Litorimonas taeanensis* G5^T, and *Hirschia baltica* DSM 5838^T, and weak activity was also found in *Maricaulis parjimensis* MCS 25^T and *Hyphomonas polymorpha* DSM 2665^T. All other species are lacking this activity. With the exception of the *Henriciella* species, the same species all showed weak activity of β-glucosidase. Only for *Hirschia maritima GSW*-2^T and *Hellea balneolensis* DSM 19091^T activity of β-galactosidase was reported. *Hirschia maritima* GSW-2^T was the only species showing α-fucosidase activity, while *Woodsholea maritima* CM 243^T was the only species possessing weak *N*-acetyl-β-glucosaminidase activity. None of the species showed any α-mannosidase or α-galactosidase activities.

Hyphomonadaceae display some characteristic fatty acids which can be used for their identification (*Table 12.1*). *Hirschia* species are the only ones containing C14:0. *Woodsholea*, *Oceanicaulis*, *Algimonas*, and *Ponticaulis* species are lacking C15:0; *Maricaulis*, *Robiginitomaculum*, and *Hellea* species have only traces of this fatty acid which is present in higher amounts in the other *Hyphomonadaceae* genera. C16:0 is usually abundant in the cells, but *Woodsholea*, *Oceanicaulis*, *Hellea*, and *Robiginitomaculum* spp. have low amounts. Usually this fatty acid is accompanied by lower amounts of its unsaturated derivative C16:1ω7c. However, *Woodsholea*, *Henriciella*, *Oceanicaulis*, *Ponticaulis*, *Hellea*, and *Litorimonas* are lacking C16:1ω7c completely. The isoforms of C17:0, iC17:0 and iC17:1ω9c, are together with C18:1ω9c abundant only in *Maricaulis* and characteristic within the *Hyphomonadaceae* for this genus. *Oceanicaulis* and *Woodsholea* species contain significantly higher amounts of C18:0 than the species of the other genera. *Henriciella* strains are the only ones showing C20:0. The branched fatty acid, *trans*-11-methyl-12-octadecenoic acid (ECL 18.080) (Abraham et al. 2008), was found in *Maricaulis*, *Hyphomonas*, *Henriciella*, *Oceanicaulis*, *Hellea*, and *Litorimonas* species with unusually high amounts in *Oceanicaulis*. The occurrence of 10-methyl-C18:0 (TSBA) in *Hellea balneolensis* is remarkable. Hydroxy fatty acids are usually esterified with the cell wall and are often used as biomarkers for bacteria. iC11:0 3OH is only present in species of *Maricaulis* and *Hellea*. While *Hyphomonas* and *Hellea* species have more C12:1 3OH than C12:0 3OH, the contrary is the case for *Henriciella* strains. *Oceanicaulis* and *Woodsholea* species possess only C12:0 3OH. C12:0 3OH and C12:1 3OH are absent in species of *Maricaulis*, *Robiginitomaculum*, *Litorimonas*, and *Algimonas*. Only *Algimonas* and *Hellea* strains show high amounts of C18:1 2OH which was also found in much lower amounts in some *Henriciella* strains. *Woodsholea* is the only genus containing considerable amounts of the unknown fatty acid ECL 15.275.

Instead of saponifying the fatty acids for the analysis, the intact polar lipids can be used for chemotaxonomy. A detailed analysis of the polar lipids using tandem-mass spectrometry revealed that they are good biomarkers for the characterization of genera of *Hyphomonadaceae*. The glycolipid fraction of the cells can best be analyzed by chemical ionization (CI) using ammonium as ionization gas. This gives mainly the molecular ions of the individual glycolipids which can be further characterized by collision-induced decay (CID) allowing the identification of the individual fatty acids and their position at the

Table 12.5
Comparison of selected characteristics of *Hyphomonas* species

	1	2	3	4	5	6	7	8
Biofilm formation	+++	Weak	+	−/+	Weak	−	−/+	++
Optimal growth temperature (°C)	25–37	25–31	37	25–37	30–37	20–30	30–37	25–45
Salt range (g l^{-1})	15–120	35–75	35–75	15–60	10–75	10–75	5–50	10–120
Optimal pH	5,7–8.7	7.6	7.6	5.7–8.1	8.0	7.6	7.0–7.4	5.7–8.9
Rosette formation	−	−	Variable	+	−	−	−	+
Nitrate reduction	+	+	+	+	+	+	−	+
Growth in glucose	−	−	−	+	−	−	−	−
G+C %	60	57	60	64	60–62	59	60–61	61

1 H. adhaerens, 2 H. hirschiana, 3 H. jannaschiana, 4 H. johnsonii, 5 H. neptunium, 6 H. oceanitis, 7 H. polymorpha, 8 H. rosenbergii. Data for 1, 4 and 8 from Weiner et al. (2000); for 2, 3, 5, 6, 7 Weiner et al. (1985)

glycerol moiety of the molecules. The pattern of the glycolipids allows a differentiation of isolates often down to the species level. In *Hyphomonadaceae* only the glycolipids α-D-glucopyranosyl diacylglycerol (MGDG) and α-D-glucuronopyranosyl diacylglycerol (GUDG) could be detected. For a similar analysis of phospho- and sulfolipids, ionization by fast atom bombardment (FAB) is more suitable. *Hyphomonadaceae* show here a rich diversity of sulfoquinovosyl diacylglycerols (SQDG), phosphatidyl diacylglycerols (PG), and α-D-glucuronopyranosyl diacylglycerol taurine amide (Tau). The distribution of SQDG, PG, and Tau is not uniform but specific for the individual taxa, and many show reduced amounts of PG which seems to be replaced by SQDG (Abraham et al. 1997). This fits to their habitats and it has been shown that limitation in phosphate leads to a shift from PG to SQDG (Batrakov et al. 1996a; van Mooy et al. 2009).

The main features of members of *Hyphomonadaceae* are listed in ❯ *Tables 12.5*, *12.6*, and *12.7*.

Hyphomonas ex Pongratz (1957), Moore et al. (1984), Weiner et al. (2000)

The description is the one given by Weiner et al. (2000) emended by our own results. *Hyphomonas* species normally generate only a single polar prosthecum and reproduce by budding (❯ *Fig. 12.3*). Members of the genus *Hyphomonas* undergo a rather complex biphasic life cycle. The life cycle consists of a swarm-cell stage that eventually metamorphoses into a benthic, reproductive cell during the biphasic developmental cycle. *Hyphomonas* cells are rod-shaped to oval, mature cells measuring 0.5–1.0 × 1.0–3.0 μm; buds are produced at the tips of polar prosthecae (❯ *Fig. 12.4*), which measure 0.2–0.3 μm in diameter and are 1.5 times the length of the cell body; and swarm cells are motile by means of a single polar to lateral flagellum located on developing buds of younger daughter cells. Gram negative, nonacid fast, aerobic, nonspore forming, and chemoorganotrophic. All strains investigated so far are catalase positive, oxidase positive, urease negative, indole negative, hydrogen sulfide negative, non-saccharolytic, and nonpathogenic for mammals. Peptone is normally required for growth. With one exception, all strains denitrify. The species catabolize amino acids for energy and growth; only *Hyphomonas johnsonii* MHS-2 T can utilize sugars. Members of *Hyphomonas*, especially the vent strains, grow along steep physicochemical gradients involving considerable temperature, pressure, and salinity variations in a broad range of environmental conditions. The optimum growth temperature range is 22–37 °C. *Hyphomonas neptunium*, *Hyphomonas polymorpha*, *Hyphomonas oceanitis*, and *Hyphomonas hirschiana* have a Q-11 ubiquinone type along with a significant amount of Q-10 (approx. 10 % of total ubiquinones) and minor amounts of Q-9 and Q-12. *Hyphomonas jannaschiana* has Q-10 as its major quinone, with trace amounts of Q-9 and Q-11. Polar lipids are 1,2-diacyl-3-α-D-glucopyranosyl-sn-glycerol, 1,2-diacyl-3-α-D-glucuronopyranosyl-*sn*-glycerol, phosphatidyl glycerol, and 1,2-diacyl-3-α-D-glucuronopyranosyl-*sn*-glycerol taurine amide (Batrakov et al. 1996a, b), while sulfoquinovosyl diacylglycerol is absent (Abraham et al. 1997). The dominant fatty acid, common to all *Hyphomonas* spp., is 18:1ω7c. The GC content of the DNA is 57–64 mol%. *Hyphomonas* species were isolated from many ocean niches, including the open ocean (the pelagic zone), mud sloughs, and different hydrothermal vent sites. They are primary colonizers of submerged marine surfaces, some species producing dense biofilms.

Maricaulis Abraham et al. (1999)

L. n. *mare -is*, the sea; L. masc. n. *caulis*, stalk; N.L. masc. n. *Maricaulis*, stalk from the sea.

The description is an emended version of the description given Abraham et al. (1999). Gram-negative cells, rod shaped, fusiform, or vibrioid, 0.4–0.5 by 1–2 μm. Cells possess a prostheca, ca. 0.15 μm in diameter and of varying length depending on the species and environmental conditions, extending from one pole as a continuation of the long axis of the cell. Adhesive material is present at the distal end of the

Table 12.6
Comparison of selected characteristics of *Maricaulis* species

Characteristic	1	2	3	4	5
Enzyme					
Trypsin	+/−	+	+		+
α-Chymotrypsin	+	+/−	+		+
Acid phosphatase	−	+	+/−		−
Naphthol-AS-BI-phosphohydrolase	+/−	+	+/−		+/−
α-Glucosidase	−	+/−	−		−
β-Glucosidase	+	+/−	−		−
Protease	+	+	−		−
β-Galactosidase	−	+	−		−
Nitrate reduction	−	−	+		+
Predominant cellular fatty acids	18:1ω9	18:1ω9	i17:1ω9c	i17:1ω9c	i17:1 ω9c
				18:1ω9	
DNA G+C content		63.0	63.3	65.2	63.0

1 M. maris, 2 M. parjimensis, 3 M. salignorans, 4 M. virginensis, 5 M. washingtonensis

Table 12.7
Comparison of selected characteristics of *Henriciella* species (Lee et al. 2011)

Characteristic	*H. aquamarina*	*H. litoralis*	*H. marina*
Colony color	Grey	Yellow	White
Growth at 40 °C	+	+	−
Esculin hydrolysis	−	+	−
Enzyme activity			
Naphthol-AS-BI-phosphohydrolase	+	Weak	+
α-Glucosidase	+	Weak	+
β-Glucosidase	−	Weak	−
Carbon utilization			
Tween 80	−	−	+
L-Arabinose	−	+	+
D-Fructose	+	+	−
α-D-Glucose	+	−	−
Raffinose	−	+	−
Sucrose	+	−	+
Glycerol	−	+	−
DNA G+C content	61.0	55.2	56.2

prostheca. Occur singly. Multiplication by binary fission. At the time of separation, one cell possesses a prostheca and the other a single polar flagellum. Each new appendage occurs at the cell pole opposite to the one formed during fission. The flagellated cell secretes adhesive material at the base of the flagellum, develops a prostheca at this site, and enters the immotile vegetative phase. Colonies circular, convex, and colorless. Chemoorganotrophic aerobes, but most of the strains tested could grow anaerobically probably using amino acid as fermentable carbon source. Nitrate is reduced to nitrite anaerobically by some strains. All strains are positive for alkaline phosphatase, esterase (C_4), esterase/lipase (C_8), leucine arylamidase, α-chymotrypsin, and naphthol-AS-BI-phosphohydrolase; trypsin activity is always present, although in some species only weakly. All species show weak activities for lipase (C_{14}) and no activities for acid phosphatase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, α-mannosidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase (API ZYM). An exception here is *Maricaulis parjimensis* MCS 25^T which has weak α-chymotrypsin and α- and β-glucosidase activities. Most strains can store carbon as poly-β-hydroxybutyric acid. Mixtures of B vitamins, amino acids, and other organic factors are required for growth. NaCl is required for optimal growth; however, only a few strains tolerate salt concentrations above 100 g NaCl l^{-1}. All strains can grow on peptone yeast extract media with 5 g NaCl l^{-1}; optimal growth between 20 and 60 g NaCl l^{-1}. Growth is inhibited or cells become deformed in media containing 1 % or more organic material. Temperature range of most isolates is 15–40 °C, while 20–30 °C is optimal. The pH range is 6.0–8.0 and optimal pH for growth is around neutral. All strains are characterized by three major fatty acids 16:0, 17:1isoω9c, and 18:1ω7 and the following minor compounds: 17:1ω8, 17:0iso, 17:0, and 18:1ω9. 11:0iso 3-OH is the main hydroxy fatty acid. Polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranosyl diacylglycerol, sulfoquinovosyl diacylglycerol, and α-D-glucuronopyranosyl diacylglycerol taurine amide. Most strains contain also phosphatidylglycerol. All isolates were obtained only from seawater. The G+C content is 62.5–65.2 mol%.

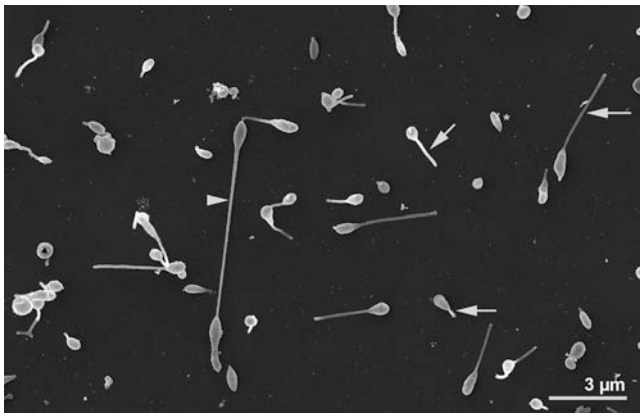

Fig. 12.3
Field emission scanning electron microscopic (FESEM) overview image of logarithmically grown *Hyphomonas jannaschiana* ATCC 33883^T depicting the diverse distinct morphological features of *Hyphomonas* like the formation of prostheca/stalks with different lengths (*arrows*), dividing cell by forming a bud from the distal end of the stalk (*arrowhead*) and swarmer cells (*star*)

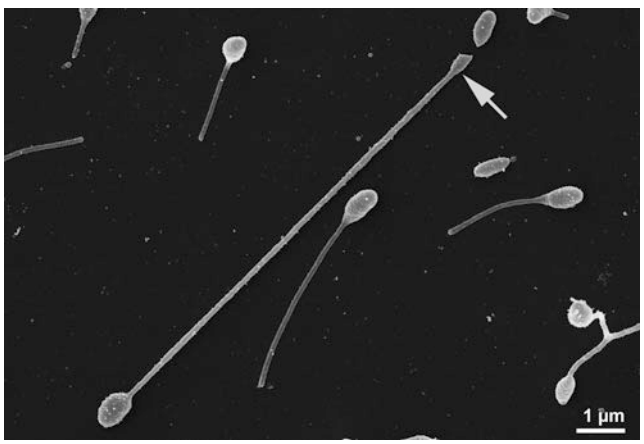

Fig. 12.4
FESEM image showing different lengths of the protheca/stalks of *Hyphomonas jannaschiana* ATCC 33883^T and starting of a budding cell from the distal end of a stalk (*arrow*)

The type species is *Maricaulis maris*. *Caulobacter halobacteroides* Poindexter 1964 was shown to be conspecific to *M. maris* by DNA–DNA hybridization.

Henriciella Quan et al. (2009); emend. Lee et al. (2011)

Henriciella (Hen.ri.ci.el'la. N. L. fem. n. *Henriciella* named after Henrici, A.T., who first described the stalked bacteria genus *Caulobacter*).

Cells are Gram-negative, aerobic, nonspore-forming, motile rods, oxidase, and catalase positive. The major respiratory quinone is Q-10. Cells are usually 0.4–0.7 µm wide and 0.7–2.8 µm long with polar flagellum. Division mode is binary fission. Some cells form mycelium, ranging from 7.8 to 8.0 µm in length. No prostheca were found in the species so far described. Good growth occurs on R2A with 1 % NaCl and MA. Colonies are translucent and shiny. Growth occurs at 10–37 °C (optimum, 20 °C), at pH 5.3–10.5 (optimum, pH 6.9–7.6), and in the presence of 10–150 NaCl g l^{-1} (optimum, 10–20 g l^{-1}). In the API ZYM system, positive for alkaline and acid phosphatase, esterase (C_4), esterase/lipase (C_8), lipase (C_{14}), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, α-glucosidase, and naphthol-AS-BI-phosphohydrolase activities; negative for cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, and N-acetyl-β-glucosaminidase activities. *Henriciella marina* differs from this scheme by showing α-fucosidase activity but lack of cystine arylamidase activity. In the API 20NE system, negative for nitrate reduction, indole production, glucose acidification, urease, β-galactosidase, and arginine dihydrolase activities. Some species show β-glucosidase activity. Species do not hydrolyze aesculin and gelatin. The major cellular fatty acids are C18:1ω7c, C16:0, C16:1ω5c, and C17:1ω8c; hydroxyl fatty acids are C12:0 3-OH, C12:1 3-OH, and C18:1 2-OH, but from *Henriciella litoralis* SD10,^{T} no hydroxylated fatty acids were reported. The DNA G+C content of type species is 55.2–62.0 mol %. The type species is *Henriciella marina*. The only species of the genus *Maribaculum*, *Maribaculum marimum*, was transferred to this genus as *Henriciella aquamarina* (Lee et al. 2011).

Hirschia Schlesner et al. (1990); emend. Park and Yoon (2012)

Hirschia (Hirsch'i.a. M.L. fem. n. *Hirschia*, honoring Peter Hirsch, a German microbiologist who is an expert on budding and hyphal bacteria).

The description is an emended version of the description given by Schlesner et al. (1990). Gram-negative, aerobic, motile or nonmotile, oxidase- and catalase-positive, nonspore-forming, prosthecate bacteria with one or occasionally two polarly inserted prostheca (0.06–0.1 µm in diameter). Reproduction occurs by bud formation at the tips of the prostheca. The buds are polarly monotrichously flagellated. Yellow- or orange-pigmented colonies. Mature cells are spherical, oval or rod shaped and measure about 0.6–1.1 × 1.2–1.3 µm. Grow at 10–30 °C (optimum, 30 °C), at pH 6.1–10.1 (optimum, pH 8.1–9.1) with 10 g l^{-1} NaCl. Produces a non-diffusible carotenoid pigment, but bacteriochlorophyll-*a* is absent. Enzyme activities for alkaline phosphatase, trypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase were positive, but esterase (C_4), esterase/lipase (C_8), lipase (C_{14}), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase were negative (API ZYM). Chemoheterotrophic, C_1 compounds are not utilized as carbon

sources. Polyhydroxybutyrate is not stored. The ubiquinone system is a Q-10 system. Main polar lipids are phosphatidylglycerol and two unidentified lipids. The major cellular fatty acids are C18:1ω7 and C16:0. Hydroxy fatty acids are 3-OH C12:0 for all known species while *Hirschia baltica* had more 3-OH C14:1. The DNA base ratio is 43.7–47 mol% G+C. The type species is *Hirschia baltica*. The genus consists currently of the species *H. baltica*, *H. maritima* Kang and Lee (2009b), and *H. litorea* Park and Yoon (2012). *H. maritima* differs from *H. baltica* by its ability to grow at 4 °C and 37 °C, its tolerance to 20 g l^{-1} NaCl, its ability to hydrolyze gelatin but not DNA, its possession of α- and β-glucosidase, and its lack of β-galactosidase and leucine arylamidase activities (Kang and Lee 2009a).

Oceanicaulis Strömpl et al. (2003)

Oceanicaulis [O.ce.an.i.cau'lis. L. masc. n. oceanus the ocean; L. masc. n. caulis stalk, referring to a prostheca; N.L. masc. n. *Oceanicaulis* stalk (ed organism) from the ocean].

The description of the genus is an emended version given by Strömpl et al. (2003). Gram negative, nonspore forming. Cells are rod shaped or vibrioid. Cultivated cells are 0.5–1 × 0.9–3 μm. In the early stage, cells are non-stalked and motile by means of a single polar flagellum. During binary fission, one cell possesses a stalk (prostheca) and the other a single polar flagellum. During binary fission, at the point of separation, one cell possesses a prostheca and the other a single polar flagellum. Adult cells are nonmotile with prostheca. Adhesive material is present at the distal end of the prostheca. Colonies on MA are round, convex, and non- or lightly pigmented. Aerobic and chemoorganotrophic. The major respiratory quinones are ubiquinone Q-10 and Q-9. Nitrate is reduced to nitrite by most strains. Strains are catalase and oxidase positive. In API ZYM tests, alkaline phosphatase, C4 esterase, C8 esterase/lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, and naphthol-AS-Bi-phosphohydrolase activities are present, but C14 lipase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are absent. In some strains poly-β-hydroxybutyrate granule accumulation is observed. Cells grow in marine broth at full strength and at 1/10 strength. Strains tested negative for lecithinase, starch, xanthine, indole, o-nitrophenylgalactoside, and Voges–Proskauer, and most tested negative for aesculin, elastinase, gelatinase, and lipase. All strains grow with 20–90 g l^{-1} NaCl and pH 6–10. Mesophilic. Optimum growth occurs at 35–40 °C, 1–2 % NaCl, and pH 7–9. Polar lipids are phosphatidyl glycerol and sulfoquinovosyl diacylglycerol. Major fatty acids are C18:1ω7, C18:0, 7-Me-C18:1ω6, and C17:0 with 3-OH C12:0 as main hydroxy fatty acid. Many strains contain large plasmids. *Oceanicaulis stylophorae* is sensitive to gentamicin, sulfamethoxazole plus trimethoprim, rifampicin, novobiocin, chloramphenicol, nalidixic acid, and tetracycline and resistant to ampicillin, kanamycin, streptomycin, and penicillin G (Chen et al. 2012b). The G+C content of the DNA is 61–62 mol%. The type species is *Oceanicaulis alexandrii*. *Oceanicaulis* comprises two species: *O. alexandrii* and *O. stylophorae* (Chen et al. 2012b). *O. stylophorae* differs from *O. alexandrii* by its temperature range of growth of 15–45 °C (*O. alexandrii* 4–35 °C), lack of C_{14} lipase, the ability to hydrolyze casein but not CM-cellulose, and the utilization of arabinose, adipate, citrate, D-fructose, L-fucose, D-galactose, L-alanine, L-phenylalanine, and L-methionine but not maltose, α-cyclodextrin, α-D-lactose, lactulose, maltose, succinamic acid, hydroxyl-L-proline, or L-threonine.

The following genera *Algimonas*, *Hellea*, *Litorimonas*, *Ponticaulis*, *Robiginitomaculum*, and *Woodsholea* are currently monospecific. Some of their properties have been covered under "short description of genera" and in *Table 12.1*.

Algimonas Fukui et al. (2012)

Algimonas (Al.gi.mo'nas. L. n. alga, seaweed; L. fem. n. *monas*, a monad, unit; N.L. fem. n. *Algimonas*, a unit (bacterium) isolated from seaweed).

The description is the one given by Fukui et al. (2012). The type species is *Algimonas porphyrae* 0C-2-2^T isolated from the red alga *Porphyra yezoensis*. Cells are Gram-negative and straight to slightly curved rods. Many cells are non-stalked and possess a polar flagellum for motility. Some cells possess a prostheca. Multiplication occurs by binary fission. Cells produce orange carotenoid pigments but bacteriochlorophyll-*a* is not found. Aerobic condition and NaCl are required for growth. The predominant isoprenoid quinone is Q-10. Polar lipids are phosphatidylglycerol, glucuronopyranosyldiglyceride, monoglycosyldiglyceride, three unidentified phospholipids, and an unidentified glycolipid. In addition to the description of the genus, the following properties are exhibited by *Algimonas porphyrae*. The cells are 2.13 μm long and 0.37 μm in diameter in MB. Colonies on MA are circular, convex, entire, and 0.22 mm in diameter. Growth of the type strain occurs at 10–30 °C (optimum, 20 °C), pH 6.0–9.0 (optimum, pH 7.0–8.0), and 10–50 g l^{-1} NaCl (optimum, 20–30 g l^{-1}). Oxidase is negative and catalase is positive. Methyl red and Voges–Proskauer reactions are negative. H_2S and indole are not produced. Aesculin and Tween 20, 40, and 80 are hydrolyzed, but agar, alginate, DNA, gelatin, and starch are not. No acid is produced from carbohydrates. Nitrate is reduced. The type strain has alkaline phosphatase, esterase (C_4), esterase/lipase (C_8), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities, but no lipase (C_{14}), cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities (API ZYM system). The type strain is susceptible to ampicillin, carbenicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, kanamycin, nalidixic acid, neomycin, norfloxacin, novobiocin, penicillin G, rifampicin, streptomycin, and vancomycin but resistant to polymyxin

B and tetracycline. The major cellular fatty acid is C18:1ω7*c*; the major hydroxy fatty acid is 2-OH C18:1. The G+C contents of the DNA are 58.5–60.2 mol%.

Hellea Alain et al. (2008)

Hellea (Hel.le'a. L. fem. n. Helle, a sea goddess in Greek mythology; N.L. fem. n. Hellea named after Helle in reference to the marine origin of the first strain).

The description is an emended version of the description given by Alain et al. (2008). Cells are Gram negative, nonspore forming, rod shaped to vibrioid, and dimorphic. They usually possess one polar stalk (prostheca) and are nonmotile and sessile, or they are nonstalked and motile by means of a polar flagellum. Cells divided by budding and are aerobic, heterotrophic, mesophilic, neutrophilic, and grow best at salt concentrations close to marine salinity. Colonies on MA medium are round, convex, brilliant, and pigmented a brick-red color. Optimal growth occurs at 30 °C, with growth at 15–37 °C. The pH optimum is close to neutral. Cells are catalase positive and oxidase negative and do not reduce nitrate. Hydrolysis of Tween 40 and Tween 80. Grows at NaCl concentrations of 0.2–50 g l^{-1}, with a clear optimum at 30 g l^{-1} NaCl. Growth occurs on acetate, citrate, propionate, pyruvate, succinate, aspartate, glutamate, L-alanine, L-asparagine, L-histidine, L-proline, casamino acids, peptone, tryptone, yeast extract, and D-mannitol. In addition, cis-aconitic acid, D-glucuronic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketoglutaric acid, methyl pyruvate, quinic acid, urocanic acid, L-pyroglutamic acid, hydroxy-L-proline, putrescine, *N*-acetylglucosamine, D-arabitol, myoinositol, and xylitol are used. The predominant quinone is Q-10. Polar lipids comprise glucuronopyranosyldiglyceride, monoglycosyldiglyceride, phosphatidylglycerol, an unidentified glycolipid, and two unidentified phospholipids. Main fatty acids are C18:1ω7c, C17:1ω6c, and C16:0; main hydroxy fatty acids are 2-OH C18:1 and 3-OH C10:0. The G+C content of the DNA of the type strain of the type species is 46.8 mol%. The type strain *Hellea balneolensis* 26III/A02/215^T was isolated from the surface microlayer of coastal water in the bay of Banyuls-sur-Mer, in the northwestern Mediterranean Sea on the coast of France.

Litorimonas Jung et al. (2011)

Litorimonas (Li.to.ri.mo'nas. L. n. litus -oris beach; L. fem. n. monasmonad, unit; N.L. fem. n. *Litorimonas* beach bacterium).

The description of the genus is an emended version given by Jung et al. (2011). Cells are Gram-reaction-negative, nonspore-forming, budding, straight to slightly curved rods. Usually nonstalked and motile by means of a polar flagellum; some cells possess one polar stalk (prostheca) and are nonmotile. Cells divide by budding. Oxidase and catalase positive. Neither nitrate nor nitrite is reduced. The only isoprenoid quinone detected is ubiquinone-10 (Q-10). The DNA G+C content of the type strain of the type species is 63.6 mol %. In addition to the characteristics described for the genus, the type species is characterized by the following properties. Cells are 0.4–0.6 × 1.6–2.0 μm, strictly aerobic, chemoheterotrophic, and moderately halophilic. Colonies on MA are yellow orange, convex, and round with entire margins. Growth occurs at 15–40 °C (optimum 25–30 °C), at pH 6–9 (optimum pH 7–8), and in 1–6 % (w/v) NaCl (optimum 2–3 %). Positive for assimilation of D-glucose but negative for assimilation of L-arabinose, D-mannose, maltose, D-mannitol, malic acid, potassium gluconate, *N*-acetylglucosamine, capric acid, adipic acid, trisodium citrate, and phenylacetic acid (API 20 NE). Positive for alkaline phosphatase, esterase/lipase (C_8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, and a-glucosidase activities but negative for lipase (C_{14}), α- and β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities. Weak enzymic activities are observed for esterase (C_4), β-glucosidase, and naphthol-ASB1-phosphohydrolase (API ZYM). Resistant to polymyxin B, streptomycin, gentamicin, kanamycin, novobiocin, oleandomycin, lincomycin, ampicillin, tetracycline, penicillin G, and carbenicillin but sensitive to chloramphenicol. Phosphatidylglycerol, monoglycosyldiglyceride, glucuronopyranosyldiglyceride, and two unidentified glycolipids are the polar lipids detected. The major cellular fatty acids are C18:1ω7c, C17:0, and C16:0; no hydroxy fatty acids were detected. The DNA G+C content of the type strain is 63.6 mol%; however, this value has recently been corrected drastically to 47.1 % (Fukui et al. 2012). The type strain *Litorimonas taeanensis* G5^T was isolated from beach sand in Taean, South Korea.

Ponticaulis Kang and Lee (2009a)

Ponticaulis (Pon.ti.cau'lis. L. neut. n. pontus the sea; L. masc. n. caulis a stalk, referring to a prostheca; N.L. masc. n. *Ponticaulis* stalk from the sea).

The type strain of *Ponticaulis koreensis* GSW-23^T was isolated from seawater taken from Gimnyeong Beach, Republic of Korea, and expresses the following properties (Kang and Lee 2009a). Colonies are colorless, circular, smooth, convex, and 0.3–0.5 mm in diameter after 5 days of incubation. Cells are 3.1–6.6 μm long and 0.4–0.5 μm wide. Cells are strictly aerobic, Gram-negative, nonspore-forming, non-budding, obligately halophilic rods or vibrioids that are motile by means of a polar flagellum. Multiplication occurs by binary fission. Some cells possess a long prostheca (0.2 μm in diameter) and a holdfast. The major isoprenoid quinone is Q-10. The major cellular fatty acids are summed feature 7 (C18:1ω9c, C18:1ω12t, and/or C18:1ω7c), C16:0, and C18:0. Growth occurs at 10–42 °C (optimum 30–37 °C), at pH 6.1–10.1 (optimum pH 7.1) and with up to 60 g l^{-1} NaCl. Hydrolyses DNA but not casein or starch. Degrades DL-tyrosine but not elastin, chitin, cellulose, hypoxanthine, or xanthine. Positive for gelatin hydrolysis but negative for nitrate reduction, indole production, glucose

fermentation, arginine dihydrolase, urease, aesculin degradation, and β-galactosidase (API 20NE). In API ZYM tests, positive for alkaline phosphatase, esterase (C_4) (weak), esterase/lipase (C_8) (weak), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C_{14}), valine arylamidase, cystine arylamidase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-glucosaminidase, α-mannosidase, and α-fucosidase. The DNA G+C content of the type strain is 53.3 mol%.

Robiginitomaculum Lee et al. (2007)

Robiginitomaculum (Ro.bi.gi.ni.to.ma.cul'um. L. n. robigo -inis rust; L. neut. n. tomaculum, a kind of sausage; N.L. neut. n. Robiginitomaculum, a rust-colored sausage).

The type strain *Robiginitomaculum antarcticum* IMCC3195^T was isolated from surface seawater of Maxwell Bay, King George Island, West Antarctica, and possesses the following properties (Lee et al. 2007). Colonies on MA are circular, smooth, convex, viscous, rusty colored and 0.3–1.0 mm in diameter. Cells are Gram-negative; 1.3–4.5 mm long and 0.4–1.0 mm wide, with a tapered end; nonmotile; non-budding; thin prostheca-producing; and obligately aerobic rods or vibrioids. Multiplication occurs by binary fission. Flagella and holdfast are not present. Some cells possess a thin prostheca that extends along the long cell axis from one pole. Carotenoid pigments are found but bacteriochlorophyll-*a* not. Chemoheterotrophic. The predominant fatty acids are C18:1ω7c, C17:1ω8c, C17:0, and C17:1ω6c and hydroxy fatty acids are 3-OH C9:0, 3-OH C10:0, and 3-OH C11:0. The major respiratory quinone is Q-10. Growth occurs at 3–25 °C, optimally at 20 °C, but not above 30 °C. Growth occurs at pH 5–10 and 5–50 g l^{-1} NaCl, optimally at pH 7 and at 20–25 g l^{-1} NaCl. Oxidase negative and catalase positive. In API 20NE strips, positive for nitrate reduction, aesculin hydrolysis, and β-galactosidase activity. Negative for urea hydrolysis, indole production, acid production from glucose, gelatin liquefaction, and arginine dihydrolase. In the API ZYM system, alkaline phosphatase, esterase/lipase (C_8), leucine arylamidase, valine arylamidase, and cystine arylamidase activities are present. Negative for esterase (C_4), acid phosphatase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase, lipase (C_{14}), trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, α-galactosidase, α-glucosidase, and α-fucosidase activities. Susceptible to chloramphenicol, erythromycin, rifampicin, and tetracycline, but resistant to ampicillin, gentamicin, kanamycin, penicillin G, streptomycin, and vancomycin. The DNA G+C content is 60.3 mol%.

Woodsholea Abraham et al. (2004)

Woodsholea (Woods.hol'e.a. N.L. fem. n. *Woodsholea* named in honor of the Woods Hole Oceanographic Institution, Massachusetts, USA).

The description of the genus is the one given by Abraham et al. (2004). The type strain of *Woodsholea maritima* CM243^T was isolated from seawater taken from Woods Hole, USA, and expresses the following properties (Abraham et al. 2004): Gram-negative cells, rod shaped and vibriod. Cells possess a stalk, varying in length depending on the strain and environmental conditions, extending from one pole as a continuation of the long axis of the cell. Adhesive material is present at the distal end of the stalk. Occur singly. Multiplication by binary fission. Colonies circular, convex, colorless. Chemoorganotrophic, aerobes, cells can store carbon as poly-β-hydroxybutyric acid. Requirement for organic growth factors is complex and not satisfied by mixtures of B vitamins and amino acids. Grows on peptone/yeast extract media with 40 g NaCl l^{-1}. Growth is inhibited or cells become deformed in media containing 1 % (w/v) or more organic material. Growth temperature range is 20–40 °C and optimal pH for growth is approximately neutral. Do not reduce nitrate, oxidize tryptophan to indole, or hydrolyze arginine, urea, aesculin, gelatin, or p-nitrophenyl-3-D-galactopyranoside. Cells show no catalase activity and are positive for alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, leucine arylamidase, acid phosphatase, esterase (C_4), esterase/lipase (C_8), oxidase, and trypsin but negative for α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, α-mannosidase, and α-fucosidase. Strains have no to weak α-chymotrypsin and *N*-acetyl-β-glucosaminidase activities; some isolates including the type strain have weak lipase activity. The genus is characterized by two major fatty acids, 18:0 and 18:1ω7c, and minor amounts of 12:0 3-OH, 16:0, 17:0, summed feature 3 (14:0 3-OH, 16:1 iso I, ECL 10.928, and/or 12:0 ALDE), and the unidentified fatty acid ECL 15.275. Polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranuronosyl diacylglycerol, sulfoquinovosyl diacylglycerol, and α-D-glucuronopyranosyl diacylglycerol taurine amide. Isolated from seawater. The G+C content is 65 mol%. The G+C content of the type strain is 65.2 mol%. The type species is *Woodsholea maritima*.

Isolation, Enrichment, and Maintenance Procedures

Cell densities of *Hyphomonadaceae* in sea samples are usually low because of the oligotrophic nature of the cells. At the same time the cells are adapted to starvation and this can be used for their isolation. For this reason old marine samples are usually enriched in these bacteria. Many *Hyphomonadaceae* stick with their holdfasts to surfaces, and these surfaces can be other bacteria but also eukaryotic cells. Therefore, filtering the sea samples before plating is not advisable and would lead to a loss of bacteria of interest. Strains of *Hyphomonadaceae* can be isolated from seawater using a standard dilution-plating method on an oligotrophic medium, e.g., R2A agar, diluted 1:10 in seawater (1/10R2A). Because all strains are coming from oligotrophic habitats, full media with more than 1 g l^{-1} soluble organic substrates should be avoided which will cause disfiguring of the cells (pleomorphism). Cell growth is slow

and colonies are visible often only after one or even more weeks. Another isolation method is keeping the seawater sample after addition of 10–30 mg l^{-1} peptone or other carbon source at room temperature for 1–2 months and plating the formed surface film (biofilm) on diluted agar. Instead of using the neuston of the samples, substrata can be submerged in the samples acting as target for the attachment of *Hyphomonadaceae*. The plates can then be removed and the attached bacteria scrapped off and plated. At least *Maricaulis*, *Woodsholea*, *Henriciella*, and *Oceanicaulis* species can also be grown in 0.05 % peptone, 0.05 % casamino acids, and 75–80 % seawater (CPS medium, Poindexter 1964) or marine *Caulobacter* medium SPYEM containing 30 g sea salts (Sigma), 0.5 g NH_4Cl, and 1 l deionized water. After autoclaving and cooling, 20 ml 50 × PYE (50 × PYE: 100 g peptone and 50 g yeast extract in 1 l deionized water (autoclaved)), 2 ml 50 % glucose, and 5 ml riboflavin (0.2 mg ml^{-1}) are added (Abraham et al. 1999). Further purification can be done on marine agar 2216 (MA) after culture at 20 °C for 2 weeks. One should keep in mind that most *Hyphomonadaceae* form clumps and the isolates have to be checked carefully for any mixed cultures. Almost all strains need complex media for growth. One exception is Hyphomonas neptunium which can be grown on GAMS medium: 125 mM of each glutamate, aspartate, serine, methionine, 0.26 µM calcium pantothenate, and 30 % seawater (Havenner et al. 1979). Most strains can be preserved as glycerol suspension (10 %, v/v) at −75 °C. Vegetative stocks should be maintained on 1 % 1/10 R2A agar slants at 4 °C and transferred every 8 or 9 weeks, incubated 2 or 3 days at 20–25 °C then kept at 4 °C. For many strains lyophilization is problematic and often not reliable.

Cell Morphology

All species in the family *Hyphomonadaceae* possess dimorphic cells (◉ *Fig. 12.3*) and reproduce either by binary fission or by budding. Meso-diaminopimelic acid is the diagnostic diamino acid of the cell-wall peptidoglycan. Sessile cells are connected via a stalk to their substrata. For replication in *Algimonas*, *Henriciella*, *Maricaulis*, *Oceanicaulis*, *Ponticaulis*, *Robiginitomaculum*, and *Woodsholea*, a cell is separated by binary fission from the progenitor cell. This sibling carries no stalk but a flagellum which propels it through the medium until a suitable site is found (◉ *Fig. 12.5*). Here the sibling then develops a stalk, becomes sessile, and sheds the flagellum. Long axis of cells can be distinctly curved, but may be straight in some species. Cells are long and slender, approximately 1.5–2.5 µm in length when not dividing, and less than 0.5 µm in diameter or approximately 1 µm in length when not dividing and 0.6 µm in diameter. Stalk length dependent on growth condition and species, one to four times the length of the cell body, diameter 0.11–0.18 µm (◉ *Figs. 12.6* and *12.7*). With the exception of cells of *Robiginitomaculum*, the stalk carries adhesive material at its distal end. *Hellea*, *Hirschia*, *Hyphomonas*, and *Litorimonas* replicate in a different manner. Here the stalk is a reproductive

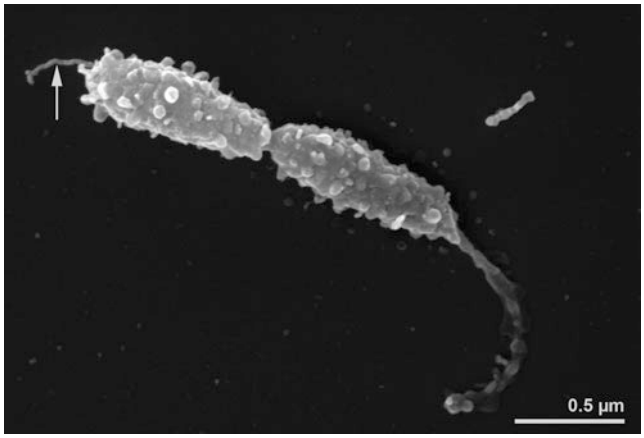

Fig. 12.5
FESEM image of a dividing *Maricaulis salignorans* MSC 18^T cell that depicts the formation of a daughter cell. A flagellum is formed from the distal end of the daughter cell

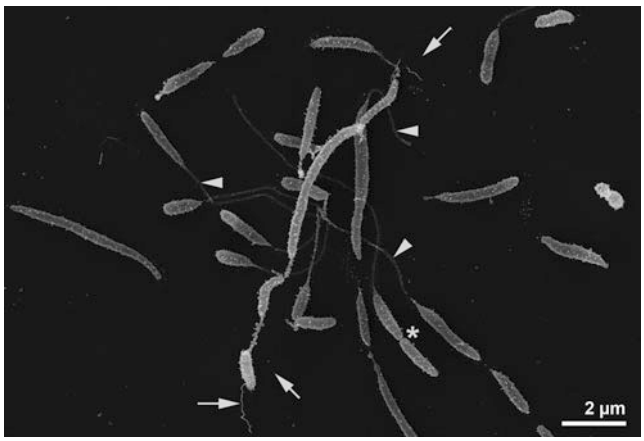

Fig. 12.6
FESEM image of logarithmically grown *Maricaulis salignorans* MSC 18^T showing the diverse distinct morphological features of formation of prostheca/stalks with different lengths (*arrowheads*) and cells with flagella (*arrows*) and a dividing cell (*star*)

structure and DNA and proteins are channeled through it to the new budding cell. Transport within the stalk is achieved in compartments, so-called pseudovesicles (Zerfas et al. 1997) (◉ *Fig. 12.8*). Cells are rod shaped to oval, 0.5–3.0 µm long, and 0.5–1.0 µm in diameter; buds are produced at the tips of polar prosthecae, which measure 0.2–0.3 µm in diameter and are one to five times the length of the cell body. Swarm cells are motile by means of a single polar to lateral flagellum located on developing buds of younger daughter cells (◉ *Fig. 12.9*).

These two groups of cells seem to differ not only in their reproduction but also in their reproductive abilities. While cells of *Maricaulis* or *Caulobacter* produce about 100 offsprings during their lifetime (Poindexter 1964; Ackermann et al. 2003), this number is limited to about 8 in budding genera (Moore 1981).

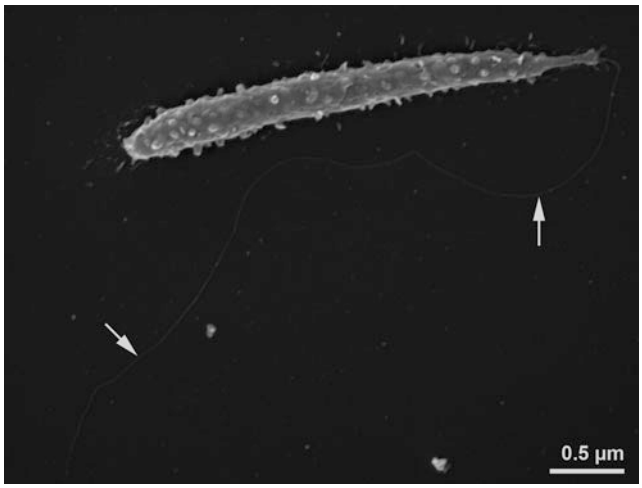

Fig. 12.7
FESEM image of a swarmer cell of *Maricaulis salignorans* MSC 18^T exhibiting a long flagellum

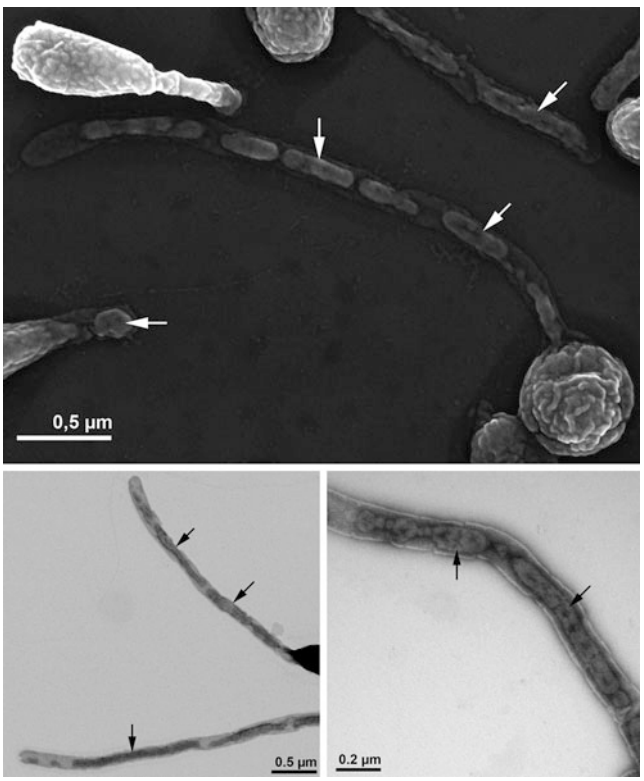

Fig. 12.8
Within the prosthecum/stalk, clearly defined pseudovesicles (*arrows*) can be distinguished by FESEM (*upper image*) or by negatively staining with 2 % uranyl acetate of logarithmically grown *Hyphomonas polymorpha* DSM 2665^T in transmission electron microscopy (*lower two images*). The pseudovesicles are covered by the outer membrane of the bacterium

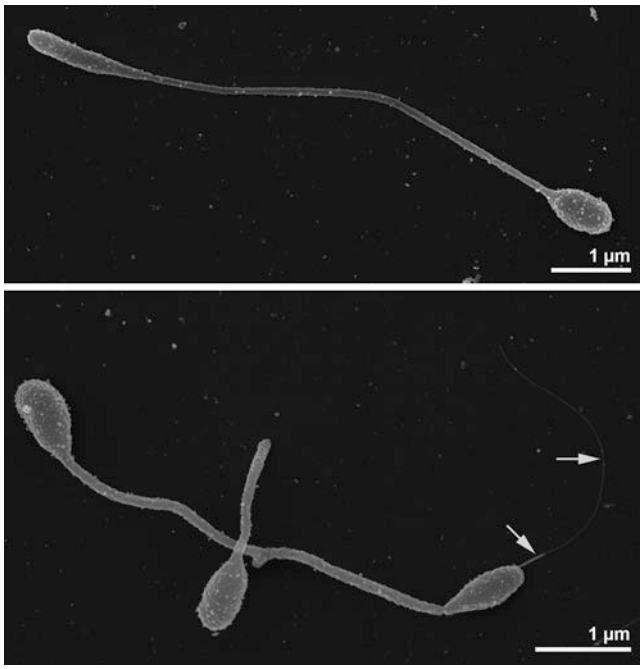

Fig. 12.9
FESEM image depicting a budding cell of *Hyphomonas jannaschiana* ATCC 33883^T from the distal end of a prosthecum/stalk (*upper image*). The formation of a flagellum (*arrows*) after dividing (*lower image*) is visible. The cell with the flagellum will be released as a swarmer cell

One way to protect cells from exoenzyme activities, antibiotics, or other noxious agents produced in biofilms is the formation of S-layers. Such an S-layer was characterized for *Hyphomonas jannaschiana* and two proteins of 29 and 116 kDa were isolated. Both proteins are glycoproteins with the heavier one being the tetramer of the smaller protein. They seemed to be linked by divalent cations because they could be solubilized by EDTA (Shen and Weiner 1998). For the stalk of *Oceanicaulis alexandrii*, it has been shown that it is also covered by an S-layer (Strömpl et al. 2003). S-layers have been found in many *Caulobacteraceae* and it can be assumed that they are also present in a number of *Hyphomonadaceae* species where they have not yet been reported.

Physiology and Metabolism

All species of *Hyphomonadaceae* are strict aerobes although many of them are able to reduce nitrate. None of the isolates have been reported to grow on any mineral medium containing a single substrate as sole source of carbon and energy. Some strains can grow on such a medium if it is supplemented by various growth factors, but most strains need complex (and often ill-defined) mixtures of growth factors or even carbon substrates. *Hyphomonas* are amino-acid-requiring isolates. *Hyphomonas johnsonii* is an outlier species in its genus because it is the only species able to use sugars.

Ecology (Main Habitats)

The main habitat of *Hyphomonadaceae* is the sea with two exceptions: *Hirschia baltica* was isolated from brackish water and *Hyphomonas polymorpha* was isolated from human nasal secretions (but the patient was a deep sea diver). The optimal growth temperature for most species is around 20–30 °C. This is also true for *Robiginitomaculum antarcticum* although it was isolated from surface seawater of Maxwell Bay, King George Island, West Antarctica. This species and species from hydrothermal vents, e.g., *Maricaulis virginensis*, however, can also grow at lower temperature which they probably experience in their special habitats. A true psychrophilic *Hyphomonas* sp. (strain SW47) was isolated from Antarctic sea ice underlining again the ability of some *Hyphomonadaceae* strains to thrive in cold habitats (Bowman et al. 1997).

Although only few reports are available, it can be expected that due to the oligotrophic nature of their habitats, cell numbers of these bacteria will be low. All *Hyphomonadaceae* have swarmer cells which are motile. The dispersal of the progenitor cells is important for species living in oligotrophic habitats because it prevents competition of the siblings for substrates. Genomic and experimental evidences indicate that at least *Hyphomonas neptunium* cells are not chemotactic. The lack of evidence for chemotaxis supports the notion that motility among *Hyphomonas* swarmer cells is a random dispersal method (Badger et al. 2006). However, for most of the *Hyphomonadaceae* species, this has not yet been investigated.

For a number of isolates, associations with eukaryotic cells were reported. *Oceanicaulis alexandrii* was isolated from the marine dinoflagellate *Alexandrium tamarense* (Lebour) Balech. *Maricaulis virginensis* VC-13 was reported to associate in cocultures with several diatoms (Poindexter 2006). The phylogenetic analysis of bacterial communities associated with leaves of the seagrass *Halophila stipulacea* revealed three clones belonging to the Hyphomonadaceae (Weidner et al. 2000). They are phylogenetically positioned between the genera *Ponticaulis* and *Henriciella*. Currently the nature of these associations is not clear, and it has been suggested that the attachment of these strains may simply be caused by the need for a surface to attach. The advantage of an attachment to eukaryotes, however, can easily exceed the mere requirement for a surface to attach. Use of exudates excreted by the eukaryotic host, consumption, and detoxification of oxygen produced by photosynthetic algae or a closed carbon cycle of oxidation of exudates to CO_2 and immediate fixation of the produced CO_2 by the photosynthetic host are only few of the many advantages such epibionts may have in the environment (Poindexter 2006). Still much more research is needed to understand the association of *Hyphomonadaceae* to their hosts.

Biological and Technological Relevance

For none of the isolates, any pathogenicity was shown. This is even the case for *Hyphomonas polymorpha* which comes from a human habitat (Pongratz 1957). Subsequent studies on the diversity of bacteria in the human nasal cavity, however, did not seem to confirm that this is a common habitat for *Hyphomonas* species (Frank et al. 2010).

Only few potential biotechnological applications for strains of the *Hyphomonadaceae* have been reported. A novel architecture has been revealed for the *N*-acetylglutamate synthase of *Maricaulis maris* MCS 10, but it is still too early to predict any biotechnological application from this finding (Shi et al. 2011). *Hyphomonadaceae* have also been shown to be major members in the degradation of municipal solid waste (Trzcinski et al. 2010), but the reported SSU RNA gene sequence is too short for identification of the genus. Strains of *Hyphomonadaceae* seem also to have abilities to degrade complex or even xenophytic organic compounds. Freshwater bacteria degrade the aromatic heterocyclic organic compound carbazole via angular dioxygenation using 1,9a-dioxygenases. A screening for marine isolates possessing this metabolic potential revealed two *Hyphomonadaceae*, strains OC-5 and OC-6 (Maeda et al. 2009). While strain OC-5 falls within the genus *Hyphomonas*, strain OC-6 seems to be a member of the genus *Henriciella*.

To form biofilms many Proteobacteria use *N*-acyl-homoserine lactones (AHLs) for communication for their quorum-sensing systems. It has been first shown for some bacteria that there are enzymes which can split these lactones quenching quorum sensing. There are enzymes cleaving AHL at the lactone site and others cleaving the acyl site chain. While cleavage at the first site is reversible, cleaving the acyl side chains destroys the bioactivity of AHL permanently. These enzymes gained more importance recently as tools to control biofilms (Abraham 2005). Kalia et al. (2011) analyzed the genomes of a number of bacteria for the presence of acyl-homoserine lactone-acylases and lactonases and found both AHL lactonase and acylase in *Hyphomonas neptunium* ATCC 15444. The positive identification of these enzymes has not only possible biotechnological applications, but it tells us also that at least this strain is possible to control biofilm formation of other bacteria by destroying their AHLs.

Concluding Remarks and Perspectives

The last decade has seen descriptions of a number of novel genera belonging to the family *Hyphomonadaceae*, and there is no reason to believe that this will come to an end. We looked at the phylogeny of clones from environmental samples which fall into the space of the *Hyphomonadaceae* to get an impression what may be in store in the future. There are a number of SSU rRNA gene sequences which do not fall into any of the described genera pointing to the existence of genera still novel to science. There are two groups of genera which will probably change if novel species will lead to a sharper definition of these genera. One of these genera is *Henriciella*. Here, only non-stalked species are known but we have isolates in

our laboratory which fall into this genus but clearly show prostheca. Although *Henriciella* is phylogenetically close to *Hyphomonas*, these isolates divide not by budding but by binary fission resembling more *Maricaulis* than *Hyphomonas* species. Another complex of genera for which the genus boundaries seem to be not well defined is formed by the genera *Maricaulis*, *Woodsholea*, and *Oceanicaulis*. Currently criteria can be extracted differentiating these genera; however, only future will tell whether we already know the whole diversity of the species of this complex. This statement is generally valid for any of the single-species genera populating the phylogenetic tree of the *Hyphomonadaceae*. From these considerations it can easily be predicted that we are still facing many exciting new discoveries in this family.

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13 The Order *Kiloniellales*

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Abstract
The *Kiloniellaceae* family is represented currently by a single genus *Kiloniella* with the species *Kiloniella laminariae* as type species. *Kiloniella laminariae* was isolated from the marine macroalga *Saccharina latissima* (former *Laminaria saccharina*) obtained from the Baltic Sea. The bacterium is a mesophilic, typical marine bacterium. It is a chemoheterotrophic aerobic bacterium with the potential of denitrification. Growth optima are at 25 °C, pH 5.5, and 3 % NaCl. The distinguished phylogenetic position separates *Kiloniella* from other alphaproteobacterial orders. The 16S rRNA gene sequence revealed a distant relationship to species of several orders of the *Alphaproteobacteria* with less than 91 % sequence similarity. This gives rise to the recognition of *Kiloniella* as a representative of a new order of the *Alphaproteobacteria*, the *Kiloniellales*. Phylogenetic analyses revealed a distinct cluster of *Kiloniella* with an uncharacterized bacterium (isolate KOPRI 13522) from hydrothermal plumes. This cluster forms a larger group together with the distantly related *Terasakiella pusilla* (88.4 % sequence similarity of the 16S rRNA gene) and the *Thalassospira* species (88.9–90.2 % sequence similarity). These genera are supposed to form separate families within the *Kiloniellales*.

Taxonomy: Historical and Current

Among bacteria associated with the marine brown alga *Saccharina latissima* (former *Laminaria saccharina*), a new bacterium not associated to any of the known alphaproteobacterial orders was isolated. The new bacterium was described as the new species and genus *Kiloniella laminariae* and was considered as the representative of a new family and order of the *Alphaproteobacteria*, the *Kiloniellaceae* and *Kiloniellales* with the genus *Kiloniella* as the type genus of the new family (Wiese et al. 2009a). Among the *Alphaproteobacteria* the *Kiloniellales* form a distinct phylogenetic branch separate (Fig. 13.1) from *Caulobacterales*, *Sphingomonadales*, *Rhizobiales*, *Rickettsiales*, *Rhodobacterales*, *Rhodospirillales*, *Kordiimonadales*, *Sneathiellales*, and *Parvularculales* (Garrity et al. 2005; Jordan et al. 2007; Kurahashi et al. 2008; Kwon et al. 2005).

Kiloniella laminariae is phylogenetically most closely related to an undescribed isolate, KOPRI 13522, and distantly related to the genera *Terasakiella* and *Thalassospira* (Wiese et al. 2009a). Characteristic of species of these two genera as well as of *Kiloniella laminariae* is the low G+C content of 48–55 % (see Table 13.1), which differentiates these bacteria from most species of the *Rhodospirillales* which exhibit a significantly higher DNA G+C content between 62 % and 67 %, in some clusters of *Acetobacteraceae* related to *Craurococcus* species even exceeding 70 % (Shi et al. 2002). Due to the distant relationship to *Kiloniella*, *Terasakiella* and *Thalassospira* may not be considered members of the *Kiloniellaceae* family but should be included into the order *Kiloniellales* as separate families (see Fig. 13.1). According to phylogenetic calculations, all three genera are distinct from *Rhodospirillales* and other alphaproteobacterial orders (Wiese et al. 2009a).

Molecular Analyses

Sequence similarity values of the 16S rRNA gene of *Kiloniella laminariae* to the seven closest validly described type strain sequences are between 90 % and 91 % including representative of the *Rhodospirillales*, the *Rhizobiales*, the *Rhodobacterales*, and *Thalassospira xianhensis* (Table 13.2). Another 30 species similar at the level of 88.4–89.7 % include members of the *Rhizobiales* and species of *Thalassospira* and *Terasakiella*.

All phylogenetic trees confirm the close relationship of *Kiloniella laminariae* to an uncharacterized isolate (strain KOPRI 13522) from hydrothermal plumes at the East Pacific Rise (Wiese et al. 2009a). The two bacteria form a distinct group with 100 % bootstrap values separate from any known alphaproteobacterial order. They form a cluster with the distantly related *Terasakiella pusilla* IFO 13613^T and the *Thalassospira* species (*T. lucentensis* DSM 14000^T, *T. profundimaris* DSM 17430^T, *T. tepidiphila* DSM 18888^T, *T. xianhensis* JCM 14850^T, *T. xiamenensis* DSM 17429^T). Though members of the genus *Thalassospira* were provisionally assigned to the family *Rhodospirillaceae* (Lopez-Lopez et al. 2002; Kodama et al. 2008; Zhao et al. 2010), the phylogenetic analysis strongly supported the

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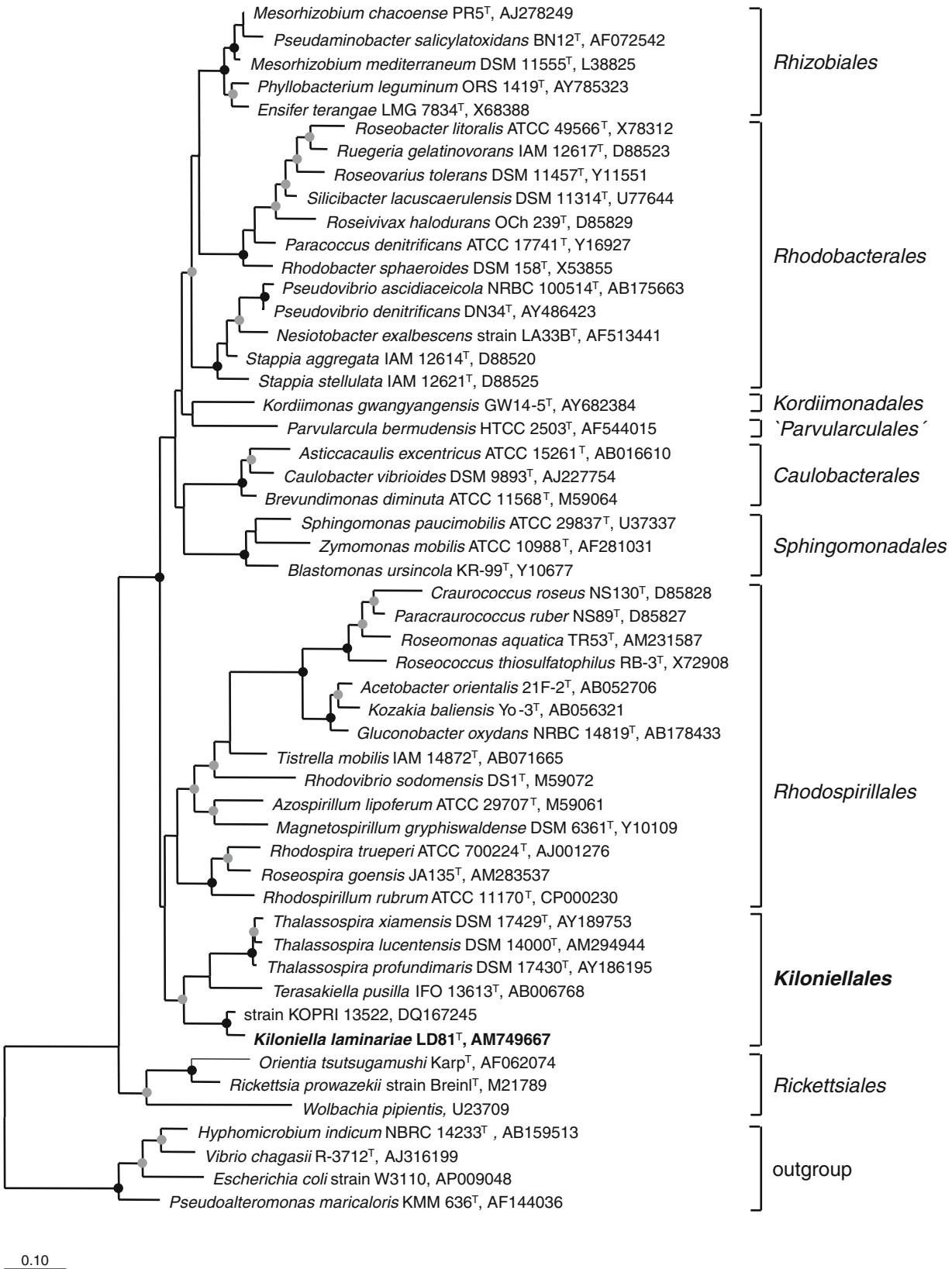

Fig. 13.1

Phylogenetic tree showing the relationship of *Kiloniella laminariae* to representative type species of the *Alphaproteobacteria*. The calculation is based on the ML, NJ, and ME methods, respectively, with 1,000 bootstraps (Modified from Wiese et al. 2009a and recalculated by V. Thiel). • >95 % bootstrap value ML, NJ, and ME method, • >50 % bootstrap value ML, NJ, and ME method

Table 13.1

Differential characteristics of *Kiloniella laminariae* to species of the genera *Terasakiella* and *Thalassospira*

Characteristic	*Kiloniella laminariae*	*Terasakiella pusilla*	*Thalassospira* spp.
Cell morphology	Spiral (occasionally rod or filamentous)	Spiral	Vibrioid to spiral
Flagella	+(single polar)	+(bipolar single)	+(single polar)/−
Pigment	−	−	+/−
Bacteriochlorophyll	−	nd	−/nd[a]
Salt tolerance (%)	Up to 8	Up to 8	Up to 17
Salt requirement	+	+	+
Catalase	+	w/−v	+
Oxidase	+	+	+
Reduction of nitrate	+(to N_2O)	+(to nitrite)	+(to nitrite)/−
Growth on carbohydrates	+	+	+
Quinone type	nd	Q10	nd/Q9[b]
G + C content (mol %)	51.1	48/51[c]	47–55[d]
Nonpolar fatty acids[e]			
C-18:1	49	58	35–45
C-16:1	31	18	3–18
C-16:0	8	15	15–25
C-18:0	3	1	2–9
3-hydroxy fatty acids[f]			
C–14:0 3–OH	64[g]	87	25–41/1[h]
C–16:0 3–OH	0	2	51–61/3[h]
C–17:0 3–OH	11	0	0
C–18:0 3–OH	25	10	8–15/1[h]
Oxygen requirement	Aerobe/anaerobe	Aerobe	Aerobe/anaerobe
Anaerobic phototrophic growth	−	nd	−/nd[i]

Data are derived from Sakane and Yokota (1994), Terasaki (1979), Satomi et al. (2002), Lopez-Lopez et al. (2002), Liu et al. (2007), Kodama et al. (2008), Wiese et al. (2009a), and Zhao et al. (2010).

+ positive, − negative, *w* weak, *nd* not determined

[a]*T. lucentensis* contains no bacteriochlorophyll. The presence of bacteriochlorophyll was not tested for *T. xiamenensis*, *T. profundimaris*, *T. tepidiphila*, and *T. xianhensis*

[b]The characteristic is given for *T. xianhensis*

[c]Sakane and Yokota (1994) reported 48 mol%, and Terasaki (1979) reported 51 mol%

[d]*T. xianhensis* showed an DNA G + C content of 61.2 ± 1.0 mol%

[e]The numbers refer to the percentage of total fatty acids

[f]The numbers refer to the percentage of the total 3-hydroxy fatty acids. Zhao et al. have given no data on the 3-hydroxy fatty acid content of *T. xianhensis*

[g]C–14:0 3OH and/or 16:1-ISOl

[h]The characteristic is given for *T. tepidiphila*

[i]*T. lucentensis* shows no anoxic photosynthetic growth. *T. xiamenensis* and *T. profundimaris* were not examined

formation of a cluster including *Thalassospira* species together with *Terasakiella pusilla* and *Kiloniella laminariae* (>90 % bootstrap values) which is clearly separated from *Rhodospirillaceae* and *Acetobacteraceae* (<91 % sequence similarity) as well as other *Alphaproteobacteria* (Fig. 13.1). Although *Kiloniella laminariae* shares less than 90.3 % 16S rRNA gene sequence similarities to species of the genera *Thalassospira* and *Terasakiella* (Table 13.2), because of the clear cluster formation in phylogenetic calculations, it is suggested to include members of the genera *Thalassospira* and *Terasakiella* together with *Kiloniella* in the order *Kiloniellales*.

Those species of other alphaproteobacterial orders found similar to *Kiloniella* at an 16S rRNA sequence level of 88–91 % revealed significantly higher G + C content of their DNA: *Pelagibius litoralis* (*Rhodospirillales* – 66.3 mol%) (Choi et al. 2009), *Mesorhizobium camelthorni*, *Ensifer terangae* and *Pseudaminobacter salicylatoxidans* (*Rhizobiales* – 61.6–63.9 %) (Chen et al. 2011; Young 2003; Kämpfer et al. 1999), and *Parvularcula bermudensis* (*Parvularculales* – 60.8 mol%) (Cho and Giovannoni 2003). On the other hand, *Kordiimonas gwangyangensis* (*Kordiimonadales*) has remarkable low values of 39.3 mol% (Kwon et al. 2005).

Table 13.2

16S rRNA gene sequence similarity values between *Kiloniella laminariae* and "next relatives" of the *Alphaproteobacteria*

Related species	Accession number	Sequence similarity (%)
Pseudovibrio denitrificans DN34^T	AY486423	90.8
Pseudovibrio ascidiaceicola NBRC 100514^T	AB175663	90.7
Thalassospira xianhensis P-4^T	EU01754611	90.2
Pelagibius litoralis CL-UU02^T	DQ401091	90.3
Mesorhizobium camelthorni CCNWXJ40-4	EU169581	90.1
Mesorhizobium chacoense PR5^T	AJ278249	90.0
Mesorhizobium alhagi CCNWXJ12-2^T	EU169578	90.0
Mesorhizobium shangrilense CCBAU 65327^T	EU074203	89.7
Ensifer terangae LMG 7834^T	X68388	89.7
Pseudaminobacter salicylatoxidans BN12^T	AF072542	89.5
Mesorhizobium alexandrii AM1V30^T	AJ786600	89.5
Aminobacter aganoensis DSM7051 ^{T}	AJ011760	89.5
Ochrobactrum rhizosphaerae PR17^T	AM490632	89.5
Ensifer kostiensis LMG 19225^T	AM181747	89.4
Ahrensia kielensis IAM12618^T	D88524	89.3
Mesorhizobium mediterraneum UPM-Ca36^T	L38825	89.3
Mesorhizobium amorphae ACCC 19665^T	AF041442	89.3
Thalassospira profundimaris WP0211^T	AY186195	89.2
Thalassospira tepidiphila 1-1B ^{T}	AB265822	89.2
Mesorhizobium temperatum SDW 018^T	AF508208	89.1
Thalassospira xiamenensis M-5^T	AY189753	89.1
Pleomorphomonas oryzae F-7^T	AB159680	89.1
Mesorhizobium septentrionale SDW 014^T	AF508207	89.0
Phyllobacterium leguminum ORS 1419^T	AY785323	88.9
Hoeflea marina LMG128^T	AY598817	88.9
Hoeflea alexandrii AM1V30^T	AJ786600	88.9
Thalassospira lucentensis SDM 14000^T	AM294944	88.9
Ensifer saheli LMG 7837^T	X68390	88.9
Pleomorphomonas koreensis Y9^T	AB127972	88.9
Mycoplana dimorpha IAM 13154^T	D12786	88.9
Mesorhizobium australicum WSM2073 ^{T}	AY601516	88.9
Mesorhizobium huakuii IAM 14158^T	D12797	88.8
Ensifer fredii ATCC 35423^T	D14516	88.8
Ensifer mexicanus ITTG-R7^T	DQ411930	88.7
Mesorhizobium opportunistum WSM2075 ^{T}	AY601515	88.6
Ensifer kummerowiae CCBAU 71714^T	AF364067	88.5
Terasakiella pusilla IFO 13613^T	AB006768	88.4

Phenotypic Analyses

Characteristic properties of *Kiloniella* and *Kiloniellaceae* according to those of *Kiloniella laminariae* (Wiese et al. 2009a) are as follows. Colonies grown on marine broth are cream colored, smooth, soft, and have a diameter between 1 and 2 mm. Cells are motile with a monopolar flagellum. They are Gram-negative, slender, slightly curved spirilla, and their size is 0.5–0.6 × 2.5–5.0 µm. Short rodlike cells and also longer filamentous cells may occasionally be observed. Pigments are not produced under any growth condition applied.

Kiloniella species grow as chemoheterotrophic aerobic bacteria in complex media and can use nitrate as an alternative

Table 13.3
Fatty acid profile of *Kiloniella laminariae* LD81^T

Fatty acid	Percentage of total
C-12:0 ALDE	1.7
C-13:1 AT 12–13	0.1
Unknown component (ECL 14.502)	0.7
C-15:1 ω8c	0.3
Unknown component (ECL 14.959)	1.2
C-15:0	0.1
C-14:0 3OH/C-16:1 ISO I	1.2
C-16:1 ω7c	30.7
C-16:0	8.5
C-17:1 ω8c	0.3
C-17:1 ω6c	0.1
C-17:0	0.9
C-18:1 ω7c	48.6
C-18:0	3.0
C-17:0 3OH	0.2
Unknown component (ECL 18.814)	0.4
C-19:0 cyclo ω8c	1.4
C-18:0 3OH	0.5
C-20:1 ω9c	0.3

ECL equivalent chain length

electron acceptor. In the case of *K. laminariae*, nitrate is reduced to gaseous products (N_2O is the major product and *nosZ*, the gene for N_2O reductase, is lacking). The temperature range for growth is from 4 °C to 40 °C with an optimum of 25 °C. The pH range is pH 3.5–9.5 with an optimum at 5.5. Typical marine and moderately halotolerant bacteria. Salt is required for growth, which occurs in media containing 0.3–8.0 % NaCl (optimum 3.0 %) or 0.3–10 % artificial sea salts (optimum 4.0 %). Catalase and oxidase reactions were positive and poly-ß-hydroxybutyrate was accumulated. Luminescence was negative. Details of the physiological characteristics of *K. laminariae* including substrate utilization and enzyme activities are given in the species description (Wiese et al. 2009a).

The dominant fatty acids are *cis*-7-octadecenoic acid (C18:1ω7c), which contributed approximately 50 % of the total amount, and *cis*-7-hexadecenoic acid (C16:1ω7c) representing 31 %. The individual fatty acids and their proportions are listed in *Table 13.3*. The G + C content of the DNA of *Kiloniella laminariae* is 51.1 mol%.

Common properties of *Kiloniella laminariae* and its phylogenetic neighbors *Terasakiella pusilla* and the *Thalassospira* species are the salt requirement and tolerance up to approx. 8–10 % NaCl, the aerobic chemoorganotrophic mode of growth, the ability to reduce nitrate, the G + C content of the DNA from 48 to 55 mol%, and the spiral to vibrioid cell shape (*Table 13.1*). Differential characteristics of *Kiloniella* to these bacteria, in addition to clear differences in 16S rRNA gene sequences (88.4–90.2 mol %), are the proportions of fatty acids, in particular the production of 3-hydroxy heptadecanoic acid by *Kiloniella laminariae* and the reduction of nitrate to N_2O by this species instead of nitrite by the other bacteria (*Table 13.3*). In addition, *Terasakiella pusilla* possesses bipolar flagella compared to the single monopolar flagellum of *Kiloniella* and *Thalassospira*.

Isolation, Enrichment, and Maintenance Procedures

Homogenized and suspended tissue of *Saccharina latissima* or material from alternative sources is diluted in sterile seawater and plated on TSB medium (3 g/l Difco tryptic soy broth, 7 g/l NaCl, 15 g/l Bacto agar, pH 7.2). The plates are incubated at 22 °C, and after good growth an overlay containing TSB medium (with 8 g/l Bacto agar) inoculated with an overnight culture of *Candida glabrata* DSM 6425 is poured on top. After incubation for 24 h at 22 °C, antibiotic-active colonies are visible due to inhibition zones against *C. glabrata* and are picked and repeatedly streaked on agar plates until pure cultures are obtained. Storage at −80 °C using the Cryobank System (Mast Diagnostica GmbH) is used for maintenance (Wiese et al. 2009a).

Ecology

The worldwide distribution of *Laminaria* and related genera of marine macroalgae (Bartsch et al. 2008) and the supposed predetermination of the microbial community composition through physiological and biochemical properties of these algae (Goecke et al. 2010) suggest also wide distribution of bacteria associated with these algae in marine habitats. *Kiloniella laminariae* was one out of approximately 100 antibiotic-active bacteria isolated from the marine macroalga *Saccharina latissima* (former *Laminaria saccharina*) from the Kiel Fjord (Baltic Sea, Germany). Although by and large the ecological role of *Kiloniella* is not known, this bacterium inhibited the growth of *Bacillus subtilis* and of *Candida glabrata* (Wiese et al. 2009b), and it is assumed that it may be able to protect the host against microbial destruents and causative agents of algal diseases by producing antibiotics. Such beneficial effects also have been proposed for *S. latissima* by associated pseudomonads (Nagel et al. 2012).

Application

Kiloniella laminariae revealed antimicrobial activity against the Gram-positive *Bacillus subtilis* and the yeast *Candida glabrata* (Wiese et al. 2009b). This indicates the potential to produce compounds that can be applied against pathogens causing algal and human diseases.

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14 The Family *Kordiimonadaceae*

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Abstract
The genus *Kordiimonas* is the only genus within the order *Kordiimonadales* (Kwon et al. Int J Syst Evol Microbiol 55:2033-2037, 2005), an order that contains a single family—the *Kordiimonadaceae*, a name we here formally propose. Currently (January 2013) the genus *Kordiimonas* contains four species: *K. gwangyangensis*, *K. lacus*, *K. aestuarii*, and *K. aquimaris*. *Kordiimonas* species form a monophyletic cluster, and can be distinguished from other groups within the *Alphaproteobacteria* by their chemotaxonomic features, especially by their cellular fatty acid composition. Branched fatty acids are seldom found in the *Alphaproteobacteria*, but high contents of iso- and anteiso-branched fatty acids were reported in *Kordiimonas* species. *Kordiimonas* is distributed globally, but has never yet been found as a dominant population. The natural habitats are mainly marine environments: seawater and marine sediments. Enrichment and isolation of *Kordiimonas* strains usually require low nutrient media and long incubation periods. No clinical reports of infections of humans or animals by *Kordiimonas* species have been published. *K. gwangyangensis* participates in polycyclic aromatic hydrocarbon degradation as well as alkane oxidation.

Taxonomy, Historical and Current

Order *Kordiimonadales* Kwon, Lee, Yang and Kim 2005, 2036[VP]

Kor.di.i.mo.na.da'les. N.L. fem. n. *Kordiimonas*, type genus of the order; suff. *–ales*, ending denoting an order; N.L. fem. pl. n. *Kordiimonadales*, the *Kordiimonas* order.

The order *Kordiimonadales*, with the genus *Kordiimonas* as the sole described genus, was proposed mainly on the basis of its phylogenetic position (Kwon et al. 2005). Thus far, no formal proposal was made to name the family in which this genus should be classified. We therefore propose here the family name *Kordiimonadaceae*.

Family *Kordiimonadaceae* (Effective publication: Xu, Wu and Oren, this publication).

Kor.di.i.mo.na.da'ce.ae. N.L. fem. n. *Kordiimonas* type genus of the family; *-aceae* ending to denote a family; N.L. fem. n. *Kordiimonadaceae* the family of *Kordiimonas*.

The descriptions of the order and the family are the same as for the genus *Kordiimonas* (see below).

The genus *Kordiimonas* (Kwon et al. 2005; emended by Xu et al. 2011; emended by Yang et al. 2013) is the only genus within the order and the family. Currently (January 2013) the genus contains four species: the type species *K. gwangyangensis* (Kwon et al. 2005), *K. lacus* (Xu et al. 2011), *K. aestuarii* (Math et al. 2012), and *K. aquimaris* (Yang et al. 2013). Several additional isolates that share >90 % 16S rRNA gene sequence similarity with *K. gwangyangensis* have been classified within the same group (Ito et al. 2011; Maeda et al. 2009, 2010). The cells are Gram-negative straight to slightly curved rods, motile by means of flagella. The G+C content of the DNA varies between 50 and 58 mol%. The predominant quinone is Q-10. The major components in the polar lipid profile are phosphatidylethanolamine and several unidentified glycolipids, aminophospholipids and amino-lipids. The major fatty acids are iso-$C_{17:1}$ $\omega 9c$, iso-$C_{15:0}$ and $C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2-OH. $C_{16:0}$, iso-$C_{17:0}$, $C_{18:1}$ $\omega 7c$ and hydroxyl fatty acids (iso-$C_{17:0}$ 3-OH and $C_{16:0}$ 3-OH) may also occur. The 16S rRNA gene signatures common to all thus far characterized strains are 150 (C), 164 (G), 409–433 (U-A), 442–492 (G-C), and 998–1043 (A-U). *Kordiimonas* species are obligatory aerobic chemoheterotrophic bacteria, like the majority of the currently described

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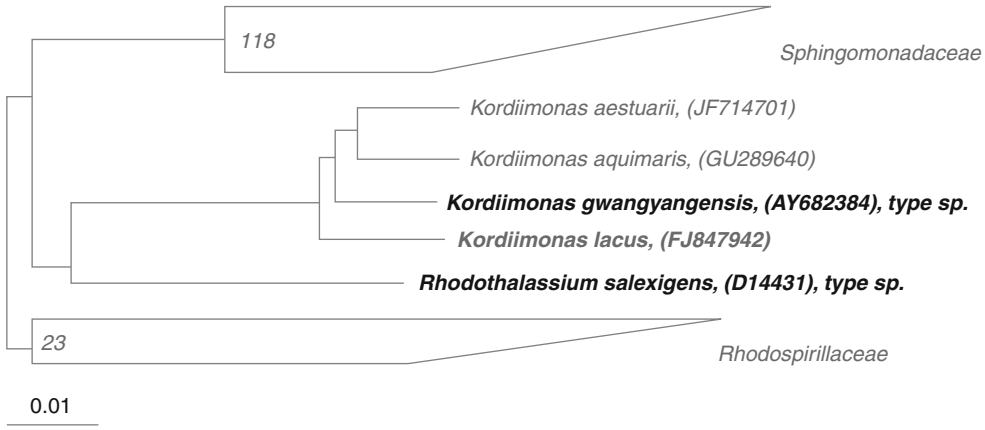

Fig. 14.1

Neighbor-joining genealogy reconstruction of the two species of the family *Kordiimonadaceae* present in the LTP_106 (Yarza et al. 2010), and two more species whose descriptions were added later. The tree was reconstructed by using a subset of sequences 767 type strains of bacteria and archaea to stabilize the tree topology. In addition, a 40 % conservational filter for the whole bacterial domain was used to remove hypervariable positions. Numbers in triangles denote number of taxa included. The bar indicates 1 % sequence divergence

strains living in marine habitats. Some participate in polycyclic aromatic hydrocarbons (PAHs) degradation as well as alkane oxidation.

Phylogenetic Structure of the Family and Its Genus

The order *Kordiimonadales* was proposed on the basis of the phylogenetic position of its representatives within the *Alphaproteobacteria* (Kwon et al. 2005). In the neighbor joining tree based on 16S rRNA sequences (Fig. 14.1), its closest relatives are *Rhodothalassium salexigens*, the family *Sphingomonadaceae*, and the family *Rhodospirillaceae*. A maximum likelihood tree shows the same topology. The four *Kordiimonas* species form a monophyletic cluster that can be distinguished from others by its chemotaxonomic features, especially the cellular fatty acid composition. Branched fatty acids are not commonly found in the *Alphaproteobacteria*, but high contents of iso- and anteiso-branched fatty acids were reported in *Kordiimonas* species. The contents of iso-branched fatty acids of type strains of *K. gwangyangensis*, *K. lacus*, *K. aestuarii* and *K. aquimaris* were 68 %, 56 %, 71 %, and 67 %, respectively (Xu et al. 2011; Math et al. 2012; Yang et al. 2013).

K. gwangyangensis GW14-5^T and *K. aestuarii* 101-1^T were isolated from marine sediments near Korea. Several other strains, isolated from seawater off the coast of Japan, were classified in the order *Kordiimonadales* on the basis of 16S rRNA gene phylogenetic analysis (Ito et al. 2011; Maeda et al. 2009). These form an independent cluster, showing a low (<93 %) 16S rRNA gene sequence similarity to the described *Kordiimonas* species, and they do not show the following 16S rRNA gene signatures of those species, 45–396 (C-G), 108 (G), 241–285 (A-U), 301 (G), 379 (G), 502–543 (U-A), 576 (C), 669–737 (G-C), 678–712 (U-A), 780 (G), 822–878 (G-C), 1122–1151 (U-A), and 1189 (U), whereas those of the unnamed isolates are 45–396 (U-A), 108 (C), 241–285 (G-C), 301 (A), 379 (C), 502–543 (C-G), 576 (A), 669–737 (U-A), 678–712 (A-U), 780 (A), 822–878 (A-U), 1122–1151 (C-G), and 1189 (C). An in-depth study of the taxonomy positions of those isolates is recommended.

Genome Analysis

No genome sequences have yet been reported for any of the species within the genus *Kordiimonas*. DNA-DNA hybridization (DDH) studies have been performed on several *Kordiimonas* type strains. *K. gwangyangensis* KACC 13321^T and *K. aestuarii* 101-1^T, sharing 97.3 % 16S rRNA gene sequence similarity, exhibit 24.8 % DDH similarity (Math et al. 2012). The DNA-DNA relatedness value between *K. lacus* JCM 16261^T and *K. aestuarii* 101-1^T (96.3 % 16S rRNA gene similarity) was 32.2 % (Math et al. 2012).

Phenotypic Analyses

The Properties of the Genus *Kordiimonas* and Its Species

Genus *Kordiimonas* Kwon, Lee, Yang and Kim 2005, 2036^{VP} (emend. Xu, Huo, Bai, Wang, Oren, Li and Wu, 2011, 424; emend. Yang, Kim, Seo, Lee, Lee, Kim and Kwon, 2013, 301).

Kor.di.i.mo'nas. N.L. fem. n. *Kordia* arbitrary name derived from the abbreviation KORDI, which stands for Korea Ocean Research and Development Institute; L. fem. n. *monas* a monad, unit; N.L. fem. n. *Kordiimonas* a microorganism described by scientists working at KORDI.

Cells are ovoid to rod-shaped, motile by means of polar or lateral flagella, and multiply by binary fission. They occur singly or in chains. Gram-negative. Strictly aerobic. No growth occurs under anaerobic conditions, although nitrate may be reduced to nitrite. The principal respiratory quinone is ubiquinone-10. Mesophilic, with optimum growth at 20–41 °C and pH 6.5–7.0. Salinity range for growth is 0–13 % NaCl. Colonies are usually smooth, translucent or opaque, raised and circular with regular borders, usually 0.5–3 mm in diameter after 3–7 days inoculation on marine 2216 agar. Colony color varies from creamy white to beige to light yellow, depending on the strain and on the age of the colonies. Oxidase and catalase positive. Chemoorganotrophic, utilizing a wide range of carbohydrates, salts of organic acids, and amino acids as sole carbon sources. Some strains hydrolyze polycyclic aromatic hydrocarbon with two to five rings. Produce an acidic reaction in mineral salts medium containing D-glucose or other carbohydrates. The polar lipid profile consists of the major components phosphatidylethanolamine and several unidentified glycolipids, amino-phospholipids, and amino-lipids. The major fatty acids are iso-$C_{17:1}$ $\omega 9c$, iso-$C_{15:0}$ and $C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2-OH. $C_{16:0}$, iso-$C_{17:0}$, $C_{18:1}$ $\omega 7c$, and hydroxyl fatty acids (iso-$C_{17:0}$ 3-OH and $C_{16:0}$ 3-OH) may also occur. Found in marine sediment and seawater. Not encountered as clinical isolates and not known to be pathogenic for animals or plants, although some strains were isolated from algae.

The mol% G+C of DNA is 50–58.

The type species is *Kordiimonas gwangyangensis* with type strain GW14-5^T (=KCCM 42021^T = JCM 12864^T) (Kwon et al. 2005).

There are four species within the genus *Kordiimonas*: *K. gwangyangensis*, *K. lacus*, *K. aestuarii* and *K. aquimaris*. All the type strains of *Kordiimonas* species were isolated from marine environments, including marine sediment, seawater, and sediment from the ballast water tank of a crude carrier. Most common characteristics of *Kordiimonas* species are listed in the above genus description as well as in *Table 14.1*. A high content of branched fatty acids (>50 %) distinguishes the *Kordiimonas* members from their closest neighbors in the phylogenetic tree.

The phenotypic characteristics that differentiate the *Kordiimonas* species are given in *Table 14.1*. The upper NaCl concentration for growth of *K. gwangyangensis* (4 %) is much lower than that of the other three *Kordiimonas* species (9–13 %). The optimum NaCl range for growth of all *Kordiimonas* species was 2–3.5 %, coinciding with the salt concentration in their habitat. *K. gwangyangensis* GW14-5^T can degrade six different polyaromatic hydrocarbons (PAHs) with two to five rings, including fluorene, phenanthrene, anthracene, pyrene, chrysene, and benzo[a]pyrene (Kim and Kwon 2010). Several isolates with high 16S rRNA gene sequence similarity with *K. gwangyangensis* GW14-5^T also have the ability to degrade PAHs (Tian et al. 2008; Wang et al. 2010). However, there are no reports of PAHs degradation by other *Kordiimonas* species.

K. lacus reduces nitrate to nitrite and utilizes a large number of amino acids as sole carbon and energy sources, including L-alanine, L-arginine, L-asparagine, L-aspartate, L-cysteine, L-glutamate, L-glutamine, L-histidine, L-ornithine, L-serine, and L-valine. *K. lacus* produces acids from a number of sugars and sugar alcohols, including cellobiose, glucose, *myo*-inositol, maltose, and trehalose.

K. aestuarii markedly differs in cell size and colony color from *K. gwangyangensis*, but resembles *K. lacus* in these properties. The DDH similarity of type strains between *K. aestuarii* and *K. gwangyangensis* (24.8 %) was lower than that between *K. aestuarii* and *K. lacus* (32.2 %) (Math et al. 2012). The three species could be distinguished from each other by enzymatic activities and sensitivity to antimicrobial agents. For example, *K. lacus* is sensitive to chloramphenicol, nitrofurantoin, novobiocin, streptomycin and tobramycin; whereas *K. gwangyangensis* is resistant to nitrofurantoin and tobramycin, and *K. aestuarii* is resistant to chloramphenicol, novobiocin and streptomycin.

K. aquimaris has a low optimum growth temperature (20 °C) and uses a greater variety of carbon sources compared to other members. *K. aquimaris* MEBiC06554^T utilized the following Biolog GP substrates (26): erythritol, D-fructose, D-galactose, α-D-lactose, lactulose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, turanose, mono-methyl succinate, cis-aconitic acid, citric acid, D-galacturonic acid, D-gluconic acid, α-ketoglutaric acid, propionic acid, quinic acid, succinic acid, succinamic acid, L-asparagine, glycyl-L-aspartic acid, L-ornithine, γ-aminobutyric acid, glucose-1-phosphate, and glucose-6-phosphate; all these compounds are not used by *K. gwangyangensis* GW14-5^T and *K. lacus* S3-22^T (Yang et al. 2013).

Isolation, Enrichment and Maintenance Procedures

Several isolation techniques for *Kordiimonas* have been described. Usually, isolation of *Kordiimonas* strains needs low nutrient medium and a long period of enrichment. In the case of *K. gwangyangensis* GW14-5^T, approximately 1 g sediment was enriched in MM2 liquid medium [18 mM $(NH_4)_2SO_4$, 1 μm $FeSO_4 \cdot 7H_2O$, and a trace of KH_2PO_4/Na_2HPO_4 buffer solution in 1 l of aged sea water, pH 7.2] supplemented with 100 ppm each of pyrene and benzo[a]pyrene and incubated for 2 years (Kwon et al. 2005). The long-term enrichment and the presence of pyrene and its derivative inhibited the growth of most other microorganisms, enabling *K. gwangyangensis* GW14-5^T to

Table 14.1
Differential metabolic and chemotaxonomic characteristics of type strains of *Kordiimonas* species

Characteristic	*K. gwangyangensis*[a]	*K. lacus*[b]	*K. aestuarii*[c]	*K. aquimaris*[d]
Type strain	KCCM 42021	CGMCC 1.9109	KACC 16184	KCCM 42940
Cell size	0.25 × 1.3–1.4	0.5–0.8 × 3.0–6.0	0.3–0.6 × 1.5–4.5	0.6–0.9 × 0.7–1.2
Color of colonies on MA	Creamy white	Light yellow	Light yellow	Beige
NaCl range for growth and optimum (%)	0.5–4.0 (2.0)	0.5–10.0 (2.0–3.0)	1.0–9.0 (2.0–3.5)	0–13.0 (3.0–3.5)
pH range for growth and optimum	6.0–8.5 (7.0)	6.0–9.0 (7.0)	5.0–8.0 (6.5–7.0)	5.0–8.0 (7.0)
Temperature range for growth and optimum (°C)	17–44 (37–41)	10–43 (30–37)	15–40 (30)	10.5–35 (20)
Oxidase/catalase activity	+/w	+/+	+/+	+/w
Nitrate reduction	–	+[c, d]	–	–
Acetoin production	–[c, d]	+	–	+
Enzyme activities (API ZYM, bioMérieux)				
N-Acetyl-β-glucosaminidase	+	+[b, c] (but –[d])	–	–
Lipase (C14)	w[b, c] (but –[d])	–	–	–
α-Glucosidase	+[d] (but –[c])	–	–	–
α-Mannosidase	–[c, d] (but +[b])	–	–	–
Assimilation of (API 20NE, bioMérieux)				
D-Glucose	+[c, e]	–[c] (but +[e])	+	+[e]
N-Acetyl-glucosamine	+[c, e]	–[c, e]	–	+[e]
Maltose	+[c, e]	–[c] (but +[e])	+	+[e]
Acid production from mannose	+[b]	–	–	NR
Sensitivity to (μg per disc)				
Chloramphenicol (30)	+[b]	+	– (100 μg)	NR
Nitrofurantoin (300)	–[b]	+	NR	NR
Novobiocin (30)	+[b]	+	– (5 μg)	NR
Streptomycin (10)	+[b]	+	– (50 μg)	NR
Tobramycin (10)	–[b]	+	NR	NR
DNA G+C content (mol%)	55.6–58.0[b, d]	54.9	53.3	50.3

All strains are positive for hydrolysis of starch and Tween 80, but negative for arginine dihydrolase and urease. Neither strain produces H_2S or indole. In API ZYM tests, acid and alkaline phosphatases, α-chymotrypsin, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-β-1-phosphohydrolase, valine arylamidase, and trypsin activities are detected in all
+ positive, – negative, w weakly positive, *NR* not reported
Data taken from [a]Kwon et al. 2005; [b]Xu et al. 2011; [c]Math et al. 2012; [d]Yang et al. 2013; [e]Data from Microlog GN2 (Biolog) plate readings from Yang et al. 2013

degrade the pyrene and benzo[a]pyrene as carbon and energy source. The suspension was then plated onto marine agar 2216 (BD), and the isolate was obtained by the serial dilution method. *K. gwangyangensis* S20-13 was isolated by similar methods, using crude and diesel oils as a carbon source and an enrichment time of 2–3 months (Wang et al. 2010). *K. aquimaris* MEBiC06554^T was obtained by the extinction-dilution cultivation method (Connon and Giovannoni 2002) after 9 months of enrichment. The culture broth was then spread onto twofold-diluted marine agar 2216 solid medium (BD) supplemented with seawater and incubated at 15 °C up to 4 months (Yang et al. 2013). Isolation of *Kordiimonas* strains may thus be achieved by streaking long-term enrichment cultures containing low levels of carbon and nitrogen sources on agar plates.

The members of *Kordiimonas* can be maintained for short-term storage on Marine 2216 agar slants at 4 °C for 2 months. For long-term preservation, cultures can be preserved in liquid nitrogen, by lyophilization or frozen at −80 °C.

Ecology

Habitat

The natural habitats of *Kordiimonas* are mainly marine environments. They are found in seawater collected offshore (Thompson et al. 2011; Yang et al. 2013) and from the ocean (Pham et al. 2008; Wang et al. 2010), in estuarine (Tian et al. 2008) and deep-sea sediment (Li et al. 2009, 2011), as well as in

ballast water tanks of commercial ships (Xu et al. 2011), hydrothermal vent chimneys (Peng et al. 2010), and marine sponges (Anderson et al. 2010). They have been reported as important components of biofilms in a reverse osmosis seawater desalination system (Bae et al. 2011). McKew et al. (2011) reported a low abundance (0.2 %) of *Kordiimonas*-related 16S rRNA gene sequences in the sediments from a creek near a salt marsh in the UK (36 ‰ salinity). *Kordiimonas* strains are distributed globally. They were found in Asia (Tian et al. 2008), Europe (McKew et al. 2011), South America (Thompson et al. 2011), the Pacific Ocean (Liao et al. 2011), the Atlantic Ocean (Wang et al. 2010), the Indian Ocean (Peng et al. 2010), and the Arctic Ocean (Li et al. 2009). However, they were never found to dominate the bacterial community. Relative abundances of *Kordiimonas* in their natural habitats are usually less than 2 %. Generally, the natural habitats of *Kordiimonas* seem to be oligotrophic environments containing salt and low concentrations of organic matter and temperatures of 1–30 °C. The ability of *K. gwangyangensis* strains to hydrolyze PAHs may be an advantage for growth and survival in oligotrophic and polluted environments.

The type strains of the four species were isolated from marine sediments collected from the Gwangyang Bay in Korea (*K. gwangyangensis*) (Kwon et al. 2005), from a ballast water tank of a ship, which can be considered to form a man-made incubator for growth of microorganisms, releasing invasive aquatic species to coastal regions (*K. lacus*) (Xu et al. 2011), from sediment from a sea-tidal flat in Korea (*K. aestuarii*) (Math et al. 2012), and from seawater near the Anmok Port in Korea (*K. aquimaris*) (Yang et al. 2013).

Pathogenicity, Clinical Relevance

No clinical reports of infections of humans or animals by *Kordiimonas* species have been published. Antibiotic sensitivity of three type strains tested, *K. gwangyangensis*, *K. lacus* and *K. aestuarii*, showed susceptibility to penicillin G, but resistance to ampicillin, carbenicillin, kanamycin, and tetracycline (Math et al. 2012; Xu et al. 2011). Susceptibility to chloramphenicol, nitrofurantoin, novobiocin, streptomycin, and tobramycin differs among the three species (*Table 14.1*). The antibiotic sensitivity of *Kordiimonas* species might be used to specifically isolate different *Kordiimonas* strains.

Application

Bioremediation

Because of their ability to degrade a wide variety of PAHs, *K. gwangyangensis* strains are of potential interest in bioremediation. PAHs with two to five rings (fluorene, phenanthrene, anthracene, pyrene, and benzo[a]pyrene) were degraded to a large extent by *K. gwangyangensis* GW14-5^T, but chrysene was not degraded within one week of incubation (Kim and Kwon 2010; Kwon et al. 2005). Among the isolates from a PAH-polluted site in a mangrove ecosystem, *K. gwangyangensis* strains capable of degrading pyrene and fluorene formed an important component (Liu et al. 2010; Tian et al. 2008). Molecular studies revealed that *K. gwangyangensis* strains possess alkane hydroxylases. The integral membrane nonheme di-iron alkane hydroxylases (e.g., AlkB and AlkM) are one of the most important enzyme systems in the terminal oxidization of medium-length alkanes in prokaryotes (Wang et al. 2010). *K. gwangyangensis* S20-13 isolated from pelagic surface water has two alkane hydroxylase genes (A*lkB*) and is able to grow on diesel oil (Wang et al. 2010). However, the industrial application of *K. gwangyangensis* has been hampered by its relatively slow growth and its ability to use of a large number of carbohydrates, which are potentially competitive substrates during degradation of PAHs or alkanes.

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15 The Family *Methylobacteriaceae*

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Abstract
The *Methylobacteriaceae* comprise a large family of *Alphaproteobacteria* within the Order *Rhizobiales* and currently contains three genera, *Methylobacterium*, *Microvirga*, and *Meganema*. The largest genus currently contains 44 validated species of *Methylobacterium*, most of which are facultative methylotrophs able to grow on methanol and other one-carbon compounds as sources of energy and carbon. Most are pink-pigmented, exhibit common fatty acid profiles, and contain ubiquinone Q-10. The eight species of *Microvirga* and the single species of *Meganema* are not methylotrophic. The phylogenetic and phenotypic properties of *Meganema* indicate that it is wrongly placed in this family. *Methylobacterium* species are ubiquitous in the natural environment, both as free-living organisms in soil and water, but also on the phylloplane of plants, and in the leaf, stem, and root tissues of plants: Some induce plant leaf and root nodule formation, and can promote plant growth by production of auxins. Some species are opportunistic human pathogens; others have been found in insect tissues. Some are important for their role in the degradation of pollutants, and they may also cause commercial problems such as the fouling of aircraft fuels. *Microvirga* species occur in diverse habitats as free-living species, and others are colonists in plant root nodules. The filamentous *Meganema* has only been recovered as an organism involved in the fouling and blocking of water filtration systems. The genomes of several *Methylobacterium* species have been sequenced, and they show considerable interstrain homology. Significant genome plasticity is indicated by the large number of insertion elements in some genomes, and some methylotrophic functions seem to have been acquired by horizontal gene transfer.

Methylobacteriaceae Garrity, Bell, and Lilburn 2005, 567

Meth.yl.o.bac.te.ri.a'ce.ae. M.L. neut. N. *Methylobacterium* type genus of the family; *-aceae* ending to denote family; M.L. fem. Pl. n. *Methylobacteriaceae* the *Methylobacteriaceae* family.

The *Rhizobiales* (Kuykendall 2005) comprise Order VI of the Class *Alphaproteobacteria*, and contains ten families, of which Family IX is the *Methylobacteriaceae* (Garrity et al. 2005). The family was defined on the basis of phylogenetic analysis of the 16S rRNA gene sequences, and currently comprises three genera, *Methylobacterium*, *Methylovirga*, and *Meganema*. All are chemoorganotrophic, most *Methylobacterium* species are facultative methylotrophs, and most species of *Methylobacterium* and *Microvirga* form pink or red colonies.

Type genus: *Methylobacterium* Patt, Cole, and Hanson 1976, 28 emend. Green and Bousfield 1983, 876. The second edition of *Bergey's Manual of Systematic Bacteriology* (Brenner et al. 2005) described only one genus in this family, *Methylobacterium* (Green 2005), with 12 species. Currently, 44 species are given on the *List of Prokaryotic names with Standing in Nomenclature*, curated by J. P. Euzéby (LPSN; http://www.bacterio.cict.fr/m/

E. Rosenberg et al. (eds.), *The Prokaryotes – Alphaproteobacteria and Betaproteobacteria*, DOI 10.1007/978-3-642-30197-1_256,

methylobacterium.html). The two further genera subsequently assigned to the family are *Microvirga* with eight validly published species (http://www.bacterio.cict.fr/m/microvirga.html), and *Meganema* (http://www.bacterio.cict.fr/m/meganema.html) with a single validated species. The links to the LPSN provide the deposition history and the culture collections which hold each species; they also provide direct links to most of the original publications describing each species, and to the 16S rRNA gene sequence accession numbers for the type strain of each species.

Genus *Methylobacterium*

Meth.yl.o.bac.te'ri.um. M.L. n. *methyl* the methyl radical; Gr. n. *bacterion* a small rod; M.L. neut. n. *Methylobacterium* methyl bacterium.

History and Taxonomy

The advent of taxonomic alignments based on 16S rRNA gene sequence homologies was extremely beneficial in establishing the relationships among the diverse pink-pigmented facultative methylotrophs (PPFMs), most of which are now classified as *Methylobacterium* species. Prior to this rationalization, PPFMs had been assigned to diverse genera, based on morphological and nutritional differences, including variation among species in the ability to grow on methylamine, glucose, fructose, fucose, xylose, arabinose, and some organic and amino acids (Green 2006). The genus was established by Patt et al. (1976 and emended by Green and Bousfield 1983), with *Mtb organophilum* established as the type species of the genus. This assignment was somewhat anomalous as (a) the original description of *Mtb organophilum* was based on the properties of a single strain (strain XX), while later recommendations were that new species descriptions should be based on more than one, and even on five, strains (Stackebrandt et al. 2002), although this criterion is commonly not observed (Drancourt and Raoult 2005); and (b) strain XX was claimed to oxidize methane, a property that was used to exclude non-methane-oxidizing PPFM strains from the genus. Strain XX subsequently "lost" (or had never had) the ability to use methane, and the genus description was changed to delete methane use as a characteristic property of *Methylobacterium* species (Green and Bousfield 1983; Green 2006). As the original basis of the genus description was flawed, it is arguably regrettable that *Mtb extorquens* was not chosen to be the type species (see below), as it was initially isolated as the first example of the genus in 1913 (but not transferred to *Methylobacterium* until 1985), whereas *Mtb organophilum* was isolated in 1974. *Mtb extorquens* is a "classic" example of the genus, strains of which have been the subject of more physiological, biochemical, and molecular studies than of any other *Methylobacterium* species, but of course *Mtb organophilum* has strict taxonomic precedence.

Only 12 species of *Methylobacterium* were recognized by Green (2005, 2006), but at the time of writing (2012) there were 44 validated species names (◉ *Table 15.1*; ◉ *Fig. 15.1*). While validly named, three of the *Methylobacterium* species have been proposed to be later synonyms of existing validly published species, with which they share essentially 100 % 16S rRNA gene sequence identity: *Mtb chloromethanicum* CM4 (McDonald et al. 2001) and *Mtb dichloromethanicum* DM4 (Doronina et al. 2000) are regarded as strains of *Mtb extorquens*; and *Mtb lusitanum* as a strain of *Mtb rhodesianum* (◉ *Table 15.1*; Kato et al. 2005). Marx et al. (2012) proposed that *Mtb populi* should also be regarded as a synonym for *Mtb extorquens*, based on whole genome comparisons of the two species. This proposal is not currently acceptable, as current phylogenetic relationships are based on 16S rRNA gene sequence comparisons, and *Mtb populi* has closer phylogenetic relatives (*Mtb thiocyanatum* and *Mtb rhodesianum*; ◉ *Fig. 15.1*) than *Mtb extorquens*. Both *Mtb populi* and *Mtb extorquens* are members of a distinct 10-species phylogenetic cluster of the genus (from *Mtb aminovorans* to *Mtb podarium* in ◉ *Fig. 15.1*), and reassignment of *Mtb populi* alone could not be justified until complete genome comparison among all ten species is possible. Reclassification based on complete genomes could result in amalgamation (or "lumping") of numerous species into single overarching species, possibly even the lumping of all the species in each of the clusters of species (up to seven in ◉ *Fig. 15.1*, and other published trees) into one species for each cluster. This would, however, be undesirable, as it would obscure the physiological and phenotypic differences among those species.

Possibly the earliest strain of a PPFM to be described was the oxalate-degrading organism isolated from earthworm excreta ("worm casts"), in the Botany Department garden of Basel University, in 1909 by Bassalik (1913). This organism was variously named *Bacillus extorquens*, *Vibrio extorquens*, *Pseudomonas extorquens*, *Pseudomonas methylica*, *Mycoplana rubra*, *Flavobacterium extorquens*, *Protaminobacter ruber*, *Protomonas extorquens*, and finally its current definition as *Methylobacterium extorquens* (Bassalik 1913; Bassalik et al. 1960; Bhat and Barker 1948; Bousfield and Green 1985; Breed et al. 1957; Den Dooren de Jong 1927, 1957; De Vries and Derx 1953; Green 1992, 2006; Green and Bousfield 1983; Janota 1950, 1956; Janota-Bassalik and Pedyk 1961; Krasil'nikov 1949; Stocks and McCleskey 1964; Urakami and Komagata 1984). Unfortunately Bassalik's original isolate perished in 1939 (as an early casualty of World War II), but a very similar strain was recovered in 1948 from soil in the Botany Department garden of Warsaw University by Ludmila Janota (Janota 1950, 1956). This neotype strain, initially named *Pseudomonas extorquens*, was deposited in the NCIMB in 1960 by Ludmila Janota-Bassilik to become the type strain of *Mtb extorquens* (NCIB 9399^T; ATCC 23326^T, DSM 1337^T, TK 0001^T).

The 12 species recognized in the second edition of *Bergey's Manual of Systematic Bacteriology* (Green 2005) were initially assigned to the genus on the basis that they were PPFMs with similar morphology, physiology, and growth properties (optimum pH, optimum temperature, ranges of organic substrates used for chemoorganotrophic growth; Green 2005, 2006). All showed similar percentages of GC in their genomic DNA (68–73 mol%) and were subsequently shown to share reasonably

Table 15.1

Type strains of the species of *Methylobacterium*, 16S rRNA gene accession numbers, and some distinguishing properties. All 44 validated names are shown (LPSN; http://www.bacterio.cict.fr/)

Species	Culture collection accession number[a]	GenBank accession number	G+C content of type strain DNA (mol %)	Isolation source of the type strain	Growth on glucose/motility
Mtb adhaesivum	CCM 7305	AM040156	63.6	Drinking water	−/+
Mtb aerolatum	KACC 11766	EF174498	73.2	Air (Korea)	(+)/+
Mtb aminovorans	JCM 8240	AB175629	68.0	Soil	−/+
Mtb aquaticum	DSM 16371	AJ6353033	67.5	Drinking water	+/+
Mtb brachiatum	NCIMB 14379	AB175649	69.5	Freshwater	−/+
Mtb bullatum[b]	LMG 24788	FJ268657	67.1	Gametophytes of *Funaria hygrometrica*	−/+
Mtb cerastii	DSM 23679	FR733885	n.a.	Leaf of *Cerastium holosteoides*	−/−
Mtb chloromethanicum[c]	VKM B-2223	CP001298	68.1	Polluted soil (Petrochemical works)	−/+
Mtb dichloromethanicum[c]	VKM B-2191	AB175631	68.0[d]	Activated sludge	−/+
Mtb dankookense	KCTC 22512	FJ155589	68.9	Drinking water	−/+
Mtb extorquens	NCIB 9399	AB175632	68.5[e]	Soil	−/+
Mtb fujisawaense	NCIB 12417	AJ250801	71.4	Fujisawa region, Japan	+/+
Mtb gnaphalii	NBRC 107716	AB627971	67.2	Leaf of *Gnaphalium spicutum*	−/+
Mtb goesingense	CCUG 56108	AY364020	n.a.	Endophyte of *Thlaspi goesingense*	−/+
Mtb gossipiicola	NRRL B-51692	EU912445	64.2	Cotton phyllosphere (*Gossipium hirsutum*)	+/+
Mtb gregans	NCIMB 14376	AB252200	70.0	Freshwater	−/+
Mtb hispanicum	DSM 16372	AJ635304	67.7	Drinking water	−/+
Mtb iners	KACC 11765	EF174497	68.0	Air (Korea)	−/+
Mtb isbiliense	CECT 7068	AJ888239	69.0	Drinking water	−/+
Mtb jeotgali[f]	KCTC 1267	DQ471331	64.9	Fermented seafood (jeotgal, Korea)	−/−
Mtb longum	DSM23933	FN868949	68.6	Leaf of *Arabidopsis thaliana*	−/+
Mtb komagatae	NCIMB 14377	AB252201	68.7	Freshwater	(+)/+
Mtb lusitanum[g]	VKM B-2239	AB175635	66.5	Sewage station	−/+
Mtb marchantiae	DSM 21328	FJ157976	n.a.	Liverwort thallus (*Marchantia polymorpha*)	−/−
Mtb mesophilicum	NCIB 11561	AB175636	69.8	Leaf surface (*Lolium perenne*)	+/+
Mtb nodulans[f]	ORS 2060	AF220763	68.4	Legume root nodule (*Crotolaria podocarpa*)	−/+
Mtb organophilum^T	ATCC 27886	AB175638	70.8	Lake sediment	+/+
Mtb oryzae	DSM 18207	AY683045	70.6	Rice stem tissue (*Oryza sativa*)	−/+
Mtb oxalidis	NBRC 107715	AB607860	70.2	Sorrel leaves (*Oxalis corniculata*)	−/+
Mtb persicinum	MCIMB 14378	AB252202	67.1	Freshwater	−/+
Mtb phyllosphaerae	LMG 24361	EF126746	66.8	Phylloplane of rice	(+)/+
Mtb platani	KCTC 12901	EF426729	68.5	Plane tree leaf (*Platanus orientalis*)	−/+
Mtb podarium	DSM 15083	AF514774	68.0	Human foot (female)	+/−
Mtb populi	NCIMB 13946	CP001029	70.4	Endophyte: hybrid poplar (*Populus deltoides x nigra*)	−/+
Mtb radiotolerans	NCIB 10815	D32227	72.3	Rice grains	+/+

Table 15.1 (continued)

Species	Culture collection accession number[a]	GenBank accession number	G+C content of type strain DNA (mol %)	Isolation source of the type strain	Growth on glucose/motility
Mtb rhodesianum	NCIB 12249	AB175642	70.5	Formaldehyde fermenter	−/+
Mtb rhodinum	NCIB 9421	AB175644	71.8	Rhizosphere of alder (*Alnus*)	(+)/+
Mtb salsuginis	NCCB 100140	EF015478	n.a.	Seawater	+/
Mtb soli	KCTC 22810	EU860984	66.2	Forest soil	+/n.a.
Mtb suomiense	NCIMB 13778	AB175645	65.8	Soil	+/−
Mtb tardum	NCIMB 14380	AB252208	69.5	Freshwater	−/+
Mtb thiocyanatum	DSM 11490	U58018	70.4	Rhizosphere of *Allium aflatunense*	+/+
Mtb variabile	DSM 16961	AJ851087	69.2	Drinking water	+/+
Mtb zatmanii	NCIB 12243	AB175647	70.3	Formaldehyde fermenter	−/+

[a]Culture collection into which the type strain was originally deposited
[b]A contemporary synonym for this species is *Mtb funariae* (Schauer and Kutschera 2011); 16S rRNA: FJ157975
[c]Kato et al. (2005) reclassified both these strains as heterotypic synonyms of *Mtb extorquens*
[d]GC value from the complete genome of *Mtb dichloromethanicum* DM4 (Marx et al. 2012)
[e]GC value from the complete genome of *Mtb extorquens* AM1 (Marx et al. 2012)
[f]These strains are non-pigmented
[g]Kato et al. (2005) showed this to be a heterotypic synonym of *Mtb rhodesianum*
n.a., Data not available; +, growth; −, no growth; (+) weak growth

similar 16S rRNA gene sequences: with 11 species showing 96–97 % sequence identity to the type species, *Mtb organophilum*. The type species and the other ten species showed 95–100 % identity to *Mtb extorquens* (authors' data). As the number of species described rose to more than 40, principally on the basis of being phylogenetically similar PPFMs, and distinguishable by DNA-DNA hybridization, the inhomogeneity of the group has become progressively apparent: Phylogenetic trees demonstrate that the species cluster in a number of distinct clades (*Fig. 15.1*; also see the 16S rRNA trees in Dourado et al. 2012a, b; Idris et al. 2006; Knief et al. 2010a; Tani et al. 2012a, b; Wellner et al. 2012). Up to seven distinct clades have been shown in this way (Dourado et al. 2012b; Idris et al. 2006; Knief et al. 2010a, b; Tani et al. 2012a, b). The genus consequently begins to resemble an organized chaos reminiscent of a Jackson Pollock masterwork.

The authors do not believe, however, that this diversity justifies splitting the genus into multiple genera, as the major physiological and biochemical properties (especially facultative methylotrophy, and in most cases the carotenoid pigment) are common to most members of the genus. The criterion of ability to use or not use specific organic compounds as growth substrates (e.g., glucose and other sugars, citrate and methylamines; Green 2006) remains a useful property, especially when distinguishing phylogenetically very similar species. Such differences can, however, be due to relatively small biochemical variations, such as absence of a gene involved in encoding a transport protein or a primary process such as sugar phosphorylation. It is interesting to note that the type strain of *Mtb extorquens* is usually reported as unable to grow on glucose (Green 2005), but in her original work after isolating the type strain, Janota (1956) used Warburg manometry to show glucose oxidation by bacteria previously grown on a meat extract/peptone/glucose medium. Using vacuum-dried bacteria she found glucose oxidation to proceed rapidly with a CO_2 produced/O_2 consumed ratio around 0.70, which is less than the ratio of 1.0 required for complete oxidation:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

In the presence of 5 mM sodium azide, however, this ratio increased to 0.93–1.07. The lower ratio would be consistent with some glucose-carbon being assimilated, but this was inhibited by azide. Moreover, the use of vacuum-dried cells by Janota would have abolished any uptake barrier preventing rapid glucose transport into the bacteria. A possibility is that the ability of the type species to use glucose was subsequently lost in culture.

The description of the genus in *Bergey's Manual* (Green 2005) is adequate, although it was based on the properties of only 12 species, but needs to be updated to note that two of the currently described 44 species are not pigmented, and that some species are capable of inducing nodulation of the leaves and roots of some plants.

Molecular Analyses

Complete genome sequencing of some *Methylobacterium* species. To date, the genomes of 13 strains of *Methylobacterium* have been sequenced, and they are available in completed or permanent draft form on the Joint Genome Institute website (http://img.jgi.doe.gov/cgi-bin/geba/main.cgi?section=FindGenomes?page=findGenomes) and the NCBI Genome database

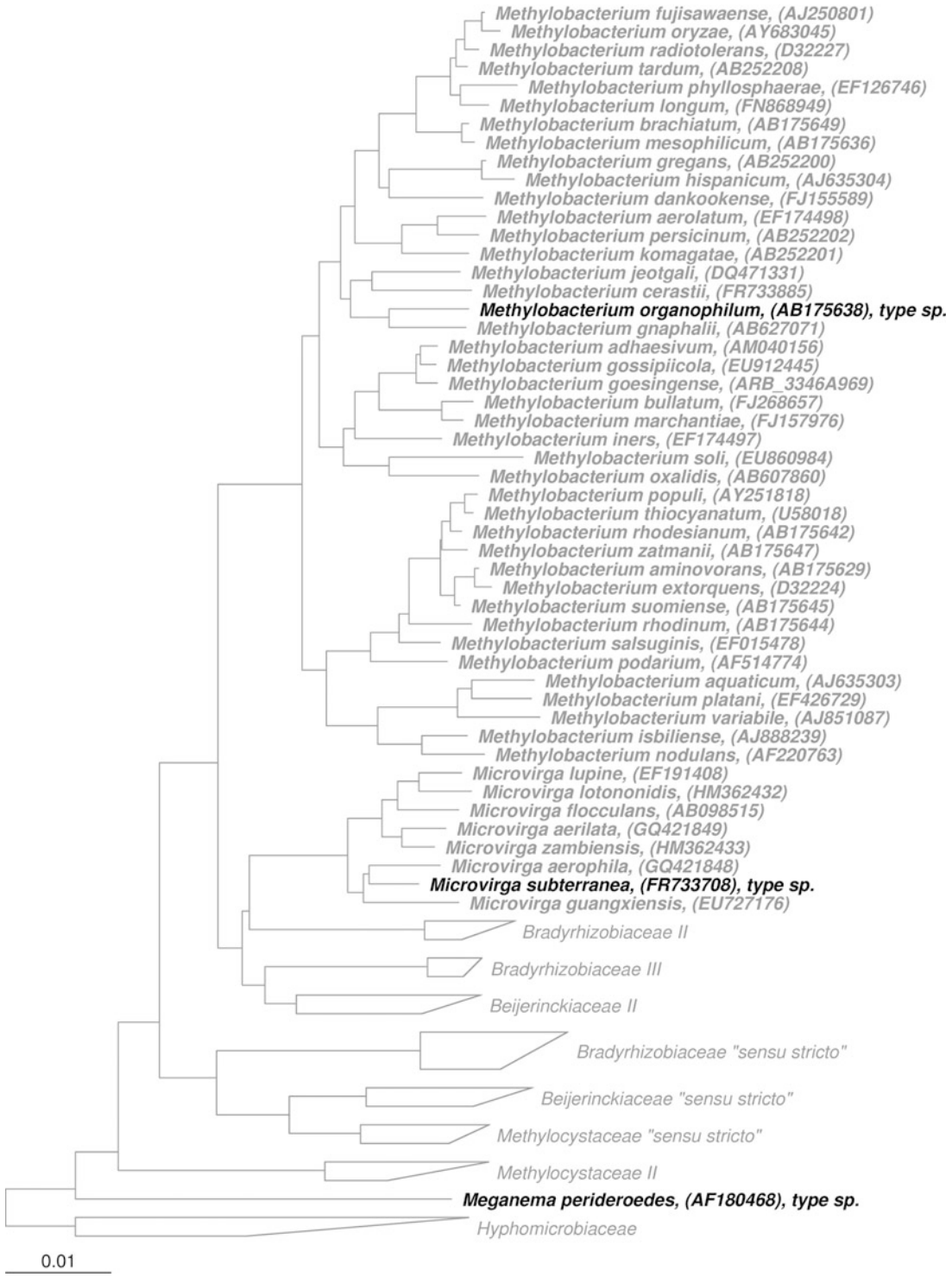

Fig. 15.1

Phylogenetic reconstruction of the family *Methylobacteriaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. All validated names for *Methylobacterium*, *Microvirga*, and *Meganema* are shown, except for *Mtb chloromethanicum* and *Mtb dichloromethanicum* (which are synonyms of *Mtb extorquens*) and *Mtb lusitanum* (a synonym of *Mtb rhodesianum*). Scale bar indicates estimated sequence divergence

(http://www.ncbi.nlm.nih.gov/genome?term=methylobacterium). The available genomes are for *Mtb extorquens* strains AM1 (NCIMB 9133), CM4, DM4, PA1, and strain F (DSM 13060), *Mtb nodulans* (ORS 2060^T), *Mtb populi* (BJ001^T), *Mtb radiotolerans* (JCM 2831^T), *Methylobacterium* sp. strain 4–46 (Marx et al. 2012), *Methylobacterium* sp. strain GXF4 (Gan et al. 2012), and strains WSM2598, 77, and 88A. Neither the genome of the type species, *Mtb organophilum*, nor that of the type strain of *Mtb extorquens* (NCIMB 9399^T) has yet been sequenced. The genomes of *Mtb mesophilicum*, *Mtb oryzae*, and *Methylobacterium* strain MB200 are included in current sequencing projects (2012). Comparative analysis of the genomes should reveal further insights into the nature of methylotrophy in this genus.

The sequenced genomes show considerable ranges of sizes (5.47–8.84 Mb), plasmid number (0–8), and plasmid size (0.01–1.26 Mb), as illustrated in Table 15.2. All the genomes possess several conserved gene clusters known to be involved in methylotrophy in *Methylobacterium* (Chistoserdova 2011). Interestingly, five strains have been shown to possess gene clusters associated with photosynthesis (Marx et al. 2012). The genomes of *Mtb extorquens* strains AM1 and DM4 (formerly *Mtb chloromethanicum*; Doronina et al. 2000) have been studied in the most detail, and exhibit considerable genome-wide homology and gene synteny (Vuilleumier et al. 2009), consistent with their being very closely related strains of the same species (Kato et al. 2005). This homology includes nearly identical genes associated with methylotrophy. Major exceptions were (1) a dichloromethane utilization (*dcm*) gene cluster (126 kb; essential for growth on dichloromethane as sole carbon and energy source) is present in strain DM4, but is absent from strain AM1; and (2) the methylamine utilization (*mau*) gene cluster is found only in strain AM1, indicating strain DM4 to use an alternative, unidentified pathway for growth on methylamine (Vuilleumier et al. 2009). The origins of these *dcm* and *mau* gene clusters are intriguing, as both are located on genomic islands in the respective chromosomes. The *dcm* cluster is located in a 1,054 kb genomic island in the DM4 chromosome (17.7 % of the chromosomal DNA), and the *mau* cluster is part of a 652 kb island in the AM1 chromosome (11.5 % of the chromosome). The presence of the *dcm* and *mau* gene clusters in genomic islands supports the redefinition of strain DM4 (formerly *Mtb dichloromethanicum*) as a strain of *Mtb extorquens* (Kato et al. 2005), as these clusters would have entered either strain DM4 or AM1 by horizontal gene transfer (HGT) and become incorporated into the respective genomes (Juhas et al. 2009; Vuilleumier et al. 2009), making the physiological differences between DM4 and AM1 the result simply of chance HGT events, rather than reflecting any deeper taxonomic difference. The GC content (60.5 %) of the 126 kb *dcm* gene cluster in strain DM4 differs markedly from the average GC content of the genome (68 %), indicative of its acquisition by horizontal transfer (Vuilleumier et al. 2009). A similar acquisition of the *cmu* gene cluster, enabling use of chloromethane by *Mtb extorquens* strain CM4 (formerly *Mtb chloromethanicum*; McDonald et al. 2001), might thus also have occurred by HGT. Apart from *dcm* and *mau* examples, few of the genomic islands appear to encode central metabolic functions or methylotrophy. Strain AM1 has a 47.5 kb genomic island that encodes a hypothetical protein polypeptide of 15,831 residues, which, if it were actually expressed, would be one of the largest-known biological proteins (Vuilleumier et al. 2009). The large sets of insertion elements identified in strains AM1 and DM4 indicate the potential for significant genome plasticity in the species.

In addition to their chromosomes, several strains contain very large plasmids (Table 15.2), the largest being that of *Mtb extorquens* AM1 at 1.26 Mb, or 18 % of the total chromosomal and plasmid DNA. The megaplasmid of strain AM1 has a 130 kb block of genes (with no known functions) which occurs on the chromosome of strain DM4, which has no megaplasmid. This synteny is consistent with the recent evolutionary divergence of the two strains. The genomes analyzed to date also contain variable numbers of pseudogenes, ranging from only four in *Methylobacterium* strain GXF4, through 47–114 in strains AM1, PA1, *Mtb populi*, and *Mtb radiotolerans*, to 206–336 in strains DM4, CM4, and 4–46, and to a remarkable 602 in *Mtb nodulans*, of which 275 (47 %) are on the two very large plasmids (Table 15.2).

The *mxaF* gene as a taxonomic tool. An *mxaF* gene is found in diverse methylotrophs in the *Alpha-*, *Beta-*, and *Gammaproteobacteria*: It encodes the large (α-) subunit of the methanol dehydrogenase protein (MxaF). While the active site of this enzyme is highly conserved (McDonald and Murrell 1997), there is considerable diversity among the amino acid sequences of the encoded MxaF protein; for example, identities (% by BLASTP or TBLASTX) for the MxaF of *Mtb organophilum* compared to *Mtb extorquens*, *Hyphomicrobium methylovorum*, *Paracoccus denitrificans*, *Methylobacillus flagellatus*, *Granulibacter bethesdensis*, and *Xanthomonas campestris* were 95.3 %, 79.2 %, 75.1 %, 61.4 %, 49.3 %, and 41.9 %, respectively (authors' data). In contrast, amino acid sequence identities for MxaF between *Methylobacterium* species were mostly in the range 90–100 % (authors' data). Phylogenetic trees of *mxaF* gene sequences from numerous *Methylobacterium* species were superficially similar to the trees for 16S rRNA gene sequences (e.g., Fig. 2 of Wellner et al. 2012), showing the *mxaF* genes to fall into seven distinct clades (Dourado et al. 2012a; Wellner et al. 2012). Comparison of the 16S rRNA and *mxaF* trees showed, however, that there was not universal congruence between the trees. Both trees were congruent for *Mtb oryzae*, *Mtb phyllosphaerae*, *Mtb fujisawaense*, and *Mtb radiotolerans*, with all four falling in the same or closely linked clades. This was also the case for the pairs *Mtb goesingense/Mtb adhaesivum*, *Mtb gregans/Mtb hispanicum*, and *Mtb suomiense/Mtb aminovorans*. Other species (including *Mtb organophilum/Mtb thiocyanatum*, *Mtb aquaticum/Mtb platani*, *Mtb rhodinum*, *Mtb aerolatum*, and *Mtb iners*) fell into very divergent clades on both trees. For example, using BLASTN, *Mtb oryzae*, *Mtb fujisawaense*, and *Mtb phyllosphaerae* showed 99.0–99.8 % 16S rRNA sequence identity and 100 % identity of their *mxaF* genes, while *Mtb aquaticum* and *Mtb platani* showed 98 % 16S rRNA sequence identity but only 89 % *mxaF* identity, and *Mtb podarium* and

Table 15.2

Properties of the sequenced genomes of several strains of *Methylobacterium*. Data were retrieved from the NCBI and JGI genome databases

Strain analyzed	Genome size (Mb)	GC%	Genes detected (total)	Protein-coding genes	rRNA genes	Pseudogenes
Mtb extorquens AM1						
Chromosome	5.51	68.7	5,071	4,953	15	38
Megaplasmid	1.26	67.6	1,177	1,162	–[a]	9
Plasmid 1	0.044	67.9	33	33	–	–
Plasmid 2	0.038	65.2	34	34	–	–
Plasmid 3	0.025	66.9	30	30	–	–
Mtb extorquens DM4						
Chromosome	5.94	68.1	5,842	5,594	15	170
Plasmid 1	0.142	65.3	105	105	–	32
Plasmid 2	0.039	63.7	41	37	–	4
Mtb extorquens CM4						
Chromosome	5.78	68.2	5,463	5,173	15	211
Plasmid 1	0.380	66.3	351	306	–	45
Plasmid 2	0.023	63.9	39	37	–	2
Mtb nodulans						
Chromosome	7.77	68.9	7,765	7,355	21	316
Large plasmid 1	0.488	65.9	506	402	–	102
Large plasmid 2	0.458	65.7	482	409	–	173
Plasmid 3	0.040	64.2	60	53	–	7
Plasmid 4	0.038	61.6	49	48	–	1
Plasmid 5	0.020	61.4	23	21	–	2
Plasmid 6	0.013	60.5	13	13	–	–
Plasmid 7	0.010	67.2	8	7	–	1
Mtb extorquens PA1						
Chromosome	5.47	68.2	4,956	4,829	15	52
Mtb populi						
Chromosome	5.80	69.4	5,492	5,314	15	102
Plasmid 1	0.025	64.9	27	25	–	2
Plasmid 2	0.023	66.8	27	26	–	1
Mtb radiotolerans						
Chromosome	6.08	71.5	5,839	5,686	13	82
Large plasmid 1	0.586	69.6	536	507	6	22
Plasmid 2	0.047	62.6	52	52	–	–
Plasmid 3	0.043	63.2	46	43	–	2
Plasmid 4	0.038	63.7	42	41	–	–
Plasmid 5	0.036	62.0	37	31	–6	
Plasmid 6	0.028	61.0	25	23	–	1
Plasmid 7	0.022	61.1	22	21	–	1
Plasmid 8	0.021	65.1	27	27	–	–
Mtb strain 4–46						
Chromosome	7.66	71.6	7,047	6,609	18	355
Plasmid 1	0.058	65.1	77	62	–	15
Plasmid 2	0.020	59.2	21	21	–	–
Mtb strain WSM 2598						
Chromosome	7.66	71.2	7,230	7,117	18	(–)[b]
Mtb strain GFX4						
Chromosome	6.12	69.6[c]	5,976	5,923	3	4

[a]No gene or pseudogene present

[b]No pseudogenes recorded separately on the JGI database

[c]Value taken from Gan et al. (2012)

Mtb aerolatum showed 100 % *mxaF* identity but only 94 % 16S rRNA identity (authors' data).

Comparing *mxaF* genes and gene products can be an alternative taxonomic tool, especially when comparing identities and properties of *Methylobacterium* strains isolated from diverse habitats (e.g., different plant species), but is not an exclusive alternative to comparison of phylogeny based on 16S rRNA genes. The congruence of 16S rRNA and *mxaF* genes in some species is indicative of a shared common history, while the divergence seen with other species is most likely due to later acquisition of the *mxaF* genes by horizontal gene transfer. The most extreme diversity is seen with the *mxaF* genes of *Mtb variabile* (EU194913) and *Mtb isbiliense* (EU194912), which show very low similarity with that of *Mtb extorquens* (69.9 % and 68.6 %, respectively), and very low or no similarity to some other *Methylobacterium* species. The *mxaF* of *Mtb variabile* shows only 69.5–71.9 % identity to those of several *Methylobacterium* species (*Mtb cerastii*, *Mtb salsuginis*, *Mtb mesophilicum*, *Mtb radiotolerans*, *Mtb organophilum*, *Mtb suomiense*, and *Mtb populi*), and 78.2 % to that of *Mtb isbiliense* (EU194912), showing the genes from *Mtb variabile* and *Mtb isbiliense* were not significantly related. The *mxaF* sequence of *Mtb variabile* does show 98.4 % identity to those of methylotrophic strains of *Mucilaginibacter* (EU912494), *Methylobacillus* (EU194909), and *Pedobacter* (EU194901), and 96.8 % to that of *Burkholderia vietnamensis* (EU194908), but 81 % identity to those of *Flavisolibacter* (EU912489) and *Pantoea* (EU439310). The *Mtb isbiliense mxaF* shows relatively low identity to any other database sequences, for example, 89.6 % to *Methylophilus* (EU19404), and only 82.8–83.5 % to *Flavisolibacter*, *Pantoea*, and *Pseudomonas* strain CBMB18 (EU439305). This extreme diversity suggests either a very different, and independent, acquisition history of the *mxaF* genes of *Mtb variabile* and *Mtb isbiliense* compared to the other *Methylobacterium* species, or raises questions about the taxonomic status of these two species.

Maldi-TOF/MS spectra were reported for some *Methylobacterium* species and provided a means of distinguishing *Mtb oxalidis* from *Mtb iners* (Tani et al. 2012a). This technique is the most recent and very promising method for distinguishing species and lineages of *Methylobacterium* strains: Tani et al. (2012d) applied whole-cell matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis (WC-MS) to assess the diversity of about 200 *Methylobacterium* type species and newly isolated strains. WC-MS spectral peaks for *Mtb extorquens* AM1 cells were due mainly to ribosomal proteins, and showed many identical proteins in *Mtb oryzae*, *Mtb phyllosphaerae*, *Mtb extorquens*, *Mtb chloromethanicum*, and *Mtb dichloromethanicum*, comprising the "*Mtb extorquens* group." Some of these proteins were shared with those of *Mtb populi* and *Mtb radiotolerans*. Strains of 37 named species were compared in this way, but no single ribosomal protein was found that was common to all the species analyzed, although the method provided a threshold similarity value for species-level discrimination, including some type strains that could not easily be discriminated solely by their 16S rRNA gene sequence similarity. Notably, *Mtb nodulans* and *Methylobacterium* sp. strain 4–46 shared some proteins, but did not share any with the *Mtb extorquens* group. Evaluating WC-MS data from 191 isolates from various plants showed that 16S rRNA gene sequencing mainly matched the identification by WC-MS, with the possibility that some isolates with unique spectra may be novel species. Strains of *Mtb extorquens*, *Mtb adhaesivum*, *Mtb marchantiae*, *Mtb komagatae*, *Mtb brachiatum*, and *Mtb radiotolerans* (and "novel lineages" related to *Mtb adhaesivum*) were the most frequent phyllosphere colonists. The WC-MS technique could thus assist identification of novel species without the requirement for 16S rRNA gene sequencing (Tani et al. 2012d). It should be recognized, however, that although the spectral differences reflect the diversity of *Methylobacterium* species, they do not necessarily indicate their evolutionary relationships, so MALDI-TOF MS cannot be used definitively for phylogenetic analyses. A joint international project is in progress to carry out MALDI-TOF analyses of a range of *Methylobacterium* species, with an emphasis on the rhizobial strains (J. K. Ardley, personal communication, 2012).

Phenotypic Analyses

Common properties of *Methylobacterium* species. The species are typically Gram-negative short rods, 0.8–1.5 × 1.0–8.0 μm, occurring singly, in pairs or aggregates, and occasionally rosettes. Rosette formation ("Sternbildung") was probably first observed for a *Methylobacterium* in the species now known as *Mtb rhodinum* (Heumann 1962).

Many species are motile (*Table 15.1*) by means of a single polar, subpolar, or lateral flagellum. Colonies of nearly all species, on methanol-agar or richer media, range from pale pink to bright orange-red or red in color, growing to 1–4 mm in diameter in 3–7 days. Methanol is reported in their original descriptions to be used for growth by all validly named species, although some species have subsequently proved capable only of poor (or no) growth on methanol, or to require a vitamin supplement for growth. These include *Mtb isbiliense*, *Mtb variabile*, *Mtb platani*, and *Mtb aquaticum* (J. K. Ardley and P. N. Green, personal communications, 2012). Many species grow on other C_1-compounds (those with no carbon-carbon bonds), including mono-, di-, and trimethylamines; formaldehyde (at 1 mM or lower); formate; chloro- and dichloromethane; and methylated sulfur compounds such as dimethylsulfide, dimethylsulfone, and methanesulfonate. Most species grow optimally around neutral pH, and rapidly between pH 6 and 9, but growth of some has been observed at the extremes of pH 4.0 and pH 10. The temperature range for most species is 15–37 °C, with an optimum between 25 °C and 30 °C, but *Mtb cerastii* has an optimum of 20 °C (Wellner et al. 2012) and some have an optimum of 37 °C, with slow growth at 4 °C and 43 °C, but not at 55 °C. Numerous organic substrates can serve for heterotrophic growth, but many species do not use glucose or some other sugars and some amino acids.

No requirement for NaCl has been shown, but most species tolerate NaCl at concentrations approaching 1 % (w/v); few will tolerate concentrations higher than 1.5 % (w/v). An exception is *Mtb podarium* (Anesti et al. 2004) which tolerates 2.5 % (w/v) NaCl. Interestingly, *Mtb salsuginis* was isolated as apparently the only example of a *Methylobacterium* from seawater (Wang et al. 2007), but it could not grow at NaCl concentrations of 1.5 % (w/v) or above. This suggests that it was not a true marine organism as seawater contains 2.8 % (w/v) NaCl (=480 mM), while *Mtb salsuginis* tolerated less than 260 mM NaCl.

Pink pigmentation. This is a characteristic of most *Methylobacterium* species and is due to water-insoluble pink to red-colored carotenoid pigments. A study of three (non-type) strains of *Mtb extorquens*, *Mtb fujisawaense*, and *Mtb mesophilicum* showed them to contain 0.23–0.45 mg carotenoids (mg dry wt)$^{-1}$ (Konovalova et al. 2006, 2007). Thin-layer and liquid chromatography and spectral analysis showed the principal carotenoids (tetraterpenoids) to be xanthophylls, particularly hydroxycarotenoids, and to include oscillixanthin and 3,4-didehydrolicopene: These accounted for about 80 % of the total cellular carotenoids (Konovalova et al. 2007). In all the species for which pigment identity has been determined, the spectral properties are similar, indicating common pigment chemistry. These include *Mtb extorquens*, *Mtb cerastii*, *Mtb gnaphalii*, *Mtb organophilum*, *Mtb oxalidis*, *Mtb fujisawaense*, *Mtb rhodinum*, *Mtb thiocyanatum*, *Mtb suomiense*, *Mtb lusitanum*, *Mtb aminovorans*, *Mtb phyllosphaerae*, *Mtb oryzae*, *Mtb populi*, and *Mtb marchantiae*, which show absorbance peaks in the ranges 461–473, 490–505, and 520–534 nm. Some also show peaks at 233 and 358–360 nm (*Mtb oryzae*, *Mtb phyllosphaerae*, *Mtb cerastii*) or 390–394 nm (*Mtb populi*, *Mtb oxalidis*). Some variations in these maxima might be due to extraction methods and the solvents used by different workers (80 % and 100 % methanol; chloroform:methanol at 1:1; acetone:methanol at 3:1 and 7:2; hexane; benzene; and carbon disulfide). Some species have apparently not been analyzed in this way, including *Mtb persicinum*, *Mtb komagatae*, *Mtb brachiatum*, *Mtb tardum*, *Mtb gregans*, *Mtb dankookense*, *Mtb iners*, *Mtb aerolatum*, and *Mtb salsuginis*, so the possible taxonomic usefulness of any pigment variation cannot be comprehensively assessed at present (Şahin 2011). Two species (*Mtb nodulans* and *Mtb jeotgali*) are non-pigmented, but this does not necessarily pose a taxonomic problem, as the loss of carotenoid pigment by *Mtb extorquens* can be induced by a single mutation in the gene encoding phytoene dehydrogenase (Van Dien et al. 2003).

Some methylobacteria have also been shown to produce chlorophyll-like pigments, including the demonstration of bacteriochlorophyll/chlorophyll *a*, and chlorophyllide synthase genes in *Mtb extorquens*, *Mtb radiotolerans*, and *Mtb populi* (http://www.ncbi.nlm.nih.gov/gene/6313745#reference-sequences), and a light-harvesting pigment encoded in the genome of *Mtb extorquens*: http://www.ebi.ac.uk/ena/data/view/ACS42880, described as a "Major Facilitator Family (MFS) transporter (Bch2-like)."

Fatty acids and quinones. Cellular fatty acid profiles (fatty acid methyl ester—FAMES—analyses) have been determined for a large number of *Methylobacterium* species to assess the taxonomic usefulness of comparative profiles. Comparing fatty acid data for 17 species showed the major fatty acid in all cases to be C18:1ω7, with lesser amounts of C18:0 and C16:0, with variable amounts of 2-OH and 3-OH hydroxyacids. The range of amounts reported for each of the three acids (as % of total fatty acids) was quite wide: 74.8–93.1 %, 1.2–8.8 %, and 1.5–4.1 %, respectively. Some determinations reported by different authors for the *same* species were, however, also quite diverse; for example, C18:1ω7 has been reported as 83.7–93.1 % for *Mtb organophilum*, 75.1–87.2 % for *Mtb phyllosphaerae*; for C16:0, 0–4.8 % in *Mtb fujisawaense* and 1.5–4.1 % in *Mtb oryzae*. Consequently differences among some minor fatty acids may have taxonomic usefulness, but variation in major acids is of limited value, and clearly requires absolute standardization of analyses. Mean values for the species analyzed to date are C18:1ω7, 89.6 %, C16:0, 4.0 %, and C18:0, 3.2 %, with quite high standard deviations around the means.

All the species analyzed contain ubiquinone Q-10, typically at 95–100 % of total quinones, including *Mtb extorquens*, *Mtb fujisawaense*, *Mtb radiotolerans*, *Mtb mesophilicum*, *Mtb komagatae*, *Mtb brachiatum*, *Mtb tardum*, *Mtb hispanicum*, and *Mtb soli*. The presence of ubiquinone Q-9 in *Mtb gnaphalii* was a feature differentiating it from its closest phylogenetic relatives (Tani et al. 2012b).

Pyrroloquinoline quinone (PQQ, an essential cofactor in the methanol dehydrogenase of *Methylobacterium*) is secreted into the culture medium in variable amounts by some species, for example, 2.3 and 24.6 μg ml^{-1} by *Mtb oxalidis* and *Mtb gnaphalii*, with a mean for 191 type and other strains of 4.6 μg ml^{-1} (Tani et al. 2012a, b, c).

Ecology, Isolation, Properties, Culture Media, and Growth Conditions for *Methylobacterium*

Methylobacterium species are seemingly ubiquitous and have been isolated from soils, dust, the air, fresh- and seawater, the Antarctic, domestic and clinical water supplies, polluted waters, sewage treatment stations, activated sludge, industrial fermentations (e.g., those degrading formaldehyde), high-temperature geological petroleum reservoirs (Orphan et al. 2000), coal fields, aviation fuels (fuel/water interfaces), fermented seafood, earthworms, from the human body (foot, mouth and eye), and as opportunistic pathogens, and from a vast range of plants (Corpe and Rheen 1989; Madhaiyan et al. 2012; Romanovskaya et al. 2009a; Trotsenko et al. 2001). There is no unique pattern determining the habitat from which any particular species may be found, as many occur in multiple locations. Consequently ecological descriptions are based on specific habitats in some of the following sections and concentrate on physiological properties relevant to their ecology, as well as the conditions required for culture of the organisms.

Media for isolation and cultivation. Numerous medium formulations have been used for the isolation, culture, and maintenance of methylotrophs. Some in common use are given below; others may be found in Atlas (2010) and Hanson (1998). Nitrogen can be supplied as either ammonium or

nitrate salts, or as thiocyanate. The pH of media can be varied by using different ratios of acid and basic phosphates (i.e., KH_2PO_4/Na_2HPO_4). The media can be used with methanol, methylamines, or multicarbon substrates. Enrichment from environmental samples can be by inoculation of samples of soil, water, air-filters, or from leaf impressions on agar, or dissected leaf tissues into media with methanol or methylamine. To prevent overgrowth of fungi, especially in the agar used for leaf-impression cultures, addition of cycloheximide (100 mg l^{-1}) has been recommended (Hanson 1998).

DSMZ Medium No. 632: Nitrate Mineral Salts Medium

(http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium632.pdf)

KNO_3	1.0 g
KH_2PO_4	0.272 g
$Na_2HPO_4{\cdot}12H_2O$	0.717 g
$MgSO_4{\cdot}7\ H_2O$	1.0 g
$CaCl_2{\cdot}6H_2O$	0.2 g
Fe(III)NH_4-EDTA	0.004 g
Trace element solution (see below)	0.5 ml
Distilled water	1,000 ml

Adjust to pH 6.8. Autoclave at 121 °C for 15 min. Methanol (1 or 2 ml) is added from a sterile stock after autoclaving, immediately before use. For solidified medium, add agar (12.5 g) before autoclaving.

Trace element solution:

Na_2-EDTA	0.5 g
$FeSO_4{\cdot}7H_2O$	0.2 g
$ZnSO_4{\cdot}7H_2O$	0.01 g
H_3BO_3	0.03 g
$CoCl_2{\cdot}6H_2O$	0.02 g
$MnCl_2{\cdot}4H_2O$	0.003 g
$Na_2MoO_4{\cdot}2H_2O$	0.003 g
$NiCl_26H_2O$	0.002 g
$CaCl_2{\cdot}2H_2O$	0.001 g
Distilled water	1,000 ml

Methylobacterium Medium with Ammonium as Nitrogen Source

NH_4Cl	0.50 g
K_2HPO_4	0.70 g
KH_2PO_4	0.54 g
$MgSO_4{\cdot}7H_2O$	1.0 g
$CaCl_2{\cdot}2H_2O$	0.2 g
$FeSO_4{\cdot}7H_2O$	0.004 g
H_3BO_3	300 μg
$CoCl_2{\cdot}6H_2O$	200 μg
$ZnSO_4{\cdot}7H_2O$	100 μg
$MnCl_2{\cdot}4H_2O$	30 μg
$CuCl_2{\cdot}2H_2O$	10 μg
$NiCl_2{\cdot}6H_2O$	20 μg
$Na_2MoO_4{\cdot}2H_2O$	60 μg
Distilled water	1,000 ml

Adjust to pH 6.8. Add sterile methanol to a concentration of 0.5 % to the sterilized medium. The medium may be solidified with 15 g agar l^{-1}.

Kelly and Wood (1998) Medium

This high phosphate medium was used for the culture of several methylotrophs and the isolation of *Mtb thiocyanatum* and *Mtb podarium* (Kelly and Wood 1998). It contains (g l^{-1}): $Na_2HPO_4{\cdot}2H_2O$ (7.9), KH_2PO_4 (1.5), NH_4Cl (0.8), $MgSO_4{\cdot}7H_2O$ (0.1), trace metal solution (10 ml l^{-1}; Kelly and Wood 1998), with separate autoclaving of the phosphates. This is supplemented with one of (mM): methanol (50), methylamine (20), dimethylamine (10), trimethylamine (10), sodium formate (25), sodium methanesulfonate (15), formaldehyde (0.5), dimethylsulfide (10, but added in 1–1.5 mM aliquots during growth because of its possible toxicity), dimethylsulfone (10), dimethylsulfoxide (10). For heterotrophic growth, the following can tested (note: some species do not use sugars): sucrose (5), glucose (10), sodium succinate (10), or sodium acetate (20). Methanol, DMS, DMSO, and formaldehyde need to be injected into flasks or tubes containing sterile medium, sealed with gas-impermeable vaccine stoppers (to prevent loss of these volatile substrates). For solid media, Oxoid Bacteriological Agar No. 1 at 1.5 % (w/v) is added. For agar-medium cultures, methanol, formaldehyde, DMS, and DMSO need to be supplied on sterile filter paper in the lids of inverted Petri plates (Anesti et al. 2005) or added to the airspace above a slant before sealing the lid. Agar plates with methanol, DMS, DMSO, or formaldehyde should be incubated in gastight containers to prevent loss of the volatile substrates.

Glucose Medium with Thiocyanate as Sole Nitrogen Source

KSCN	0.25 g
$MgSO_4{\cdot}7H_2O$	0.10 g
K_2HPO_4	1.5 g
$Na_2HPO_4{\cdot}2H_2O$	7.9 g

Glucose	4.5 g
2 % $FeSO_4 \cdot 7H_2O$	1 ml
Distilled water	1,000 ml

Glucose can be replaced with 0.2 %, 0.5 %, or 1 % (v/v) methanol.

Heterotrophic Media

The following have been widely used for culture and maintenance. Commercial nutrient agar is not successful with all species.

R2A Medium

This has been used at full strength or in various dilutions (10 % and 50 %) and contains (g) (Reasoner and Geldreich 1985):

Yeast extract	0.5
Difco Proteose Peptone No. 3	0.5
Difco Casamino acids	0.5
Glucose	0.5
Soluble starch	0.5
K_2HPO_4	0.3
Sodium pyruvate	0.3
$MgSO_4 \cdot 7H_2O$	0.05
Agar	15
Distilled water	1,000 ml

Adjust to pH 7.2 by adding K_2HPO_4 or KH_2PO_4, and add agar before autoclaving.

Glycerol Peptone (GP) Agar

This can be used for maintaining stocks of pure cultures and contains (g):

Agar	15
Glycerol	10
Difco Peptone	10
Distilled water	1,000 ml

Adjust to pH 7.0 before autoclaving.

Methylobacterium and Plants

Several species associate with plants (monocotyledons, dicotyledons, ferns, mosses, and liverworts), both as epiphytes on the phylloplane and rhizoplane, and as endophytes in internal tissues. These can have symbiotic or growth-promoting roles, and in some cases induce root nodulation and enable nitrogen fixation. Earlier work on the relationship of methylobacteria and plants focused on temperate and tropical crop plants, where the bacteria promoted seed germination and increased crop yield, resistance to pathogens, and tolerance of drought stress (Irvine et al. 2012; Raja et al. 2008). Recent studies have identified methylobacteria in "wild" higher plants, liverworts, mosses, and ferns. Among the species found as commensals with plants are *Mtb extorquens*, *Mtb mesophilicum*, *Mtb rhodinum*, *Mtb radiotolerans*, *Mtb bullatum*, *Mtb thiocyanatum*, *Mtb populi*, *Mtb suomiense*, *Mtb aminovorans*, *Mtb fujisawaense*, *Mtb gossipiicola*, *Mtb hispanicum*, *Mtb nodulans*, *Mtb oxalidis*, *Mtb marchantiae*, *Mtb zatmanii*, *Mtb oryzae*, *Mtb gnaphalii*, *Mtb cerastii*, and *Mtb longum*. Interestingly, in a study of the epiphytic methylobacterial community of the surfaces of sunflower plants, *Mtb extorquens* comprised 36 % of the community, *Mtb mesophilicum*, 18–32 %, *Mtb radiotolerans*, 3–10 %, but with 22–43 % being unidentified *Methylobacterium* species (Schauer and Kutschera 2008). Similarly, a phylogenetic study of the bacterial community on the rehydrating phylloplane of the Resurrection Fern (*Polypodium polypodioides*) showed numerous isolates of *Methylobacteriaceae*, including some related to *Mtb aquaticum*, *Mtb hispanicum*, and *Mtb radiotolerans*, as well as at least 12 novel isolates (Jackson et al. 2006).

Phyllosphere colonists. *Methylobacterium* species are among the principal members of the bacterial flora of plant surfaces (Delmotte et al. 2009; Knief et al. 2010a, b; Poonguzhali et al 2008; Tani et al. 2012a, b; Vorholt 2012), in some cases accounting for up to 80 % of the bacterial colonists on leaves (Corpe and Rheen 1989). Several studies have reported the predominant species as *Mtb extorquens* (on more than 50 plant species; Corpe 1985; Trotsenko et al. 2001), *Mtb bullatum* strain F3.2, *Mtb mesophilicum*, and *Mtb rhodinum* (Irvine et al. 2012). A study of plants in the Ukraine showed phyllosphere methylobacteria in more than 200 species of agricultural, decorative, medicinal, and wild plants (Romanovskaya et al. 1996; Trotsenko et al. 2001). Phyllosphere methylobacteria exhibit properties valuable for survival in the potentially extreme conditions that leaves can be subjected to, showing resistance to dehydration, UV and ionizing radiation, and high temperature (Romanovskaya et al. 1998; Trotsenko et al. 2001). For example, *Mtb extorquens* and *Mtb mesophilicum* (typically the most abundant epiphytes) tolerated UV radiation in excess of 250 J m^{-2}; their LD_{90} and $LD_{99.9}$ doses for γ-radiation were 2.0–3.4 and 4.4–6.0 kilogray (J g^{-1}), respectively (Romanovskaya et al. 2002, 2009b). Leaf temperatures in summer can exceed 30 °C even in temperate climates, and can exceed 40 °C in tropical and arid regions, but methylobacteria can survive these conditions. An extreme example of their temperature resilience is seen in the method employed to prepare a soil sample for isolation of *Mtb soli* (Cao et al. 2011): The soil sample was dried at room temperature for 7 days, then heated for 1 h at 80 °C, after which the dry soil was suspended in sterile distilled water, and aliquots spread on agar medium, resulting in the successful isolation of *Mtb soli*.

Studies by Knief et al. (2008, 2010a, b, 2012) on *Arabidopsis thaliana*, and 25 other plant genera, have shown that

colonization efficiency by different *Methylobacterium* species was linked to their phylogeny, rather than to the geographical origin or the plant genus being colonized, with *Mtb extorquens* and *Mtb tardum* being the most aggressive phyllosphere colonists. Dourado et al. (2012a) reported a large study of 120 DNA sequences of partial 16S rRNA and *mxaF* gene sequences from 60 endophytic *Methylobacterium* strains (including 23 strains identified as *Mtb extorquens*, *Mtb fujisawaense*, *Mtb hispanicum*, *Mtb mesophilicum*, *Mtb populi*, and *Mtb radiotolerans*) isolated from six plant species: *Borreria* (12 strains), *Capsicum* (7 strains), *Citrus* (18 strains), *Coffea* (8 strains), a *Eucalyptus* hybrid (7 strains), and *Saccharum* (8 strains). Some *Methylobacterium* strains were "generalists" able to colonize more than one host plant (e.g., for named strains: *Mtb fujisawaense* from *Saccharum*, *Citrus*, and *Capsicum*). Other strains were exclusive to only one plant species. Comparing 16S rRNA and *mxaF* genes from these revealed seven phylogenetic groups for each of 16S rRNA genes and *mxaF* genes, and showed that congruence between host and colonist phylogeny was higher in the *mxaF* gene groups than for the 16S rRNA gene sequences. Some *mxaF* groups showed significant specificity for particular plant species: Thus *mxaF* groups IV and V were only with *Citrus*, and group VI mainly with *Borreria*, while *mxaF* group VII contained isolates from five of the six plant species. Dourado et al. (2012a) concluded that such "niche specialization" results from a function-based selection whereby the plant species interact with potential *Methylobacterium* colonists to select strains most suited to endophytic colonization. Discrimination among different endophytic species has relied mainly on identification by standard phylogenetic analyses, but Lacava et al. (2006) described a quantitative PCR-based method specific for detection and monitoring of the development in *Citrus* plants of endophytic *Mtb mesophilicum*. Primers for the intergenic region of the ribosomal operon of *Mtb mesophilicum* produced a 390 bp amplicon (sensitive to around 130 bacterial cells per assay) with *Mtb mesophilicum*, but did not react with *Mtb extorquens*, *Mtb radiotolerans*, *Mtb zatmanii*, or *Mtb fujisawaense*. Such an assay for other species would need to be equally specific.

Symbiosis and growth-promoting secretions from *Methylobacterium*. Green (2006) commented that while extra- and/or intracellular symbiotic or mutualistic associations apparently existed between plants and some strains of *Methylobacterium*, there was no further evidence at that time to support the theory of a symbiotic association. Corpe and Basile (1982) had suggested that the PPFM bacteria present on mosses and liverworts might produce growth-stimulatory substances for those plants. Since the completion of Green's review, the nature of some of the symbioses has become better understood. Examples of stimulated germination, growth, and yield by methylobacteria are common (Holland 1997; Madhaiyan et al. 2007; Mattilä 2001; Pirttilä et al. 2000; Trotsenko et al. 2001), as are examples of the deleterious effect of the *absence* of methylobacteria from seed or plant surfaces. For example, heating soya bean seeds reduced the population of symbiotic bacteria by up to 97 % and lowered subsequent plant biomass production by 75 %. This effect was not due to damage to the seeds, as the effects were prevented by inoculation, after heating, with methylobacteria, which increased germination rates and biomass production over those of control seeds (Trotsenko et al. 2001). Spraying corn, spinach, beet, and soya bean plants with methanol solutions augmented yield, an effect attributed to the doubling of methylobacteria numbers following spraying (Trotsenko et al. 2001). This artificial stimulation by exogenous methanol parallels the demonstration by Sy et al. (2006) that a competitive advantage in phyllosphere colonization of *Medicago trunculata* by *Mtb extorquens* was provided by the methanol excreted by the plant, as wild-type bacteria were more effective colonizers than mutants defective in methanol oxidation. Seedlings of *Ginkgo biloba* (the maiden hair tree; *Ginkgophyta*) grew more rapidly in the presence of methylobacteria compared to an aseptic control, suggesting the epiphytic *Methylobacterium* might provide stimulatory phytohormones (Hellmuth and Kutschera 2008; Kutschera and Niklas 2009). In a study of methylobacteria on strawberry leaves (*Fragaria* × *ananassa* cv. Elsanta), Abanda-Nkpwatt et al. (2006) isolated strains of *Mtb extorquens* and *Methylobacterium* sp. strain ME4, all of which promoted the growth of various plants: Seedling weight and shoot length of *Nicotiana tabacum*, *Lycopersicon esculentum*, *Sinapis alba*, and *Fragaria vesca* were all increased by the methylotrophs. Bacterial culture supernates also stimulated germination, suggesting the production of a growth-promoting agent. Bacterial growth was correlated with methanol production by the plants and consequent growth promotion: The output of methanol from *Nicotiana tabacum* seedlings, without methylobacteria, was up to 0.7 ppbv, but was decreased to 0.005–0.01 ppbv when the seedlings were cocultivated with *Mtb extorquens* ME4, showing consumption of the methanol by the bacteria. This indicated a truly symbiotic "cross-feeding" between plant and bacteria.

Direct evidence for the production and secretion of specific hormones by *Methylobacterium* species includes the secretion of zeatin (cytokinin) by *Mtb mesophilicum* (Ivanova et al. 2000; Koenig et al. 2002; Lee et al. 2006), and in the cells of methylotrophs isolated from *Arabidopsis*, maize, barley, and soya beans (Trotsenko et al. 2001). *Trans*-zeatin secretion and enhancement of wheat seed germination by the culture filtrates from two new *Methylobacterium* isolates was observed by Meena et al. (2012), who showed that four new isolates and *Mtb extorquens* all secreted *trans*-zeatin, which they suggested resulted from tRNA turnover, rather than from de novo synthesis.

Other work has shown that the auxin, indole-3-acetic acid (IAA), which has roles in promoting cell division, expansion, differentiation, root formation, leaf fall, and fruit ripening in plants is produced by methylobacteria, including *Mtb mesophilicum* (Doronina and Trotsenko 2002; Hornschuh et al. 2006; Ivanova et al. 2001; Omer et al. 2004). Also, the presence of the *IpdC* gene encoding indolepyruvate decarboxylase in *Methylobacterium extorquens* AM1 (Fedorov et al. 2010) indicated the presence of an IAA-biosynthetic pathway. Knockout of

the *IpdC* gene decreased IAA secretion by more than 50 %, while complementing the mutation increased IAA secretion into the culture medium 6.3-fold compared to the mutant. Radha (2007) studied numerous plant-associated methylobacteria, which secreted soya-bean-growth-promoting compounds into the medium: IAA up to 28 μg (ml culture filtrate)$^{-1}$, cytokinin (zeatin) up to 8 μg ml^{-1}, and gibberellic acid up to 70 μg ml^{-1}. Tani et al. (2012c) in a survey of 191 type and non-type *Methylobacterium* strains found their average auxin production to be 1.28 μg ml^{-1}.

The availability of the complete genome sequences of several *Methylobacterium* species has provided molecular evidence for auxin secretion by the bacteria: For example, the proteosomes of *Methylobacterium* sp. strain 4–46 (http://www.ncbi.nlm.nih.gov/nuccore/NC_010511.1), *Mtb populi* strain BJ001 (NCIMB 13946), *Mtb extorquens* strains PA1, AM1, DSM 13060, and CM4, *Mtb radiotolerans*, and *Mtb nodulans* ORS 2060 (LMG 21967) all contain sequences predicted to encode membrane-integrated auxin-efflux-carrier proteins of 309–322 amino acids. The predicted proteins for the four *Mtb extorquens* strains and *Mtb populi* show 96–100 % amino acid sequence identity to each other, but only 27–33 % identity to those of *Mtb nodulans*, *Mtb radiotolerans*, and *Methylobacterium* strain 4–46 (determined by the authors, using the BLASTP algorithm from the NCBI database). The predicted protein sequence for *Mtb radiotolerans* shows 44 % identity to that for strain 4–46, but only around 30 % to the other species. There is obviously species-related diversity among the efflux proteins, possibly indicative of acquisition of the encoding genes from diverse sources by horizontal gene transfer. It is interesting to note that some plants (including *Arabidopsis*) secrete chloromethane (Anderson et al. 2010; Nadalig et al. 2011), which can be a substrate for *Mtb extorquens* strain CM4, a property not shared with *Mtb extorquens* strain AM1 or with the dichloromethane-using strain DM4 of *Mtb extorquens*.

Stimulation of liverworts and mosses by methylobacteria is a well-established phenomenon (Hornschuh et al. 2002, 2006; Kutschera and Koopmann 2005; Schauer and Kutschera 2011). Thallus growth of liverworts (*Marchantiophyta*, bryophytes) and moss development show symbiotic stimulation by the phytohormones (cytokinins, auxins) secreted by the methylobacteria (Hornschuh et al. 2002; Kutschera 2007). Growth of the moss *Racomitrium* is stimulated by a methanol-mediated symbiosis with several strains of *Methylobacterium*, phylogenetically most similar to *Mtb extorquens*, *Mtb aquaticum*, *Mtb oryzae*, *Mtb tardum*, and *Mtb zatmanii* (Tani et al. 2011, 2012a, b, c). This mutualism has been exploited practically in the use of *Racomitrium* to create roof-coverings of the moss to reduce roof and building temperatures, save energy (e.g., air-conditioning) in hot latitudes, and in urban storm-water management (Anderson et al. 2010; Tani et al. 2012c). *Racomitrium* is a primary plant colonizer in hostile environments, resistant to extreme desiccation and very sunny conditions, commonly known as the "woolly moss" and "woolly fringe-moss" found abundantly in the natural environment (Tani et al. 2012c; Woolly moss Iceland: http://icelandairhotels.com/sites/default/files/imagefield_thumbs/IH_natturu_31x42_230511_LQ.pdf; Woolly fringe-moss on a dry stone wall, Scotland: http://icelandairhotels.com/sites/default/files/imagefield_thumbs/IH_natturu_31x42_230511_LQ.pdf).

While novel species, some as yet unnamed, of *Methylobacterium* are present on/in the phylloplane, rhizosphere, and tissues of numerous plants, a novel species has been well characterized from a "lower plant," the moss *Funaria*. *Methylobacterium* sp. strain F3.2 was isolated and described as an epiphytic growth-promoter of protonemal filaments of the moss *Funaria hygrometrica* (Hornschuh et al. 2002; Schauer and Kutschera 2011). Strain F3.2 was separately given new species names by two separate groups, as *Mtb funariae* (Schauer and Kutschera 2011) and as *Mtb bullatum* (Hoppe et al. 2011). *Mtb bullatum* was, however, the name validated for strain F3.2 (http://www.bacterio.cict.fr/m/methylobacterium.html), with *Mtb funariae* as a synonym. Identical 16S rRNA gene sequences are thus available in GenBank as FJ268657 (*Mtb bullatum*; 1,448 bp; Hoppe et al. 2011) and FJ157975 (*Mtb funariae*; 1,421 bp; Schauer and Kutschera 2011). Phylogenetically, *Mtb bullatum* is closely related to *Mtb marchantiae* (Schauer and Kutschera 2011), as their 16S rRNA gene sequences are 99.3 % identical; its next nearest phylogenetic neighbor is *Mtb adhaesivum* (at only 97.9 % sequence identity). Interestingly, *Mtb marchantiae* was isolated as a growth-promoter of another "living fossil" plant, the liverwort *Marchantia polymorpha* (Kutschera and Koopmann 2005; Schauer et al. 2011).

Strains highly similar to *Mtb bullatum* strain F3.2 have notably also been found on the phyllosphere and root zones of higher plants (Irvine et al. 2012; Schauer and Kutschera 2008), including *Methylobacterium* strain 5a.1.5 from sunflower, which showed only one nucleotide difference in its 16S rRNA gene sequence (FJ157965) from that of the type strain of *Mtb bullatum*.

The phytosymbiotic association between methylobacteria and mosses and liverworts is likely to be an extremely ancient one (300–400 Ma BCE), of considerable evolutionary significance (Hornschuh et al. 2002, 2006; Kutschera, 2006; Kutschera and Koopmann 2005; Schauer and Kutschera 2011), and probably significant in the establishment of commensal associations of methylobacteria with higher plants. Tani et al. (2012d) have shown that 18 out of 57 new isolates from bryophytes appeared, from WC-MS analysis, to be new lineages within the *Methylobacterium* genus, consistent with a very long-standing relationship between this bacterial group and the bryophytes.

Endophytic colonization and nodulation of plant roots and leaves. The earliest isolation of a nodule-inducing methylotroph from a plant was of *Mycoplana rubra* (now *Mtb extorquens* strain RP4, NCIMB 10409) from surface-sterilized leaf nodules of the Zimbabwean evergreen tree, *Psychotria mucronata* (*Rubiaceae*; now *P. kirkii*), by M. E. Rhodes-Roberts (cited by Green 2006; Miller et al. 1983). Several reports have demonstrated that *Methylobacterium* species could be isolated from root nodules of some leguminous plants (Jaftha et al. 2002; Sy et al. 2001a, b), and a study of a homogeneous group of 72

isolates of non-pigmented *Methylobacterium* from three species of *Crotalaria* resulted in the description of the novel species, *Mtb nodulans* from *C. podocarpa* (Dakar, Senegal; Jourand et al. 2004; Sy et al. 2001b). This species contains the nodulation gene *nodA*, the *nifH* gene, encoding nitrogen fixation, and *mxaF* for a methanol dehydrogenase subunit. It is able to grow on methanol, formaldehyde, and formate, and promotes nodulation and nitrogen fixation in *Crotalaria* (Jourand et al. 2004, 2005; Renier et al. 2011). In comparison, *Mtb extorquens*, *Mtb organophilum*, *Mtb radiotolerans*, *Mtb rhodinum*, *Mtb mesophilicum*, *Mtb rhodesianum*, and *Mtb zatmanii* were unable to induce root nodule formation in legumes (Sy et al. 2001b). In addition, eight nodulating bacteria were isolated from *Lotononis* species and were called *Rhizobium* or *Methylobacterium* strains xct7-xct17 (Jaftha et al. 2002). Ardley et al. (2009) assessed 11 pink-pigmented *Methylobacterium* strains from the Western Australian Soil Microbiology (WSM) collection (Murdoch University, Western Australia; Yates et al. 2007), as well as several reference strains. Ten of these were from *Lotononis* root nodules, including two (WSM 2598 and 3035) from *L. bainesii*, six from *L. listii*, and two from *L. solitudinis*. In addition, *Methylobacterium* sp. strain 4–46 was apparently effective in fixing nitrogen in association with nodules of *L. bainesii* (J. K. Ardley, personal communication, 2012). None of the WSM strains could grow on glucose (Ardley et al. 2009).

The availability of the fully sequenced genomes of *Mtb nodulans*, *Methylobacterium* strain 4–46, and *Methylobacterium* WSM 2598 enabled comparison of their *nodA* nodulation genes and the encoded NodA proteins. Comparative sequence analysis (by the authors) of the predicted NodA amino acid sequences (using pairwise BLASTP) of *Mtb nodulans* (http://www.ebi.ac.uk/ena/data/view/CAN84682), *Methylobacterium* 4–46 (http://www.ebi.ac.uk/ena/data/view/ACA18208), and *Methylobacterium* WSM 2598 (http://www.ebi.ac.uk/ena/data/view/AEQ76835) showed them all to be 214–220 amino acids in length. The highest sequence identity (99 %) was between strains 4–46 and WSM 2598, but the strain 4–46 and WSM sequences showed only 80–81 % identity to that of *Mtb nodulans*. This illustrates that there are host-plant determinants in the nodulation mechanisms that are specific for each bacterial strain in the case of *Lotononis*, and indicates that the WSM strains are apparently distinct from *Mtb nodulans* and remote from most other *Methylobacterium* species by 16S rRNA phylogeny. A further striking similarity between strains 4–46 and WSM2598 is their almost identical genome size, compared to most other *Methylobacterium* species (◗ *Table 15.2*). Their chromosomes are both 7.66 Mb, compared to an average of 5.8 Mb for most of the species in ◗ *Table 15.2*, and to 7.77 Mb for *Mtb nodulans*. Their total genome sizes (including plasmids) are 7.74 and 7.66 Mb, compared to 8.84 for *Mtb nodulans*, and an average of 6.34 Mb for the other species (◗ *Table 15.2*). While *Mtb nodulans* clearly differs from all the other species, it seems a characteristic of these three legume-nodulating strains to have considerably larger genomes than the other species.

Phylogenetic relationship of the nodulating strains and *Methylobacterium*. As discussed above, numerous strains can induce root nodule formation: *Mtb nodulans* (isolated from *Crotalaria*), *Methylobacterium* sp. strain 4–46, 17 strains from *Lotononis* species, including nine *Rhizobium* strains: xct7, 8, 9, 10, 12, 13, 14, 16, and CB376 and eight WSM strains: WSM 2598, 2693, 2799, 3032, 3950, 3960, 3962, and 3966. Strain CB376 was isolated from *Listia bainesii* root nodules in Kenya in 1954 by J. P. Botha (Ardley et al. 2009; Yates et al. 2007). It is the current Australian commercial inoculant for *L. bainesii* and is synonymous with strain xct9, also isolated from *L. bainesii* in South Africa (Jaftha et al. 2002; personal communication from Ian Law, ARC Plant Protection Research Institute, Pretoria, to J. K. Ardley). Most of the listed strains are pink-pigmented, except *Mtb nodulans* which is pigmentless. Strain CB376 has a GC content of 68.9 % (Godfrey 1972), in the range for other *Methylobacterium* species. Its carotenoid pigments have been thoroughly studied (Godfrey 1972; Kleinig and Broughton 1982) and shown to be identical to those of the strain of *Pseudomonas rhodos* which is now the type strain of *Mtb rhodinum* (Kleinig and Broughton 1982). Of particular interest is that its carotenoid biosynthesis was suppressed when inoculated into *L. bainesii* (Godfrey 1972), compared to *Mtb nodulans* which always appears pigmentless. The original source of *Methylobacterium* sp. strain 4–46 is uncertain. It was believed to be one of four PPFM strains originally isolated from soil in Bayreuth, Germany, by Heinz Stolp (Heumann et al. 1984), and variously coded Stolp 37 and PPFM 37 (Wolfrum et al. 1986), before being redesignated by Wolfram Heumann as strain 4–46 (D. Nayak, personal communication, 2012). Strain 4–46 was later reported as being synonymous with the South African strains CB376 and xct9 (Fleischman and Kramer 1998). It seems possible that all three strains might have been independent isolations of the same organism, or that strain 4–46 was actually strain CB376 and not Stolp's PPFM 37 (J. K. Ardley, personal communication, 2012). The latter view is supported by the observation that the original strain PPFM 37 grows on methanol, but strain 4–46 does not (P. N. Green, personal communication, 2012); strain 4–46 induced nodulation in *Listia bainesii* and could be re-isolated from those nodules (J. K. Ardley, personal communication, 2012). Strain 4–46 is described on its NCBI genome web page as a "methanol-utilizing bacterium" (http://www.ncbi.nlm.nih.gov/genome/13594?project_id=58843), but this is not confirmed by the available published literature. The medium used for its original isolation and culture contained methanol with 0.05 % (w/w) yeast extract, but it was subsequently found unable to grow on methanol or methylamine alone (C. Marx and S. Vuilleumier, personal communications, 2012). The xct strains of Jaftha et al. (2002) were described as able to grow on 1 % (v/v) methanol, but this could not be confirmed for strain xct9, or for the ten WSM strains, which were all unable to oxidize or grow on methanol, and lacked the typical *mxaF* gene for the small subunit of methanol dehydrogenase (Ardley et al. 2009). BLASTN and TBLASTP of the strain 4–46 genome detected an *mxaF*-like gene encoding a "methanol-ethanol family PQQ-dependent dehydrogenase." This showed only 74 % nucleotide and 53 % amino acid sequence identity to the *mxaF* and MxaF of

Mtb extorquens strain AM1 (our data), but showed 94 % nucleotide identity to the *xoxF* gene of strain AM1. The strain 4–46 genome also contains two further *xoxF* homologues. This is intriguing as the *xoxF1* and *xoxF2* homologues are jointly essential for the expression of methanol dehydrogenase activity (Skovran et al. 2011), whereas the complete genomes of strains 4–46 and WSM 2598 lack an *mxa* operon. This operon is carried in the genome of most *Methylobacterium* species: An exception is *Mtb nodulans* in which the *mxa* operon is carried on its PMNOD02 plasmid, rather than the chromosome (Marx et al. 2012; J. K. Ardley, personal communication, 2012).

The closest named phylogenetic relatives of *Methylobacterium* strain 4–46 are *Mtb isbiliense* and *Mtb nodulans* (at 97.9 and 97.6 % 16S rRNA gene sequence identities), but it shows only 96.4 % and 95.8 % identity to *Mtb variabile*, and *Mtb rhodinum* (our BLAST determinations). Strain 4–46, xct strains 7–10, 13, 14, and 16, and WSM strains 2598, 2693, 2799, 3032, 3950, 3960, 3962, and 3966 share 97–100 % 16S rRNA gene sequence identity with each other, but only 95–97.6 % to *Mtb nodulans* and *Mtb isbiliense*, and only 92–96 % identity to all other current *Methylobacterium* species (authors' data; Ardley et al. 2009; Jaftha et al. 2002; Yates et al. 2007). The phylogenetic homogeneity of these strains suggests they could be strains of a single (novel) species, or might be closely related species of a novel genus. This possibility would need further clarification of the failure of the xct and WSM strains, and strain 4–46, to grow on methanol or methylamine. Proof of failure of these strains (and some type species) to grow on methanol (or other methylated C_1-compounds) inevitably suggests that these strains should perhaps not be classified as *Methylobacterium*, as a tenet of the genus definition is "methylotrophy." It is conjectural to propose that this clade, albeit closely related to *Methylobacterium*, arose by loss of the ability for methylotrophy: By analogy with *Mtb nodulans*, if the *mxa* operon of their ancestral form was plasmid-borne, loss of the plasmid could produce the phenotype exhibited by these strains. This also possibly makes *Mtb nodulans* a descendent of the precursor to these strains, retaining the plasmid-borne *mxa* operon, but having lost the characteristic pink pigmentation of most *Methylobacterium* species. A number of anomalies are clearly in need of resolution.

Methylobacterium and Animals

Methylobacterium species appear to be normal, stable rather than transient, commensals with humans as part of the foot and mouth microbiota (Anesti et al. 2004, 2005; Hung et al. 2011, 2012). Their occurrence in the earthworm gut and as human opportunistic pathogens appear to be transient or opportunistic events, the former probably from earthworm consumption of plant débris. There are several examples of apparent symbioses between *Methylobacterium* strains and insects. One is of an endosymbiotic strain of *Methylobacterium* from the small parasitic wasp *Tamarixia rediata* (which is a parasitoid of the plant pathogen *Diaphorina citri*; Meyer and Hoy 2008). This strain showed 100 % 16S rRNA gene sequence identity to *Mtb lusitanum* (NCIMB 13379) and 99.6 % identity to *Mtb rhodesianum* (NCIMB 12249). A second example is from *Tetraponera binghami*, ants which live only in nest cavities in the hollow internodes of bamboo. These cavities contain numerous *Kermicus wroughtoni* pseudococcids, which provide the ants with amino acid-deficient honeydew. The ants are believed to obtain amino acids produced by bacteria in the dense aggregations of bacterial symbionts located in a pouch-shaped organ located at the junction of the midgut and intestine of the ants (Van Borm et al. 2002). Among these bacteria is a strain provisionally identified as a *Methylobacterium* by Van Borm et al. (2002). The 16S rRNA gene sequence of this organism (AF459799) shows about 96 % identity to each of *Mtb oryzae*, *Mtb radiotolerans*, and *Mtb fujisawaense* (authors' data). An area needing further study is clearly to determine how widespread insect/invertebrate/*Methylobacterium* symbioses may be.

***Methylobacterium* species as human pathogens.** *Methylobacterium* strains have been increasingly reported as infectious agents in humans (Borsali et al. 2011; Holton et al. 1990; Lambert et al. 1983; Sanders et al. 2000; Truant et al. 1998), with 30 clinical cases reviewed by Sanders et al. (2000). Clinically recovered *Methylobacterium* strains are typically regarded as opportunistic pathogens in immunocompromised patients, such as those on chemotherapy (Engler and Norton 2001; Sanders et al. 2000), and with immunosuppression due to alcoholism, renal failure, diabetes, tuberculosis, or (uncommonly) from patients with no previous underlying clinical condition (Gilardi and Faur 1984; Lai et al. 2011; Lambert et al. 1983; Liu et al. 1997; Strazzi et al. 1992; Truant et al. 1998). Symptoms due to *Methylobacterium* infections range from bacteremia, fever, sepsis, empyema, pneumonia, peritonitis, to eye or urinary tract infection (Borsali et al. 2011; Brown et al. 1996; Fernandez et al. 1997; Furuhata et al. 2006; Hiraishi et al. 1995; Lee et al. 2004; Sanders et al. 2000).

Methylobacterium species have been isolated from blood, lymph nodes, bone marrow, corneal scrapings, peritoneal, pleural effusion, ascitic and cerebrospinal fluids, synovia, skin ulcers, and bronchial washings (Flournoy et al. 1992; Sanders et al. 2000). A frequent isolate from infected patients has been *Mtb mesophilicum* (Engler and Norton 2001; Holton et al. 1990; Kaye et al. 1992; Sanders et al. 2000), which has also been shown as the cause of a postoperative septicemia case (Barriere et al. 2008). Other species (including *Mtb radiotolerans*, *Mtb aminovorans*, *Mtb thiocyanatum*, *Mtb extorquens*, *Mtb zatmanii*, *Mtb fujisawaense*, *Mtb lusitanum*, and *Mtb podarium*) have also been reported (Borsali et al. 2011; Fanci et al. 2010; Furuhata et al. 2006; Hornei et al. 1999; Lai et al. 2011; Truant et al. 1998), with *Mtb extorquens* earning the clinical nickname "the red phantom" (Holton et al. 1990). It has been suggested that infection rates may be underestimated because of the difficulty of identifying *Methylobacterium* strains which grow only slowly on commonly used clinical media (Hornei et al. 1999), and underreporting of infection by *Methylobacterium* species due to difficulties in identification using standard clinical practices (Blomqvist et al. 1997; Lee et al. 2004). It is also likely that

infections involving *Methylobacterium* species, prior to recognition of the genus, and progressive identification of its species, have been diagnosed only as "caused by Gram-negative rods" (Blomqvist et al. 1997; Korvick et al. 1989), possibly "uncharacterized *Pseudomonas*," or as organisms which were subsequently reclassified as *Methylobacterium*. Examples are the fortuitous recovery of *Mtb fujisawaense* after long incubation of mycobacterial blood cultures (Fanci et al. 2010), infections attributed to *Pseudomonas mesophilica* (Gilardi and Faur 1984; Rutherford et al. 1988), *Protomonas extorquens* (Hayashi et al. 1990; Holton et al. 1990), and *Vibrio extorquens* (Lambert et al. 1983).

The ubiquitous distribution of *Methylobacterium* poses problems in determining sources and how infection can occur, and in eliminating external contamination (of patients, patient samples, or equipment) as a source during handling of clinical samples or preparation and cleaning of equipment such as catheters, bronchoscopes, endoscopes, and so on. It has been exhaustively demonstrated that *Methylobacterium* is present in domestic water systems (Gallego et al. 2005a, b, c, 2006; Rice et al. 2000), shower curtains and bath rails (Kelley et al. 2004; Rutherford et al. 1988), clinical and dental unit water supplies (Barbeau et al. 1996; Flournoy et al. 1992; Thomas et al. 2006), and in the biofilms formed on bronchoscopes, endoscopes, and in small-bore waterline tubing (Barbeau et al. 1996; Flournoy et al. 1992; Imbert et al. 2005; Kovaleva et al. 2012, 2013). A common route for entry of the infection is via a catheter, with the contaminant potentially coming from hospital tap water, which has been reported to contain *Mtb fujisawaense*, *Mtb aquaticum*, *Mtb mesophilicum*, *Mtb radiotolerans*, *Mtb zatmanii*, and other species (Furuhata et al. 2006; Hornei et al. 1999). Flournoy et al. (1992) described a "pseudo-outbreak of *Methylobacterium mesophilica*" in which the organism *Mtb mesophilicum* was transmitted to seven patients (or samples from them) as a contaminant in the hospital tap water during bronchoscopy. A further consideration is that several *Methylobacterium* strains are found in or on the human body, being found in the mouth, and on the foot, and skin (Anesti et al. 2004, 2005; Greub et al. 2004; Hung et al. 2011, 2012; Strazzi et al. 1992).

In many of the cases of *Methylobacterium* infection, antibiotic treatment eradicated the organism and cured the symptoms, providing strong but circumstantial evidence that the two are linked: the organism being the cause of the infection. This link was probed by Borsali et al. (2011) in considering the diagnostic difficulty of relating the presence of *Mtb podarium* to the eye infection reported by them. It is not simple to consider the *Methylobacterium* case in terms of Koch's postulates: The first postulate ("The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms") was abandoned as a universal rule by Koch on discovering asymptomatic carriers of cholera and typhoid fever, and asymptomatic carriers are now known to be a common feature of many infectious diseases, and could be the case for the commensal strains of *Methylobacterium*. The second postulate has been fulfilled frequently ("The microorganism must be isolated from a diseased organism and grown in pure culture"). The third and fourth postulates have not been tested in humans or to our knowledge in any animal model ("The cultured microorganism should cause disease when introduced into a healthy organism" and "the microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent"), although observed resurgence of the infection (and organism) in patients partly supports those criteria. Probably a convincing early example of *Mtb extorquens* as a primary pathogen was the chronic progressive skin nodulation and ulceration seen in a previously healthy individual, 15 years after hysterectomy and radiation therapy, in which the patient developed a high agglutination titer to the organism (Lambert et al. 1983). This infection was completely resolved after 18 months treatment with a regime of ethambutol, isoniazid, rifampin, and kanamycin, and did not recur.

It is notable that clinical and laboratory studies have shown marked antibiotic resistance among *Methylobacterium* strains, with some infections proving difficult to eradicate. In one example, intervention with five antibiotics was needed to cure the infection (Hornei et al. 1999). Aminoglycoside antibiotics (imipenem, ticarcillin-clavulanic acid, sulfamethoxazole, and trimethoprim), tetracycline, rifamycin, and gentamycin have been reported to be effective against *Methylobacterium*, but a number of studies have shown resistance of some strains to antibiotics, including cephalosporins, ampicillin, piperacillin, carbenicillin, spectinomycin, puromycin, cefuroxime, erythromycin, vancomycin, chloramphenicol, fosfomycin, piperacillin, cefazolin, cefotetan, cerfuroxime, ceftazidime, ceftriaxone, ciprofloxacin, meropenem, nalidixic acid, teicoplanin, gentamycin, amikacin, nitrofurantoin, and *p*-fluorophenylalanine (Borsali et al. 2011; Brown et al. 1992; Furuhata et al. 2006; Goodwin et al. 1988; Korvick et al. 1989; Lee et al. 2004; Zaharatos et al. 2001). Brown et al. (1992) showed the majority of 15 clinical isolates of *Methylobacterium* were resistant to β-lactam drugs, with the exceptions of ceftriaxone and ceftizoxime, and that six of these were β-lactamase-negative with a minimum inhibitory concentration for ampicillin of 4 μg ml^{-1}, whereas nine β-lactamase-positive isolates were resistant to 8–32 μg ml^{-1} (or more). Significantly, they found that even β-lactam drugs usually resistant to β-lactamase inactivation had very little activity against the *Methylobacterium* isolates, suggesting that mechanisms other than drug inactivation by lactamases might be involved in the resistance. Genes encoding β-lactamase have been predicted in the completely sequenced genomes of *Mtb radiotolerans* JCM 2831 (http://www.uniprot.org/uniprot/B1LXB8), *Methylobacterium* sp. strain 4–46 (http://www.uniprot.org/uniprot/B0UJG8), and *Mtb extorquens* strain CM4 (http://www.uniprot.org/uniprot/B7KSG2).

A potentially useful variability thus exists in the effectiveness of different antibiotics against various strains of *Methylobacterium*. This was further illustrated by Lai et al. (2011) using clinical isolates of five different species: Two *Mtb thiocyanatum* isolates were resistant to aztreonam and

ciprofloxacin; two *Mtb lusitanum* isolates were resistant to aztreonam, ceftazidime, and ciprofloxacin; and *Mtb radiotolerans* was resistant to aztreonam, ceftazidime, cefepime, and piperacillin/tazobactam (a combination in which tazobactam is a β-lactamase inhibitor). The sixth isolate, *Mtb aminovorans*, was resistant to all the antimicrobial agents tested. It might be possible for clinical microbiology laboratories to be able to infer the species identity of a *Methylobacterium* isolate from antibiotic sensitivity and thereby recommend an optimal regime of antibiotic treatment. Differential sensitivity has also been suggested as a means of phenotypic identification at the genus level of clinical isolates as *Methylobacterium* (Zaharatos et al. 2001). This conjecture was based on differential sensitivity of four clinical isolates and ATCC cultures of *Mtb extorquens*, *Mtb organophilum*, and *Mtb mesophilicum* to imipenem (extreme susceptibility) and meropenem (high resistance).

The definitive method for identification of clinical isolates of *Methylobacterium* must, however, be comparative sequencing of the 16S rRNA genes of the isolates (Borsali et al. 2011; Fanci et al. 2010; Hornei et al. 1999; Lai et al. 2011; Zaharatos et al. 2001).

Methylobacterium in Biotechnology and Biodeterioration

The importance of *Methylobacterium* strains in damaging or assisting man-made systems, and in their potential for biotechnological exploitation has been recognized increasingly in recent years. *Methylobacterium* strains have been applied commercially to fermentation processes in the manufacture of coenzyme Q-10, lysine, tyrosine, phenylalanine, vitamin B12, enzymes, bioactive peptides, bio-insecticides, biopolymers such as poly-β-hydroxybutyrate (PHB), and copolymeric polyalkanoates (Bormann et al. 1997; Bourque et al. 1995; Desmarteaux et al. 2012; Green 2006; Höfer et al. 2011; Kim et al. 1999; Yezza et al. 2006). *Methylobacterium* strains were never regarded as likely candidates for the commercial production of single-cell-protein as a foodstock, or for biosynthesis of deuterated and $^{13}C/^{15}N$-labeled ribonucleotides, as their growth yields (protein produced/mol methanol consumed) were inferior to those of methylotrophs such as *Methylophilus methylotrophus*, which used the Quayle cycle for C_1-assimilation, rather than the serine pathway found in *Methylobacterium* (Batey et al. 1996; Schrader et al. 2009; Westlake 1986).

A strain of *Methylobacterium* related to *Mtb extorquens*, isolated from contaminated groundwater, was shown to have commercial potential as a producer of polyhydroxyalkanoates of high yield and quality (Yezza et al. 2006). This strain yielded PHB from 0.5 % (v/v) methanol at 40 % (w/w) of the dry biomass produced. It could be induced also to synthesize the co-polyester poly-3-hydroxybutyrate-poly-3-hydroxyvalerate (PHB/PHV) when grown with methanol + valeric acid (7:1 w/w/ratio): This resulted in PHB/PHV accumulation reaching 30 % of the dry biomass, with PHV at twice the PHB content (Yezza et al. 2006). Potassium-limitation of *Mtb organophilum* had also been shown previously to enhance co-polyalkanoate formation (Kim et al. 1999). Using both feeding with different n-alkenoic acids and genetic modification of a proprietary *Methylobacterium* strain could enable production of the "functionalized" polyhydroxyalkanoates required as advanced materials for tissue engineering, biocomposites, biodegradable polymers, and several medical uses (Höfer et al. 2010, 2011).

Methylobacterium strains have also been implicated as beneficial to bioremediation of diverse contaminated environments. Very high tolerance to toxic metals (e.g., cadmium and lead, arsenate and chromate) and to naphthalene, xylene, styrene, phenol, and other organic pollutants led De Marco et al. (2004) to describe two strains of *Mtb extorquens* and one of *Mtb fujisawaense* as "super-bugs." Synergism in increasing biodegradation of methyl t-butyl ether (MTBE) and trichloroethylene was seen when mixtures of degrader strains and these metal-resistant strains were present together (Fernandes et al. 2009). Multiple-metal resistance has been found in 109 isolates of strains of *Methylobacterium* (related to *Mtb fujisawaense*, *Mtb radiotolerans*, *Mtb oryzae*, *Mtb populi*, *Mtb aminovorans*, *Mtb thiocyanatum*, *Mtb extorquens*, and several uncharacterized *Methylobacterium* species) present as endophytes of three mangrove species involved in the remediation of oil-polluted mangrove ecosystems (Dourado et al. 2012b). MTBE is a potentially serious pollutant of wastewater and groundwater as it is added (at 5–15 % w/v) to vehicle fuels (petrol/gasoline). MTBE is resistant to degradation in soil and activated sludge (Fujiwara et al. 1984; Jensen and Arvin 1990; Mo et al. 1997), but was efficiently degraded by a strain of *Methylobacterium* isolated from *Ginkgo biloba*.

A potential pollutant, from some natural and industrial sources, is dimethyl isophthalate (used as a polyacrylate resin comonomer and as a perfume fixative), which can be degraded by consortial activity of *Mtb mesophilicum* and *Klebsiella oxytoca* (Li and Gu 2007). Waste treatment bioreactors are used to remove the highly toxic formaldehyde from waste liquors, and one study has shown a *Methylobacterium* strain to have been the organism responsible (Chongcharoen et al. 2005). That strain (BIP) showed 98 % 16S rRNA gene sequence similarity to that of *Mtb thiocyanatum* and could be acclimatized to an input concentration of 100 mM formaldehyde in a substrate-limited continuous flow chemostat (Chongcharoen et al. 2005). Formaldehyde is normally tolerated as a growth substrate by *Methylobacterium* species in batch culture only up to about 1 mM.

"Phytoremediation" of toxic pollutant nitro-substituted explosives (TNT, RDX, and HMX) involving *Methylobacterium* strains has also been reported (Fournier et al. 2005; Van Aken et al. 2004, 2011). Degradation of these explosives is catalyzed by hybrid poplar trees (*Populus deltoides x nigra* DN34), and this property was attributed to the phytosymbiotic role of endophytic *Mtb populi* strain BJ001^T (Van Aken et al. 2011). Pure cultures of strain BJ001 completely degraded these explosives, with over 50 % of RDX- and HMX-carbon converted to carbon dioxide. During aerobic RDX degradation, a ring-cleavage metabolite can accumulate as a "dead-end" product

(Fournier et al. 2002): This is 4-nitro-2,4-diazobutanal (NDAB), which can also arise from hydrolysis of HMX. NDAB was degraded as a nitrogen source for growth by *Methylobacterium* strain JS178 isolated from sludge out of an aerobic treatment plant at a US air force base (Fournier et al. 2005). This strain was also identified as *Mtb populi* and indicates that both endophytic and "free-living" strains are effective in the degradation of these complex molecules. The further application of *Methylobacterium* species for targeted bioremediation (in soils, activated sludge, fluidized beds, etc.) merits more study: For example, Green (2006) suggested that cyanate and thiocyanate wastes might be degraded by some *Methylobacterium* strains, such as *Mtb thiocyanatum* (Wood et al. 1998), and that strains showing radiation-tolerance comparable to that of *Deinococcus radiodurans*, might be suitable as indicator organisms for quality-control monitoring in the food and packaging industries.

In addition to the beneficial effects ascribed to methylobacteria, they have been implicated in corrosion and fouling. A particular problem is the fouling of fuels and fuel delivery systems by bacteria capable of growth on hydrocarbons, particularly those of chain lengths less than C_{18} (Green 2006; Passman and McFarland 1997). Aviation fuel (a middle-distillate fuel) is subject to fouling, where storage tanks, aircraft wing tanks, fuel filters, and fuel injectors are all affected (Brown et al. 2010). Analysis of 16S rRNA clone libraries showed *Methylobacterium* strains to be the dominant organisms in samples of one fuel type (Jet A) and highly significant in another (JP-8), accounting for 55 % and 18 %, respectively, of the total genera identified (Brown et al. 2010). Besides fouling, fuel tanks are also subject to microbial corrosion as a consequence of biofilm formation, likely to involve methylobacteria (Brown et al. 2010; McNamara et al. 2005; Rauch et al. 2006).

Physiology

All species are facultative heterotrophs, capable of growth on a wide range of multicarbon substrates, with sugars being poorly or not used by several species. The most-studied species, *Mtb extorquens*, can express enzymes for the serine cycle (for one-carbon-unit assimilation for biosynthesis), a complete Krebs tricarboxylic cycle, a glyoxylate-regeneration cycle, the ethylmalonyl CoA pathway, the tetrahydomethanopterin (H_4MPT)-dependent formaldehyde oxidation pathway, and enzymes for glycolysis, amino acid, fatty acid, and alcohol metabolism (Chistoserdova 2011; Chistoserdova et al. 2003, 2009; Lidstrom 2006; Vuilleumier et al. 2009).

Metabolism of one-carbon compounds. Most of the validly published species of *Methylobacterium* oxidize methanol, and can grow on it as the source of energy and carbon. Many, but not all, can also grow on methylamine, and on a wide range of other C_1-compounds, including dimethylamine, trimethylamine, methanethiol, dimethylsulfide, dimethylsulfoxide, dimethylsulfone, methanesulfonate, chloromethane, dichloromethane, formaldehyde, and formate. Most of these compounds are ubiquitous in the natural environment, with methanol, dimethylsulfide, methanesulfonate, and trimethylamine being important atmospheric or biogenic products from plants, animals, and bacteria (Fall and Benson 1996; Gout et al. 2000; Jourdain and LeGrand 2001; MacDonald and Fall 1993; Murrell and Kelly 1996; Nemecek-Marshall et al. 1995; Schink and Zeikus 1980; Yang and Xianfeng 2006). Methanol comprises 40–60 % of the total atmospheric volatile organic carbon (Trotsenko et al. 2001), and its input to the atmosphere from living plants and plant decay, principally from pectin degradation, is about 151 Tg $year^{-1}$ (Jacob et al. 2005). Methanol emission by 11 different plant species (including *Populus deltoides*, Russian olive, and several bean genera) was analyzed in detail by Nemecek-Marshall et al. (1995), who showed emission rates of up to about 100 μg h^{-1} (g dry weight)$^{-1}$ and showed internal leaf pools of up to about 60 μg methanol (g fresh weight)$^{-1}$. Forests are likely to be a major source of atmospheric methanol. An unquantifiable amount of the methanol produced by living plants, especially from young growing leaves, is intercepted by the epiphytic and endophytic populations of methylobacteria.

The biochemistry and genetics of C_1-compound oxidation and assimilation have been exhaustively studied for over 40 years (Anthony 1982; Chistoserdov et al. 1994; Chistoserdova 2011; Chistoserdova and Lidstrom 1994, 1997; Chistoserdova et al. 2003; 2009; Kirikova 1970; Machlin et al. 1988; McDonald and Murrell 1997). The pathway of methanol oxidation is common to many methylotrophic genera and is initiated by the periplasmic quinoprotein, methanol dehydrogenase, which has pyrroloquinoline quinone (PQQ) as its prosthetic group, requires Ca^{2+} for activity, and has cytochrome c_L as the electron acceptor. The active enzyme protein is a $\alpha_2\beta_2$ tetramer, with a large α-subunit (66 kDa polypeptide encoded by the *mxaF* gene) and a small β-subunit polypeptide (8.5 kDa polypeptide encoded by the *mxaI* gene). The 17 or more gene-encoding proteins for methanol oxidation by *Mtb extorquens* are located in three different places on the chromosome, with a single operon of 14 genes encoding the complex of methanol dehydrogenase polypeptides (*mxaFJGIRSACKLDEHB*). Chistoserdova et al. (2003) have shown that methylotrophs express over 100 genes to enable oxidation of C_1-compounds and biosynthesis from them, and that these belong to specialized "metabolic modules" with some genes in the modules located in "methylotrophy islands," and others are located as "singular entities" in the chromosome (Chistoserdova et al. 2003; Lidstrom 2006). Several enzymes catalyzing the primary oxidation of methylamines by methylotrophs are known (Anthony 1982; Chistoserdova et al. 2003; Lidstrom 2006): These include methylamine, dimethylamine, and trimethylamine dehydrogenases, dimethylamine and trimethylamine monooxygenases, and methylamine oxidase. The methylamine dehydrogenase of *Mtb extorquens* is a periplasmic tryptophan-tryptophylquinone (TTQ)-dependent tetrameric quinoprotein ($\alpha_2\beta_2$), encoded by *mauA* (small subunit), *mauB* (large subunit), and *mauC* (the amicyanin acceptor), which are part of an 11-gene operon (*mauFBEDACJGLMN*). The primary product of methanol and methylamine oxidation (and of methanesulfonate, oxidized by

a methanesulfonate monooxygenase) is formaldehyde. This is the metabolic branch point for oxidation and biosynthesis from the serine cycle. Other C_1-compounds must also undergo formaldehyde-producing metabolism for these purposes. Formaldehyde can be oxidized by several enzyme systems, producing formate, which is further oxidized to carbon dioxide, or is channeled into the serine cycle (Chistoserdova et al. 2003; Kelly and Murrell 1999; Lidstrom 2006).

Metabolism of oxalate. Oxalate is found in all plants, primarily in leaves, but also found in some plant stems and roots, although in an extreme range of concentrations. It is produced from glucose and Krebs' cycle intermediates via glycolate and glyoxylate, and is essentially an obligatory end product of metabolism, as an excretion of carbon dioxide in its mildly reduced form, HOOC-COOH. It is thus a ubiquitous natural product, both in the plant and released into the soil. The most productive accumulators are *Oxalis* species (ca. 900 spp., including sorrels and shamrocks), with *O. pes-caprae* accumulating up to 16 % of its dry weight as oxalic acid (Millerd et al. 1963). Rhubarb leaves are also well known as oxalate accumulators, with up to 5.3 g per kg fresh weight. Concentrations (g per kg) in common vegetables range from 0.4 to 1.0 for parsnip, pea, okra, onion, potato, and tomato and up to 6.1–17.0 for beet leaves, spinach, cassava, chives, purslane, and parsley (USDA 1984).

Numerous bacteria have been reported as oxalotrophs, degrading oxalate as a source of energy and carbon. The earliest report of an oxalate-degrader was the *Bacillus extorquens* (*Vibrio extorquens*, now *Mtb extorquens*) described by Bassalik (1913). This organism should not be confused with the unrelated *Vibrio oxalaticus* (Bhat and Barker 1948), which, like *B. extorquens*, was isolated from the excreta of earthworms: It is a member of the *Betaproteobacteria* and was successively reclassified as *Pseudomonas oxalaticus*, *Ralstonia oxalatica*, and finally as *Cupriavidus oxalaticus* (Vandamme and Coenye 2004). The re-isolation of *Mtb extorquens* (as *Pseudomonas extorquens*) by Janota (1956), using a mineral medium with 1.2 g ammonium oxalate per liter, enabled further study of oxalate metabolism by *Methylobacterium*. She demonstrated by Warburg manometry that oxalate oxidation initially proceeded with a (carbon dioxide output/oxygen uptake) ratio of about 2, which fell to a long-term (4 h) steady-state ratio of 0.94 ± 0.3. The complete oxidation of oxalate would require a balance of:

$$2(COOH)_2 + O_2 \rightarrow 4\ CO_2 + 2\ H_2O\ [CO_2/O_2 = 4.0]$$

The observation of maximum ratio of 2.0 and a steady-state ratio of only about 1.0 indicates incomplete oxidation and the likelihood of formation of partially oxidized intermediates and fixation of some oxalate-carbon into biomass.

Subsequently the pathways of oxidation and assimilation of oxalate were established by Blackmore and Quayle (1970), using (as they thought) four different species of pink-pigmented methylotrophs: *Pseudomonas AM1*, *Pseudomonas* AM2, *Protaminobacter ruber*, and *Protomonas extorquens* (all of which are *Mtb extorquens*). During growth on oxalate, all the organisms were shown to contain oxalyl-CoA decarboxylase, formate dehydrogenase, and oxalyl-CoA reductase. This was consistent with oxidation of oxalate involving oxalyl-CoA, formyl-CoA and formate as intermediates, and the reduction of oxalyl-CoA to glyoxylate. The oxalate-grown organisms also contained L-serine-glyoxylate aminotransferase and hydroxypyruvate reductase, indicating that synthesis of C_3-compounds from oxalate occurred by a variant of the serine pathway "used by *Pseudomonas* AM1 during growth on C_1-compounds" (Blackmore and Quayle 1970). This metabolism was subsequently confirmed for other *Methylobacterium* species and for *Mtb extorquens* strain AM1 (Schneider et al. 2012), and the mechanism of oxalate oxidation and assimilation is available on the MetaCyc database for *Mtb extorquens* (http://metacyc.org/META/NEW-IMAGE?type=NIL&object=PWY-6696) and *Mtb chloromethanicum* (http://biocyc.org/MCHL440085/NEW-IMAGE?object=Oxalate-Degradation).

Şahin et al. (2008) tested 12 oxalotrophic strains, isolated from soil or plant tissues, which fell into four groups of strains, which were closest to the type strains of *Mtb thiocyanatum*, *Mtb aminovorans*, *Mtb extorquens*, and *Mtb organophilum*. They also tested 20 type cultures of *Methylobacterium* for their use of oxalate: *Mtb radiotolerans*, *Mtb aminovorans*, *Mtb fujisawaense*, *Mtb thiocyanatum*, *Mtb rhodesianum*, *Mtb rhodinum*, *Mtb zatmanii*, *Mtb mesophilicum*, *Mtb dichloromethanicum*, *Mtb chloromethanicum*, *Mtb suomiense*, *Mtb lusitanum*, *Mtb organophilum*, *Mtb hispanicum*, *Mtb nodulans*, *Mtb adhaesivum*, *Mtb isbiliense*, and *Mtb podarium* were all oxalate-users, but *Mtb variabile* and *Mtb aquaticum* were not. Oxalotrophy is thus a common feature of the majority of species tested to date.

Oxidation of thiosulfate. Some *Methylobacterium* strains have been shown to gain energy from the oxidation of thiosulfate to sulfate, which increased growth yields in mixotrophic culture with methanol or succinate as the carbon source (Anandham et al. 2007, 2009). This was demonstrated for *Mtb fujisawaense*, *Mtb goesingense*, *Mtb oryzae*, and *Mtb thiocyanatum*, but not for *Mtb suomiense* or *Mtb rhodinum*. *Mtb phyllosphaerae*, previously grown on a yeast extract + thiosulfate medium oxidized tetrathionate, trithionate, sulfite, and elemental sulfur as well as thiosulfate (Anandham et al. 2008).

Genus *Microvirga*

Mi.cro.vir'ga Gr. adj. *mikros* small; L. fem. n. *virga* rod; N.L. fem. n. *Microvirga* a small rod. Type species *Mv subterranea*: sub.ter. ra'ne.a L. fem. adj. underground, subterranean.

Taxonomy

Microvirga was assigned to the *Methylobacteriaceae* in the second edition of *Bergey's Manual of Systematic Bacteriology*, but no description was given (Garrity et al. 2005). The eight validly published species form a self-contained cluster on the basis of their 16S rRNA gene sequences, which is distinct from *Methylobacterium*, but is clearly related to it as a separate

Table 15.3

Type strains of the species of *Microvirga*, 16S rRNA gene accession numbers, and some distinguishing properties. All validated names are shown (LPSN; http://www.bacterio.cict.fr/)

Species	Culture collection accession number	GenBank accession number	GC content of type strain DNA (mol %)	Isolation source of the type strain	Growth on glucose/nitrate reduction	Motility/ colony color
Mv aerilata	KACC 12744	GQ421849	61.5	Atmosphere (Korea)	−/−	−/Pink
Mv aerophila	KACC 12743	GQ421848	62.2	Atmosphere (Korea)	−/−	−/Pink
Mv flocculans	ATCC BAA-817	AB098515	64.0	A hot spring (Japan)	−/−	−/White
Mv guangxiensis	JCM 15710	EU727176	64.3	Soil (rice field, China)	+/+	+/Pink
Mv lotononidis	LMG 26455	HM362432	62.9	N_2-fixing nodules of *Listia angolensis* (Zambia)	+/+	+/Pink
Mv lupini	LMG 26460	EF191408	61.9	N_2-fixing nodules of *Lupinus texensis* (Texas)	+/−	−/Orange
Mv subterranea^T	ATCC BAA-295	FR733708	63.5	Geothermal bore hole	−/+	+/Pink
Mv zambiensis	LMG 26454	HM362433	62.6	N_2-fixing nodules of *Listia angolensis* (Zambia)	+/+	+/Cream

genus of the *Methylobacteriaceae* (Fig. 15.1; Ardley et al. 2012; Weon et al. 2010). Like most *Methylobacterium* species, some of its species produce pink colonies, have similar rod-shaped morphology, similar major fatty acid profiles, and contain ubiquinone Q-10. Unlike *Methylobacterium*, *Microvirga* species do not use methanol or other C_1-compounds for energy or carbon.

The original genus description (Kanso and Patel 2003) has been subject to two emendations to date, as new species were characterized (Weon et al. 2010; Zhang et al. 2009) and can now be updated as below.

Strictly aerobic, small, Gram-negative rod-shaped cells; different species produce light-pink, pale orange, white, or cream colonies. The optimum temperature for growth is 37–45 °C, with a species-dependent temperature range for growth between 10 °C and 45 °C. Four of the eight species cannot grow on glucose. They all reduce nitrate to nitrite. Cells are susceptible to antibiotics that inhibit members of domain *Bacteria*. Bacteriochlorophyll is absent but carotenoids are present; PHB is produced. Thiosulfate does not stimulate growth. Positive for catalase, but negative for hydrolysis of casein, chitin, carboxymethylcellulose, and for xanthine, indole production, glucose fermentation, and arginine dihydrolase. The predominant isoprenoid quinone is Q-10. The major fatty acid is C18:1ω7c (63.3–73.8 % of total fatty acids). The polar lipids consist of phosphatidylcholine and phosphatidyl-ethanolamine as major components, and phosphatidyl-monomethyl-ethanolamine, phosphatidyl-dimethyl-ethanolamine, diphosphatidyl-glycerol and phosphatidyl-glycerol in moderate amounts. The GC content of the DNA is 61.5–64.3 mol%. 16S rRNA gene sequence analysis indicates that it is a member of the *Alphaproteobacteria*, placed almost equidistantly between *Chelatococcus asaccharovorans* and *Bosea thiooxidans*, respectively, members of the families *Beijerinckiaceae* and *Bradyrhizobiaceae*, as the nearest phylogenetic relatives. The type species is *Microvirga subterranea*.

Molecular Analyses

The genomes of *Mv lupini* strain Lut6 and *Mv lotononidis* strain WSM 3557 have been sequenced http://www.ncbi.nlm.nih.gov/genome/13788?project_id=66529 and http://www.ncbi.nlm.nih.gov/genome/13788?project_id=167863. Lut6 contains 9,633,614 bp; with 61.58 mol% GC; and only one gene each for 5S, 16S and 23S rRNA. WSM 3557 contains 7,082,538 bp; 63.08 mol% GC; three genes encoding 5S rRNA, one for 16S rRNA, and two for 23S rRNA.

Phenotypic Analyses

Some distinguishing features of the eight species are summarized in Table 15.3. Four of the species have cell sizes (μm) of 0.4−0.8 × 1.0−2.2, but the other four are larger: *Mv subterranea*, *Mv flocculans*, *Mv aerophila*, and *Mv aerilata* being 1.0 × 1.5−4.0; 0.5−0.7 × 1.5−3.5; 0.8−1.1 × 1.6−4.2; and 1.2−1.5 × 1.6−3.3, respectively. Only three species (*Mv lotononidis*, *Mv zambiensis*, and *Mv flocculans*) are motile by means of polar flagella. Their optimum temperatures and pH for growth are in the range 35–45 °C and pH 7.0–8.5. None will tolerate more than 2 % (w/v) NaCl. Some will grow in minimal media while some require yeast extract as an absolute requirement, and all grow with complex media. Growth with ranges of multicarbon substrates is detailed in the original species descriptions, as is antibiotic sensitivity for some species. The three root nodule species (Ardley 2011; Ardley et al. 2010, 2012) grow on glucose, fructose, L-arabinose, and mannitol, and are oxidase negative, but the other species are mainly negative for these substrates (or have not been tested), and four of the five other species are oxidase positive. Only the root nodule

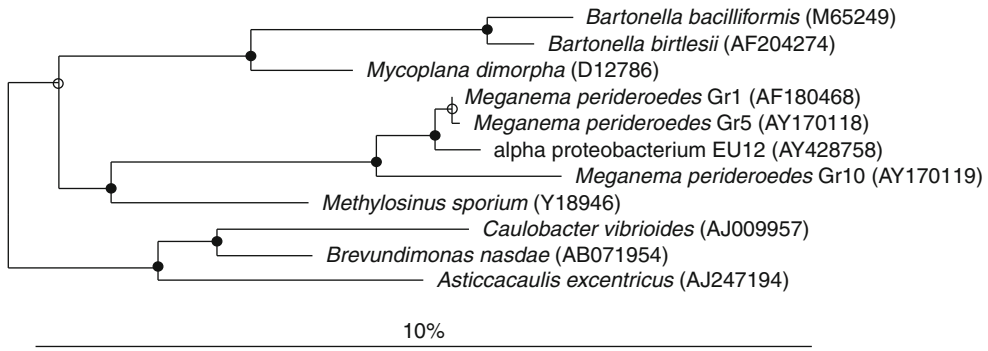

Fig. 15.2

Phylogenetic analysis of 16S rRNA gene sequences from *Meganema perideroedes* (ATCC BAA-740^T; DSM 15528^T), other *Meganema* strains, and the closest phylogenetic relatives of the type strain. Sequences were aligned using the ARB-SILVA database (Ludwig et al. 2004), and phylogenetic trees were constructed from 1,190 bp of aligned sequence using neighbor-joining (NJ) in the ARB software environment. The scale bar represents 10 % sequence divergence, and bootstrap values, from 100 replicates, are represented at the tree nodes: ● >90 % and ○ 70–90 % similarity

species (*Mv lotononis*, *Mv lupini*, and *Mv zambiensis*) have been shown to fix nitrogen: The other five species have not been tested.

Ecology, Isolation, Properties, Culture Media, and Growth Conditions for *Microvirga*

Microvirga species have been isolated from water (a thermal spring and a thermal aquifer), air, and soil and as endosymbionts of the legumes *Listia* (formerly *Lotononis*) and *Lupinus* (Ardley et al. 2012; Kanso and Patel 2003; Takeda et al. 2004; Weon et al. 2010; Zhang et al. 2009). The type species, *Mv subterraneae*, came from free-flowing geothermal water from a borehole (source depth, 295 m; Kanso and Patel 2003).

The medium used by Kanso and Patel (2003) for the type species is suitable for all the current *Microvirga* species, and contains (g):

Yeast extract	1
Peptone	5
$MgSO_4 \cdot 7H_2O$	0.20
$CaCl_2$	0.05
Ferric ammonium citrate	0.15
$MnSO_4 \cdot 4H_2O$	0.05
$FeCl_3 \cdot 4H_2O$	0.01
Vitamin solution	10 ml
Trace element solution	1 ml
Distilled water	1,000 ml

For solid medium, 17 g agar was added; adjusted to pH 7.1 with NaOH. Vitamin solution was that used by Wolin et al. (1963), and the trace element solution was that of Zeikus et al. (1979). Other media used have been half-strength lupin agar (½ LA; Yates et al. 2007), and R2A agar (described above). Glucose, yeast extract-malt extract media, and Luria Broth also support growth (Zhang et al. 2009).

Genus *Meganema*

Me.ga.ne'ma. Gr. nom. neut. adj. *mega* big; Gr. nom. neut. n. *nema* thread; N.L. neut. n. *Meganema* large thread-like microorganism. *Meganema perideroedes*: pe.ri.de.ro'ed.es N.L. neut. adj. necklace-like.

Ecology, Isolation, Culture Media, Growth Conditions, Taxonomy, Molecular and Physiological Properties of *Meganema*

The type strain of *Mn perideroedes* was isolated by micromanipulation from mixtures of filamentous bacteria in the activated sludge biomass causing bulking in a wastewater treatment plant (Thomsen et al. 2006). To date, the only other named isolates were from the same source, comprising five strains that form a monophyletic cluster, and show 97.7–99.6 % sequence identity for their 16S rRNA genes, but the most dissimilar strains (Gr1^T and Gr10) still showed 71.5 % DNA/DNA hybridization. All five were thus strains of a single species (*Figs. 15.1* and *15.2*). The GC content of its genomic DNA, determined by the DSMZ, was reported as 42.9 % (Thomsen et al. 2006), but sequencing of its genome has shown this figure to be incorrect, and the true GC content is 67.2 %.

Mn perideroedes was described after completion of the second edition of *Bergey's Manual of Systematic Bacteriology* and was assigned to the *Methylobacteriaceae* (http://www.bacterio.cict.fr/m/meganema.html) without further discussion. The family was circumscribed for *Bergey's Manual* "on the basis of phylogenetic analysis of 16S rRNA sequences" (Garrity et al. 2005).

Table 15.4

Comparative sequence identities of the 16S rRNA gene sequence of *Meganema perideroedes* and species of other genera of *Rhizobiales* and *Caulobacteraceae*, and comparison with the genomes and GC content (mol%) of the other genera

Genus/species	Genome size (Mb)	GC content (%) of genomic DNA	GenBank accession number (16S rRNA)	% sequence identity to *Meganema perideroedes*
Rhizobiales				
Meganema perideroedes	3.41	67.2	AF180468	100
Bartonella bacilliformis	1.45	38.2	NR_044743	87.0
Bartonella birtlesii	1.88	37.0	NR_025051	87.0
Mycoplana dimorpha	3.15	64.5	NR_043388	86.9
Methylocystis parva	4.48	63.3	Y18945	90.0
Methylosinus sporium	na	66.0	Y18946	90.0
Methylosinus trichosporium	4.85	66.0	Y18947	89.7
Hyphomicrobium vulgare	na	61.3	AB543807	88.8
Hyphomicrobium denitrificans	3.64	61.0	CP0020803	87.1
Xanthobacter autotrophicus	5.63	67.4	X94201	89.1
Methylobacterium organophilum	na	70.8	AB175638	86.9
Methylobacterium extorquens	6.88	68.0	AB175633	86.9
Caulobacteraceae				
Caulobacter vibrioides	4.30	67.0	AJ009957	86.8
Asticcacaulis excentricus	4.31	59.5	AJ247194	88.3
Brevundimonas nasdae	na	66.5	NR_028633	89.4
Brevundimonas subvibrioides	3.45	68.4	NR_037107	88.3
Brevundimonas diminuta	3.24	67.1	NR_040805	88.7

na, Genome sequence not yet available

Its cells are 1.5–2.0 µm in length in filaments about 1.5 µm in width, Gram-negative, with PHB and Neisser-positive granules; the major respiratory quinone is Q-10, and the predominant fatty acid is C18:1ω7c (86.4 % for the type species). Its cells are nonmotile and grow between 15 °C and 35 °C, with an optimum between 25 °C and 30 °C; it will not grow in defined mineral media, and requires a relatively rich medium (R2A, described above) for growth; cannot grow anaerobically with nitrate; tolerates 2 % but not 3 % (w/v) NaCl.

The genome of *Mn perideroedes* has been sequenced as part of the Genomic Encyclopedia of Type Strains (http://www.ncbi.nlm.nih.gov/genome?term=meganema; http://img.jgi.doe.gov/cgi-bin/w/main.cgi?section=TaxonDetail&page=taxonDetail&taxon_oid=2515154196). The genome contains 3,409,949 bp, of which 89.68 % are coding bases, and contains 3,092 genes, of which 98.09 % are protein coding. The genome size (*Table 15.4*) is thus much larger than those of *Bartonella* species, and half or less of those of *Methylobacterium* and *Microvirga* species, but similar to those of some genera within the *Rhizobiales* (e.g., 3.2–3.6 Mb for *Brucella*, *Mycoplana*, and *Hyphomicrobium*) and the *Caulobacteraceae* (3.2–4.3 Mb for *Brevundimonas*, *Asticcacaulis*, and *Caulobacter*).

In our view, *Meganema* has been erroneously placed in the *Methylobacteriaceae*. Unlike most members of the other genera in the family, *Methylobacterium* and *Microvirga*, its cells form filaments as its normal morphology, and its colonies are white, with no pink pigmentation (Thomsen et al. 2006). The phylogenetic tree presented in the primary description of *Meganema* included no species of *Methylobacterium* or *Microvirga* as phylogenetic relatives (Thomsen et al. 2006). Its nearest neighbors (*Fig. 15.2*) were apparently *Brevundimonas*, *Asticcacaulis*, and *Caulobacter* (in Order V, *Caulobacterales*, Family *Caulobacteraceae*), and *Mycoplana* (Order VI, *Rhizobiales*, Family *Brucellaceae*). Its closest phylogenetic relative was reportedly *Brevundimonas*, but at only 89.8 % 16S rRNA gene sequence similarity. BLASTN comparison of the 16S rRNA gene sequence of *Meganema* with *Methylobacterium organophilum*, *Mtb extorquens*, *Microvirga subterranea*, and *Mv aerilata* showed only 86.9–87.8 % sequence identity, compared to 92.7–93.4 % identity between the sequences of the *Methylobacterium* and *Microvirga* species. The supposed affiliation of *Meganema* with *Methylobacterium/Xanthobacter* (Thomsen et al. 2006) was based on the major fatty acid being C18:1ω7c, but this (and other C18:1 fatty acids) is also significant in the other genera including *Brevundimonas*, so this property is not a distinguishing criterion. Our phylogenetic tree (*Fig. 15.1*) shows rather distant relationships of *Meganema* to *Hyphomicrobiaceae* and *Methylocystaceae*, but BLAST2 comparisons of its 16S rRNA gene sequences showed only 88–90 % identity to *Hyphomicrobium vulgare*, *Methylocystis parvus*, and

Methylosinus species (Fig. 15.2; Table 15.4). Comparing the 16S rRNA gene sequences to a range of possibly related *Alphaproteobacteria* indicated *Meganema* to have no close phylogenetic relatives, as all the sequence identities were in the range 86.8–90 % (Table 15.4). The principal ubiquinone in most of the compared genera was Q-10, but some genera are distinguished from *Meganema* by containing Q-8 (*Methylocystis* and *Methylosinus*) or Q-9 (*Hyphomicrobium*). Several online taxonomic assignment databases can be interrogated for the position of *Meganema*. Also, the principal fatty acid in *Meganema* is C18:1ω7c, but C18:1ω8c in *Methylosinus*. Using the SILVA database project (http://www.arb-silva.de/) the assignments predicted by SILVA, RDP, and EMBL are all to the *Methylobacteriaceae*, but the *Greengenes Taxonomy* analysis unequivocally places *Meganema* in the *Caulobacterales* (http://greengenes.lbl.gov/cgi-bin/nph-browse.cgi#focus), specifically the *Caulobacteraceae* (http://www.arb-silva.de/browser/ssu/AF180468). While three genera of the *Caulobacteraceae* are prosthecate, the 4th, *Phenylobacterium*, is not. The DNA of strains of *Phenylobacterium* contains 65–68.5 % GC, its 16S rRNA gene (Y18216) shows 87 % identity to that of *Meganema*, and its cells (0.7–1.0 × 1.0–2.0 µm) become pleomorphic in old cultures, forming filament-linked long chains. The two genera thus share several structural properties, which suggest that both could be genera of the *Caulobacteraceae*.

Numerous other strains showing 97.9–99.8 % 16S rRNA gene sequence identity to the type strain of *Meganema* have been reported, as well as many uncultured clones (NCBI database, as revealed by BLASTN of the NCBI database with the *Meganema* sequence). This indicates that strains of this filamentous genus occur widely, especially in activated sludge and water treatment plants (Levantesi et al. 2004; Thomsen et al. 2006; Jiang et al. 2009).

The genus *Meganema* should thus either be reassigned to the *Caulobacterales* as a novel genus within the *Caulobacteraceae*, or if retained in the *Rhizobiales*, needs to be established as a new Family, the *Meganemaceae*, initially with the one genus and species, represented by *Meganema perideroedes*.

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16 The Family *Methylocystaceae*

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Abstract
Methylocystaceae, a family belonging to the order *Rhizobiales*, within the class *Alphaproteobacteria*, consist of genera *Albibacter*, *Hansschlegelia*, *Methylocystis*, *Methylopila*, *Methylosinus*, *Pleomorphomonas*, and *Terasakiella*. Most members of the family *Methylocystaceae*, with the exception of the genera *Pleomorphomonas* and *Terasakiella*, are type II methanotrophs, which assimilate C_1 carbon compounds (most commonly methane and methanol) via the serine pathway. All known species of these genera are gram-negative with the G+C content ranging from 50.7 to 70.4 mol%. The phylogenetic clustering within the group is somewhat weak, and as further strains are identified and classified to the family, some reorganization may occur.

Taxonomy: Historical and Current

Short Description of the Family

Methylocystaceae, Bowman (2005)

Me.thy.lo.cys.ta'ce.ae. N.L. masc. (sic) n. *Methylocystis*, type genus of the family; suff. *-aceae*, ending to denote family; N.L. fem. pl. n. *Methylocystaceae*, the *Methylocystis* family (Bowman 2006).

Methylocystaceae belongs to the order *Rhizobiales*, within the class *Alphaproteobacteria*. The family is classified as type II methanotrophs, in which the species belonging to the family are able to utilize methane and its derivatives as the carbon sources through the serine pathway. Many of these methanotrophic bacteria perform important environmental functions as they are a link in the global carbon cycle, act as nitrogen fixers, and have the ability to degrade a variety of organic contaminants (Gulledge et al. 2001). Currently, the family *Methylocystaceae* contains seven established genera, namely, *Albibacter*, *Hansschlegelia*, *Methylocystis*, *Methylopila*, *Methylosinus*, *Pleomorphomonas*, and *Terasakiella*.

Phylogenetic Position

Based on the 16S rRNA phylogenetic tree, the nearest families that are related to *Methylocystaceae* are *Beijerinckiaceae*, *Bradyrhizobiaceae*, *Methylobacteriaceae*, *Xanthobacteriaceae*, and *Hyphomicrobiaceae*. The 16S rRNA phylogenetic trees were constructed based on maximum likelihood (*Fig. 16.1*) and neighbor-joining (not shown) algorithms, and both agreed that the genera *Terasakiella* and *Pleomorphomonas* may be more distantly related to the other members of *Methylocystaceae*. Also, the family *Methylocystaceae* appeared not to be

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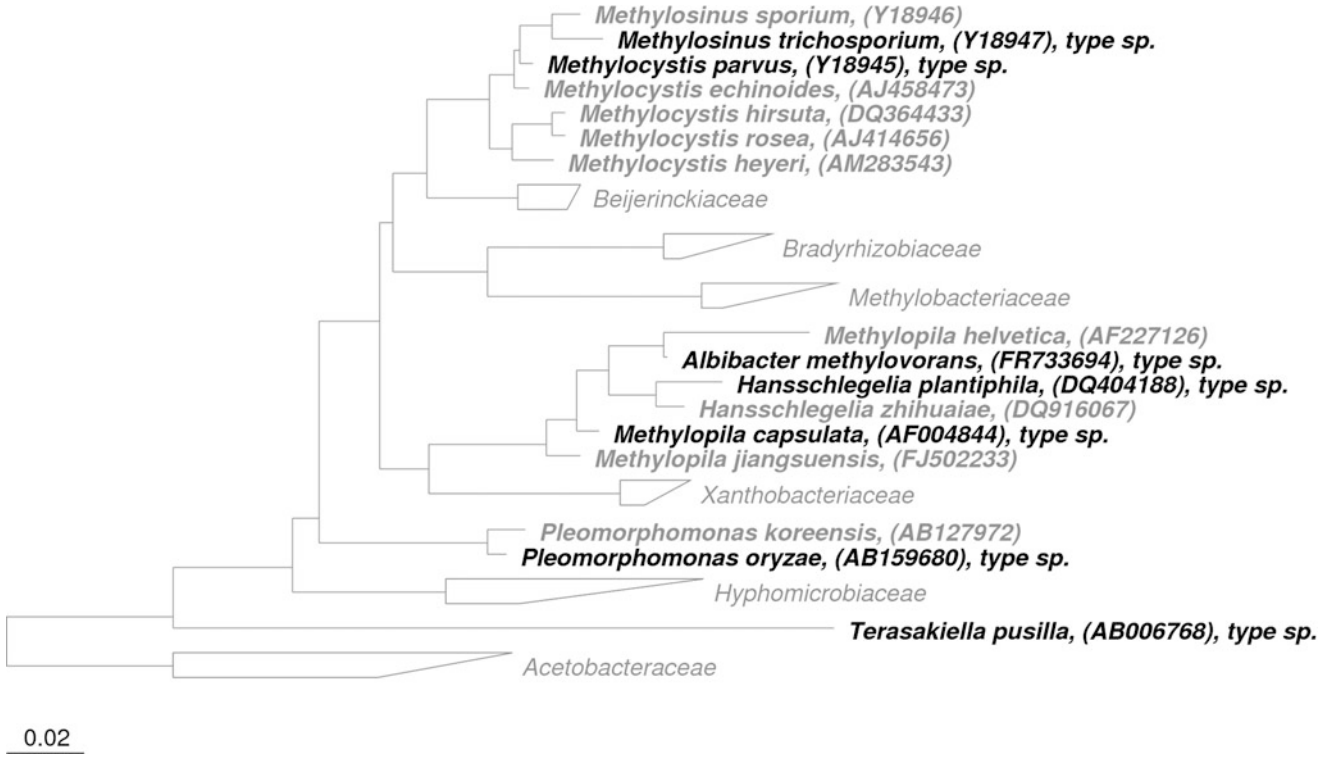

Fig. 16.1

Phylogenetic reconstruction of the family *Methylocystaceae* based on 16S rRNA gene sequences and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010); http://www.arb-silva.de/projects/living-tree. Representative sequences from closely related taxa were used as out-groups. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

monophyletic and has two branches, one associated with *Methylocystis* and the other with *Methylopila*. The position of *Methylopila helvetica* suggests that it may be more closely related to the genus *Albibacter*.

The following is a list of *Methylocystaceae* type strains used for dendrogram construction: *Albibacter methylovorans* DSM 22840^T, *Hansschlegelia plantiphila* S1^T, *Hansschlegelia zhihuaiae* S113^T, *Methylocystis echinoides* IMET 10491^T, *Methylocystis heyeri* H2^T, *Methylocystis hirsuta* CSC1^T, *Methylocystis parvus* NCIMB 11129^T, *Methylocystis rosea* SV97^T, *Methylopila capsulata* IM1^T, *Methylopila helvetica* DM9^T, *Methylopila jiangsuensis* JZL-4^T, *Methylosinus sporium* NCIMB 11126^T, *Methylosinus trichosporium* NCIMB 11131^T, *Pleomorphomonas koreensis* Y9^T, *Pleomorphomonas oryzae* F-7^T, and *Terasakiella pusilla* IFO 13613^T.

Molecular Analyses

Only a few studies have reported on DNA-DNA hybridization (DDH) results for members of the family *Methylocystaceae*. Only two pairs of species have been demonstrated to possess considerable similarity (>40 %) between their genomes; *Hansschlegelia plantiphila* and *H. zhihuaiae* were shown to share 44.9 % of their genomes, and *Methylocystis hirsuta* and *M. rosea* share 41.1 %.

Phenotypic Analyses

Methylocystis, Whittenbury, Phillips, and Wilkinson (1970) and Emend. Bowman, Sly, Nichols, and Hayward (1993)

N.Gr. n. *methyl* (from Gr. n. *methu* wine and Gr. n. *hulê*wood) methyl group; N.L. fem. n. *cystis* (from Gr. fem. N. *kustis*) the bladder and, in biology, a cyst; N.L. fem. n. *Methylocystis* methyl cyst (Whittenbury et al. 1970; Bowman et al. 1993).

Cells belonging to the genus *Methylocystis* are gram-negative reniforms, coccobacilli, or rods and are 0.3–1.2 μm wide by 0.5–4.0 μm long in size. Most of the species belonging to the genus were grown on nitrate mineral salts (NMS) medium, with the exception of *M. heyeri* which was grown on medium M2. Colonies vary from white, highly raised, and slimy to opaque, smooth, low convex, circular with entire edges, butyrous consistency, and pale pink to tan/white color. *M. parvus* also produces a brown diffusible pigment.

Methylosinus, Whittenbury, Phillips, and Wilkinson (1970) and Emend. Bowman, Sly, Nichols, and Hayward (1993)

N.L. n. *methylum* (from French *méthyle*, back-formation from French *méthylène*, coined from Gr. n. *methu*, wine and Gr. n. *hulê*, wood), the methyl radical; N.L. pref. *methylo-*, pertaining to the methyl radical; L. masc. n. *sinus*, a bent surface, curve; N.L. masc. n. *Methylosinus*, methyl bender (Whittenbury et al. 1970; Bowman et al. 1993).

The cells of *Methylosinus* are gram-negative, pyriform or vibrioid, which range in size from 0.5 to 1.0 μm wide by 1.5 to 3.0 μm long. Colonies grown on NMS agar are opaque with smooth surfaces, low convex, and circular with entire edges; have a butyrous consistency; and are white to buff or buff to tan. *M. sporium* may also produce diffusible brown pigment or a bright red prodigiosine-like pigment.

Methylopila, Doronina, Trotsenko, Krausova, Boulygina, and Tourova (1998)

N.L. n. *methylum* (from French *méthyle*, back-formation from French *méthylène*, coined from Gr. n. *methu*, wine and Gr. n. *hulê*, wood), the methyl group; N.L. pref. *methylo-*, pertaining to the methyl radical; L. fem. n. *pila*, ball or sphere; N.L. fem. n. *Methylopila*, methyl-using sphere (Doronina et al. 1998).

Methylopila cells are gram-negative rods, 0.5–1.2 μm wide by 0.8–2.0 μm long in size. Colonies grown on methanol-salt agar are white, semitransparent, convex, and circular with entire edges. *M. capsulata* and *M. jiangsuensis* were also grown well in complex media, e.g., nutrient agar, Luria-Bertani agar, or trypticase soy medium. Granules of poly-β-hydroxybutyric acid (PHB) are observed in cells within this genus.

Albibacter, Doronina, Trotsenko, Tourova, Kuznetsov, and Leisinger (2001)

L. adj. *albus*, white; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Albibacter*, white rod (Doronina et al. 2001).

Albibacter cells are gram-negative rods, 0.9–1.0 μm wide by 1.2–1.8 μm long in size. Colonies on methanol or peptone yeast glucose (PYG) agar are circular, white, convex, translucent to opaque, and mucoid and are 1–2 mm in diameter. To date, only one species has yet been described within this genus.

Terasakiella, Satomi, Kimura, Hamada, Harayama, and Fujii (2002)

N.L. fem. dim. n. *Terasakiella*, named to honor Y. Terasaki, the Japanese microbiologist who has made many contributions to our understanding of the classification and identification of spiral-shaped bacteria (Satomi et al. 2002).

Cells belonging to *Terasakiella* are gram-negative spirilla, ranging in size between 0.3 and 0.5 μm wide by 1.2–4.0 μm long. Colonies grown on medium 325 semisolid agar at 48 h are punctiform, entire, granular, convex or pulvinate, and glistening; yellowish-white butyrous consistency was observed after 7 days of incubation. *T. pusilla* is the only species belonging to this genus to date.

Pleomorphomonas, Xie and Yokota (2005)

N.L. masc. adj. *pleomorphus* (from Gr. adj. *pleos*, full, and Gr. n. *morphê*, form, shape), pleomorphic; Gr. fem. n. *monas*, monad, unit; N.L. fem. n. *Pleomorphomonas*, pleomorphic monad (Xie and Yokota 2005).

Pleomorphomonas cells are gram-negative pleomorphs or rods, with the size ranging from 0.3 to 1.0 μm wide by 1.0–5.0 μm long. The colonies of *P. oryzae* grown on the nitrogen-free agar are colorless, while the colonies of *P. koreensis* grown on the R2A agar are smooth, low convex, circular, translucent, non-shiny, and white in color.

Hansschlegelia, Ivanova, Doronina, and Trotsenko (2007) and Emend. Euzéby (2010)

N.L. fem. n. *Hansschlegelia*, named after Prof. Hans Schlegel, the famous German microbiologist known for his classic studies on autotrophic bacteria (Ivanova et al. 2007; Euzéby 2010).

Cells belonging to the genus *Hansschlegelia* are rods/cocci, 0.6–0.9 μm wide by 1.0–1.5 μm long in size. *H. plantiphila* forms round, low-convex, colorless, entire-edge, semitransparent, and granulous colonies when grown on agar medium supplemented with CH_3OH, while *H. zhihuaiae* forms circular and white colonies when grown on yeast-tryptone-$CaCl_2$ (YTC) agar (*Tables 16.1* and *16.2*).

Isolation, Enrichment, and Maintenance Procedures

Methylocystis

The species of *Methylocystis* were isolated from different sources as well as from different geographical regions as in *Table 16.3*. All species were cultivated on nitrate mineral salt (NMS) medium, except *M. heyeri* which was obtained using liquid medium M2 at pH 5.5 (Dedysh et al. 1998), and pure culture isolated from gellan gum (Gel-Gro, ICN Biomedicals) agar.

Methylosinus

The source of *Methylosinus* species was from NCIMB. They were grown under a methane-air-CO_2 (5:4:1) atmosphere, at 28 °C, on NMS agar.

Table 16.1
Phenotypic properties of the genera belonging to *Methylocystaceae*

	Genus						
	Albibacter	*Hansschlegelia*	*Methylocystis*	*Methylopila*	*Methylosinus*	*Pleomorphomonas*	*Terasakiella*
Shape	Rod shaped	Rods/cocci	Reinforms, coccobacilli/rods	Rod shaped	Pyriform/vibrioid	Pleomorphs/rods	Spirilla anticlockwise helix
Size (μm)	0.9–1.0 × 1.2–1.8	0.6–0.9 × 1.0–1.5	0.3–1.2 × 0.5–4.0	0.5–1.2 × 0.8–2.0	0.5–1.0 × 1.5–3.0	0.3–1.0 × 1.0–5.0	0.3–0.5 × 1.2–4.0
Motility	−	−	−	v	+	−	+
Pigment	−	−	−/pink-red/brown	−	−/brown/bright red	White	ND
Respiration	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	ND	Aerobic
Temp. (°C)	10–37	12–37	5–40	10–37	20–37	15–42	6–40
NaCl	< 3 %	< 2 %	−	0–2.5 %	−	ND	0.5–0.8 %
pH	6.0–9.0	5.0–9.0	4.5–9.0	5.0–10.0	5.5–9.0	6.0–8.0	6.0–9.0
G + C (mol %)	66.7	65.7–68.5	61.5–67.0	66–70.4	63–67	62.1–65.1	48
Nitrate reduction	+	+	+	+	−	+	+
Catalase	+	+	+	v	+	+	v
Oxidase	W	+	+	+	+	+	+
Urease	+	+	v	+	v	+	ND
Major quinone	Q-10	Q-10	Q-8	Q-10	Q-8	Q-10	Q-10
Major fatty acids	$C_{18:\ 1}\omega7$ $C_{16:0}$	$C_{18:1}\omega7$ $C_{16:0}$ $C_{19:0}$cyclo	$C_{18:1}\omega8c$ $C_{16:1}\omega8c$	$C_{18:1}\omega7$ $C_{18:0}$ $C_{16:0}$	$C_{18:1}\omega8c$	$C_{18:1}\omega7c$ $C_{19:0cyclo}\omega8c$ $C_{16:0}$	ND
Metabolism	Chemolithoheterotrophic and facultatively methylotrophic	Restricted facultative methylotrophic and chemolithotrophic	Chemolithotrophic	Chemoorganotrophic	Chemoheterotrophic	Phototrophic	Chemoheterotrophic

+ positive, − negative, *w* weakly positive, *v* variable, *ND* not determined

Table 16.2
Substrate utilization of genera belonging to the family *Methylocystaceae*

Substrate	Genus						
	Albibacter	*Hansschlegelia*	*Methylocystis*	*Methylopila*	*Methylosinus*	*Pleomorphomonas*	*Terasakiella*
Acetate	+	v		–		v	+
L-Alanine	+	–	+	–	+	–	
L-Arginine			+	–	+	–	
Citrate	+	–		–			+
Dimethylamine	–	–	–		–		
Ethanol	+	+		v		–	
Formate	+	+	–	+	–		
Fructose	+	–		v		+	
Fumarate	+	w		+		+	+
Glucose	+	–	–	v	–	+	
L-Glutamate	+	–	+	v	+	–	
Glycerol	+	+		+		+	+
L-Lysine			v	–	+	–	
Malate	+	–		v		v	+
Maltose	+	–		+		v	
D-Mannose	+	–		–		+	
Methane	–	–	+	–	+		
Methanol	+	+	+	+	+	–	
Methylamine	+	+	–	+	v		
Pyruvate	+	v		+		+	+
D-Sorbitol	+	–		v		+	
Succinate	+	w		+		–	+
Sucrose	+	v		v		+	
Trimethylamine	–	–	–	+	–		
D-Xylose	+	–		–		+	

Methylopila

The type species of the genus *M. capsulata* was isolated from Uzbekistan. The culture was incubated with medium K and maintained on medium K solidified with Difco Bacto agar or PYG agar. The strains of *M. helvetica* were isolated from soil and water samples in the Netherlands, Germany, and Switzerland. They were grown on medium K at 29 °C and stored in the freeze-dried form with skim milk at −80 °C for long-term storage. *M. jiangsuensis* was isolated from an activated sludge of a synthetic pyrethroid-manufacturing wastewater treatment facility on medium K. Colonies were maintained on the medium K supplemented with 0.5 % methanol at 30 °C.

Albibacter

Albibacter methylovorans was first isolated in Switzerland from groundwater samples contaminated with DCM. Colonies were isolated on DCM agar, and pure cultures were maintained on minimal medium K (2 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 0.025 g $MgSO_4.7H_2O$, and 0.5 g NaCl, pH7.2).

Terasakiella

T. pusilla strains are typically maintained by stab culture in medium 325 (IFO 1996) semisolid agar. The medium contains 1.0 % (w/v) polypeptone (Difco), 0.2% yeast extract (Difco), 0.05 % $MgSO_4.7H_2O$, 75 % (w/v) seawater, 25 % (w/v) distilled water, and 0.2 % agar. The pH should be adjusted to 7.2 by the addition of 1 MNaOH. Cultures require approximately 3 days of incubation at 20 °C.

Pleomorphomonas

The type species of the genus *P. oryzae* was isolated from *Oryza sativa* in Japan in 1982. Strains were grown in nitrogen-free medium (10 g glucose, 0.1 g $CaCl_2.2H_2O$, 0.1 g $MgSO_4.7H_2O$,

Table 16.3
Source and isolation site for genus *Methylocystis*

Species	Strain(s)	Source or isolation site
M. echinoides	IMET 10491^T	IMET[a]
M. heyeri	H2^T = DSM 16984^T = VKM B-2426^T	Lake Teufelssee, Germany
M. hirsuta	CSC1^T = ATCC BAA-1344^T = DSM 18500^T	Uncontaminated groundwater aquifer, Moffett Naval Air Station, Mountain View, CA, USA
M. parvus	ACM 3309^T = NCIMB 11129^T = ATCC 35066^T = IMET 10483^T JB21 JB22, JB47, JB48, JB51, JB52 JB29, JB30 JB33 JB190 JB191, JB192, JB196, JB198	NCIMB[a] Coal mine drainage water Soil Raw water Lake sediment Marsh mud Creek mud
M. rosea	SV97^T = DSM 17261^T = ATCC BAA-1196^T	Soil core, high Arctic wetland near the Ny-Ålesund settlement (78° 56′ N 11° 53′ E), Svalbard Islands, Norway

[a]Abbreviations: *IMET* Institute of Medical and Experimental Therapy, Jena, Germany; *NCIMB* National Collection of Industrial and Marine Bacteria, Aberdeen, UK

0.9 g K_2HPO_4, 0.1 g KH_2PO_4, 5 g $CaCO_3$, 10 mg $FeSO_4.7H_2O$, and 5.0 mg $Na_2MoO_4.2H_2O$, pH 7.3) at 25 °C. *P. koreensis* was isolated from a contaminated culture of the phototrophic bacterium R. palustris by direct plating on R2A agar. Colonies were maintained on R2A agar/broth at 30 °C and stored as glycerol suspension (20 %, w/v) at −70 °C for long-term storage.

Hansschlegelia

The type species of the genus *H. plantiphila* was first isolated from the linden *Tilia cordata* L. and lilac *Syringia glauca* L. buds, as well as the needles of the blue spruce *Picea pungens var. glauca* L., in February 2003, from Pushchino City, Moscow, at −17 °C. Plant samples were first incubated with medium K (2 g $(NH_4)_2SO_4$, 2 g KH_2PO_4, 0.5 g NaCl, 0.125 g $MgSO_4.7H_2O$, 0.02 g $FeSO_4.7H_2O$, 0.2 mL yeast autolysate, and 10 mL methanol, pH 7.2) at 29 °C for 5 days. Pure cultures were obtained after subculturing twice and exhaust plating on K medium agar. On the other hand, the second species of the genus *H. zhihuaiae* was isolated from sulfonylurea herbicide-contaminated farmland soil in Jiangsu, China. Soil samples were incubated with mineral salt medium (MSM) (1 g NaCl, 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.1 g $MgSO_4.7H_2O$, 0.025 g $FeSO_4$, and 10 mL trace element solution, pH 7.0) supplemented with glucose and 50 mg metsulfuron-methyl as the sole carbon source at 30 °C for 7 days. Cultures were obtained after the fifth subculturing, followed by serial dilutions in MSM for the enrichment of strains that were capable of degrading metsulfuron-methyl. Pure cultures were

Table 16.4
Antibiotic sensitivity/resistance of selected species of *Methylocystaceae*

	Albibacter methylovorans	*Hansschlegelia zhihuaiae*	*Methylopila jiangsuensis*
Ampicillin	S	R	R
Chloramphenicol	ND	S	ND
Doxycycline	ND	S	ND
Erythromycin	ND	R	ND
Gentamicin	S	S	R
Kanamycin	S	S	S
Lincomycin	S	R	ND
Nalidixic acid	S	ND	ND
Neomycin	S	S	ND
Novobiocin	S	ND	ND
Penicillin	R	R	ND
Polymyxin B	ND	S	ND
Rifampicin	R	S	ND
Streptomycin	ND	S	R
Tetracycline	S	S	ND
Vancomycin	ND	R	ND

S sensitive, *R* resistant, *ND* not determined

obtained by selecting the cultures that still exhibit metsulfuron-methyl degradation capability from the highest dilution and preserved in 25 % (v/v) glycerol solution in distilled water at −80 °C (Huang et al. 2007).

Pathogenicity and Clinical Relevance

There is only little information regarding the pathogenicity or clinical relevance on species belonging to family *Methylocystaceae*. However, a small amount of data is available on the antibiotic sensitivity for selected species (◉ *Table 16.4*).

Applications

The members of the family *Methylocystaceae* are known for their methanol-utilizing abilities as well as the use of the serine pathway for formaldehyde assimilation. *Albibacter methylovorans* and *Methylopila helvetica* have been reported to be able to utilize dichloromethane (DCM) or methylene chloride (CH_2Cl_2), industrial waste compounds which are toxic, mutagenic, and potentially carcinogenic (Doronina et al. 2000, 2001). *Hansschlegelia zhihuaiae* has been isolated for its degradation capability on a variety of sulfonylurea herbicides, and a novel sulfonylurea herbicide de-esterification esterase gene (*SulE*) was successfully identified from this strain (Hang et al. 2012). All *Methylocystis* species, with the exception of *M. parvus* OBB^T and *M. rosea* $SV97^T$, are able to express a soluble form of methane monooxygenase (sMMO) at low copper concentrations (Lindner et al. 2007). Recently, unidentified nitrogen-fixing *Pleomorphomonas* strains have been reported to be able to methylate mercury and produce hydrogen sulfide (Achá et al. 2012); however, the isolated strains are yet to be validly described.

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17 The Family *Parvularculaceae*

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Abstract
Parvularculaceae, a family within the order *Parvularculales* in the *Alphaproteobacteria*, embraces the genus *Parvularcula*. It forms a deep branch in the *Alphaproteobacteria*, on the basis of 16S rRNA analysis, distinct from other orders. The genus *Parvularcula* currently comprises three species: *Parvularcula bermudensis,* a marine bacterium isolated from the western Sargasso Sea using dilution-to-extinction culturing; *Parvularcula lutaonensis,* a moderately thermotolerant marine bacterium recovered from a coastal hot spring; and *Parvularcula dongshanensis,* recently isolated from soft coral. *Parvularcula* isolates are Gram-negative, strictly aerobic, chemoheterotrophic, slightly motile short rods with a single flagellum. Colonies on marine agar are very small (0.3–0.8 mm in diameter), yellowish-brown, and very hard. They are oxidase positive and catalase negative. Phylogenetically, the genus forms a novel seventh order of the *Alphaproteobacteria*. The type species of the genus is *Parvularcula bermudensis*.

Taxonomy, Historical and Current

Short Description of the Family

Par.vu.lar.cu.la.' ce.ae. M. L. fem.n. *Parvularcula* type genus of the family; −aceae ending to denote a family; M. L. fern. pl.n. *Parvularculaceae* the *Parvularcula* family.

Phylogenetically the sole member of the order *Parvularculales*, the seventh order in the class *Alphaproteobacteria*, the family *Parvularculaceae* was circumscribed on the basis of phylogenetic analysis of 16S rRNA sequences (Cho and Giovannoni 2003). Distantly related to the orders *Rhodobacterales* and *Rhizobiales* in the neighbor-joining phylogenetic tree, the 16S rRNA sequence similarities of *Parvularculaceae* strains to the other members of these orders were less than 89.4 % (*Fig. 17.1*). The family comprises a single genus *Parvularcula* proposed by Cho and Giovannoni (2003).

According to the phylogenetic branching of 72 completely or nearly completely sequenced *Alphaproteobacteria* in a species tree produced from a concatenation of alignments for 104 well-behaved protein families, *Parvularculaceae* is related to *Caulobacterales* and *Hyphomonadaceae* (Williams et al. 2007). A characteristic linking the *Hyphomonadaceae* and Caulobacterales, a proliferation of TonB-dependent receptors (Badger et al. 2006), is shared by *Parvularcula*; however, this characteristic may not be highly distinctive (Blanvillain et al. 2007). The robust specie tree for *Alphaproteobacteria* suggest a unification of *Caulobacterales* and *Hyphomonadaceae* within the order *Caulobacterales*, additionally including the Parvularculaceae, with the abandonment of the order *Parvularculales* (Williams et al. 2007). Comprehensive comparative genome analysis of the single *Parvularcula* genome will be a significant next step in considerate the family phylogenetic affiliation.

Uncultured *Parvularculaceae* bacteria clones identified by 16S ribosomal RNA have been recently documented in several environmental samples, exemplified by marine sediment, *Siderastrea stellate* coral bleached colonies (Lins-de-Barros et al. 2012), conventional activated sludge processes from petroleum refineries (Silva et al. 2010), shoreline environments affected by the prestige oil spill (Alonso-Gutierrez et al. 2009), and biofilms (D'Auria et al. 2010).

Molecular Analyses

Genome Structure

Presently only the complete genome sequence of *Parvularcula bermudensis,* the type strain branching within the 16S rRNA gene tree of *Parvularculaceae*, has been released (Oh et al. 2011). The 2,902,643 bp long single replicon genome is part of the Moore Foundation Microbial Genome Sequencing Project.

E. Rosenberg et al. (eds.), *The Prokaryotes – Alphaproteobacteria and Betaproteobacteria*, DOI 10.1007/978-3-642-30197-1_375,

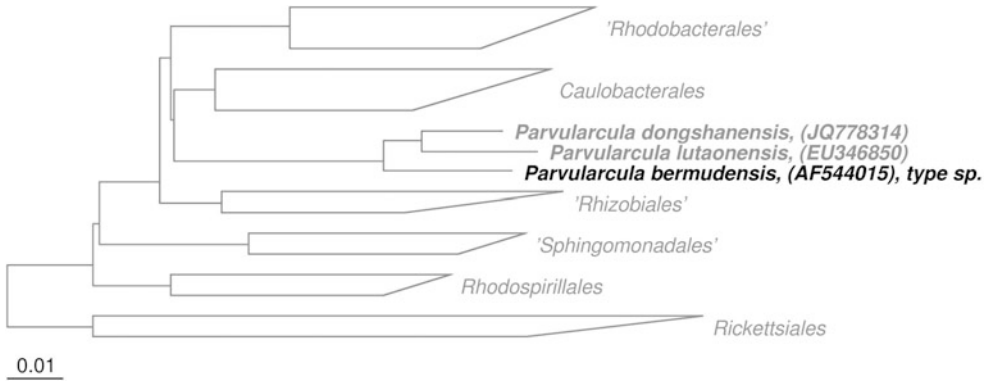

Fig 17.1
Neighbor-joining tree showing the phylogenetic positions of *Parvularcula* representatives *Parvularcula dongshanensis* (JQ778314), *Parvularcula lutaonensis* (EU346850), *Parvularcula bermudensis* (AF544015), type sp., and representatives of the α-Proteobacteria, inferred from 16S rRNA gene sequence analyses

The complete genome sequence of *P. bermudensis* HTCC2503^T is 2,902,643 bp long, contains 2,687 open reading frames, including one 16S-23S-5S rRNA operon with 43 tRNA genes, and the mol% G+C of DNA is 60.0 %. *Strain HTCC2503T* is predicted to hold complete metabolic pathways, including glycolysis, the pentose phosphate pathway, tricarboxylic acid cycle, and amino acid synthesis. The genome also codes for genes for DMSP demethylase, type IV secretion/conjugal transfer systems, and Ton and Tol transport systems as well as a plethora of genes for cobalt-zinc-cadmium resistance. In accord with the previous specie description (Cho and Giovannoni 2003), the *P. bermudensis* genome is predicted to code for genes for carotenoid biosynthesis, lactamase, flagella synthesis, and various catalytic enzymes for utilizing carbon compounds. *Parvularcula bermudensis* replicon is represented in ❯ *Fig. 17.2*, where the distribution of two-component system (TCS) genes is summarized. The TCS is the dominant prokaryotic-signaling pathway, typically comprised of a pair of signal-transduction proteins - a histidine kinase (HK) and partner response regulator (RR) – both of which are multi-domain proteins, which regulate a wide range of physiological processes. P2CS, a database of prokaryotic two-component systems (Barakat et al. 2011), predicted 39 HK and 33 RR proteins in the *P. bermudensis* genome (❯ *Fig. 17.2*).

Phenotypic Analyses

The family *Parvularculaceae* currently comprises a single genus, *Parvularcula.*

Parvularcula (Par.vu.lar'cu.la. L. adj. parvulus very small; L. fem. n. arcula a jewel-casket; N.L. fem. n. Parvularcula a very small jewel-casket) forms a deep branch in *Alphaproteobacteria.* The cells are Gram-negative, strictly aerobic short rods that occur singly, are sometimes coccoid, multiply by binary fission, and are slightly motile with a single flagellum (❯ *Fig. 17.3*). Endospores and poly-β-hydroxybutyrate granules are not formed. Colonies on marine agar are very small (0.3–0.8 mm in diameter), yellowish-brown, circular, dry, and very hard. They produce carotenoid pigments but not bacteriochorophyll a, and they are catalase-negative and oxidase-positive. Nitrate is reduced to nitrite. Urea and gelatin are hydrolysed, but aesculin is not. Chemoheterotrophic and moderately halophilic, they require NaCl for growth. Cellular fatty acids are even-numbered monounsaturated or saturated fatty acids. The major fatty acid is cis-7-octadecenoic acid (73.3 %) (Cho and Giovannoni 2003).

The genus comprises three species based on 16S rRNA gene sequences phylogenetic analysis (❯ *Fig. 17.1*). The genus type species is *Parvularcula bermudensis,* named for the Bermuda Islands, the geographical origin of the type strain of the species (Cho and Giovannoni 2003). *Parvularcula* also encompass the species *Parvularcula lutaonensis*, named for Lutao, a small volcanic island off the eastern coast of Taiwan (Arun et al. 2009), and *Parvularcula dongshanensis,* named for Dongshan, a city in Fujian, China, where the type strain was first isolated (Yu et al. 2012).

The main phenotypic characteristics as well as the differences among *Parvularcula* members—*Parvularcula bermudensis*, *Parvularcula lutaonensis,* and *Parvularcula dongshanensis*—are given in ❯ *Table 17.1*.

Isolation, Enrichment, and Maintenance Procedures

All *Parvularculaceae* members were isolated from marine environments. The first described member of the family, *Parvularcula bermudensis,* was isolated using high-throughput culture methods that rely on dilution to extinction in very-low-nutrient media (Connon and Giovannoni 2002). Oligotrophic seawater was diluted to 10 cells ml^{-1} in low-nutrient heterotrophic medium (0.2 mm-filtered and autoclaved sea water, modified with 1.0 mMNH_4Cl and 0.1 mMKH_2PO_4), supplemented with 0.001 % (w/v) D-glucose, D-ribose, succinic acid, pyruvic acid, glycerol and N-acetyl-D-glucosamine, 0.002 % (v/v) ethanol and Va vitamin solution at 10^{-4} dilution

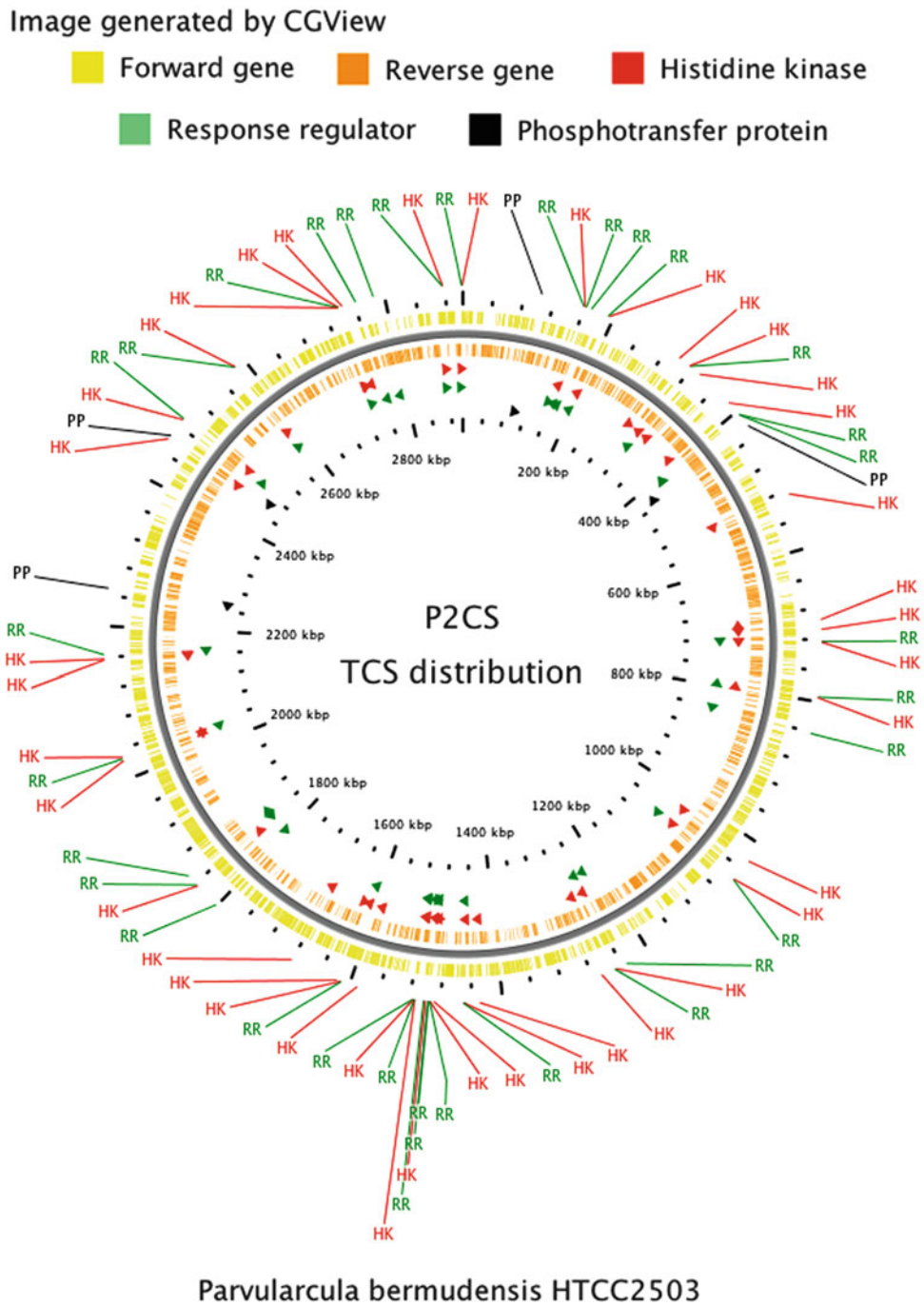

Fig. 17.2
Distribution of two-component system (TCS) genes, the dominant prokaryotic-signaling pathway, around *Parvularcula bermudensis* replicon. CGView representation generated using P2CS (database of prokaryotic two-component systems) (Barakat et al. 2011)

(Davis and Guillard 1958). Microtiter dish wells were scored for growth by microscopic examination, using a procedure for creating cell microarrays (Connon and Giovannoni 2002). Positive cultures were spread onto marine agar (MA), incubated at 25 °C for 14 days, purified by subsequent streaking onto MA, and stored as 10 % (v/v) glycerol suspensions in liquid nitrogen (Cho and Giovannoni 2003).

The moderately thermotolerant marine bacterium *Parvularcula lutaonensis* was isolated from a bacterioplankton community of a coastal hot spring (temperature 55 °C; pH 7.3; salinity 32 %). Isolation and maintenance was performed on marine agar after 37 °C for 48 h incubation (Arun et al. 2009). The last member currently described within *Parvularculaceae* family, *Parvularcula dongshanensis,* was isolated from soft

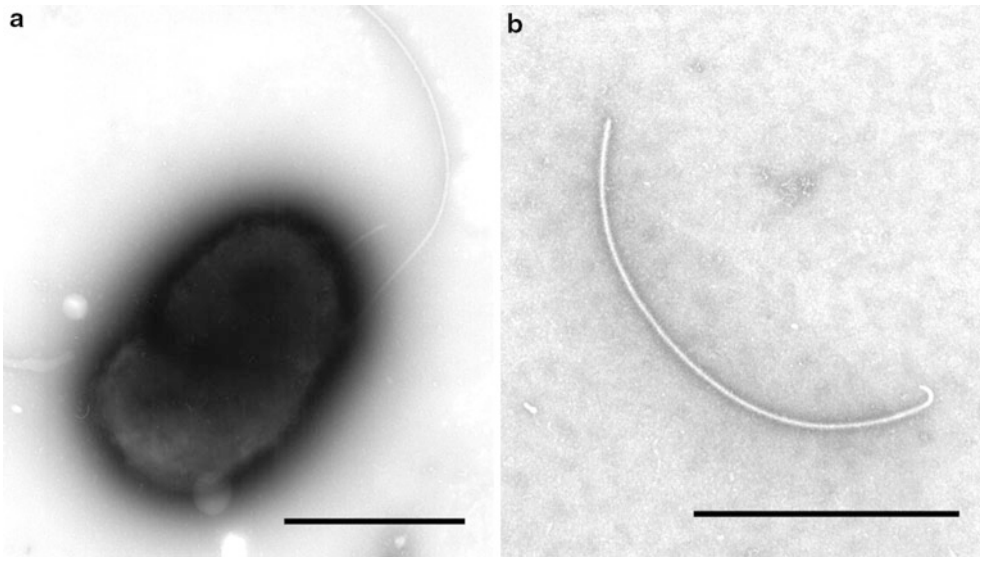

Fig 17.3
Electron micrographs of negatively stained cells of *Parvularcula bermudensis* (strain HTCC2503T). (a) Cell with single flagellum and fimbrae; (b) detached flagellum with hook at one end. Bars, 1 μm (Cho and Giovannoni 2003)

Table 17.1
Differentiating phenotypic properties of *Parvularcula* strains

Characteristic	P. bermudensis	P. lutaonensis	P. dongshanensis
Cell size (μm)	0.4–1.3 × 0.6–1.8	0.3–0.8 × 1–3	0.8–0.9 × 1.4–1.5
NaCl range for growth (%, w/v) (optimum)	0.75–25 (3)	0.5–6 (3)	0–12 (1–3)
Temperature range for growth (°C) (optimum)	10–37(30)	25–50(37)	16–41 (28)
Cystineaminopeptidase, leucineaminopeptidase, lipase (C14), valineaminopeptidase	w	w	–
α-Galactosidase	–	+	+
α-Glucosidase, β-Galactosidase, β-Glucosidase	–	+	+
β-Glucuronidase	–	w	+
Esterase (C4)	w	+	w
Reduction of nitrate to nitrite	+	–	–
Urease	+	–	–
D-Glucose	+	+	–
D-Mannitol, L-arabinose	+	–	–
Malic acid	–	w	–
Potassium gluconate	–	w	+
Adipic acid	–	–	w
Susceptibility to antibiotics			
Ciprofloxacin (5 μg), gentamincin (10 μg), kanamycin (30 μg), ofloxacin (5 μg), tetracycline (30 μg), vibramycin (30 μg)	+	+	–
Lincomycin (2 μg), penicillin G (10 μg), clindamycin (2 μg)	+	–	+
Polymyxin B (30 IU)	+	–	–
DNA G+C content (mol %)	60.8	59.0	61.8

Characteristics are scored as: w, weak; +, positive; –, negative

coral. Small pieces of coral tissue extensively washed with sterilized seawater were macerated with 0.85 % sterilized NaCl solution, spread onto MA plates, and incubated aerobically for 7 days at 25 °C without light (Yu et al. 2012).

Ecology

Habitat

The main habitat of the Parvularcula group is the marine environment. However, each species currently described was recovered from a distinct characteristic site. The type species of the genus, *Parvularcula bermudensis*, was isolated from an oligotrophic region in the western Sargasso Sea, Atlantic Ocean. Two strains of *Parvularcula bermudensis* (HTCC2503^T and HTCC2517) were isolated from 10-m depth seawater at the Bermuda Atlantic Time Series (BATS) station using dilution-to-extinction culturing (Cho and Giovannoni 2003). *Parvularcula lutaonensis* (strain CC-MMS-1T), a thermotolerant bacterium, was recovered from water samples collected from a rare coastal hot spring located in a small volcanic island off the east coast of Taiwan (Arun et al. 2009). A single symbiotic species belonging to *Parvularcula* genus was identified at present. *Parvularcula dongshanensis* (strain SH25^T) was isolated from soft coral collected from Dongshan Island, Fujian Province, China (Yu et al. 2012).

The sampling of diverse environments, such as soil and marine sediment, has recovered several uncultivable members of *Parvularculaceae* family. The apparent rarity of this order may be due to most species being uncultured, rather than an actual scarcity in the environment. Frequently, numerous environmental nongrowers require growth factors from other bacteria. In a siderophore-based approach, D'Onofrio and colleagues (2010) have enabled the culturing of previously uncultured isolates from marine sediment biofilm grow on a Petri dish in the presence of cultured organisms from the same environment. A single *Parvularculaceae* isolate KLE1250 was recovered in this study, showing 93.7 % identity by 16S rRNA gene sequence to *Parvularcula bermudensis* KCTC 1208T, was suggested to represent a new genus within this putative order (D'Onofrio et al. 2010).

Culture Independent-Studies

Nonculture molecular techniques have been increasingly applied in order to characterize the microbial communities (bacteria and fungi) associated with diverse eukaryotic hosts. Comparative metagenomics of the community structure and functional profile of bacteria on healthy and diseased thalli of the red seaweed *Delisea pulchra* have shown that the phylogenetic differences between healthy and bleached communities mainly reflected relative changes in few taxa, including Parvularcula (Fernandes et al. 2012). Taxonomically, healthy algae tissue was characterized by the abundance of bacteria belonging to the genera *Parvularcula* and *Haliscomenobacter* and the family *Rhodobacteraceae*. While the ecological roles of these bacterial associates that dominate the community of healthy *D. pulchra* is not clear, it was proposed they might be involved in the biogeochemical cycling of sulfur because the genus *Parvularcula* also harbors genes for the degradation of the abundant algal osmolyte dimethylsulfoniopropionate (DMSP) (Oh et al. 2011) and are known to oxidize inorganic sulfur metabolites (Sorokin 2005), such as the byproducts of DMSP degradation (Wagner-Döbler and Biebl 2006). Additionally, pyrosequencing of bacterial symbionts within *Axinella corrugata* sponges have highlighted taxa not typically previous found in sponge symbiont surveys, including *Parvularcula sp.* (White et al. 2012).

Still, the characterization of the bacterial and fungal communities associated with different lesion sizes of dark spot syndrome occurring in the coral *Stephanocoenia intersepta* has pointed to a possible pathogenic role for *Parvularcula* symbionts (Sweet et al. 2013). In this study, only four bacteria were absent from healthy tissues, increased in apparently healthy tissues, and were dominant in all the diseased tissues; these included ribotypes related to a *Corynebacterium sp.* (KC190237), an *Acinetobacter sp.* (KC190251), a *Parvularculaceae sp.* (KC190270), and an *Oscillatoria sp.* (KC190271).

Pathogenicity, Clinical Relevance

Currently, all described *Parvularculaceae* strains were recovered from environmental studies. No information on human pathogens is reported. However, a recent metagenomic study has highlighted an uncultured *Parvularculaceae sp.* as a potential coral pathogen (Sweet et al. 2013).

P. bermudensis is sensitive by the agar diffusion method to ciprofloxacin, gentamincin, kanamycin, ofloxacin, tetracycline, vibramycin, lincomycin, penicillin G, clindamycin, and Polymyxin B.

P. lutaonensis cells are susceptive to ciprofloxacin, gentamincin, kanamycin, ofloxacin, tetracycline, vibramycin, but resistant to lincomycin, penicillin G, clindamycin, and Polymyxin B.

P. dongshanensis cells are susceptive to lincomycin, penicillin G, and clindamycin. Cells are resistant to ciprofloxacin, gentamincin, kanamycin, ofloxacin, tetracycline, vibramycin, and Polymyxin B.

Applications

Evidence that the *Parvularculaceae* family may be involved in biotechnological applications originates from a study by Silva et al. (2010), where the bacterial diversity of petroleum refineries wastewater treatment systems were investigated through 16S rRNA gene libraries. Sequencing and phylogenetic analysis showed that the bacterial community composition of

conventional activated sludge (CAS) clones were mostly related to class *Alphaproteobacteria*, represented by uncultured bacteria related to the order *Parvularculales* (Silva et al. 2010). *Parvularcula* sp. clone identification at higher taxonomic ranks was not possible by comparison of 16SrRNA gene sequences with databases and subsequent phylogenetic analyses, suggesting that the corresponding organisms may represent new taxa.

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18 The Family *Phyllobacteriaceae*

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Abstract
The family *Phyllobacteriaceae* belongs to the order *Rhizobiales* in the *Alphaproteobacteria* and currently comprises the 72 species in 13 genera: *Ahrensia*, *Aliihoeflea*, *Aminobacter* (including *Chelatobacter*), *Aquamicrobium* (including *Defluvibacter*), *Chelativorans*, *Hoeflea*, *Lentilitoribacter*, *Mesorhizobium*, *Nitratireductor*, *Phyllobacterium*, *Pseudahrensia*, *Pseudaminobacter*, and *Thermovum*. They form a single cluster within the 16S rRNA gene phylogeny. The family consists of environmental (soil, water) and plant-associated bacteria that have a heterotrophic respiratory metabolism with oxygen as terminal electron acceptor. One *Aquamicrobium* species can use nitrate as an alternative terminal electron acceptor. One *Mesorhizobium* species is facultatively chemolithotrophic using thiosulfate or elemental sulfur as sole energy source. *Candidatus Liberibacter*, a group of uncultivated phloem-inhabiting bacteria that are associated with various plant diseases in citrus and *Solanaceae* or are endophytic in pear plants, is also associated with the family. However, comprehensive phylogenetic analyses indicate the position of this group as a member of the *Phyllobacteriaceae* is uncertain.

Taxonomy, Historical and Current

Short Description of the Family

Phyl.lo.bac.te.ri.a'ce.ae. N.L. neut. n. *Phyllobacterium*, type genus of the family; suff. *-aceae*, suffix to denote a family; N.L. fem. pl. n. *Phyllobacteriaceae*, the *Phyllobacterium* family.

The family *Phyllobacteriaceae* belongs to the *Rhizobiales* order in the *Alphaproteobacteria* class of the phylum Proteobacteria. It was proposed by Mergaert and Swings (2005a) in *Bergey's Manual of Systematic Bacteriology* and was validated in 2006. At the time the family comprised six genera and one *Candidatus* genus: *Phyllobacterium, Aminobacter*, *Aquamicrobium*, *Defluvibacter*, *Candidatus Liberibacter*, *Mesorhizobium*, and *Pseudaminobacter*. *Defluvibacter* has since been transferred to *Aquamicrobium* (Kämpfer et al. 2009). *Aminobacter* includes *Chelatobacter heintzii* which is regarded as a later subjective synonym of *Aminobacter aminovorans* (Kämpfer et al. 2002). The basis for the proposal of this family was that these genera form a cluster in the 16S rRNA gene phylogeny. The description of the family (Mergaert and Swings 2005a) is rather brief: "Rod-shaped, ovoid, or reniform cells when cultured in vitro. Nonsporeforming. Gram negative. Aerobic. Cells cultured in vitro are motile by means of polar, subpolar, or lateral flagella. Strains grow well on complex solid media at 28 °C. Occur in leaf nodules and the rhizosphere of higher plants. The mol % G+C of the DNA is 60–62". With the inclusion of the additional genera *Ahrensia*, *Chelativorans*, *Hoeflea*, *Lentilitoribacter*, *Nitratireductor*, *Pseudahrensia*, and *Thermovum* to the *Phyllobacteriaceae* cluster, most of this definition still applies except that cells can also be nonmotile and members of the family can also occur in seawater, marine sediments, activated sludge, and soil and

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thermophilic members are found in compost. The range of the G+C content of DNA is 48–65 %. Comprehensive phylogenetic analysis reveals that the position of *Candidatus Liberibacter* as a member of the *Phyllobacteriaceae* cluster is uncertain (see below).

Phylogenetic Structure of the Family and Its Genera

In the 16S rRNA gene phylogeny, the *Phyllobacteriaceae* family forms a single cluster in the phylum Alphaproteobacteria, and inside this large cluster, the different species generally group together per genus, in support of the current taxonomy (Fig. 18.1).

An important exception is the genus *Mesorhizobium*: these species make up several groups and separate lineages grouping in between the other genus clusters (Fig. 18.1). The type species *Mesorhizobium loti* forms tight subcluster with *Mesorhizobium ciceri*, *Mesorhizobium australicum*, *Mesorhizobium shangrilense*, *Mesorhizobium sangaii*, and *Mesorhizobium qingshengii*. *Mesorhizobium chacoense* forms a separate but related lineage, and the *Aminobacter* cluster is their nearest neighbor. Nineteen species form the largest subcluster: *Mesorhizobium metallidurans*, *Mesorhizobium temperatum*, *Mesorhizobium mediterraneum*, *Mesorhizobium gobiense*, *Mesorhizobium tarimense*, *Mesorhizobium caraganae*, *Mesorhizobium robiniae*, *Mesorhizobium muleiense*, *Mesorhizobium tianshanense*, *Mesorhizobium tamadayense*, *Mesorhizobium amorphae*, *Mesorhizobium septentrionale*, *Mesorhizobium huakuii*, *Mesorhizobium plurifarium*, *Mesorhizobium silamurunense*, *Mesorhizobium opportunistum*, *Mesorhizobium abyssinicae*, *Mesorhizobium hawassense*, and *Mesorhizobium shonense*. *Mesorhizobium albiziae* groups at the periphery of this subcluster as does *Mesorhizobium thiogangeticum*. The position of the two latter species, however, varied depending on the filter applied. In most trees, it constitutes a separate lineage at some distance from other *Mesorhizobium* subclusters or other genera of the family. *Mesorhizobium thiogangeticum* was not recovered from legume nodules but was isolated from soil by enrichment using reduced sulfur compounds as sole electron sources; it is the only *Mesorhizobium* species reported to be facultatively chemolithoautotrophic. Two other species, *Mesorhizobium camelthorni* and *Mesorhizobium alhagi*, make up a further subcluster that groups most closely to the *Chelativorans* cluster. The 16S rRNA gene phylogeny of *Mesorhizobium* is thus polyphyletic, and the genus may in the future require taxonomic rearrangements if further evidence would support these observations.

Ahrensia and *Lentilitoribacter* group together with *Hoeflea* species, but, according to branch length, are clearly distinct.

Nitratireductor species form a single cluster, except for *Nitratireductor basaltis* which is located separately.

Candidatus Liberibacter, consisting of psyllid-transmitted, as yet uncultured, phloem-limited bacteria associated with greening disease or huanglongbing disease of citrus and yellows disease of various *Solanaceae* plants or endophytic in pear plants, was initially placed inside the *Phyllobacteriaceae* based on a limited phylogenetic indications (Mergaert and Swings 2005a; Garnier 2005). A more comprehensive analysis performed for this chapter revealed that the position of *Candidatus Liberibacter* as a member of the *Phyllobacteriaceae* cluster is uncertain. Although it does group on a long branch inside the *Phyllobacteriaceae* cluster in Fig. 18.1, in most other trees calculated using other filters and including other neighboring taxa, *Candidatus Liberibacter* grouped outside the family and occupied a separate position in the *Alphaproteobacteria*. Its membership of the family therefore seems not strongly supported by 16S rRNA phylogeny.

Comments on the Membership of the Family

Although the genus *Ahrensia* is was classified in the *Rhodobacteraceae* in *Bergey's Manual of Systematic Bacteriology* (Garrity et al. 2005) based on the phylogenetic analysis of 16S rRNA genes, since then more taxa have been described in the *Rhizobiales*, and current 16S rRNA gene phylogeny places *Ahrensia* in the *Phyllobacteriaceae* (Living Tree Project, release 111). It is therefore included in this chapter.

"*Aliihoeflea aestuarii*" gen. nov., sp. nov. was described for a bacterium isolated from tidal flat sediments (Roh et al. 2008). Its 16S rRNA gene sequence was reported to cluster with members of the *Phyllobacteriaceae*. Fatty acid data, quinone, and DNA G+C content data are also in agreement with the family characteristics; therefore, although *Aliihoeflea aestuarii* has as yet not been included in a validation list, it is included here in the chapter on *Phyllobacteriaceae*.

Molecular Analyses

DNA-DNA Hybridization Studies

In all multispecies genera of the family, DNA-DNA hybridizations with existing species have been performed to justify proposals of new species.

Other Sequence Analyses

Genes other than the 16S rRNA gene have been reported in *Mesorhizobium* where recA sequences are available for all species and a number of other genes including *atpD*, *gyrB*, *dnaK*, and *rpoB* have also been reported for several of the *Mesorhizobium* species. However, for other *Phyllobacteriaceae* genera, only in a few cases have other genes been reported and used for phylogenetic purposes: for three of six *Aminovorans* species sequences are available for *atpD*, *dnaK*, and *recA* (Maynaud et al. 2012); for four of eight *Phyllobacterium* species, an *atpD* sequence has been reported, as well as a *recA* sequence for one species

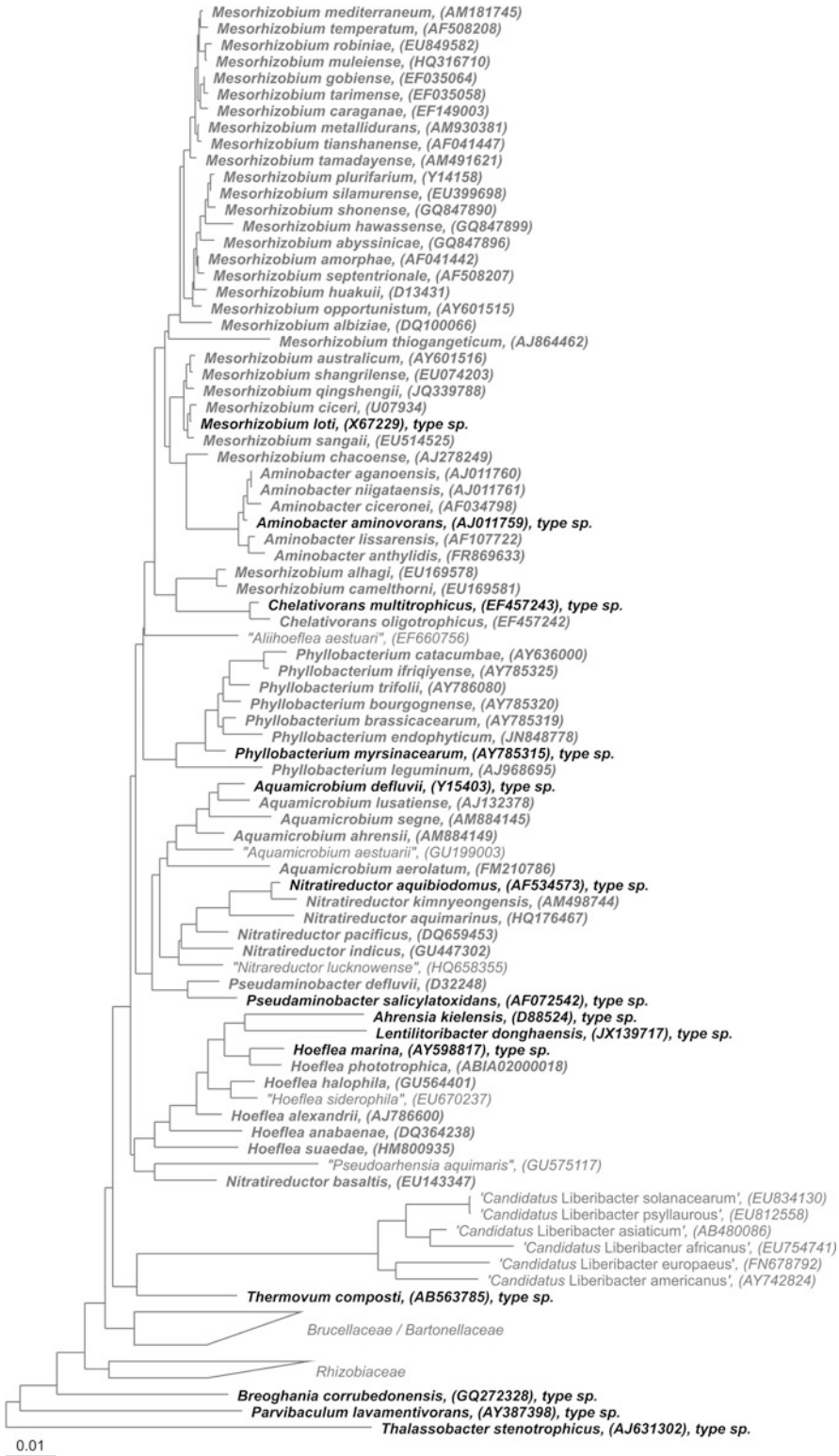

Fig. 18.1

Phylogenetic reconstruction of the family *Phyllobacteriaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (*LTP*) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

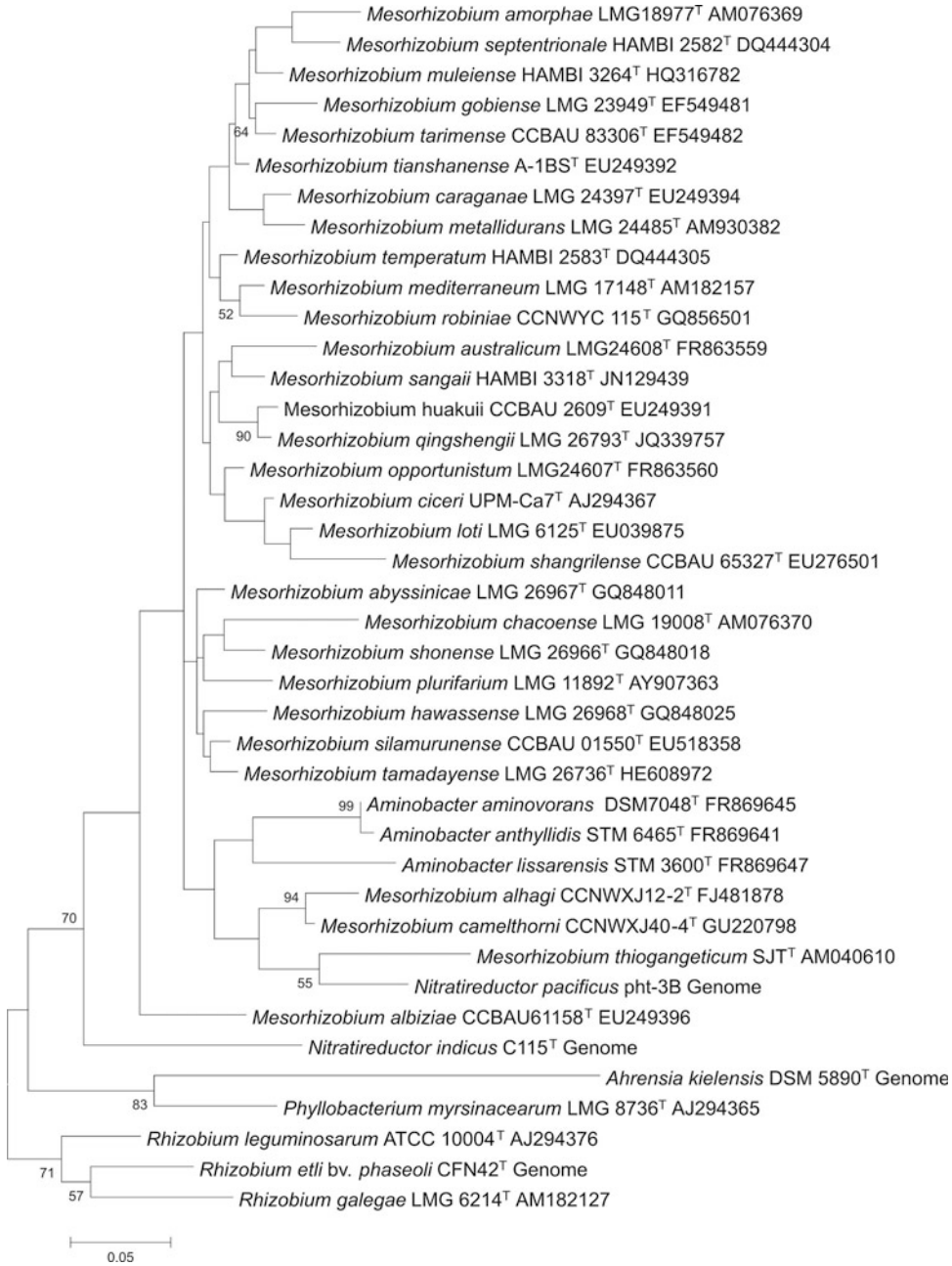

Fig. 18.2

Phylogenetic reconstruction of the family *Phyllobacteriaceae* based *recA* sequences. The tree was constructed using the maximum likelihood method and general time-reversible model in MEGA (Tamura et al. 2011). A bootstrap analysis with 500 replicates was performed to assess the support of the clusters; values above 50 % are shown at the nodes

(Mantelin et al. 2006b), and for two of six *Nitratireductor* species, an *rpoD* sequence is available (unpublished data available through NCBI dataportal). Given this lack of data for all genera, it is currently not possible to comprehensively assess the phylogeny of the family using a housekeeping gene other than the 16S rRNA gene. At present most data are available for *recA* where 6 of the 13 genera are represented (three of these are extracted from total genome information). Given that *Mesorhizobium* is represented with 30 species versus only 8 species from other genera, this tree (*Fig. 18.2*) does not permit a comprehensive comparison with the 16S rRNA gene phylogeny (*Fig. 18.1*). It thus remains to be established whether the subclusters of *Mesorhizobium* in the latter phylogeny are confirmed by *recA* or other gene phylogenies.

Table 18.1

Overview of genome sequences available for type strains of the *Phyllobacteriaceae*

Organism	Genome structure	Size (Mb)	GC%	Genes	Proteins	GenBank	Reference
Ahrensia kielensis DSM 5890^T	16 Contigs	3.36	48.0	3,287	3,233	ARFW01000000	JGI Project ID: 406534
Candidatus Liberibacter asiaticus psy62	1 Ch.	0.99	36.5	1,162	1,109	NC_012985	Duan et al. 2009
Candidatus Liberibacter solanacearum CLso-ZC1	1 Ch.	1.26	35.2	1,246	1,192	NC_014774	Lin et al. 2011
Chelativorans sp. NBC1	1 Ch., 3 pl.	4.94	61.1	4,684	4,543	CP000390.1, CP000389.1, CP000391.1, CP000392.1	JGI Project ID: 10690
Hoeflea phototrophica DFL-43 (DSM 17068)	22 Contigs	4.46	59.8	4,407	4,357	ABIA02000000	The Gordon and Betty Moore Foundation Marine Microbial Genome Sequencing Project; J. Craig Venter Institute
Mesorhizobium alhagi CCNWXJ12-2^T	375 Contigs	6.97	62.6	7,244	7,195	AHAM00000000	Zhou et al. 2012
Mesorhizobium amorphae CCNWGS0123	274 Contigs	7.29	62.9	7,136	7,084	AGSN00000000	Hao et al. 2012
Mesorhizobium australicum WSM2073^T	1 Ch.	6.2	62.8	6,075	5,792	CP003358.1	JGI Project ID: 47287
Mesorhizobium ciceri bv. *biserrulae* WSM1271	1 Ch., 1 pl.	6.69	62.6	6,532	6,264	CP002447.1, CP002448.1	JGI Project ID: 48991
Mesorhizobium loti MAFF303099	1 Ch., 2 pl.	7.6	62.5	7,333	7,272	BA000012.4, BA000013.4, AP003017.1	Kaneko et al. 2000
Mesorhizobium opportunistum WSM2075^T	1 Ch.	6.88	62.9	6,746	6,508	CP002279.1	JGI Project ID: 33861
Nitratireductor aquibiodomus RA22	95 Contigs	4.59	61.3	4,293	4,241	AJXZ00000000	Singh et al. 2012
Nitratireductor indicus C115^T	75 Contigs	4.99	60.8	4,872	4,824	AMSI01000000	Lai et al. 2012b
Nitratireductor pacificus pht-3B^T	51 Contigs	4.47	65.5	4,246	4,197	AMRM01000000	Lai et al. 2012a

Data obtained from the NCBI genome pages (http://www.ncbi.nlm.nih.gov/genome)

Genome Comparisons

Only in recent years have some complete genome sequences been reported or drafts made available. An overview of the type strains and a few other strains is given in *Table 18.1*. Unpublished draft genomes are available in public database online for *Ahrensia kielensis*, *Chelativorans* sp., *Hoeflea phototrophica*, *Mesorhizobium australicum*, *Mesorhizobium ciceri* bv. *biserrulae*, and *Mesorhizobium opportunistum* (*Table 18.1*).

The genome of *Mesorhizobium loti* MAFF303099 was reported more than 10 years ago (Kaneko et al. 2000); however, later this strain was shown to be a representative of another *Lotus* symbiont, *Mesorhizobium huakuii* bv. *loti* (Turner et al. 2002). Its genome consists of one chromosome (7 Mb) and two megaplasmids (352 kb and 208 kb); a transmissible symbiotic island containing 580 protein-encoding genes including genes for nodulation and nitrogen fixation was identified on the chromosome, inserted into the phe-tRNA gene as in other *Mesorhizobium loti* strains (Kaneko et al. 2000). The genomes of several other *Mesorhizobium* strains have been sequenced and had a similar size between 6.2 and 7.6 Mb with one circular chromosome and either no, one or two megaplasmids (*Table 18.1*). *Mesorhizobium amorphae* CCNWGS0123 is a copper-resistant rhizobium that contributes to survival of the

host plant in copper-, zinc-, and chromium-containing environments. Its genome was found to harbor numerous genes involved in copper resistance including a copper efflux system and multicopper oxidases, as well as various genes for plant growth promotion that most rhizobia share. In addition genes involved in the biosynthesis of a number of antibiotics and in chloramphenicol resistance were present (Hao et al. 2012). *Mesorhizobium alhagi* CCNWXJ12-2^T is very resistant to salt (0.8 M) and alkali (pH 12). Its genome was found to encode various systems contributing to salt resistance and osmoregulation including multiple membrane transport system (Zhou et al. 2012).

The genomes have been reported for three *Nitratireductor* strains. For the type species, *Nitratireductor aquibiodomus* strain RA22 from a marine water sample in India has been sequenced; its 16S rRNA gene was reported as 100 % identical to that of the type strain. Annotation revealed genes for iron acquisition, ammonia and sulfur assimilation, biosynthesis of ectoine and betaine, and uptake of choline and betaine, indicative of its marine habitat requiring osmotic stress tolerance. Genes for catabolism of aromatic compounds, including genes for the chloroaromatic degradation pathway, correspond with the observation that many *Nitratireductor* strains were obtained from sources contaminated with pyrene, crude oil, or pesticide (Singh et al. 2012). *Nitratireductor pacificus* pht-3B^T, although isolated from a pyrene-degrading consortium from deep-sea sediments, is unable to utilize pyrene, and this was confirmed by the absence of polycyclic aromatic hydrocarbon (PAH)-degrading dioxygenase in its genome (Lai et al. 2012a). *Nitratireductor indicus* C115^T originates from a crude oil-degrading consortium from deep seawater; however, it cannot degrade *n*-alkanes or PAHs as sole carbon source. Also here, the genome sequence confirmed the absence of any alkane-degrading monooxygenase or PAH-degrading dioxygenase (Lai et al. 2012b).

Two complete genomes have been reported representing *Candidatus Liberibacter*, a group of uncultured bacteria associated with *citrus* and *Solanaceae* plant diseases; genomes were obtained from DNA isolated from the phloem-feeding psyllid vectors that transmit the pathogen (Duan et al. 2009; Lin et al. 2011). As can be expected for obligate intracellular endophytes, the genomes are small and have a low GC content: 0.99 Mb and 36.5 mol % and 1.26 Mb and 35.2 mol % for *Candidatus Liberibacter asiaticus* psy62 and *Candidatus Liberibacter solanacearum* CLso-ZC1, respectively. *Candidatus Liberibacter asiaticus* psy62 harbored few genes for the biosynthesis of compounds that can be obtained from the host and more genes for motility such as type IV pili and flagellar genes; it had no transposons or insertion elements but did have some phage-related genes (Duan et al. 2009). Its genome also revealed the absence of several key components required for oxidative phosphorylation and several terminal oxidases, pointing to a limited potential for aerobic respiration; genome analysis suggests that the organism cannot reduce sulfur compound, but instead anaerobic respiration is coupled to nitrogen metabolism. The presence of an active TCA cycle suggests that a range of amino acids (present in phloem fluid) may serve as energy sources (Duan et al. 2009). *Candidatus Liberibacter solanacearum* shares 884 protein-encoding genes with *Candidatus Liberibacter asiaticus*. Comparison of both genomes revealed many rearrangements and gene losses/gains (Lin et al. 2011). *Candidatus Liberibacter solanacearum* also contained several small and two large phage-derived segments, one of which was similar to a segment in *Candidatus Liberibacter asiaticus*. The analysis of its gene repertoire suggests it can take up glucose but not sucrose or fructose and has limited capacity for aerobic respiration and for the biosynthesis of amino acids and lacks a complete restriction-modification system. It has several transport systems for amino acids and a system (NttA) for the uptake of ATP and ADP from the host. The comparison further revealed that *Candidatus Liberibacter solanacearum* has reduced capacity for nucleic acid modification, increased potential for amino acid and vitamin biosynthesis, and a high-affinity iron transport system (Lin et al. 2011). Lin et al. (2011) point out that the approach of extracting bacterial genome information from the vector does not exclude that other genetic components such as plasmids or linear chromosomes could be present.

Based on the complete genome (Duan et al. 2009; Tyler et al. 2009), a computational analysis of the *Candidatus Liberibacter asiaticus* proteome has been performed, and the results predicting 3D structure, function, cellular localization, and potential virulence factors are publically available (http://prodata.swmed.edu/liberibacter_asiaticus/curated/) as a tool for further study of this pathogen (Cong et al. 2012).

Phenotypic Analyses

A comparison of some general features of the members of the *Phyllobacteriaceae* is given in *Table 18.2*.

Ahrensia Uchino et al. 1999, 1^{VL}

Ah.ren'si.a. N.L. fem. n. *Ahrensia*, named in honor of R. Ahrens, a German microbiologist, for his contribution to the taxonomy of marine species of *Agrobacterium*.

The genus *Ahrensia* comprises rod-shaped cells that do not form spores. They are motile with polar flagella. Aerobic and oxidase and catalase positive. The major quinone is ubiquinone Q10; the major fatty acid is C18:1; the main hydroxy fatty acid is C12:0 3-OH. No 2-hydroxy fatty acids are present. The G+C content of the DNA is 48 mol %. The type species is *Ahrensia kielensis*.

The following description of *Ahrensia kielensis* is based on those from Uchino et al. (1998) and from Rüger and Höfle (1992). The species is able to grow at 5 °C, but not at 37 °C. Na^+ is required. Cells are motile rods, 0.6–1.0 × 2.0–4.0 μm. Hardly any carbon sources are used: the type strain tested negative for 12 carbohydrates, 11 carboxylic acids, 3 alcohols, 7 amino acids, and putrescine (Rüger and Höfle 1992). H_2S is produced from cysteine; hydrolysis of gelatin and starch is negative. Nitrate is not reduced to nitrite or gas. Acids are

Table 18.2

Morphological and chemotaxonomic characteristics of the genera of *Phyllobacteriaceae*

Genus	Source	# species	Morphology	Motility	Metabolism	Temperature preference	Major fatty acids	Major hydroxy fatty acids	G+C content
Ahrensia	Seawater	1	Rods	Polar flagella	Aerobic respiration	Mesophilic	C18:1 ω7c	C12:0 3-OH	48
Aliihoeflea	Tidal flat sediment	1	Rods	Not reported	Aerobic respiration	Mesophilic	C18:1 ω7c, C19:cyclo ω8c	–	53.4
Aminobacter	Soil, root nodules	6	Rods	Subpolar flagella	Aerobic respiration	Mesophilic	SF7 (C18 :1 ω7c/C18:1 ω9t/C18:1 ω12t)	C12:0 3-OH	62.5–63.8
Aquamicrobium	Air from a duck shed, biofilter for the treatment of animal rendering waste gas, activated sludge	6	Rods	+	Aerobic respiration; one species also uses nitrate as respiratory electron acceptor	Mesophilic	C18:1 ω7c	–	58.7–61.7
Chelativorans	Soil, activated sludge, sewage sludge	2	Rods	+/–	Aerobic respiration	Mesophilic	SF7 (C18:1 ω7c/C18:1 ω9t/C18:1 ω12t), C19:0 cyclo ω8c	–	60.8–63.1
Hoeflea	Seawater, in cultures of marine dinoflagellates or cyanobacteria, root surface of a halophyte	7	Rods	One polar flagellum or nonmotile	Aerobic or microaerophilic respiration	Mesophilic	C18:1 ω7c	None or C12:0 3-OH	53.1–59.7
Lentilitoribacter	Seawater	1	Rods, short rods	–	Aerobic respiration	Mesophilic	C18 :1 ω7c, 11-methyl-C18:1 ω7c, SF3 (iso-C15:0 2-OH and/or C16:1 ω7c)	C10:0 3-OH	49.3
Mesorhizobium	Nodules of legume plants or soil	30	Rods	+	Aerobic respiration; one species is fac. chemolithotrophic using thiosulfate or elemental sulfur	Mesophilic	C18:1 ω7c		57.9–65.1
Nitratireductor	Marine sediment, seawater, beach sand, dried seaweed sample	7	Rods or coccoid cells	+/–	Aerobic respiration	Mesophilic	C18:1 ω7c/ω6c	–	56.7–63
Phyllobacterium	Root or leaf nodules, tuff volcanic rock	8	Rods	+	Aerobic respiration	Mesophilic	C16:0, C18:1 ω7c, C18:1 ω7c 11-Me, C19:0 cyclo ω8c	C16:0 3-OH, C18:1 2-OH	52–58.5
Pseudahrensia	Seawater	1	Ovoid to rod shaped	–	Aerobic respiration	Mesophilic	C18:1 ω7c	C18:0 3-OH, iso-C13:0 3-OH	60.1
Pseudaminobacter	River water, activated sludge	2	Coccoid to rod shaped	+	Aerobic respiration	Mesophilic	C18:1 (ω7c/ω9t/ω12t), C19:0 cyclo ω8c	C15:0 iso 3-OH	62.9–63.9
Thermovum	Compost	1	Ovoid	–	Aerobic respiration	Thermophilic	C18:1 ω7c, C19:0 ω8c and C18:0	–	63.4

Data taken from the original descriptions and references as given in the main text

produced from fructose, maltose, xylose, and glycerol after 4–6 weeks of incubation. Negative in the following tests: indole production, methyl red, Voges-Proskauer, lysine and ornithine decarboxylase, and hydrolysis of casein, chitin, and alginate.

The major fatty acid is C18:1 ω7c; C12:0 3-OH and iso-C13:0 3-OH are present, but 2-hydroxy fatty acids are absent (Uchino et al. 1998; Park et al. 2013). The G+C content of the DNA is 48 mol %.

The type strain IAM 12618^T was isolated from seawater of the Baltic Sea.

Aliihoeflea Roh et al. 2008

A.li.i.ho.e.fle'a, L. adj. and pronoun *alius*, other, another, different; N.L. fem. n. *Hoeflea*, a bacterial genus name; N.L. fem. n. *Aliihoeflea*, the other *Hoeflea*.

Aliihoeflea comprises rod-shaped cells that are catalase and oxidase positive. The major quinone is ubiquinone Q10; the major fatty acids are C18:1 ω7c and C19:0 cyclo ω8c. G+C content is approximately 53 mol %. The type species is *Aliihoeflea aestuarii*.

The following description of the phenotype is based on the description of the strain N8^T, thus far the only strain of *Aliihoeflea aestuarii* (Roh et al. 2008). Cells are rod shaped (0.50–0.75 μm × 1.25–1.50 μm). Colonies on MA are circular with entire margin, convex, shiny, and cream colored. Growth is also possible on Trypticase soy agar, SA, LA, and yeast mannitol agar, but not on R2A. Temperature range for growth is 17–37 °C; the optimal growth temperature is 30 °C. Optimal NaCl concentration is 1 % (w/v), although NaCl is not required and up to 8 % is tolerated during growth. Nitrates are not reduced to nitrites or nitrogen. Indole is not produced. Glucose is not fermented, and hydrolysis of starch, esculin, gelatin, and PNPG (*p*-nitrophenyl-β-D-galactopyranoside) is negative. Urease positive and arginine dihydrolase negative. Glycogen, Tween 80, L-arabinose, D-fructose, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, D,L-lactic acid, succinic acid, succinamic acid, L-alaninamide, D-alanine, L-alanine, L-glutamic acid, glycyl-L-glutamic acid, L-leucine, L-serine, inosine, uridine, and thymidine can be used as sole carbon sources. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase. Negative for lipase (C14), acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. The genomic DNA G+C content is 53.4 mol %. The type strain KCTC 22052^T was isolated from tidal flat sediment in Yeosu (34°47′26″ N 127°34′01″ E), Republic of Korea.

Aminobacter Urakami et al. 1992, 90^{VP}

Am.i.no.bac'ter, N.L. n. *aminum*, amine; N.L. masc. n. *bacter*, rod; N.L. masc.n. *Aminobacter*, amine rod.

The genus *Aminobacter* comprises non-spore-forming rod-shaped cells that can utilize methylamine. Cells are motile by means of subpolar flagella. They multiply by budding. Poly-β-hydroxybutyrate granules are accumulated in the cells. Good growth in nutrient broth and PYG broth. No water-soluble fluorescent pigment is produced. No growth factors are required. Oxidase and catalase positive and urease negative. Aerobic respiratory metabolism, not fermentative (Urakami et al. 1992).

The following tests are negative: methyl red, Voges-Proskauer, indole production, hydrogen sulfide production, hydrolysis of gelatin and starch, denitrification, litmus milk, and fermentation of sugars.

Ammonia is produced. Acids are produced from sugars oxidatively. Monomethylamine, trimethylamine, trimethylamine-N-oxide, and sugars are utilized. Methanol, methane, and hydrogen are not utilized. Ammonia, nitrate, urea, peptone, and methylamine are utilized as nitrogen sources.

Good growth occurs at pH 6.0–8.0 and at 30–37 °C. No growth above pH 9.0 and below pH 5.0 at 42 °C and in the presence of 3 % NaCl (Urakami et al. 1992).

The type strains of all species can utilize L-arabinose and L-alanine; none can use adonitol (McDonald et al. 2005; Maynaud et al. 2012). McDonald et al. (2005) performed a biochemical characterization of all *Aminobacter* species except *Aminobacter anthyllidis* and found that all type strains could utilize *N*-acetyl-D-glucosamine, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, D-ribose, D-xylose, i-inositol, D-mannitol, D-sorbitol, acetate, 4-aminobutyrate, DL-3-hydroxybutyrate, DL-lactate, oxoglutarate, L-histidine, L-leucine, L-ornithine, and L-proline and hydrolyze bis-*para*-nitrophenyl (pNP)-phosphate, pNP-phenyl-phosphonate, L-alanine-*para*-nitroanilide (pNA), and L-proline-pNA. None of the strains could utilize *p*-arbutin, α-D-melibiose, salicin, maltitol, putrescine, cis-aconitate, trans-aconitate, adipate, azelate, citrate, fumarate, itaconate, mesaconate, suberate, L-phenylalanine, 3-hydroxybenzoate, and phenylacetate. All type strains used sucrose. None of the strains could hydrolyze esculin, pNP-β-D-galactopyranoside, pNP-β-D-glucuronide, 2-deoxythymidine-5′-pNP-phosphate, and L-glutamate-γ-3-carboxy-pNA and none produced acid from lactose, adonitol, rhamnose, methyl D-glucoside, erythritol, and melibiose. Additional features and differentiating characteristics of the species are shown in *Table 18.3*.

Only strains of *Aminobacter ciceronei* and *Aminobacter lissarensis* utilize several methyl halides as sole carbon sources (*Table 18.3*). Of all *Aminobacter* strains tested, two strains of *Aminobacter ciceronei* (ER2 and C147; not the type strain) were the sole *Aminobacter* strains that could degrade atrazine and carbofuran (McDonald et al. 2005). *Aminobacter anthyllidis*, which is capable of nodulation and was isolated from a Zn-Pb

Table 18.3

Comparison of selected characters of the species of *Aminobacter*

Characteristic	*Aminobacter anthyllidis* LMG 26462^T	*Aminobacter aganoensis* DSM 7051^T	*Aminobacter aminovorans* LMG 2122^T	*Aminobacter ciceronei* CCUG 50580^T	*Aminobacter lissarensis* CCUG 50579^T	*Aminobacter niigataensis* DSM 7050^T
References	Maynaud et al. 2012	Urakami et al. 1992; McDonald et al. 2005	Urakami et al. 1992; McDonald et al. 2005	McDonald et al. 2005	McDonald et al. 2005; Maynaud et al. 2012	Urakami et al. 1992; McDonald et al. 2005
Source	Soil from a Zn-Pb mine, after trapping with *Anthyllis vulneraria* subsp. *pyrenaica*	Soil enrichment on trimethylammonium hydroxide	Soil enrichment cultures containing various amines	CH_3Br-fumigated soil	Soil from beech woodland	Soil enrichment on *N,N*-dimethylformamide
Motility		Subpolar flagella	Subpolar flagella	Motile	Motile	Subpolar flagella
Cell shape	Rods	Rods with rounded ends	Rods with rounded ends	Rods	Rods	Rods with rounded ends
Cell size		0.5–0.9 × 1.0–3.0 μm	0.5–0.9 × 1.0–3.0 μm	0.6 × 1.3 μm	0.5–0.6 × 1.3–1.5 μm	0.5–0.9 × 1.0–3.0 μm
Colony pigmentation	Cream	White to light yellow	White to light yellow	Unpigmented	Faint pink	White to light yellow
Metabolism		Aerobic respiration	Aerobic respiration	Aerobic	Aerobic	Aerobic respiration
Temperature range for growth (°C)	Up to 37	30–37	30–37			30–37
Growth at 42 °C	–	–	–			–
Optimal growth (°C)	28			28–30	25	
Growth below pH 5 and above pH 9	+ (5–11)	–	–			–
Optimal pH		6.0–8.0	6.0–8.0	6.5–7.5	6.7–7.2	6.0–8.0
Growth with 3 % NaCl	–	–	–		+	–
Oxidase		+	+			+
Catalase		+	+			+
Urease		–	–			–
Major fatty acids (>10 %)		SF7 (C18:1 ω7c/C18:1 ω9t/C18:1 ω12t), unknown (ECL 18.081)	SF7 (C18:1 ω7c/C18:1 ω9t/C18:1 ω12t), C19:0 cyclo ω8c, unknown (ECL 18.081)	11-Me-C18:1 ω7t, SF7 (C18:1 ω7c/C18:1 ω9t/C18:1 ω12t)	11-Me-C18:1 ω7t, SF7 (C18:1 ω7c/C18:1 ω9t/C18:1 ω12t)	SF7 (C18:1 ω7c/C18:1 ω9t/C18:1 ω12t), C19:0 cyclo ω8c, unknown (ECL 18.081)
Important fatty acids (5–10 %)		C16:0		C16:0, C19:0 cyclo ω8c	C16:0, C19:0 cyclo ω8c	C16:0
Hydroxy fatty acids		C12:0 3-OH	C12:0 3-OH	C12:0 3-OH	C12:0 3-OH	C12:0 3-OH
DNA G+C content (mol %)	62.6	63.8	62.5	62.0–63.7	62.5	63.2
Reduction nitrate to nitrite		w	–			w
Indole production = tryptophanase		–	–			–
Hydrolysis of:						

Table 18.3 (continued)

Characteristic	*Aminobacter anthyllidis* LMG 26462^T	*Aminobacter aganoensis* DSM 7051^T	*Aminobacter aminovorans* LMG 2122^T	*Aminobacter ciceronei* CCUG 50580^T	*Aminobacter lissarensis* CCUG 50579^T	*Aminobacter niigataensis* DSM 7050^T
Gelatin, starch		–	–			–
pNP-α-D-glucopyranoside		+	+	w	–	+
pNP-β-D-glucopyranoside		w	w	–	–	w
pNP-phosphorylcholine		w	+	w	–	–
Acid production from:						
Glucose		w	w	w	w	w
D-Mannitol	+	w	w	w	w	w
Sucrose		w	w	–	w	w
Dulcitol, salicin		–	w	–	–	w
Inositol	+	w	–[a]	–	–[a]	w
Sorbitol	+	w	–[a]	–	w	w
L-Arabinose		–	w	w	w	w
Raffinose, cellobiose		–	w	–	–	–
Maltose	–	w	w	w	–	w
D-Xylose	–	w	w	w	w	w
Trehalose		–	w	–	–	w
D-Arabitol	w	w	w	–	w	w
M-Mannose	+	w	w[b]	w	w	w
Erythritol, L-xylose, L-sorbose, D-tyranose, D-lyxose	–		–		+	
D-Adonitol, D-lactose	–		–		–	
D-Galactose, D-fructose	+		+		+	
Fucose	w		+		+	
L-Rhamnose	+		–		+	

Utilization of:						
Gluconate		−	−	w	−	−
L-Rhamnose		−	+	+	+	+
D-Trehalose		+	−	+	+	+
Propionate	−	+	+[b]	+	+[b]	−
Glutarate		+	+	+	+	−
L-Malate	+	+	−	−	−	−
Pyruvate	+	+	−	+	+	+
L-Aspartate	w	+	+[b]	+	+	+
L-Serine	w	+	+[b]	+	+[b]	+
β-Alanine		+	−	+	+	+
L-Tryptophan		−	−	+	+	−
4-Hydroxybenzoate		+	−	−	−	+
H_2S production		−	−			−
Methyl red		−	−			−
Voges-Proskauer		−	−			−
Denitrification		−	−			−
Utilization as sole carbon and energy source of:						
CH_3Br, CH_3Cl		−	−	+	+[c]	−
CH_3I				+	−	
CH_3F				−	−	
Methylamine				+	+[c]	
PHB accumulation		+	+			+

Data taken from the descriptions in the references that are listed in the table. Fatty acid data from McDonald et al. (2005)

[a]Positive according to Maynaud et al. (2012)

[b]Negative according to Maynaud et al. (2012)

[c]Requires the presence of cyanocobalamin (1 $mg.l^{-1}$)

mining site through trapping with *Anthyllis vulneraria*, can tolerate 1–2 mM of Zn and 0.3–1 mM of Cd in YEM broth after 1 week (Maynaud et al. 2012).

The DNA base composition ranges from 62 to 64 mol % G + C. The main cellular fatty acids include C18:1, and the main hydroxy fatty acids include C12:0 3-OH. The ubiquinone system is ubiquinone Q10.

The type species is *Aminobacter aminovorans*, originally described as *Pseudomonas aminovorans* (Urakami et al. 1992).

Aquamicrobium Bambauer et al. 1998, 631[VL] emend. Lipski and Kämpfer 2012

A.qua.mi.cro'bi.um, L. n. *aqua*, water; N.L. neut. n. *microbium*, a microbe; N.L. neut. n. *Aquamicrobium*, a bacterium living in water/wastewater.

This description is based on the emended description of Lipski and Kämpfer (2012). *Aquamicrobium* consists of pleomorphic or regularly shaped short rods that are mesophilic and grow best at pH 6–9. They can tolerate up to 7 % NaCl (w/v) and utilize sugars, carbonic acids, amino acids, and alcohols for growth. Major quinone is Q10, major fatty acid is C18:1 *cis*-11, major polyamine is spermidine, and main polar lipids are phosphatidylglycerol, phosphatidylcholine, and phosphatidylethanolamine. G+C content of the DNA is 57–65 mol %. The type species is *Aquamicrobium defluvii*.

A number of phenotypic and other characteristics of the *Aquamicrobium* type strains are listed in Table 18.4. All species are oxidase and catalase positive. *Aquamicrobium defluvii* is able to utilize thiophene-2-carboxylate as sole carbon source in the presence of molybdate (Bambauer et al. 1998). In addition, acetate, propionate, butyrate, crotonate, glucose, fructose, mannose, xylose, mannitol, and sorbitol are used for growth with oxygen or nitrate as electron acceptors. Nitrate is reduced to nitrite. No growth was observed with thiophene-2-acetate, thiophene-3-carboxylate, thiophene-3-acetate, thiophene-2-carbaldehyde, thiophene-2-methanol, thiophene-2-mandelate, thiophene-2-acrylate, thiophene, benzothiophene, dibenzothiophene, pyrrole-2-carboxylate, furan-2-carboxylate, pyridine, nicotinate, benzoate, phenylacetate, phthalate, galactose, ribose, sorbose, maltose, saccharose, cellobiose, and lactose. Hydrolysis of gelatin, arginine dihydrolase, lysine decarboxylase, and urease is negative (Bambauer et al. 1998). *Aquamicrobium lusatiense* is able to degrade 4-chlorophenol, 2,4-dichlorophenol, and phenol, and this capacity was not lost over repeated transfers and attempts at curing. Indeed, genes for chlorocatechol 1,2-dioxygenase and 2,4-dichlorophenol hydroxylase were shown to be located on the chromosome rather than on a megaplasmid (Fritsche et al. 1999). Hydrolysis of urea, starch, gelatin, casein, DNA, Tween 80, and esculin is negative (Fritsche et al. 1999). *Aquamicrobium aerolatum* is positive for phosphatase and L-alanine aminopeptidase (Kämpfer et al. 2009).

Small amounts of 12:0 3-OH were reported for *Aquamicrobium defluvii* and *Aquamicrobium lusatiense* and iso-15:0 3-OH for *Aquamicrobium aerolatum* by Kämpfer et al. (2009), but a later study comprising all species did not find hydroxy fatty acids (Lipski and Kämpfer 2012).

Chelativorans Doronina et al. 2010, 1047[VP]

Che.la'ti.vo.rans. N.L. n. *chelatum*, a chelate; L. part. adj. *vorans*, devouring; N.L. masc. n. *Chelativorans*, a bacterium digesting metal chelates.

Chelativorans strains are non-spore-forming rods. The genus was described as nonmotile (Doronina et al. 2010), although flagella were later reported for *Chelativorans multitrophicus* DSM 9103^T and several *Chelativorans* sp. strains (Kaparullina et al. 2011). They often occur as pairs and multiply by binary fission. They form small white colonies on EDTA/mineral salt agar (diameter 0.1–0.3 mm after 7 days at 30 °C). Optimal NaCl concentration for growth is 1.5 %. No PHB inclusions; electron dense inclusions are thought to consist of calcium and magnesium phosphates and are absent in cells grown on fumarate. Oxidase and catalase positive; indole is produced; no nitrate reduction to nitrite; no nitrogen fixation. Optimal temperature and pH for growth are 25–35 °C and 6.5–7.5. Aerobic respiratory metabolism; able to use EDTA as carbon, nitrogen and energy source, either facultatively (*Chelativorans multivorans*) or obligately (*Chelativorans oligotrophicus*). No autotrophic or methylotrophic growth; unable to use methanol or methylated amines as carbon, nitrogen, or energy source (Doronina et al. 2010). Unable to use alcohols, amines, malate, pyruvate, L-alanine, and L-serine as carbon and energy sources (Kaparullina et al. 2011). *Chelativorans oligotrophicus* has several defective or missing enzymes in the central carbon metabolism. The tricarboxylic acid cycle lacks α-ketoglutarate dehydrogenase activity, and 6-phosphofructokinase (ATP/PPi) is also absent (Doronina et al. 2010). The major cellular fatty acids are summed feature 7 (C18:1 ω7c, C18:1 ω9t and/or C18:1 ω12t) and C19:0 cyclo ω8c. Hydroxy fatty acids C12:0 3-OH, C13:0 3-OH, and C15:0 iso 3-OH are absent. The major ubiquinone is Q10. Predominant polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidyldimethylethanolamine, phosphatidylmonomethylethanolamine, and diphosphatidylglycerol. *Mesorhizobium*-specific ornithine lipid is absent. sym-Homospermidine is the main polyamine with small amounts of spermidine and putrescine present. The DNA G+C content is 60–64 mol %. The type species is *Chelativorans multitrophicus* (Doronina et al. 2010).

Additional characters and differentiating features of both species are shown in Table 18.5.

Hoeflea Peix et al. 2005, 1165[VP]

Hoef.le.a'. N.L. fem. n. *Hoeflea* honoring Manfred Höfle, German microbiologist, in recognition of his contribution to the taxonomy of marine bacteria.

Table 18.4

Comparison of selected characters of the species of *Aquamicrobium*

Characteristic	*Aquamicrobium aerolatum* DSM 7051^T	*Aquamicrobium aestuarii* KACC 14931^T	*Aquamicrobium ahrensii* DSM 19730^T	*Aquamicrobium defluvii* CCUG 50580^T	*Aquamicrobium lusatiense* CCUG 50579^T	*Aquamicrobium segne* DSM 19714^T
References	Kämpfer et al. 2009; Lipski and Kämpfer 2012	Jin et al. 2013	Lipski and Kämpfer 2012	Bambauer et al. 1998; Lipski and Kämpfer 2012	Fritsche et al. 1999; Kämpfer et al. 2009; Lipski and Kämpfer 2012	Lipski and Kämpfer 2012
Source	Air collected in a duck shed	Crude oil-contaminated tidal flat	Biofilter for the treatment of animal rendering waste gas	Activated sludge from the municipal sewage plant of Regensburg (Germany)	Activated sludge of an industrial wastewater treatment plant	Biofilter for the treatment of animal rendering waste gas
Cell shape	Rods	Ovoid rods	Rods	Rods	Short rods	Rods
Cell size	0.3–0.5 × 1.5–2.0 μm	0.5–07 × 0.8–1.4 μm		0.5–0.8 × 1.5–2.5 μm	0.6–0.8 × 1.5–3 μm	
Motility	Motile	Two polar flagella		Motile	Polar flagellum	
Colony morphology	Smooth, greyish white, translucent, shiny with entire margin	Round, entire margin, convex, ivory	Circular, whitish	Circular, white, convex	Circular, white-greyish	Circular, whitish
Metabolism	Aerobic	Aerobic	Aerobic	Respiration with oxygen or nitrate electron acceptor	Aerobic	Aerobic
Temperature range for growth (°C)		15–45			12–44	
Growth at 41 °C		+		+		
Optimal growth (°C)		30–35	30	30–37	30–37	30
pH range	5.5–10	5.5–9.0			6.0–9.2	
Optimal pH		6.5–7.5	6–9	7.5–8.5	7.0–7.5	6–9
Growth with 2 % NaCl	+	+		+		
Nitrate reduction		+		+	−	
Vitamins required				+	+	
Major fatty acids (>10 %)	C18:1 ω7*c*, C17:0, C19:0 cyclo 11–12	C18:1 ω7*c*/ω6*c*	C18:1 ω7*c*	C18:1 ω7*c*	C18:1 ω7*c*	C18:1 ω7*c*, C16:0, C19 :0 cyclo ω8c
Important fatty acids (5–10 %)		C19:0 cyclo ω8c			C19:0 cyclo ω8c	C18:0
Polyamines	Putrescine, spermidine, spermine	Putrescine, spermidine, *sym*-homospermidine	Putrescine, spermidine, *sym*-homospermidine, spermine		Spermidine	Putrescine, spermidine, spermine
Polar lipids[a]	PG, PC, DPG, PE, UAL	PG, DPG, PE, PC, PME, UAL, UPL	PG, PC, DPG, PE, PME, UAL	PG, PC, DPG, PE, PME, UAL	PG, PC, DPG, PE, PME, UAL	PG, PC, DPG, PE, UAL
DNA G+C content (mol %)		56.9	60.6	61.7	61.4	58.7

Table 18.4 (continued)

Characteristic	*Aquamicrobium aerolatum* DSM 7051^T	*Aquamicrobium aestuarii* KACC 14931^T	*Aquamicrobium ahrensii* DSM 19730^T	*Aquamicrobium defluvii* CCUG 50580^T	*Aquamicrobium lusatiense* CCUG 50579^T	*Aquamicrobium segne* DSM 19714^T
Reaction in Biolog GN tests:						
Tween 40	–	+	w	–	–	–
Tween 80	–	–	w	w	w	–
N-Acetyl-D-galactosamine, adonitol, L-ornithine	–	–	–	–	+	–
α-Ketoglutaric acid	–	+	–	–	+	–
N-Acetyl-D-glucosamine, D-mannose, formic acid, L-alanine, L-threonine	–	–	–	+	+	–
myo-Inositol	–	+	–	+	+	–
D-Arabitol, D-mannitol	–	–	–	+	–	–
α-Ketobutyric acid	–	+	–	+	–	–
D-Fructose, D-alanine, L-glutamic acid, L-histidine, L-leucine, γ-Aminobutyric acid	–	–	+	+	+	–
α-D-Glucose, DL-lactic acid, L-proline	–	+	+	+	+	–
Gentiobiose	–	–	–	–	w	–
D-Sorbitol	–	–	–	w	–	–

Methylpyruvate	+	+	+	+	–	–
Inosine, uridine, thymidine	+	–	–	–	–	–
Monomethyl succinate, succinamic acid	+	+	–	–	–	–
Acetic acid, L-aspartic acid, 2-aminoethanol	–	–	–	w	+	–
D-Gluconic acid	+	–	+	+	+	–
Urocanic acid, glycerol	+	+	+	+	+	–
α-Hydroxybutyric acid	–	+	+	w	w	–
β-Hydroxybutyric acid	w	+	–	+	+	–
γ-Hydroxybutyric acid	w	+	–	–	–	–
α-Ketovaleric acid, hydroxy L-proline	–	–	w	+	+	–
L-Alanyl glycine, L-asparagine, L-serine	–	–	w	w	+	–
Propionic acid	–	+	w	w	+	–
Succinic acid	–		+	–	+	–
L-Alaninamide	+	+	–	–	+	–
Glycyl-L-aspartic acid	–		w	–	+	–
Glycyl-L-glutamic acid	+		w	–	+	–
DL-Carnitine	–		w	w	–	–

Data taken from the descriptions in the references is listed in the table. Fatty acid data were taken from Lipski and Kämpfer (2012). This study did not detect hydroxy fatty acids

[a]*PG* phosphatidylglycerol, *PC* phosphatidylcholine, *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PME* phosphatidylmonomethylethanolamine, *UAL* unidentified aminolipid, *UPL* unidentified phospholipid

Table 18.5
Characteristics of the species of *Chelativorans*

Characteristic	*Chelativorans multitrophicus* DSM 9103^T	*Chelativorans oligotrophicus* DSM 19276^T
Source	Mixture of soil extracts and activated sludge samples taken from various industrial wastewater treatment plants	Municipal sewage sludge samples
Cell size	0.5–1.0 × 0.7–2.0 μm	0.4–0.5 × 1.0–2.0 μm
Utilization of EDTA	Facultative	Obligate
Optimal growth temperature (°C)	30	32–34
Optimal pH	7.0	6.8–7.2
Vitamin requirements	Biotin, thiamine	Biotin
DNA G+C content (mol %)	63.1	60.8
Utilization as sole carbon and energy source:		
Glucose, lactate, glutamate, fumarate, succinate, acetate, nitrilotriacetate, iminodiacetate, N,N9-ethylenediaminediacetate, and ethylenediamine disuccinate	+	–
Diethylenetriamine pentaacetate, hydroxyethylethylene-diaminetriacetate	–	
Generation time on EDTA under optimal growth conditions	14h	7h
Degradation of uncomplexed EDTA, Ca^{2+}-, Ba^{2+}-, Mg^{2+}-, Mn^{2+}-, or Zn^{2+}-EDTA complexes	+	+
Degradation of Pb^{2+}- and Cu^{2+}-EDTA complexes	+	–
Degradation of Fe^{3+}-EDTA complexes	–	–
Growth on dilute complex media	Good but slow	–
Resistance to oxacillin, ampicillin, and lincomycin	+	+
Resistance to novobiocin, nalidixic acid, and neomycin	–	+
Resistance to gentamicin and streptomycin	–	–
α-Ketoglutarate dehydrogenase	+	–

Data from Doronina et al. (2010) and Kaparullina et al. (2011)

Cells are non-spore-forming, motile short rods. They are aerobic chemoorganotrophs and are oxidase and catalase positive except for *Hoeflea alexandrii* which was described as oxidase negative. Cells do not require NaCl; however, they can grow in the presence of up to 5 % NaCl. Growth is possible at a temperature of 18–33°C although some species can grow at higher and lower temperatures (*Table 18.6*). pH range for growth is 6–8 or 9, and *Hoeflea suaedae* has a more wide pH range of 5–10. No nitrate reduction to nitrite or nitrogen except for *Hoeflea suaedae* which was reported to reduce nitrate to nitrite.

The main fatty acid is C18:1 ω7c, and other important fatty acids (>3 %) are C16:0, 11-Me C18:1 ω7c, and C19:0 cyclo ω8c. Only small amounts of hydroxy fatty acid are present. Ubiquinone Q10 is the major quinone; the main polar lipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, and sulfoquinovosyldiacylglyceride, although the latter polar lipid was not reported from *Hoeflea anabaenae*. The G+C content of the DNA ranges from 53 to 60 mol %. The type species is *Hoeflea marina*.

Additional characters and differentiating features of the five *Hoeflea* species are shown in *Table 18.6*.

Hoeflea anabaenae cells attach to *Anabaena* heterocysts; of the other *Hoeflea* species, only *Hoeflea phototrophica* has been observed to do this, although at a much lower frequency (Stevenson et al. 2011). *Hoeflea siderophila* is the only species reported to be iron oxidizing, using FeS, $FeSO_4$, or $FeCO_3$ for lithotrophic growth while depositing iron oxides on the cell surface. It also has a facultative anaerobic metabolism. In anaerobic, iron-oxidizing conditions, it uses nitrate or N_2O as terminal electron acceptor. Nitrate is converted to nitrite which inhibits growth as it accumulates. In those conditions, nitrite can chemically oxidize up to 20 % of Fe(II). When N_2O is given as an electron acceptor, there is virtually no chemical Fe(II) oxidation, and N_2 is formed. The organism is also able to grow mixotrophically or

Table 18.6

Characteristics of *Hoeflea* species

Characteristic	*Hoeflea alexandrii* DSM 16655^T	*Hoeflea anabaenae* CCUG 56626^T	*Hoeflea halophila* KTCT 23107^T	*Hoeflea marina* LMG 128^T	*Hoeflea phototrophica* DSM 17068^T	*Hoeflea siderophila* DSM 21587^T	*Hoeflea suaedae* KACC 14911^T
References	Palacios et al. 2006; Stevenson et al. 2011	Stevenson et al. 2011	Jung et al. 2013	Peix et al. 2005; Stevenson et al. 2011; Chung et al. 2013	Biebl et al. 2006; Stevenson et al. 2011; Chung et al. 2013	Sorokina et al. 2012	Chung et al. 2013
Source	A culture of *Alexandrium minutum* AL1V (marine planktonic dinoflagellate)	Culture of *Anabaena*, attached to heterocysts	Marine sediment	Baltic Sea, water	A culture of *Prorocentrum lima* (marine dinoflagellate)	Brackish, iron-rich spring	Root surface of the halophyte *Suaeda maritima*
Shape	Rods	Irregular club-shaped rods; star-shaped aggregates	Straight to curved rods	Short rods	Small rods	Thin, slightly curved rods	Short rods
Cell size	0.8 × 2.5 μm	0.2 × 2–5 μm	0.7–0.8 × 2.5 μm	0.7–0.9 × 1.1–1.4 μm	0.3–0.5 × 0.7–2.0 μm	0.4 × 0.9–2.2 μm	0.3–0.5 × 1.3–1.4 μm
Motility	+, single polar flagellum	–	+, polar flagellum	+	+, single polar flagellum or single bipolar flagella	+, single polar flagellum	+, single polar flagellum
Pigmentation colonies	Light brown (marine agar)	None	Beige	White-cream (nutrient agar)	Light beige (marine agar) to wine red (1/10 marine agar)	Orange (due to Fe oxides)	White-cream (marine agar)
Bacteriochlorophyll a	–	–	–	–	+	–	–
Carotenoid pigment	–	–	–		+		
Metabolism	Aerobic respiration	Aerobic to microaerophilic respiration	Aerobic respiration	Aerobic respiration	Microaerophilic respiration	Fac. iron oxidizing; fac. mixo- or heterotrophic in anaerobic or microaerobic conditions	Aerobic respiration
Temperature range for growth (°C)	10–42	18–34	15–30	4–37	15–33	9–38	10–42
Optimal growth temperature (°C)	30	30	25–28	28	31	30	30–37
pH range for growth	6–9	6–8	6–9	6–8	6–9	6.2–8.5	5.0–10.0
Optimal pH	7	6.5	7.5	7		7.5	6.5–7.5

Table 18.6 (continued)

Characteristic	*Hoeflea alexandrii* DSM 16655^T	*Hoeflea anabaenae* CCUG 56626^T	*Hoeflea halophila* KTCT 23107^T	*Hoeflea marina* LMG 128^T	*Hoeflea phototrophica* DSM 17068^T	*Hoeflea siderophila* DSM 21587^T	*Hoeflea suaedae* KACC 14911^T
Optimal salt conc. (% w/v)	0–6.8	1.25–1.75	0.5–1	3	ND	1	0–7
Salt range (%)	0–11.8	0.5–2.5	0–5	Up to 5	0.5–7	0.1–8.5	0–9.5
Growth on NaCl instead of sea salt		–					
Growth factors required		Yeast extract	Yeast extract		Yeast extract		
Major fatty acids (>10 %)	C18:1 ω7c	C18:1 ω7c/ω9t/ω12t, ECL 17.603	C18:1 ω7c 11Me, C18:1 ω7c/ω6c	C18:1 ω7c	C18:1 ω7c, C18:1 ω7c 11Me	C18:1ω7c, C16:0, C18:1ω7c 11Me	C16:0, C18:1 ω7c
Important fatty acids (5–10 %)	C16:0	C18:1 ω9c, ECL 18.846/ C19:1 ω6c	C16:0	C18:1 ω7c 11Me, C19:0 cyclo ω8c	C16:0		C18:1 ω7c 11Me, C19:0 cyclo ω8c
Hydroxy fatty acids	–	–	–	–	–	–	C12:0 3-OH
Polar lipids[a]	PG, DPG, PEA, PMMEA, PC, SQVDG, UPGL, UAL, UGL	PG, DPG, PEA, PMMEA, PC, UPL1, UPL2, UAL	PG, PEA, SQVDG, PC, PMMEA	PG, PEA, PMMEA, SQVDG, DPG, UPL, UPGL, PC	PG, PEA, PMMEA, PC, SQVDG, DPG, UAL		PG, PEA, PMMEA, SQVDG, PC, UGL, UL
Polyamines	2-Hydroxyputrescine, putrescine	2-Hydroxyputrescine, putrescine, spermidine		ND	ND		ND
DNA G+C content (mol %)	59.7	58.1	57.8	53.1	59.3	57.5	53.7
Oxidase	–		+	+	+	+	+
Catalase	+		+	+	+	w	+
Inhibition of *Pythium ultimum* and *Phytophtora capsici*	–	ND		–	–		+
Nitrate reduction to nitrite	–	ND		–	–	+	+
Nitrate reduction to nitrogen	–	ND		–	–	–	ND
Arginine dihydrolase	–			–			

Indole production	–				–		
Hydrolysis of gelatin	–			Weak, slow	–		–
Hydrolysis of esculin	+			+			+
Hydrolysis of starch					–		–
Hydrolysis of alginate					–		
Hydrolysis of Tween 80	+			+	–[f]		–
Hydrolysis of Tween 20	+			+	–		–
Urease	–			+	ND		+
β-Galactosidase	+	+	+	+[c]	+[c]		–
Alkaline phosphatase, leucine arylamidase, α-glucosidase	+	+	+	+	+		+
Esterase (C4)	+	+	+	+	+		–
Esterase lipase (C8)	+	–	+	+	+		–
Naphthol-AS-BI-phosphohydrolase	+[c]	–	–	–	–		+
Valine arylamidase	+[c]	–	+	–	–		–
Cystine arylamidase	+	–	+	–	–		–
β-Glucosidase	+	+	+	+	–		–
Trypsin	–	+	–	–	–		+
Acid phosphatase	–[d]	–	–	–	–		+
α-Galactosidase	–[d]	–	+	–	–		+
Lipase (C14), α-chymotrypsin, α-mannosidase, α-fucosidase	–	–	–	–	–		–
N-Acetyl-β-glucosaminidase,	–	–	–	–	–		+
β-Glucuronidase	–	–	–	–	–		

Table 18.6 (continued)

Characteristic	*Hoeflea alexandrii* DSM 16655^T	*Hoeflea anabaenae* CCUG 56626^T	*Hoeflea halophila* KTCT 23107^T	*Hoeflea marina* LMG 128^T	*Hoeflea phototrophica* DSM 17068^T	*Hoeflea siderophila* DSM 21587^T	*Hoeflea suaedae* KACC 14911^T
Growth in mineral medium plus:							
Yeast extract			+		+	+	
Succinate					w	+	
Citrate			+		w	+	
Acetate, pyruvate	+	+	–	+	+	+	
Malate	+	+		+	+	+	
Fumarate	+	+	+	+	+	+	
Fructose	+	–		+	+	+	
Sucrose	+	–	+	+	+	+	
Glucose, lactate	+	–	–	+	+	+	
L-Arabinose	+	–	+	+	+		
Glutamate	+	–	–	+	+	–	
Glycerol	–	–	–	+	–[b]	+	
Butyrate	–	–	–	–	+	+	
Ethanol	–	–		–	–	–	
Methanol	–	–		–	–		
Utilization of:							
Salicin, L-fucose	+[e]			–	–		+
D-Xylose	+[e]		–	–	–	+	+

D-Arabinose	–	+	+	–	–		+
Erythritol	–			–	–		+
D-Glucose	–		–	+	w	+	
D-Mannose	–		+	+		+	
L-Arabinose	–		+	+			
D-Mannitol	–		+	+			
D-Maltose	–		+	+		+	
N-Acetylglucosamine	–			–		+	
Malate	–			+	+	+	
Gluconate	–						
Gentiobiose	+			–			
Caproate	–			–			
Adipate	–			–			
Citrate	–			–		+	
Phenylacetate	–			–			

[a]*PEA* Phosphatidylethanolamine, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PMMEA* phosphatidylmonomethylethanolamine, *PC* phosphatidylcholine, *SQVDG* sulfoquinovosyldiacylglyceride, *UAL* unidentified aminolipid, *UPL* unidentified phospholipids, *UPGL* unidentified phosphoglycolipid
[b]Weak positive according to Biebl et al. (2006)
[c]Negative according to Stevenson et al. (2011)
[d]Positive according to Stevenson et al. (2011)
[e]Negative according to Chung et al. (2013)
[f]Positive according to Chung et al. (2013)

organotrophically in microaerobic or anaerobic condition, using nitrate or N_2O as electron acceptors. Nitrite, ClO_4^-, S^0, thiosulfate, and $Fe(OH)_3$ are not used as electron acceptors, and H_2 oxidation is not possible (Sorokina et al. 2012).

Lentilitoribacter Park et al. 2013, 2365[VL]

Len.ti.li.to.ri.bac'ter. L. masc. adj. *lentus*, slow, delayed; L. n. *litus-oris*, the seashore, coast; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Lentilitoribacter*, slowly growing rod from the coast).

Lentilitoribacter cells are non-spore-forming, nonmotile, and rods to short rods. They are catalase and oxidase positive and do not reduce nitrate to nitrite. Aerobic. The predominant ubiquinone is Q10. The major fatty acids are C18:1 ω7c, 11-methyl-C18:1 ω7c, and summed feature 3 (iso-C15:0 2-OH and/or C16:1 ω7c). The major polar lipids are phosphatidylglycerol and phosphatidylmonomethylethanolamine. The DNA G+C content is 49.3 mol %. The type species is *Lentilitoribacter donghaensis*.

Lentilitoribacter donghaensis cells are rods, 0.3–0.6 × 0.6–4.0 μm. Colonies on marine agar are circular, slightly convex, smooth, whitish yellow and less than 0.5 mm in diameter after 10 days at 25 °C. Optimal growth temperature is 25 °C; growth occurs at 4 and 30 °C, but not at 35 °C. Optimal pH is between 7.0 and 7.5; growth occurs at pH 5.5, but not at pH 5.0. Grows in the presence of 1.0–5.0 % NaCl (bstl growth with 2.0 % NaCl). Requires Mg^{2+} ions for growth. Hydrolyzes Tween 20, 40, 60, and 80, but not esculin, casein, gelatin, hypoxanthine, L-tyrosine, starch, and xanthine. Acid is produced from D-xylose, but not from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, myo-inositol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, and D-trehalose. In the API ZYM tests, alkaline phosphatase, esterase lipase (C8), and leucine arylamidase are positive, while esterase (C4), trypsin, and acid phosphatase activities are weakly present, and lipase (C14), valine arylamidase, cysteine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, or α-fucosidase activities are negative. The major fatty acids (>10 %) are C18:1 ω7c, 11-methyl-C18:1 ω7c, and summed feature 3 (iso-C15:0 2-OH and/or C16:1 ω7c). C10:0 3-OH is the only hydroxy fatty acid detected.

The type strain CCUG 62792^T was isolated from seawater from the coast around Baekdo harbor in the East Sea, South Korea. Its DNA G+C content is 49.3 mol %.

Mesorhizobium Jarvis et al. 1997, 897[VP]

Me.so.rhi.zo'bi.um. Gr. adj. *mesos*, middle; N.L. neut. n. *Rhizobium*, bacterial genus name; N.L. neut.n. *Mesorhizobium*, rhizobia, phylogenetically intermediate between the genera *Bradyrhizobium* and *Rhizobium*. This etymology is given in the original description (Jarvis et al. 1997); alternatively in the List of Prokaryotic names with Standing in Nomenclature (www.bacterio.cict.fr), the name *Mesorhizobium* is said to refer to the growth rate of the bacteria which is intermediate between that of the genera *Rhizobium* and *Bradyrhizobium*.

The genus *Mesorhizobium* comprises 30 species, most occurring as nitrogen-fixing endosymbionts in root nodules of various legume plants. The species *Mesorhizobium thiogangeticum* was isolated from the soil adjacent to the roots of the legume *Clitoria ternatea*, by enrichment using reduced sulfur compounds as sole carbon and energy source (Ghosh and Roy 2006).

All species comprise rod-shaped cells that form creamy, white, or colorless colonies on agar media. They are aerobic organotrophs; only *Mesorhizobium thiogangeticum* is capable of facultative chemolithotrophic growth using thiosulfate or elemental sulfur as energy source (Ghosh and Roy 2006). Optimal temperature for growth is around 28 °C, and optimal pH is about 7. Three species have been reported to grow at 4°C: *Mesorhizobium ciceri*, *Mesorhizobium sangaii*, and *Mesorhizobium shonense* (Zhou et al. 2013); *Mesorhizobium ciceri* is the only species reported to grow at 40 °C (Jarvis et al. 1997; Zhou et al. 2013). Several species can grow in the presence of 1 or 2 % NaCl (*Table 18.7*); for *Mesorhizobium shangrilense*, even growth with 3 % NaCl was reported (Lu et al. 2009).

The fatty acid C18:1 ω7c is present in all species in large amounts (at least 10 % detected in itself or as part of a summed feature), while C16:0, 11-Me C18:1 ω7c, and C19:0 cyclo ω8c are also important (at least 5 %) in more than two thirds to half of the species. Hydroxy fatty acids have been reported at more than 1 % in 7 of the 30 species and comprise mostly C12:0 3-OH and/or iso-C13:0 3-OH; only in *Mesorhizobium albiziae* and *Mesorhizobium temperatum* has iso-C15:0 3-OH been reported at more than 1 % (Wang et al. 2007). Polar lipids have been reported for six of the species (*Table 18.7*): all comprised phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylcholine, while five also contained diphosphatidylglycerol and several unidentified phospholipids (Zhang et al. 2012; Zheng et al. 2013). The DNA G+C content ranges from 57.9 % to 65.1 %.

The type species is *Mesorhizobium loti*. Additional characters and differentiating features of *Mesorhizobium* species are shown in *Table 18.7*.

Nitratireductor Labbé et al. 2004, 54[VP]

Ni.tra.ti.re.duc'tor. N.L. masc. n. *nitras*, nitrate; L. v. *reducere*, to bring back, to reduce; N.L. masc. n. *nitratireductor*, nitrate-reducing bacterium.

The genus *Nitratireductor* comprises six species that occur in various marine habitats. All species are aerobic chemoorganotrophs; nitrate reduction varies between strains. Cells are rods, short rods, or coccoid. Motility is variable. Optimum temperature for growth is 25–35 °C. No growth below 10 °C. pH range is 5–12. All species are oxidase and catalase positive. Major quinone is ubiquinone Q10. The main fatty acid is C18:1 ω7c/ω6c. DNA G+C content is 56.7–63 mol %. The type species is *Nitratireductor aquibiodomus*.

Table 18.7

Characteristics of the type strains of species of *Mesorhizobium*

(Part 1)								
Characteristic	*Mesorhizobium abyssinicae* LMG 26967^T	*Mesorhizobium albiziae* LMG 23507^T	*Mesorhizobium alhagi* HAMBI 3019^T	*Mesorhizobium amorphae* LMG 18977^T	*Mesorhizobium australicum* LMG 24608^T	*Mesorhizobium camelthorni* HAMBI 3020^T	*Mesorhizobium caraganae* LMG 24397^T	*Mesorhizobium chacoense* LMG 19008^T
References	Degefu et al. 2013	Wang et al. 2007	Chen et al. 2010	Wang et al. 1999	Nandasena et al. 2009	Chen et al. 2011	Guan et al. 2008	Velazquez et al. 2001
Source	Root nodules of *Acacia abyssinica* and *Acacia tortilis*	Root nodules of *Albizia kalkora*	Root nodules of *Alhagi sparsifolia*	Root nodules of *Amorpha fruticosa*	Root nodules *Biserrula pelecinus*	Root nodules of *Alhagi sparsifolia*	Root nodules of *Caragana microphylla*	Root nodules of *Prosopis alba*
Motility	+	+	+			+	+	+
Cell size (μm)	0.3–0.4 × 2.2–3.0	0.3–0.5 × 1–3	0.5–0.7 × 1.3–1.5	0.4–0.7 × 0.5–1.7		0.3–0.6 × 1.0–1.2	0.5 × 2–3	
Growth with 1 % (w/v) NaCl	−	v	+	v			v	−
Growth with 2 % (w/v) NaCl			+	−		+	−	
Growth at pH 5		−	−	v		−	−	−
Growth at pH 9				−				
Growth at pH 10		−	+	−		−	+	−
Growth at 4 °C								
Growth at 10 °C						−	+	
Growth at 37 °C	−			−			+	
Growth at 39 °C							+	
Growth at 40 °C							+	
Survival at 60 °C (10 min)							+	
Temperature range (°C)	Max. 35			<37			Max. 40	
pH range	4,5–9,0			< 9	5,5–9,0	6–11		
Major fatty acids (>10 %)	C16:0, C18:1 ω7c	C18:1 ω7c/ω9c/ω9t/ω12t	11-Me C18:1 ω7c, C18:1 ω7c, C19:0 cyclo ω8c	C16:0, 11-Me C18:1 ω7c, C18:1 ω7c/ω9t/ω9c/ω12t, C19:0 cyclo ω8c	C16:0, C18:1 ω7c, 19:0 cyclo ω8c	11-Me C18:1 ω7c, C18: ω7c	C16:0, C18:1 ω7c/w6c	C16:0, C19:0 cyclo ω8c, C18:1 ω7c/ω9c/ω12t
Important fatty acids (5–10 %)		iso-C15:0, 11-Me C18:1 ω7c	iso-C17:0	C18:0		iso-C17:0, C19:0 cyclo ω8c	C18:0	C18:0, 11-Me C18:1 ω7c
Important hydroxy fatty acids (>1 %)	C12:0 3-OH, iso-C13:0 3-OH	iso-C15:0 3-OH						
DNA G+C content (mol %)	63.5	59.0	60.4	64	63.0	63.7	59.7	61.7

Table 18.7 (continued)

(Part 1)								
Characteristic	*Mesorhizobium abyssinicae* LMG 26967^T	*Mesorhizobium albiziae* LMG 23507^T	*Mesorhizobium alhagi* HAMBI 3019^T	*Mesorhizobium amorphae* LMG 18977^T	*Mesorhizobium australicum* LMG 24608^T	*Mesorhizobium camelthorni* HAMBI 3020^T	*Mesorhizobium caraganae* LMG 24397^T	*Mesorhizobium chacoense* LMG 19008^T
Polar lipids[a]								
Nitrate reduction							+	
Catalase								
Oxidase				+				
Urease								
Reduction of nile blue								
Reduction of methyl blue				–				
Utilization as sole carbon source:								
L-Arabinose		–	+	+				+
D-Arabinose		–	+	–		+	+	–
D-Galactose			+					
Glucose				+				
D-Fructose		v	+	+		+	+	v
Lactose		+		v				–
D-Mannose				–				
L-Rhamnose		–	+	v		+	+	v
D-Ribose		–		–			+	–
Sucrose		+		+	+		+	v
Sorbose		v		–	–		v	–
Trehalose		+		+	+		+	–
L-Xylose		+		+				
D-Xylose		–	+	v		+	+	–
D-Maltose		v	+	+	+	+	+	v
D-Raffinose		+		+			+	+
D-Melibiose	+	v	v	+	+	–	–	v
Turanose								
Melezitose				–				
Calcium gluconate				–			–	

Inulin		+		−			+	+
D-Sorbitol		−	+	+		+	+	−
Dulcitol		−		v	−		−	−
Inositol		+	+	+		+	+	+
Glycerol	+							
Sodium pyruvate		−		−			+	−
Salicin		−		−			v	−
Dextrin	+	−		−			+	−
Amygdalin		−		−			+	−
Sodium formate	+	−		−			−	−
Sodium acetate		−		−			+	−
Sodium citrate	+	−	+	v		+	−	−
Sodium D-gluconate	+			−			−	
Sodium hippurate		−	−	−		−	+	v
Sodium tartrate		−	v	−		−	−	v
L-Malate		+		+				+
DL-Sodium malate		+		+			+	−
Succinate	+	+	+	+		+	+	−
Adipic acid								
Malonate	+	−		v				+
Itaconate	+							
Soluble starch							−	
Vanillic acid							−	
Glycine		+		−			−	−
L-Serine	+							
L-Methionine		−		−			+	−
L-Histidine	+							
L-Arginine		−		−			−	−
DL-Asparagine							−	
L-Proline								
L-Glycine							−	
L-Threonine	+	−		−			+	−
N-Acetyl-D-glucosamine	+							

Table 18.7 (continued)

(Part 1)								
Characteristic	*Mesorhizobium abyssinicae* LMG 26967^T	*Mesorhizobium albiziae* LMG 23507^T	*Mesorhizobium alhagi* HAMBI 3019^T	*Mesorhizobium amorphae* LMG 18977^T	*Mesorhizobium australicum* LMG 24608^T	*Mesorhizobium camelthorni* HAMBI 3020^T	*Mesorhizobium caraganae* LMG 24397^T	*Mesorhizobium chacoense* LMG 19008^T
Utilization as sole nitrogen source								
L-Phenylalanine	+	+	+	v		+	+	–
L-Valine		+		v			+	+
L-Methionine		+		+			+	+
D-Arginine		+	+	+		–	+	–
L-Arginine		+		+				
L-Cystine		v	+	v			+	–
DL-α-Alanine		+		–				
DL-Histidine		–	–	–		–		–
Glycine		–	+	–		+		–
DL-α-Aminopropionate		–		+			+	–
Hypoxanthine		–		+			+	–
L-Isoleucine		+		+			+	+
L-Lysine		+	+	+		+	+	+
D-Threonine		+		–			–	–
L-Threonine		+		+			+	–
D-Aspartate		+	–	+			+	–
L-Aspartic acid	+		–	–		–		
D-Glutamic acid		–		v			+	–
L-Glutamic acid	+	v		+			+	–
Resistance to (μg/ml)								
Ampicillin (5)				v			–	
Ampicillin (50)		+	–	–	–	–	–	–
Ampicillin (100)		+		–			–	–
Ampicillin (300)		+		–			–	–
Ampicillin sodium (5)		+		–				–
Ampicillin sodium (50)		+		–				–
Ampicillin sodium (300)		+		–				–

Bacitracin (5)		+		+			+	–
Bacitracin (50)		+		+			+	–
Bacitracin (100)		–		–			–	–
Chloramphenicol (5)		+	–	v			–	–
Chloromycetin (100)		+		–			–	+
Chloromycetin (50)		+		–			–	+
Chloromycetin (5)		+	+	+		+		+
Erythromycin (5)		+	+	+		+	+	–
Erythromycin (50)		+	–	+		–	+	–
Erythromycin (100)		+		v			–	–
Erythromycin (300)		+		+			v	–
Gentamicin (50)							–	
Gentamicin (5)		+	–	+		–	–	–
Kanamycin sulfate (50)	–	–		–	+		v	–
Kanamycin (100)		–		–				–
Kanamycin (50)		+		–				–
Kanamycin sulfate (5)		+		+			+	–
Neomycin (50)							+	
Neomycin sulfate (5)		+		+			+	–
Spectinomycin (5)	–	+		–			+	–
Spectinomycin (50)					–		+	
Streptomycin (50)	–			–				
Streptomycin (5)			–	–		–		
Streptomycin sulfate (5)		+	–	–			+	–
Streptomycin sulfate (50)		+		–			–	–
Streptomycin sulfate (100)		+		–			–	–
Streptomycin sulfate (300)				–			–	–
Dihydrostreptomycin (5)		+		–				+

Table 18.7 (continued)

(Part 1)								
Characteristic	*Mesorhizobium abyssinicae* LMG 26967^T	*Mesorhizobium albiziae* LMG 23507^T	*Mesorhizobium alhagi* HAMBI 3019^T	*Mesorhizobium amorphae* LMG 18977^T	*Mesorhizobium australicum* LMG 24608^T	*Mesorhizobium camelthorni* HAMBI 3020^T	*Mesorhizobium caraganae* LMG 24397^T	*Mesorhizobium chacoense* LMG 19008^T
Dihydrostreptomycin (50)		+		–				–
Dihydrostreptomycin (100)		+		–				–
Dihydrostreptomycin (300)		–		–				–
Bromothymol blue (0.2 %)		+	–	+		–		+
Neutral red (0.2 %)		–	v	–		–		–

(Part 2)								
Characteristic	*Mesorhizobium ciceri* LMG 14989^T	*Mesorhizobium gobiense* LMG 23949^T	*Mesorhizobium hawassense* LMG 26968^T	*Mesorhizobium huakuii* LMG 14107^T	*Mesorhizobium loti* LMG 6125^T	*Mesorhizobium mediterraneum* LMG 17148^T	*Mesorhizobium metallidurans* LMG 24485^T	*Mesorhizobium muleiense* HAMBI 3264^T
Reference	Nour et al. 1994; Jarvis et al. 1997	Han et al. 2008	Degefu et al. 2013	Chen et al. 1991; Jarvis et al. 1997	Jarvis et al. 1982, 1997	Nour et al. 1995; Jarvis et al. 1997	Vidal et al. 2009	Zhang et al. 2012
Source	Root nodules of *Cicer arietinum*	Root nodules of *Oxytropis glabra*	Root nodules of *Sesbania sesban*	Root nodules of *Astragalus sinicus*	Root nodules of *Lotus corniculatus*	Root nodules of *Cicer arietinum*	Root nodules of *Anthyllis vulneraria*	Root nodules *Cicer arietinum*, China
Motility			+	One polar or subpolar flagellum	One polar or subpolar flagellum			+
Cell size (μm)		0.3–0.6 × 1–3	0.28–0.32 × 2.25–3.15	0.5–0.9 × 1.2–3.0				0.46–0.61 × 0.91–2.40
Growth with 1 % (w/v) NaCl	+	+		+	v	v	+	–
Growth with 2 % (w/v) NaCl	+	v			–	v	–	–
Growth at pH 5	+	+		–	–	–		
Growth at pH 9		+			+	+		
Growth at pH 10	v	v		v	v	v	+	–
Growth at 4 °C					–		–	–
Growth at 10 °C		–				–	+	–
Growth at 37 °C		+	–		–	v	+	–
Growth at 39 °C				–		+	–	
Growth at 40 °C	+				–	+		

Survival at 60 °C (10 min)		–				–	–	–
Temperature range (°C)	Max. 40		Max. 35			Max. 40	Max. 37	
pH range	5.0–10.0	5–9	4.5–10.0	5.0–9.5	4.0 to <10.0	5.0–9.5	5–11	6–9
Major fatty acids (>10 %)	C16:0, C19:0 cyclo ω8c, 11-Me C18:1 ω7c, C18:1 ω7c/ω6c	C16:0, C19:0 cyclo ω8c, 11-Me C18:1 ω7c, C18:1ω7c/ω6c	C16:0, C18:1 ω7c	C16:0, C18:1 ω7c, C19:0 cyclo ω8c	C16:0, C19:0 cyclo ω8c, 11-Me C18:1 ω7c, C18:1 ω7c/ω6c	C16:0, C19:0 cyclo ω8c, C18:1ω7c/iso-C17:1ω9c	C16:0, C19:0 cyclo ω8c, C18:1ω7c/iso-C17:1ω9c	C16:0, C19:0 cyclo ω8c, C18:1ω7c/iso-C17:1ω9c
Important fatty acids (5–10 %)		iso-C17:0	iso-C17:0	iso-C17:0		C18:0, 11-Me C18:1 ω7c	C18:0, 11-Me C18:1 ω7c	C18:0, 11-Me C18:1 ω7c
Important hydroxy fatty acids (> = 1 %)			iso-C13:0 3-OH	C12:0 3-OH, iso-C13:0 3-OH				
DNA G+C content (mol %)	63.0	57.9	62.5	63.5	62.7	62.5		61.2
Polar lipids[a]	DPG, PEA, PG, PC, 6UPL				DPG, PEA, PG, PC, 5UPL		DPG, PEA, PG, PC, 6UPL	PC, PEA, PG
Nitrate reduction	+	v			+	–	+	–
Catalase		+						+
Oxidase	+	+			+	+		+
Urease	–	–			–			
Reduction of Nile blue	–	–			–			
Reduction of methyl blue	–	+			v	–		
Utilization as sole carbon source:								
L-Arabinose	+			+	+	+	+	+
D-Arabinose	+	+		v	v	v		+
D-Galactose	+	+		+	+	+	+	+
Glucose	v	+		+	+	+	+	+
D-Fructose	+	+		+	v	+	+	+
Lactose	v	+	+	v	+	+	+	+
D-Mannose	+	+		+	+	+		+
L-Rhamnose	v	+		+	+	+	+	+
D-Ribose	+			+	v	v	v	+
Sucrose	+	+		+	+	+		+
Sorbose	–	–		–	–	–	+	–
Trehalose	v	+		+	+	+		+
L-Xylose	+			+	–	–	+	
D-Xylose	v	+		+	v	v		+
D-Maltose	+	+		+	+	+	+	
D-Raffinose	–	–		+	v	–	v	–

Table 18.7 (continued)

(Part 2)								
Characteristic	*Mesorhizobium ciceri* LMG 14989^T	*Mesorhizobium gobiense* LMG 23949^T	*Mesorhizobium hawassense* LMG 26968^T	*Mesorhizobium huakuii* LMG 14107^T	*Mesorhizobium loti* LMG 6125^T	*Mesorhizobium mediterraneum* LMG 17148^T	*Mesorhizobium metallidurans* LMG 24485^T	*Mesorhizobium muleiense* HAMBI 3264^T
D-Melibiose	v		+	v	v	–	+	
Turanose	+	+			+	+	+	
Melezitose	–	–			–	–	–	–
Calcium gluconate	–	v			–	–	–	v
Inulin	v	–		v	–	–	–	+
D-Sorbitol	v			+	v	v	–	+
Dulcitol	v	+		v	–	v	v	–
Inositol	+	v		v	+	+	+	–
meso-Erythritol	+	+		–	v	v	–	+
Glycerol	+		+	w	+	+	+	
Sodium pyruvate	v	+		v	v	v	–	–
Salicin	–	–		v	–	v	v	–
Dextrin	v	+	+	+	v	v	–	
Amygdalin	v	–		–	–	–	–	
Sodium formate	v	v	w	v	v	v	–	–
Sodium acetate	v	–		v	v	v	–	+
Sodium citrate	v	+	+	v	v	–	–	
Sodium D-gluconate	v	v	+	+	v	v	–	–
Sodium hippurate	–	–		v	–	–	–	
Sodium tartrate	–	–		v	–	–	–	
L-Malate	+			+	+	+		
DL-Sodium malate	v	+		+	+	–		
Succinate	+	+	+	v	v	v	–	
Adipic acid	–	–			–	–		–
Malonate	v		w	v	v	–	–	
Itaconate	v		+	w	v	–	–	
Soluble starch	–	–		–	–	–	–	W
Vanillic acid		–		–		–	–	–
Glycine	–	–		+	–	–	+	–
L-Serine	v		w	w	v	–	–	
L-Methionine	–	–		+	–	v		

L-Histidine	+		+	w	+	+		
L-Arginine	−			+	−	−	−	
DL-Asparagine		−		−		−	−	−
L-Proline	+			−	v	+	v	+
L-Glycine	+	−			−	−	v	−
L-Threonine	v	+	+	v	v	−	−	−
N-Acetyl-D-glucosamine	+		+	w	+	+		
Utilization as sole nitrogen source:								
L-Phenylalanine	v	v	+	v	+	+	+	−
L-Valine	v	v		v	v	v	−	+
L-Methionine	+	−		+	+	v	+	+
D-Arginine	+			+	+	−		
L-Arginine	−	+		+	−	+	−	+
L-Cystine	v	+		+	v	v		+
DL-α-Alanine	−	+			−	−	−	+
DL-Histidine	−			−	−	−		
Glycine	−	+		−	−	+		
DL-α-Aminopropionate	+			+	+	−		
Hypoxanthine	v	+		+	v	+	−	+
L-Isoleucine	+	+		+	v	v	−	+
L-Lysine	+	+		+	v	v	−	+
D-Threonine	−	+		+	−	v	−	
L-Threonine	+	+		+	+	−		+
D-Aspartate	+			+	+	−		
L-Aspartic acid	v	+	+	+	+			−
D-Glutamic acid	+	+		+	v	v	−	−
L-Glutamic acid	v	+	+	+	v	+	−	−
Resistance to (μg/ml):								
Ampicillin (5)	v	+		−	+	+	v	−
Ampicillin (50)	v	−		−	v	v	+	−
Ampicillin (100)	−			−	v	−	−	
Ampicillin (300)	−			−	−	−		
Ampicillin sodium (5)	−			−	+	−		
Ampicillin sodium (50)	−			−	+	−		
Ampicillin sodium (300)	−			−	−	−		
Bacitracin (5)	+			+	+	+		

Table 18.7 (continued)

(Part 2)								
Characteristic	*Mesorhizobium ciceri* LMG 14989^T	*Mesorhizobium gobiense* LMG 23949^T	*Mesorhizobium hawassense* LMG 26968^T	*Mesorhizobium huakuii* LMG 14107^T	*Mesorhizobium loti* LMG 6125^T	*Mesorhizobium mediterraneum* LMG 17148^T	*Mesorhizobium metallidurans* LMG 24485^T	*Mesorhizobium muleiense* HAMBI 3264^T
Bacitracin (50)	+			+	+	–		
Bacitracin (100)	–			+	–	–		
Chloramphenicol (5)	v	+		v	v	v		
Chloromycetin (100)	–	+		–	–	v	+	–
Chloromycetin (50)	–	+		–	–	v	+	–
Chloromycetin (5)	+			+	–	–		+
Erythromycin (5)	+	+		+	v	+		
Erythromycin (50)	+			+	v	v		
Erythromycin (100)	+			+	+	–		
Erythromycin (300)	+	–		+	+	–	+	+
Gentamicin (50)		–				+	+	–
Gentamicin (5)	+			+	+	–		+
Kanamycin sulfate (50)	–	–		v	+	v	+	–
Kanamycin (100)	–			+	+	–		
Kanamycin (50)	–			+	+	–		
Kanamycin sulfate (5)	+			+	+	–		+
Neomycin (50)		–				+	+	–
Neomycin sulfate (5)	+			+	+	–		
Spectinomycin (5)	–	+	–	+	+	v	+	–
Spectinomycin (50)		–				–	+	–
Streptomycin (50)	–	–	–		+	–		
Streptomycin (5)	+	+			+	+		
Streptomycin sulfate (5)	v	–		+	+	v	+	–
Streptomycin sulfate (50)	–			+	+	–		
Streptomycin sulfate (100)	–			+	+	–		
Streptomycin sulfate (300)	–				+	–		
Dihydrostreptomycin (5)	+			+	+	–		
Dihydrostreptomycin (50)	–			–	+	–		

Dihydrostreptomycin (100)	–			–	+	–		
Dihydrostreptomycin (300)	–			–	+	–		
Bromothymol blue (0.2 %)	+			+	–	+		
Neutral red (0.2 %)	–			–	+	+		

(Part 3)

Characteristic	*Mesorhizobium opportunistum* LMG 24607^T	*Mesorhizobium plurifarium* LMG 11892^T	*Mesorhizobium qingshengii* LMG 26793^T	*Mesorhizobium robiniae* HAMBI 3082^T	*Mesorhizobium sangaii* HAMBI 3318^T	*Mesorhizobium septentrionale* HAMBI 2582^T	*Mesorhizobium shangrilense* LMG 24762^T	*Mesorhizobium shonense* LMG 26966^T
Reference	Nandasena et al. 2009	de Lajudie et al. 1998	Zheng et al. 2013	Zhou et al. 2010	Zhou et al. 2013	Gao et al. 2004	Lu et al. 2009	Degefu et al. 2013
Source	Root nodules of *Biserrula pelecinus*	Root nodules of *Acacia senegal*	Root nodules of *Astragalus sinicus*, China	Root nodules of *Robinia pseudoacacia*	Root nodules of *Astragalus luteolus*, China	Root nodules of *Astragalus adsurgens*	Root nodules of *Caragana bicolor*	Root nodules of *Acacia abyssinica*
Motility		+		+	+			+
Cell size		0.5–0.7 × 1–3	0.38–0.52 × 1.75–2.12	0.3–0.6 × 1–2.5	0.3–0.6 × 1–3		0.3–0.6 × 1–3	0.3–0.4 × 2.1–3.1
Growth with 1 % (w/v) NaCl		v	+	+	+	v	+	–
Growth with 2 % (w/v) NaCl		+		+	–		v	
Growth at pH 5		–				–	+	
Growth at pH 9				+		–		
Growth at pH 10		v		v	–	–	+	
Growth at 4 °C			–				–	
Growth at 10 °C			+	+			+	
Growth at 37 °C		+	–	+		–	+	–
Growth at 39 °C							–	
Growth at 40 °C		+		+	–			
Survival at 60 °C (10 min)				+				
Temperature range (°C)		Max. 42	10–35				10–37	Max. 35
pH range	5.5–9.0	4.5–9.0	5.5–9.0	6–11	5–9		5–10	4.5–10.5
Major fatty acids (>10 %)	C16:0, C18:1ω7c	C16:0, C18:1 ω7c, C19:0 cyclo ω8c	C16:0, 11-Me C18:1 ω7c, C18:1 ω7c/ω6c	C19:0 cyclo ω8c, C18:1 ω7c/ω9c/ ω9t/ω12t	C16 :0, C18:1 ω7c/ ω9c/ω9t/ω12t, 11-Me C18:1 ω7c, C19:0 cyclo ω8c	C16:0, 11-Me C18:1 ω7c, C18:1 ω7c/ω9t/ ω9c/ω12t	C16:0, 11-Me C18:1 ω7c, C19:0 cyclo ω8c, C18:1ω7c/ω6c	C16:0, C18:1 ω7c
Important fatty acids (5–10 %)	11-Me C18:1 ω7c	iso-C17:0		C16:0, iso-C17:0, 11-Me C18:1 ω7c		C18:0		C19:0 cyclo ω8c
Important hydroxy fatty acids (> = 1 %)		iso-C13:0 3-OH	iso-C13:0 3-OH	iso-C13 3-OH				C12:0 3-OH, iso-C13:0 3-OH

Table 18.7 (continued)

(Part 3)								
Characteristic	*Mesorhizobium opportunistum* LMG 24607^T	*Mesorhizobium plurifarium* LMG 11892^T	*Mesorhizobium qingshengii* LMG 26793^T	*Mesorhizobium robiniae* HAMBI 3082^T	*Mesorhizobium sangaii* HAMBI 3318^T	*Mesorhizobium septentrionale* HAMBI 2582^T	*Mesorhizobium shangrilense* LMG 24762^T	*Mesorhizobium shonense* LMG 26966^T
DNA G+C content (mol %)	63.2	64.4 or 62.8	59.5	61.5	58.3	59.4 or 63.12	61.4	62.2
Polar lipids[a]			DPG, PEA, PG, PC, 5UPL					
Nitrate reduction				+	–		v	
Catalase			+		+		+	
Oxidase					+	+	v	
Urease			+		+		–	
Reduction of Nile blue					–		–	
Reduction of methyl blue					–	–	+	
Utilization as sole carbon source:								
L-Arabinose		+		+		+		
D-Arabinose		+	+		+	–	+	
D-Galactose			+	+			–	
Glucose		+	+	+	+	v	+	
D-Fructose		v	+	+	+	v	+	
Lactose	+	v	–	+	+	+	–	
D-Mannose		+	+	+	+	v	+	
L-Rhamnose		+	–	+	+	+	+	
D-Ribose		+	+	+	+	+	–	
Sucrose	+	+	+	+	+	+	v	
Sorbose	–	+	–	v		–	–	
Trehalose	+	+				+	+	
L-Xylose		+				+		
D-Xylose		+	+	+	+	v	+	
D-Maltose	+	+	+	+	+	+	+	
D-Raffinose	–	+	–	+	+	+	–	
D-Melibiose	+	v	+		+	v	–	+
Turanose	+					+	–	
Melezitose						–	–	
Calcium gluconate			–	+		–	–	
Inulin		+	–			+	+	

D-Sorbitol		+	–		+	+	+	
Dulcitol	–	–	+	+	+	+	–	
Inositol		+	+	+		+	+	
meso-Erythritol		–	+			v	–	
Glycerol		w						+
Sodium pyruvate		v		+		v	+	
Salicin		+	–	+		–	–	
Dextrin		v	–			v	–	+
Amygdalin		–	–			v	–	
Sodium formate		v	–	+		v	–	+
Sodium acetate		+	–			v	–	
Sodium citrate		+	–		–	–	+	+
Sodium D-gluconate		w	–	+		–	–	+
Sodium hippurate		+	–	+	–	+		
Sodium tartrate		–	–	–	–	–	–	
L-Malate		+				+		
DL-Sodium malate		+	+			–	+	
Succinate		+	+	+		v	+	+
Adipic acid			–				–	
Malonate		v	–	+	–	–		+
Itaconate		w						+
Soluble starch			–	+	–		–	
Vanillic acid				+	–		–	
Glycine		–		+		–	–	
L-Serine		w						+
L-Methionine		–	–			–	–	
L-Histidine		w						+
L-Arginine		v	+			–	–	
DL-Asparagine			–	+			–	
L-Proline		+	+				–	
L-Glycine			+	+	–			
L-Threonine		+	+	+		+		+
N-Acetyl-D-glucosamine	+	w						+
Utilization as sole nitrogen source:								
L-Phenylalanine		+		+	+	v	+	+

Table 18.7 (continued)

(Part 3)								
Characteristic	*Mesorhizobium opportunistum* LMG 24607^T	*Mesorhizobium plurifarium* LMG 11892^T	*Mesorhizobium qingshengii* LMG 26793^T	*Mesorhizobium robiniae* HAMBI 3082^T	*Mesorhizobium sangaii* HAMBI 3318^T	*Mesorhizobium septentrionale* HAMBI 2582^T	*Mesorhizobium shangrilense* LMG 24762^T	*Mesorhizobium shonense* LMG 26966^T
L-Valine		+	+	+	+	v	+	
L-Methionine		+	+	+	+	+	+	
D-Arginine		+				−		
L-Arginine			−	+	+	+	+	
L-Cystine		+	+	+	−	v	+	
DL-α-Alanine			−		−	−	−	
DL-Histidine		−				+		
Glycine		−			−	+	v	
DL-α-Aminopropionate		+				−		
Hypoxanthine		+	−	+		v	+	
L-Isoleucine		+	+			+	−	
L-Lysine		+	+		−	v		
D-Threonine		+	−			v	−	
L-Threonine		+		+	+	+	+	
D-Aspartate		+		+		+		
L-Aspartic acid		+				−	+	+
D-Glutamic acid		+	+			v		
L-Glutamic acid		+	+	+	−	−	+	+
Resistance to (μg/ml):								
Ampicillin (5)		+	−	+	−	v	+	
Ampicillin (50)	−	−		+	−	+	+	
Ampicillin (100)		−	−	+		+		
Ampicillin (300)		−		+		+		
Ampicillin sodium (5)		+				−		
Ampicillin sodium (50)		−				−		
Ampicillin sodium (300)		−				−		
Bacitracin (5)		+				−		
Bacitracin (50)		+				−		
Bacitracin (100)		+				−		
Chloramphenicol (5)		−	+			v	+	
Chloromycetin (100)		−	+	+		−		

Chloromycetin (50)		–		+		–		
Chloromycetin (5)		–				–		
Erythromycin (5)		+				v	+	
Erythromycin (50)		+				v	–	
Erythromycin (100)		+				–		
Erythromycin (300)		+	+	+		–		
Gentamicin (50)				+				
Gentamicin (5)		+				–		
Kanamycin sulfate (50)	+	v		+		–	–	
Kanamycin (100)		–				–		
Kanamycin (50)		+				–		
Kanamycin sulfate (5)		+				–	+	
Neomycin (50)				+				
Neomycin sulfate (5)		+				–		
Spectinomycin (5)		–		+		+		–
Spectinomycin (50)	–			+				
Streptomycin (50)					–	–	–	–
Streptomycin (5)				+		–		
Streptomycin sulfate (5)		+		+	–	v	+	
Streptomycin sulfate (50)		–				–	+	
Streptomycin sulfate (100)		–				–		
Streptomycin sulfate (300)		–				–		
Dihydrostreptomycin (5)		+				–		
Dihydrostreptomycin (50)		–				–		
Dihydrostreptomycin (100)		–				–		
Dihydrostreptomycin (300)		–				–		
Bromothymol blue (0.2 %)		+		+		+		
Neutral red (0.2 %)		–				–		

(Part 4)

Characteristic	*Mesorhizobium silamurunense* LMG 24822^T	*Mesorhizobium tamadayense* LMG 26736^T	*Mesorhizobium tarimense* LMG 24338^T	*Mesorhizobium temperatum* HAMBI 2583^T	*Mesorhizobium thiogangeticum* LMG 22697^T	*Mesorhizobium tianshanense* LMG 18976^T
Reference	Zhao et al. 2012	Ramirez-Bahena et al. 2012	Han et al. 2008	Gao et al. 2004	Ghosh and Roy 2006	Chen et al. 1995; Jarvis et al. 1997
Source	Root nodules of *Astragalus membranaceus*	Root nodules of *Anagyris latifolia*	Root nodules of *Lotus frondosus*	Root nodules of *Astragalus adsurgens*	Soil, by enrichment using reduced S compounds as sole C and energy source	Root nodules of *Glycyrrhiza pallidiflora*

Table 18.7 (continued)

(Part 4)						
Characteristic	*Mesorhizobium silamurunense* LMG 24822^T	*Mesorhizobium tamadayense* LMG 26736^T	*Mesorhizobium tarimense* LMG 24338^T	*Mesorhizobium temperatum* HAMBI 2583^T	*Mesorhizobium thiogangeticum* LMG 22697^T	*Mesorhizobium tianshanense* LMG 18976^T
Motility	+	One subpolar flagellum				Peritrichous flagella
Cell size	1.2–1.8 × 2.1–4.2	0.6–0.7 × 1.1–1.2	0.3–0.6 × 1–3		0.2–0.4 × 1.2–1.5	0.2–0.9 × 1.2–3.0
Growth with 1 % (w/v) NaCl	−	+	v	v		v
Growth with 2 % (w/v) NaCl		−	+	−		−
Growth at pH 5			+	−		−
Growth at pH 9			+	+		−
Growth at pH 10	+		+	−		v
Growth at 4 °C						
Growth at 10 °C		−	−	−		+
Growth at 37 °C		−	v	v	−	−
Growth at 39 °C						
Growth at 40 °C	+					
Survival at 60 °C (10 min)	+		−	−		−
Temperature range (°C)	Max. 40	11–36				
pH range		5–9			5.5–8.5	
Major fatty acids (>10 %)	C16:0, C18:1 ω7c/ ω6c		C16:0, C18:1 ω7c/ ω6c	C16:0, C18:0, C19:0 cyclo ω8c, C18:1ω7c/iso-C17:1ω9c	C18:1 ω7c	C16:0, C19:0 cyclo ω8c, 11-Me C18:1 ω7c, C18:1ω7c/iso-C17:1ω9c
Important fatty acids (5–10 %)	C19:0 cyclo ω8c		C18:0, 11-Me C18:1 ω7c	C18:1 ω9c, 11-Me C18:1 ω7c	iso-C15:0	C18:0
Important hydroxy fatty acids (> = 1 %)				iso-C15:0 3-OH		
DNA G+C content (mol %)	62.4	60.3	57.9	65.1 or 62.22	59.6 or 60.72	61 or 62.51
Polar lipids[a]						DPG, PEA, PG, PC, 12UPL
Nitrate reduction	−	−	v	+		+
Catalase	+		+		+	
Oxidase	−		−	+	−	+

Urease	–	+	–			
Reduction of Nile blue	+		–			
Reduction of methyl blue	+		–	–		–
Utilization as sole carbon source:						
L-Arabinose		+		–	+	
D-Arabinose	–		v	+		v
D-Galactose	+		v		+	v
Glucose	+	+	+	–	+	+
D-Fructose	+		+	v	–	+
Lactose	–		v	+	–	+
D-Mannose	+	+	+	–	–	v
L-Rhamnose	+	+	v	+	–	v
D-Ribose		+	–	+		v
Sucrose	+	+	+	v	–	+
Sorbose	–		v	v		–
Trehalose	+		+	–		+
L-Xylose				–	–	+
D-Xylose	+		v	+	–	v
D-Maltose	+	+	+	v	–	–
D-Raffinose	–		v	v	–	–
D-Melibiose	+	+		v		v
Turanose			+	–		
Melezitose	–		–	–		–
Calcium gluconate	–		v	–		v
Inulin	–		–	+		v
D-Sorbitol	+	+	+	v		v
Dulcitol			+	v		v
Inositol	+	+	+	v		v
meso-Erythritol	+		v	v	–	v
Glycerol					–	+
Sodium pyruvate	–		+	v		v
Salicin	–	w	v	–		v
Dextrin	–		+	–		v
Amygdalin	–		–	–		–
Sodium formate	–		–	v		v

Table 18.7 (continued)

(Part 4)						
Characteristic	*Mesorhizobium silamurunense* LMG 24822^T	*Mesorhizobium tamadayense* LMG 26736^T	*Mesorhizobium tarimense* LMG 24338^T	*Mesorhizobium temperatum* HAMBI 2583^T	*Mesorhizobium thiogangeticum* LMG 22697^T	*Mesorhizobium tianshanense* LMG 18976^T
Sodium acetate	+	–	–	+	–	v
Sodium citrate	–	–	–	v	–	v
Sodium D-gluconate	–	–	v	v		v
Sodium hippurate	–		–	–		v
Sodium tartrate			–	–		–
L-Malate				–	–	–
DL-Sodium malate	+	+	+	–		v
Succinate	+		v	–	+	+
Adipic acid	–	–	–			
Malonate		–	–	+		v
Itaconate		–				
Soluble starch	–		v	–		–
Vanillic acid			v	–		–
Glycine			+	–		–
L-Serine		–			–	
L-Methionine	–		–	–		–
L-Histidine		v			+	
L-Arginine	–		–	–	–	v
DL-Asparagine	–		+	–		–
L-Proline	–	+	+			+
L-Glycine	–		v	–		v
L-Threonine			v	v	–	v
N-Acetyl-D-glucosamine		+				
Utilization as sole nitrogen source:						
L-Phenylalanine	+		v	v		v
L-Valine	+		v	–		v
L-Methionine			–	+		v
D-Arginine				–		+
L-Arginine	+		+	+		+
L-Cystine	+		–	–		+

DL-α-Alanine			–	–		v
DL-Histidine				–		–
Glycine			–	+		+
DL-α-Aminopropionate				–		+
Hypoxanthine	+		v	v		+
L-Isoleucine	+		–	v		+
L-Lysine	+		+	v		v
D-Threonine			–	v		v
L-Threonine	+		+	+		+
D-Aspartate				–		–
L-Aspartic acid	+		+			v
D-Glutamic acid	+		–	–		v
L-Glutamic acid	+		v	–		+
Resistance to (μg/ml):						
Ampicillin (5)	+		v	v		v
Ampicillin (50)	–		+	–		–
Ampicillin (100)			–	–		–
Ampicillin (300)				–		–
Ampicillin sodium (5)				+		–
Ampicillin sodium (50)				–		–
Ampicillin sodium (300)				–		–
Bacitracin (5)				–		+
Bacitracin (50)				–		+
Bacitracin (100)				–		–
Chloramphenicol (5)	–		–	v		v
Chloromycetin (100)			+	–		–
Chloromycetin (50)			+	–		–
Chloromycetin (5)				+		+
Erythromycin (5)			+	v		+
Erythromycin (50)				–		v
Erythromycin (100)	+			–		v
Erythromycin (300)	–		–	–		v
Gentamicin (50)	–		+	–		–

Table 18.7 (continued)

(Part 4)						
Characteristic	*Mesorhizobium silamurunense* LMG 24822^T	*Mesorhizobium tamadayense* LMG 26736^T	*Mesorhizobium tarimense* LMG 24338^T	*Mesorhizobium temperatum* HAMBI 2583^T	*Mesorhizobium thiogangeticum* LMG 22697^T	*Mesorhizobium tianshanense* LMG 18976^T
Gentamicin (5)	+			−		−
Kanamycin sulfate (50)	−		−	v		v
Kanamycin (100)				−		−
Kanamycin (50)				−		−
Kanamycin sulfate (5)	+			−		+
Neomycin (50)	−		+	+		−
Neomycin sulfate (5)				−		−
Spectinomycin (5)			+	v		v
Spectinomycin (50)			+	−		+
Streptomycin (50)			−	−		−
Streptomycin (5)			−	−		−
Streptomycin sulfate (5)	−		+	v		v
Streptomycin sulfate (50)				−		−
Streptomycin sulfate (100)				−		−
Streptomycin sulfate (300)				−		−
Dihydrostreptomycin (5)				+		−
Dihydrostreptomycin (50)				−		−
Dihydrostreptomycin (100)				−		−
Dihydrostreptomycin (300)				−		−
Bromothymol blue (0.2 %)				+		+
Neutral red (0.2 %)				−		+

For substrate utilization and resistance, only characters with data for at least 10 species are listed; data were compiled from the original descriptions; *v* different report differ in this character, *w* weak positive. Empty cells indicate no data available

[a]*PEA* Phosphatidylethanolamine, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PC* phosphatidylcholine, *UPL* unidentified phospholipids

All species are positive for leucine arylamidase (API ZYM tests) and for the use of D-glucose and N-acetyl-glucosamine (API 20NE); all are negative for indole production (API 20NE). Further characteristics and differentiating features of the species are given in *Table 18.8*.

Phyllobacterium (*ex* Knösel 1962) Knösel 1984, 356[VP]

Phyl.lo.bac.te'ri.um. Gr. neut. n. *phyllon*, leaf; L. neut. n. *bacterium*, rod; N.L. neut. n. *phyllobacterium*, leaf bacterium (occurring in leaf nodules of higher plants).

Cells are rods and motile by means of polar, subpolar, or lateral flagella. The optimal growth temperature is 28 °C, and there is no growth at 40 °C. Growth occurs in 1 % NaCl. Glucose metabolism is oxidative. Oxidase positive; urease is positive except for *Phyllobacterium endophyticum*. Indole production, β-galactosidase, and gelatinase are negative for all species. Some other enzyme activities that were originally included in the genus description, however, have not been reported for all species. These include DNase (negative, but no data for *P. endophyticum* and *P. trifolii*), hydrolysis of Tween 80 (negative, but not tested for *P. catacumbae*, *P. endophyticum*, and *P. trifolii*), starch (negative for *P. myrsinacearum* and *P. catacumbae*), pectin and cellulose (both only reported as negative for *P. myrsinacearum*), nitrate reduction (positive for *P. myrsinacearum*, negative for *P. catacumbae*, *P. endophyticum* and *P. trifolii*). Esculin is hydrolyzed (weak reaction for *P. trifolii*). 3-Ketolactose test is negative (no data for *P. endophyticum* and *P. trifolii*). Assimilation of D-glucose, D-mannose, L-arabinose, D-mannitol, and N-acetylglucosamine is positive for all species. Maltose is used by all species except *P. endophyticum*. Quinones have only been reported for *Phyllobacterium endophyticum* and comprised Q10 (88 %) and Q9 (12 %) (Flores-Felix et al. 2013). Additional characteristics of the *Phyllobacterium* species are given in *Table 18.9*.

The G+C content of the DNA ranges from 51 to 61 mol % (Tm). The type species is *Phyllobacterium myrsinacearum*.

Pseudahrensia Jung et al. 2012, 2059[VP]

Pseu.dah.ren'si.a. Gr. adj. *pseudes*, false; N.L. fem. n. *Ahrensia*, a bacterial genus name; N.L. fem. n. *Pseudahrensia*, the false *Ahrensia*.

Pseudahrensia cells are aerobic, non-spore-forming, nonmotile, and ovoid to rod shaped. Catalase, oxidase, and nitrate reduction are positive. The predominant ubiquinone is Q10. The major fatty acid is C18:1 ω7c. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine. The DNA G+C content of the type strain of the type species is 60.1 mol %. The type species is *Pseudahrensia aquimaris*.

Pseudahrensia aquimaris cells are nonmotile, ovoid to rod shaped, and 0.5–1.0 × 1.0–7.0 µm. Colonies on MA are circular, convex, smooth, glistening, cream colored and 1.0–1.5 mm in diameter after 5 days at 30 °C. Temperature range for growth is 4–32 °C; optimal growth at 30 °C, pH 7–8, and 2–3 % NaCl. Grows at pH 5.5, but not pH 5. Can grow in 10 % NaCl, but not in 11 % or without NaCl. Na^+ and Mg^{2+} ions are required for growth. No anaerobic growth on marine agar. Nitrate is reduced to nitrite. Gelatin is hydrolyzed. H_2S is not produced. Esculin; casein; hypoxanthine; starch; Tween 20, 40, 60, and 80; L-tyrosine; urea; and xanthine are not hydrolyzed. Acid is positive from D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-ribose, and sucrose; no acid production from L-arabinose, cellobiose, myo-inositol, D-mannitol, melezitose, melibiose, raffinose, L-rhamnose, D-sorbitol, trehalose, or D-xylose. Susceptible to ampicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, penicillin G, polymyxin B, and streptomycin; resistant to carbenicillin, lincomycin, oleandomycin, and tetracycline. The following enzymes are present (API ZYM): alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, esterase lipase (C8) (weak), and trypsin (weak); the following enzymes are absent: lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase (Jung et al. 2012).

Major fatty acid (>10 %) is C18:1 ω7c; no other fatty acids are present at more than 5 %; the only hydroxy fatty acid detected is C18:0 3-OH, apart from the possible presence of C14:0 3-OH as part of summed feature 2 (Jung et al. 2012). However, Park et al. (2013) report the presence of iso-C13:0 3-OH and the absence of C18:0 3-OH.

The type strain (CCUG 60023^T) was isolated from seawater, Yellow Sea of the island of Hwang-do, Korea.

Pseudaminobacter Kämpfer et al. 1999, 894[VP]

Pseud.ami.no.bac'ter. Gr. adj. *pseudos*, false; N.L. masc.n. *Aminobacter*, bacterial genus name; N.L. masc. n. *Pseudaminobacter*, false *Aminobacter*.

Pseudaminobacter cells are rod shaped and motile. Obligate aerobic heterotrophs. They have an oxidative metabolism and can use D-glucose, D-ribose, D-xylose, acetate, propionate, pyruvate, β-alanine, N-acetyl-D-glucosamine, 4-aminobutyrate, DL-3-hydroxybutyrat, DL-lactate, oxoglutarate, L-alanine, L-histidine, L-leucine, and L-proline as sole carbon source. Growth occurs on nutrient agar (Oxoid), Caso agar, R2A agar (Oxoid), and TSB agar (BBL). Colonies are circular, entire, slightly convex and smooth, glistening, and pale beige on nutrient agar at 25 °C. Oxidase and catalase positive. Main ubiquinone is Q10. The major polyamines are spermidine, sym-homospermidine, and putrescine. Polar lipids include phosphatidylcholine, phosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, and diphosphatidylglycerol in nearly the same amounts. Main fatty acids are C18:1 and C19:0 cyclo ω8c. The only hydroxy fatty acid is C15:O iso 3-OH. The G+C content of the

Table 18.8
Characteristics of the type strains of *Nitratireductor* species

Characteristic	*Nitratireductor aquibiodomus* DSM 15645^T	*Nitratireductor aquimarinus* JCM 17288^T	*Nitratireductor basaltis* JCM 14935^T	*Nitratireductor indicus* LMG 25540^T	*Nitratireductor kimnyeongensis* JCM 14851^T	*Nitratireductor lucknowense* DSM 24322^T	*Nitratireductor pacificus* LMG 25541^T
Reference	Labbé et al. 2004	Jang et al. 2011	Kim et al. 2009	Lai et al. 2011b	Kang et al. 2009	Manickam et al. 2012	Lai et al. 2011a
Source	Marine denitrification system	Culture of the marine diatom *Skeletonema costatum*	Black beach sand	Deep seawater crude oil enrichment culture	Dried seaweed samples	Lindane-contaminated soil	Deep-sea sediment pyrene-degrading consortium
Shape	Rods	Rods	Coccoid cells or rods	Rods	Rods	Rods	Short rods
Motility		1 polar flagellum	Nonmotile	1 polar flagellum	Motile		Motile
Cell size	1 × 2–3 μm	0.4–0.7 × 1.3–2.0 μm	0.6–0.7 × 0.6–2.0 μm	1.3 × 3.0 μm	0.4–0.5 × 1.2–2.7 μm		0.8–0.9 × 1.4–1.5 μm
Colonies	White	Cream	Cream	Gray	Light yellow	Straw yellow	Gray
Temperature range		20–40	15–45	10–43	10–40	20–42	10–41
Optimal temperature for growth (°C)	30–35	30–35		25–30	30	30	25–30
pH range		6.5–9.0	5.5–10.0		6.1–12.1	5.2–11.0	
Optimal pH for growth	7–7.5	7.7–8.2				8.0	
NaCl range for growth (%)		1–7	0–8	0–7	1–7	0–5	0–7
Optimal NaCl range for growth (%)		3–4		3	ND	2	3
Major cellular fatty acids (>10 %)	C18:1 ω7c/ω6c, C19:0 ω8c cyclo	C18:1 ω7c/ω6c, C19:0 ω8c cyclo	C18:1 ω7c/ω6c	C18:1 ω7c/ω6c	C18:1 ω7c/ω6c, C19:0 ω8c cyclo	C18:1 ω11c/ω9t/ω6t	SF8 (C18:1 ω7c/ω6c), C19:0 ω8c cyclo
Important fatty acids (5–10 %)	–	–	C18:0	C18:0	–	C16:0, C18:0, C19:0 ω8c cyclo	–
Hydroxy fatty acids (Jang et al. 2011)	–	iso-C15:0 3-OH, C18:0 3-OH	–	–	–	–	–
Major polar lipids[a]	PC, PG, DPG, PEA, UAPL1, UAPL2, UAL, UL1, UL2, UL3	PC, PG, DPG, PEA, UAPL1, UAPL2, UAL, UL1, UL2, UL3				PMMEA, PEA, DPG, PG, PC	
DNA G+C content (mol %)	57	56.7	56.7	59	60.4	62.4	63
Nitrate reduction	+	+	+	+	–	+	+
API ZYM tests:							
Alkaline phosphatase	+	+	+	+	+	–	+

Esterase C4, valine arylamidase, phosphoamidase	+	+	+	+	+		+
Lipase C8	+	+	+	–	+	+	+
Lipase C14	–	–	–	+	–	–	+
Cystine arylamidase	+	w	–	+	+	+	+
Trypsin	+	+	w	+	+		+
Chymotrypsin	–	–	–	+	–		–
Acid phosphatase	+	+	–	+	+		+
α-Galactosidase, β-galactosidase	–	–	+	–	–	–	w
β-Glucuronidase	–	–	w	–	–		–
α-Glucosidase	+	–	–	+	+	–	+
β-Glucosidase	–	–	–	–	–		+
N-Acetyl-β-glucosaminidase	+	+	–	–	–	–	+
α-Mannosidase, α-fucosidase	–	–	–	–	–		–
API 20 NE tests:							
Reduction nitrate to nitrite	+		+				
Reduction nitrate to nitrogen	–		–			+	
Fermentation of glucose	–	–	–	–	–		–
Hydrolysis of gelatin	–	–	–	–	–	+	–
Arginine dihydrolase	w	–	–	+	–	–	–
Urease	–	+	–	+	–	+	–
Hydrolysis of esculin	–	–	–	+	–	–	+
β-Galactosidase	–	+	+	+	–	–	w
Use of:							
L-Arabinose	+	+	+	–	–		–
D-Mannose	+	+	–	+	–	w	w
D-Mannitol	–	–	+	–	+		–
D-Maltose, potassium gluconate	–	–	+	–	–		–
Capric acid	–	–	–	–	–	–	+
Adipic acid	w	–	–	+	–	–	w

Table 18.8 (continued)

Characteristic	*Nitratireductor aquibiodomus* DSM 15645^T	*Nitratireductor aquimarinus* JCM 17288^T	*Nitratireductor basaltis* JCM 14935^T	*Nitratireductor indicus* LMG 25540^T	*Nitratireductor kimnyeongensis* JCM 14851^T	*Nitratireductor lucknowense* DSM 24322^T	*Nitratireductor pacificus* LMG 25541^T
Malic acid	w	–	–	+	–	–	–
Trisodium citrate	+	+	–	+	–	–	+
Phenylacetate	–	–	–	+	–	–	–
Biolog GN2:							
Acetic acid	w		+	w	–	–	+
Bromosuccinic acid, turanose	–		–	w	–	–	–
cis-Aconitic acid, succinic acid	–		w	+	–	–	–
DL-α-Glycerol phosphate, propionic acid	–		–	+	–	–	–
D-Alanine, L-alanine	+		+	+	–	–	+
D-Arabitol, D-mannose	–		–	–	+		–
Dextrin, itaconic acid	–		w	–	–		–
Thymidine	–		w	–	–	–	–
D-Fructose, D-mannitol	–		+	–	+		–
D-Galactose	+		w	–	+		–
D-Glucosaminic acid	+		–	–	–		–
D-Sorbitol, inosine, L-arabinose, uridine	–		+	–	–		–
Tween 40	–		+	–	–	+	–
Glycogen, γ-hydroxybutyric acid	–		+	–	–	–	–
Trehalose	+		–	+	+	–	w
Glycerol	+		+	w	+	–	+

Glycyl-L-aspartic acid, L-alanyl glycine	+		w	−	w	−	+
Glycyl-L-glutamic acid	w		+	+	w	−	+
Hydroxyl-L-proline	+		+	w	+	−	w
L-Alaninamide	+		+	−	−	−	+
L-Asparagine	+		w	+	+	−	+
L-Aspartic acid	−		+	w	+	−	−
L-Fucose	+		w	−	−		−
L-Histidine, L-ornithine	+		−	w	+	−	−
L-Leucine	−		+	w	−	−	+
L-Phenylalanine	+		−	−	−	−	+
L-Threonine	+		+	w	+	−	−
Maltose	−		+	−	+	−	w
Pyruvic acid methyl ester	+		w	+	+		w
myo-Inositol	+		−	+	w	−	−
Succinic acid monomethyl ester	+		−	+	+		+
N-Acetyl-D-glucosamine	+		−	+	+	−	+
N-Acetyl-D-galactosamine	+		−	−	w		−
Succinamic acid	−		+	+	w	−	−
Tween 80	−		+	−	−	+	w
Urocanic acid	−		−	+	+	−	+
α-D-Glucose	+		+	+	+	+	−
α-Hydroxybutyric acid	+		w	+	w	−	−
α-Ketobutyric acid	−		−	w	−	+	w
α-Ketoglutaric acid	w		w	+	+		+
γ-Aminobutyric acid	w		+	+	w	−	w

[a]*PEA* Phosphatidylethanolamine, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PMMEA* phosphatidylmonomethylethanolamine, *PC* phosphatidylcholine, *UAPL* unidentified aminophospholipid, *UAL* unidentified aminolipid, *UL* unidentified lipids

Table 18.9

Comparison of selected characters of the species of *Phyllobacterium*

Characteristic	*Phyllobacterium bourgognense* LMG 22837^T	*Phyllobacterium brassicacearum* LMG 22836^T	*Phyllobacterium catacumbae* LMG 22520^T	*Phyllobacterium endophyticum* LMG 26470^T	*Phyllobacterium ifriqiyense* LMG 22831^T	*Phyllobacterium leguminum* LMG 22833^T	*Phyllobacterium myrsinacearum* LMG 8736^T	*Phyllobacterium trifolii* LMG 22712^T
Reference	Mantelin et al. 2006b; Flores-Felix et al. 2013	Mantelin et al. 2006b; Flores-Felix et al. 2013	Jurado et al. 2005	Flores-Félix et al. 2013	Mantelin et al. 2006b	Mantelin et al. 2006b	Mergaert et al. 2002; Mergaert and Swings 2005b	Valverde et al. 2005
Source	Root of *Brassica napus* cv. Eurol	Root of *Brassica napus* cv. Eurol	Tuff volcanic rock in catacombs	Root nodule of *Phaseolus vulgaris*	Root nodule of *Lathyrus numidicus*	Root nodule of *Astragalus algerianus*	Leaf nodules of *Rubiaceae* and *Myrsinaceae*	Root nodule of *Trifolium repens*
Shape	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Motility	Motile with flagella	Motile with flagella	Motile with a polar tuft of flagella	Motile with a polar flagellum	Motile with flagella	Motile with flagella	Motile with flagella	Motile with a polar flagellum
Cell size			0.4–0.7 × 0.4–1.9 μm				0.4–0.8 × 0.8–2.0 μm	
Colonies	White/cream	White/cream	Beige	Pearly white	White/cream	White, highly convex	Beige	Small, pearly white
Temperature range (°C)				5–39				
Temperature max (°C)	Below 35	Below 35		39	37	37	36	4–37
Optimal temperature for growth (°C)	28	28	28	28	28	28	28–34	28
Growth at 37 °C	−	−	+	+	+	+	−	+
pH range	5–10	5–10		6–8	5–10	5–10	4–10	6–8
Optimal pH for growth				7				7
Growth with 1 % NaCl	+	+			+	+	+	
Growth with 2 % NaCl	−	+	−	+	+	+	+	+
Growth with 3 % NaCl	−	−			+	−	+	+
NaCl max for growth (%)	1	2		2	3	2		0–3
Major cellular fatty acids (>10 %) (Jang et al. 2011)	C16:0, SF8 (C18:1 ω6c/C18:1 ω7c), C19:0 cyclo ω8c	C16:0, SF8 (C18:1 ω6c/C18:1 ω7c)	C16:0, C18:1 ω7c, C18:1 ω7c 11-Me, C19:0 cyclo ω8c	C16:0, C19:0 cyclo ω8c, SF8 (C18:1 ω6c/C18:1 ω7c)			C16:0 3-OH, C18:1 ω7c, C18:1 2-OH	C16:0, C18:1 ω7c, C18:1 ω7c 11-Me, C19:0 cyclo ω8c
Important fatty acids (5–10 %)	C18:1 ω7c 11-Me, C18:0	C19:0 cyclo ω8c, C18:1 ω7c 11-Me		C18:1 ω7c 11-Me			C16:0, C20:0	
Hydroxy fatty acids	C16:0 3-OH	C16:0 3-OH, C18:0 3-OH	C16:0 3-OH, C18:1 2-OH	C16:0 3-OH, C18:1 2-OH, C18:0 3-OH			C15:0 3-OH, C16:0 2-OH, C16:0 3-OH, C18:1 2-OH, C18:0 3-OH	C16:0 3-OH, C18:1 2-OH

DNA G+C content (mol %)	54	55.5	55.9	52	52	57	58.5	56.4
Oxidase	+	+	+		+	+	+	
Urease	+	+	+	–	+	+	–[a]	w
Growth in LB broth	–	+			+	+	+	
Arginine dihydrolase			+	–			–	–
Hydrolysis of esculin				+				w
Use of:								
3-O-Methyl-D-glucopyranose	–	–			–	–	–	
3-Phenylpropionate	–	–			–	–	–	
5-Keto-D-gluconate	+	+	+	+	+	–	+	+
Adonitol	+	–			+	–	+	
Esculin	+	+			+	+	+	
α-Ketoglutarate	–	+			–	–	+	
Benzoate	–	–			–	–	–	
Betaine	+	+			+	–	+	
β-Gentiobiose	+	+			+	+	+	w
β-Glucuronide	–	–			–	–	–	
Caprylate	–	–			–	–	–	
cis-Aconitate	+	+			+	–	+	
Citrate	+	+	–	–	–	–	+	–
D-Alanine	–	–			–	–	–	
D-Arabitol	+	+			+	+	+	
D-Cellobiose	+	+			+	+	+	
D-Galactose	+	+			+	+	+	
D-Galacturonate	–	–			+	+	+	
D-Gluconate	+[b]	+[b]	+	–	+	+	+	+
D-Glucosamine	+	+			+	+	+	
D-Glucuronate	–	–			+	+	+	
DL-α-Amino-n-butyrate	–	+			+	–	+	
DL-β-Hydroxybutyrate	+	+	+	+	+	–	+	+
DL-Glycerate	–	+			+	–	+	
DL-Lactate	+	+		+	+	+	+	
D-Lyxose	+	+			+	+	+	
D-Malate	+	+	+	+	+	–	+	+
D-Melezitose	–	–			–	–	–	

Table 18.9 (continued)

Characteristic	*Phyllobacterium bourgognense* LMG 22837^T	*Phyllobacterium brassicacearum* LMG 22836^T	*Phyllobacterium catacumbae* LMG 22520^T	*Phyllobacterium endophyticum* LMG 26470^T	*Phyllobacterium ifriqiyense* LMG 22831^T	*Phyllobacterium leguminum* LMG 22833^T	*Phyllobacterium myrsinacearum* LMG 8736^T	*Phyllobacterium trifolii* LMG 22712^T
D-Melibiose	–	–		–	–	–	–	
D-Raffinose	–	–			–	–	–	
D-Saccharate	+	–			–	+	–	
D-Sorbitol	+	+		+	+	+	+	
D-Tagatose	+	–			+	–	+	
D-Tartrate	–	–			–	–	–	
D-Trehalose	+	+			+	+	+	
Dulcitol	+	+			+	–	+	
D-Xylose	+	+			+	+	+	
Ethanolamine	–	+			+	–	+	
Fructose	+	+			+	+	+	
Fumarate	+	+			+	–	+	
Gentisate	–	–			–	–	–	
Glutarate	–	–			–	–	+	
Histamine	–	–			–	–	–	
Hydroxyquinoline	–	–			–	–	–	
i-Erythritol	–	–			+	–	–	
L-Alanine	+[b]	+	+	+	+[b]	–	+	w
L-Arabitol	+	–			+	–	+	
L-Aspartate	+	+			+	–	+	
L-Malate	+	+			+	–	+	
L-Serine	–	+		–	+	–	+	
L-Tartrate	–	–			–	–	–	
L-Tryptophan	–	–			–	–	–	
L-Tyrosine	–	–			–	–	+	
Malonate	–	+	w	w	+	–	–	–
Maltitol	+	+			+	–	+	
Maltotriose	+	+			–	–	+	

m-Coumarate	–	–			–	–	–	
meso-Tartrate	–	–			–	–	–	
Methyl α-D-glucopyranoside	+	–			–	–	+	
Methyl β-D-galactopyranoside	–	+			+	+	+	
Mucate	–	–			–	+	–	
Palatinose	+	+			+	–	+	
Phenylacetate	–	–		–	–	–	–	–
p-Hydroxybenzoate	–	+	–	–	–	–	+	–
Propionate	–	–		–	–	–	–	
Protocatechuate	+	+			–	–	+	
Putrescine	–	–			–	–	–	
Quinate	+	+			–	–	+	
Succinate	+	+			+	–	+	
trans-Aconitate	+	+			+	–	+	
Tricarballylate	–	–			–	–	–	
Trigonelline	+	+			–	–	+	
Tryptamine	–	–			–	–	–	
Xylitol	–	–			+	–	+	
3-Hydroxybenzoate	–	–		–	–	–	–	
Itaconate	–	–		–	–	–	–	
L-Fucose	+	+		+	+	+	+	
L-Histidine	–	–		w	–	–	–	
L-Proline	+	+		+	+	+	+	
L-Rhamnose	+	+		+	+	+	+	
Sucrose	+	+	+	–		–	+	+
Valerate	+	+	+	–	v	–	+	+
Capric acid	–	–		–	–	–	–	
D-Maltose	+	+	+	–	+	v	+	+

[a]Positive according to Mantelin et al. (2006b)
[b]Negative according to Flores-Felix et al. (2013)

Table 18.10
Characteristics of the two *Pseudaminobacter* species. Data taken from Kämpfer et al. (1999)

Characteristic	*Pseudaminobacter defluvii* NBRC 14570^T	*Pseudaminobacter salicylatoxidans* DSM 6986^T
Source	Activated sludge	6-Aminonaphthalene-2-sulfonate-degrading consortium from river water
Cell shape	Coccoid to rod shaped	Rods
Cell size	0.8–0.8 × 0.8–1.2 μm	0.5–0.8 × 1–1.5 μm
Temperature range	10–40	20–40
Major fatty acids (>10 %)	C18:1 (ω7c/ω9t/ω12t), C19:0 cyclo ω8c	C18:1 (ω7c/ω9t/ω12t), C19:0 cyclo ω8c
Important fatty acid (5–10 %)		C17:0
Hydroxy fatty acids	C15:0 iso 3-OH	C15:0 iso 3-OH
DNA G+C content (mol %)	62.9	63.9
Acid produced from D-mannitol, dulcitol, and melibiose	–	w
Assimilation of D-maltose, D-trehalose, adonitol, D-mannitol, D-sorbitol, *cis*-aconitate, glutarate, L-malate, L-aspartate, and 4-hydroxybenzoate	–	+
Assimilation of L-ornithine and L-serine	+	–

DNA is 62.9–63.9 mol %. The type species is *Pseudaminobacter salicylatoxidans*.

One additional species was described, *Pseudaminobacter defluvii*. Both species produce acid weakly from glucose, but not from lactose, sucrose, salicin, inositol, sorbitol, L-arabinose, raffinose, maltose, D-xylose, trehalose, cellobiose, D-arabitol, mannose, adonitol, rhamnose, methyl D-glucoside and erythritol. Both species hydrolyze bis-para-nitrophenyl (pNP)-phosphate, pNP-phenyl-phosphonate, L-alanine-para-nitroanilide (pNA), and L-proline-pNA, but not pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-phosphorylcholine, esculin, pNP-β-D-galactopyranoside, pNP-β-D-glucuronide, 2-deoxythymidine-5′-pNP-phosphate, and L-glutamate-γ-3-carboxy-pNA. They do not assimilate p-arbutin, D-melibiose, salicin, maltitol, putrescine, trans-aconitate, adipate, azelate, fumarate, itaconate, mesaconate, suberate, L-tryptophan, 3-hydroxybenzoate, and phenylacetate. Additional characteristics of the *Psedaminobacter* species are given in Table 18.10.

Thermovum Yabe et al. 2012, 2994^{VP}

Ther.mo'vum. Gr. n. *thermê*, heat; L. neut. n. *ovum*, egg, oval; N.L. neut. n. *Thermovum*, a heat(-loving) oval-shaped organism.

Thermovum comprises Gram-positive ovoid cells that do not form spores. Thermophilic. Major fatty acids (>10 %) are C18:1 ω7c, C19:0 ω8c, and C18:0. Polar lipids comprise phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylinositol, phosphatidylmonomethylethanolamine, an unknown glycolipid, and a ninhydrin-positive phospholipid. The main quinone is ubiquinone Q10. The type species is *Thermovum composti*.

Thermovum composti cells are nonmotile, ovoid shaped, and 0.9 μm × 1.4 μm (after 2 days at 50 °C). Catalase and oxidase positive. Growth occurs at 23–57 °C, with optimal growth at 50 °C, at pH 5.9–8.8 (optimum, pH 7.0) and in the presence of 0–4 % (w/v) NaCl. In addition to the major fatty acids listed above in the genus description, C16:0 is a further important fatty acid (5–10 %) in *Thermovum composti*, while no hydroxy fatty acids were reported. Negative for gelatinase, urease, and indole production. Positive for nitrate reduction and for the utilization of D-arabinose, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, cellobiose, lactose, melibiose, gentiobiose, D-fucose, and potassium 5-ketogluconate; negative for the utilization of glycerol, erythritol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, maltose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, L-fucose, DL-arabitol, potassium gluconate, and potassium 2-ketogluconate. The following enzyme activities were present (API ZYM): esterase C4, esterase C8, leucine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase; the following were absent: alkaline phosphatase, lipase C14, cystine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, mannosidase, and α-fucosidase. The type strain JCM 17863^T was isolated from compost. The G+C content of its DNA is 63.4 mol %.

If appropriate, the description of metabolic pathways and/or physiology may deserve an individual heading.

Isolation, Enrichment, and Maintenance Procedures

The genera and species of the family have an aerobic respiratory metabolism and originate from a wide range of habitats. No single isolation or enrichment procedure is available to selectively obtain all or most members of the family, and therefore the genera are discussed separately below.

The only species of the genus *Ahrensia*, *Ahrensia kielensis*, was isolated from the Baltic Sea during studies of star-shaped-aggregate-forming bacteria and was originally named *Agrobacterium kielense* (Ahrens 1968). Because Dr. Renata Ahrens later withdrew the proposal, these species were not documented elsewhere in the following years, and no details on specific isolation conditions are available in recent literature (Rüger and Höfle 1992). With the recent sequencing of the genome of this organism, it may become possible in future to propose suitable isolation or enrichment strategies. The organism can be cultivated on regular marine media (e.g., Difco Marine Broth) at 26 °C and can be freeze-dried for long-term preservation.

Aliihoeflea was isolated from tidal flat sediment samples by plating on marine agar 2216 (Difco). Circular colonies—convex with entire margin, shiny, and cream colored—were 0.5–1.0 mm in diameter after 2 days incubation at 30 °C. Growth also occurs on trypticase soy agar (TSA, Difco), Luria agar (Difco), and yeast extract mannitol agar (YMA, per liter, 10 g D-mannitol, 0.5 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 4 g $CaCO_3$, 0.4 g yeast extract, 15 g agar; pH 6.8–7.0) (Roh et al. 2008).

All *Aminobacter* species were isolated from the soil using various enrichment or trapping methods. *Aminobacter anthyllidis* was isolated from the nodules of *Anthyllis vulneraria* that was used as a trapping plant and was grown in soil from a zinc and lead mining site (Maynaud et al. 2012). The surface-sterilized nodules were crushed in sterile water, and the bacteria were isolated by streaking the suspension on YMA (Vincent 1970; recipe as listed above) and incubating at 28 °C.

Aminobacter aganoensis, *Aminobacter aminovorans*, and *Aminobacter niigatensis* were isolated from soil by enrichment using methylamine compounds (mono-, di-, tri-, or tetramethylamine, trimethylamine-*N*-oxide, or tetramethylammonium hydroxide) or methylformamide compounds (*N*-methylformamide or *N*,*N*-dimethylformamide) (Urakami 2005). For routine growth of PYG medium, pH 7.0 can be used at 30 °C (Urakami et al. 1992).

Aminobacter ciceronei and *Aminobacter lissarensis* are methylotrophic species. *Aminobacter ciceronei* was isolated from CH_3Br-fumigated soil in the USA by enrichment on a mineral salt medium under a modified atmosphere of air plus CH_3Br (Miller et al. 1997). *Aminobacter lissarensis* strain CC495 was isolated from the top 5 cm of soil in a beech wood in County Down, Northern Ireland, by enrichment with CH_3Cl as the sole carbon and energy source. One gram of soil was added to 100 ml of minimal medium in 500-ml flasks containing 0.125 g of CH_3Cl. The minimal medium had the following composition (in grams per liter): KH_2PO_4 (4.5), K_2HPO_4 (10.5), $MgSO_4 \cdot 7H_2O$ (0.15), and NH_4NO_3 (1.5), pH adjusted to 7.2 with 6 M NaOH; a trace element solution was added (10 ml. l^{-1}) containing (in $mg.l^{-1}$) H_3BO_3(500), $CuSO_4 \cdot 5H_2O$ (40), KI (100), $FeSO_4 \cdot 7H_2O$ (200), $MnSO_4 \cdot 7H_2O$ (400), $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ (200), and $ZnSO_4$ (400). In the pure cultures, the medium was additionally supplemented with a vitamin solution (5 $ml.l^{-1}$) containing (in milligrams per liter) folic acid (4), p-aminobenzoic acid (200), and cyanocobalamin (200). CH_3Cl (0.15 g) was added as an aqueous solution to give a concentration in the culture medium, after equilibration of the gaseous and aqueous phases, of 11.8 mM (30 mM if partitioning is neglected and the total CH_3Cl present is expressed as a concentration in the aqueous phase) (Coulter et al. 1999).

All *Aminobacter* species can be stored in broth medium plus 20 % glycerol at −80 °C or can be lyophilized and stored at 4 °C.

Two *Aquamicrobium* species were isolated from activated sludge, *Aquamicrobium defluvii* and *Aquamicrobium lusatiense* (Bambauer et al. 1998; Fritsche et al. 1999; Kämpfer et al. 2009). The former species originated from a municipal wastewater plant and was obtained on a mineral medium with thiophene-2-carboxylate as the sole source of carbon and nitrate as the electron acceptor. The mineral salt medium (Bambauer et al. 1998), also used for cultivation, contained per l 3.56 g $Na_2HPO_4 \cdot 2H_2O$, 0.4 g NH_4Cl, and 0.07 g K_2SO_4. After autoclaving, 1 l of medium was supplemented with 2 ml of a sterile solution containing per l 100 g MgCl2, 25 g CaCl2, 10 ml vitamin solution (Balch et al. 1979), and 1 ml trace element solution (Widdel et al. 1983). Thiophene-2-carboxylate (2–30 mM final concentration) was added from a sterile, tenfold concentrated stock solution. For anaerobic growth, the medium was supplemented with 5–20 mM KNO_3 (Bambauer et al. 1998).

Three other species were isolated from air or waste gas in a duck shed and an animal rendering plant: *Aquamicrobium aerolatum*, *Aquamicrobium ahrensii*, and *Aquamicrobium segne* (Kämpfer et al. 2009; Lipski and Kämpfer 2012). The latter two species were isolated on Antibiotic Sulfonamide Sensitivity-test agar (Merck 1.05392) (Ahrens et al. 1997). *Aquamicrobium aerolatum* was isolated collecting bioaerosol samples by filtration over gelatin filters and isolation on trypticase soy agar incubated at 26 °C. The organism can also be grown on nutrient agar (Kämpfer et al. 2009). *Aquamicrobium aestuarii* was isolated from crude oil-contaminated sediments of a tidal flat (Jin et al. 2013) by incubating approximately 10 g of sediment with 100 ml of 0.2 µm filtered seawater containing 3 ml crude oil in 500-ml Erlenmeyer flask at 25 °C. The enrichment was aerated (180 rpm) and was transferred (1:20) four times every 2 weeks. For isolation, the enrichment was plated on marine agar 2216 (BD) plates and incubated under aerobic conditions at 25 °C for 5 days. In addition, the species grows well on R2A agar (BD), Luria-Bertani agar, trypticase soy agar, and marine agar (Jin et al. 2013).

Chelativorans strains were obtained from sludge samples. *Chelativorans multitrophicus* was isolated from a mixed microbial culture enriched in a column packed with activated carbon that was continuously fed with a mineral medium containing EDTA as sole source of carbon, nitrogen, and energy. The original inoculum of the column was activated sludge from various industrial wastewater treatment plants and soil extracts. For the isolation, further aerobic enrichment in continuous culture on a column packed with glass beads and fed with mineral medium (per liter, 1.0 g MgSO4 · $7H_2O$, 0.2 g $CaCl_2$ · $2H_2O$, 0.13 g KH_2PO_4, and 0.615 g Na_2HPO_4, 2 ml of Widdel trace element solution (Pfennig et al. 1981) and 1 ml of a vitamin solution (Egli et al. 1988)) containing 200–300 $mg.l^{-1}$ EDTA as well as batch cultures to establish optimal growth conditions were used (Weilenmann et al. 2004). The best conditions for growth were 30 °C, initial EDTA concentration in the range of 1–1.5 $g.l^{-1}$, $CaCl_2$ · $2H_2O$ concentration in the mineral medium of 0.4 $g.l^{-1}$ and an initial pH of 7.0. Pure cultures were obtained by successive plating on Plate Count Agar and liquid culture in the mineral medium (Weilenmann et al. 2004). *Chelativorans multitrophicus* was obtained by enrichment from municipal sludge samples: 10 g of sample was suspended in 100 ml of medium (per liter, 1.0 g EDTA, 1.0 g MgSO4 · $7H_2O$, 0.4 g $CaCl_2$ · $2H_2O$, 0.26 g KH_2PO_4, and 0.83 g Na_2HPO_4 · $12H_2O$ and trace elements and vitamins (Egli et al. 1988), pH 7.0). The medium was incubated in a 750-ml flask on a shaker (150–200 rpm) at 28 °C for 2 weeks. Five milliliters of this enrichment then inoculated into a 750-ml flask with 100 ml of fresh medium and cultivated for 2 weeks. After five such transfers, pure colonies were picked from plates of the same medium plus agar (Chistyakova et al. 2005).

Hoeflea species have been isolated from different aquatic environments. *Hoeflea marina* comprises one strain, LMG 128^T, that was originally classified as *Agrobacterium ferrugineum* (other strains of this species have been renamed as *Pseudorhodobacter ferrugineus*, a member of the *Rhodobacteraceae*). *Hoeflea marina* was isolated from water from the Baltic Sea, off the coast of Germany, during a study of star-forming bacteria (Ahrens 1968; Peix et al. 2005). *Hoeflea phototrophica* was isolated from cultures of the marine dinoflagellates *Alexandrium lusitanicum* and *Prorocentrum lima*. Wine red colonies were obtained by plating washed single dinoflagellate cells onto 1/10-strength Difco marine agar. Pigmentation was found to depend on the salt concentration with cultures with 3, 6, or 9 $g.l^{-1}$ sea salts being very pink, while at 35 $g.l^{-1}$ cultures were colorless (Biebl et al. 2006). *Hoeflea alexandrii* was purified from cultures of another marine dinoflagellate, *Alexandrium minutum*. In this case, the washed dinoflagellate cells were sonicated prior to plating on full- and half-strength Difco marine agar and incubation during 7 days at 15 °C. Brown-pigmented colonies were obtained. Marine agar or broth was used for routine cultivation at 30 °C (Palacios et al. 2006). *Hoeflea anabaenae* was isolated from a culture of the cyanobacterium *Anabaena* under heterotrophic conditions in the brackish marine purity liquid medium (per liter, 20 g NaCl, 17 g AC broth (Difco), 8 g $MgSO_4 \cdot 7H_20$, 1.5 g $CaCl_2 \cdot 2H_20$ (Stevenson and Waterbury 2006)) from a culture in which it was attached almost exclusively to *Anabaena* heterocysts. It is also able to grow aerobically at 30 °C in full- and half-strength marine broth (Difco) and marine agar and liquid or solid PY medium (20 g sea salts, 3 g peptone, and 0.5 g yeast extract per liter (Biebl et al. 2005)). *Hoeflea suaedae* was isolated from the root surface of the halophyte *Suaeda maritima*. Surface-sterilized and dried root pieces (1 g) were ground in 9 ml of autoclaved filtered seawater (AFS) with a sterile mortar and pestle. Dilution series were plated in triplicate on one-tenth-strength R2A (1/10 R2A) medium in filtered seawater and supplemented with 50 μg/ml cycloheximide. Plates were incubated at 28 °C for 2–3 weeks. Routine maintenance is on 1/10 R2A medium in filtered seawater, and the organism can be stored with 15 % glycerol at −70 °C (Bibi et al. 2012).

Hoeflea siderophila was isolated from fresh ochreous sediments collected near the outlet of an iron-rich brackish spring using dilution plating on the following medium (g per liter): NaCl, 20; NH_4Cl, 0.3; $CaCl_2 \cdot 6H_2O$, 0.3; $MgCl_2 \cdot 7H_2O$, 3; $NaHCO_3$, 0.5; 10 % phosphate buffer (pH 7.0), 0.1; Hepes buffer (pH 7.2), 3.0; KNO_3, 0.3; CH_3COONa, 0.15; vitamins and trace elements (Pfennig and Lippert 1966); Difco agar, 5.0; and pH 7.0. Before inoculation, the medium was supplemented with fresh sterile FeS suspension (Hanert 1981) (0.2 mL per 10 mL of medium). Inoculated media were incubated for 2–3 weeks at 28 °C. Growth consisted of dense spherical colonies, orange-colored due to the formation of iron oxides. In liquid medium, iron oxidation results in an ochreous precipitate (Sorokina et al. 2012).

Hoeflea hydrophila was isolated from marine sediments by serial dilution in filter-sterilized natural seawater containing 0.1 % yeast extract. After aerobic incubation at 25 °C for 2 weeks, a sample from the lowest dilution showing growth was plated on the same medium, and after incubation at 25 °C for 2 weeks, single colonies that were beige, circular, and convex with regular edges were purified on marine agar 2216 (Difco). This species can be routinely grown on marine broth or marine agar. Marine broth cultures plus 20 % glycerol can be stored at −80 °C (Jung et al. 2013).

Lentilitoribacter was isolated from coastal seawater by dilution plating on marine agar 2216 (Becton–Dickinson) at 25 °C. These conditions were also used for routine cultivation. For short-term preservation, marine agar cultures can be stored at 4 °C, while for long-term preservation, glycerol suspensions (20 %) can be stored at −80 °C (Park et al. 2013).

Mesorhizobium comprises soil bacteria that can live endosymbiotically in root nodules on various legume plants where they can fix atmospheric nitrogen contributing to plant nutrition. A widely used approach to isolate rhizobia is through the use of legume plants to trap the bacteria from a particular soil. Surface-sterilized seeds are allowed to germinate in the soil, and after the plants develop, nodules are harvested for isolation of the bacteria (Vincent 1970). A similar approach is also used to verify the nodulation capacity of a strain with a particular host species. Isolation from surface-sterilized and crushed nodules is performed using yeast mannitol agar (YMA, per liter, 10 g

D-mannitol, 0.5 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 4 g $CaCO_3$, 0.4 g yeast extract, 15 g agar, pH 6.8–7.0). Incubation at 28 °C will result in the formation of creamy, entire, and convex mucoid colonies after 3–4 days. This procedure can result in growth of not only *Mesorhizobium* strains but also other rhizobia. Phenotypic distinctions are not straightforward: *Mesorhizobium* members can in some cases be distinguished in that they have a moderately fast growth rate (generation time 4–15 h) compared to *Rhizobium* (<6 h) and *Bradyrhizobium* (>6 h). Also they produce acid on YMA (as do *Rhizobium* strains), while *Bradyrhizobium* strains produce alkali (Chen et al. 2005). More certain genus assignment, however, requires verification of the partial 16S rRNA gene sequence.

All *Mesorhizobium* species have been isolated using the trap legume method except for one species, *Mesorhizobium thiogangeticum*, which was obtained from soil adjacent to the root of the legume *Clitoria ternatea* through enrichment using reduced sulfur compounds as sole carbon and energy source. Soil was supplemented with $Na_2S_2O_3 \cdot 5H_2O$ (5 %), Na_2S (1 %), and elemental sulfur powder (5 %) and incubated at 30°C for 2 weeks with intermittent sprinkling of sterile water. After this enrichment, soil samples (1 %, w/v) were incubated on a rotary shaker at 30 °C in mineral salt thiosulfate yeast extract liquid medium (20 mM $Na_2S_2O_3 \cdot 5H_2O$ supplemented with 5 g yeast extract per liter, pH 7.0–7.5) in mineral salt solution that contained (per liter of distilled water) 1 g NH_4Cl, 4 g K_2HPO_4, 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, and 5.0 ml trace metal solution (Vishniac and Santer 1957). When the color of the phenol red indicator had changed to yellow, serial dilutions were plated on the same agar medium for the isolation of pure cultures (Ghosh and Roy 2006).

Nitratireductor strains have been isolated from diverse marine sources, often by standard dilution plating techniques. *Nitratireductor aquibiodomus* was isolated from a marine aquarium denitrification system fitted with cellulose carriers. For the isolation, cellulose carriers were homogenized, and a dilution series was plated onto trypticase soy agar and R2A and incubated at room temperature for 3 weeks. *Nitratireductor aquibiodomus* was one of the several organisms that were picked up (Labbé et al. 2003); its colonies were white, smooth, circular, and convex (Labbé et al. 2004). *Nitratireductor basaltis* was isolated from black sand from Soesoggak beach, Jeju Island, Korea, by dilution plating onto marine agar 2216 (Difco) and incubating at 30–37 °C. It is not reported whether other organisms were able to grow in these conditions; the colonies of *Nitratireductor basaltis* were creamy, circular, convex, and smooth (Kim et al. 2009). *Nitratireductor kimnyeongensis* was isolated from a dried seaweed sample from Kimnyeong beach in Jeju, Republic of Korea (Kang et al. 2009), by transferring a piece of dried seaweed directly transferred onto isolation medium (WAT-SW agar) consisted of 0.05 % $MgSO_4.7H_2O$, 0.05 % $CaCl_2.2H_2O$, and 1.5 % agar in 60 % natural seawater and 40 % distilled water (pH 7.3) (Lee 2006). The organism can also be conveniently grown on trypticase soy agar where, after 5 days of incubation, colonies are small (0.5–1 mm in diameter), light yellow, circular, convex, smooth, and entire (Kang et al. 2009). *Nitratireductor aquimarinus* was isolated from exponential cultures of the marine diatom *Skeletonema costatum* by plating a 10-µl sample on marine agar 2216 (Difco) and incubating aerobically for 1 week. Colonies are creamy, smooth, circular, and convex. Growth is also good on trypticase soy agar at 35 °C. Strains can be preserved in trypticase soy broth supplemented with 30 % glycerol and −80 °C (Jang et al. 2011). *Nitratireductor indicus* was isolated from a deep-sea water sample taken at a depth of 2,488 m taken with Niskin bottles attached to a CTD (conductivity, temperature, and depth) sampler at 25.3217°S 70.0405°E in the southwestern part of the Indian Ridge. The seawater was enriched with 1 % sterilized crude oil, and after two months, bacteria were isolated by using the plating on 216L medium (per liter seawater: 1.0 g CH_3COONa, 10.0 g tryptone, 2.0 g yeast extract, 0.5 g sodium citrate, and 0.2 g NH_4NO_3; pH 7.5) (Lai et al. 2009; Lai et al. 2011b). On marine agar, colonies are unpigmented, smooth gray, and slightly raised in the center and have a regular margin (Lai et al. 2011a). *Nitratireductor pacificus* was also isolated from deep-sea water samples, this time from a pyrene-degrading enrichment (described in Wang et al. 2008) by using phthalate as sole carbon source in mineral medium (MM, comprising per liter: 3.5 g $MgSO_4 \cdot 7H_2O$, 0.05 g $CaCl_2$, 24 g NaCl, 0.35 g KCl, 1.0 g NH_4NO_3, 1.0 KH_2PO_4, 1.0 g K_2HPO_4, 0.01 g $FeCl_3$, 0.0001 g $ZnSO_4 \cdot 7H_2O$, 24 mg $SrCl_2 \cdot 6H_2O$, and 0.08 g KBr, adjusted to pH 7.4) (Wang et al. 2008; Lai et al. 2011a). On marine agar, colonies are smooth gray, nonpigmented with a regular margin, and slightly raised in the center (Lai et al. 2011a).

Most recently, a new species, *Nitratireductor lucknowense*, was published, though not yet validated. It was isolated from pesticide-contaminated soil from a γHCH (lindane) manufacturing site in India. Five-gram soil samples collected from three different locations were mixed together in 50 ml of sterile mineral medium (Senoo and Wada 1989). After the slurry had settled, the liquid phase was used to enrich for bacteria on 0.34 M lindane. On trypticase soy agar, the new species produces colonies that are straw yellow, smooth, circular, glistening, opaque, and convex with an entire margin (Manickam et al. 2012). NaCl is tolerated up to 2 % (Manickam et al. 2012), considerably less than most other *Nitratireductor* species which can tolerate up to 7 or 8 % (❯ *Table 18.8*).

Most *Phyllobacterium* species are plant associated, and while the first species were isolated from tropical ornamental plants, these bacteria have since also been isolated from other plants elsewhere and from non-plant sources such as volcanic rock used for construction. Different isolation procedures have been used and are summarized in the following overview. The selectivity in most cases is not documented.

Phyllobacterium myrsinacearum and its junior subjective synonym *Phyllobacterium rubiacearum* (Mergaert et al. 2002) have been isolated from leaf nodules of members of the plant families *Rubiaceae* (*Pavetta zimmermanniana*) and *Myrsinaceae* (*Ardisia crispa*, *Ardisia crenata*). Washed leaf pieces carrying nodules were macerated by rubbing and placed in saline. After shaking, dilutions were plated onto carrot juice agar containing yeast extract (fresh carrot juice, 500 ml; water, 500 ml;

$FeSO_4 \cdot 7H_2O$, 0.1 g; $MnSO_4 \cdot H_2O$, 0.1 g; agar, 15 g; pH 7.2; the medium is sterilized by fractional sterilization). After incubation at 28 °C, typical nonpigmented to beige, slimy, and circular colonies that are translucent to opaque in the center are transferred into liquid carrot juice medium. After 24- to 48-h phase, contrast microscopy can be used to verify the formation of star-shaped clusters. Stock cultures can be kept on trypticase soy agar at 5 °C for 1–2 months, and cultures can be lyophilized for long-term preservation (Knösel 1984). Isolates of this species have also been obtained from the root surface of sugar beet by using trypticase soy broth agar as a nonselective medium (Lambert et al. 1990; Mergaert et al. 2002).

Phyllobacterium trifolii was isolated from the nodules of *Trifolium pratense* as described above for *Mesorhizobium* species. Colonies on YMA are white, mucoid, translucent, and convex. The growth rate of this *Phyllobacterium* species (generation time 2 h) is faster than most mesorhizobia. Growth is also possible on nutrient agar (Valverde et al. 2005).

A further five species were also obtained using the same procedure, this time from roots of *Brassica napus* cv. Eurol (*Phyllobacterium bourgognense*, *Phyllobacterium brassicaearum*), root nodules of *Lathyrus numidicus* and *Astragalus algerianus* (*Phyllobacterium ifriqiyense*), root nodules of *Astragalus algerianus* and *Argyrolobium uniflorum* (*Phyllobacterium leguminum*) (Mantelin et al. 2006b), and root nodules of *Phaseolus vulgaris* (Flores-Felix et al. 2013). Colonies are circular, white or cream colored with regular margins, and in most strains highly mucoid (Mantelin et al. 2006b).

Phyllobacterium catacumbae does not originate from a plant-associated source. It was isolated from tuff, volcanic rock used in the walls of the Roman catacombs of Saint Callixtus, Rome, Italy. The B-4 medium used for isolation contained (per liter) 2.5 g calcium acetate, 4 g yeast extract and 15 g agar, pH 8, and incubation was at 28 °C. Colonies are circular, smooth, and beige. Growth is also good on trypticase soy agar (Jurado et al. 2005).

Phyllobacterium species can be stored at −80 °C in broth medium plus 20 % glycerol or at 4 °C lyophilized.

Pseudahrensia was isolated from seawater from the Yellow Sea, Korea, by the standard dilution plating on marine agar 2216 (Difco) and incubating at 25 °C. Colonies are circular, convex, smooth, glistening, and cream colored. This organism can be routinely cultivated on marine agar at 30 °C (Jung et al. 2012).

Although no precise isolation media have been published, *Pseudaminobacter* strains have been isolated by exploiting specific degradation capacities. The *Pseudaminobacter salicylatoxidans* type strain was isolated as a degrader 6-aminonaphthalene-2-sulfonate from a microbial consortium degrading this substrate and originating from the river Elbe, Germany (Nortemann et al. 1986; Kämpfer et al. 1999). The type strain of *Pseudaminobacter defluvii* was isolated from activated sludge which was enriched with thiocyanate (Katayama-Fujimura et al. 1983; Kämpfer et al. 1999). Growth is possible on several media including nutrient agar, trypticase soy agar, trypticase soy broth plus 1.5 % agar, and R2A (Oxoid) (Kämpfer 2005).

Thermovum composti was isolated from mature compost produced by a field-scale composter used for the treatment of livestock excreta. Dilution series of 1 g of compost in saline solution were plated onto isolation medium composed of (per liter distilled water) 1 g yeast extract, 2 g tryptone, 1 g NaCl, 1 g $MgSO_4 \cdot 7H_2O$, 20 g agar, 20 mg trimethoprim, 10 mg nalidixic acid, and 20 mg kanamycin, pH 7.0. After incubation at 50 °C for 7 days, colonies were picked and repeatedly transferred for purification. Colonies on nutrient agar are cream colored. Stock cultures in trypticase soy broth, grown for 2 days at 50 °C, can be supplemented with 20 % glycerol and stored at −80 °C (Yabe et al. 2012).

Ecology

Members of the *Phyllobacteriaceae* are versatile environmental bacteria that occur in diverse habitats that often are polluted or nutritionally rather rich. These habitats can be marine (*Aliihoeflea*, *Ahrensia*, *Aquamicrobium*, *Hoeflea*, *Lentilitoribacter*, *Nitratireductor*, *Pseudahrensia*) or polluted freshwater (*Pseudaminobacter*, *Aquamicrobium*, *Chelativorans*) systems, soil (*Aminobacter*, *Chelativorans*, *Mesorhizobium*, *Nitratireductor*, *Phyllobacterium*, *Thermovum*), or air (*Aquamicrobium*). Several genera are plant associated or associated with dinoflagellates or cyanobacteria (*Hoeflea*, *Mesorhizobium*, *Phyllobacterium*).

Aquamicrobium species have been isolated from diverse polluted environments (activated sludge, oil-polluted sediments, air/waste gas from poultry/animal rearing) and have degradative capabilities that may significantly contribute to the breakdown of pollutants: *Aquamicrobium defluvii* is able to degrade thiopene-2-carboxylate (Bambauer et al. 1998), *Aquamicrobium lusatiense* can utilize phenol and chlorophenols such as 4-chloro-2-methylphenol, 2,4-dichlorophenol, and 4-chlorophenol (Fritsche et al. 1999), and *Aquamicrobium aestuarii* was enriched from marine sediments using crude oil (Jin et al. 2013).

Chelativorans species have been found in municipal and industrial sludge samples and are able to degrade EDTA, a chelating agent with many applications that is generally recalcitrant to biodegradation. *Chelativorans multitrophicus and Chelativorans oligotrophicus* are able to use EDTA as sole carbon, nitrogen, and energy source, facultatively and obligately, respectively. They may have a significant role in the clearing of EDTA pollution in surface waters (Doronina et al. 2010).

Hoeflea phototrophica contains bacteriochlorophyll at reduced salt concentrations, but not at the concentration seawater (3.5 %); it also contains a carotenoid pigment thought to be spheroidenone and was found to contain *pufL* and *pufM* genes coding for proteins of the photosynthetic reaction center. However, *Hoeflea phototrophica* does not grow anaerobically in light or dark conditions, and conditions under which it may

live phototrophically have not been described in detail (Biebl et al. 2006).

Hoeflea siderophila is able to grow mixotrophically and organoheterotrophically. It is the only species of the genus that is capable of fac. chemolithotrophic growth through iron oxidation at neutral pH, in anaerobic conditions with nitrate or N_2O as terminal electron acceptor, and microaerobically with oxygen. It is one of the rare species that is capable of neutrophilic lithotrophic iron oxidation (Sorokina et al. 2012).

Mesorhizobium strains are soil bacteria that can, in the vicinity of a compatible legume host species, enter into a molecular dialogue with the plant, resulting in the formation of root nodules on the plant that can be occupied by the bacteria. The bacteria receive a safe habitat and food, while they in turn can fix atmospheric nitrogen and thus contribute to plant nutrition. Most *Mesorhizobium* species (29 of 30) have been described as symbiotic with various legume species (*Table 18.11*). The only species that was not obtained from nodules, *Mesorhizobium thiogangeticum* obtained through enrichment from the soil adjacent to the legume *Clitoria ternatea*, was not able to nodulate this host nor *Pisum sativum* or *Cicer arietinum* (Ghosh and Roy 2006). *Mesorhizobium* has occasionally also been reported from marine systems (Sfanos et al. 2005; Krick et al. 2007) and from aquatic microbial mats in Antarctica (Peeters et al. 2012).

Several *Phyllobacterium* species have been isolated from leaf or root nodules of plants and contribute to plant growth promotion (*Table 18.12*). For *Phyllobacterium myrsinacearum*, there is no direct evidence that it actively induces nodule formation in leaves. Nodulation has been confirmed for *Phyllobacterium trifolii* (Valverde et al. 2005),

Unspecified *Phyllobacterium* strains have also been reported from the rhizosphere of *Lotus* spp. (Oger et al. 2004), associated with roots in *Brassica napus* (Bertrand et al. 2001), as endophytes in *Ipomea batatas* (Khan and Doty 2009), and in root nodules of many legumes including *Dalbergia louvelii* (Rasolomampianina et al. 2005), *Lathyrus gmelinii* (Baymiev et al. 2011), *Acacia* sp. (Hoque et al. 2011), *Sophora alopecuroides* (Zhao et al. 2010), *Vicia* sp. (Lei et al. 2008), and *Ononis tridentata* (Rincon et al. 2008). They have also been found as free-living bacteria in water (Mergaert et al. 2001) and associated with unicellular organisms (Gonzalez-Bashan et al. 2000).

The possible symbiotic function of phyllobacteria is reported to be the production of plant growth hormones, protective antibacterial and antifungal activity (Lambert et al. 1990), phosphate solubilization (Chen et al. 2006), root hair elongation (Galland et al. 2012), and nitrogen fixation (Valverde et al. 2005).

Both *Pseudaminobacter* species have been isolated from polluted aquatic environments (Kämpfer et al. 1999). *Pseudaminobacter salicylatoxidans* can degrade substituted naphthalenesulfonates and substituted salicylates. One strain has also shown to be a facultative sulfur chemolithotroph that can oxidize $S_2O_3^{2-}$, $S_4O_6^{2-}$, SO_3^{2-}, S_2^{2-}, and S^0 directly to SO_4^{2-} without any intermediate formation (Ghosh and Dam 2009). In polluted oligotrophic aquatic systems, these bacteria may play an important role in biodegradation.

Thermovum is the only genus of the family that is thermophilic (maximum growth temperature 60 °C). It was isolated from mature compost; its role in the compost ecosystem is not documented (Yabe et al. 2012).

Pathogenicity, Clinical Relevance

Mesorhizobium amorphae has been reported as an amoeba-associated bacterium that may be involved in nosocomial pneumonia through contaminated water supplies (La Scola et al. 2003; Berger et al. 2006). As no recent reports confirming these observations were found, the significance of *Mesorhizobium amorphae* as a nosocomial pathogen is not clear. No other animal or human pathogens are among the current members of the *Phyllobacteriaceae*.

Many species, particularly of *Mesorhizobium* and *Phyllobacterium*, are plant endophytes, rhizoplane or rhizosphere bacteria that have plant beneficial effects (see next section below).

No plant pathogenic effects have been reported for most members of the family *Phyllobacteriaceae*. *Candidatus Liberibacter* is, however, a serious plant pathogen. It is included here, although the membership of this group is currently uncertain (see above under Sect. 1.2, "Phylogenetic Structure of the Family and Its Genera"). The trivial name "liberobacter" (*sic*) (from the Latin liber [bark] and bacter [bacteria]) was proposed in 1994 for a phloem-limited bacterium-like organism associated with citrus greening disease, also known as huanglongbing disease or yellow dragon disease, a severe and widespread citrus disease that is transmitted by the Asian citrus psyllid (*Diaphorina citri*) and the African citrus psyllid (*Trioza erytreae*) (Jagoueix et al. 1994). The disease causes yellowing and blotchy mottling of the leaves, production of bitter, small and misshapen fruits, and ultimately death of the tree (http://www.aphis.usda.gov/plant_health/plant_pest_info/). Two species, *Candidatus Liberibacter asiaticus* (originally Liberobacter asiaticum) and *Candidatus Liberibacter africanus* (originally Liberobacter africanum), were proposed for the Indian and South African liberibacters, respectively, which can be distinguished based on temperature sensitivity (in Africa symptoms occur only in cooler regions), serology, and genomic properties (Jagoueix et al. 1994). Garnier et al. (2000) corrected the spelling of the genus name and proposed a separate subspecies, *Candidatus Liberibacter africanus* subsp. capensis, for a South African liberibacter in the ornamental rutaceous tree, *Calodendrum capense*. The citrus disease was later also reported from Brazil, and the pathogen recognized as a new species, *Candidatus Liberibacter americanus*, spread by the vector *Diaphorina citri* (Texeira et al. 2005). Although the species epithet originally referred to the geographic occurrence of the group, *Candidatus Liberibacter asiaticus* has also been found in the Americas (Raddadi et al. 2011). In the USA, the Asian citrus psyllid,

Table 18.11

Host plants nodulated by *Mesorhizobium* species

Species	Host plants nodulated	References
Mesorhizobium abyssinicae	*Acacia abyssinica, Acacia tortilis, Acacia tortilis*	Degefu et al. 2013; Wolde-meskel et al. 2005
Mesorhizobium albiziae	*Albizia kalkora, Albizia julibrissin, Glycine max, Leucaena leucocephala, Phaseolus vulgaris*	Wang et al. 2007
Mesorhizobium alhagi	*Alhagi sparsifolia, Sophora alopecuroides, Glycyrrhiza inflata, Medicago sativa, Indigofera endecaphylla, Vicia cracca, Sophora flavescens*	Chen et al. 2010
Mesorhizobium amorphae	*Amorpha fruticosa, Cicer arietinum*	Rivas et al. 2007; Wang et al. 1999
Mesorhizobium australicum	*Biserrula pelecinus, Astragalus membranaceus, Macroptilium atropurpureum*	Nandasena et al. 2009
Mesorhizobium camelthorni	*Alhagi sparsifolia, Sophora alopecuroides, Glycyrrhiza inflata, Medicago sativa*	Chen et al. 2011
Mesorhizobium caraganae	*Caragana microphylla, Caragana intermedia*	Guan et al. 2008
Mesorhizobium chacoense	*Prosopis alba*	Velazquez et al. 2001
Mesorhizobium ciceri	*Cicer arietinum*	Nour et al. 1994
Mesorhizobium gobiense	*Astragalus filicaulis, Lotus frondosus, Lotus tenuis, Oxytropis glabra*	Han et al. 2008
Mesorhizobium hawassense	*Sesbania sesban*	Degefu et al. 2013
Mesorhizobium huakuii	*Astragalus sinicus, Robinia pseudoacacia*	Chen et al. 1991; Ulrich and Zaspel 2000
Mesorhizobium loti	*Lotus* spp., *Anthyllis vulneraria, Lupinus densiflorus, Robinia pseudoacacia*	Jarvis et al. 1982; Ulrich and Zaspel 2000
Mesorhizobium mediterraneum	*Cicer arietinum*	Nour et al. 1995
Mesorhizobium metallidurans	*Anthyllis vulneraria*	Vidal et al. 2009
Mesorhizobium muleiense	*Cicer arietinum*	Zhang et al. 2012
Mesorhizobium opportunistum	*Biserrula pelecinus, Astragalus adsurgens, Astragalus membranaceus, Lotus peregrinus, Macroptilium atropurpureum*	Nandasena et al. 2009
Mesorhizobium plurifarium	*Acacia* spp., *Leucaena* spp., *Prosopis juliflora, Chamaecrista ensiformis*	de Lajudie et al. 1998
Mesorhizobium qingshengii	*Astragalus sinicus*	Zheng et al. 2013
Mesorhizobium robiniae	*Robinia pseudoacacia*	Zhou et al. 2010
Mesorhizobium sangaii	*Astragalus luteolus*	Zhou et al. 2013
Mesorhizobium septentrionale	*Astragalus adsurgens, Phaseolus vulgaris, Glycine max, Leucaena leucocephala, Macroptilium atropurpureum, Lotus corniculatus, Robinia pseudoacacia*	Gao et al. 2004; Han et al. 2008
Mesorhizobium shangrilense	*Caragana* spp., *Glycyrrhiza uralensis, Astragalus adsurgens, Vigna unguiculata, Vigna radiata, Phaseolus vulgaris*	Lu et al. 2009
Mesorhizobium shonense	*Acacia abyssinica*	Degefu et al. 2013
Mesorhizobium silamurunense	*Astragalus membranaceus*, *Astragalus adsurgens*, *Caragana intermedia*	Zhao et al. 2012
Mesorhizobium tamadayense	*Anagyris latifolia*, *Lotus berthelotii*	Ramirez-Bahena et al. 2012
Mesorhizobium tarimense	*Lotus frondosus, Lotus tenuis*	Han et al. 2008
Mesorhizobium temperatum	*Astragalus adsurgens, Phaseolus vulgaris, Vigna unguiculata, Glycine max, Leucaena leucocephala, Medicago sativa, Lotus corniculatus*	Gao et al. 2004
Mesorhizobium thiogangeticum	None reported. Isolated from the rhizosphere of *Clitoria ternatea* although it did not nodulate this host	Ghosh and Roy 2006
Mesorhizobium tianshanense	*Glycyrrhiza, Sophora, Caragana, Halimodendron, Swainsonia, Glycine, Cicer arietinum*	Chen et al. 1995; Rivas et al. 2007

Table 18.12
Sources of isolation and nodulation capacity reported for the different *Phyllobacterium* species

Species	Source	Nodulation capacity	References
Phyllobacterium bourgognense	Root surface of *Brassica napus* vc. Eurol	No nodulation data	Mantelin et al. 2006b
Phyllobacterium brassicacearum	Root surface of *Brassica napus* vc. Eurol; root nodules of *Caragana microphylla*	No nodulation data	Mantelin et al. 2006b; Dai et al. 2012
Phyllobacterium catacumbae	Volcanic tuff stone used in the Roman catacombs of Saint Callixtus, Rome, Italy	No nodulation data	Jurado et al. 2005
Phyllobacterium endophyticum	Root nodules of *Phaseolus vulgaris*	Unable to nodulate *Phaseolus vulgaris*	Flores-Felix et al. 2013
Phyllobacterium ifriqiyense	Isolated from root nodules of *Lathyrus numidicus* and *Astragalus algerianus*	No nodulation data	Mantelin et al. 2006b
Phyllobacterium leguminum	Isolated from root nodules of *Astragalus algerianus* and *Argyrolobium uniflorum*	No nodulation data	Mantelin et al. 2006b
Phyllobacterium myrsinacearum	Leaf nodules of *Pavetta zimmermannia*, *Ardisia crispa*, and *Ardisia crenata;* root surface of sugar beet	No nodulation data	Knösel 1984; Lambert et al. 1990; Mergaert et al. 2002
Phyllobacterium trifolii	*Trifolium pratense, Trifolium repens, Lupinus albus*	Nodulates *Trifolium pratense, Trifolium repens*, and *Lupinus albus*; nodD gene present	Valverde et al. 2005

Diaphorina citri, has been present in Florida since 1998, and *Candidatus Liberibacter asiaticus* was found in Florida in early September 2005. In 2010, the USDA imposed a plant quarantine in several states and territories in the USA to stop the spread of citrus greening (http://www.aphis.usda.gov/plant_health/plant_pest_info/). The disease was reported in South California in 2012 (www.californiacitrusthreat.com). The European and Mediterranean Plant Protection Organization (EPPO) has placed *Liberibacter africanus*, *Liberibacter asiaticus*, and *Liberibacter americanus* and the vector *Diaphorina citri* on its A1 list of pests recommended for regulation as quarantine pests. This list comprises pests regarded as absent from the EPPO region. The vector *Trioza erytreae* was included on the A2 List of pests recommended for regulation as quarantine pests that are locally present in the EPPO region.

Citrus huanglongbing disease is regarded as a pest of urgent phytosanitary concern for southern parts of the EPPO region where citrus is grown. Several PCR and real-time PCR tests have been developed for the detection of these *Candidatus Liberibacter* sp. in plants and in the psyllid vectors (Morgan et al. 2012 and references therein). To eliminate or suppress *Candidatus Liberibacter asiaticus*, a combination of penicillin and streptomycin administered by trunk injection or root soaking was shown to be effective in citrus plants (Zhang et al. 2011).

Several additional species have more recently been reported to affect *Solanaceae* plants. *Candidatus Liberibacter psyllaurous* is associated with psyllid yellows disease of potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) in North America and is transmitted by *Bactericera cockerelli* (Hansen et al. 2008). *Candidatus Liberibacter solanacearum* was reported from tomato, capsicum (*Capsicum annuum*), potato, tamarillo (*Solanum betaceum*), cape gooseberry (*Physalis peruviana*), and chilli (*Capsicum* sp.) from New Zealand; it is transmitted by the psyllid *Bactericera cockerelli*. It has also been detected in the USA in potatoes affected by zebra chip disease (Liefting et al. 2009). *Candidatus Liberibacter solanacearum* and its vector *Bactericera cockerelli* have been placed on the EPPO A1 and A2 lists, respectively, and are considered of particular concern for southern and central parts of the EPPO region and for areas with mild winters in the northern part (http://www.eppo.int/QUARANTINE/quarantine.htm).

Candidatus Liberibacter europaeus, the most recently described species, is the only one presently considered to be an endophyte rather than a plant pathogen. It was reported from Italy and is found in the midgut lumen, salivary glands, and Malpighian tubules of the pear psyllid pest *Cacopsylla pyri* and has been detected in pear plant tissue, both in laboratory-inoculated plants and in field-collected samples. However, the plants remained free of disease symptoms (Raddadi et al. 2011).

As the family *Phyllobacteriaceae* comprises many soil bacteria and other free-living bacteria, it is not surprising that many species show diverse resistance patterns to a number of antibiotics which they can be expected to encounter in their natural habitat. These characters can often be used for phenotypic differentiation and have therefore been included above in the Sect 3, "Phenotypic Analyses".

Application

Most strains of the genus *Mesorhizobium* are capable of nitrogen-fixing symbiosis in root nodules of legume plants, allowing the host plant to grow in soils with lower nitrogen content than other plants. Several agricultural crops, including chickpea

(*Cicer arietinum*), alfalfa or lucerne (*Medicago sativa*), and several *Lotus* species (Table 18.11), are nodulated by mesorhizobia, and these bacteria thus make an important contribution to the success of these crops. Interest to introduce new *Lotus* forage species in Australia has stimulated research into compatible *Mesorhizobium* strains (Howieson et al. 2011). *Mesorhizobium* strains are also important as symbionts of legume trees such as *Acacia* and *Prosopis* spp. used in tropical agroforestry systems (de Lajudie et al. 1998; Bala et al. 2003; Degefu et al. 2013).

In addition to symbiotic nitrogen fixation, several strains of *Mesorhizobium* and *Phyllobacterium* are recognized for other plant growth-promoting effects. Mesorhizobia can exert these effects by producing the plant hormone indole acetic acid or ammonia (Ahmad et al. 2008). *Mesorhizobium mediterraneum* PECA21 was shown to solubilize phosphorus from tricalcium phosphate and improve growth and phosphorus content in chickpea (Peix et al. 2001). *Mesorhizobium loti* MP6 was shown to increase yield and resistance to white rot (*Sclerotinia sclerotiorum*) in *Brassica campestris* in India (Chandra et al. 2007). Other strains are resistant to heavy metals (Maynaud et al. 2013). *Phyllobacterium brassicacearum* STM 196 was shown to promote the growth of oilseed rape and *Arabidopsis* and to stimulate root hair elongation possibly through activation of the ethylene signalling pathway (Mantelin et al. 2006a; Galland et al. 2012).

A strategy for the regeneration of disused mining site is through revegetation to allow sustainable plant cover to stabilize the site and limit wider environmental impact. In France, *Anthyllis vulneraria* is one of the legume species tolerant of these contaminated sites (Mahieu et al. 2011). It is nodulated by two members of the *Phyllobacteriaceae*: *Aminobacter anthyllidis* and *Mesorhizobium metallidurans.* Both species are tolerant to high concentrations of heavy metals and contribute through this symbiosis to the success of the legume species in the process of revegetation of contaminated sites (Vidal et al. 2009; Maynaud et al. 2012). The combined inoculation of legumes with plant growth-promoting rhizobacteria and *Mesorhizobium loti* strains that have the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which hydrolyzes the precursor to the plant hormone ethylene was shown to have a synergistic positive effect on plant growth, nutrition, and adaptation to metal-polluted soils. The ACC-hydrolyzing bacteria eliminate the plant growth inhibition caused by increased ethylene production and can thus be valuable participants in the phytostabilization of contaminated mining sites (Safronova et al. 2012). In an application of the same mechanism, a strain of *Mesorhizobium ciceri* transformed to express exogenous ACC deaminase was shown to improve yields of chickpea and reduce susceptibility to root rot disease (Nascimento et al. 2012). One highly chromium-tolerant *Mesorhizobium* strain was shown to improve the yield and decrease chromium uptake in chickpea (Wani et al. 2008).

Aminobacter strains were reported to be responsible for the degradation of 2,6-dichlorobenzamide, a frequent groundwater pollutant that is a degradation product of the herbicide 2,6-dichlorobenzonitrile (dichlobenil). A specific PCR test targeting the 16S rRNA genes was designed to monitor the distribution of *Aminobacter* strains (Sjoholm et al. 2010). *Aminobacter aminovorans* strains previously classified as *Chelatobacter heintzii* are able to degrade nitrilotriacetate and EDTA (Auling et al. 1993; Nortemann 1999). *Chelativorans* species are also able to degrade EDTA and particularly *Chelativorans oligotrophicus* has been applied in biofilters to remove EDTA and EDTA-metal complexes (Kuvichkina et al. 2012; Kaparullina et al. 2012).

Pseudaminobacter strains have been investigated for use in bioremediation of soil contaminated with atrazine and one strain was reported to use as sole carbon and nitrogen source (Topp 2001). They have also been implicated in the degradation of methyl parathion (Zhang et al. 2005).

The salicylate 1,2-dioxygenase from *Pseudaminobacter salicylatoxidans* BN12T has been characterized extensively (Hintner et al. 2004; Matera et al. 2008; Ferraroni et al. 2012). A soil isolate very similar to *Pseudaminobacter salicylatoxidans* was shown to be a sulfur chemolithotroph (Bagchi et al. 2005; Mandal et al. 2007).

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19 The Family *Rhizobiaceae*

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Abstract
Rhizobiaceae is a family of *Rhizobiales* order into *Alphaproteobacteria* class that presents genera associated with soil and planta hosts. *Rhizobium* is the type genus and encompasses the largest number of species into the family. Taxonomy is mostly supported by phylogenetic analyses based on 16S rRNA sequences and nomenclature in *Rhizobiaceae* is one issue that has caused much discussion. Bacteria are phenotypically heterogeneous, predominately aerobic, and Gram-negative rod-shaped. Many species present large plasmids which harbor a large proportion of genome generally including genes involved in interaction with specific hosts. Some members of the *Rhizobiaceae* family are characterized by their ability to establish symbiotic associations with host plants and develop the process of biological nitrogen fixation. In contrast, others are able to establish pathogenicity against plants. Both skills can be exploited for applied purposes. The selection of efficient strains from *Rhizobium* and *Ensifer* designed to plant inoculants is one of important research viewing the production of microbial inoculants to help plant development. Considering *Agrobacterium tumefaciens*, the management of its natural ability to transform plants is directed to obtaining disarmed strains and clone vectors widely applied to recombinant DNA technology and plant biotechnology. Finally, some genera in *Rhizobiaceae* family have intriguing metabolisms which allow degradation of potentially toxic molecules and thus could be applied as biomarkers or in bioremediation.

Taxonomy: Historical and Current

The family *Rhizobiaceae* Conn 1938 (order *Rhizobiales*, class *Alphaproteobacteria*) is composed of at least nine genera: *Rhizobium* (type genus), *Agrobacterium*, *Carbophilus*, *Chelatobacter*, *Ensifer*, *Sinorhizobium*, *Allorhizobium*, *Kaistia*, and *Shinella* (Conn 1938; Kuykendall 2005; Euzéby 1997; Parte 2014). Currently, the *Rhizobiaceae* taxonomy is based on 16S rRNA gene sequence analysis, and it is a phenotypically heterogeneous family of aerobic and Gram-negative, rod-shaped bacteria. The family included only four genera (*Rhizobium*, *Bradyrhizobium*, *Agrobacterium*, and *Phyllobacterium*) in the 1984 edition of *Bergey's Manual of Systematic Bacteriology*, and the separation of these genera was predominantly based on the ability to stimulate the production of root or leaf nodules in host plant species (Jordan 1984).

More recently, it became evident that the symbiotic trend and range of host plants are not closely and easily correlated with kinship based on phylogenetic comparisons of the ribosomal marker. It was particularly noted that *Rhizobium* and *Shinorhizobium* are more closely related to *Agrobacterium* and more distantly related to *Bradyrhizobium* (family *Bradyrhizobiaceae*—Garrity et al. 2005) and *Phyllobacterium* (family *Phyllobacteriaceae*—Mergaert and Swings 2006).

Studies on the microbiology and taxonomy of *Rhizobiaceae* began with the classical works of Hellriegel and Wilfarth (1988), who determined that microbes allowed some leguminous to obtain nitrogen from the atmosphere in their root nodules. Subsequently, Beijerinck (1888) isolated and cultured those bacteria, thereby confirming their role in the nitrogen fixation process. These bacteria were dubbed *Bacillus radicicola*. Finally, Frank (1889) changed the name of this bacterium species to *Rhizobium leguminosarum*, which became the type species for the genus *Rhizobium* (Willems 2006).

The original genus *Rhizobium* (from *rhiza*—*bios*: which lives in a root) underwent several subsequent changes in recent years, giving rise to many other taxa. Nodulation tests on host plants to determine cross-inoculation groups (Fred et al. 1932) were extensively used in the early twentieth century to identify and characterize bacteria belonging to this genus. Subsequently,

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starting in the second half of the century, traditional phenotypic methods were also applied to identify and characterize these bacteria, including analysis of colony morphology in culture medium; intrinsic antibiotic resistance; biochemical, metabolic and nutritional characteristics; bacteriophage susceptibility; and serological reactions (Graham 1963; Graham 1964; Moffet and Colwell 1968; Vincent and Humphrey 1970). Finally, simple characteristics based on DNA molecular analysis were added to the numerical taxonomic studies (De Ley and Rassel 1965; Somasegaran and Hoben 1994; Zakhia and de Lajudie 2001).

The systems of classification have acquired a phylogenetic nature because of the advancement of novel molecular biology techniques applied to taxonomic studies, particularly the development of the Polymerase Chain Reaction (PCR), DNA-DNA and DNA-RNA hybridization, G+C content determination, and the sequencing of genetic markers (Somasegaran and Hoben 1994; Albuquerque et al. 2009). Unlike the phenetic classification that groups organisms based on their phenotypic properties without considering their evolutionary course, phylogenetic taxonomy organizes taxa based on their ancestral evolutionary lineages. Even microorganisms that have evolved differently and are evolutionary distantly related may develop similar characteristics (Atlas 1997). As a result, there was a steady increase in the number of genera and species recognized as rhizobia and other soil bacteria (Graham et al. 1991; Bécquer 2004; Willems 2006; Zakhia and de Lajudie 2006).

The sequencing and analysis of the 16S rRNA gene has been one of the most important resources contributing to the phylogenetic diversity of *Rhizobiaceae*, as well as of any other bacterial group (Peter et al. 1996); however, in some cases, the data contrast those obtained using phenetic analysis. Phylogenetic studies led to a revision and dismemberment of the genus *Rhizobium* and its relatives of the class *Alpha-proteobacteria*. *Rhizobium*, *Agrobacterium*, and *Allorhizobium* (*Rhizobiaceae*) form more a closely related group (i.e., with a higher degree of kinship), whereas *Sinorhizobium* (*Rhizobiaceae*), *Bradyrhizobium* (*Bradyrhizobiaceae*), *Mesorhizobium* (*Phyllobacteriaceae*), and *Azorhizobium* (*Hyphomicrobiaceae*) form separate groups (Willems 2006).

A recent discussion of a proposal to incorporate the closely related (although phenotypically distinct) genera *Agrobacterium* and *Allorhizobium* exclusively into the genus *Rhizobium*, and *Sinorhizobium* into *Ensifer*, has caused a division in the scientific community (Young et al. 2001, 2003; Farrand et al. 2003; Willems et al. 2003; Tindall 2008; Young 2010). There also have been disagreements regarding whether the genera *Ensifer* and *Sinorhizobium* (*Fig. 19.1*) should belong to a single taxon. The subcommittee on the taxonomy of *Agrobacterium* and *Rhizobium* prioritizes the genus *Ensifer* (Lindström and Martinez-Romero 2002); however, this position also found no general support and required consideration by a Judicial Commission. Formally, the genera recognized in *Rhizobiaceae* are *Rhizobium, Ensifer, Carbophilus, Kaistia*, and *Shinella*. From the four genera and 10 species reported in 1984 (Jordan 1984), the more recent taxonomic studies in *Rhizobiaceae* led to a total of five genera and over 100 species being recognized (Euzéby 1997; Kuykendall 2005; Young 2010; Parte 2014).

The application of polyphasic taxonomy has had greatest impact of the various techniques for resolving the taxonomic uncertainties and defining the taxonomy of *Rhizobiaceae* and other families (Cardoso et al. 2012). The polyphasic classification is based on the combination of phenotypic data obtained by the various available methods, consistent with the phylogenetic classification using sequence data from 16S rRNA or 23S rRNA. The accepted consensus classification must have a minimum of contradictions (Vandamme et al. 1996). However, the conclusive certification of a new species relies on comparative genomics, which is currently easier to apply due to the expansion of whole genome sequencing projects.

Young (2013) conducted a survey of the nomenclature used in several publications from 1982 to 2012 for species related to the genus *Ensifer*. Due to the controversies that genus transfer have caused in the scientific community regarding the taxonomy and nomenclature of some bacterial species, terms designating synonymous, equivalent names, common names, and scientific names for bacteria remain (LPSN; Taxonomy Browser/NCBI; UniProtKB taxonomy). In this chapter, we chose to maintain the common name for some species.

Molecular Analyses

The *Rhizobiaceae* family contains a fast growing number of species that may be saprophytic or able to establish beneficial or deleterious plant interactions (Ferreira et al. 2011). Due the absence of phenotypic information to differentiate the rhizobial species, the identification of members of these families is mainly based on gene sequencing (Ferreira et al. 2011). Decades ago, gene sequencing was a very expensive and time-consuming method, and this fact was considered to be one of the major hurdles for the study of the *Rhizobiaceae* family at molecular level. Different approaches were used during the 1980s and 1990s, and along with biochemical assays, molecular techniques such as Multilocus Enzyme Electrophoresis (MLEE), DNA fingerprinting, and DNA-DNA hybridization were well employed for the identification and analysis of *Rhizobiaceae* members (Van Berkun et al. 1998). Even today, MLEE is still considered to be a marker with perfectly known Mendelian inheritance, which is applicable for multilocus analysis of most living organisms and can be applied at a reasonable cost (Tibayrenc 2009). Recently, these out-of-fashion techniques have been replaced by more sophisticated approaches, such as matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, and phylogeny based on gene sequencing. Indeed, molecular approaches, especially those that rely on PCR amplification and sequencing, provide outstanding advantages (Tibayrenc 2009). For instance, both multiplex PCR and recA-based PCR were used to assess the diversity of the *Rhizobium*/*Agrobacterium* species (Puławska et al. 2006; Shams et al. 2013).

Currently, with the development of next-generation sequencing instrumentation, phylogenetic analysis based on gene and genome sequencing is considered to be one of the most trustworthy methods to study the *Rhizobiaceae* family at

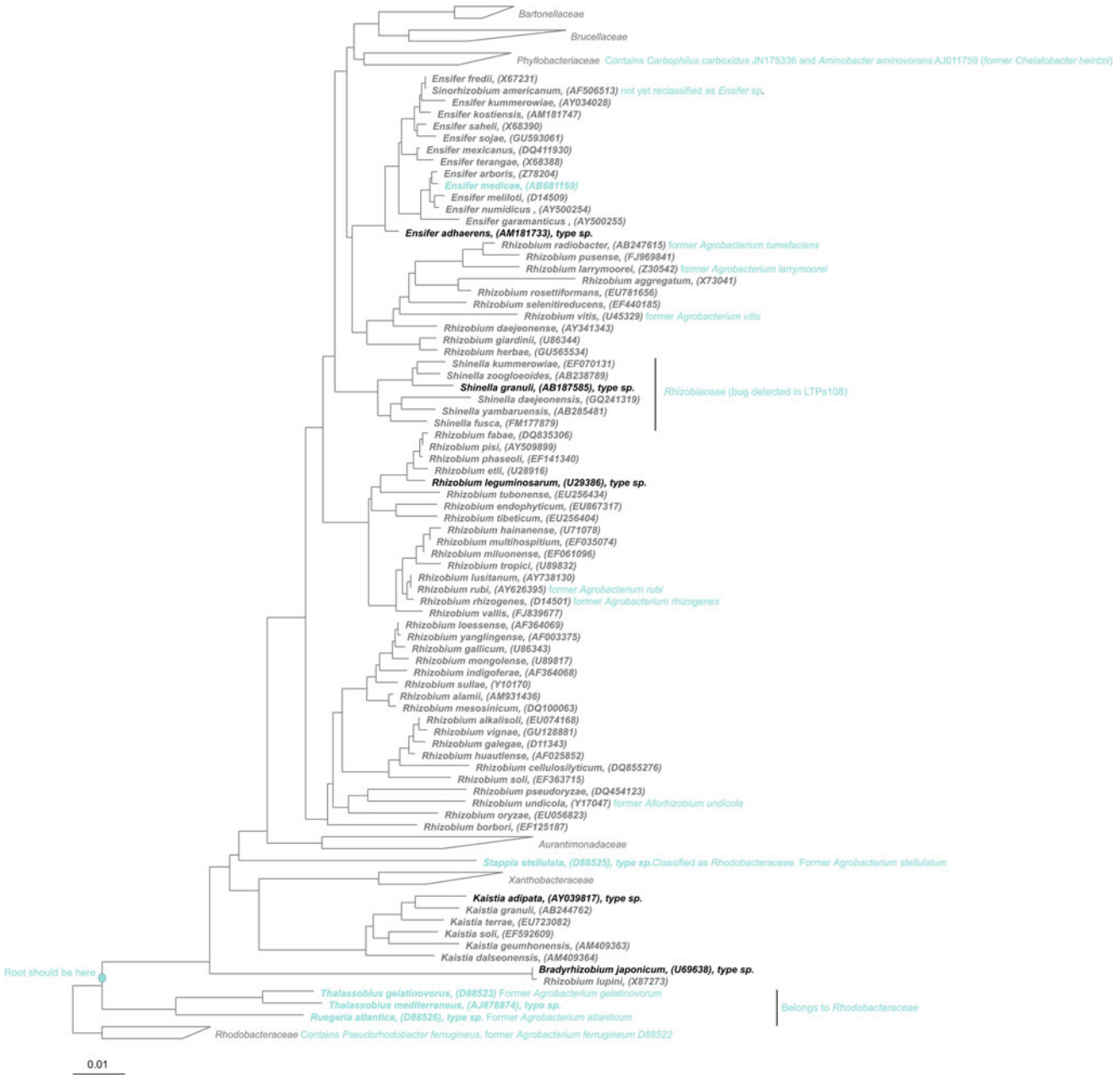

Fig. 19.1

Phylogenetic reconstruction of the family Rhizobiaceae based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes–Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010, http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

the molecular level. However, for wide population identification and classification, MALDI-TOF mass spectrometry is also considered to be a reliable and alternative method that yields similar results as those obtained using gene sequencing and phylogenetic analysis (Ferreira et al. 2011).

The employment of these different molecular techniques allowed for the identification of different *Rhizobiaceae* genera over the past several years. Indeed, the *Rhizobiaceae* diversity is vast, and the deluge of genomic data generated by different genome and environmental sequencing projects over the last 10 years has created new challenges for *Rhizobiaceae* researchers to analyze and classify both genus and species levels. Therefore, the identification, classification, and differentiation of the members of this family are still in progress. Different research groups interested in *Rhizobiaceae* biology are working to classify new members, and this often results in new arrangements in

the family classification. For instance, the family can be described in the literature as containing seven genera: *Rhizobium* and *Agrobacterium, Ensifer* and *Sinorhizobium, Carbophilus, Kaistia,* and *Shinella.* However, during the last decade, species have been arranged into five genera, *Rhizobium, Ensifer, Carbophilus, Kaistia*, and *Shinella* (Lin et al. 2008; An et al. 2006). The genera *Agrobacterium* and *Allorhizobium* are now included in the *Rhizobium* genus (Young et al. 2001), and *Sinorhizobium* is currently named *Ensifer* (Judicial Commission 2008; Young 2010).

In addition, for identification and classification purposes, genome sequencing has delivered many new insights about the biology of the family, particularly for the genome evolution and structure. For instance, genomic comparisons among sequenced strains using bioinformatics approaches indicated that the members of the *Rhizobiaceae* family have a diverse genomic structure and architecture. The number of replicons is varied, including single chromosomes, extrachromosomal replicons (ERs), and plasmids of various size (Slater et al. 2009).

Interestingly, a large proportion of the *Rhizobiaceae* genomes are located in the ERs (Althabegoiti et al. 2014). The ERs are represented in the genomic databases as a "second chromosome" or "megaplasmid." These carry a sufficient number of distinct and consistent properties that allow researchers to distinguish them from chromosomes and plasmids (Harrison et al. 2010). For instance, they carry essential genes for growth under most conditions, together with plasmid-type replication and partitioning systems. Moreover, these extrachromosomal replicons do not use a chromosome-type replication system, and the majority of their genes are genus-specific genes that confer accessory functions, such as pathogenesis, symbiosis, metabolism, and antibiotic resistance. Therefore, these secondary replicons are not chromosomes and should not be referred to as such (Harrison et al. 2010). Recently, it was suggested that the term "chromid" or "ER" be used to distinguish them from the chromosomes and plasmids (Harrison et al. 2010; López-Guerrero et al. 2012).

The chromids/ERs are often found in the *Rhizobium/Agrobacterium* and *Ensifer/Sinorhizobium* genomes, and they represent at least half of these genomes (López-Guerrero et al. 2012; Althabegoiti et al. 2014). Interestingly, some of the nodulation and nitrogen fixation genes may reside in these chromids (Harrison et al. 2010; Althabegoiti et al. 2014). Another remarkable feature is that the chromid-carrying bacteria from the genus *Rhizobium/Agrobacterium* and *Ensifer/Sinorhizobium* are able to grow faster in culture than those that do not carry chromids (Harrison et al. 2010).

In contrast to the diversity provided by the chromids/ERs, important genetic mechanisms are also observed and considered to be key agents for the diversity of the *Rhizobiaceae* genomes. Their large capacity to adapt to different plant hosts may be an indicator of the acquisition of fitness genes via lateral gene transfer (Martínez-Romero 2009). In particular, it is proposed that the *Rhizobiaceae* nodulation ability originated recently during the coevolution with the plant hosts and other rhizobia populations (Martínez-Romero 2009; Provorov and Vorobyov 2008). This fact suggests that lateral gene transfer has a key role in this process. Indeed, some of the symbiotic islands that are common in the chromosome, chromids/ERs, and plasmids have a different evolutionary history than the chromosomal "core" genome, which is a clear indication that these genes were acquired by lateral transfer events (Martínez-Romero 2009).

Aside from the diverse genomic structure and architecture caused by lateral gene transfer and by the presence of chromids/ERs observed in the *Rhizobiaceae* family, genome sequencing and its associated bioinformatics tools have also yielded important insights regarding the biology of the group. Indeed, whole genome sequencing is an important tool that is used to explore the biology of many life forms on Earth. For instance, the atrazine-degrading genes (atzABC and trzD) of *Chelatobacter* are located on three plasmids with sizes varying from 44 to 178 kb. Moreover, the atzABC and trzD genes are located in the vicinity of Insertion Sequences (ISs). These ISs are transposons that facilitate the spread of the atrazine catabolic genes via the lateral gene transfer and enhance the degradation of atrazine between members of the soil microbial community (Rousseaux et al. 2002).

In addition, several different species of the genus *Rhizobium/Agrobacterium* and *Ensifer/Sinorhizobium* were already partially and fully sequenced according both the GenBank and Genomes Online databases (Pagani et al. 2012). Genomic information is accumulating on the public databases, and our knowledge regarding the biology of the family is increasing each year. For instance, the *Rhizobium leguminosarum* is subdivided into biovars that nodulate peas and common beans (biovar viciae), clovers (biovar trifolii), or kidney beans (biovar phaseoli) (Young et al. 2006). These biovars are closely related based on DNA-DNA reassociation experiments that determined that the level of DNA relatedness among these strains varies between 58 % and 67 % (Berkum et al. 1996). Currently, the GenBank database accounts for up to 39 draft genomes, whereas 14 are represented by biovar trifoli and viciae and only one is from biovar phaseoli. Moreover, three *R. leguminosarum* genomes were already fully sequenced, whereas two and one are from the *trifolii* and *viciae* biovars, respectively (Young et al. 2006; Reeve et al. 2010). The *trifolii* biovars were isolated from Mediterranean and South America clovers, whereas the viciae were isolated from peas from the United Kingdom (Young et al. 2006; Reeve et al. 2010). Interestingly, all fully sequenced strains bear more than three copies of distinct plasmids, corroborating previous observations that indicated that at least three different plasmids are necessary to carry the genes necessary for symbiosis (Hynes and McGregor 1990).

A survey on the genome structure and architecture of the *R. leguminosarum* bv. viciae strain 3841 (Rlv3841) has determined a genome of 7.75 Mb that is composed of six large plasmids in addition to a large chromosome, with 61 % of G+C overall (Young et al. 2006). The whole genome encodes for a total of 7,263 genes, and the essential protein-encoding genes, such as the housekeeping genes, all three rRNA operons, and 52 tRNA genes, are mainly located on the large chromosome

(Young et al. 2006). Moreover, the Rlv3841 genome has two main components: a "core," which is higher in G+C content, is mostly chromosomal, and shares at least 2,056 genes with closely related organisms such as *A. tumefaciens*, and an "accessory" component composed of 90 % of the 7,263 genes and is not universally shared even among closely related species, is lower in G+C content, and has a sporadic distribution along the plasmids and chromosomal islands (Young et al. 2006). Several of these genomic islands are flanked by tRNA genes, suggesting that they may be mobile elements that target tRNA genes (Young et al. 2006). That observation has also been described for other symbiosis islands located in the genome of the closely related *M. loti* from the *Phyllobacteriaceae* family (Kaneko et al. 2000) and in many genomic and pathogenicity islands identified in other bacteria genera (Young et al. 2006). In summary, the "accessory" genes include mobile genetic elements, such as phages and transposons and the most well-known symbiosis-related genes. Young and collaborators (2006) proposed that these "accessory" genomes hold the key to unraveling bacterial adaptation and specialization. Indeed, lateral gene transfer is a key agent for *Rhizobium*/*Agrobacterium* and *Ensifer*/*Sinorhizobium* genome evolution.

In a second survey, the soil-borne and biotroph pathogen *A. tumefaciens* (currently named as *R. radiobacter*) was found to alter the physiology and morphology of infected host plants, causing crown gall disease (Wood et al. 2001). Over the last decade, plant scientists have disclosed an impressive portfolio of agrobacterial infection strategies, and the plant defense mechanisms counteracting these strategies are equally diverse and impressive (Pitzschke 2013). The genomic research employed to study the biology of *A. tumefaciens* led scientists to develop "disarmed" *Agrobacterium* strains with the aim of creating efficient delivery systems for the genetic manipulation of plants (Pitzschke 2013). This manipulation has been vastly explored in the development of modern plant genetics and agricultural biotechnology as a means of inserting foreign genes into plants (see section "Application").

Genomes from all three *Agrobacterium* biovars are now available (Slater et al. 2009): the genomes of the biovar I strain *A. tumefaciens* C58; *Agrobacterium radiobacter* K84, an avirulent biovar II strain; and *A. vitis* S4 (S4), a virulent biovar III strain (Slater et al. 2009). According the genomic databases, the genome size varies from 5.67 Mb (C58) to 7.27 Mb (K84), encoding from 5,385 to 6,752 genes and containing an overall 59 % G + C content (Slater et al. 2009). Moreover, the genome replicon compositions also vary between the biovars. The C58 genome consists of a circular chromosome I, a linear chromosome II, and two plasmids, whereas both the K84 and S4 genomes consist of two circular chromosomes (I and II) in addition to four and five plasmids, respectively (Slater et al. 2009).

Recent studies have shown that chromosome II of C58 and S4 and the 2.65-Mbp megaplasmid of K84 originated from intragenomic transfers from primary chromosomes to ancestral plasmids, whereas large blocks of DNA, including rRNA operons and other essential genes, have transferred from the chromosome I (Slater et al. 2009). Although they share a common origin and common properties, these three replicons are indeed chromids/ERs by the definition criteria (Slater et al. 2009; Harrison et al. 2010). The only differences between these chromids/ERs are that the K84 chromid does not have any ribosomal RNA genes (Harrison et al. 2010). In contrast, the virulence and inter-kingdom gene transfer capability is encoded in the transfer DNA (T-DNA) region, which is carried by the tumor-inducing (Ti) plasmid (Pitzschke 2013). The virulence proteins carried by the T-DNA regions include VirD2, VirE2, VirE3, VirD5, and VirF, and they are inserted in the host cell using a type IV secretion system (Pitzschke 2013; Cascales and Christie 2003). Another remarkable genomic feature found in the genomes of *Agrobacterium* biovars are the genomic islands, which are spread throughout the genome replicons (Slater et al. 2009). These islands are common associates of transposases and phage-related genes (Varani et al. 2013). Along with these islands, at least six and nine regions resemble events of prophage infection in C58 and S4, respectively (Varani et al. 2013). In addition, the chromids/ERs have a nucleotide composition close to that of chromosome I (Harrison et al. 2010), whereas the genomic islands and prophages have a variable nucleotide composition similar to that of the chromosome (Varani et al. 2013). Interestingly, the sequenced genomes from the *Ensifer*/*Sinorhizobium* group follow the trends observed in the *Rhizobium*/*Agrobacterium* group. They are composed of a diverse number of replicons, including the chromosome, chromids/ERs, and plasmids (Harrison et al. 2010).

In summary, this section/topic described the molecular aspects employed to identify and classify the *Rhizobiaceae* family over the last several decades; in particular, the advancements made by new techniques related to gene and genome sequencing were noted, and the main discoveries in the biology of the *Rhizobiaceae* family that were found using genome sequencing were explored. Both gene and genome sequencing have shown that plant—rhizobium interactions are directly related to and dependent on the genome constitution, structure, and architecture. The bacterial chromid/ER gene constitution and the impact and effects of lateral gene transfer in the chromosome, chromids/ERs, and plasmids are the main features of *Rhizobiaceae* genomes, and these topics are currently being explored by the scientific community.

Phenotypic Analyses

The family *Rhizobiaceae* has been described in the literature as consisting of nine genera: *Rhizobium, Agrobacterium, Allorhizobium, Ensifer, Sinorhizobium, Carbophilus, Kaistia, Shinella*, and *Chelatobacter*. However, during the last decade, species were arranged into seven genera: *Rhizobium, Ensifer, Carbophilus, Kaistia, Shinella, Allorhizobium, and Chelatobacter* (Lin et al. 2008; An et al. 2006). The genus *Agrobacterium* is now included in genus *Rhizobium* (Young et al. 2001), and *Sinorhizobium* is currently named *Ensifer* (Judicial Commission 2008; Young 2010). The genera *Allorhizobium* and *Chelatobacter*

have been proposed to be in *Rhizobium* and *Aminobacter*, respectively (Young et al. 2001; Kampfer et al. 2002). However, the International Committee on Systematics of Prokaryotes did not recognize this reclassification. Thus, we will present the biochemical, morphological, and microbiological characteristics of the seven genera formerly belonging to *Rhizobiaceae*, considering that two of them are currently formed by the fusion of two genera (Table 19.1). This topic will be covered due to several controversies regarding this new classification and to properly encourage the reader to compare the genera through these traditional approaches.

The identification of members of the family *Rhizobiaceae* is necessarily based on gene sequencing because there is no phenotypic information that allows the differentiation and identification of rhizobial species. This family is currently composed of fast-growing species of bacteria that may be saprophytic or able to establish beneficial or deleterious plant interactions. The vast majority of species were isolated from soil or plant tissues and are Gram-negative, aerobic bacteria, exhibiting the morphological, biochemical, and microbiological characteristics briefly described in Table 19.2, which were mostly obtained from Kuykendall (2005).

The bacteria within the *Rhizobium/Agrobacterium* cluster, despite different opinions (Farrand et al. 2003), are officially classified in the genus *Rhizobium*, regardless of their symbiotic characteristics or rhizogenic or tumorigenic ability. These species are rod-shaped, and their sizes range from 0.5 to 1.0 μm in width and from 1.5 to 3.0 μm in length.

Rhizobium species exhibit a Y shape when they are present in the root nodules of leguminous, but they form colonies in medium containing Congo Red assuming the traditional normal rod-shaped. All species from the genus are motile by polar or peritrichous flagella and produce copious water-soluble exopolysaccharide gum. This component has an important role during the development process of nodules in the roots of leguminous plants (Masson-Boivin et al. 2009). The colony is similar between the species, as is the cultivation temperature and optimal pH for development; in this respect, the symbiotic species demonstrate resistance to a broader pH range (pH 4–10). Species previously classified as *Rhizobium* contain poly-β-hydroxybutyrate granules, and the pathogenic *Agrobacterium* form cellulose fibrils during their attachment to plants in vitro. The DNA C+G content of the species of the two groups classified as belonging to the genus *Rhizobium* is similar, ranging from 57 % to 66 %.

Species of the *Rhizobium/Agrobacterium* cluster use a large variety of carbohydrates and salts from organic acids as their carbon source and use ammonium salts, nitrate, and several amino acids as their nitrogen source (Kuykendall 2005). These bacteria are not able to perform carbon assimilation; however, the *Rhizobium* fix nitrogen in symbiosis with leguminous plants. In culture, there is a single difference between strains of *Rhizobium* and *Agrobacterium*: the former does not grow in medium with 2 % NaCl, while the latter tolerate this salt concentration. In addition, *Agrobacterium* species are capable of growing under anaerobic conditions in the presence of nitrate or under reduced oxygen stress in plants.

A revision accepting the inferred phylogeny of the taxon that takes account the supporting phenotypic data, which show *Allorhizobium undicola* to be indistinguishable from *Rhizobium*, would have this species included in the genus *Rhizobium*. Although both perform symbiotic nitrogen fixation, these two genera have both similar and distinct characteristics (Table 19.2). De Lajudie et al. (1998) considered the *Neptunia* isolate (*A. undicula*) to be a separate genus because it had some phenotypic and genotypic characteristics that were distinct from *R. leguminosarum*. However, there are no single or multiple phenotypic characteristics reported in the generic circumscriptions of *Rhizobium*, *Agrobacterium*, *Allorhizobium*, or *Sinorhizobium* (other nitrogen fixation bacterium in this family) by which these taxa, as genera, can be differentiated. The taxonomy of rhizobia, which are bacteria capable of nodulating leguminous plants, has changed considerably over the last 25 years and needs to be defined.

Another group with controversial classification is the genus *Ensifer*. This genus is composed of two old genera, the *Ensifer* and the *Sinorhizobium* (Table 19.1). The morphological, biochemical, and microbiological characteristics of these bacteria are separated in Table 19.2 to help the reader observe why there are disagreements with the classification of this bacterium genus. When the characteristics of these two groups of bacteria are compared, we can conclude that none of them are similar between previously classified *Ensifer* and *Sinorhizobium* microorganisms.

Ensifer have important features (Table 19.2), including cell division by budding at one pole, asymmetric polar growth, and binary fission; the mother and daughter cells are the same size or the latter are slightly smaller. The cells have a length of approximately 1–2 μm and form a large and grayish-white colony (10–15 mm), and a film and/or sediment can be observed in broth medium. A bar of darkly stained material is visible using electron microscopy; its chemical composition has not been determined, and it extends between the bacteria and its host (Casida 1982 to see images). The *Ensifer* attaches end-wise to various Gram-negative and Gram-positive host bacteria and causes lysis; however, it is not an obligate predator.

On the other hand, the *Sinorhizobium* group is composed of rods that are up to 3 μm in length, and they form colonies that are 2–4 mm diameter and grow quickly (high turbidity) in aerobic or agitated broth. These bacteria have strains that can tolerate temperatures from 10 °C to 44 °C and a pH of 10.5 and can grow in YM medium with 1–4.5 % NaCl. The organisms are able to form nodules in the roots of temperate-zone and tropical-zone leguminous plants and fix nitrogen as a microsymbiont cell, with pleomorphic forms and named bacteroides. Using phenotypic characteristics, the *Sinorhizobium* bacteria have not been distinguished from the genus *Rhizobium*. Instead, researchers use the quantitative fatty acid composition differences of and 16S rDNA sequences (Tighe et al. 2000). Recently, the sequenced genome of the *E. medicae* strain WSM419 demonstrated higher similarity between 16S rRNA gene sequences between strains from *E. meliloti* and *E. fredii* than with other related species of *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* (Reeve et al. 2010).

Table 19.1

Type Species of *Rhizobiaceae* family and local of isolation

Genera	[a]*Rhizobium/Agrobacterium* group		*Allorhizobium*	[b]*Ensifer/Sinorhizobium* group		*Carbophilus*	*Kaistia*	*Shinella*	*Chelatobacter*
	Rhizobium	*Agrobacterium*		*Ensifer*	*Sinorhizobium*				
Reference	Frank 1889	Smith and Townsend 1907; Young et al. 1978	De Lajudie et al. 1998 and Young et al. 2001	Casida 1982	Scholla and Elkan 1984 and Chen et al. 1988	Meyer et al. 1993	Im et al. 2004	An et al. 2006	Auling et al. 1993 and Kampfer et al. 2002
Type species	*R. leguminosarum*	*A. tumefaciens*	*A. undicola*	*E. adhaerens*	*S. fredii*	*C. carboxidus*	*K. adipata*	*S. granuli*	*C. heintzii*
Number of species[c]	70	11	1	14	11	1	8	6	1
Origin	Soil or nodule from root nodules of leguminous plants	Soil and crown gall tissues	Isolates from *Neptunia natans*, an indigenous stem nodulated tropical legume found in waterlogged areas	Isolated from soil where had a predatory activity in indigenous gram-negative bacterium	Soil and root nodules of leguminous plants	Garden soil at the city of Moscow	Soil sample collected in an industrial stream	Isolated from an upflow anaerobic sludge blanket reactor	Soil

[a]The type species of *Agrobacterium* was transferred to *Rhizobium* by Young et al. (2001). Consequently, the genus *Agrobacterium* would be included in the genus *Rhizobium*. However, this transfer is highly controversial (Farrand et al. 2003; Young et al. 2003)

[b]"In response to a Request for an Opinion, the Judicial Commission (Opinion 84) confirmed the earlier synonym, *Ensifer*, as the correct name for the genus better known as *Sinorhizobium*. The Judicial Commission considered that this decision would not cause sufficient confusion to justify the conservation of the latter name. The Subcommittee on the Taxonomy of *Agrobacterium* and *Rhizobium* of the International Committee on Systematics of Prokaryotes (ICSP) publicly disagreed with this conclusion" (Young 2010)

[c]Number of species cited in the List of Prokaryotic names with Standing in Nomenclature (http://www.bacterio.net/index.html)

Table 19.2

Morphological, microbiological, biochemistry and molecular characteristics of type species from genera of *Rhizobiaceae*

Genera	*Rhizobium/Agrobacterium cluster*		*Allorhizobium*	*Ensifer/Sinorhizobium cluster*		*Carbophilus*	*Kaistia*	*Shinella*	*Chelatobacter*
	Rhizobium	*Agrobacterium*		*Ensifer*	*Sinorhizobium*				
Morphology	Rods (0.5–1.0 × 1.2–3.0 μm). Withe colonies on YMA agar plate with 0.025 % Congo Red	Rods, single or pair (0.6–1.0 × 1.5–3.0 μm)	Rods (0.5–0.7 pm × 2.0–4.0 μm)	Rods, single or pair (0.7–1.1 × 1.0–1.9 μm). Cell division is by budding at one pole, asymmetric polar growth and binary fission	Rods pleomorphics under adverse growth conditions (0.5–1.0 × 1.2–3.0 μm)	Rod-shaped (0.7–0.9 × 0.9–1.7 μm)	Short rod to coccus-shaped bacterium. 0.7–0.9 mm long	Rod-shaped (0.2–0.5 × 4.0–6.0 μm)	Rod with rounded ends (0.7–0.9 × 1.0–2.0 μm). Pleomorphic, L-, Y- or X-shapedCells occur singly
Special internal structures, storage and important compounds	Poly-β-hydroxybutyrate and water-soluble extracellular polysaccharide	Copious extracellular polysaccharide slime. Cellulose-containing fibrils are formed by pathogenic strains during their attachment to plant cell, in vitro	Produce extracellular polysaccharide	A bar of darkly stained material is visible by electron microscopy, between this bacteria and its hosts	Poly-β-hydroxybutyrate granules in older cells. Produce copious extracellular polysaccharide slime in carbohydrate medium	None	None	None	All isolates exhibit an outer membrane closely associated with the underlying murein layer
Motility	Motile (1 or 2 polar flagella or 1–6 peritrichous flagella)	Motile (1 or 4 peritrichous flagella)	Motile (subpolar or peritrichous flagella)	Motile (tuft of 3–5 subpolar flagella)	Motile (1 polar or sub-polar flagellum or 1–6 peritrichous flagella)	Motility variable, whem present is by mean of 5 peritrichous flagella (maximal in late exponential growth phase)	Non-motile	Motility by multiple polar flagella	Motile (2 or 3 subpolar flagella)
DNA G+C %	57.0–66.0	57.0–63.0	60	63	57–66	63.8	67.4	64–66	62–63
Colony size, appearance and color	2–4 mm diameter (3–5 days incubation). Colony is circular, and convex, opaque or semi-translucent, white or beige	Colony is convex, circular, smooth, nonpigmented to light beige	0.5–3 mm diameter (1–2 days on YMA). Colonies are round, creamy, convex to drop-like, beige coloured; margin and surface have a smooth aspect	10–15 mm diam (6 days incubation on heart infusion agar diluted 10 times), Colony is grayish white, circular with undulated margins, convex, slimy, moist and opaque. Pelicule and sediment can be observed in broth medium	2–4 mm diameter (3–5days incubation) and pronounced turbidity after 2–3 days on aerated or agitated broth. Colonies are white, circular, convex, usually opaque (some translucent), raised, and mucilaginous	White to cream colored colony	Ivory-pigmented and round, raised with greasy surface	Amorphous or finger-like flocculent growth occurs in liquid media	2–3 mm (4–5 days incubation on PCA medium). Round and smooth, beige-brown colony. The colony morphology can change in different medium
Optimal temperature	25–30 °C (maximum growth temperature 38 °C)	25–28 °C (not grow above 30 °C)	28 °C	27 °C (range 20–37 °C)	25–30 °C optimal, same species can tolerate 10 °C, 35 °C, 42–44 °C	30 °C	10–37° (optimal 37 °C) not grow at 42 °C	4–40 °C	30 °C and 37 °C, but does not occur at 42 °C
Optimal pH	6–7 (range 4–10)	7.1 (range 5–9)	Not determined	6.0–7.0	6.0–8.0 optimal, same strains can grow at pH 5.0 and others tolerate pH10.5	Not determined	6.0–7.0	pH 6–10	pH 6.0–8.0

Metabolic and cultural characteristics	Aerobic	Aerobic some strains are capable of anaerobic in presence of nitrate and most strains are able to growth under reduced oxygen tension in plant	Aerobic, Chemoorganotrophic	Aerobic with oxidative metabolism of glucose and galactose	Aerobic, with the oxygen as a terminal electron acceptor	Aerobic, with the oxygen as a terminal electron acceptor	Aerobic. Chemoorganotrophic. This strain was resistant to 1 mM phenol and 4-chlorophenol concentrations for 1 week culturing	Aerobic and Grows in 1–4 % NaCl	Aerobic. Metabolism is strictly respiratory and not fermentative
	Chemoorganotrophic	Grows in medium with 2 % NaCl	Pronounces turbidity after 1–2 days in agitated yeast mannitol mineral salts broth	Chemoorganotrophic. Tthe growth is inhibited in 4 % but not in 2.5 % of NaCl. Definite but slow grow on soil extract agar	Chemoorganotrophic, Tolerate 1 % NaCl and some strains grow on YM medium with 4.5 % NaCl	Facultatively Chemolithoautotrophic			Grow in presence of 1 % NaCl and no grow occurred with 10 %
	Not grows in medium with 2 % NaCl								
Main carbon source	Use wide range of carbohydrate and salts of organic acids as a carbon source	Use wide range of carbohydrate and salts of organic acids as a carbon source	Use some carbohydrates, organic acids and amino acids that can be discriminatory to the specie	Utilizes a variety of organic carbon sources	Several carbohydrates and salts of organic acids as a carbon source	Use several salts of organic acids (except citrate), amino acids, sugars and sugars alcohol	Utilized a broad spectrum of carbon sources	Several carbohydrates, organic acids and some amino acids as a carbon source	Grow abundantly in nutrient broth and peptone, yeast extract, glucose broth. Can use nitriloacetate as a unic source of carbon, energy and nitrogen
Main nitrogen source	Ammonium salts, nitrate and amino acids	Ammonium salts, nitrate and amino acids	Not determined	Nitrite and nitrate are reduced	Ammonium salts, nitrate, nitrite and most of amino acids	Ammonia, Hydroxyamine, nitrate and urea	Not determined	Not determined	Ammonia, nitrate, urea and peptone
N_2 fixation	In root nodules the bacteria occurs as a entophytic, bacteroides, which reduced or fix atmospheric nitrogen that can be used by the plant	No	In root nodules the bacteria occurs as a entophytic, bacteroides, which reduced or fix atmospheric nitrogen, is host specific	No	Yes in symbiosis with temperate-zone and tropical-zone leguminous plant	No	No	No	No
C assimilation	No	No	No	No	No	Yes, CO as a energy source and CO_2 as a carbon source with H_2. (Cypionka et al. 1980)	No, reduces nitrate to nitrite	No	No
Biological characteristics	All species induced hypertrophisms in plant root nodules with or without symbiotic nitrogen fixation	Strains of some species invade the crown, roots and stems of a great variety of plants causing transformation of the plant and autonomously proliferating tumor cells	Induce nitrogen-fixing nodules on their original host *N. natans* and can also nodulate *M. sativa* and other tropical-zone leguminous plants	Attaches end-wise to various gram-negative and gram-positive host bacteria and causes lysis	Form nodules in root of leguminous plant and fix nitrogen as a microsymbiont cell	The type specie is isolated from the soil, it's only bacteria that can oxidizing CO that has the inability to growth on H_2 and CO_2 chemolhitoautotrophic conditions	Bacteria resistant to phenolic compounds	Isolated from an upflow anaerobic sludge blanket reactor	A nitrilotriacetate-utilizing organism

The genus *Carbophilus* is represented by a unique species, *C. carboxidus*. This bacterium was isolated from the soil near a stream in Moscow, Russia. This species was originally named *Achromobacter carboxydus* and later *Alcaligenes carboxydus*, as described by Meyer (2005). *C. carboxidus* is able to grow with CO as the energy source and CO_2 as the carbon source under aerobic chemolithotrophic conditions. This species is separated from the other aerobic carboxidotrophic bacteria (*Oligotropha*, *Bradyrhizobium*, and *Zavarzia*) because it cannot grow chemolithotrophically on H_2 and CO_2. This bacterium species is rod-shaped and has variable motility. It displays white- to cream-colored colonies and uses several organic molecules as a carbon source (except citrate). Ammonia, hydroxylamine, and nitrate can be used as a nitrogen source, and it cannot fix dinitrogen. The optimal pH and the tolerance to pH have not been determined.

The other two genera from the *Rhizobiaceae* family are *Kastia* and *Shinella*, described by Im et al. (2004) and An et al. (2006), respectively. *Kastia* is a genus that includes strains such as *K. adipata*, a bacterium isolated from soil samples collected in an industrial stream near an industrial complex in Korea. This bacterium could not degrade phenolic compounds but instead was resistant to such compounds. The cells are rod- and cocci-shaped with sizes varying from 0.7 to 0.9 mm long. Colonies formed on a nutrient agar plate are ivory-pigmented, round, and raised with a greasy surface. The cells are non-motile and can grow at 10–37 °C but not at 42 °C. The optimum temperature for growth is 37 °C at a pH of 6–7. They grow aerobically and utilize a broad spectrum of carbon sources such as mannitol, D-glucose, salicin, D-melibiose, L-fucose, D-sorbitol, L-arabinose, 2-ketogluconate, L-proline, rhamnose, *N*-acetyl-glucosamine, D-ribose, inositol, D-sucrose, maltose, lactate, L-alanine, 5-ketogluconate, glycogen, and mannose. This species also reduces nitrate to nitrite.

Shinella is a genus of motile rods characterized by an amorphous or finger-like flocculent growth in liquid media. The type species is *Shinella granuli* (An et al. 2006). These cells are 0.2–0.5 μm in width and 4–6 μm in length. Colonies on R2A agar media are glistening, convex with an entire margin, viscous, and pale yellow. Growth occurs at 4–40 °C, with 1–4 % NaCl and at pH 6–10. The bacteria use several carbohydrates, organic acids, and some amino acids as a carbon source and can perform nitrate reduction. The genus *Shinella* comprises six recognized species: *S. granuli* and *S. zoogloeoides* (An et al. 2006), *S. kummerowiae* (Lin et al. 2008), *S. yambaruensis* (Matsui et al. 2009) and *S. fusca* (Vaz-Moreira et al. 2010), and *S. daejeonensis* (Lee et al. 2011). These species have been isolated from a variety of locations including an upflow anaerobic sludge blanket reactor, sewage treatment systems, root nodules of herbal legumes, soil with sulfur, kitchen refuse compost, and the sludge from a leachate treatment plant. Furthermore, they have several characteristics that permit their use in biotechnological applications.

The genus *Chelatobacter* is another controversial genus in the *Rhizobiaceae* family. At first, this genus was composed of a single species, *Chelatobacter heintzii*. This species was classified as a new genus in the *Rhizobiaceae* family (Auling et al. 1993) based on its cell and colony morphology; cultural, nutritional, and physiological characteristics; and some biochemical components (ubiquinone Q-10 and polyamines patterns). *Chelatobacter heintzii*, which was described as a nitrilotriacetate-utilizing organism, was re-investigated, and on the basis of 16S rDNA sequence comparisons, this species clusters phylogenetically with species of the genus *Aminobacter*. The fatty acid patterns, polar lipid profiles, polyamine patterns, quinone system, and DNA–DNA similarity studies supported this placement (Kampfer et al. 2002). The name *Chelatobacter heintzii* is considered to be synonymous with Aminobacter aminovorans (Notification List IJSEM 2002).

The most important feature of *Chelatobacter* is its ability to grow with the metal-chelating aminopolycarboxylic acid nitrilotriacetic acid (NTA) as the sole source of carbon, energy, and nitrogen. Another interesting characteristic of this genus is the colony morphology and growth in different media. On PCA media, *Chelatobacter* exhibited round and smooth, beige-brown colonies of 2–3 mm after 4–5 days of growth. When 0.2 × PCA medium was used, the colonies were round, light-beige, translucent, and smaller than the colonies grown in PCA media. The colonies were round and volcano or fried-egg-like and can have rings around the outside. Alternatively, the bacteria grown on NTA-containing medium were white, tough, and pin-point in size at early growth time points. Later, a brown center appeared, and after 4–5 days, the colony turned black and was polygonal in shape. The growth on diluted PCA broth is faster than with the original conditions. The growth in batch culture with NTA increased the pH and inhibited growth. Notably, growth in NTA and other carbon sources such as glucose is faster than with NTA alone.

Isolation, Enrichment, and Maintenance Procedures

Bacteria from *Rhizobium* and *Agrobacterium* (*Rhizobium* Genus), *Sinorhizobium* (*Ensifer* Genus), and *Allorhizobium* can be isolated from soil, root nodules, or gall tissue. *Rhizobium* and *Sinorhizobium*, both symbiotic species, are best isolated from freshly excised legume root nodules. Their isolation directly from soil is difficult, and generally, this process needs trap hosts (Abd-Alla et al. 2012). On the other hand, *Agrobacterium* is isolated from gall tissue or soil or from still water samples (Kumar et al. 2013).

Root material must be washed in running tap water to remove any adherent soil. Individual nodules are dissected from the roots using a flame-sterilized scalpel and tweezers. If the nodules are very small, a small amount of root tissue can be left attached to either side of the nodule. Nodules need to be washed thoroughly in sterile water and non-ionic surfactant such as Tween-80 (100 ml/l) to remove all traces of soil. The nodules are then transferred to sterile Petri dishes and surface sterilized by immersion in 5 % sodium hypochlorite for 10–30 min, depending on the nodule size. This is followed by incubation in 70 % ethanol for 1 min and subsequently rinsing

three times with sterile water. Individual nodules may be crushed using a glass rod and then suspended in a liquid medium. Large nodules can be sliced using a sterile scalpel blade, and portions of the interior can be removed with a needle. The exudates obtained are aseptically streaked onto surface-dried yeast extract mannitol agar (YEMA) plates (Vincent 1970). Agar plates are incubated at 30 °C for 3–4 days, and individual colonies appearing over this period are re-streaked onto YEMA plates.

Isolating of *Agrobacterium* from gall tissue is a similar process to that used for the nodule. The difference is that the gall tissues must be finely chopped. These pieces are placed into a culture tube containing sterile distilled water or buffer, vortexed, and allowed to stand for at least 30 min. Using an inoculating loop, the suspension is streaked on medium and incubated at 25–27 °C. Several types of medium have been used to isolate *Agrobacterium*. The most common are Medium of Schroth et al. (1965), Medium 1A and 2E (Brisbane and Kerr 1983), or, more recently, YEMA medium (Kumar et al. 2013).

To isolate these bacteria (i.e., *Rhizobium*, *Agrobacterium*, *Sinorhizobium*, and *Allorhizobium*) directly from soil samples, it is necessary to prepare a dilution series of the sample in water or buffer and culture the sample on medium at 27 °C. The isolates can be maintained on agar slants in a screw-capped vials at 4 °C for 2 months or in 80 % Glycerol in water at −20 °C or −80 °C. Lyophilized cultures stored at 4 °C remain viable for at least 25 years.

The *Ensifer* adhaerens (*Ensifer* genus) are frequently obtained from soil. These isolates have predatory activity in indigenous Gram-negative bacterium. The enrichment and isolation procedures can use a host organism, and frequently, the host is *Agromyces ramosis*, *Micrococcus luteus*, *Staphylococcus aureus*, or some *Myxococcus* or *Streptomyces* soil isolates. To isolate these bacteria using a host, there are two procedures. In the first, the host organism must be added to natural soil, followed by the addition of water to adjust the soil water content to 50 % or 60 %, and the sample is incubated for 4–6 days at 25–27 °C. After incubation, soil dilutions are plated on desoxycholate agar or MacConkey agar. The soil dilution may also be spread on surface plates using pre-grown lawns of the host organism. The plates are then incubated to isolate a characteristic colony (grayish white, circular with undulated margins, convex, slimy, moist, and opaque). In the second isolation procedure, the host organism is smeared on a sterile glass slide, the cells are dried at room temperature, and the slide is placed in contact with the soil sample. Water is added to the soil sample. After incubating at 27 °C for 3–4 days, the lawn of host organisms is streaked by a loop, and the material is placed onto the surface of plates containing desoxycholate or MacConkey agar (Balkwill 2005).

The *Ensifer* genus consists of bacteria that are not obligate predators, and they grow well on most media. Currently, there are several studies that isolated *E. adhaerens* directly from biological samples, such as from the gut of *Holotrichia parallela* larvae (Huang et al. 2012b), from water biofilm (Huang et al. 2012c), or directly from soil samples (Guang-can Zhou et al. 2013).

The *Carbophylus* genus is represented by the species *Carbophylus carboxidus*. This species grows with CO as the energy source and CO_2 as the carbon source under aerobic, chemolithoautotrophic conditions (Meyer 2005). The metabolic characteristics of this bacterium are similar to other aerobic carbon monoxide-oxidizing microorganisms, such as *Oligotropha* from the *Bradyrhizobiaceae* Family. The most important factor in the process to enrich and isolate these bacteria is to use the appropriate gas atmosphere composition. Normally, culturing is performed in mineral medium incubated in an atmosphere composed of 5 % CO_2, 45 % CO, and 50 % air or alternatively 5 % CO_2, 10 % O_2, and 85 % CO. The enrichment is performed in batch cultures, with or without shaking, at 30 °C in the dark. Isolation and maintenance can be performed in mineral agar medium supplied with nutrient broth in the same atmosphere composition. These bacteria are slow-growing microorganisms; fast-growing colonies are generally CO-tolerant contaminants.

The two newest genera from the *Rhyzobiaceae* family, *Kastia* and *Shinella*, are composed of aerobic chemorganotrophic species. Therefore, these bacteria are isolated using a non-selective complex medium such as R2A agar plates, plate count agar, nutrient agar, or AG medium (Matsui et al. 2001). Cultures are incubated at 27 °C to 30 °C in the same isolation medium and cryopreserved at −80 °C in broth supplemented with 15 % (v/v) glycerol (Lee et al. 2007; Vas-Moreira et al. 2010).

Strains from the *Chelatobacter* genus can be isolated from soil, wastewater, or surface waters. These bacteria can be isolated in media with NTA as the only carbon and nitrogen source. Maintenance must be in freeze-dried conditions or alternatively in culture grown with NTA, glycerol (15 %, v/v), or DMSO (50 %, v/v). The cultures should be revived directly onto NTA agar plates, and the cultures must be transferred to new plates every week because growth leads to increased pH, which leads to growth inhibition.

Ecology

Some members of the family *Rhizobiaceae* are characterized by their ability to establish symbiotic associations with host plants to develop the process of biological nitrogen fixation (BNF). This ability is not limited to members of this family; it extends to a diverse group of bacteria collectively called rhizobia. Rhizobia can be differentiated by morphophysiological characteristics, growth time, and pH reaction in medium containing yeast extract, mannitol, mineral salts, and agar (YMA—Vincent 1970). Their members are distributed across six genera: *Rhizobium*, *Sinorhizobium/Ensifer*, and *Allorhizobium/Rhizobium* (*Rhizobiaceae*), which contains predominantly fast-growing and acidifying strains; *Mesorhizobium* (*Phyllobacteriaceae*), which contains intermediate- to fast-growing and acidifying strains; *Bradyrhizobium* (*Bradyrhizobiaceae*), which is represented by slow-growing and alkalinizing strains; and *Azorhizobium* (*Xanthobacteraceae*), which consists of fast-growing and alkalinizing strains (Moreira and Siqueira 2006).

Although they might grow in any region, fast-acidifying strains predominate in temperate regions, whereas the slow-alkalinizing strains predominate in tropical climates (Norris 1965).

Nitrogen-fixing organisms provide a source of nitrogen that can be assimilated by other organisms such as plants and animals, and the nitrogen can be incorporated into amino acids and other essential organic compounds. This ability is restricted to organisms that exhibit nitrogenase, an enzyme complex capable of catalyzing the molecular transformation of atmospheric nitrogen (N_2) into ammonia (NH_3) (Rees and Howard 2000). Despite the diversity of nitrogen-fixing bacteria, there is high homology between the fixing systems based on the nitrogenase complex (Burris 1991). BNF through symbiosis with members of the family *Rhizobiaceae* is almost exclusively restricted to leguminous plants (Fabaceae), with the exception of *Parasponia* (Ulmaceae) (van Rhijn and Vanderleyden 1995; Crawford et al. 2000).

Bacterial strains of any genus exhibit different specificities to host plants depending on the type of nodulation (Nod) factors produced, and *Rhizobium* is one of the genera that displays the greatest promiscuity (Pueppke and Broughton 1999). The plant signals associated with the expression of these Nod factors are plant-specific flavonoids. For example, the flavone luteolin and the chalcone 4,4′-dihydroxy-2′-methoxychalcone, which are capable of stimulating *Sinorhizobium meliloti*, are found in plants of the genus *Medicago*, whereas *S. fredii* is stimulated by the isoflavone daidzein produced by *Glycine max*. Naringenin is a flavanone found in plants of the genus *Vicia*, and it is capable of stimulating *R. leguminosarum* bv. *viciae* (Crawford et al. 2000). The structural organization of the genes for nodulation and nitrogen fixation is usually a grouped one that forms symbiotic islands. These islands are located in megaplasmids denoted as "pSym" in bacteria of the genera *Rhizobium* and *Sinorhizobium* (Batut et al. 1985; Renalier et al. 1987; Galibert et al. 2001).

Plants and bacteria create a species-specific root nodule environment through endophytic colonization, which becomes able to support symbiosis, ATP synthesis, and the BNF process in a microaerophilic environment. The bacterium turns into a pleomorphic form called a "bacteroid," which is the morphophysiologically active type located inside the root nodules exhibiting nitrogenase activity (Jones et al. 2007; Oldroyd and Downie 2008). The plant's metabolism produces organic acids that serve as food for the bacteroid and provides carbonate skeletons for the organic nitrogen compounds produced in return (generally in the form of amides (glutamine and asparagine) or ureides (allantoin and allantoic acid)). In turn, these compounds are produced from the metabolic interrelation of the macro-and microsymbiont after the reduction of N_2 into NH_3 and its subsequent acidification into ammonium (NH_4^+) in the interior of the bacteroids (Lodwig and Poole 2003; Lodwig et al. 2003).

C. carboxidus, the only species of the genus *Carbophilus*, was defined as a carboxydotrophic bacterium capable of using carbon monoxide (CO) as the sole source of carbon and energy both in aerobic and denitrifying conditions (Meyer et al. 1993). Bacteria with this ability are considered to be primary colonizers on recent volcanic deposits and are metabolically versatile; therefore, they are potential residents in inhospitable conditions with primordial chemolithotrophic features. The presence of the carbon monoxide dehydrogenase gene enables CO oxidization and allowed this bacterium to be present in the early and subsequent stages of ecological succession (Weber and King 2010).

The genus *Chelatobacter* comprises one single species, *C. heintzii*, which has the ability to degrade nitriloacetate (NTA) under aerobic conditions. This activity allows this bacterium to take part in the mineralization of NTA (i.e., removing this chelating agent from the environment) (Kampfer et al. 2002; Yuan and Van Briesen 2006). NTA, as well as EDTA, are agents employed in the removal of metal residues from contaminated environments; however, their indiscriminate use allows their accumulation and transformation into a potential pollutant. *Chelatobacter henintzii* assimilates NTA and generates three intermediates: iminodiacetate (IDA), glyoxylate, and glycine. These latter two are innocuous, contributing to bacterial growth and complete mineralization of NTA. IDA is the only intermediate product that retains complex metal characteristics, and the effects of initial chelation may persist in the environment (Yuan and Van Briesen 2006).

The genus *Kaistia* contains species isolated from environmental samples such as soil and sediments, including those originating from turf and anaerobic sludge blanket reactors. The latest isolate of this genus was obtained from the skin of *Hirudo verbana*, a leech with medical applications (Glaeser et al. 2013). The ability to reduce nitrate was detected for some species of this genus (Kim et al. 2010).

The genus *Shinella* also has species with ability to reduce nitrate and nitrite. Some of these bacteria were found when searching for microorganisms with industrial potential and were originally isolated from sludge reactors or waste treatment stations (An et al. 2006; Vaz-Moreira et al. 2010; Lee et al. 2011). The concentration of organic matter is relatively high in these environments, thereby contributing to an increase in the nitrogen content. Thus, the transformations performed by bacteria of this genus contribute to ammonia production and maintenance of the degradation process of these residues in addition to the maintenance of the microbial community in anaerobic environments (Franke-Whittle and Insam 2013; Murto et al. 2004; De Vrieze et al. 2013). One species of this genus, *S. kummerowiae*, is able to establish symbiosis with the leguminous *Kummerowia stipulacea*, having been isolated from the root nodules of this plant in a province of China. This strain contains the *nodC*, *nod*, and *nifH* genes, which share high levels of similarity with those of *R. tropici*, demonstrating possible potential for nitrogen fixation (Lin et al. 2008).

Pathogenicity: Clinical Relevance

Agrobacterium is the most important genus of *Rhizobiaceae* regarding pathogenicity, with representatives that are even characterized by this criterion. Three disease-causing agents in plants

are noteworthy: the species *A. tumefaciens* (causative agent of crown gall tumors), *A. rhizogenes* (causative agent of hairy root disease), and *A. vitis* (causative agent of tumors and necrotic disease on grapevines) (Matthysse 2006). The range of dicotyledonous host plants varies according to species; however, the plant *Helianthus annuss* is susceptible to the largest range of tumorigenic strains. Conversely, *A. vitis* and *A. rubi* have limited host ranges (Young et al. 2005).

The chromosomal genes involved in pectin breakdown are the main virulence factors in *A. vitis*. The presence of the bacterium is generally limited to the phloem or to tissues directly beneath the bark of grapevines. The tumors caused by *Agrobacterium* are self-proliferative and can be transmitted by grafting. The induction and maintenance of tumors is due a tumor-inducing plasmid (Ti-plasmid) present in *A. tumefaciens* and *A. vitis*, whereas hairy root induction is due to a root-inducing plasmid (Ri-plasmid) present in *A. rhizogenes* (Young et al. 2005; Matthysse 2006).

The infection of plant tissues by parasites occurs through different injuries suffered by the plant. Through these injuries, the plant exudes phenolic compounds, sugars, and amino acids that perform positive chemotactism, and the bacterium invades the injured surface usually in the interface between the soil and the shoots. The bacterium is required for tumor induction, and the chromosomal genes *chvA*, *chvB*, and *pscA* are needed for the initial attachment of *Agrobacterium* onto the plant cell (Sheng and Citovsky 1996; Tzfira and Citovsky 2000). However, the tumor is maintained as a result of a genetic transformation in the plant cells through the transfer of some genes contained in the Ti plasmid in a region named T-DNA (transferable portion of the Ti genome to the nucleus of the plant). The release and transfer of T-DNA into the plant genome occurs through a diverse class of virulence genes (*vir*). Plant phenolic compounds such as p-hydroxybenzoic acid and vanillin induce the expression of the *vir* genes (Maury et al. 2010; Wise et al. 2010).

The T-DNA encodes enzymes involved in the biosynthesis of cytokinins and auxins and is expressed at its integration site, thereby promoting hormonal imbalance in the plant. The chaotic proliferation of transformed plant cells originates a tumor known as "crown gall" (Yamaguchi et al. 2012; Gohlke et al. 2013). For *A. rhizogenes*, the expressed oncogenes induce the proliferation of hairy roots at the injury site (Georgiev et al. 2012). Additionally, T-DNA encodes for enzymes responsible for the synthesis of opines, such as nopaline, octopine, or agropine, which vary depending on the group of *Agrobacterium* that carry them. The opines produced by the transformed plant cells are derivatives of amino acids and sugars and are the chemical intermediates of parasitism; they are used as a nutritional source by the bacteria (Tzfira and Citovsky 2000). Some species of *Agrobacterium* are susceptible to agricins, which are antibiotic-like compounds produced by certain *A. radiobacter* strains. However, this does not seem to be an efficient method for biological control of plant parasitic strains, specifically for *A. vitis* (Matthysse 2006).

With less important pathogenicity, the genus *Ensifer* comprises the species *E. adhaerens*. This species lives in the soil and was originally described as a non-obligatory predator of *Micrococcus luteus* (Casida 1982). The range of bacteria susceptible to attack by *Ensifer* is usually limited to certain bacteria that were added to the soil, including *Agromyces ramosus*, *Staphylococcus aureus*, and strains C2 and 34 of *Streptomyces*. Both Gram-positive and Gram-negative cells may be lysed by *Ensifer*, which becomes an obligatory predator in conditions of nutrient limitation (Germida and Casida 1983; Zeph 1986; Zeph and Casida 1986).

Application

In quantitative terms, the symbiosis between nitrogen-fixing bacteria and their leguminous host plants can be considered to be one of the most productive systems when the BNF is taken into account. Many important agro-economic plants worldwide are leguminous species, such as alfalfa, clover, beans, and soybeans. A major portion of the global process of BNF lies on these cultivated leguminous, and this is a crucial step in the nitrogen cycle that annually adds 50–70 million tons (Tg) of nitrogen to the soils (Herridge et al. 2008; Lindström et al. 2010).

Although microbial inoculants based on bradyrhizobia for soybean are the most widespread due to the great importance of soybean worldwide, the inoculation of other leguminous grain crops, green manure, foragers, and trees have drawn particular attention to the group of rhizobia, which belongs to the family *Rhizobiaceae* (Moreira et al. 1993; Lindström et al. 2010). Among these host species, *Phaseolus vulgaris* (common bean) is considered to form one of the most important symbiotic associations with rhizobia of the genera *Rhizobium* and *Sinorhizobium* (Ribeiro et al. 2009; Mnasri et al. 2012).

The industry and market for inoculants for bean crops is considerably smaller than those for soybean; however, the importance and benefit of inoculation for bean productivity is not ignored (Hungria et al. 2000; Lindström et al. 2010). Maximization of the BFN process in leguminous crops can be compromised by several limiting properties to the establishment of symbiosis and nitrogen fixation efficiency. These include biotic factors such as competition with native soil bacteria, as well as abiotic factors such as temperature, humidity, and pH (Valentine et al. 2010). The selection of efficient strains capable of performing BNF with agriculturally important plants has been the subject of extensive research, and in it rests the success of inoculant application in these crops (Thies et al. 2001; Pinto et al. 2007; Ribeiro et al. 2009; Olivares et al. 2013).

BNF is not exclusively an applied process that can be exploited by members of *Rhizobiaceae*. In addition to nitrogen fixation, interaction with rhizobia also enables the rhizosphere of leguminous plants to benefit from other molecules able to stimulate plant growth, form other symbiotic associations, and control phytopathogens (Dakora 2003). Some bacteria of the genus *Rhizobium* colonize the rhizosphere and the rhizoplane of cultivated plants and promote plant growth by mechanisms that include the production of phytohormones of the gibberellins, auxins, and cytokinins classes. Therefore, these bacteria act as

Plant Growth-Promoting Rhizobacteria (PGPR) (Lugtenberg and Kamilova 2009) and can be exploited as inoculants directed to other agroecological properties.

Certain polymers produced by bacteria belonging to the family *Rhizobiaceae* may also have industrial applications, particularly the extracellular polysaccharides denominated exopolysaccharides (EPS). These are strain-specific homo- or hetero-polysaccharides with biological functions such as recognition and adhesion to host plant cells (Sutherland 2001; Skorupska et al. 2006; Castellane and Lemos 2007). The bacterial EPS has been shown provide bacteria protection from various environmental insults such as desiccation, predation, and the effects of antibiotics (Donot et al. 2012). The EPS originated in rhizobia has several functions in nature including participation as receptor molecules in cell-cell interactions and triggering of the nodulation process (Kirichenko et al. 2004); induction of the formation of nodules and micro-colonies (Becker and Pühler 1998); participation in the development of the nodule (Kosenko et al. 2001); and acting as ion exchange resins to concentrate minerals and nutrients around the cell (Whitfield 1988).

The interest in EPSs has increased considerably in recent years because these compounds are candidates for many commercial applications, including as food products, pharmaceuticals, bioemulsifiers (Xie et al. 2013), bioflocculants (Sathiyanarayanan et al. 2013), chemical products (Wang et al. 2008; Shah et al. 2008), and antibiofilm agents (Rendueles et al. 2013) and in the biosorption of heavy metals (Mohamad et al. 2012). These EPSs can be applied as thickeners, stabilizers, emulsifiers, coagulants, biofilms, lubricants, gelling agents, dispersants, and suspending agents (Sutherland 1998).

A number of microorganisms have been demonstrated to produce polysaccharides and other biopolymers that exhibit metal-binding properties (Gutnick and Bach 2000), such as the water-soluble and amphipathic exopolysaccharides (EPS). Rhizobial exopolysaccharides have been studied extensively for their role in plant host specificity (Dudman 1984); however, only recently has their metal sorption capacity been investigated (Wu et al. 2010). Several works illustrate metal-binding properties from the bacteria of the *Rhizobium* genus. Douka and Xenoulis (1988) reported a significant reduction in radioactive metal concentration by nodulated pasture legumes after the Chernobyl radioactive fallout. A study by Abd-Alla et al. (2012) reported that the *R. leguminosarum bv. viciae* isolate can be useful as an inexpensive and efficient bioremediation technology to remove and can recover heavy metal (Co^{2+} and Cd^{2+}) ions from an aqueous solution. *Mesorhizobium amorphae* isolated from the root of *Robinia pseudoacacia* in tailings from plumber and zinc mines in Gansu (China) was used to help host plants to survive in environments contaminated with copper, zinc, and chromium, indicating a potential application in the remediation of heavy metals (Mohamad et al. 2012). Cells of this bacterium exhibited a good ability to bind Cr ions, with a desorption efficiency of 70 % for Cr (III) and 76 % for Cr (VI), suggesting a potential application in the bioremediation field (Mohamad et al. 2012; Xie et al. 2013). However, there are few studies available on the ability of rhizobia and EPS being used as biosorbents of ions in an aqueous environment, particularly containing chromium and copper.

Studies have demonstrated the action of EPSs produced by a variety of Gram-negative bacteria as a potential bioemulsifier in the degradation of hydrocarbons. Huang et al. (2012a) tested the optimization of EPS production from three strains isolated from roots, namely *Rhizobium miluonense* (CC-B-L1), *Burkholderia seminalis* (CC-IDD2w), and *Ensifer adhaerens* (CC-GSB4), by adjusting culture medium components, as well as by testing the effect of EPS production on the performance of diesel oil emulsification. The study demonstrated that EPS production by the three strains was approximately 200 mg L^{-1}, and the emulsification index (E24) was on average 60 %. Changes in culture conditions resulted in increased EPS production and greater efficiency of the emulsification process.

Castellane and Lemos (2007) cultured *Rhizobium tropici* SEMIA 4077 and SEMIA 4080 in different carbon sources for exopolysaccharide production and observed that exopolysaccharides consisted of glucose and galactose residues with traces of mannose, rhamnose, and uronic acids. However, the proportions of sugar in these biopolymers displayed variations in each exopolysaccharide produced. Rheological properties and the effects of salt addition on the viscosity of exopolysaccharides produced by these bacteria showed that the EPS molecules can be applied in food sectors (Aranda-Selverio et al. 2010).

There is evidence that the EPS synthesis is inversely proportional to the synthesis of poly-3-hydroxybutyrate (PHB), another important polymer. PHB is the most well-known among the bacterial biodegradable polymers denominated by polyhydroxyalkanoates. This polymer aroused much interest in the petrochemical industry because the structural similarity of PHB with polypropylene attracted attention from various industries seeking to find a substitute for plastics with a petrochemical origin, as well as in the medical field in which biodegradability and biocompatibility are both important factors. The production of both polymers (EPS and PHB) by the same cells requires a biochemical regulation of bacteria because both metabolic pathways are large drains on carbon skeletons. *Rhizobium* is not yet used for PHB industrial production; however, Paganelli et al. (2011) demonstrated that some strains have potential for this purpose. These strains demonstrated the ability to accumulate PHB in relatively high amounts with sucrose.

Bacteria of the genus *Ensifer*, in addition to having the ability to produce EPS, can participate in important environmental and biotechnological processes. Thiamethoxam (THIA), a second-generation neonicotinoid insecticide in the thianicotinyl subclass, is used worldwide. Environmental studies revealed that microbial degradation is the major mode of removal of this pesticide from the soil. The nitrogen-fixing and plant-growth-promoting rhizobacterium *E. adhaerens* TMX-23, which is able to degrade THIA, has the potential for bioaugmentation as well as for promoting the growth of field crops in THIA-contaminated soil (Guang-can Zhou et al. 2013). A strain of *E. adhaerens* with the ability to produce alliinase was isolated from a soil sample. The enzyme was purified to characterize its general properties and to evaluate its application in

on-site production of allicin-dependent fungicidal activity. Allicin is best-known as the active compound in freshly crushed garlic extract, and it is known to possess a vast variety of biological effects, including antimicrobial, anti-inflammatory, antithrombotic, anticancer, and antiatherosclerotic activities (Yutani et al. 2011).

The natural ability of *Agrobacterium* to transfer and stably integrate DNA into plant genomes has led to an extensive exploration of this model for use as vectors for genetic transformation of plant cells using recombinant DNA technology (Tzfira and Citovsky 2006). Certain strains are used to obtain disarmed strains, aiming to integrate *Agrobacterium* in genetic engineering and biotechnology methodologies. In these lineages, the original Ti plasmid retains only the *vir* region, whereas the border sequences and the T-DNA are deleted along with the oncogenes. The chromosomal genes required for virulence also remain in the disarmed strains (Zambryski et al. 1983; Kiyokawa et al. 2009).

To obtain the cloning vectors, usually by double recombination, the original T-DNA has its oncogenes removed and replaced by selection markers, multiple cloning sites, and promoters for heterologous expression in plants. Thus, these modified plasmids retain the ends of the T-DNA required for their transfer, and genes of interest can be cloned in between. Vectors carrying the border sequence and the genes of interest can be transferred via triparental mating by *Escherichia coli* into the disarmed *A. tumefaciens* or *A. rhizogenes*, which will be used for infection and transformation of the plants of interest (Zambryski et al. 1983; Matthysse 2006; Kiyokawa et al. 2009). Plant explants used for transformation are selected using the appropriate antibiotic, and the transformed plant is regenerated using tissue culture, whereas the *Agrobacterium* is eliminated with the application of cefotaxime (Valvekens et al. 1988; Dhekney et al. 2008; Marcondes and Hansen 2008).

Finally, the most relevant application proposed for *Carbophilus*, *Chelatobacter*, *Kaistia*, and *Shinella* according to their environmental origins consists of exploiting their metabolism for use in bioremediation and biodegradation. In addition to the degradation of organic matter, the chemolithotrophic metabolisms capable of degrading carbon monoxide, nitrate, nitrite, and metal chelating agents can be exploited both under aerobic or anaerobic conditions in some cases. Thus, bacteria of the family *Rhizobiaceae* can aggregate the microbial consortia applied for the detoxification of industrial effluents and for the treatment of organic waste (Ilyin et al. 2004; Murto et al. 2004; De Vrieze et al. 2013; Franke-Whittle and Insam 2013).

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20 The Family *Rhodobacteraceae*

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Abstract

The family *Rhodobacteraceae* can be considered a paradigm of modern taxonomy of prokaryotes. Taking into account the number of species and genera that conforms the family, together with the knowledge about their abundance and vast global distribution, it surprises that most of them have been described relatively recent to our days. Two notable exceptions are *Rhodonostoc capsulatum* (Molisch, Die purpurbakterien nach neuen untersuchungen, vols i–vii. G. Fischer, Jena, pp 1–95, 1907) and *Micrococcus denitrificans* Beijerinck and Minkman (Zentbl Bakteriol, Parasitenkd, Infektionskr Hyg. Abt II 25:30–63, 1910), early basonyms of *Rhodobacter capsulatus* and *Paracoccus denitrificans*, respectively. The fact that so many descriptions within this family are recent means that some studies have been concomitant and pose a challenge not only for pure taxonomic studies but also for interpreting other studies in which a rapidly evolving nomenclature had to be used anyway. The metabolic and ecological diversity of the group adds further complexity. In spite of all these difficulties, the picture is far from being a chaos and it can be considered an exciting and important bacterial group to study.

Rhodobacteraceae are, fundamentally, aquatic bacteria that frequently thrive in marine environments. They comprise mainly aerobic photo- and chemoheterotrophs but also purple non-sulfur bacteria which perform photosynthesis in anaerobic environments. They are deeply involved in sulfur and carbon biogeochemical cycling and symbiosis with aquatic micro- and macroorganisms.

One hundred genera are currently recognized as members of the family although the *Stappia* group, *Ahrensia*, *Agaricicola*, and *Rhodothalassium* do not belong, phylogenetically, to the family. The 90 other genera are distributed in 5 phylogenetic groups (the *Rhodobacter*, the *Paracoccus*, the *Rhodovulum*, the *Amaricoccus*, and the *Roseobacter* clades) that might be considered a family on its own.

Taxonomy, Historical and Current

Rho.do.bac.ter.a'ce.ae. M.L. masc. n. *Rhodobacter*, type genus of the family; -aceae ending to denote family; M.L. fem. pl. n. *Rhodobacteraceae*, the *Rhodobacter* family.

The family *Rhodobacteraceae* was first established by Garrity et al. (2005) as the sole member of the order *Rhodobacterales* (class *Alphaproteobacteria*, phylum Proteobacteria) in the 2nd edition of *Bergey's Manual of Systematic Bacteriology*. Both names were validated in Validation List 107 (Euzéby 2006). The circumscription of the taxon was based on the phylogenetic analysis of 16S rRNA gene sequences of their members, which, at that time, comprised 31 genera (*Rhodobacter*, the type genus, plus *Ahrensia*, *Albidovulum*, *Amaricoccus*, *Antarctobacter*, *Gemmobacter*, *Hirshia*, *Hyphomonas*, *Jannaschia*, *Ketogulonicigenium*, *Leisingera*, *Maricaulis*, *Methylarcula*, *Octadecabacter*, *Pannonibacter*, *Paracoccus*, *Pseudorhodobacter*, *Rhodobaca*, *Rhodothalassium*, *Rhodovulum*, *Roseibium*, *Roseinatronobacter*, *Roseivivax*, *Roseobacter*, *Roseovarius*, *Rubrimonas*, *Ruegeria*, *Sagittula*, *Staleya*, *Stappia*, and *Sulfitobacter*). In the same year, Lee et al. described the family *Hyphomonadaceae*, removing the prosthecate genera *Hirshia*, *Hyphomonas*, and *Maricaulis* from *Rhodobacteraceae* (Lee et al. 2005) among other proposals for the subdivision of *Alphaproteobacteria* that were also based on 16S rRNA gene analysis. These authors did not recognize *Rhodobacterales* as an order, but included both the families *Rhodobacteraceae* and *Hyphomonadaceae* in the order *Caulobacterales*, along with *Caulobacteraceae*. Whatever the hierarchy considered, *Hyphomonadaceae* is a neighboring family to *Rhodobacteraceae* (*sensu stricto*) (see *Fig. 20.1*, General tree of the family *sensu lato*). The family *Hyphomonadaceae* contains a dozen of genera of prosthecate marine bacteria. The next closer neighbor is represented by the family *Kordiimonadaceae* that includes only the genus *Kordiimonas*, with two marine and one terrestrial species. However, phylogenetic analysis using different data sets and methods, as the concatenated alignments for 104 well-behaved protein families (Williams et al. 2007), splits *Hyphomonadaceae* apart from the order *Rhodobacterales*, forming an expanded group with *Caulobacterales* that also includes *Parvularcula*.

According to Rule 51b (1) of the *Bacteriological Code*, the family name *Rhodobacteraceae* (Garrity et al. 2005) is illegitimate because the family contains the genus *Hyphomonas* (ex Pongratz 1957, Moore et al. 1984) which is the type of the family *Hyphomonadaceae* (Lee et al. 2005; Euzéby 2006).

Since the publication of these studies, and following an increase of taxonomic attention to the marine microbiota, dozens of new genera have been described into the family *Rhodobacteraceae*. To date it comprises 99 genera and 288 species, being one of the most "populated" families of the Domain Bacteria (*Table 20.1*).

As already stated at the time of its establishment, the family is phenotypically, metabolically, and ecologically diverse, including photoheterotrophs able to grow photoautotrophically or chemotrophically, aerobic and facultative anaerobic chemoorganoheterotrophs, and facultative methylotrophs. Several members are aerobic anoxygenic phototrophs (AAP) while others, as the type genus of the family, are classical purple non-sulfur photosynthetic bacteria, whose phototrophic ability is restricted to anaerobic conditions. The vast majority of the species contained in the family are aquatic, and many of them require sodium ion or combined salts for growth. Their cells are Gram negative and multiply by binary fission or by budding, following monopolar growth. When motile, they exhibit flagella, usually

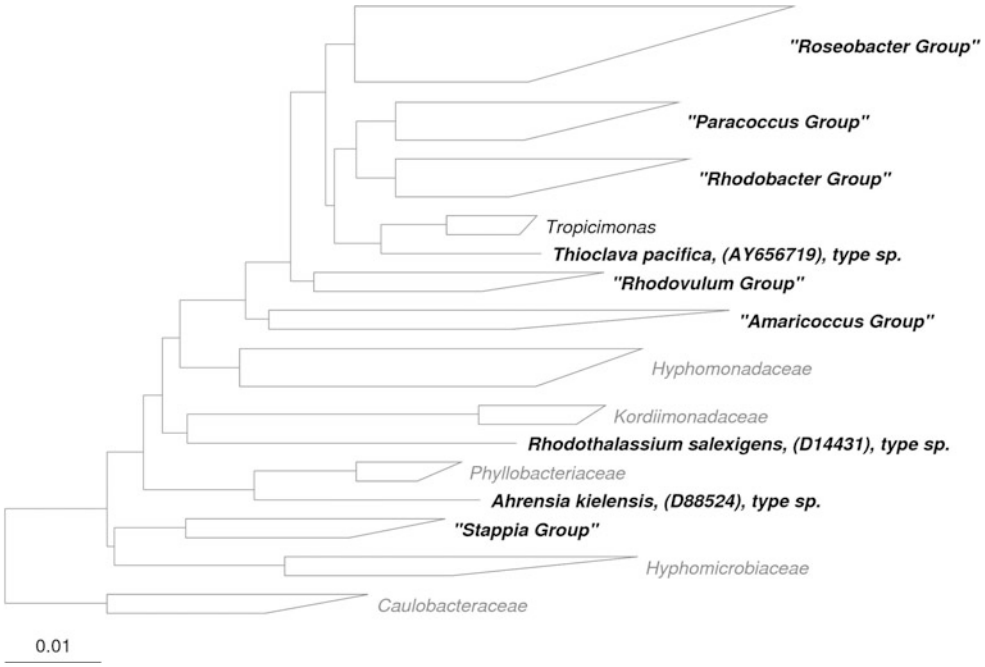

Fig. 20.1

Phylogenetic reconstruction of the family *Rhodobacteraceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence data sets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

polar. Most species are positive for oxidase. Pigmentation does occur, not only in photosynthetic members (that present Bchl *a* and carotenoids of spheroidene class) but also in non-phototrophic members. Carbon reserve material is formed by some members as polyhydroxyalkanoates (PHA), commonly polyhydroxybutyrate (PHB). The major or only respiratory quinone is ubiquinone 10 (as occurs almost universally in the Class *Alphaproteobacteria*), and their cellular fatty acids are usually dominated by C18:1 ω7c, which very often constitutes more than 50 % of the total. Polar lipids may include phosphatidyl glycerol, diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl choline, and several amino-, phospho-, and glycolipids in different combinations. The mol% G+C content of their DNA is above 50 %, with only one exception known (*Pelagicola*, 47 mol%), and being the most common range 55–70 mol% and the total range 47–76 mol%. A summary of the chemotaxonomic features of the genera is included as *Table 20.2*.

After introducing the common traits of the family, it should be stressed that its internal heterogeneity is not just a matter of metabolic or physiological diversity and/or versatility but also a matter of lack of phylogenetic homogeneity: as can be observed in *Fig. 20.1*, a bunch of genera formally included in the family lay far away from the large clade that comprises the true rhodobacters (family *Rhodobacteraceae sensu stricto*). These outsider genera fall in three groups: a well-defined clade comprising seven genera and sixteen species, labeled as "*Stappia* group," the genus *Ahrensia* (related to *Phyllobacteriaceae*), the genus *Agaricicola* (not shown), and the purple non-sulfur genus *Rhodothalassium*, which forms a distinct lineage in this part of the alphaproteobacterial tree. The separate position of this genus was already underlined in a Bergey's Editorial Note and by placing it as incertae sedis in the manual (Imhoff 2005). In fact, it was stated that *Rhodothalassium* could constitute a family on its own, but the scarcity of sequence data available prevented the proposal at that time.

Thus, the family *Rhodobacteraceae* formally contains, up to date, these ten genera (http://www.bacterio.cict.fr/classifgenerafamilies.html#Rhodobacteraceae) although they could certainly constitute two additional families, "*Stappiaceae*" (with *Labrenzia*, *Nesiotobacter*, *Pannonibacter*, *Polymorphum*, *Pseudovibrio*, *Roseibium*, and *Stappia*) and *Rhodothalassiaceae* (monogeneric) [Note: During the writing of the chapter, the proposal of *Rhodothalassiaceae* fam. nov. and *Rhodothalassiales* ord. nov. was published by Venkata Ramana et al. (2013), with an emended description of the genus *Rhodothalassium*]. On the other hand, *Ahrensia* could be transferred to the family *Phyllobacteriaceae*, where it is placed with *Hoeflea* species in more detailed trees (see the last release of the Living Tree Project 16S rRNA gene tree, LTP111, Yarza et al. 2010), and *Agaricicola*,

Table 20.1

List of genera in the family *Rhodobacteraceae sensu lato* (=order *Rhodobacterales*: 99 genera, 288 species), distributed by groups (tentative new families) by phylogenetic assignment

***Rhodobacter* group = *Rhodobacteraceae* (*sensu stricto*): 11 genera, 35 species**			
Catellibacterium[a]	*Defluviimonas*	*Gemmobacter*	*Haematobacter*
Pararhodobacter	*Pseudorhodobacter*	*Rhodobaca*	***Rhodobacter***
Roseibaca	*Roseicitreum*	*Roseinatronobacter*	*Thioclava*
***Rhodovulum* group = *Rhodovulaceae*: 3 genera, 18 species**			
Albidovulum	*Jhaorihella*	***Rhodovulum***	
***Amaricoccus* group = *Amaricoccaceae*: 5 genera, 9 species**			
Albimonas	***Amaricoccus***	*Oceanicella*	*Rubribacterium*
Rubrimonas			
***Paracoccus* group = *Paracoccaceae*: 2 genera, 42 species**			
Methylarcula	***Paracoccus***	*Thiosphaera*[a]	
***Roseobacter* group = *Roseobacteraceae*: 68 genera, 164 species**			
Actibacterium	*Antarctobacter*	*Celeribacter*	*Citreicella*
Citreimonas	*Dinoroseobacter*	*Donghicola*	*Epibacterium*
Gaetbulicola[a]	*Hasllibacter*	*Huaishuia*	*Hwanghaeicola*
Jannaschia	*Ketogulonicigenium*	*Leisingera*	*Lentibacter*
Litoreibacter	*Litorimicrobium*	*Loktanella*	*Lutimaribacter*
Mameliella	*Maribius*	*Marinovum*	*Maritimibacter*
Marivita	*Nautella*	*Nereida*	*Oceanibulbus*
Oceanicola	*Oceaniovalibus*	*Octadecabacter*	*Pacificibacter*
Palleronia	*Pelagibaca*	*Pelagicola*	*Pelagimonas*
Phaeobacter	*Planktotalea*	*Pontibaca*	*Ponticoccus*
Poseidonocella	*Primorskyibacter*	*Profundibacterium*	*Pseudoruegeria*
Roseibacterium	*Roseicyclus*	*Roseisalinus*	*Roseivivax*
Roseobacter	*Roseovarius*	*Rubellimicrobium*	*Ruegeria*
Sagittula	*Salinihabitans*	*Salipiger*	*Sediminimonas*
Seohaeicola	*Shimia*	*Silicibacter*[a]	*Staleya*[a]
Sulfitobacter	*Tateyamaria*	*Thalassobacter*	*Thalassobius*
Thalassococcus	*Tranquillimonas*	*Tropicibacter*	*Tropicimonas*
Vadicella	*Wenxinia*	*Yangia*	
***Stappia* group = *Stappiaceae*: 7 genera, 17 species**			
Labrenzia	*Nesiotobacter*	*Pannonibacter*	*Polymorphum*
Pseudovibrio	*Roseibium*	***Stappia***	
Unaffiliated: 3 genera, 3 species			
Agaricicola	*Ahrensia*	*Rhodothalassium*	

[a]Empty genera, all their species have been reclassified into other genera

which is related to *Prosthecomicrobium pneumaticum* (LTP111), should be also excluded from the family *Rhodobacteraceae*.

The remaining 89 genera (268 species) form a monophyletic clade that may be considered the true *Rhodobacteraceae* family, from a strictly phylogenetic point of view. Phylogenetic analysis, based on 16S rRNA gene sequences, allows the recognition of five well-defined groups among these 89 genera. Lee et al. (2005) already recognized these five main groups, although their work included less than 20 % (46 out of 268) of the currently established species. In *Figs. 20.1*, *20.2*, *20.3*, *20.4*, *20.5*, and *20.6* and *Table 20.1*, each of these groups is named after the senior genus contained. *Table 20.1* also reflects a suggestion for the nomenclature to be used if these groups are finally recognized as new families, a proposal that, in our opinion, merits consideration but is outside of the aim of this chapter. As it can be observed, the distribution of genera

Table 20.2

Chemotaxonomic traits of the genera in the family *Rhodobacteraceae sensu lato*, distributed by groups (tentative new families). Empty cells indicate that no information could be retrieved for that particular genus and trait. +, positive; −, negative; *v*, variable

Genus	Quinone	Polar lipids	Pigments	PHB/PHA	GC mol%	Other
***Rhodobacter* group**						
Rhodobacter	Q10	PG, PE, PC, AL, PL, L	Bchl *a* +, carotenoids[a]		62–73	
Defluviimonas	Q10		None		65	
Gemmobacter	Q10	PG, PE, PC, AL, DPG[b]	None	v	61–69.5	*m*-DAP[c]
Haematobacter			None		65	
Pararhodobacter	Q10		None		68	
Pseudorhodobacter	Q10	PC, PG, ALs, APLs	Bchl *a* −, *pufLM* -		57–62	
Rhodobaca			Bchl *a* +, demethylspheroidene and demethylspheroidenone	yes	58–60	
Roseibaca	Q10	DPG, PE, PG, PC	Bchl *a* +	yes	61	
Roseicitreum	Q10	PG, PE, PC, AL	Bchl *a* +	yes	63	
Roseinatronobacter			Bchl *a* +, spheroidene	yes	59–62	
Thioclava	Q10		Bchl *a* −		63	
***Rhodovulum* group**						
Rhodovulum	Q10	PE, PG, L, 2SL[d], SQD[e], DPG[e], APL[e]	Bchl *a* +, spheroidene		58–69	
Albidovulum	Q10	PG, PE, PC[f]	Bchl *a* −		63–71	
Jhaorihella	Q10	PC, PG, PE, DPG, PLs, ALs		yes	65	
***Amaricoccus* group**						
Amaricoccus[g]	Q10	PE, PC, PG, 2AL, GL		yes	51–63	
Albimonas	Q10	PG, DPG, PE, PC	Bchl *a* −		72	
Oceanicella	Q10	PC, PG, AL, DPG				
Rubribacterium			Bchl *a* +, spheroidene 60 % and spirilloxanthin 38 %	yes	70	
Rubrimonas	Q10	PG, PC, ALs, Ls, PL[h]	Bchl *a* +, *pufLM* +, carotenoid			
***Paracoccus* group**						
Paracoccus	Q10[i]	PG, PC, DPG (common), PE (rare), ALs, PLs	carotenoids when pigmented	most spp.	58–71	
Methylarcula	Q10	PE, PC, PG, DPG	carotenoids −	yes	57–61	Ectoine (main compatible solute)
***Roseobacter* group**						
Roseobacter	Q10	PG, DPG, PC, AL, PL, [PE absent]	Bchl *a* +, spheroidenone		56–60	
Actibacterium	Q10	PG, 2AL, 3L, 2PL, GL	Bchl *a* −	no	61.3	
Antarctobacter	Q10	PG, PC, AL, PL	Bchl *a* −	yes	62–63	*m*-DAP
Celeribacter	Q10	PG, AL, L, PE[j], PC[j], LPE[j]	Bchl *a* −	no	59–62	
Citreicella	Q10		Bchl *a* −		67–69	
Citreimonas			Bchl *a* −		67	
Dinoroseobacter	Q10	PG, DPG, AL, 8L	Bchl *a* +, spheroidene	yes	65	
Donghicola	Q10		Bchl *a* −		59–62	
Epibacterium	Q10	PG, PC, 2AL, 4PL	Bchl *a* −		52–53	
Hasllibacter	Q10				71.6	
Huaishuia	Q10	PG, PLs, AL, L		no	60	

Table 20.2 (continued)

Genus	Quinone	Polar lipids	Pigments	PHB/PHA	GC mol%	Other
Hwanghaeicola	Q10				61	
Jannaschia	Q10	PG, PC, PE, AL, DPG[k]	Bchl *a* −[l]	v	63–68	
Ketogulonicigenium					53–54	
Leisingera	Q10	PG, PE, PL, AL, L, [PC absent]	Bchl *a* −	v	60–62	
Lentibacter	Q10	PG, PE, PC, L, AL	Bchl *a* −	yes	55	
Litoreibacter	Q10	PC, PG, PE, L, AL, DPG[m]	Bchl *a* −	v	56–60	
Litorimicrobium	Q10	PG, DPG, PC, AL, PL, L			62	
Loktanella	Q10	PG, PC, PE[n], DPG[o]	Bchl *a* +[p], *pufLM* +[q]		55–69	
Lutimaribacter	Q10	PC, PG, PE, AL, 2PL	Bchl *a* −		63.5	
Mameliella	Q10			yes	63–64	
Maribius	Q10		Bchl *a* −	yes	66–70	
Marinovum	Q10	PG, PE, PC, DPG, AL, PL, L	Bchl *a* −	yes		
Maritimibacter	Q10	PE, PG, PC, [DPG absent]	Bchl *a* −	no	61–64	
Marivita	Q10	PC, PG, PE, DPG, AL, L	Bchl *a* v, *pufLM* +	v	58–65	
Nautella				yes	61	
Nereida				no	56	
Oceanibulbus	Q10	PG, PC, PE, DPG, AL	Bchl *a* −	yes	60	
Oceanicola	Q10	PC, PG, PE, AL, L	Bchl *a* −	yes	64–73	
Oceaniovalibus	Q10	PG, DPG	Bchl *a* −	no	62	
Octadecabacter			Bchl *a* −	no	56–57	
Pacificibacter	Q10	PC, PG, DPG, 2L	Bchl *a* −		52.6	
Palleronia	Q10		Bchl *a* −	yes	64	
Pelagibaca	Q10		Bchl *a* −	no	65	
Pelagicola	Q10	PC, PG, PE, AL, 3L	Bchl *a* −	no	47	
Pelagimonas	Q10	PC, PG, PE, DPG, PMME, AL, PL, L	Bchl *a* −, *pufLM* −		55	
Phaeobacter	Q10	PG, PE, PC, AL, L, PL[r]	Bchl *a* −	no[s]	56–65	
Planktotalea	Q10	PC, PG, AL, PL	Bchl *a* −, *pufLM* +		53–54	
Pontibaca	Q10	PC, PG, AL, PL, 3L	Bchl *a* −		65	
Ponticoccus	Q10	PC, PG, PE, 2AL, GL, L	Bchl *a* −, pufLM −	yes	68	
Poseidonocella	Q10	PC, PG, DPG, PA, AL, Ls	Bchl *a* −		60–65	
Primorskybacter	Q10	PC, PE, PG, DPG, L	Bchl *a* −		60–62	
Profundibacterium	Q10	PG, PE 2PL		no	64	
Pseudoruegeria	Q10	PG, PE, GL, DPG[t], PL[t], PC[u], AL[u], L[u]			67–73	
Roseibacterium	Q10		Bchl *a* +	v	68–76	
Roseicyclus			Bchl *a* +		66	
Roseisalinus	Q10	DPG, PG, PC	Bchl *a* +	yes	67	*m*-DAP
Roseivivax	Q10	PG, PE, PC, DPG, SQDG, 2-3PL, AL, L	Bchl *a* v	v	59–69	
Roseovarius	Q10	PG, PC, PE, DPG[v], AL, 1-2PL	Bchl *a* v		55–64	*m*-DAP
Rubellimicrobium	Q10	DPG, PC, AL, PG, PE[w]	Bchl *a* −, carotenoid +	yes	69–72	Polyamine pattern: putrescine, spermidine and *sym*-homospermidine
Ruegeria	Q10	PC, PG, Ls, AL[x], DPG[y], PL[z], PE[aa]	Bchl *a* −	v	55–68	

Table 20.2 (continued)

Genus	Quinone	Polar lipids	Pigments	PHB/PHA	GC mol%	Other
Sagittula			Bchl *a* −	yes	65	
Salinihabitans	Q10		Bchl *a* −		63.5	
Salipiger	Q10		Bchl *a* −	yes	64.5	
Sediminimonas	Q10	DPG, PG, PC, 4PL	Bchl *a* −	no	63–64	
Seohaeicola	Q10	PC, PG, PE, L			63.4	
Shimia	Q10		Bchl *a* −	no	55–57	
Sulfitobacter	Q10	PC, PG, PE, DPG[ab], AL[ac], PL[ad]	Bchl *a* −	v	55–62	
Tateyamaria	Q10	PG, PE, PC, L	Bchl *a* v		56–62	
Thalassobacter		PG, DPG, PC, 2PL, AL, PE[ae]	Bchl *a* +	yes	59	
Thalassobius	Q10	PC, PG, PE, L	*pufLM* −[af]	yes	57–61	
Thalassococcus	Q10	PG, PC, Ls, AL[ag], PE[ah]		v	57.8–58	
Tranquillimonas	Q10		Bchl *a* −	yes	69	
Tropicibacter	Q10	PE, PG, PC, AL, 4PL	Bchl *a* −	yes	58–65	
Tropicimonas	Q10 and Q9	PC, PG, DPG, AL, Ls, PL[ai]	Bchl *a* −	yes	66.5–69.6	
Vadicella	Q10	PC, PG, PA, AL, L, PE	Bchl *a* −		56–60	
Wenxinia	Q10	PG, PC, GL, PE, PL	Bchl *a* −	yes	69.4	
Yangia	Q10		Bchl *a* −	yes	63	
***Stappia* group**						
Stappia	Q10	DPG, PC, PG, PE, PMME[aj], AL[ak], PL[al]	Bchl *a* v		59–65.9	Major polyamines: spermidine and spermine
Labrenzia	Q10	DPG, PC, PG, PE, PMME, ALs, SQDG	Bchl *a* v		56–60	
Nesiotobacter			Bchl *a* −		61	
Pannonibacter	Q10	DPG, PG, PE, PC[am], PMME[am], 2AL[am], PS[an], PL[an]	Bchl *a* −		63–64.6	*m*-DAP
Polymorphum	Q10	DPG, PMME, PG, PC, AL, PLs, SQDG	Bchl *a* −		65.6	
Pseudovibrio			Bchl *a* −		50–52	
Roseibium	Q10	PG, DPG, PE, PC, SQDG, PMME, AL	Bchl *a* +		57.6–63.4	
Unaffiliated						
Ahrensia	Q10		Bchl *a* −	no	48	
Agaricicola	Q10	DPG, PC, PG, PE	Bchl *a* −	yes	62.7	
Rhodothalassium	Q10, MK10	DPG, PG, OL, PL, AL, L	Bchl *a* +, carotenoids: spirilloxanthine series		60–62.8	Contains aminopropylhomospermidine

Quinone systems: MK10, menaquinone 10; Q9, ubiquinone 9; Q10, ubiquinone 10

Polar lipids: *AL* aminolipid, *APL* aminophospholipid, *DPG* diphosphatidylglycerol, *GL* glycolipid, *L* lipid, *LPE* lysophosphatidylethanolamine, *OL* ornithine lipid, *PA* phosphatidic acid, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PL* phospholipid, *PMME* phosphatidylmonomethylethanolamine, *PS* phosphatidylserine, *SL* sulfonolipid, *SQD* sulphoquinovosyldiglyceride, *SQDG* sulphoquinovosyldiacylglycerol

Other: *m*-DAP, meso-diamino pimelic acid in peptidoglycan

[a]spheroidene and spheroidenone/neurosporene in *R. viridis*; [b]DPG only in *G. caeni* and *G. nanjingensis*; [c]in *G. aquatilis*; [d]2SL *in R. bhavnagarense*; [e]SQD, DPG (cardiolipin) and APL in *R. euryhalinum* and *R. tesquicola*; [f]PC only in *A. xiamenensis*; [g]chemotaxonomic information, except for G+C mol%, only investigated in *A. kaplicensis*; [h]PL only in *R. shengliensis*; [i]ubiquinone 8 in *P. yeei*; [j]only in *C. neptunius*; [k]except in *J. seosinensis* and *J. donghaensis*; [l]except for *J. seohaensis*; [m]DPG in *L. janthinus*; [n]in *L. atrilutea*, *L. maricola*, and *L. tamlensis*; [o]DPG only in some species of *Loktanella*; [p]Bchl *a* + in *L. maricola*; [q]*pufLM* + in *L. vestfoldensis*; [r]PL in *P. gallaeciensis*, *P. inhibens*, *P. daeponensis*; [s]PHA/PHB in *P. caeruleus*; [t]DPG and PL in *P. aquimaris*; [u]PC, AL, and L in *P. lutimaris*; [v]DPG absent in *R. litoreus*; [w]PE in *R. aerolatum*; [x]AL absent in *R. marina*; [y]DPG in *R. conchae*, *R. faecimaris*, *R. halocynthiae*, and *R. atlantica*; [z]PL in *R. conchae* and *R. marina*; [aa]PE in *R. marina*; [ab]DPG absent in *S. guttiformis*; [ac]AL in *S. brevis* and *S. guttiformis*; [ad]PL in *S. brevis* and *S. donghicola*; [ae]PE in *T. arenae*; [af]*pufLM* in *T. aestuarii*; [ag]AL in *T. lentus*; [ah]PE in *T. halodurans*; [ai]PL absent in *T. sediminicola*; [aj]PMME in *S. indica* and *S. stellulata*; [ak]AL in *S. indica*; [al]PL in *S. taiwanensis*; [am]PC, PMME, and 2AL in *P. indica*; [an]PS and PL in *P. phragmitetus*

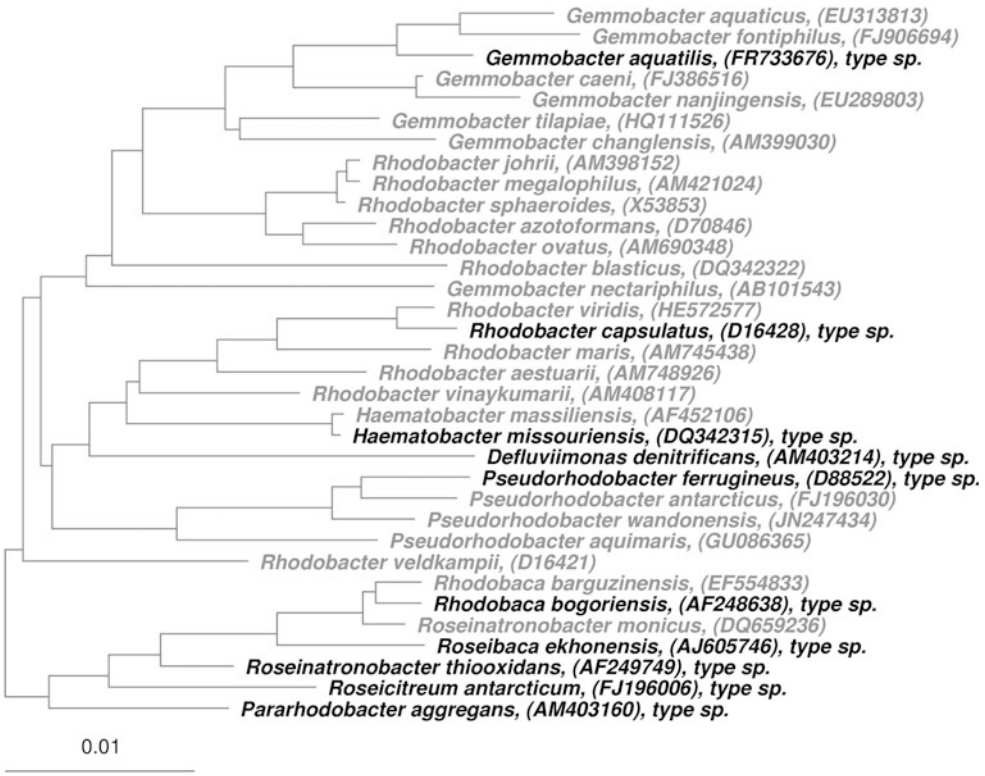

Fig. 20.2

Phylogenetic reconstruction of the *Rhodobacter* group based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence data sets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

among these groups is not even, but heavily biased to the *Roseobacter* group, that contains nearly 70 genera and more than 160 species. Metabolic, physiological, and ecological traits may differ between groups, giving a base for their suggested recognition as new families. For example, the *Rhodobacter* group contains mainly species from freshwater and terrestrial habitats, with few showing salt requirements, while *Rhodovulum* group is dominated by halophilic, marine species. On the other hand, the *Roseobacter* group contains mostly aerobic chemoorganoheterotrophs, occasionally AAP, isolated from marine habitats, but no purple non-sulfur photosynthetic genera, which are dominant in *Rhodobacter* and *Rhodovulum* groups. The *Paracoccus* group is composed almost exclusively by species of this genus, which thrives commonly in aquatic environments, including sewage and sewage treatment plants and is widely known for the denitrification activity displayed by some of its members.

Strains of the *Rhodobacteraceae* are successfully preserved in 20 % (w/v) glycerol suspension at −80 °C. Long-term preservation by freeze-drying gives good results. In our experience at CECT, the best protecting agent for lyophilization is 5 % (w/v) inositol. Some strains need the addition of salts for maintaining the ionic stability in the suspension. This could be achieved by adding the recommended culture broth to the cryoprotectant solution in a proportion of 1 to 1. Some strains could not be lyophilized successfully. Preservation and storage of these strains in liquid nitrogen is possible with 5 % (v/v) DMSO as cryoprotectant.

The *Rhodobacter* Group

This group comprises the following genera: *Defluviimonas*, *Gemmobacter*, *Haematobacter*, *Pararhodobacter*, *Pseudorhodobacter*, *Rhodobaca*, *Rhodobacter*, *Roseibaca*, *Roseicitreum*, *Roseinatronobacter*, and *Thioclava*.

The group contains a diverse collection of metabolic lifestyles, as purple non-sulfur photosynthetic bacteria (*Rhodobacter*, the type genus of the family, plus *Rhodobaca*), aerobic anoxygenic photoheterotrophs (*Roseibaca*, *Roseicitreum*, and *Roseinatronobacter*), chemoorganoheterotrophs (most *Gemmobacter* species, *Defluviimonas*, *Haematobacter*, *Pararhodobacter*, and *Pseudorhodobacter*), and facultative sulfur chemolithotrophs (*Thioclava*).

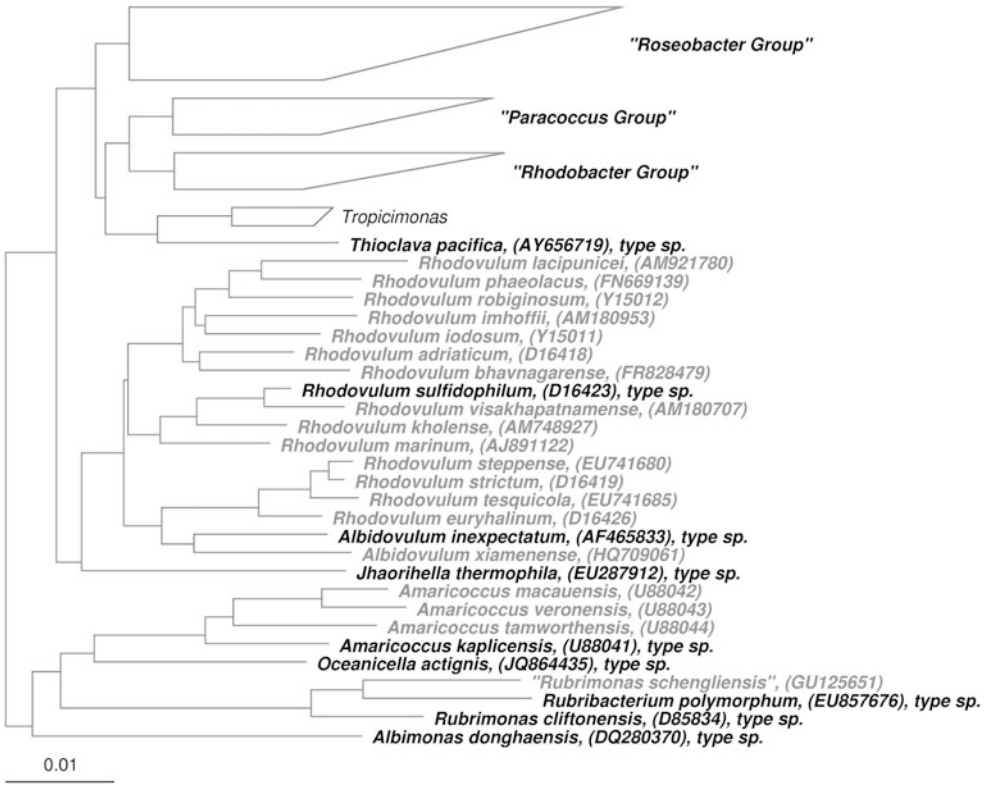

Fig. 20.3

Phylogenetic reconstruction of the *Rhodovulum* and *Amaricoccus* groups based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence data sets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

All of them, except for *Thioclava*, are grouped in a well-defined clade, whose nearest neighbor is the *Paracoccus* group (*Fig. 20.2*). *Thioclava* does not merge with the clade in the NJ analysis, but is clearly included when ML is used (not shown), having *Defluviimonas* as closest relative. The internal phylogenetic structure of the group does not reflect a separation of lifestyles or habitats, as a close relationship is found, for example, between the clinical chemoorganotrophic *Haematobacter* species and a subset of *Rhodobacter* species that include the three isolated from marine samples. An exception is the grouping of all AAPs together with the two *Rhodobaca* species, as most of them share hypersaline soda lake water as a common habitat (*Table 20.3*).

Rhodobacter

The type genus of the family, *Rhodobacter*, has been thoroughly treated by Imhoff (2005, 2006). The reader is addressed to those chapters for more detailed information on this genus (as well as *Rhodobaca* and *Rhodovulum*). A summary of its general characteristics is given here together with information on those species described after the reference work of Imhoff cited above.

Rhodobacter is defined, among all the phototrophic alphaproteobacteria, by a set of characters that include cellular morphology and division mode, intracytoplasmic membrane type, pigment composition, optimal phototrophic conditions, polar lipid content, saline preferences, and major products of sulfide oxidation. *Rhodobacter* cells are ovoid or short rods, with polar flagella (when motile) that divide by binary fission (sometimes forming chains) and exhibit vesicular intracytoplasmic membrane systems when grown phototrophically (an exception is *R. blasticus*, formerly *Rhodopseudomonas blastica*, which forms peripheral lamellae and divide by budding).

They present Q-10 as predominant quinone and have C18:1 ω7c as dominant cellular fatty acid, accompanied by C18:0 and C16:0 (Girija et al. 2010). Polar lipids may include PE and sulfolipids in addition to PC and PG. The DNA G+C content is 62–73 mol%.

Their preferred metabolism is photoorganoheterotrophy, with light and organic carbon and electron sources in anaerobic

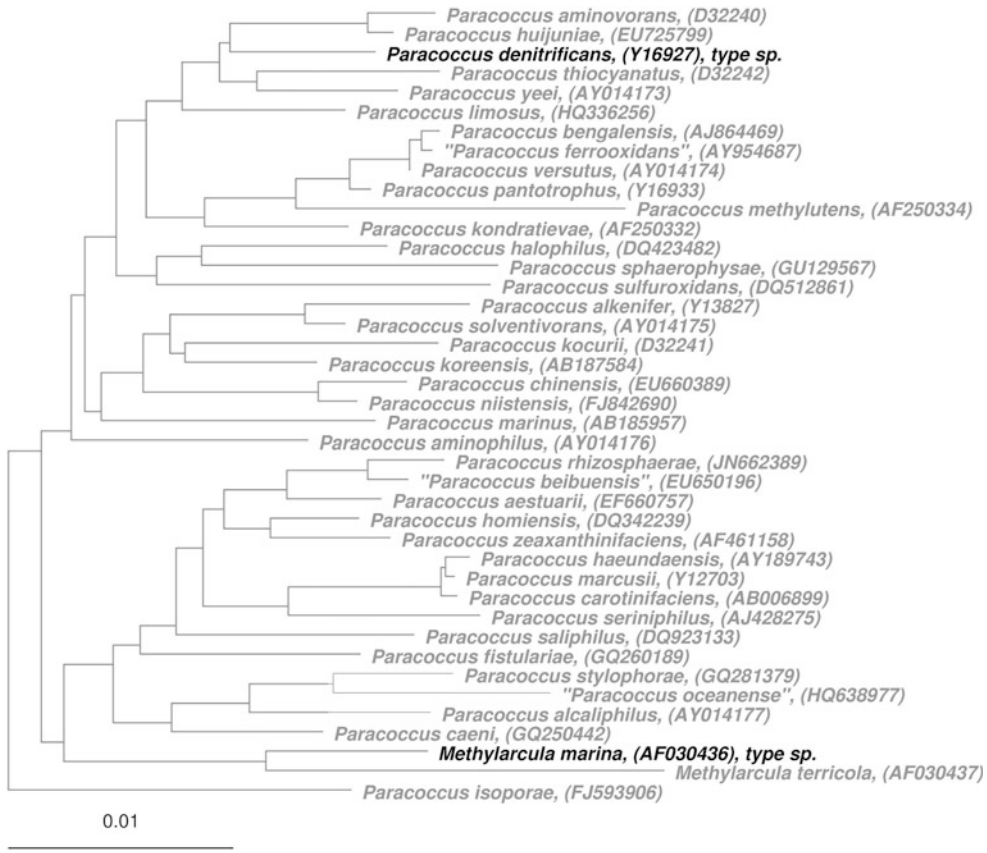

Fig. 20.4

Phylogenetic reconstruction of the *Paracoccus* group based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence data sets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

conditions. Phototrophically grown cultures are yellow brown to yellow green, but turn pink or red when exposed to oxygen. Main photosynthetic pigments are Bhcl *a* and carotenoids of the spheroidene series, with spheroidene and spheroidenone as the major components, except for *R. viridis* (Shalem Raj et al. 2013) which presents neurosporene as major carotenoid. In addition to the photoheterotrophic growth, some rhodobacters are able to thrive as photolithoautotrophs, using reduced sulfur compounds (sulfide, thiosulfate) or hydrogen as electron donors and bicarbonate/CO_2 as carbon source (fixed through Calvin cycle). Elemental sulfur is the common end product of sulfide oxidation, although some species can oxidize it to sulfate (*R. veldkampii*). Chemoheterotrophic growth in the dark by aerobic respiration is also common, being pyruvate, succinate, lactate, and other organic acids and sugars usually used. In anaerobic conditions, denitrification, other anaerobic respiration processes (with TMAO or DMSO), or fermentation (on pyruvate or sugars) could support chemoheterotrophic growth of some species. Chemolithoautotrophic growth is also possible with hydrogen being used by some species. Metabolic versatility, is, thus, considered a prominent character of this genus. Nevertheless, ability for photolithoautotrophic growth is restricted to only two out of the seven species described since 2007 and chemolithoautotrophy is even rarer. Thus, the current balance is that most *Rhodobacter* species are unable to growth autotrophically.

All *Rhodobacter* species have vitamin requirements (vitamin B12, biotin, niacin, thiamin, and/or *p*-aminobenzoic acid). Ammonium salts are the best nitrogen source, but they are also able to fix molecular nitrogen, both in phototrophic and in chemotrophic regimes (in this later case, with microoxic conditions). Sulfate is used as sulfur source by most species but some require reduced sulfur compounds.

Species of *Rhodobacter* are inhabitants of freshwater environments and are mesophilic and non-halophilic, being this later trait one that distinguish *Rhodobacter* from the members of *Rhodovulum*. While this assertion is true for the older members of the genus, however, three of the newest species come from marine samples and one of them displays saline requirements, as it needs NaCl for growth (*R. vinaykumarii*, Srinivas et al. 2007a).

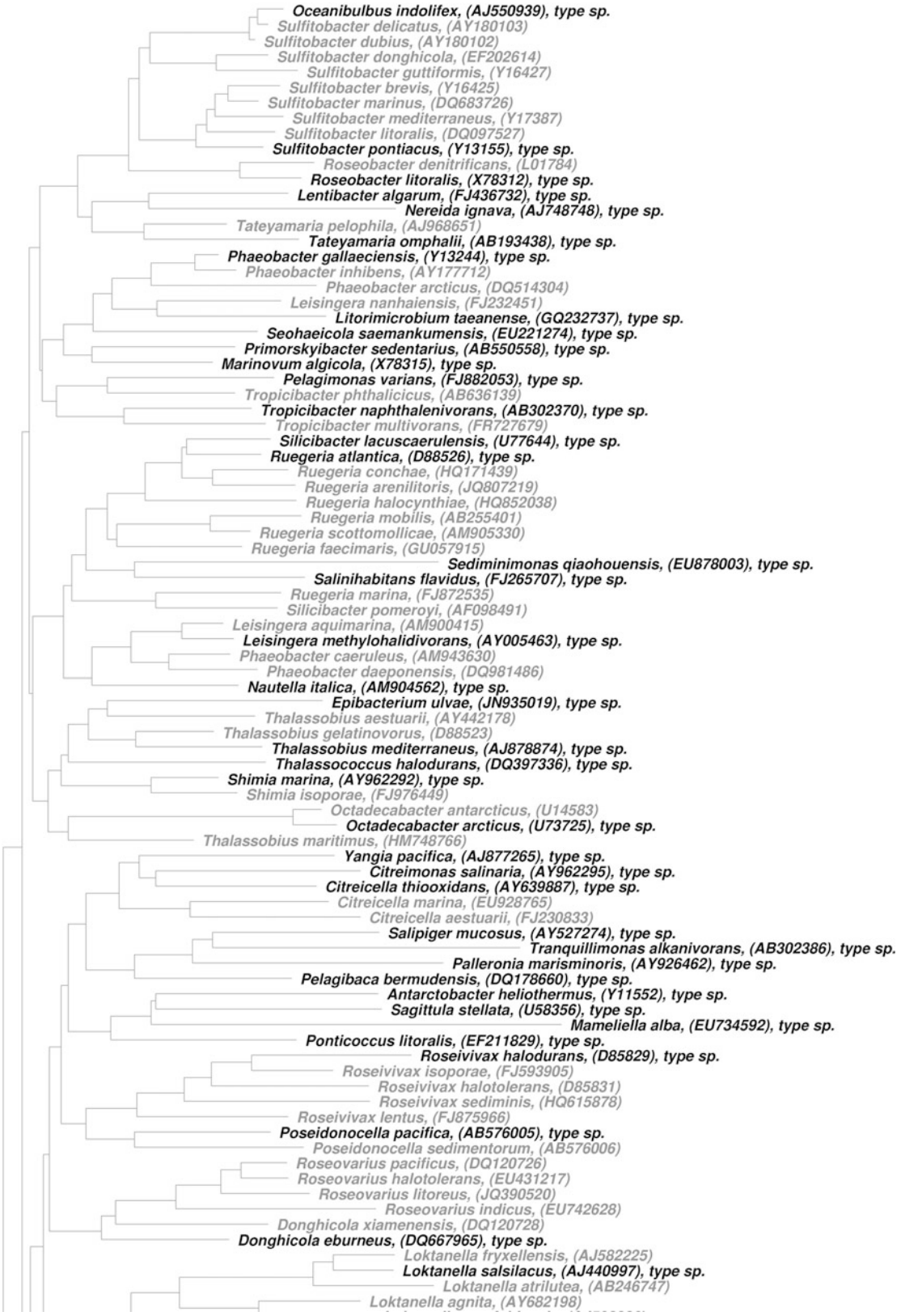

Fig. 20.5 (Continued)

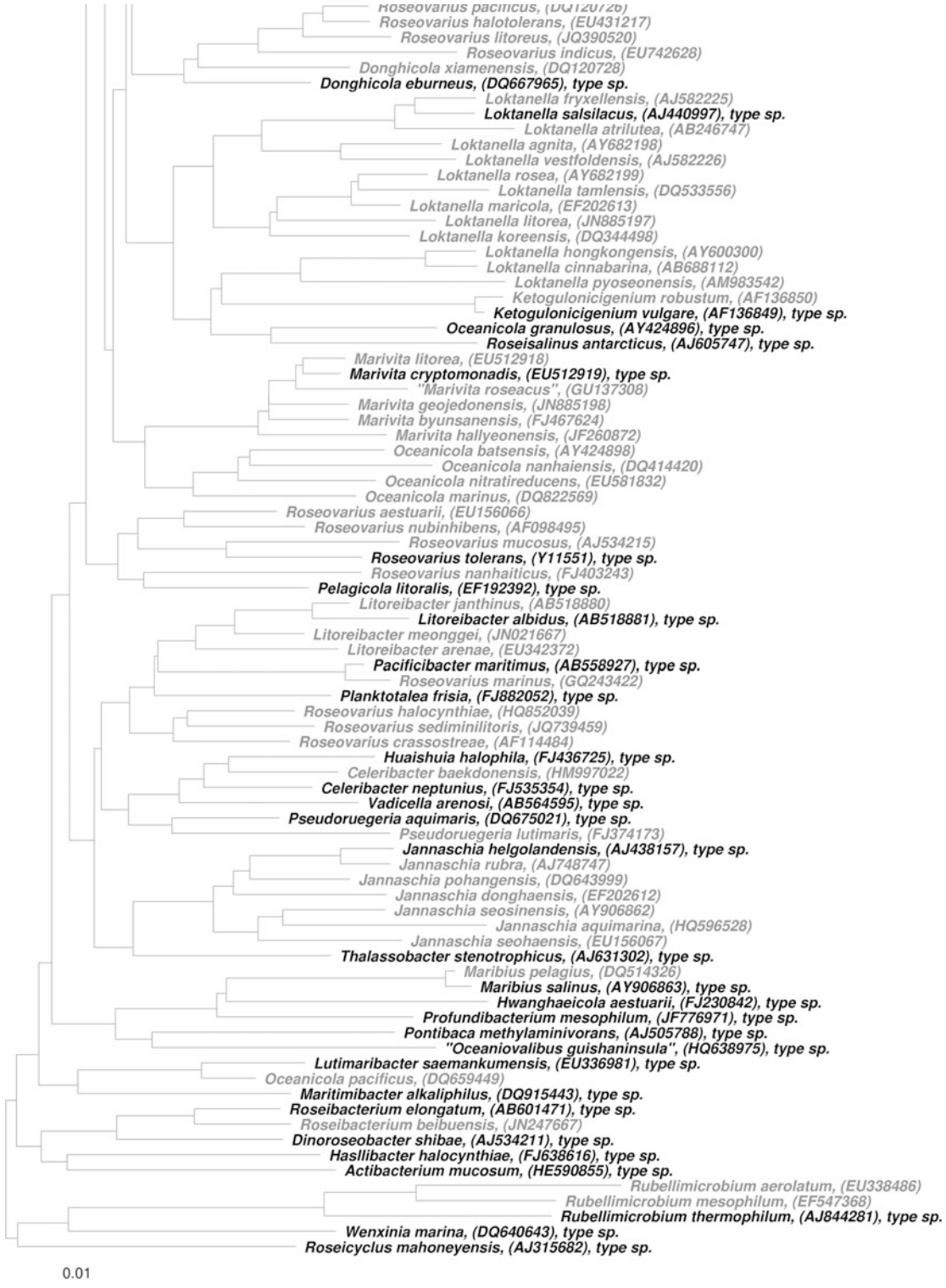

Fig. 20.5

Phylogenetic reconstruction of the *Roseobacter* group, based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence data sets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

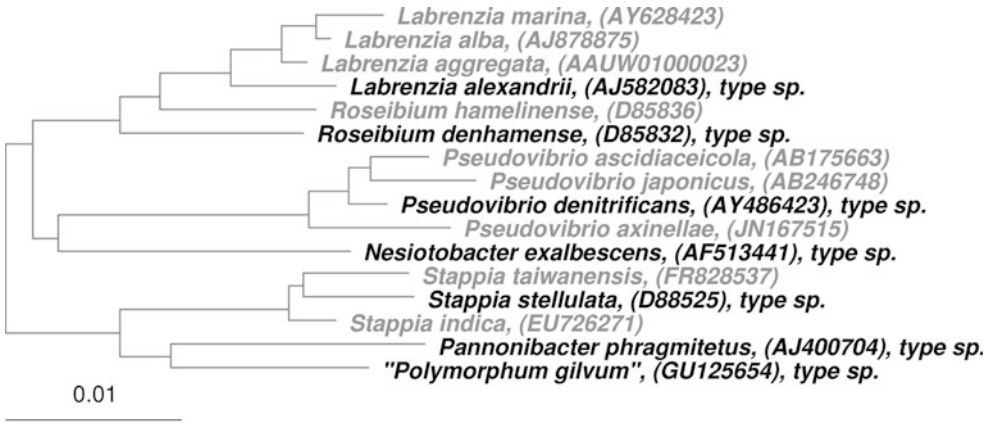

Fig. 20.6

Phylogenetic reconstruction of the *Stappia* group based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence data sets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

Other rhodobacters of marine origin are *R. maris* and *R. aestuarii* (Venkata Ramana et al. 2008, 2009), although they do not require NaCl for growth. The remaining recently recognized species have been isolated from soil (*R. megalophilus*, Arunasri et al. 2008; *R. jhorii*, Girija et al. 2010) or freshwater environments (*R. ovatus*, Srinivas et al. 2008; *R. viridis*, Shalem Raj et al. 2013).

A remarkable finding in one of the newly described species, *R. johrii*, is the ability to form endospores (Girija et al. 2010). This species was isolated from pasteurized rhizosphere soil and, after careful assessment of purity both by cultural and molecular methods, the presence and characteristic features of endospores were confirmed: dipicolinic acid content, staining behavior, induction upon stress, thermoresistance, thermal activation, ultrastructural appearance (not typical). To the best of our knowledge, genes encoding this complex ability have not been investigated; thus the ability is pending confirmation as in the case of other alleged endospore-formers outside *Firmicutes* (e.g., *Mycobacterium marinum*, Ghosh et al. 2009; Traag et al. 2010).

The complete genome sequence of *R. capsulatus* SB 1003 has been reported (Strnad et al. 2010). It consists of a 3.7 Mb chromosome and a 133 kb plasmid. The genome encodes genes for photosynthesis, nitrogen fixation, utilization of xenobiotic organic substrates, and synthesis of polyhydroxyalkanoates. In the case of *R. sphaeroides*, four strains have been fully sequenced (strains 2.4.1^T, ATCC 17019, ATCC 17025, and KD131) having two chromosomes, except strain ATCC 17019 with only one, one to five plasmids, and 4.5–4.7 Mb total size (Choudhary et al. 2007; Lim et al. 2009). In a recent comprehensive analysis of genomic islands in 70 selected marine bacterial genomes, Fernández-Gómez et al. found that *R. sphaeroides* ATCC 17025 had the highest ratio (0.12, genomic islands size/genomic size) of all.

Species of *Rhodobacter* separate in two clades (◉ *Fig. 20.2*), both in NJ and ML trees (ML not shown) suggesting a polyphyletic nature for the genus. In one hand, *R. capsulatus* joins *R. aestuarii*, *R. maris*, *R. vinaykumarii*, and *R. viridis* in a group that is more closely related to *Haematobacter* spp. than to the rest of *Rhodobacter* spp. On the other hand, the species *R. azotoformans*, *R. johrii*, *R. megalophilus*, *R. ovatus*, and *R. sphaeroides* form another clade that is closer to *Gemmobacter* species. *R. blasticus* is marginally related to this group and *R. veldkampii* occupies an isolated position among the main lineages.

Defluviimonas

Defluviimonas is a recently described monospecific genus, whose only species; *D. denitrificans* was isolated from a fluidized bed reactor from a recirculating marine aquaculture system (Foesel et al. 2011). *Defluviimonas* presents as Gram-negative rods that may be motile by polar flagella and divide by binary fission. They are chemoorganotrophic and facultatively anaerobic, with a strictly respiratory metabolism. Oxidase and catalase are positive. It is nonpigmented and non-phototrophic. *D. denitrificans* is able to grow anaerobically by denitrification, with N_2 as end product of the process. NaCl is not needed for growth, but it is tolerated up to 5 %. Q10 is the predominant quinone and C18:1 ω7c the major cellular fatty acid. DNA G+C content is 65 mol%.

Gemmobacter

The genus *Gemmobacter*, as currently defined, contains *G. aquatilis*, the type species (Rothe et al. 1987), *G. fontiphilus*

Table 20.3
Species in the *Rhodobacter* group

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
Rhodobacter capsulatus (*Rhodopseudomonas*)	Stagnant freshwater exposed to the light with reduced oxygen, USA and Cuba	PNS	No	Imhoff et al. 1984
R. aestuarii	Microbial mat at mangrove forest, India	PNS	No	Venkata Ramana et al. 2009
R. azotoformans	Photosynthetic sludge from wastewater treatment, Japan	PNS	No	Hiraishi et al. 1997
R. blasticus (*Rhodopseudomonas*)	Stagnant freshwater exposed to the light with reduced oxygen, UK	PNS	No	Kawasaki et al. 1993
R. johrii	Pasteurized rhizosphere soil, India	PNS	No	Girija et al. 2010
R. maris	Coastal marine sediment, India	PNS	No	Venkata Ramana et al. 2008
R. megalophilus	Soil, Himalayas, India	PNS	No	Arunasri et al. 2008
R. ovatus	Sediment of industrially polluted pond, India	PNS	No	Srinivas et al. 2008
R. sphaeroides	Stagnant freshwater exposed to the light with reduced oxygen, the Netherlands and USA	PNS	No	Imhoff et al. 1984
R. veldkampii	Stagnant freshwater exposed to the light with reduced oxygen, Netherlands	PNS	No	Hansen and Imhoff 1985
R. vinaykumarii	Tidal seawater, India	PNS	Yes	Srinivas et al. 2007a
R. viridis	Stream water and mud, India	PNS	No	Shalem Raj et al. 2013
Defluviimonas denitrificans	Biofilter, marine aquaculture recirculating system, Israel	COH	No	Foesel et al. 2011
Gemmobacter aquatilis	Forest pond, USA	COH fac an	No	Rothe et al. 1987
G. aquaticus (*Catellibacterium*)	Water reservoir, China	COH	No	Chen et al. 2013a; Liu et al. 2010
G. caeni (*Catellibacterium*)	Activated sludge, China	COH	No	Chen et al. 2013a; Zheng et al. 2011a
G. changlensis (*Rhodobacter*)	Snow, Himalayas, India	PH	No	Chen et al. 2013a; Anil Kumar et al. 2007
G. fontiphilus	Freshwater spring, Taiwan	COH	No	Chen et al. 2013a
G. nanjingensis (*Catellibacterium*)	Activated sludge, China	COH	No	Chen et al. 2013a; Zhang et al. 2012a
G. nectariphilus (*Catellibacterium*)	Activated sludge, Japan	COH	No	Chen et al. 2013a; Tanaka et al. 2004
G. tilapiae	Freshwater fish culture pond, Taiwan	COH	No	Sheu et al. 2013a
Haematobacter missouriensis	Clinical samples (blood, wounds), USA	COH a	No	Helsel et al. 2007
H. massiliensis (*Rhodobacter*)	Amoebal coculture from nasal swab, France	COH a	No	Helsel et al. 2007; Greub and Raoult 2003
Pararhodobacter aggregans	Biofilter, marine aquaculture recirculating system, Israel	COH a	No	Foesel et al. 2011
Pseudorhodobacter ferrugineus (*Agrobacterium*)	Seawater, Baltic Sea, Germany	COH a	No	Lee et al. 2013a; Chen et al. 2013b; Jung et al. 2012a; Uchino et al. 2002
P. antarcticus	Intertidal sandy sediment, Antarctica	COH a	No	Chen et al. 2013b
P. aquimaris	Seawater, S. Korea	COH a	Yes (+ Mg^{2+})	Jung et al. 2012a
P. wandonensis	Wood falls at South sea, S. Korea	COH a	No	Lee et al. 2013a
Rhodobaca bogoriensis	Water and sediment of alkaline soda lakes, Rift Valley, Kenya	PNS	No, alkaliphilic	Milford et al. 2000

Table 20.3 (continued)

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
R. barguzinensis	Sediment of alkaline soda lake, Russia	PNS	Yes, alkaliphilic	Boldareva et al. 2008
Roseibaca ekhonensis	Hypersaline Ehko Lake, Antarctica	AAP	Yes, alkalitolerant	Labrenz et al. 2009
Roseicitreum antarcticum	Intertidal sandy sediment, Antarctica	AAP	No	Yu et al. 2011
Roseinatronobacter thiooxidans	Siberian steppe soda lakes, Russia	AAP	Yes, alkaliphilic	Sorokin et al. 2000
R. monicus	Hypersaline soda lake, Mono Lake, USA	AAP	No, alkaliphilic	Boldareva et al. 2007
Thioclava pacifica	Coastal sulfidic seep, Papua New Guinea	fac CLA	Yes	Sorokin et al. 2005a

[a]*AAP* aerobic anoxygenic photoheterotroph, *COH* chemoorganoheterotroph, *COH a* chemoorganoheterotroph aerobic, *COH fac an* chemoorganoheterotroph facultative anaerobic, *fac CLA* facultatively chemolithoautotroph, *PH* photoheterotroph, *PNS* purple non-sulfur anoxygenic photoheterotroph

(Chen et al. 2013a), and *G. tilapiae* (Sheu et al. 2013a), and five species transferred from the genus *Catellibacterium* as new combinations (Chen et al. 2013a): *G. aquaticus*, *G. caeni*, *G. changlensis*, *G. nectariphilus*, and *G. nanjingensis*. As it can be observed in *Fig. 20.2*, the phylogenetic relationship within this group supports the combination of all species in one genus, with only some concern about the doubtful position of *G. nectariphilus*. *Gemmobacter* stands as a close relative of the "sphaeroides" clade of *Rhodobacter*.

After several emended descriptions of both *Catellibacterium* and *Gemmobacter* genera, the recent reclassification of Chen et al. (2013a) leaves the following general properties as characteristic of the newly defined *Gemmobacter*: Gram-negative rod-shaped cells that sometimes reproduce by budding and do not form endospores. Most species are nonmotile (except for *G. aquaticus*, with one polar flagellum). Chemoheterotrophs (*G. changlensis* is photoheterotroph able to live as chemoheterotroph in aerobic, dark conditions) use mainly carbohydrates aerobically (*G. aquatilis*, *G. nanjingense*, and *G. fontiphilus*, also anaerobically) as carbon and energy sources. Oxidase and catalase are positive. Main cellular fatty acid is C18:1 ω7c. Common polar lipids are PG, PE, PC, and one unidentified aminolipid. Q10 is the dominant ubiquinone. G+C content of DNA is 61–69.5 mol%. All species of *Gemmobacter* have been isolated from freshwater environments, including activated sludge and aquaculture facilities. They are non-halophilic and some have a low tolerance to NaCl (e.g., the maximum salinity tolerated by *G. fontiphilus* is 0.5 %, while *G. aquaticus* and *G. tilapiae* only tolerate 1 %). They are mesophilic and neutrophilic. One species isolated from snow at the Himalayas, *G. changlensis*, is able to grow at low temperatures (5 °C), but optimum is 25–30 °C.

DNA-DNA hybridization (DDH) experiments have been used to define the position of several close species of the group: type strains of *G. nanjingensis* and *G. caeni* show low levels of DDH (14–19 %, Zhang et al. 2012a); *G. tilapiae* and *G. aquatilis* are related by values of 45–48 % (Sheu et al. 2013a), *G. fontiphilus* and *G. aquatilis* show 46–52 %, *G. fontiphilus* and *G. aquaticus* 52–57 %, and *G. fontiphilus* and *G. caeni* 40–53 % DDH (Chen et al. 2013a).

The draft genome of *G. nectariphilus* DSM 15620^T has 4.52 Mb.

Haematobacter

Haematobacter was described in 2007 from a context atypical for members of *Rhodobacteraceae*, the clinical environment. In fact, it is the only genus being involved to some extension in pathogenicity, as it has been related to opportunistic infections. The genus name is taken from the most common source of isolates, human blood samples, although one species has been obtained from a nasal swab. The genus is defined as Gram negative, nonmotile, nonspore-forming, non-fermentative, and pleomorphic. Cultural properties have been determined as usual for clinical isolates, assuming chemoorganotrophy as the sole metabolic lifestyle; thus no information about other potential metabolisms is available. They are aerobic, oxidase and catalase positive, and not pigmented. DNA G+C content is around 65 mol% and main cellular fatty acids are C18:1 ω7c (50–90 %), C10:0 3OH, C16:1 ω7c, C16:0, C18:0, C18:1 ω9c, and C19:0 cyclo ω8c. There are two named species, *H. missouriensis* and *H. massiliensis*, related by interspecies DDH values of 40–55 % and one unnamed genospecies that shows 63–64 % DDH with *H. massiliensis* and *H. missouriensis* strains (Helsel et al. 2007). Buscher et al. (2010) have isolated a *Haematobacter*-like monoculture from blood of a patient with endocarditis which is only 96.7 % similar in its 16S rRNA gene sequence to the closer *Haematobacter* species. *H. massiliensis* was formerly described as a *Rhodobacter* species

(Greub and Raoult 2003) and was isolated through amoebal coculture; it was lately confirmed as able to resist amoeba grazing.

Aminoglycosides, fluoroquinolones, carbapenems, tetracycline, and chloramphenicol have good activity against *Haematobacter* isolates, while aztreonam and piperacillin have higher MIC values (Helsel et al. 2007).

Pararhodobacter

Pararhodobacter was described for accommodating one isolate obtained from the same environment that rendered *Defluviimonas denitrificans* (Foesel et al. 2011; see above). It was defined by its proximity to *Rhodobacter*, although its position in the complete *Rhodobacteraceae* tree seems closer to the *Rhodobaca* clade (Fig. 20.2). *Pararhodobacter* comprises Gram-negative, nonmotile, nonspore-forming rods that divide by binary fission and are chemoorganotrophic and strictly aerobic. Non-phototrophic. Catalase is positive but oxidase is negative. Similarly to *Defluviimonas denitrificans*, *Pararhodobacter aggregans*, the only species described so far, does not need NaCl for growth but tolerates up to 5 %. It grows with several organic acids and a few amino acids and sugars. It does not reduce nitrates or nitrites. DNA G+C content is 68 mol%. Q10 is the predominant quinone and C18:1 ω7c the main fatty acid.

Pseudorhodobacter

Pseudorhodobacter currently contains four species of chemoorganotrophs, unable to synthetize Bchl *a* and with a strictly respiratory type of metabolism. The type species *P. ferrugineus*, formerly one of the so-called marine *Agrobacterium* species (Rüger and Höfle 1992; Uchino et al. 1997), was reclassified to this new genus after an extensive study of the features common and differential from *Rhodobacter*, its closer neighbor (Uchino et al. 2002). The later descriptions of a second (*P. aquimaris*, Jung et al. 2012a) and a third species (*P. antarcticus*, Chen et al. 2013b) both including emended genus descriptions had modified slightly the original profile of the genus, which currently encompasses Gram-negative rods that may be motile or nonmotile, oxidase and catalase positive, with optimal growth at temperatures from 15 °C to 30 °C and with 1–3 % of NaCl. They contain Q10 as major quinone, C18:1 ω7c as predominant fatty acid, and C10:0 3OH as major hydroxylated fatty acid. Polar lipids include PG, PC, two unidentified aminophospholipids, and one unidentified aminolipid. G+C content is 57–62 mol%. *P. aquimaris* requires NaCl and Mg ions for growth, while the other species of the genus grow optimally in the presence of 1–3 % NaCl and do not have an absolute requirement of marine cations. The draft genome of *P. ferrugineus* DSM 5888^T contains 3.43 Mb. DNA-DNA relationship among *Pseudorhodobacter* species has been determined for *P. ferrugineus* to *P. antarcticus* (56 %; Chen et al. 2013b) and to *P. wandonensis* (12 %; Lee et al. 2013a). The four species form a well-defined clade in the vicinity of *Rhodobacter*, to which they associate, but not as closely as *Gemmobacter* or *Haematobacter* (Fig. 20.2).

Rhodobaca

The genus *Rhodobaca* comprises two species of alkaliphilic purple non-sulfur bacteria that have been isolated from distant soda lakes. The type species *R. bogoriensis* was described upon the study of several strains isolated from water and sediment samples from two African soda lakes located in the Rift Valley, Kenya (Milford et al. 2000), while *R. barguzinensis* corresponds to isolates obtained from a shallow, small soda lake in Siberia, Russia (Boldareva et al. 2008). Both species share a common metabolic profile that includes the ability for photoorganotrophic growth in anaerobic, light conditions, using several organic acids and some sugars, and also as chemoorganotrophs, in aerobiosis with almost the same carbon sources. None of them are able, apparently, of autotrophic growth, as they lack RubisCo gene nor are they able to fix N_2. They do not use nitrate as N source, but ammonium salts, urea, serine, or glutamate. Sulfide is oxidized to sulfur under photoheterotrophic growth conditions. Interestingly, both species are able to reduce tellurite and selenite to Te and Se, respectively, which are accumulated outside the cells. They synthetize Bchl *a* and carotenoids of the spheroidene series, being the major carotenoid demethylspheroidenone in the case of *R. bogoriensis* and demethyl spheroidene for *R. barguzinensis*. Cells of *Rhodobaca* species are motile, coccoid to short rods, and accumulate PHB-resembling granules. Under phototrophic conditions, they present vesicular intracytoplasmic membranes that are scarce and located in the peripheral zone of the cell. *R. barguzinensis* produces abundant slime and, unlike *R. bogoriensis*, divides by unequal fission. Biomass produced in anaerobic, light conditions is yellow brown (*R. bogoriensis*) or beige (*R. barguzinensis*), turning pink in both cases after exposure to oxygen or when cultured in aerobiosis.

Rhodobaca species behave as alkaliphiles: they do not grow at pH 7.0; their range starts at 7.5 and extends up to 10. The optimal pH of the type species is 9.0 while for *R. barguzinensis* is 8.2. They are mesophilic but *R. bogoriensis* has optimal and minimal temperatures for growth notoriously higher than the Siberian species (minimum 30, optimum 39 °C). Both are stimulated by NaCl but only *R. barguzinensis* has an absolute need of Na ion for growth.

Their major cellular fatty acids are C18:1 ω7c accompanied by C16:0, 11-methyl C18:1 ω7c, C16:1 ω7c, and C14:1 ω7c. The DNA G+C content is 58.8–59.8 mol%.

Rhodobaca-like isolates have been identified among the dominant bacteria obtained during the late-flooded phase of ephemeral hypereutrophic playa lakes in the Mojave Desert (Navarro et al. 2009).

Rhodobaca species form a coherent clade that also includes the following three genera, all of them aerobic, bacteriochlorophyll-containing heterotrophs isolated from soda lakes or

hypersaline lakes around the world (Table 20.3). The *Rhodobaca* clade lays at the edge of the *Rhodobacter* group (Fig. 20.2).

Roseibaca

Roseibaca contains a single species, *R. ekhonensis*, isolated from Ehko Lake, a hypersaline, meromictic, heliothermal lake in Antarctica (Labrenz et al. 2009). This species is similar to *Roseinatronobacter*, but alkalitolerant instead of alkaliphilic. It has been characterized more deeply in its chemotaxonomic features: it contains Q10 as predominant quinone, presents DPG, PE, PG, and PC as main polar lipids, meso-diaminopimelic acid in its peptidoglycan, and has a G+C content of 61 mol% in its DNA. The main fatty acids are C18:1 ω7c, C14:1 3OH, C16:1 ω9c, and C18:1 ω9c. *R. ekhonensis* is a strict aerobe and heterotroph, which synthetizes Bchl *a* and carotenoids, producing red to pink colonies. Cells are rod shaped, with a narrower end that suggests budding division, produce fimbria, and sometimes stemlike structures. The cells are often associated forming rosettes. They are nonmotile and accumulate PHB. It has an optimum temperature of 16 °C but grows from 10 °C to 30 °C and has a wide range of pH for growth (from as low as 5.5, in contrast with *Roseinatronobacter* and *Rhodobaca* species) with optimum between 7.0 and 9.0. It has an absolute requirement for Na ion and grows optimally at 2.5 % NaCl. It is positive for oxidase and gives a weak catalase reaction. Growth of *R. ekhonensis* requires thiamine and vitamin B12. Carbon sources used for growth include several organic acids and a large list of carbohydrates.

Roseicitreum

Roseicitreum is another AAP genus, currently containing a sole species, *R. antarcticum*, which was isolated from intertidal, sandy sediments in the coastal area of East Antarctica (Yu et al. 2011). Among its chemotaxonomic features, it contains the common quinone system of the family (Q10); its polar lipid composition includes PG, PE, PC, and one unidentified aminolipid; and its major fatty acids are C18:1 ω7c, C10:0 3OH, C16:0, C17:0 cyclo, and C19:0 cyclo ω8c. The DNA G+C content is 63 mol%. *R. antarcticum* cells are nonmotile, lemon shaped (hence the generic name), and accumulate PHB. They produce a small quantity of peripheral vesicles, similar to *Rhodobaca* cells. Colonies are pink to red. Bchl *a* is produced but *Roseicitreum* is unable to growth as photoautotroph or as anaerobic photoheterotroph. It is positive for oxidase and catalase tests. It uses carbohydrates and some organic acids as carbon and energy sources for growth. Preferred temperatures are in the mesophilic range (25–27 °C), but it is able to grow from 0 °C to 33 °C. Although Na ions are not required, growth is optimum at 7–8 % and occurs up to 15 % NaCl. The pH range is 5–9.5, with an optimum at 7.0. Thus, it is psychrotolerant and non-alkaliphilic.

Roseinatronobacter

Roseinatronobacter is the eldest of these *Rhodobaca*-associated soda lake AAPs. It was described by Sorokin et al. (2000) after the study of one pink pigmented, alkaliphilic strain isolated from a microbial mat sample obtained in a steppe soda lake in Siberia. The strain was able not only to synthetize Bchl *a* but also to oxidize thiosulfate to sulfate, using it as additional electron donor during heterotrophic growth. The species *R. thiooxidans* is also able to oxidize other sulfur compounds (sulfide, elemental sulfur, sulfite) producing sulfate. A second species was described in 2007, *R. monicus*, based on two strains isolated from Mono Lake, California (Boldareva et al. 2007). The cells of *R. thiooxidans* are elongated while *R. monicus* has oval cells, but both are nonmotile, divide by binary fission, and accumulate PHB. They are strictly aerobic and heterotrophic and able to oxidize thiosulfate, sulfide, polysulfide, and elemental sulfur. They synthetize Bchl *a* and carotenoids of the spheroidene series. Light inhibits synthesis of bacteriochlorophyll, and in the case of *R. thiooxidans*, full oxygen tension also inhibits bacteriochlorophyll and pigmentation. The highest pigment content is obtained in media rich in organic nitrogen compounds, micro-oxic, and in the dark. Both species are mesophilic, slightly halophilic (but do not have a strict Na ion requirement), and obligately alkaliphilic, with a pH range of 8.5 to 10.4 and optima at 9.0–10.0. They contain C18:1 ω7c as major cellular fatty acid, accompanied by C18:2 ω6,9c, 11-methyl C18:1 ω7c, C16:0, C14:1 ω7c, and C16:1 ω7c. G+C content is 59–61.5 mol% (Boldareva et al. 2007). The two species described so far are related by levels of DDH of 22–25 %. It is noteworthy that, based on 16S rRNA gene comparison, *R. monicus* is closer to *Rhodobaca* species than to *R. thiooxidans* (Fig. 20.2).

Roseinatronobacter-like isolates and phylotypes have been detected in soda lakes of other Russian regions (Gorlenko et al. 2010) and European countries (Borsodi et al. 2013).

Thioclava

The genus *Thioclava* does not show a definite affiliation to the *Rhodobacter* group when 16S rRNA gene sequences are analyzed through NJ method (Figs. 20.1 and 20.2) but relates to *Tropicimonas* species. However, its link to the *Rhodobacter* group is clear in ML analysis (not shown). Former phylogenetic analysis showed *Thioclava* grouped with two *Rhodobacter* species, *R. maris* and *R. aestuarii* (LTP111). Thus, *Thioclava* has been included in the *Rhodobacter* group. The genus is monospecific (A second species of the genus *Thioclava*, *T. dalianensis*, has been recently proposed for an isolate unable to grow chemolithoautotrophically on inorganic sulfur compounds. Its description is accompanied by an emended description of the genus *Thioclava* (Zhang et al. 2013)) since its description (Sorokin et al. 2005a) and contains *T. pacifica*, a species of facultative autotrophic, sulfur-oxidizing marine bacteria that was isolated from a coastal sulfidic hydrothermal area in Papua New Guinea. *T. pacifica* forms cells with widely different

Table 20.4
Species in the *Rhodovulum* group

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
Rhodovulum sulfidophilum	Mud from intertidal flats, the Netherlands	PNS	No	Hiraishi and Ueda 1994; Hansen and Veldkamp 1973
R. adriaticum	Coastal lake, Eastern Adriatic Sea	PNS	Yes	Hiraishi and Ueda 1994
R. bhavnagarense	Sediment of pink pond, India	PNS	Yes	Srinivas et al. 2012
R. euryhalinum	Shallow saline waters, Russia	PNS	Yes	Hiraishi and Ueda 1994; Kompantseva 1985
R. imhoffii	Aquaculture pond water, India	PNS	Yes (low)	Srinivas et al. 2007b
R. iodosum	Coastal sediment, North Sea, Germany	PNS	Yes	Straub et al. 1999
R. kholense	Mangrove mud, India	PNS	Yes	Anil Kumar et al. 2008
R. lacipunicei	Saline purple pond water, India	PNS	No	Chakravarthy et al. 2009
R. marinum	Seawater, India	PNS	Yes (low)	Srinivas et al. 2006
R. phaeolacus	Sediment of brown pond, India	PNS	No	Lakshmi et al. 2011
R. robiginosum	Coastal sediment, North Sea, Germany	PNS	Yes	Straub et al. 1999
R. steppense	Steppe soda lakes, Siberia, Russia	PNS	Yes	Kompantseva et al. 2010
R. strictum	Tidal and seawater pools, coast of Japan	PNS	Yes	Hiraishi and Ueda 1995
R. tesquicola	Steppe soda lakes, Siberia, Russia	PNS	Yes	Kompantseva et al. 2012
R. visakhapatnamense	Tidal water, Bay of Bengal, India	PNS	No	Srinivas et al. 2007c
Albidovulum inexpectatum	Marine hot spring, Azores	COH	Yes, slightly thermophilic	Albuquerque et al. 2002
A. xiamenense	Terrestrial hot spring, Fujian, China	COH	Yes, slightly thermophilic	Yin et al. 2012
Jhaorihella thermophila	Coastal hot spring, Taiwan	COH	Yes	Rekha et al. 2011

[a]*COH* chemoorganoheterotroph, *PNS* purple non-sulfur anoxygenic photoheterotroph

morphologies depending of the media and growth conditions, from long filaments with swollen ends to short rods. This later morphology may exhibit motility by a polar flagellum. Oxidase and catalase are positive. *Thioclava* is mesophilic, neutrophilic, and requires NaCl for growth (1–8 %, optimum 3–4 %). It does not synthetize Bchl or carotenoids and is a strictly aerobic chemotroph, able to grow chemoautotrophically by thiosulfate (or sulfide) oxidation (CO_2 is then fixed through Calvin cycle) or chemoheterotrophically, using organic acids, carbohydrates, or amino acids as carbon and energy sources. It requires thiamine and biotin. It is also able to oxidize thiosulfate in mixotrophic conditions (acetate + thiosulfate), producing sulfate as terminal by-product. It does not ferment glucose and does not denitrify. The RubisCo produced by *Thioclava* is the green form of type I and its gene sequence (cbbL gene) relates specifically (but not very closely) to the corresponding genes of *Rhodobacter* spp., *Rhodovulum* spp., and *Hydrogenophilus thermoluteus* (a betaproteobacterium).

T. pacifica presents Q10 as sole quinone. The G+C content of its DNA is 63 mol%.

The *Rhodovulum* Group

Members of this group include the marine purple non-sulfur photosynthetic genus *Rhodovulum* that currently contains 15 species, along with the chemoorganotrophic, unpigmented, and slightly thermophilic species of *Albidovulum* and *Jhaorihella* (*Table 20.4*). *Albidovulum* is closely related to one of the subclades of *Rhodovulum* (*R. euryhalinum*, *R. steppense*, *R. strictum*, *R. tesquicola*), while *Jhaorihella* is marginal to the whole group (*Fig. 20.3*). Altogether, they form a clade that relates with the combined *Roseobacter* plus *Paracoccus* plus *Rhodobacter* groups, as can be seen in *Figs. 20.1* and *20.3*.

Rhodovulum

Rhodovulum is the largest genus in this group and the only one behaving as purple non-sulfur photosynthetic bacteria. The genus was created to accommodate three former *Rhodobacter* species of marine origin, with salt requirements for optimal

growth. Not only salt requirement but also sulfide tolerance, final product from sulfide oxidation, and polar lipid composition allow the differentiation of the species *R. sulfidophilum*, *R. adriaticum*, and *R. euryhalinum* from the freshwater, true *Rhodobacter* species (Hiraishi and Ueda 1994). Only 1 year later, the same authors described a fourth species, *R. strictum* (Hiraishi and Ueda 1995), which was followed by the recognition of *R. iodosum* and *R. robiginosum*, two ferrous iron-oxidizing new species (Straub et al. 1999). These six species were included in the excellent chapter on phototrophic Alphaproteobacteria (Imhoff 2006), where a full account for taxonomy, phylogeny, habitats, methods of isolation, culture and preservation, physiology, and metabolism of the whole group is given. The reader is addressed to this chapter for wider treatment of genus *Rhodovulum*, in all these aspects. Its general properties are summarized here, after including the nine *Rhodovulum* species described later, with special emphasis in those features that differed from the commonly accepted for the genus.

Rhodovulum includes purple non-sulfur anoxygenic photoheterotrophs that inhabit marine or saline shallow water masses or their sediments and form oval- to rod-shaped cells that are mainly nonmotile (when motile, they exhibit polar flagella). Cellular size is 0.5–0.9 × 0.9–3.8 μm. They are typical Gram-negative cells and display intracytoplasmic membrane invaginations of the vesicular type when grown in phototrophic regime. Division takes place by binary fission. They synthetize Bchl *a* and carotenoids of the spheroidene series (spheroidene, spheroidenone, demethylspheroidene, hydroxyspheroidene, neurosporene) in different proportions. They are mesophilic, with optimum temperatures between 25 °C and 35 °C, and slightly halophilic, growing best at salinities 1–7.5 %. Most of them require NaCl for growth. Two species *R. steppense* and *R. tesquicola*, isolated from shallow soda lakes, are alkaliphilic, with optimum growth at pH 8.5–9.0, and unable to grow at pH 7.0. They are closely related to *R. strictum*, also slightly alkaliphilic, to which they show moderate DDH values (40–53 %) (Kompantseva et al. 2010, 2012).

Preferred metabolism is photoorgano- or photolitho-heterotrophic, in anaerobiosis with light, (sulfide/thiosulfate) and organic carbon sources, but most can also thrive as chemoorganoheterotrophs, with oxygen and organic carbon in the dark (*R. euryhalinum*, *R. adriaticum*, *R. phaeolacus*, and *R. bhavnagarense* are exceptions, as they require sulfide or thiosulfate in addition to the organic compound). Some can also develop as photo- or chemo-lithoautotrophs, using reduced sulfur compounds (sulfide, thiosulfate, and sulfur), hydrogen, or ferrous iron as electron donors and bicarbonate as carbon source. Sulfur compounds are oxidized to sulfate (with a transitory deposition of extracellular elemental sulfur during sulfide oxidation in *R. steppense* and *R. tesquicola*, Kompantseva et al. 2010, 2012). Several species are unable to grow autotrophically: *R. marinum*, *R. lacipunicei*, *R. phaeolacus*, *and R. bhavnagarense*. None of the species described so far is able to grow fermentatively.

The preferred organic carbon sources for growth are organic acids such as pyruvate, lactate, Krebs cycle intermediates, and short-chain fatty acids. Some amino acids and sugars, as well as formate, may be used by some species. Vitamin requirement is a general rule for all species, which are variable for the given combination they need.

Species of *Rhodovulum* have C18:1 ω7c as major cellular fatty acids, with minor amount of C18:0, C16:0, 11-methyl C18:1 ω7c, and C10:0 3OH. The polar lipid composition includes PE, PG, several sulfolipids (including sulfoquinovosil diglyceride, SQD), and other lipids, but excludes PC. Although not regularly investigated, the two species that have been analyzed for this trait, *R. bhavnagarense* (Srinivas et al. 2012) and *R. tesquicola* (Kompantseva et al. 2012), confirm this general behavior. The major ubiquinone is Q10. The DNA G+C content, reported to be 62–69 mol% in the genus description (Hiraishi and Ueda 1994), has to be lowered, as *R. imhoffii* shows 58 mol% (Srinivas et al. 2007a) and *R. visakhapatnamensis* has 61.2 mol% (Srinivas et al. 2007b).

Recently, Khatri et al. (2012) have reported the 4.8 Mb draft genome of *Rhodovulum* sp. strain PH10 isolated from a mangrove soil sample. It contains 3 copies of the rRNA genes, 47 aminoacyl tRNA synthetase genes, and a DNA G+C content of 69.7 mol%.

Rhodovulum species (*R. sulfidophilum* in particular) have been investigated for the interest of some of its activities: hydrogen production (Cai and Wang 2013), PHB production (Cai et al. 2012), or anti-viral RNA aptamer secretion for aquaculture improvement (Hwang et al. 2012; Suzuki et al. 2010).

Albidovulum

The genus *Albidovulum* was described by Albuquerque et al. (2002) as a non-photosynthetic, thermophilic relative of *Rhodovulum*, with a single species, *A. inexpectatum*. Recently, a second species *A. xiamenense* (Yin et al. 2012) has been described and corresponds also to an inhabitant of a coastal hot spring. Similarity in 16S rRNA gene sequence between both species is around 96 %. Main characteristics of the genus include the absence of Bchl *a* and carotenoid production, the chemoorganotrophic, strict aerobic nature of its metabolism, and the slightly thermophilic character, with temperature optimum for growth at 50–58 °C (ranges: 28–65 °C for *A. xiamenense*, 35–60 °C for *A. inexpectatum*). Their cells are rod shaped and only *A. xiamenense* shows polar flagella and motility, while *A. inexpectatum* is nonmotile. They are positive for oxidase and catalase tests, reduce nitrates to nitrites (but are unable to grow with nitrate in anaerobiosis), do not ferment carbohydrates, and use a variety of organic compounds as carbon sources (sugars, polyols, amino acids, and organic acids) but are unable to fix CO_2. *A. inexpectatum* is facultatively chemolithoorganotrophic on reduced sulfur compounds, as it is able to oxidize thiosulfate to sulfate, deriving energy for increased growth, in the presence of organic carbon sources. Thiosulfate oxidation was not tested on *A. xiamenense*.

Table 20.5
Species in the *Amaricoccus* group

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
Amaricoccus kaplicensis	Laboratory-scale sequence batch reactor, Czech Republic	COH a	No	Maszenan et al. 1997
A. macauensis	Activated sludge plant domestic waste, Macau	COH a	No	Maszenan et al. 1997
A. tamworthensis	Industrial plant treating malting waste, Australia	COH a	No	Maszenan et al. 1997
A. veronensis	Activated sludge plant, domestic waste, Italy	COH a	No	Maszenan et al. 1997
Albimonas donghaensis	Sea water, East Sea, S. Korea	COH a	No	Lim et al. 2008
Oceanicella actignis	Shallow marine hot spring, Azores, Portugal	COH	Yes, slightly thermophilic	Albuquerque et al. 2012
Rubribacterium polymorphum	Soda lake sediment, Siberia, Russia	PNS	Yes, alkaliphilic	Boldareva et al. 2009
Rubrimonas cliftonensis	Saline lake, Australia	AAP	yes	Suzuki et al. 1999a
R. shengliensis	Oil-polluted saline soil, China	AAP	No	Cai et al. 2011

[a]*AAP* aerobic anoxygenic photoheterotroph, *COH* chemoorganoheterotroph, *COH a* chemoorganoheterotroph aerobic, *PNS* purple non-sulfur anoxygenic photoheterotroph

Jhaoriella

Jhaoriella thermophila is the only recognized species of the genus *Jhaoriella* (Rekha et al. 2011). In spite of the specific name and isolation source, it is not a true thermophile, as it grows optimally at 37–45 °C and is able to grow from 25 °C up to 55 °C. The genus was defined on the basis of its deep lineage within the *Rhodobacteraceae*. It was found distantly related to some members of the *Roseobacter* clade and was compared to them in the description. It is a rod-shaped, unpigmented, nonmotile bacterium which shows Q10 as major respiratory quinone; PC, PG, and PE as major identified polar lipids; and cellular fatty acids dominated, as usual, by C18:1 ω7c. It requires seawater or combined sea salts for growth (2–6 %, with optimum at 3–4 %) and is obligately aerobic, unable to grow with nitrate in anaerobic conditions. It is positive for oxidase and catalase tests and PHB accumulation. The only known strain was isolated from a coastal hot spring and has a DNA G+C content of 65 mol%.

The *Amaricoccus* Group

In this group, five genera are recovered as part of a clade that always represents the deepest rooted and more distantly related clade to the rest of the family (*sensu stricto*) (*Fig. 20.3*). It includes *Amaricoccus* (with four species), *Albimonas* (one species), *Oceanicella* (one species), *Rubribacterium* (one species), and *Rubrimonas* (two species). Again, the group contains a purple non-sulfur photosynthetic member (*Rubribacterium*) and also aerobic anoxygenic phototrophs (*Rubrimonas* sp.), along with chemoorganotrophic, non-phototrophic members (*Table 20.5*).

Amaricoccus

The four species that constitute the genus *Amaricoccus* (Maszenan et al. 1997) were isolated after micromanipulation from activate sludge biomass obtained in geographically distant wastewater treatment plants. The aim of the study by Maszenan and coworkers was the isolation and characterization of the so-called G-bacteria, a morphotype common to the sludge and particularly difficult to cultivate. This morphotype corresponded to Gram-negative cocci arranged in tetrads and sheets. After trying a wide range of media, the isolates could be grown only in medium GS (Williams and Unz 1985) that contains (per liter) 0.15 g glucose, 0.50 g $(NH_4)_2SO_4$, 0.10 g $CaCO_3$, 0.10 g $Ca(NO_3)_2$, 0.05 g KCl, 0.05 g K_2HPO_4, 0.05 g $MgSO_4{\cdot}7H_2O$, 0.187 g $Na_2S{\cdot}9H_2O$, 15 g bacteriological agar (Difco), and 1.0 ml of a 10x vitamin stock solution. The new genus was described as large, Gram-negative, nonspore-forming cocci (mean cell diameter, 1.3–1.8 μm), usually arranged in tetrads. The cells were not motile and did not accumulate polyphosphate granules, either when growing in the sludge or in axenic lab cultures. They are aerobic chemoheterotrophs that use a large variety of carbohydrates and organic acids as substrates. They are oxidase positive, mesophilic (20–37 °C), and grow at pH between 5.5 and 9.0. The DNA G+C content is 51–63 mol%. According to Falvo et al. (2001), *A. kaplicensis* is able to store acetate as PHB at high rates. In a recent study, Albuquerque et al. (2012) report that this species is able to grow without NaCl addition and contains PE, PC, PG, and two unidentified aminolipids and one phospholipid as major polar lipids. These authors also report ubiquinone 10 as dominant quinone system, although its detection was difficult.

The four species can be distinguished by the differential use of several carbon sources, ability to reduce nitrate to nitrite,

some enzymatic activities (as determined in API ZYM strips), and the G+C content. *Amaricoccus kaplicensis* type species was isolated in Czech Republic, while *A. tamworthensis*, *A. veronensis*, and *A. macauensis* were isolated from Australian, Italian, and Macau wastewater samples, respectively. Up to date, activated sludge seems to be the only habitat of species of this genus that have been studied in different wastewater treatment systems by using in situ detection with FISH (Maszenan et al. 2000). They occur in large numbers not only in enhanced biological phosphate removal (EPBR) treatment plants (in which their abundance was suspicious of a detrimental role by competing phosphate removing bacteria) but also in conventional plants. They were particularly abundant in aerobic-anaerobic systems, although no evidence of that detrimental role has been found (Seviour et al. 2000). The effect of periodic acetate feeding in sequencing batch reactor on substrate uptake and storage rates by a pure culture of *A. kaplicensis* was investigated by Aulenta et al. (2003), who found that different cycle lengths resulted in different lengths of famine periods and different specific PHB contents at the end of each cycle. Moreover, as cycle length increased, flocculation and settleability of the culture significantly worsened, whereas the observed yield increased, showing that substrate removal was mainly due to oxidation and storage, whereas the growth played a minor role. Thus, a longer famine period caused a higher capacity of cells to answer quickly to sudden change of substrate availability.

Albimonas

Albimonas is a genus marginally related to the *Amaricoccus* group, from which it separates in some of the phylogenetic analysis performed. It is the deepest branch in the NJ analysis (◉ *Fig. 20.3*) but relates closely with the *Rubribacterium-Rubrimonas* subclade when ML is used for tree reconstruction. *Albimonas* contains one species, *A. donghaensis* (Lim et al. 2008), isolated in Marine Agar from seawater of the East Sea. It is an aerobic chemoorganotroph that does not synthetizes Bchl *a* and is not pigmented. Cells are short, nonmotile rods. It is positive for oxidase and catalase and negative for nitrate reduction to nitrite. Q10 is the major isoprenoid quinone, C18:1 ω7c and its 11-methyl derivative are the major cellular fatty acids, and PG, DPG, PE, and PC the major polar lipids. The DNA G+C molar content of the type strain is 72.0.

A. donghaensis grows as a mesophile, between 10 °C and 38 °C, with an optimum at 28–30 °C and pH 6.0–9.5 (optimum 7–8). It does not require sodium ions for growth, which is possible at salinities of 0–14 % (optimum 2–5 %).

Oceanicella

The genus *Oceanicella* (Albuquerque et al. 2012) has been recently described as the closer relative to *Amaricoccus*, with which it shares little resemblance in metabolic or ecological features. The pairwise similarities between *Amaricoccus* and *Oceanicella* 16S rRNA gene sequences are 93–95 %, and the relationship is supported by a bootstrap value of 96 % in the original phylogenetic analysis reported in *Oceanicella* description. *Oceanicella actignis*, the only species of the genus, is a slightly thermophilic and halophilic bacterium that forms pleomorphic, nonmotile cells. It was isolated from a shallow marine hot spring on a beach of Azores Islands. The genus is defined as organotrophic, strict aerobic, and oxidase and catalase positive. The major cellular fatty acids are C18:1 ω7c and 11-methyl C18:1 ω7c, while the major polar lipids are PC, PG, and one aminolipid. Q10 is the major respiratory quinone and the DNA G+C content of the type species is 71.2 mol%.

The temperature range for growth of *O. actignis* is 25–57.5 °C, with an optimum at 50 °C. Optimum pH is 7.5–8.0 and optimal salinity is 2–5 % (range, 1–9 %). The species does not grow without added NaCl. It reduces nitrate to nitrite, degrades gelatin and DNA, but not casein or starch. A few carbohydrates (glycerol, mannitol), several organic acids (2-oxoglutarate, lactate, acetate, pyruvate, succinate, malate, and fumarate), and several amino acids (aspartate, glutamate, alanine, asparagine, serine, leucine, proline, glutamine, phenylalanine, and isoleucine) are assimilated. *O. actignis* grows as unpigmented colonies in Degryse medium 162 (Degryse et al. 1978), containing 0.25 % yeast extract, 0.25 % tryptone, and 2 % NaCl.

Rubribacterium

In contrast to the previous genera, *Rubribacterium* and *Rubrimonas*, the remaining members of *Amaricoccus* group are phototrophic. Namely, *Rubribacterium* was described as purple non-sulfur anoxygenic photosynthetic bacterium, while *Rubrimonas* is an aerobic anoxygenic photoheterotroph (AAP) that contains two species of pink pigmented bacteria. The genus *Rubribacterium* (Boldareva et al. 2009) was established to account for a new alkaliphilic, phototrophic isolate from a soda lake in Siberia, described as a purple non-sulfur bacterium that lacks RuBisCo and grows better in aerobic than anaerobic conditions under illumination. The key difference between *Rubribacterium* and *Rubrimonas* is the ability of the former to grow photo-heterotrophically and form vesicular type of photosynthetic membranes in anaerobic conditions. *Rubribacterium polymorphum* cells are oval in young cultures, but become polymorphic with age and the accumulation of large storage granules interpreted as PHB. They may be motile by polar flagella. Carotenoids of both spheroidene and spirilloxanthin groups are formed. C18:1 ω7c is the major cellular fatty acid, accompanied by its 11-methyl derivative, C18:0, and C16:0. DNA G+C base content is 70 mol%, in the same range that of *Rubrimonas*.

Rubribacterium is a facultative anaerobe, able to grow (poorly) in anaerobiosis as photoheterotroph, with glucose, fructose, sucrose, pyruvate, malate, casein hydrolysate, yeast extract, and soyotone. These substrates also support good chemoheterotrophic growth in aerobic conditions in the dark. Nitrate is reduced to nitrite, but this activity does not support

growth. Ammonium salts are used as nitrogen source. *Nif* genes and RuBisCo activity are absent.

Rubribacterium polymorphum is a moderate alkaliphile, unable to grow at neutral pH, and shows optimal growth at 8.5–9.5. NaCl is required (0.5–4.0 %, optimum 1.0 %). Good growth occurs between temperatures of 20 °C and 35 °C.

Rubrimonas

Rubrimonas cliftonensis, the type species of the genus *Rubrimonas*, was isolated from a saline lake in Australia (Suzuki et al. 1999a). The genus was defined as aerobic and chemoheterotrophic, with the ability to synthesize Bchl *a* under aerobic conditions. Carotenoid pigments are also produced. Chemotaxonomic features include the presence of Q10 as ubiquinone system, C18:1 ω7c as dominant cellular fatty acid, and a 74–75 mol% G+C content of DNA. This later value has to be extended to a lower limit of 68 mol% with the recent recognition of a second species *R. shengliensis* (Cai et al. 2011), isolated from oil-contaminated saline soil. These authors reported data on the polar lipid composition of both species, which include PG, PC, and unidentified lipids and aminolipids. They are mesophilic (optimum 27–30 °C, but *R. shengliensis* has a wide temperature range for growth, 4–50 °C), neutrophilic (optimum pH 7–8), and slightly halophilic, with *R. cliftonensis* requiring NaCl for growth (range 0.5–7.5 %) and *R. shengliensis* being able to grow without NaCl, but growing optimally at 1 % (range 0–10 %). The cells of *R. cliftonensis* are polarly flagellated rods while *R. shengliensis* presents oval, nonmotile cells. Both form pink colonies, are positive for catalase, but differ in oxidase, nitrate reduction (see Cai et al., ❯ *Table 20.1*), gelatinase and amylase production, and ability to produce acid from several carbohydrates. Species of *Rubrimonas* show 95.7 % similarity on their 16S rRNA gene sequences, but also display a moderate similarity to *Rubribacterium polymorphum* sequence, the third member of the subgroup.

The *Paracoccus* Group

The clade that contains the forty species of the genus *Paracoccus* also groups the facultative methylotroph genus *Methylarcula*, with two species (❯ *Table 20.6*). They have the *Rhodobacter* group as closest relative (❯ *Fig. 20.4*). The close phylogenetic relationship between *Rhodobacter* and *Paracoccus* groups is also revealed by phylogenomic analysis of signature proteins, as reported by Gupta and Mok (2007) who suggest that both *Rhodobacter* and *Paracoccus* form a distinct clade that appears as the outgroup of other *Rhodobacterales* species.

In contrast with the previous groups in this chapter, *Paracoccus* encompasses strictly chemotrophic species, with no example of phototrophic activity, either aerobic or anaerobic.

Paracoccus

The genus *Paracoccus* was the subject of a thorough chapter by Kelly and colleagues (Kelly et al. 2006a) in the 3rd edition of The Prokaryotes, and the interested reader is addressed to this chapter for information on all the general aspects of this genus and the summary of the fourteen species included at that time. By then, a publication note came out (Kelly et al. 2006b) to clarify the taxonomic status of the culture collection strains of two key species of *Paracoccus*, the type species *P. denitrificans* and *P. pantotrophus*, which had been used for many years in fundamental biochemical studies and as reference species in the identification of new isolates of *Paracoccus*. Here, the complementary information on the 26 species described thereafter is developed, along with the emendation of the genus (Liu et al. 2008), underlying those aspects that complement, extend, or modify the previous information.

Paracocci have Gram-negative cells described as coccoid, coccobacilli, or short rods, usually nonmotile. When motile, they may present polar flagella (*P. homiensis*, *P. oceanense*, and *P. versutus*) or peritrichous (*P. carotinifaciens*). *P. chinensis* and *P. rhizosphaerae* are described as motile without reference to the flagellar arrangement. The remaining 34 species are nonmotile. They divide by binary fission and occur as single cells, pairs, short chains, or irregular clusters. They commonly contain PHB as carbon reserve material. Several species display pigmented colonies, most commonly orange to red, due to the production of carotenoids.

They are catalase and oxidase positive and contain ubiquinone 10 (Q10) as sole or dominant quinone, sometimes accompanied by small amounts (<5 %) of Q9 and Q11. Only one species differs from this pattern, *P. yeei*, as it has been reported to contain Q8 as major quinone (Daneshvar et al. 2003).

Major cellular fatty acids (FAs) are C18:1 ω7c, C18:0, 19:0 cyclo ω7c, and 16:0, and among the hydroxylated, C10:0 3OH is commonly present. C18:1 ω7c represents usually more than 70 % of the total FAs detected. Exceptions to this behavior are *P. caeni* (54 %, Lee et al. 2011a), *P. halophilus* (60 %, Liu et al. 2008), and *P. oceanense* with no C18:1 ω7c detectable (instead, it contains 39 % of C18:1 ω6c, Fu et al. 2011). C10:0 3OH is undetectable in *P. fistulariae* and *P. sulfuroxidans*, both containing other hydroxylated FAs (Kim et al. 2010a; Liu et al. 2006), and in *P. oceanense*, with no hydroxylated FAs reported (Fu et al. 2011).

Information on polar lipid (PL) composition is scarcer than other chemotaxonomic features. Kelly et al. (2006a) do not report PL composition of the fourteen *Paracoccus* species available, and the pattern is included only in part of the more recent descriptions. The information briefed here comes from the analysis of less than a half of the currently recognized species. Major PL recorded in all species reported are phosphatidyl glycerol (PG) and phosphatidyl choline (PC), while diphosphatidyl glycerol (DPG) is detected in most, but not all, the species tested. Phosphatidyl ethanolamine (PE) is present in *P. huijuniae*, *P. kondratievae*, and *P. rhizosphaerae*, out of the 15 species compositions known. One sphingoglycolipid is detected

Table 20.6
Species in the *Paracoccus* group

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
Paracoccus denitrificans	Soil, sewage, manure, mud	fac CLA	No	Rainey et al. 1999; Ludwig et al. 1993; Nokhal and Schlegel 1983
P. aestuarii	Tidal flat sediment, S. Korea	COH	No	Roh et al. 2009
P. alcaliphilus	Soil	COH	No	Urakami et al. 1989
P. alkenifer	Biofilters treating waste gas from animal rendering plant	COH	No	Lipski et al. 1998
P. aminophilus	Soil, Japan	COH	No	Urakami et al. 1990
P. aminovorans	Soil, Japan	COH	No	Urakami et al. 1990
P. beibuensis	Seawater, S. Korea	COH	No	Zheng et al. 2011b
P. bengalensis	Rhizosphere of leguminose (*Clitoria*), India	fac CLA	No	Ghosh et al. 2006
P. caeni	Sludge from disposal plant, S. Korea	COH	No	Lee et al. 2011a
P. carotinifaciens	Soil, Japan	COH	No	Tsubokura et al. 1999
P. chinensis	Sediment from eutrophic water reservoir, China	COH	No	Li et al. 2009
P. ferrooxidans	Denitrifying fluidized bed bioreactor, the Netherlands	fac CLA	No	Kumaraswamy et al. 2006
P. fistulariae	Intestine of marine fish (*Fistularia*), S. Korea	COH	Yes (or Mg^{2+} at 0 % NaCl)	Kim et al. 2010a
P. haeundaensis	Seawater, S. Korea	COH	No	Lee et al. 2004
P. halophilus	Marine sediment, South China Sea, China	COH	Yes	Liu et al. 2008
P. homiensis	Sea sand, S. Korea	COH	No	Kim et al. 2006a
P. huijuniae	Activated sludge of a wastewater biotreatment facility, China	COH	No	Sun et al. 2013
P. isoporae	Reef-building coral (*Isopora*), Taiwan	COH	No	Chen et al. 2011a
P. kocurii	Activated sludge system for wastewater of semiconductor manufacturing process, Japan	COH	No	Ohara et al. 1990
P. kondratievae	Maize rhizosphere, Russia	COH	No	Doronina et al. 2002; Doronina and Trotsenko 2000
P. koreensis	Anaerobic sludge blanket reactor, S. Korea	COH	No	La et al. 2005
P. limosus	Activated sludge, sewage treatment plant, S. Korea	COH	No	Lee and Lee 2013
P. marcusii	Contaminant of agar plate	COH	No	Harker et al. 1998
P. marinus	Coastal seawater, Japan	COH	Yes	Khan et al. 2008
P. methylutens	Ground water contaminated with dichloromethane, Switzerland	COH	No	Doronina et al. 1998
P. niistensis	Forest soil, India	COH	No	Dastager et al. 2011
P. oceanense	Seawater, West Pacific Ocean	COH	Yes	Fu et al. 2011
P. pantotrophus (*Thiosphaera*)	Denitrifying, desulfurizer effluent treatment system, the Netherlands; soil (GB)	fac CLA	No	Rainey et al. 1999; Robertson and Kuenen 1983
P. rhizosphaerae	Rhizosphere of plant (*Crossostephium*), Taiwan	COH	Yes	Kämpfer et al. 2012
P. saliphilus	Saline soil near Ebinur Lake, China	COH	Yes (optimum 8 % NaCl)	Wang et al. 2009a
P. seriniphilus	Bryozoan (*Bugula*), North Sea, Germany	COH	Yes	Pukall et al. 2003
P. solventivorans	Soil at natural gas company site, Germany	COH	No	Lipski et al. 1998; Siller et al. 1996
P. sphaerophysae	Root nodules of leguminose (*Sphaerophysa*), China	COH	No	Deng et al. 2011

Table 20.6 (continued)

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
P. stylophorae	Reef-building coral (*Stylophora*), Taiwan	COH	No	Sheu et al. 2011
P. sulfuroxidans	Activated sludge of a wastewater treatment bioreactor, China	fac CLA	No	Liu et al. 2006
P. thiocyanatus	Activated sludge with thiocyanate, Japan	fac CLA	No	Katayama et al. 1995
P. tibetensis	Permafrost, Tibet plateau, China	COH	No, alkaliphilic	Zhu et al. 2013
P. versutus	Soil, USA	fac CLA	No	Katayama et al. 1995; Harrison 1983
P. yeei	Clinical samples (wounds, bile, blood, eye), USA, Canada	COH	No	Daneshvar et al. 2003
P. zeaxanthinifaciens	Seaweed, Red Sea	COH	No	Berry et al. 2003
Methylarcula marina	Estuary seawater, Azov Sea, Russia	fac M	Yes	Doronina et al. 2000
M. terricola	Coastal salty soil, Black Sea, Russia	fac M	Yes	Doronina et al. 2000

[a]*COH* chemoorganoheterotroph, *fac CLA* facultatively chemolithoautotroph, *fac M* facultatively methylotroph

in *P. oceanense* (Fu et al. 2011) and several unidentified amino-phospho- and glycolipids are present in some species.

The molar % C+G content is 63–71 in Kelly et al. (2006) and Spanning et al. (2005), but several species with lower values have been described afterwards: *P. caeni*, 58.7 (Lee et al. 2011a); *P. oceanense*, 59.5 (Fu et al. 2011); *P. saliphilus*, 60.3 (Wang et al. 2009a); *P. sulfuroxidans*, 61.3 (Liu et al. 2006); *P. yeei*, 62 (Daneshvar et al. 2003); and *P. aestuarii*, 62.0 (Roh et al. 2009). Thus, the range of molar % G+C extends now from 58 to 71.

Other chemotaxonomic features, such as diamino acid on peptidoglycan or polyamine pattern, have been determined only for few species. *P. rhizosphaerae* (Kämpfer et al. 2012) contains *m*-diaminopimelic acid as diagnostic diamino acid and spermidine (SPE) and putrescine (PUT) as major polyamines, with cadaverine (CAD) and diaminopropane (Dap) as minor components. Hamana and Matsuzaki (1992) had previously determined the presence of PUT and SPE as the dominant polyamines in five species analyzed (*P. denitrificans*, *P. alcaliphilus*, *P. aminophilus*, *P. aminovorans*, and *P. kocurii*).

Species in the genus *Paracoccus* are chemoorganoheterotrophic, with a respiratory metabolism which uses oxygen as terminal electron acceptor. Some species can use, alternatively, nitrate which is reduced to nitrite or to molecular nitrogen. Nitrous oxide is also used by some species. Denitrification is not limited, when present, to chemoorganotrophic growth, but is also performed under chemolithoautotrophic conditions by some species (Kumaraswamy et al. 2006). None of the species is able to grow anaerobically by fermenting carbohydrates, although acid is produced aerobically from some of them. None of the species has been found to contain bacteriochlorophyll a, but carotenoids are common (astaxanthin, zeaxanthin, β-carotene). The range of organic substrates oxidized and used as carbon sources by paracocci is wide, including carbohydrates, organic acids, amino acids, and alcohols. In addition to this general mode of metabolism, several species in the genus, including the type species *P. denitrificans*, are able to grow as facultative chemolithoautotrophs, using reduced sulfur compounds as substrates (thiosulfate, tetrathionate, sulfur, or sulfide), and some are also able to oxidize hydrogen or ferrous iron. Finally, some species are able to live as facultative methylotrophs, using methylamines or methanol as substrate. During autotrophic growth, the Calvin-Benson cycle is used to fix CO_2. The chemoautotrophic ability is not always investigated when describing new species, so there is incomplete information about this trait in several of the 26 species not included in Kelly et al. (2006). In any case, at least two (*P. bengalensis* and *P. sulfuroxidans*) are able to thrive by oxidizing reduced sulfur compounds (Ghosh et al. 2006; Liu et al. 2006; Ghosh and Roy 2007) and one oxidizes Fe^{2+} (*P. ferrooxidans*, Kumaraswamy et al. 2006).

Some species have a requirement for growth factors when grown in minimal media (thiamine, biotin, B12, or unknown factors that may be covered with yeast extract).

Paracoccus species are mesophilic, growing optimally at 25–37 °C. The upper limit was established in 42 °C, but among the species recently described, *P. saliphilus* is able to grow up to 55 °C and *P. sphaerophysae* up to 60 °C. The optimal pH for most species is between 6.5 and 8.5, but there are moderate alkaliphilic species, as *P. alcalophilus*, that grows up to 9.5 (with optimum at 8–9) and a truly obligate alkaliphile, *P. tibetense*, which grows in the range 8.5–13.0 (optimum 9.5).

Paracocci are ubiquitous and ecologically diverse. While the earlier 14 species of *Paracoccus* were isolated from terrestrial or continental aquatic habitats (see *Table 20.6*), and none of

them displayed saline requirements for growth (Kelly et al. (2006)), most of the newly described species come from marine environments (seawater, tidal flat sediment, sand, and different marine organisms), and some are strictly halophilic (*P. halophilus*, *P. marinus*, *P. oceanense*, *P. rhizosphaerae*, *P. saliphilus*, and *P. seriniphilus* do not growth without NaCl). Others, although able to grow without added NaCl, have optimal growth at salinities ≥2 %. This motivated the emended description of the genus (Liu et al. 2008), aimed to account for the existence of halophilic and halotolerant species.

Three main environments have been the source of almost all the new descriptions of *Paracoccus* spp.: first, the already mentioned marine habitats; second, activated sludge from bioreactors in wastewater treatment plants; and finally, the rhizosphere and other plant-related sources. Interestingly, one species has been isolated from clinical sources (*P. yeei*), and since its description, it has been found in relation to opportunistic infections in the USA and Canada (Daneshvar et al. 2003; Funke et al. 2004; Schweiger et al. 2011).

The new species have been delineated with the aid of 16S rRNA gene sequence analysis and DNA-DNA hybridization (DDH) experiments. Thus, DDH values around 36 % relate *P. aminovorans* and *P. huijiniae* DNAs, *P. bengalensis* and *P. versutus* show values around 45 %, *P. chinensis* and *P. niistensis* have 44 % relatedness, *P. haeundaensis* and *P. marcusii* present 47 %, and *P. ferrooxidans* relates to *P. versutus*, *P. denitrificans*, and *P. pantotrophus* by 32–43 %. The former pairs are the closest in the tree, as can be seen in Fig. 20.4. However, some critical values are still missing, as the ones relating *P. carotinifaciens* with *P. marcusii* and *P. haeundaensis*. But being the type and only strain of *P. carotinifaciens* (IFO 16121^T) a patented strain, deposited only in one culture collection, it has not been possible to assess their DDH values. This fact also puts in question the status of the species *P. carotinifaciens*, according with rule 30 of the International Code of Nomenclature of Bacteria (http://www.bacterio.cict.fr/code.html). Other pairs of close species for which there are no DDH figures are *P. oceanense-P. stylophorae* (97.1 % similarity in 16S rRNA gene sequences) and *P. beibuensis-P. aestuarii* (97.2 %).

The genus *Paracoccus* is one of the most diverse carriers of insertion sequences within the *Alphaproteobacteria*. Dziewit et al. (2012) used trap plasmids (enabling positive selection of transposition events) to identify transposable elements residing in 25 strains representing 20 species of the genus *Paracoccus*. As a result, 41 elements were captured representing (i) insertion sequences, (ii) an IS-driven composite transposon, and (iii) non-composite transposons of the Tn3 family. By analyzing the functional transposable part of the mobilome, not only the dynamics of the process of transposition is better understood, but also its role in the dissemination of diverse genetic information (possibly of adaptative value) by HGT.

The genome of strain PD1222, a genetically modified version of strain *P. denitrificans* DSM 413^T, consists of two chromosomes and one plasmid totaling 5.24 Mb. The partial genome (contig assembly level) of *P. denitrificans* SD1 sourced from coal mine tailings in India and capable of *N*,*N*-dimethylformamide degradation has also been reported (Siddavattam et al. 2011). Yet, another draft genome sequence has been reported from a strain interesting for xenobiotic biodegradation and metabolism, *Paracoccus* sp. strain TRP isolated from activated sludge, that could completely biodegrade chlorpyrifos and 3,5,6-trichloro-2-pyridinol (Li et al. 2011a).

Paracoccus aminophilus JCM 7686^T has one chromosome and eight plasmids (total size 4.87 Mb) whereas the genome sequence of *P. zeaxanthinifaciens* ATCC 21588^T is only available as draft (3.05 Mb).

Methylarcula

The genus *Methylarcula*, the second component of the *Paracoccus* group, includes two species of facultative methylotrophs isolated from coastal saline environments (Doronina et al. 2000). Its separation by 16S rRNA sequence analysis from the genus *Paracoccus* is not as evident now as it was at the time of its description, although it occupies a marginal position in the clade, with only *P. isoporae* being more external to the main group (Fig. 20.4). The main characteristics of the genus are Gram-negative, nonmotile rods (0.5–0.8 × 1.0–2.0 μm in size), asporogenous, not pigmented; they divide by binary fission and accumulate PHB (up to 10–40 % of their total dry cell weight). Common cellular fatty acids are C18:1 ω7c (73–74 %), C18:0 (14–15 %), C19:0 cyclo (5–6 %), and C17:0 (2–3 %). Major PLs are PE and PC, with minor amounts of PG and DPG. The quinone system is Q10. Major compatible solute is ectoine. Molar % G+C is 57–61.

Both species require NaCl for growth, which is optimal at 3–8 % NaCl. Salinities over 12 % (*M. marina*) and 14 % (*M. terricola*) inhibit growth. Temperature range for growth is 10–42 °C with optimum at 30–35 °C and pH 7.5–8.5. They are oxidase positive, aerobic chemoorganotrophs, and facultatively methylotrophic, using methylamine, fructose, glucose, maltose, lactose, mannose, ribose, trehalose, galactose, xylose, sucrose, succinate, pyruvate, and acetate as carbon and energy sources. No growth is observed with $CO_2/H_2/O_2$ or CH_4/O_2. Growth factors are not required. Gelatin and starch are not hydrolyzed. Nitrate is not used as nitrogen source (ammonia and methylamine are) nor is it reduced to nitrite or further. Doronina et al. (2000) confirmed the presence of the (isocitrate lyase-negative) serine pathway for formaldehyde assimilation in methylamine-grown cells. The DDH values relating both species of this genus are 25–32 %.

The *Roseobacter* Group

It is the largest group in the family and the one that keeps enlarging at a fastest rate. At the moment of writing this chapter, 69 genera (comprising one hundred sixty species) have been described that join the phylogenetic group around the genus *Roseobacter*, the first described aerobic anoxygenic phototroph on this family (Fig. 20.5).

The clade is dominated by species of marine origin, chemoorganoheterotrophic, non-fermentative, most of them requiring Na ion (or sometimes combined marine salts) for growth. Thirteen of the genera contain at least one species that synthetize Bchl *a* (*Dinoroseobacter*, *Jannaschia*, *Loktanella*, *Marivita*, *Roseibacterium*, *Roseicyclus*, *Roseisalinus*, *Roseivivax*, *Roseobacter*, *Roseovarius*, *Sulfitobacter*, *Tateyamaria*, and *Thalassobacter*), and one more has been described as possessing *pufLM* genes, although Bchl *a* is not detected in the isolates grown at the lab (*Planktotalea*). No classical purple non-sulfur photosynthetic bacteria (anaerobic photoheterotrophs such as *Rhodovulum*) are included in this group, which also lacks any known autotrophic member. Some of them are able to oxidize sulfur compounds (sulfite, thiosulfate) with energy gain; thus, they may be defined as chemolithoheterotrophs. Carbon monoxide oxidation is a common ability of members of this group as well as the ability to degrade dimethylsulfoniopropionate (DMSP), either by the cleavage pathway or by the demethylation/dethionation pathway (Wagner-Döbler and Biebl 2006). González et al. (2000) reported that estimated abundance of *Roseobacter*-related bacteria was positively correlated with the concentration of DMSP in samples from a DMSP-producing algal bloom in the North Atlantic.

As already noted, the habitat of most of its members is the marine environment, including seawater, sea ice, estuarine, coastal and deep marine sediments, phytoplankton, algal surfaces, and marine invertebrates (sponges, ascidians, corals, mollusks, echinoderms), sometimes as biofilms attached to inert or living surfaces. A smaller group has been isolated from hypersaline environments (solar saltern water and sediments, salt lakes, salt mine sediment), and a minority comes from terrestrial (natural or man-made) environments: *Ketogulonicigenium* from soil samples and *Rubellimicrobium* from air, soil, and paper industry samples (❯ *Table 20.7*).

The so-called *Roseobacter* group/clade/lineage is usually one of the outstanding groups of marine bacteria detected in culture-independent studies, accounting for as much as 20 % of the total bacterial signal (Buchan et al. 2005; Wagner-Döbler and Biebl 2006; Brinkhoff et al. 2008; Giebel et al. 2011; Lenk et al. 2012). Its role on carbon and sulfur cycles in the marine environment as well as their contribution to other key activities (AAP, symbiotic relationships and pathogenesis, production of secondary metabolites) in the marine environment have been summarized and revised by Wagner-Döbler and Biebl (2006), and an excellent condensed overview on diversity, ecology, and genomics of the group was published by Brinkhoff et al. (2008). Recently, a comparative genomic approach (Chen 2012) revealed that many marine *Roseobacter* clade bacteria have the genetic potential to utilize methylated amines as alternative nitrogen sources since about half of the genomes available contained the key genes involved in this metabolism. Representative species bearing such potential were tested to confirm their abilities to use methylated amines. Moreover, trimethylamine monooxygenase (*tmm*) and gammaglutamylmethylamide synthetase (*gmaS*), two of the key enzymes, were chosen and successfully used as functional markers for detecting methylated amines-utilizing marine *Roseobacter* clade bacteria in the environment. These results suggest that methylated amines may serve as important nitrogen sources for *Roseobacter* clade bacteria in the marine environment and help to explain their numerical prevalence in the oceans.

The relative abundance of *Roseobacter* clade sequences among the alphaproteobacteria was quite different between two similarly hypersaline coastal lagoons as revealed through a metagenomic approach. At Mar Menor (Spain), they represented 30 % of all alphaproteobacterial reads, whereas at Punta Cormoran in Galapagos Islands (Ecuador), they were the most abundant representatives (Ghai et al. 2011, 2012). The study of coastal bacterioplankton community dynamics over an 8-month period, which encompassed a large storm event, revealed that during the natural perturbance, common bacterioplankton community members such as marine *Synechococcus* sp. and members of the SAR11 clade of *Alphaproteobacteria* decreased in relative abundance in the affected coastal zone, whereas several lineages of *Gammaproteobacteria*, *Betaproteobacteria*, and members of the *Roseobacter* clade increased (Yeo et al. 2013).

The nearly 70 genera that currently constitute the *Roseobacter* group have been defined (mainly) on a phylogenetic basis using 16S rRNA gene sequence analysis, and genomic relatedness based on DDH is commonly applied for species delimitation at high 16S rRNA sequence similarity (>97.0 %). A few descriptions also include an additional gene (*gyrB*) for inferring phylogeny but Multilocus Sequence Analysis (MLSA) as taxonomic tool is uncommon. Determination of alternative genomic parameters, as ANI values, is seen in the most recent species descriptions (Lucena et al. 2013). The accelerated pace of the new genera and species descriptions in the last few years is causing misclassifications, particularly affecting members of polyphyletic genera, as *Roseovarius* or *Oceanicola*.

Phylogenomics using protein signatures or other inference tools has been applied on data from complete genomes (Gupta and Mok 2007; Tang et al. 2010; Newton et al. 2010), with interesting results about the internal structure of the clade, but the limited number of representative taxa leave wide areas of shadow. These studies revealed five to six subclades within the group and are helpful to elucidate ecological strategies and evolutionary events.

Genome sequences and features of members of the *Roseobacter* group are reported and updated at the site Roseobase-Genomic Resource for Marine Roseobacters (http://www.roseobase.org/index.html).

Roseobacter

Roseobacter contains two species, *R. litoralis* and *R. denitrificans*. In spite of being the oldest named genus of the group, no other species has been added since the genus description (Shiba 1991). This description was based on the study of one strain, formerly identified as *Erythrobacter* sp., plus seven additional isolates from surface of seaweeds. In contrast with the orange pigmented

Table 20.7
Species in the *Roseobacter* group

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
Roseobacter litoralis	Surface of marine algae, Japan	AAP	Yes	Shiba 1991
R. denitrificans	Surface of marine alga (*Enteromorpha*), Japan	AAP	Yes	Shiba 1991
Actibacterium mucosum	Seawater, Western Mediterranean Sea, Spain	COH	Yes	Lucena et al. 2012a
Antarctobacter heliothermus	Water, hypersaline Ekho Lake, Antarctica	COH	Yes	Labrenz et al. 1998
Celeribacter neptunius	Seawater, Australia	COH	Yes	Lee et al. 2012a; Ivanova et al. 2010
C. baekdonensis	Seawater, East Sea, S. Korea	COH	Yes	Lee et al. 2012a
Citreicella thiooxidans	O_2/H_2S interface Black Sea, Russia	COH/CLH ($S_2O_3^{2-}$ ox.)	Yes	Sorokin et al. 2005b
C. aestuarii	Tidal flat, S. Korea	COH/CLH	Yes	Park et al. 2011
C. marina	Deep sea sediment, Indian Ocean Ridge	CLH ($S_2O_3^{2-}$ ox.)	Yes	Lai et al. 2011a
Citreimonas salinaria	Solar saltern water, S. Korea	COH	Yes	Choi and Cho 2006a
Dinoroseobacter shibae	Cultured marine dinoflagellate (*Prorocentrum*), Germany	AAP	Yes	Biebl et al. 2005a
Donghicola eburneus	Seawater, East Sea, S. Korea	COH	Yes	Yoon et al. 2007a
D. xiamenensis	Seawater, Taiwan		Yes	Tan et al. 2009
Epibacterium ulvae	Marine algae (*Ulva*) surface, Australia	COH	Yes	Penesyan et al. 2013
Hasllibacter halocynthiae	Ascidian (*Halocynthia*), S. Korea	COH	Yes (+ Mg ion)	Kim et al. 2012a
Huaishuia halophila	Seawater during algal bloom, China	COH	Yes	Wang et al. 2012
Hwanghaeicola aestuarii	Tidal flat sediment, S. Korea	COH	Yes (+ Mg ion)	Kim et al. 2010b
Jannaschia helgolandensis	Seawater, North Sea, Germany	COH	Yes (complex)	Wagner-Döbler et al. 2003
J. aquimarina	Seawater, S. Korea	COH	Mg	Park and Yoon 2012a
J. donghaensis	Seawater, East Sea, S. Korea	COH	Yes	Yoon et al. 2007b
J. pohangensis	Seashore sand, S. Korea	COH	Yes (complex)	Kim et al. 2008a
J. rubra	Seawater, Mediterranean coast, Spain	COH	Yes (+ Mg ion)	Macián et al. 2005a
J. seohaensis	Tidal flat sediment, Yellow Sea, S. Korea	AAP	Yes	Yoon et al. 2010a
J. seosinensis	Hypersaline water, solar saltern, S. Korea	COH	Yes (complex)	Choi et al. 2006
Ketogulonicigenium vulgare	Soil samples, USA	COH fac an	No	Urbance et al. 2001
K. robustum	Cotton field soil, USA	COH fac an	No	Urbance et al. 2001
Leisingera methylohalidivorans	Tide pool seawater, California, USA	COH/fac M	Yes	Vandecandelaere et al. 2008a; Martens et al. 2006; Schaefer et al. 2002
L. aquimarina	Marine electroactive biofilm, Genoa Port, Italy	COH	Yes	Vandecandelaere et al. 2008a
L. nanhaiensis	Marine sandy sediment, South China Sea, China	COH	Yes	Sun et al. 2010
Lentibacter algarum	Seawater during algal bloom, China	COH a	Yes	Li et al. 2012
Litoreibacter albidus	Marine snail (*Umbonium*), sediment of Sea of Japan, Russia	COH a	Yes	Kim et al. 2012b; Romanenko et al. 2011a

Table 20.7 (continued)

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
L. arenae (*Thalassobacter*)	Sea sand, S. Korea	COH a	Yes	Kim et al. 2012b; Kim et al. 2009
L. janthinus	Sediment, Sea of Japan, Russia	COH a	Yes	Romanenko et al. 2011a
L. meonggei	Ascidian (*Halocynthia*), South Sea, S. Korea	COH a	Yes (+ Mg ion)	Kim et al. 2012b
Litorimicrobium taeanense	Sandy beach, S. Korea	COH a	Yes	Jin et al. 2011
Loktanella salsilacus	Microbial mats in Antarctic lakes	COH	No	Tsubouchi et al. 2013; Lee 2012; Moon et al. 2010; Van Trappen et al. 2004
L. agnita	Seawater, Sea of Japan, Russia	COH	Yes	Ivanova et al. 2005
L. atrilutea	Seawater, Japan	COH	No	Hosoya and Yokota 2007a
L. cinnabarina	Deep sea floor sediment, Japan	COH	Yes	Tsubouchi et al. 2013
L. fryxellensis	Microbial mats in Antarctic lakes	COH	No	Van Trappen et al. 2004
L. hongkongensis	Marine biofilms, Hong Kong	COH	Yes	Lau et al. 2004
L. koreensis	Sea sand, S. Korea	COH	Yes	Weon et al. 2006
L. litorea	Seawater, South Sea, S. Korea	COH	Yes	Yoon et al. 2013a
L. maricola	Seawater, East Sea, S. Korea	AAP	Yes	Yoon et al. 2007c
L. pyoseonensis	Beach sand, S. Korea	COH	Yes	Moon et al. 2010
L. rosea	Marine sediment, Sea of Japan, Russia	COH	Yes	Ivanova et al. 2005
L. tamlensis	Seawater, S. Korea	COH	Yes	Lee 2012
L. vestfoldensis	Microbial mats in Antarctic lakes	COH	No	Van Trappen et al. 2004
Lutimaribacter saemankumensis	Tidal flat sediment, Yellow Sea, S. Korea	COH fac an	Yes	Yoon et al. 2009a
Mameliella alba	Seawater, South China Sea, China	COH	Yes	Zheng et al. 2010a
Maribius salinus	Hypersaline water, solar saltern, S. Korea	COH	Yes (+ Mg ion)	Choi et al. 2007
M. pelagius	Seawater, Sargasso Sea	COH	Yes (+ Mg ion)	Choi et al. 2007
Marinovum algicola (*Roseobacter*, *Ruegeria*)	Cultured dinoflagellate (*Prorocentrum*), Atlantic Ocean, Spain	COH a	Yes	Martens et al. 2006; Uchino et al. 1998; Lafay et al. 1995
Maritimibacter alkaliphilus	Seawater, Sargasso Sea	COH a	Yes, alkalitolerant	Lee et al. 2007a
Marivita cryptomonadis	Cryptomonas culture, S. Korea	AAP	Yes (complex)	Yoon et al. 2012; Hwang et al. 2009
M. byunsanensis (*Gaetbulicola*)	Tidal flat sediment, S. Korea	COH a	Yes	Yoon et al. 2012; Yoon et al. 2010b
M. geojedonensis	Coastal seawater, S. Korea	COH a	Yes	Yoon et al. 2013b
M. hallyeonensis	Seawater, S. Korea	COH a	Yes (+ Mg ion)	Yoon et al. 2012
M. litorea	Coastal seawater, S. Korea	AAP	Yes (complex)	Hwang et al. 2009
M. roseacus	Estuarine water, Chesapeake Bay, USA	AAP	Yes	Budinoff et al. 2011
Nautella italica	Marine electroactive biofilm, Italy	COH a	Yes	Vandecandelaere et al. 2009a
Nereida ignava	Seawater, Mediterranean Sea, Spain	COH a	Yes (complex)	Pujalte et al. 2005a
Oceanibulbus indolifex	Seawater, North Sea, Germany	COH a	Yes (complex)	Wagner-Döbler et al. 2004
Oceanicola granulosus	Seawater, BATS station, Sargasso Sea	COH a	Yes	Cho and Giovannoni 2004
O. batsensis	Seawater, BATS station, Sargasso Sea	COH a	Yes	Cho and Giovannoni 2004

Table 20.7 (continued)

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
O. marinus	Coastal seawater, Taiwan	COH fac an	Yes	Lin et al. 2007
O. nanhaiensis	Marine sediment, South China Sea, China	COH a	No	Gu et al. 2007
O. nitratireducens	Surface seawater, South China Sea, China	COH a	Yes	Zheng et al. 2010b
O. pacificus	Pyrene-degrading consortium from marine sediment, Western Pacific Ocean	COH a	Yes	Yuan et al. 2009
O. litoreus	Seashore sediment, S. Korea	COH fac an	Yes (+ Mg ion)	Park et al. 2013a
Oceaniovalibus guishaninsula	Seawater, Taiwan	COH	Yes	Liu et al. 2012
Octadecabacter arcticus	Polar marine ice, Alaska, USA	COH a	Yes, psychrophilic	Gosink et al. 1997
O. antarcticus	Polar marine ice, Antarctica	COH a	Yes, psychrophilic	Gosink et al. 1997
Pacificibacter maritimus	Sandy marine sediment, Sea of Japan, Russia	COH a	Yes	Romanenko et al. 2011b
Palleronia marisminoris	Hypersaline soil near saltern, Spain	COH a	Yes (complex)	Martínez-Checa et al. 2005
Pelagibaca bermudensis	Seawater, BATS station, Sargasso Sea	COH fac an	Yes	Cho and Giovannoni 2006
Pelagicola litoralis	Coastal seawater, S. Korea	COH a	Yes	Kim et al. 2008b
Pelagimonas varians	Seawater during phytoplankton bloom, North Sea, Germany	COH a	Yes	Hahnke et al. 2013a
Phaeobacter gallaeciensis (*Roseobacter*)	Seawater of *Pecten* larvae culture, Atlantic Ocean, Spain	COH a	Yes	Yoon et al. 2007d; Martens et al. 2006; Ruiz-Ponte et al. 1998
P. arcticus	Marine sediment, Arctic Sea	COH a	Yes	Zhang et al. 2008
P. caeruleus	Marine electroactive biofilm, Genoa Port, Italy	COH a	Yes	Vandecandelaere et al. 2009b
P. daeponensis	Tidal flat, Yellow Sea, S. Korea	COH a	Yes	Vandecandelaere et al. 2008a; Yoon et al. 2007d
P. inhibens	Surface water, tidal flat, North Sea, Germany	COH a	Yes	Vandecandelaere et al. 2008a; Martens et al. 2006
Planktotalea frisia	Seawater during phytoplankton bloom, North Sea, Germany	COH a	Yes	Hahnke et al. 2012
Pontibaca methylaminovorans	Coastal sediment, enrichment with TMA, East Sea, S. Korea	COH fac an	Yes	Kim et al. 2010c
Ponticoccus litoralis	Coastal seawater, S. Korea	COH a	Yes (complex)	Hwang and Cho 2008
Poseidonocella pacifica	Shallow marine sediment, Sea of Japan, Russia	COH a	Yes	Romanenko et al. 2012
P. sedimentorum	Shallow marine sediment, Sea of Japan, Russia	COH a	Yes	Romanenko et al. 2012
Primorskyibacter sedentarius	Shallow marine sediment, Sea of Japan, Russia	COH a	Yes	Romanenko et al. 2011c
Profundibacterium mesophilum	Sea floor sediment, Discovery Deep, Red Sea	COH a	Yes	Lai et al. 2013
Pseudoruegeria aquimaris	Seawater, East Sea, S. Korea	COH a	Yes	Jung et al. 2010a; Yoon et al. 2007e
P. lutimaris	Tidal flat sediment, S. Korea	COH fac an	Yes (+ Mg ion)	Jung et al. 2010a

Table 20.7 (continued)

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
Roseibacterium elongatum	Coastal sand, west coast of Australia	APP	Variable	Suzuki et al. 2006
R. beibuensis	Surface seawater, South China Sea, China.	AAP	No	Mao et al. 2012
Roseicyclus mahoneyensis	Saline meromictic lake, Canada	APP	Yes	Rathgeber et al. 2005
Roseisalinus antarcticus	Hipersaline, meromictic Ekho lake, Antarctica	APP	Yes (complex)	Labrenz et al. 2005
Roseivivax halodurans	Charophytes, saline lake, Australia	AAP	No	Chen et al. 2012a; Park et al. 2010; Suzuki et al. 1999b
R. halotolerans	Epiphytes on stromatolite, saline lake, Australia	AAP	Yes	Suzuki et al. 1999b
R. isoporae	Ree-building coral (*Isopora*), Taiwan	AAP	No	Chen et al. 2012a
R. lentus	Tidal flat, S. Korea	COH a	Yes	Park et al. 2010
R. sediminis	Sediment of crystallizer pond, salt mine, China	COH a	Yes	Xiao et al. 2012
Roseovarius tolerans	Hipersaline, meromictic Ekho lake, Antarctica	AAP	Yes	Labrenz et al. 1999
R. aestuarii	Tidal flat, Yellow Sea, S. Korea	COH a	Yes	Yoon et al. 2008
R. crassostreae	Juvenile oysters, NE coast USA	COH a	Yes	Boettcher et al. 2005
R. halocynthiae	Ascidian (*Halocynthia*), S. Korea	COH a	Only Mg ion	Kim et al. 2012c
R. halotolerans	Deep seawater, East Sea, S. Korea	COH a	Yes	Oh et al. 2009
R. indicus	Deep seawater, Indian Ocean	AAP	Yes	Lai et al. 2011b
R. litoreus	Seawater, S. Korea	COH a	Yes	Jung et al. 2012b
R. marinus	Seawater, Yellow Sea, S. Korea	COH a	Yes (+ Mg ion)	Jung et al. 2011
R. mucosus	Cultured dinoflagellate (*Alexandrium*), Germany	AAP	Yes	Biebl et al. 2005b
R. nanhaiticus	Sandy sediment, South China Sea	COH a	Yes	Wang et al. 2010
R. nubinhibens	Surface seawater, Caribbean Sea	COH a	Yes	González et al. 2003
R. pacificus	Deep sea sediment, W Pacific	COH a	Yes	Wang et al. 2009b
R. sediminilitoris	Seashore sediment, South Sea, S. Korea	COH a	Yes (+ Mg ion)	Park and Yoon 2013
Rubellimicrobium thermophilum	Slime on paper machines, Finland	COH a	No, thermophilic	Denner et al. 2006
R. aerolatum	Air sample, S. Korea	COH a	No	Weon et al. 2009
R. mesophilum	Soil, S Korea	COH a	No (sensitive to salinity)	Dastager et al. 2008
R. roseum	Forest soil, China	COH a	No (sensitive to salinity)	Cao et al. 2010
Ruegeria atlantica (*Agrobacterium*)	Marine sediment, NW Atlantic Sea	COH a	Yes	Uchino et al. 1998; Rüger and Höfle 1992
R. arenilitoris	Seashore sand, S. Korea	COH a	Yes	Park and Yoon 2012b
R. conchae	Ark clam (*Scapharca*), S. Korea	COH a	Yes	Lee et al. 2012b
R. faecimaris	Tidal flat, Yellow Sea, S. Korea	COH a	Yes	Oh et al. 2011a
R. halocynthiae	Ascidian (*Halocynthia*), S. Korea	COH a	Yes	Kim et al. 2012d
R. lacuscaerulensis (*Silicibacter*)	Geothermal lake, Iceland	COH a	Yes	Yi et al. 2007; Petursdottir and Kristjansson 1999

Table 20.7 (continued)

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
R. marina	Marine sediment, East China Sea, China	COH a	No	Huo et al. 2011
R. mobilis (*R. pelagia*)	Biofilm, surface seawater, coelenterate (*Hexacorallia*), Sargasso Sea	COH a	No	Vandecandelaere et al. 2008b; Muramatsu et al. 2007
R. pomeroyi (*Silicibacter*)	Seawater, Georgia, USA	COH a	Yes	Yi et al. 2007; González et al. 2003
R. scottomollicae	Marine electroactive biofilm, Italy	COH a	Yes	Vandecandelaere et al. 2008b
Sagittula stellata	Seawater from salt marsh, Georgia, USA	COH a	Yes	Lee et al. 2013b; González et al. 1997
S. marina	Seawater, S. Korea	COH a	Yes	Lee et al. 2013b
Salinihabitans flavidus	Marine solar saltern, S. Korea	COH a	Yes	Yoon et al. 2009b
Salipiger mucosus	Hypersaline soil, Spain	COH a	Yes	Martínez-Cánovas et al. 2004
Sediminimonas qiaohouensis	Salt mine sediment, China	COH a	Yes	Wang et al. 2009c
Seohaeicola saemankumensis	Tidal flat, Yellow Sea, S. Korea	COH a	Yes	Yoon et al. 2009c
Shimia marina	Biofilm, coastal fish farm, S. Korea	COH a	Yes	Choi and Cho 2006b
S. isoporae	Coral (*Isopora*), Taiwan	COH a	Yes	Chen et al. 2011b
Sulfitobacter pontiacus	O_2/H_2S interface, Black Sea	COH/CLH ($S_2O_3^{2-}$, SO_3^- ox.)	Yes	Yoon et al. 2007f; Sorokin 1995
S. brevis	Hypersaline heliothermal Ekho lake, Antarctica	COH/CLH ($S_2O_3^{2-}$, SO_3^- ox.)	Yes	Labrenz et al. 2000
S. delicatus	Starfish (*Stellaster*), S. China Sea	COH/CLH ($S_2O_3^{2-}$, SO_3^- ox.)	Yes	Ivanova et al. 2004
S. donghicola	Seawater, East Sea, S. Korea	COH	Yes	Yoon et al. 2007f
S. dubius	Sea grass (*Zostera*), Sea of Japan	COH/CLH ($S_2O_3^{2-}$, SO_3^- ox.)	Yes	Ivanova et al. 2004
S. guttiformis (*Staleya*)	Hypersaline heliothermal Ekho lake, Antarctica	COH	Yes	Yoon et al. 2007f; Labrenz et al. 2000
S. litoralis	Seawater, East Sea, S. Korea	COH/CLH ($S_2O_3^{2-}$, SO_3^- ox.)	Yes	Park et al. 2007
S. marinus	Seawater, East Sea, S. Korea	COH	Yes	Yoon et al. 2007g
S. mediterraneus	Seawater, Mediterranean Sea, France	COH/CLH ($S_2O_3^{2-}$, SO_3^- ox.)	Yes	Pukall et al. 1999
Tateyamaria omphali	Molluscan (*Omphalius*), Japan	AAP, COH fac an, CLH ($S_2O_3^{2-}$, SO_3^- ox.)	Yes	Sass et al. 2010; Kurahashi and Yokota 2007
T. pelophila	Tidal flat sediment, North Sea, Germany	COH	Yes	Sass et al. 2010
Thalassobacter stenotrophicus (*Jannaschia cystaugens*)	Seawater, W Mediterranean Sea, Spain	AAP	Yes (complex)	Pujalte et al. 2005b; Macián et al. 2005b
Thalassobius mediterraneus	Seawater, Mediterranean Sea, Spain	COH a	Yes (complex)	Arahal et al. 2005
T. aestuarii	Tidal flat sediment, S. Korea	COH a	Yes	Yi and Chun 2006
T. gelatinovorus (*Ruegeria*)	Seawater, Baltic Sea, Germany	COH a	Yes	Arahal et al. 2005; Uchino et al. 1998
T. maritimus	Seawater, South Sea, S. Korea	COH a	Only Mg ion	Park et al. 2012

Table 20.7 (continued)

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
Thalassococcus halodurans	Marina sponge (Halichondria), WA, USA	COH a	Yes	Lee et al. 2007b
T. lentus	Seawater, seaweed farm, S. Korea	COH a	Yes (+ Mg ion)	Park et al. 2013b
Tranquillimonas alkanivorans	Seawater, Semarang Port, Indonesia	COH a	Yes	Harwati et al. 2008
Tropicibacter naphthalenivorans	Seawater, Semarang Port, Indonesia	COH a	Yes	Harwati et al. 2009a
T. multivorans	Seawater, Mediterranean Sea, Spain	COH a	Yes (+ Mg or Ca ions)	Lucena et al. 2012b
T. phathalicicus	Seawater, Japan	COH a	Yes	Iwaki et al. 2012a
T. litoreus	Seawater, Mediterranean Sea, Spain	COH a	Yes	Lucena et al. 2013
T. mediterraneus	Seawater, Mediterranean Sea, Spain	COH a	Yes (+ Mg ion)	Lucena et al. 2013
Tropicimonas isoalkanivorans	Seawater, Semarang Port, Indonesia	COH a	Yes	Harwati et al. 2009b
T. aquimaris	Seawater, South Sea, S. Korea	COH a	Yes	Oh et al. 2012
T. sediminicola	Marine sediment of a cage-cultured ark clam farm, S. Korea	COH a	No	Shin et al. 2012
Vadicella arenosi	Sandy sediment, Sea of Japan, Russia	COH a	Yes	Romanenko et al. 2011d
Wenxinia marina	Sediment at oilfield, South China Sea, China	COH a	Yes	Ying et al. 2007
Yangia pacifica	Coastal sediment, East China Sea, China	COH a	Yes	Dai et al. 2006

[a]*AAP* aerobic anoxygenic photoheterotroph, *CLH* chemolithoheterotroph, *COH* chemoorganoheterotroph, *COH a* chemoorganoheterotroph aerobic, *COH fac an* chemoorganoheterotroph facultative anaerobic, *fac M* facultatively methylotroph

Erythrobacter, these strains were pink pigmented, contained a particular bacteriochlorophyll-protein complex and carotenoids not found in *Erythrobacter*, and showed unappreciable levels of DDH to the type strain of *E. longus* (3–5 %). The pink strains formed two genospecies, according to DDH values, one including seven isolates with 74–98 % intragroup relatedness and a second with only one strain, which showed 41–59 % DDH to the various strains in the former group. Thus, the new genus was described as containing two species, with the following general properties (Shiba 1991; Shiba and Imhoff 2005): cells are Gram negative, ovoid to rod shaped, motile by subpolar flagella, and divide by binary fission. They synthetize Bchl *a* (only in aerobic conditions) and spheroidenone as major carotenoid pigments. The major quinone is Q10 (menaquinones are absent), polar lipids include PG and DPG, and the dominant cellular fatty acid is C18:1 ω7c. DNA G+C content is 56–60 mol%. *Roseobacter* species are aerobic chemoheterotrophs that require biotin, thiamine, and nicotinic acid, as well as sodium ions, for growth. Preferred carbon sources are organic acids (acetate, pyruvate, succinate, malate, and citrate), some sugars (glucose) and amino acids (glutamate), but not methanol. They are proteolytic on gelatin, hydrolyze Tween 80, and are positive for both oxidase and catalase. Light inhibits respiration, decreasing O_2 consumption but not growth. The stronger suppression of respiration by light is observed when organic substrates are scarce (Shiba and Imhoff 2005). Bacteriochlorophyll synthesis is suppressed by anaerobiosis and by continuous light.

They are mesophilic (optimum 20–30 °C), neutrophilic (optimum pH 7–8), and slightly halophilic (optimum 2.5 %). The two named species differ, basically, in the denitrification activity, which gives name to *R. denitrificans* and confers to it the ability to thrive anaerobically, a lifestyle that is not possible for the type species *R. litoralis*. In addition to denitrification, *R. denitrificans* can also develop in anaerobiosis by using TMAO as electron acceptor.

A lytic siphovirus, designated RDJLΦ1, infecting *R. denitrificans* OCh 114^T has been characterized (Zhang and Jiao 2009), its genome fully sequenced (Huang et al. 2011), and the response to the infection of the host has been analyzed through real-time atomic force microscopy and proteomics (Zhang et al. 2012b).

The type strains of *R. litoralis* and *R. denitrificans* have their genomes fully sequenced (Swingley et al. 2007; Kalhoefer et al. 2011). A comparison indicates that major differences between them are due to lateral gene transfers and genome rearrangements. *R. litoralis* Och 149^T contains one chromosome (4.5 Mbp) and three plasmids (63.5–93.5 Kbp) with 4,537 protein-coding genes predicted. *R. denitrificans* OCh 114^T

genome is composed of one chromosome of 4.1 Mbp and four plasmids ranging from 5.8 to 106.5 Kbp, with a total of 4,129 predicted protein-coding genes. Genes shared by both type strains account for 3,415, a 75 % of *R. litoralis*' total genes. Photosynthetic genes are plasmid encoded in *R. litoralis*, but chromosomic in *R. denitrificans* (Kalhoefer et al. 2011; Pradella et al. 2004). The plasmid location of *pufML* genes also occurs in *Sulfitobacter* (*Staleya*) *guttiformis* (Pradella et al. 2004). Scaffolds or contigs of five additional *Roseobacter* sp. strains (AzwK-3b, CCS2, GAI101, MED193, and SK209-2-6) are also available at public repertories.

Roseobacter species form a compact grouping with members of the genus *Sulfitobacter* and *Oceanibulbus* in all assayed phylogenetic trees, and these three genera represent one of the few stable clades within this branch of the *Rhodobacteraceae*.

Actibacterium

Actibacterium is a recently described genus containing one species, *A. mucosum* (Lucena et al. 2012a), isolated from coastal seawater. *Actibacterium* is a strictly aerobic chemoorganotroph with complex ionic requirements. Cells do not accumulate PHB and contain PG, an unidentified aminolipid and a lipid as major polar lipids. Q10 is the predominant quinone and C18:1 ω7c the major fatty acid. It is oxidase and catalase positive and its DNA G+C content is around 61 mol%. *A. mucosum* is not pigmented and its cells are ovoid to rod shaped and nonmotile. It is mesophilic (15–37 °C) and halophilic (1.7–5 % total salinity) and requires a mixture of marine salts that include Na, K, Ca, Mg, and sulfate ions for optimal growth or the addition of seawater to the medium. It does not reduce nitrates and does not have growth factor requirements, as it is able to grow on defined medium with several carbohydrates, organic acids, and amino acids as sole carbon and energy sources. Its cellular fatty acids include, in addition to the dominant C18:1 ω7c, C16:0, C10:0 3-OH, and C12:0 3OH. *Actibacterium* was found to constitute an independent lineage, with no close relatives, in 16S rRNA gene-based trees and also in *gyrB* gene trees (Lucena et al. 2012a). In the complete tree of ❯ *Fig. 20.5*, *Actibacterium mucosum* relates distantly (but stably) to the newly described *Hasllibacter halocynthiae*, both lying near the edge of the *Roseobacter* group.

Antarctobacter

Antarctobacter and its sole species *A. heliothermum* described in the 1990s were one of the first members of the *Roseobacter* clade (Labrenz et al. 1998) from water samples of the hypersaline Antarctic Ehko Lake. It is a Gram-negative, budding bacterium that forms rosettes and accumulates PHB. Cells may be motile (one to three polar flagella). Colonies are brownish yellow or brownish red, depending upon the medium, and have a wide range of growth temperatures (from less than 3 °C to 43 °C) and pHs (5.3 to 9). NaCl is required for growth (optimum 2–6 %). Not phototrophic, Bchl *a* is not synthetized. Oxidase and catalase are positive. Peptidoglycan contains *m*-diaminopimelic acid and is direct cross-linked (A1γ). It contains Q10 as respiratory quinone; its main polar lipids are PG, PC, and unknown phospho- and aminolipids. C18:1 ω7c is the dominant fatty acid and C12:1 3OH is a diagnostic one. The DNA G+C content is 63 mol%. The species, *A. heliothermus*, has the following additional characteristics: requires thiamin and nicotinic acid; reduces nitrate, at least to nitrite; hydrolyzes gelatin and DNA; and grows with small organic acids (acetate, pyruvate, butyrate, malate, and succinate) and with glucose or glutamate.

Celeribacter

Celeribacter was described by Ivanova et al. (2010) as a new genus containing the species *C. neptunius*. Lee et al. (2012a) described a second species *C. baekdonensis* and emended the genus description. Currently, *Celeribacter* contains ovoid- to rod-shaped Gram-negative cells that may be motile by a single polar or subpolar flagellum or nonmotile. Q10 is the predominant quinone; C18:1 ω7c is the major fatty acid, accompanied by 11-methyl C18:1 ω7c, C18:0, C16:0, and C10:0 3OH. The polar lipid profile was somewhat under dispute, but Lee et al. (2012a) determined for both species that common polar lipids were PG, one unidentified aminolipid and one lipid. The G+C content was 59–61 mol%. *Celeribacter* species are chemoorganotrophic, do not produce Bchl *a* nor other pigments, do not accumulate PHB, and are able to grow anaerobically on Marine Agar. Although *C. neptunius* was described as oxidase negative, Lee et al. (2012a) found a positive response for this test in both species that differ in Na ion requirement and in the ability for nitrate reduction, proteolytic activity, and production of acid from a large number of carbohydrates. They show low levels of DDH (17 %). Both species are mesophilic (5–35 °C, optimum 25–30 °C), neutrophilic (with optimum at pH 7–8), and slightly halophilic, with *C. baekdonensis* having a range from 0 % to 13 % NaCl (optimum: 2 %) and requiring Mg ion and *C. neptunius* growing between 1 % and 8 % and requiring Na ion. *Celeribacter* species are phylogenetically close to *Huaishuia halophila*, *Vadicella arenosi*, and both species of *Pseudoruegeria* in the *Roseobacter* detailed tree (❯ *Fig. 20.5*). The draft genome of *C. baekdonensis* B30 has 4.33 Mb.

Four strains (YSCB1, YSCB2, YSCB3, and YSCB4) were found to produce unique intracellular chromium-rich aggregates. This remarkable capacity makes them model bacteria for studies of chromium metabolism and its biological function and suitable candidates for chromium decontamination and environmental remediation (Gao et al. 2006). These strains exhibit yet another interesting feature, since they produce spinae that are built from proteolysis-resistant filaments winding into a tubular architecture by a mechanism different from those of other bacterial appendages. An excellent study, including many striking micrographs, was published recently (Bernadac et al. 2012). Although reported as *Roseobacter* sp., they can be now

identified as members of the genus *Celeribacter*, probably *C. baekdonensis*, according to 16S rRNA data.

One of the few known roseophages is a *Celeribacter* phage, P12053L. Its complete genome has been sequenced recently (Kang et al. 2012).

Citreicella

The study of strains isolated from the oxygen-sulfide interface of the Black Sea served Sorokin and colleagues to describe a new genus of lithoheterotrophic sulfur-oxidizing bacteria *Citreicella* that presently contains three species: *C. thiooxidans* (Sorokin et al. 2005b), *C. marina* (Lai et al. 2011a), and *C. aestuarii* (Park et al. 2011). The genus is defined as Gram-negative, nonmotile (or sometimes motile by lateral flagella, as in *C. aestuarii*) cells with lemon-shaped morphology, obligately heterotrophic, and aerobic. Carbon sources used for chemoheterotrophic growth are short-chain organic acids, hexoses, and amino acids. Catalase and oxidase are positive. Citreicellas do not synthetize Bchl *a* or other pigments; they form PHB granules and oxidize, at least, thiosulfate to sulfate (*C. thiooxidans* also oxidizes sulfide and sulfur), gaining energy from this activity during its heterotrophic growth. Its predominant quinone is Q10, C18:1 ω7c is the major fatty acid (with C16:0 and C19:0 cyclo ω8c also abundant), and the DNA G+C content is between 67 and 69 mol%. They prefer neutrophilic and mesophilic conditions (25–30 °C is the optimal range) but could grow from 4 °C (*C. marina*) to 40 °C (*C. thiooxidans*). They require sodium ion (at least 0.5–1 % NaCl) and grow optimally with 2–7 % NaCl, with a maximum of up to 12 % in *C. marina*, the more halotolerant and 6 % in *C. thiooxidans*, the less halotolerant. *C. marina* and *C. thiooxidans*, the closest species, are related by DDH values of 48 % (Lai et al. 2011a). Recently, a draft genome sequence of a strain (not the type strain) of *C. aestuarii* isolated from a petroleum contaminated beach has been reported (Suarez-Suarez et al. 2012): it contains, among the genes for major metabolic pathways, sox genes (sulfite oxidation) and cox genes (carbon monoxide oxidation), and some 120 protein-coding genes were found that may be involved in the metabolism of aromatic compounds.

All three species of *Citreicella* form a well-defined clade whose nearest neighbors are *Yangia pacifica* and *Citreimonas salinaria*.

Citreimonas

Citreimonas and its sole currently recognized species *C. salinaria* were isolated and described from samples of solar saltern water (Choi and Cho 2006a). Although it is next to *Citreicella* spp. in the 16S rRNA-based tree, they do not show the thiosulfate-oxidizing ability that characterizes *Citreicella* species. *Citreimonas salinaria* is, as *Citreicella*, obligately heterotrophic, aerobic, positive for catalase (but it is negative for oxidase), and unable to synthetize Bchl *a* or carotenoids. Cells are ovoid to rod shaped and nonmotile. They do not accumulate PHB. The two major fatty acids are C18:1 ω7c and C19:0 cyclo ω8c and the DNA G+C content is in the same range than *Citreicella* (67.3 % mol%). The ranges for temperature and salinity are also similar (15–40 °C and 1–10 % total salts, with optima at 30–35 °C and 5–6 %, respectively). It grows with a large variety of sugars and some organic acids and amino acids as carbon and energy sources in a basal medium supplemented with low levels of yeast extract. It reduces nitrate and is proteolytic on gelatin.

Dinoroseobacter

The genus *Dinoroseobacter* (Biebl et al. 2005a) contains a single species, *D. shibae*, isolated from the marine dinoflagellate *Prorocentrum lima*. Members of the genus are Gram-negative cocci or ovoid rods, motile by a single polar to subpolar flagellum. Pigmentation of cultures is pink to wine red, provided that the incubation is performed in the dark or with intermittent illumination. They are strict aerobes, non-fermentative heterotrophs, which synthetize Bchl *a* and carotenoids (spheroidene). They require at least 1 % sea salts for growth and have Q10 as predominant quinone. The species *D. shibae* requires biotin, nicotinic acid, and 4-aminobenzoic acid for growth and uses acetate, succinate, fumarate, malate, lactate, citrate, pyruvate, and some simple carbohydrates (glucose, fructose, glycerol) and glutamate as carbon and energy sources (but no butyrate, ethanol, or methanol). Nitrate is reduced to N_2 and gelatin and Tween 80 are degraded. Cells of *D. shibae* contain PG, DPG, one unidentified aminolipid, and other lipids as principal polar lipids. As usual in the family, C18:1 ω7c is the dominant fatty acid, with minor amounts of C18:0, C12:1 ω5c, C10:0 3OH, C14:1 3OH, and C19:0 cyclo ω8c. DNA G+C content is 65 mol%. *Dinoroseobacter* is consistently located next to *Roseibacterium* species in the *Roseobacter* phylogenetic tree, either using NJ (*Fig. 20.5*) or ML (LTP108) methods.

D. shibae is an algal symbiont that lives attached to the surface of different algal partners (*Prorocentrum*, *Alexandrium*, *Isochrysis*), some of them toxic and responsible of diarrheic shellfish poisoning (Wagner-Döbler et al. 2010). A symbiotic relationship in which the bacterium provides the algae with growth-limiting vitamins (B12) and the bacterium thrives on the organic matter synthetized by the alga has been suggested, based in physiological and field data and also in the information provided by the study of the complete genome of *D. shibae* type strain (Wagner-Döbler et al. 2010). Moreover, research in metabolic pathways of *D. shibae* has served as a model for the understanding of the whole *Roseobacter* group in terms of the general pattern of carbon fluxes (Fürch et al. 2009). *D. shibae* DFL12^T has a genome composed of one chromosome and five plasmids (total 4.4 Mbp), containing around 4,200 protein-encoding genes. In common with known *Roseobacter* spp. genomes, it harbors information for important biogeochemical metabolic abilities, such as anoxygenic photosynthesis

(*pufLM* genes), oxidation of CO (*cox* genes), degradation of aromatic compounds (*pcaGH* and *boxC* genes), sulfur oxidation (*soxB*), dimethylsulfoniopropionate use (*dmdA*), denitrification (*nirS*/*K*), and also several families of transposases/integrases and site-specific recombinases/resolvases, which indicates a large potential for DNA exchange. Through functional analysis, Fürch et al. (2009) demonstrated that *D. shibae* metabolizes glucose exclusively through the Entner-Doudoroff pathway, has an active cyclic respiratory TCA cycle, and incorporates CO_2 through a mechanism that does not involve PEP synthesis from pyruvate, as previously thought. Tomasch et al. (2011) suggest that *D. shibae* might use the 3-hydroxypropionate cycle for CO_2 fixation, in addition to showing the changes in photoheterotrophic behavior in response to light regime and organic carbon availability.

Donghicola

Donghicola is an inconspicuous genus of chemoheterotrophic, aerobic, nonpigmented, nonmotile bacteria which was described on an isolate from seawater (Yoon et al. 2007a). Cells are coccoid to rod shaped and do not synthetize Bchl *a* or carotenoids. Basic chemotaxonomic features (quinone system, major fatty acids) are the common in the group and the DNA G+C content is 59–62 mol%. Two species have been recognized, *D. eburneus* (Yoon et al. 2007a), the type species, and *D. xiamenensis* (Tan et al. 2009), that differ mainly in the pattern of carbon source utilization. Both species are catalase and oxidase positive, slightly halophilic, requiring NaCl for growth (optimum 1–3 %, tolerance up to 11 %), mesophilic, and neutrophilic. Their closest phylogenetic relatives are a group of four *Roseovarius* species that do not include the type species of *Roseovarius*, but the relationship is not maintained in other methodological conditions. The draft genomes of *D. xiamenensis* DSM 18339^T and *Donghicola* sp. S598 have 4.73 and 3.22 Mb, respectively.

Epibacterium

Epibacterium ulvae, the type and sole species of *Epibacterium* (Penesyan et al. 2013), is rod shaped and motile by a polar flagellum. Generic features include, in addition to cell morphology, presence of catalase and oxidase activities and requirement of aerobic conditions and sodium for growth. Polar lipids are PG and PC plus two unidentified aminolipids and four unidentified phospholipids. Cellular fatty acids include C18:1 ω7c, C16:0, C18:2, C10:0 3OH, C12:0, C20:1 2OH, C16:0 2OH, C12:0 3OH (amide linked), and C18:0. Q10 is the sole respiratory quinone. The type strain of *E. ulvae* has a DNA G+C content of 52.6 mol %. Strains of this species, isolated from the surface of the marine alga *Ulva australis*, are brown-black pigmented but do not produce Bchl *a* or possess *pufLM* genes. Its optimal growth conditions are 25 °C, neutral pH, and 2–3 % NaCl. Growth is produced on a variety of carbon sources that include hexoses, disaccharides, and organic acids. *E. ulvae* strains are able to inhibit the growth of other marine bacteria through production of antibacterial compounds of unknown chemical nature. The species is related to the *Thalasobium-Thalassococcus* subclade within the *Roseobacter* group.

Hasllibacter

Hasllibacter is another recently described genus, interesting because of its ability to produce cholic acid derivatives (Kim et al. 2012a, e), including nutriacholic acid. Strains of the only described species *H. halocynthiae* are Gram negative, ovoid, or rod shaped, with a single polar flagellum, that may reproduce by budding. They have Q10 as predominant quinone and C18:1 ω7c as major fatty acid (plus C18:0, C10:0 3OH, C19:0 cyclo ω8c, and C20:1 ω7c). They are strict aerobes and have an absolute requirement for Na and Mg ions (optimal growth: 1–5 % NaCl). Their colonies are light-red pigmented (but no data on Bchl *a* or carotenoid synthesis is given in the description). The G+C content is 71.6 mol%. On 16S rRNA trees, it forms a deep lineage distantly related to *Actibacterium mucosum*.

Huaishuia

Huaishuia contains a single species, *H. halophila*, isolated from seawater during a massive green algal bloom (dominated by *Enteromorpha prolifera*) (Wang et al. 2012). It is Gram negative, nonmotile, strictly aerobic, and slightly halophilic. PHB accumulation is not observed. Catalase and oxidase are positive. Nitrate is not reduced. Major fatty acids are C18:1 ω7c, C18:1 ω6c, and 11-methyl C18:1 ω7c. It presents PG, one unidentified aminolipid, and two unidentified phospholipids as major polar lipids and a DNA G+C content of 60 mol%. Requires sodium ion and grows between 0.5 % and 11 % NaCl and between 4 °C and 45 °C. *H. halophila* appears among the members of the *Celeribacter* subclade (which groups the two *Celeribacter* species, plus *Vadicella arenosi* and *Huaishuia halophila*, and is close to *Pseudoruegeria* spp.).

Hwanghaeicola

Kim et al. (2010b) described *Hwanghaeicola aestuarii* as a new genus and species from an isolate obtained from a tidal flat. It is entirely typical in its chemotaxonomic and biochemical features: contains Q10 (major quinone), C18:1 ω7c, plus C16:0 and C10:0 3OH (major fatty acid), is a strict aerobe with oxidase and catalase activities, not able to reduce nitrate and with a DNA G+C content of 61 mol%. The strain requires Na and Mg ions for growth, which is optimal at 2–3 % NaCl (range: 1.5–6 %) and 25–30 °C (range: 15–35 °C) and neutral pH (6.0–8.0). Colonies of *H. aestuarii* are pale pink, but no information on Bchl *a* synthesis is given in the description. It shows a distant relationship with *Maribius* species.

Jannaschia

Jannaschia is a well-defined, coherent genus that currently contains seven species isolated from seawater of distant geographic areas, seashore sand, tidal flat sediment, and solar saltern water. The type species *J. helgolandensis* (Wagner-Döbler et al. 2003) and the remaining six species *J. rubra*, *J. seosinensis*, *J. donghaensis*, *J. pohangensis*, *J. seohaensis*, and *J. aquimarina* constitute a stable clade in all analyses performed on 16S rRNA gene sequences so far, an unusual case among the multispecies genera of the *Roseobacter* group. The genus, as originally defined (Wagner-Döbler et al. 2003), contains Gram-negative, irregular rods, with a tendency to form chains, that grow optimally at 30 °C, at pH of 7.0–8.0, and have an absolute requirement of sea salts, not fulfilled by the sole addition of NaCl to the medium (salinity range: 1–7 % sea salts). They are heterotrophic, strict aerobes, non-fermentative, not able to reduce nitrates, and give a weak response to the oxidase test. Main fatty acids are C18:1 ω7c, C18:0, C19:0 cyclo, C17:0, C10:0 3OH, C14:0, C14:0 3OH/C14:1 3OH, and C12:1. They contain Q10 as predominant respiratory quinone and PG, DPG, PC, PE and one aminolipid as major polar lipids. DNA G+C content of the type species is 63 mol%. The remaining six species generally fulfill this description with only a few exceptions: nitrate reduction to nitrite is performed by some species; the salinity range may be slightly wider, 11-methyl C18:1 ω7c is a major part of the fatty acid profile of most species. The polar lipid profile does not include DPG or AL in some species (*J. donghaensis*, *J. seosinensis*). In addition, the G+C of the genus expands up to 68 mol%. Most species are pigmented in various shades of orange, yellow, or red but only one of them has been confirmed as synthetizing Bchl *a* (*J. seohaensis*). Reports on oxidase activity are sometimes contradictory: *J. seosinensis* is described as negative (Choi et al. 2006) but reported as positive in a later study (Yoon et al. 2010a). Motility is variable among species, but when present it is due to flagella (usually several) located polarly. Levels of DDH between different species are low: *J. pohagensis* shows 21 % against *J. helgolandensis*, a 38 % against *J. rubra*, and 24 % against *J. seosinensis* (Kim et al. 2008a), *J. rubra* is related to *J. helgolandensis* by a value of 42 % DDH (Macián et al. 2005a), and *J. seosinensis* and *J. seohaensis* show only 17 % relatedness (Yoon et al. 2010a). The genus is phylogenetically close to *Thalassobacter stenotrophicus*, which currently contains the former *Jannaschia cystaugens* (Adachi et al. 2004). *J. cystaugens* was found to be a heterotypic synonym of *T. stenotrophicus* (Pujalte et al. 2005b).

Despite the fact that only one of the seven species of *Jannaschia* is able to produce Bchl *a*, *Jannaschia*-related clones are a substantial part of the AAP bacteria in some extensively studied environments, as the Central Baltic Sea (Salka et al. 2008), where they account for 25–30 % of the total AAP clones obtained and occur in all locations investigated. *Jannaschia* sp. and *Sulfitobacter brevis* are two of the prominent members of experimental microcosms that show an increase after exposition to crude oil contamination (Jung et al. 2010b).

The first complete *Jannaschia* genome obtained corresponds to strain CCS1, a *Jannaschia* sp. isolated from Pacific coastal seawater and Bchl *a* producer: it consist of one chromosome (4.3 Mbp) and one plasmid (86 Kbp). Among others, it contains information for phototrophy, CO oxidation, aromatic compound degradation, nitrate assimilation, and DMSP demethylation. *Jannaschia* sp. CCS1 possesses six distinct ring-cleaving pathways, all of them chromosomally encoded (Moran et al. 2007). An enzyme produced by this strain, a D-hydantoinase is of interest, due to the application of these enzymes to the industrial production of pure amino acids (Cai et al. 2009).

Ketogulonicigenium

The genus *Ketogulonicigenium* is atypical within the *Roseobacter* group because its natural habitat is not the marine environment but continental and soil related. The two species included in the genus were obtained after a search for strains able to efficiently convert L-sorbose to 2 keto L-gulonic acid (2-KLG) from soils of different locations in the USA. This conversion is of great interest for the industrial production of L-ascorbic acid (vitamin C), and in fact strains of the type species *K. vulgare* are used for this purpose in coculture with *Bacillus megaterium* (Zhang et al. 2011a).

The genus is defined as containing Gram-negative, facultatively anaerobic cells, ovoid to rod shaped, that may form flagella and fimbriae. They produce a tan pigmentation that is diffusible in agar cultures of 48 h or more. Catalase and oxidase are positive. Best growth is observed at 27–31 °C, pH 7.2–8.5, and 117–459 mM Na^+, although this ion is not required for growth. They are chemoheterotrophs which use a wide range of carbohydrates for growth (inositol, mannitol, glycerol, sorbitol, lactose, and arabinose are preferred). All strains produce 2-KLG from L-sorbose. Major cellular fatty acids are C18:1 ω7c and C16:0. DNA G+C content is 52–54 mol%, according to the genus description, but complete genome sequencing of various strains gives somewhat higher figures (≈61–62 mol%). Both species, *K. vulgare* and *K. robustum*, could be distinguished by motility, intensity of pigmentation, and DDH values of 37–40 % (reciprocal values are much lower, 11–18 %). The type strains *K. vulgare* DSM 4025^T and *K. robustum* X6L^T are patented strains (Urbance et al. 2001); thus, according to Rules 27(3) and 30 of the Bacteriological Code, these names are not validly published (see notes for the genus at Euzéby's site, now http://www.bacterio.net/).

Complete genome sequences of two industrial *K. vulgare* strains (Y25 and WSH-001) reveal that they are composed of a circular chromosome and two plasmids (sizes: ≈2.77 Mbp, 267–268 Kbp, and 242–243 Kbp for the plasmids). Genes encoding sorbose dehydrogenase are chromosomic and are in multiple (four) copies, hence the highly efficient conversion of sorbose exhibited by these strains (Xiong et al. 2011; Liu et al. 2011).

Leisingera

Leisingera is a genus described in 2002 by Schaefer and col., which currently contains three species: *L. methylohalidivorans*, *L. aquimarina* (Vandecandelaere et al. 2008a), and *L. nanhaiensis* (Sun et al. 2010). The most outstanding activity of *Leisingera* species is their ability to oxidize methyl halides (methyl chloride, methyl bromide) with the concomitant liberation of halide atoms into the atmosphere (where they contribute to ozone destruction). At the time of the description of *L. methylohalidivorans*, the type species of the genus, the knowledge of marine methyl halide-oxidizing strains was scarce. Strain MB2^T was obtained from enrichment cultures inoculated with tide pool seawater after more than 3 years of maintenance with MeBr as sole carbon and energy source. The strain grows as regular straight rods with methyl halides, methionine, or DMS on mineral medium but forms elongated rods and filaments when grown on yeast extract or glycine betaine. It is Gram negative, motile, and not pigmented. Additional genus features are as follows: obligate aerobic, moderately halophilic, and growing by oxidation of methyl halides or selected methylated substrates, such as methionine. The DNA G+C content of the type strain is 60 mol%. The emendation to the genus description by Martens et al. (2006) added the following information: Q10 is the predominant quinone; PG, PE, one phospholipid, one aminolipid, and two other lipids are produced; but PC is absent in the polar lipid profile; major fatty acids are C18:1 ω7c, C16:0 2OH, C16:0, 11-methyl C18:1 ω7c, C18:1 ω9c, C14:1, C10:0 3OH, and C12:0 3OH (amide linked). In addition, the type species is defined as having temperature and pH optima of 27 °C and 7.7, respectively, and requiring Na^+ for growth that occurs only with more than 1 % NaCl. It is catalase and oxidase positive, not able to respire with nitrate as alternative electron acceptor, and shows a narrow number of carbon sources, which include methyl halides, DMS, methionine, and glycine betaine as methylotrophic substrates, and excludes carbohydrates, amino acids (other than methionine), and small organic acids. Growth is possible on yeast extract and Casamino acids-based media and in Marine Broth.

L. aquimarina is closely related to the type species, to which it shows a 56 % value of DDH. In contrast to *L. methylohalidivorans*, it is unable to grow on methionine and it is pigmented (dark beige pink).

A third species was described recently, *L. nanhaiensis* (Sun et al. 2010), which uses both methionine and betaine and shows a profile of polar lipids, fatty acids, and DNA G+C content compatible with the genus description. However, it is not unambiguously linked to the other two species and shows a closer relationship with *Litorimicrobium taeanense*, another recently described member of the *Phaeobacter* subclade.

Leisingera-like isolates have been obtained as a part of the culturable microbiota of Chinese sea anemones (Du et al. 2010).

Lentibacter

The genus *Lentibacter* was recently described (Li et al. 2012) as containing the species *L. algarum*. The strains were isolated from a seawater sample obtained during a massive algal bloom dominated by *Enteromorpha prolifera* in China. The genus is defined as Gram negative, aerobic, not flagellated, and lacking Bchl *a*. Additional features included in the genus description are a narrow range of growth temperatures (22–28 °C) and slow growth; a salinity range of 3–9 %; oxidase, catalase, and PHB production; and ability to reduce nitrate to nitrite. Major fatty acids are C18:1 ω7c, C18:0, and C16:0. Major polar lipids are PG, PE, PC, one amino lipid, and one lipid. Q10 is the predominant respiratory quinone. The DNA G+C content is between 54 and 57 mol%. The species is notable for its wide pH range (from 2.0 to 9.0) enabling growth—the lowest pH registered in the whole *Roseobacter* group.

In addition to its original isolation, *Lentibacter* sp. has been simultaneously isolated and detected as a dominant band in DGGE from seawater samples obtained during algal blooms in the North Sea (Hahnke et al. 2013b).

Lentibacter algae forms a moderately deep lineage in the *Roseobacter* group, with *Nereida ignava* as relative.

Litoreibacter

Litoreibacter (Kim et al. 2012b; Romanenko et al. 2011a) contains four species: *L. albidus*, *L. janthinus*, *L. meonggei*, and *L. arenae* (formerly, *Thalassobacter arenae*). They have been isolated from marine invertebrates and sediments and they are defined by the following traits: Gram negative, strictly aerobic, oxidase and catalase positive, and rod-shaped bacteria which divide by budding. Chemoorganoheterotrophic. Sodium ion is essential for growth. The predominant isoprenoid quinone is Q10. Common polar lipids are PC, PG, PE, an unidentified lipid, and an aminolipid. The predominant fatty acid is C18:1 ω7c. The G+C content is 56–60.4 mol%. In addition to these characters, species of the genus lack Bchl *a*, do not reduce nitrate to nitrite, and are psychrotolerant (temperature minimum is 4–5 °C, optimum being 25–30 °C). Some species produce PHB granules (*L. meonggei* and *L. arenae*) and most are pigmented (*L. janthinus*, grayish violet; *L. arenae*, brown; and *L. meonggei*, yellow) and nonmotile (exception is *L. arenae*, which forms polar flagella).

DDH levels between *Litoreibacter* species are low: Romanenko et al. (2011a) reported levels of 15 % between type strains of *L. albidus* and *L. janthinus*. Kim et al. (2012b) found values of 12 %, 14 %, and 9 % when testing *L. meonggei* type strain against *L. albidus*, *L. janthinus*, and *L. arenae* type strains, respectively. The four species form a well-defined clade, with *Pacificibacter marinus* and *Roseovarius marinus* (that might be considered a misclassified species) as nearest neighbors. The draft genome of *L. arenae* DSM 19593^T has 3.69 Mb.

Litorimicrobium

The genus *Litorimicrobium* (Jin et al. 2011) currently contains the single species *L. taeanense*. It contains Gram-negative, nonmotile, ovoid rods, which are oxidase and catalase positive and reduce nitrates to nitrites. They present Q10 as predominant quinone and C18:1 ω7c as major fatty acid, followed by its 11-methyl derivative, C12:1 3OH, C16:0 2OH, and C16:0. Its most abundant polar lipids are PG, DPG, PC, an unidentified aminolipid, a phospholipid, and a lipid. DNA G+C content is 62.4 mol%.

The closest relative to *L. taeanense* in the 16S rRNA gene trees is *Leisingera nanhaiensis*, followed by several *Phaeobacter* species and *Seohaeicola saemankumensis*.

Loktanella

Loktanella is the most populated genus in the *Roseobacter* group, with a total of 13 species described so far (*Roseovarius* equals this figure but the taxonomic status of some of its species deserves a reevaluation). The genus was proposed by Van Trappen et al. (2004) after studying isolates from microbial mats of Antarctic lakes (Fryxell Lake, Ace Lake, Organic Lake, Pendant Lake) and has been further enlarged to encompass species isolated from diverse marine samples (seawater, sediments, and beach sand) around the world. Emended descriptions of the genus accompanied the descriptions of *L. pyoseonensis* (Moon et al. 2010), *L. tamlensis* (Lee 2012), and *L. cinnabarina* (Tsubouchi et al. 2013). The following are currently considered the basic properties of the genus: cells are Gram negative, rod shaped, nonspore-forming, strictly aerobic, chemoorganotrophic, and moderately halotolerant. Oxidase and catalase are positive. Motility is variable among species. Colony color is also variable (white, pink, beige, light orange). Optimal temperature is around 25 °C. The most abundant fatty acid is C18:1 ω7c, Q10 is the predominant quinone, and major polar lipids are DPG, PC, and PG. DNA G+C content is 55–69 mol%.

The genomes of two strains of *L. vestfoldensis* (DSM 16212^T and SKA53) have been sequenced: the draft genomes are 3.7 and 3.1 Mbp in size and have 61.8 and 60.0 mol% G+C content, respectively. *L. cinnabarina* LL-001^T and *L. hongkongensis* DSM 17492^T have similar genome sizes (3.9 Mb and 3.2 Mb, respectively) but higher G+C content (66.7 and 68.4 mol%, respectively).

Data from DDH experiments give the following figures for the species that have been submitted to this determination: *L. vestfoldensis*, *L. fryxellensis*, and *L. salsilacus* type strains are interrelated by values from 10 % to 18 % (Van Trappen et al. 2004); *L. vestfoldensis* and *L. agnita* show a 35 % relatedness (Ivanova et al. 2005); Hosoya and Yokota (2007a) reported a 28–36 % relatedness between *L. atrilutea* and *L. salsilacus* and an 11–31 between *L. atrilutea* and *L. fryxellensis*; *L. tamlensis* (Lee 2012) is related to *L. rosea* by 11–15 % values and by just 8 % to *L. maricola* (Yoon et al. 2007c); finally, *L. cinnabarina* and *L. hongkongensis* show 41–44 % DDH values (Tsubouchi et al. 2013).

The thirteen *Loktanella* species recognized so far differ in several traits, in addition to motility (three species are motile, *L. atrilutea*, *L. pyoseonensis*, and *L. tamlensis*) and pigmentation: one of them is the requirement of Na^+ for growth, which occurs in all but four species (*L. salsilacus*, *L. fryxellensis*, *L. vestfoldensis*, and *L. atrilutea*), nitrate reduction (positive only for *L. agnita*, *L. koreensis*, and *L. pyoseonensis*), psychrotolerant character (all but *L. litorea* and *L. cinnabarina* are able to grow at 4–8 °C), use of carbohydrates (present only in *L. hongkongensis*, *L. maricola*, *L. atrilutea*, *L. pyoseonensis*, and *L. litorea*), and Bchl *a* synthesis, found in *L. maricola* (although *L. vestfoldensis* also contains photosynthetic genes).

All *Loktanella* species are included in a single clade (*Fig. 20.5*) except for three: *L. hongkongensis*, *L. pyoseonensis*, and *L. cinnabarina* (all with DNA G+C content in the upper limit of the genus) that merge with the genus *Ketogulonicigenium*.

In addition to the isolation sites of the type strains, Loktanellas have been isolated and/or detected among the microbiota of hypersaline, cold environments (Jiang et al. 2010; Niederberger et al. 2010) and have been found associated to some members of marine fauna: tentacles of cnidarians (Doepke et al. 2012) and sea anemones (Du et al. 2010). *Loktanella* AAP clones have also been found in high proportions as a part of the AAP bacteria in localized basis of Central Baltic Sea (Salka et al. 2008). They are also a major part of clone libraries obtained at the surface of tropical South Pacific Ocean, along with SAR86 and unclassified Flavobacteria (Stevens and Ulloa 2008), components of biofilms formed in marine coastal water, after the first 24–36 h colonization (Lee et al. 2008), and members of summer community of ephemeral desert playa lakes (Costa et al. 2008).

L. rosea has raised interest for its highly unusual LPS composition, which includes an atypical lipid A composed of a trisaccharide backbone lacking the phosphate groups (two β-glucosamines plus an α-galacturonic acid) and a core region with unusual composition (Ieranò et al. 2010) not seen in other Gram-negative marine bacteria studied so far (Nazarenko et al. 2011).

Lutimaribacter

Lutimaribacter saemankumensis is the type and only species of the genus *Lutimaribacter* (Yoon et al. 2009a). They are Gram-negative, non-flagellated rods, positive for oxidase and catalase, and facultatively anaerobic. Q10 is the predominant quinone and C18:1 ω7c plus its 11-methyl derivative are the major cellular fatty acids. The species shows the following traits: it is pigmented (pale yellow), grows in Marine Agar in anaerobic conditions, does not reduce nitrates, requires Na^+ for growth, tolerates up to 10 % NaCl, and does not synthetize Bchl *a*. Its major polar lipids are PC, PG, PE, an unidentified aminolipid, and two phospholipids. The DNA G+C content is 63.5 mol%. The closest neighbor of *L. saemankumensis* is *Oceanicola pacificus*, one of the *Oceanicola* species that appears to be misclassified.

Mameliella

Mameliella (Zheng et al. 2010a) contains a single species, *M. alba*, isolated from seawater. The genus is described as follows: Gram-negative, nonmotile rods that multiply by binary fission and accumulate PHB granules. The predominant respiratory quinone is Q10. It is oxidase and catalase positive but negative for urease, hydrolysis of starch and gelatin, and indole production. Forms white colonies in RO (Rich Organic) medium containing (in grams per liter) yeast extract, 1.0; Bacto Peptone, 1.0; sodium acetate, 1.0; KCl, 0.3; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.05; NH_4Cl, 0.3; K_2HPO_4, 0.3; and NaCl, 20.0; plus 20 μg of vitamin B12, 200 μg of nicotinic acid, 80 μg of biotin, 400 μg of thiamine, and 1.0 ml of a trace element solution. The species is described as mesophilic and neutrophilic and is able to grow with 1–10 % NaCl in the medium and able to reduce nitrates and to use several carbohydrates and some organic acids. Contains C18:1 ω7c, C18:0, C12:1 3OH, C16:0, and 11-methyl C18:1 ω7c as major fatty acids. Its DNA G+C content is 63.7 mol%. In spite of its general lack of phenotypic distinctiveness, *M. alba* forms a distinct lineage in the 16S rRNA tree, forming a long branch connected to the pair *Antarctobacter heliothermus-Sagittula stellata*.

Maribius

The genus *Maribius* (Choi et al. 2007) contains two species *M. salinus* and *M. pelagius* isolated from distant saline waters (a Chinese solar saltern and Sargasso Sea seawater). They form a tight group, related to *Hwanghaeicola aestuarii* and to the newly described *Profundibacterium mesophilum*. The main characteristics of the genus are: rod-shaped, nonmotile, Gram-negative cells. They are obligate aerobic heterotrophs, positive for oxidase and catalase, and require Na^+ and Mg^{2+} for growth. They accumulate PHB and have Q10 as predominant quinone and C18:1 ω7c plus C19:0 cyclo ω8c as major fatty acids. Colonies on Marine Agar are opaque and beige in color and do not contain Bchl *a*. The DNA G+C content is 66–70 mol%. None of the species reduces nitrate to nitrite or hydrolyzes gelatin, but both are amylolytic. Surprisingly, the species isolated from seawater tolerates higher salinities (up to 15 %) than the one isolated from solar saltern water, *M. salinus* (up to 10 %).

Marinovum

A long path took the former *Roseobacter algicola* (Lafay et al. 1995) and then *Ruegeria algicola* (Uchino et al. 1998) to be finally recognized in a separate genus *Marinovum* (Martens et al. 2006) as *M. algicola*. The strains that served to describe the species were isolated from cultured, toxin-producing dinoflagellate *Prorocentrum lima*. The phylogenetic position of *Marinovum algicola* as an independent branch, separated from true *Roseobacter* and *Ruegeria* species, is clear in all analyses.

The genus contains Gram-negative, ovoid cells, motile through flagella that are subpolarly inserted. Colonies develop a pinkish-beige pigmentation after a few days incubation. They do not accumulate PHB but Lafay et al. (1995) show electron micrographs in which refringent spherical bodies can be seen inside the cells. They are strict aerobes, non-fermentative, unable to denitrify, and oxidase, catalase, and gelatinase positive. Do not synthetize Bchl *a*. They are mesophilic (optimum temperature 25–30 °C) and require Na^+, thiamine, and biotin for growth. They use several carbohydrates (hexoses, disaccharides) and some organic acids (pyruvate, malate, and citrate) as carbon sources. The major quinone is Q10; dominant fatty acids are C18:1 ω7c, 11-methyl C18:1 ω7c, C18:0, C12:0 3OH, and C10:0 3OH (amide linked); and major polar lipids are PG, PE, PC, an unidentified phospholipid, one lipid, and one aminolipid. The G+C content is not included in any of the three papers that concern the taxonomy of the genus. Pradella et al. (2010) determined the structure of the genome in several strains of *M. algicola*, including the type strain: it is composed of one circular chromosome (3.60–3.74 Mbp) and 9–12 plasmids (7–477 Kbp), giving a total genome size of 5.0–5.35 Mbp.

Maritimibacter

Maritimibacter alkaliphilus, the single species of the genus *Maritimibacter*, was isolated by applying dilution-to-extinction, high-throughput culturing methods to Sargasso Sea seawater (Lee et al. 2007a). The strain that served to describe the new genus and species is a Gram-negative, nonmotile, rod-shaped bacterium that does not accumulate PHB and does not synthetize Bchl *a* or carotenoids. It is chemoheterotrophic, strict aerobe and requires sodium ion for growth. Its dominant fatty acids are C16:0 2OH, C16:0, C18:1 ω7c (in lower proportion than is usual in the family), 11-methyl C18:1 ω7c, and C18:1 2OH. Q10 is the predominant quinone. Major polar lipids are PC, PE, and PG. In addition to these generic characteristics, the species presents the following traits: mesophilic (16–37 °C, optimum 30 °C), slightly halophilic (0.5–7.5 % NaCl, optimum 2.5–3.0 %), and alkaliphilic (4–12, optimum pH 10). Catalase, oxidase, and urease are positive. Although no nitrate reduction is detected in the API strips, the draft genome of the strain has been obtained, and it contains a complete nitrate reduction pathway to N_2. Other specific genes include two putative Na^+/H^+ antiporters, considered essential for alkaliphilic behavior. Genome size of *M. alkaliphilus* HTCC2654^T is 4.53 Mbp and its G+C content is 64 mol%. It contains 49 tRNA genes and one each of 5S rRNA, 16S rRNA, and 23S rRNA genes (Thrash et al. 2010a).

The position of *M. alkaliphilus* in the *Roseobacter* group is isolated, with no close relative and by the edge of the clade.

Marivita

The genus *Marivita* was proposed to accommodate two species, *M. cryptomonadis* and *M. litorea*, isolated from a culture of *Cryptomonas* sp. and a coastal seawater sample, respectively

(Hwang et al. 2009), and nowadays it contains six. Among the species added later, *M. byunsanensis* corresponds to a reclassification of the former *Gaetbulicola byunsanensis* (Yoon et al. 2010b) (so the genus *Gaetbulicola* is now "empty") and the remaining three have been isolated from seawater (*M. hallyeonensis*, *M. geojedonensis*) or estuarine water (*M. roseacus*). The genus has been emended twice (Budinoff et al. 2011; Yoon et al. 2012).

Marivita species form one of the few well-defined, tightly delimited, generic clades in the *Roseobacter* group, with all the six species closely located at the end of a rather long branch that joins to a clade of misplaced *Oceanicola* spp. (see below). Pairs of *Marivita* species for which DDH has been determined show low levels of relatedness: *M. cryptomonadis* and *M. litorea* show 13 % DDH value (Hwang et al. 2009); *M. hallyeonensis* shows 15, 17, and 11 % to *M. cryptomonadis*, *M. litorea*, and *M. byunsanensis*, respectively (Yoon et al. 2012); *M. roseacus* shows 30–33 % against *M. litorea* and 19–20 to *M. cryptomonadis* (Budinoff et al. 2011). Finally, *M. geojedonensis* presents values between 17 % and 22 % to *M. cryptomonadis*, *M. litorea*, *M. byunsanensis*, and *M. hallyeonensis* (Yoon et al. 2013b).

The genus, as presently recognized, contains Gram-negative, strictly aerobic, heterotrophic bacteria that contain genes for Bchl *a* synthesis (although some of them do not produce the pigment). Oxidase and catalase are positive. Cell morphology varies from short rods to long rods associated in chains or pleomorphic cells. May be motile by polar flagella or nonmotile. Major fatty acid is C18:1 ω7c. Q10 is the predominant quinone, while major polar lipids are PC, PG, PE, and an unidentified aminolipid. The DNA G+C content is 58–65 mol%.

Species of *Marivita* differ in motility (although there are contradictory data in different studies, see Budinoff et al. (2011) on motility and nitrate reduction activity of *M. cryptomonadis* and *M. litorea*), PHB production, nitrate reduction, pigmentation (from cream to pink or light yellow), and the use of different carbon sources (if any). All species require Na^+ for growth, some also Mg^{2+}, yet others combined sea salts. They are mesophilic, neutrophilic, and able to grow with 0.5–1.0 to a maximum of 6–10 % salinity, depending of the species. *M. cryptomonadis*, *M. litorea*, and *M. roseacus* have *puf* genes encoding phototrophic ability, but only *M. roseacus* synthetizes Bchl *a* in standard growth conditions. The rest of the species have not been tested for *puf* genes.

Nautella

The genus *Nautella* (Vandecandelaere et al. 2009a) contains one species, *N. italica*, isolated from a marine electroactive biofilm grown on a stainless steel cathode exposed to natural seawater. *Nautella* is defined as Gram-negative, motile, rod-shaped, strictly aerobic and moderately halophilic, with a positive response to catalase and oxidase tests and able to grow in the range of 4–45 °C.

The species is characterized by the following properties: cells are motile by a polar flagellum and accumulate PHB. Colonies are beige. They require Na^+ and grow from 1 % to 7 % NaCl (optimum 2–3 %). Temperature optimum is 20–28 °C and optimum pH is between 6.0 and 8.0. They are non-fermentative and do not reduce nitrate to nitrite or hydrolyze gelatin, casein, starch, chitin, or DNA. They contain C18:1 ω7c, C16:0 2OH, 11-methyl C18:1 ω7c, C10:0 3OH, C18:0 2OH, C12:0 3OH, C16:0, and C18:0 as major fatty acids. Their DNA G+C content is 61 mol% (Vandecandelaere et al. 2009a).

At the moment of its description, the closer relatives to *Nautella* were *Phaeobacter* species. Thus, in addition to the intraspecific level of DDH between the five isolates included in the species description, the relatedness with some *Phaeobacter* species (*P. daeponensis*, *P. inhibens*, and *P. gallaeciensis*) was also determined and found to be low (7–17 %).

Nautella-like isolate R11 (formerly *Ruegeria* sp. R11) has been characterized as an algal pathogen, causing bleaching disease in the marine red alga *Delisea pulchra* (Case et al. 2011). Sequencing and analysis of its genome has revealed clues for understanding the pathogenicity of the strain: adhesion mechanisms, transport of algal metabolites, oxidative stress protection, cytolysins, and other pathogen-related activities were found coded in the genome of *Nautella* sp. R11 (Fernandes et al. 2011). The genome of this strain is composed of a circular chromosome of 3.62 Mbp and a plasmid of 197 Kbp. Other closely related strains show detrimental activities in different marine organisms, as *Raphidophyceae* algae, sea urchins, and corals.

Nereida

Nereida was described as a genus containing Gram-negative, strictly aerobic, chemoorganotrophic, slightly halophilic bacteria (Pujalte et al. 2005a). They are positive for oxidase and catalase, mesophilic, require combined sea salts for growth and do not ferment carbohydrates or reduce nitrates. They do not form gas vesicles or PHB granules in the cells, which are nonmotile, coccoid, elongated, or tear-shaped rods that divide by budding. They contain C18:1 ω7c, C18:0, and C16:0 as main fatty acids and have a G+C content of 56 mol%.

The only species so far recognized in the genus is *N. ignava*, related to the sequences of uncultured symbiotic bacteria from the galls of the alga *Prionitis lanceolata* and neighbor to the recently described *Lentibacter algarum*. The species shows undetermined nutritional requirements and uses a narrow range of carbon sources, provided that the basal medium is supplemented with low amounts of yeast extract.

Oceanibulbus

Oceanibulbus indolifex is the sole species of the genus *Oceanibulbus* (Wagner-Döbler et al. 2004). This species is a member of the subclade containing *Roseobacter* and *Sulfitobacter* species. In fact, it is so closely related to the species *Sulfitobacter delicatus* and *S. dubius* (99.5 % 16S rRNA sequence

similarity, according to Ivanova et al. 2004) that they might well be members of a single genus, if only phylogenetic information were used to define the rank. *Oceanibulbus* is Gram-negative and nonmotile and the cells are irregular rods with swollen ends and inclusion bodies (PHB). They do not synthetize Bchl *a*. They are strictly aerobic, non-fermentative heterotrophs that require sea salts (NaCl alone does not support growth) and are able to develop in media with 1–7 % sea salts content. Optimum temperature and pH are 25–30 °C and 7.0–8.0, respectively. Oxidase reaction is weak. Do not reduce nitrates to nitrites. They contain Q10 as predominant quinone. PG, DPG, PC, PE, and an aminolipid are the main polar lipids and C18:1 ω7c, C18:0, C16:1 ω7c, C16:0, C10:0 3OH, and C12:1 3OH as major fatty acids. *O. indolifex* has a 60 mol% G+C content. It shows 21 % relatedness on DDH experiments to *Sulfitobacter mediterraneus* (Wagner-Döbler et al. 2004). To the best of our knowledge, the DDH levels between *O. indolifex* and *S. delicatus* and *S. dubius* have not been tested.

O. indolifex produces some interesting secondary metabolites, as indole and several indole derivatives, some cyclic dipeptides and tryptanthrin (Wagner-Döbler et al. 2004). It also produces sulfur volatiles during metabolism of S-containing compounds, as sulfides and thioesters, some of them were new natural compounds as 5-methyl phenylethanethioate and butyl methanesulfonate (Thiel et al. 2010). It was one of the species included in the development of genetic tools for investigation of regulatory and metabolic networks in the *Roseobacter* group (Piekarski et al. 2009). The genome of the type strain has an estimated size of 4.11 Mbp and contains 4,153 ORFs, with a G+C content of 59 mol%.

Oceanicola

The genus *Oceanicola* currently contains seven species: *O. granulosus*, the type species, and *O. batsensis*, both isolated by high-throughput culturing methods from seawater (Cho and Giovannoni 2004); *O. nanhaiensis*, obtained from deep (1,100 m) marine sediments (Gu et al. 2007); *O. marinus*, from seawater (Lin et al. 2007); *O. pacificus*, also from deep marine sediments (Yuan et al. 2009); *O. nitratireducens*, from seawater (Zheng et al. 2010b); and *O. litoreus* (Park et al. 2013a) from seashore sediment. The phylogenetic relationships among the members of the genus are, however, dubious, as the type species separates from the larger *Oceanicola* clade (*O. batsensis*, *O. nanhaiensis*, *O. nitratireducens*, *O. marinus*) and associates with *Roseisalinus antarcticus*, while *O. pacificus* groups very close to *Lutimaribacter saemankumensis* (both with NJ and ML). Given that the genus seems polyphyletic (as it was also recognized and reported by Thrash et al. (2010b) and Newton et al. (2010)), the generic assignment of all (but type) species is pending of a reevaluation taking into account all the neighboring new taxa recognized since the genus was first described. This situation is not uncommon; similar problems affect the genus *Roseovarius* and *Phaeobacter*. Despite the large separation in the 16S rRNA tree, the type strains of *O. granulosus* and *O. batsensis* displayed 48 % relatedness in DDH determinations (Wagner-Döbler et al. 2004). DDH values relate *O. marinus* to *O. granulosus* by 7 % and to *O. batsensis* by 26 % (Lin et al. 2007). *Oceanicola* comprises Gram-negative, nonmotile short rods that multiply by binary fission and accumulate PHB granules. They do not synthetize Bchl *a*. Their metabolism is chemoorganotrophic and aerobic to microaerotolerant (*O. marinus* and *O. litoreus* are described as facultative anaerobes due to their ability to grow on Marine Agar in anaerobic conditions, but they do not ferment glucose nor do they respire with nitrate). Oxidase test is positive but they are negative for denitrification, glucose acidification, arginine dihydrolase, indole production, and gelatinase. Main fatty acids are C18:1 ω7c, C16:0, and C 19:0 cyclo (except for *O. nitratireducens* and *O. nanhaiensis*). DNA G+C content is 64–73 mol%. Predominant quinone is reported to be Q10 for *O. nanhaiensis*, *O. nitratireducens*, and *O. litoreus*. Polar lipid composition is reported only for *O. litoreus* and *O. granulosus* (Park et al. 2013a) and consists of PC, PG, PE, an unidentified aminolipid, and a lipid for *O. litoreus*, while the type species lacks PE. Species of *Oceanicola* are neutrophilic and mesophilic but display a wide range of growth temperatures, with four of the species growing from 4 °C to 40 °C (*O. granulosus*, *O. batsensis*, *O. marinus*, and *O. litoreus*). All, except *O. nanhaiensis*, need Na^+ for growth (*O. litoreus*, also requires Mg^{2+}) and grow from 0.5 to 7–10 % salinity. They could be differentiated by the carbon sources used, presence of urease, minimum growth temperature, gelatin and esculin hydrolysis, and the percentage of the fatty acid C10:0 cyclo. As many other roseobacters, *Oceanicola* sp. participate in the transformation of DMSP in DMS, as documented by Curson et al. (2008) and Mou et al. (2005).

The genomes of *O. granulosus* and *O. batsensis* type strains have been sequenced (Thrash et al. 2010b). The genome of the type species comprises 4.04 Mbp, 3,855 ORFs and has a G+C content of 70.4 mol%. It contains genes for the Calvin cycle including the large (but not the small) RuBisCo subunit. The genome of *O. batsensis* comprises 4.44 Mbp, 4,261 ORFs and has a G+C content of 66 mol%. It does not contain *che* gene homologs, in contrast *to O. granulosus* genome. Both have putative genes for PHB synthesis. The draft genome of a third *Oceanicola* sp. strain able to degrade xylan has been reported (Kwon et al. 2012).

Oceaniovalibus

Oceaniovalibus and its single species *O. guishaninsula* (Liu et al. 2012) occupy a detached position, next to *Pontibaca methylaminivorans*, in the *Roseobacter* clade. It contains Gram-negative, ovoid to coccoid cells and is nonmotile and unable to produce PHB or synthetize Bchl *a*. Pigmentation of the colonies grown in Rich Organic medium or Marine Agar is pink. Catalase, oxidase, and nitrate reduction to nitrite are positive. They contain C18:1 ω7c, C19:0 cyclo, and C16:0 as major fatty acids and Q10 as predominant quinone.

O. guishaninsula was isolated from surface seawater. Its DNA G+C content is 62 mol%. It is neutrophilic (pH 4–10, optimum 6–9), mesophilic (16–40 °C, optimum 20–30 °C), and slightly halophilic (0.5–12 % NaCl, optimum 4–5 %). It is amylolytic but not gelatinolytic, uses as carbon sources some carbohydrates (L-arabinose, D-cellobiose, D-mannitol, and D-glucose), organic acids, and amino acids. Major polar lipids are PG and DPG. A draft genome (2.9 Mb) of the type strain JLT2003^T has been reported (Tang et al. 2012).

Octadecabacter

Octadecabacter is one of the oldest members of the *Roseobacter* group, described by Gosink et al. (1997) from sea ice samples obtained at the two polar regions. It contains heterotrophic, psychrophilic, gas vacuolated, nonpigmented bacteria distributed in two species: *O. arcticus* from Arctic ice samples and *O. antarcticus* from Antarctic sea ice. The two species maintain a moderate relatedness, a 42 % level of DDH (Gosink et al. 1997). Their distribution at the poles was the basis for discussion about biogeography of sea ice bacteria (Staley and Gosink 1999; Brinkmeyer et al. 2003).

Octadecabacter comprises Gram-negative, nonmotile rods with gas vesicles. They are aerobic to microaerophilic and do not reduce nitrates. Bacteriochlorophyll *a* is not synthetized. Catalase test is positive, but oxidase is negative (an uncommon trait in the *Roseobacter* group). True psychrophilic, they grow down to 4 °C (with maxima at 10–15 °C). No growth is observed without Na^+; the salinity growth range is 1.7 –7 %. They have nutritional requirements that may be covered by yeast extract, but even with the addition of this supplement, turbidity of the cultures is always low. Carbon sources used include L-glutamate, glycerol, and Casamino acids. The dominant fatty acid is C18:1 ω7c and the DNA G+C content is 56–57 mol%. *O. arcticus* requires thiamine, nicotinic acid, and pantothenic acid and grows up to 15 °C, while *O. antarcticus* grows only up to 10 °C. Both species contain, in addition to C18:1 ω7c, C16:1 ω7c, C16:0, and C10:0 3OH as major fatty acids.

The two species form a tight, isolated clade in the 16S rRNA tree.

Genomes of the types of both species have been finished and manually annotated (Vollmers et al. 2013). The genome size of *O. arcticus* 238^T is 5.20 Mbp (with 4,683 protein-coding genes) and 4.88 Mbp for *O. antarcticus* 307^T (4,492 protein-coding genes), both having the same DNA G+C content, 55 mol%, and exhibiting a high genome plasticity caused by an unusually high density and diversity of transposable elements. Interestingly, genes representing a new subgroup of xanthorhodopsins as an adaptation to icy environments were found in both *Octadecabacter* strains that differed from the previously characterized xanthorhodopsins of *Salinibacter ruber* and *Gloeobacter violaceus* in phylogeny, biogeography, and the potential to bind 4-keto-carotenoids. Biochemical characterization of the *Octadecabacter* xanthorhodopsins revealed that they function as light-driven proton pumps (Vollmers et al. 2013).

Pacificibacter

Pacificibacter (Romanenko et al. 2011b) accounts for marine bacteria which display the following properties: they are Gram-negative budding rods, strictly aerobic, chemoorganotrophic, and oxidase and catalase positive. They require sodium ions for growth. Q10 is the predominant quinone and their polar lipids contain PC, PG, DPG, and unidentified lipids as major components. The most abundant fatty acids are C18:1 ω7c, C16:0, C10:0 3OH, and C12:1 3OH. The only species of *Pacificibacter* currently recognized is *P. maritimus* (although the close position of *Roseovarius marinus* in the 16S rRNA tree suggests that it might be reclassified as a second species of *Pacificibacter*), which is nonmotile, unpigmented, does not synthetize Bchl *a*, and grows at 0.5–6 % salinity, at 2–36 °C (optimum 25–30 °C), and at pHs of 5.5–9.5 (6.5–8.5 optimum). It uses a few sugars (glucose, maltose, cellobiose, melibiose) and organic acids (acetate, lactate, and citrate) as carbon sources. *P. maritimus* is unable neither to reduce nitrates nor to hydrolyze gelatin, casein, or DNA. The DNA G+C content is 52.6 mol%.

Palleronia

Palleronia is one of the few genera in the *Roseobacter* clade that have been isolated from a non-marine habitat: its type species *P. marisminoris* was obtained from a saline soil sample in the surroundings of a solar saltern in Murcia, Spain (Martínez-Checa et al. 2005). *Palleronia* is defined as Gram negative, rod shaped, nonmotile, chemoheterotrophic, and aerobic. It does not synthetize Bchl *a* and it is unable to grow anaerobically either by fermentation, nitrate or fumarate reduction, or photoheterotrophy. Oxidase test is negative. They produce pink-pigmented colonies, are strictly halophilic, and require sodium, magnesium, and potassium ions for growth. They have a low nutritional versatility. Chemotaxonomic markers include Q10 as dominant quinone and C18:1 ω7c plus C19:0 cyclo ω8c as major fatty acids.

P. marisminoris is an exopolysaccharide-producing species that forms capsulated cells and mucoid colonies. Salinity growth range is 0.5–15 %, with an optimum at 5 %. Mesophilic, it grows from 20 °C to 37 °C and at pH from 5 to 10. It is negative for nitrate reduction to nitrite and for hydrolytic activities on gelatin, casein, lecithin, starch, Tween 80, or DNA. No growth was obtained in any of 35 carbon sources tested (Martínez-Checa et al. 2005). The cells accumulate polyhydroxyalkanoate granules. The DNA G+C content is 64 mol%.

The species is located next to *Tranquillimonas alkanivorans* and *Salipiger mucosus* (another taxon isolated from hypersaline soil).

Pelagibaca

Pelagibaca bermudensis is the only named species of the genus *Pelagibaca* (Cho and Giovannoni 2006). Isolated from Sargasso

Sea water by high-throughput culturing involving dilution to extinction, it is another new taxon recovered along with *Maritimibacter alkaliphilus*, *Oceanicola granulosus*, and *O. batsensis*. The genus is chemoheterotrophic and facultatively anaerobic. Cells are ovoid, nonmotile, divide by binary fission, and do not produce PHB or exopolysaccharides. Bchl *a* and carotenoids are absent. Able to reduce nitrate and nitrite. They require sodium ion for growth and are slightly halophilic. Glucose is acidified. They use a wide variety of sole carbon sources for growth. Their predominant fatty acid is C18:1 ω7c and its 11-methyl derivative. The only respiratory quinone is Q10 and the G+C content of DNA is 65.4 mol%.

P. bermudensis displays the following specific traits: grows between 10 °C and 40 °C (optimum 30–33 °C), at pH 5.5–10.5 (optimum 8.5) and from 0.25 % to 15 % salinity (optimum 3.0 %). It is oxidase and catalase positive; hydrolyzes gelatin, urea, and esculin; uses a large number of carbohydrates, organic acids, and amino acids as sole carbon and energy sources, including methanol. In addition to the fatty acids cited above, it contains more than 1 % of C16:0 and C12:0 3OH.

It is marginally related to the loose clade that contains *Saligipiger mucosus*, *Palleronia marisminoris*, and *Tranquillimonas alkanivorans*. At the time of its description, *Salipiger mucosus* was the nearest taxon, so the levels of DDH with its type strain were determined, resulting in a value of 26 % (Cho and Giovannoni 2006).

Pelagibaca sp. has been isolated, among other genera of the *Roseobacter* clade, as phthalate-degrading bacteria from seawater off the coast of Japan (Iwaki et al. 2012b).

A draft genome sequence of *P. bermudensis* HTCC2601^T has been obtained (Thrash et al. 2010a): it comprises 5.43 Mbp, 5,522 ORFs and has a G+C content of 66.4 mol%. It contains fifty-six tRNA genes, five 5S rRNA genes, four 16S rRNA genes, and five 23S rRNA genes. In addition to the general information for central metabolic pathways (complete glycolysis, Entner-Doudoroff pathway, TCA cycle), pathways for oxidation of C1 compounds are present, as well as a complete RubisCo complex, unique to the currently sequenced roseobacters. Other information includes biosynthesis of most essential amino acids and some vitamins, assimilatory nitrate reduction pathway, several type VI secretion genes, complete *sec* pathways, and a large number (362) of ABC transporters. These later gene groups are similarly present in other *Roseobacter* clade representatives sequenced so far.

Pelagicola

The genus *Pelagicola* and is type species *P. litoralis* (Kim et al. 2008b) are similar in most traits to other members of the group, except for a low G+C content, 47 mol%, which is the lowest recorded in the whole group and the only one below 50 mol%. Other traits are typical of roseobacters: they are Gram negative, nonmotile, strict aerobic and the cells are club shaped. Do not accumulate PHB. Catalase and oxidase are positive. Bchl *a* is absent. Q10 is the predominant quinone and C18:1 ω7c the major fatty acid. It contains PC, PG, PE, an unidentified aminolipid, and three lipids as major polar lipids. *Pelagicola litoralis*, which is close to the main *Roseovarius* clade, is not pigmented, grows with 2–6 % sea salt concentration (optimum 3–4 %), at 20–30 °C, and pH 6–8. Hydrolyzes starch and Tween 80 but not gelatin and does not reduce nitrate to nitrite. Sole carbon sources include acetate, betaine, glucose, L-lysine, L-proline, and L-serine. The type strain was isolated from costal seawater.

Pelagimonas

Pelagimonas is a recently described genus (Hahnke et al. 2013a) related to the *Sulfitobacter* subclade (in the paper) or to *Tropicibacter* genus (NJ tree, ❯ *Fig. 20.5*). The type strain of the type and only species *P. varians* was isolated from a seawater sample taken during an algal bloom at the North Sea. It is Gram negative, aerobic, chemoorganotrophic, catalase positive but oxidase negative. The cells are irregular rods. It does not synthetize Bchl *a* or other pigments, and *puf* genes for bacterial photosynthesis are absent. They require vitamins and sodium ion for growth. Major quinone is Q10. Dominant fatty acids are C18:1 ω7c and C18:2, accompanied by C10:0 3OH, C12:1, C14:1 3OH, C16:0, C18:0, and 11-methyl C18:1 ω7c. Major polar lipids are PC, PG, PE, DPG, PME, an unidentified aminolipid, one PL, and one lipid. The species is characterized by the presence of at least one flagellum, the ability to grow from 4 °C to 37 °C (optimum 28–32 °C), at pH 6.0–9.5 (optimum 7.0–8.5) and with salinities from 1.25 % to 8 % (optimum 1.25–5 %). It requires nicotinic acid amide. Does not reduce nitrate to nitrite. It grows with several amino acids, sugars, and organic acids as carbon sources. The G+C content of the DNA is 55 %.

Phaeobacter

The genus *Phaeobacter* currently contains five species: *P. gallaeciensis* (formerly *Roseobacter gallaeciensis*), the type species (Martens et al. 2006; Ruiz-Ponte et al. 1998), *P. inhibens* (Martens et al. 2006), *P. daeponensis* (Yoon et al. 2007d), *P. arcticus* (Zhang et al. 2008), and *P. caeruleus* (Vandecandelaere et al. 2009b). Phaeobacters are often regarded as efficient surface colonizes and as producers of inhibitory compounds that enable them to antagonize invertebrate settlement and algal or microbial growth.

General properties of the genus, as described by Martens et al. (2006) and emended by Yoon et al. (2007b) are as follows: cells are Gram negative, ovoid, and multiply by binary fission. They are motile by polar flagella and have a tendency to aggregate in liquid medium forming rosettes. Colonies are generally pigmented, brown/ochre, yellow, or blue. May produce a diffusible brownish pigment. Bchl *a* is absent. They do not grow photoheterotrophically or with methyl halides or DMS. Metabolism is chemoheterotrophic and obligately

aerobic. Oxidase and catalase are positive, but amylase, gelatinase, and tweenase are negative. The major respiratory lipoquinone is Q10. Polar lipids comprise PG, PE, PC, one aminolipid, and two phospholipids. Fatty acids comprise C18:1 ω7c, 11-methyl C18:1 ω7c, C16:0, C16:0 2OH, C18:0, C10:0 3OH, C14:1, C12:0 3OH (amide linked), and C14:1 3OH (amide linked).

The currently recognized species of *Phaeobacter* display a DNA G+C content of 55–65 mol%.

DDH experiments between different type strains of these species have been published in some of the original descriptions, for example, *P. gallaeciensis* and *P. inhibens* are related by figures of 16–20 % (while *P. gallaeciensis* showed less than 5 % against *Leisingera methylohalidivorans*, *Roseobacter* spp., and *Ruegeria*—now *Marinovum—algicola*). *P. daeponensis* was related to *P. gallaeciensis* and *P. inhibens* by values of 15 % and 18 %, respectively. *P. arcticus* showed 33 % relatedness to *P. inhibens*, the only species with more than 97 % 16S rRNA gene similarity at that time. Finally, *P. caeruleus* exhibits DDH levels of 25 % to *P. inhibens*, 28 % to *P. daeponensis*, and 40 % to *P. gallaeciensis*, well in the range for congeneric species. However, the values shown by this species towards type strains of closely related genera are, sometimes, even higher, and specially with two species of the genus *Leisingera*, the type species *L. methylohalidivorans* (55 %) and *L. aquimarina* (35 %). Also noticeable are the levels displayed with *Ruegeria atlantica* (23 %), *Silicibacter lacuscaerulensis* (29 %), and *Silicibacter pomeroyi* (18 %). These values are understandable in the light of the phylogenetic relationships revealed by comparative 16S rRNA analysis: as it can be observed in ❯ *Fig. 20.5*, the five *Phaeobacter* species are distributed in two subclades, one of them, that includes *P. gallaeciensis*, *P. inhibens*, and *P. arcticus*, relates with some recently described genera as *Seohaeicola* and *Litorimicrobium*, as well as to one misplaced *Leisingera* species, *L. nanhaiensis*, while the other two *Phaeobacter* spp., *P. daeponensis* and *P. caeruleus*, both merge with *Leisingera methylohalidivorans* and *L. aquimarina*, to which *P. caeruleus* showed the high DDH levels commented above. Clearly, a revision of the generic assignment of several species of the *Roseobacter* clade is pending, and among them *Phaeobacter* spp.

The draft genomes of three *P. gallaeciensis* and one *Phaeobacter* sp. strains are available: strain ANG1 (Collins and Nyholm 2011), strain DSM 17395^T, strain 2.10 (Thole et al. 2012) and strain Y4I (Roseobase). The estimated genome sizes are 4.16–4.59 Mbp, with 3,723–4,358 ORFs. G+C content is 59–62.6 mol% in *P. gallaeciensis* strains and 64 mol% in *Phaeobacter* sp. Y4I (an indigoidine producer).

An outstanding feature of some members of *Phaeobacter* is the ability to synthetize the antibiotic tropodithietic acid (TDA), which occurs also in members of the genera *Silicibacter* and *Ruegeria* (Brinkhoff et al. 2004). The antibiotic seems to have a key role in the interactions with algae and bacteria. TDA, a biologically active sulfur-containing tropolone compound, is synthetized in response to quorum sensing signals: *N*-acyl homoserine lactones (Berger et al. 2011) and TDA itself (Geng and Belas 2010) can act as autoinducers. Synthesis of the brown pigment is also controlled through the same regulatory mechanism and, consequently, they are enhanced in biofilms (and other aggregative growth forms, as rosettes or colonies).

Other bioactive compounds that have been studied among the secondary metabolites produced by phaeobacters are the roseobacticides (Seyedsayamdost et al. 2011a, b), compounds pertaining to the troponoid family, as TDA, which are produced and act as algaecides in response to algal senescence factors, changing the mutualistic behavior that maintain algal surface-colonizing phaeobacters to an opportunistic parasitic lifestyle. Another antimicrobial secondary metabolite indigoidine is produced by *Phaeobacter* sp. Y4I and has been demonstrated that contributes to competitive surface colonization and inhibition of *Vibrio* spp. (Cude et al. 2012). Indigoidine is synthetized via a nonribosomal peptide synthase (NRPS)-based biosynthetic pathway, one of the several identified in members of the *Roseobacter* clade (Martens et al. 2007).

As a consequence of its ability to efficiently adhere and grow on surfaces (Thole et al. 2012) and to secrete inhibitors active on other potentially harmful colonizers (pathogenic bacteria, fouling organisms), *Phaeobacter* spp. are candidates to be used as probiotics in marine aquaculture, where they have been studied as fish probiotics (Porsby et al. 2008; Prol et al. 2009; Pintado et al. 2010; D'Alvise et al. 2013) and bivalve probiotics (Prado et al. 2009; Kesarcodi-Watson et al. 2012).

In addition to the close link with algae and phytoplankton members, to which *Phaeobacter* cells associate (*Emiliania huxleyi*, e.g., Seyedsayamdost et al. 2011b), an intimate association has been documented between *P. gallaeciensis* and the accessory nidamental gland of the bobtail squid *Euprymna scolopes*, where its cells represent the dominant fraction of bacterial consortia (Collins and Nyholm 2011).

Planktotalea

Planktotalea (Hahnke et al. 2012) contains a single species, *P. frisia*, isolated from filtered seawater during a phytoplankton bloom in the North Sea. Although *Pelagicola litoralis* is cited as the nearest phylogenetic neighbor of *P. frisia* in its description, the analysis shown in ❯ *Fig. 20.5* presents *Planktotalea* as a distinct lineage in the subclade defined by *Litoreibacter* spp. and *Pacifibacter maritimus*.

Members of *Planktotalea* are Gram negative, irregular small rods. They are aerobic, oxidase and catalase positive, require vitamins and sodium ion for growth, and grow poorly on media with single (carbon) substrates. They possess *pufLM* genes. Major respiratory lipoquinone is Q10; polar lipids are PC, PG, an aminolipid, and a phospholipid, and the major fatty acids comprise C18:1 ω7c, C18:2, C16:0, C18:0, C12:1, C10:0 3OH, C12:1 3OH, and 11methyl C18:1 ω7c.

P. frisia forms small, unpigmented colonies on Saltwater Medium. Flagella are not observed and swimming motility was not clearly determined, although the cells exhibit wobbling. It grows in the ranges 4–32 °C (optimum 20–25 °C), pH 6.0–9.5 (optimum 7.5–9.0), and 1.25–8.0 % salinity (optimum 3.75 %).

It requires pantothenic acid and nicotinic acid amide. It is negative for denitrification, gelatin, and starch hydrolysis but positive for Tween-80 hydrolysis. It utilizes several amino acids, sugars, and organic acids as carbon and energy sources. Despite the presence of *pufLM* genes, Bchl *a* is not produced in laboratory cultures. The DNA G+C content is 53.8 mol%.

Pontibaca

Pontibaca methylaminovorans, the type species of the genus *Pontibaca* (Kim et al. 2010c), was isolated after an enrichment culture of a shallow marine sediment sample with trimethylamine in anaerobic conditions: as a result, two facultatively anaerobic strains were isolated which served to describe the new genus and species. Phylogenetically, *P. methylaminovorans* is loosely related to *Oceaniovalibus* and the subclade containing *Maribius* spp. (NJ) or to *Donghicola* (ML, not shown), but no close relative is evident in the trees.

Pontibaca is described as containing Gram-negative, nonmotile ovoid rods, which are slightly halophilic and facultatively anaerobic. They reduce nitrates and nitrites and do not synthetize Bchl *a*. Catalase and oxidase tests are positive. The major polar lipids are PC and PG. The dominant fatty acids include C18:1 ω7c, C16:0, and C19:0 cyclo ω8c. Q10 is the predominant respiratory quinone. The species is defined as unpigmented, mesophilic (15–37 °C, optimum 30 °C), neutrophilic (pH 6–10, optimum 7–8), and slightly halophilic (0.5–10 % NaCl, optimum 2–3 %). It presents urease activity and produces acid from several carbohydrates but does not hydrolyze gelatin, casein, or starch. Trimethylamine, dimethylamine, methyl amine, and tetramethylammonium are used as sole carbon sources both in aerobic and anaerobic conditions. It also uses acetate, 3-hydroxybutyrate, and L-serine. The DNA G+C content is 65 mol%.

Ponticoccus

Ponticoccus (Hwang and Cho 2008) contains a single species, *P. litoralis*, isolated from coastal seawater. The species joins to the base of the clade formed by *Antarctobacter heliothermus*, *Sagittula stellate*, and *Mameliella alba* but shows not close relatedness with them or the rest of the *Roseobacter* clade. The genus does not have a clear set of distinctive features and shows the same general profile of other roseobacters: Q10 as predominant quinone, C18:1 ω7c as major fatty acid (plus 11methyl C18:1 ω7c, C16:0, C18:0, and C12:1 3OH), and polar lipids that include PC, PG, PE one glycolipid, two aminolipids, and a lipid. The G+C content is 68 mol%. Cells are coccoid to rod shaped and nonmotile, strictly aerobic, and oxidase and catalase positive. No Bchl *a* is detected in the cultures. In addition, the species *P. litoralis* is characterized by the following traits: it is positive for PHB accumulation, mesophilic (10–37 °C, optimum 20 °C), neutrophilic (pH 6–8, optimum 7), and slightly halophilic (1–15 %, optimum 3–5 %), with ionic requirements that are not fulfilled only by sodium ion. It is positive for gelatin and starch hydrolysis but negative for Tween-80 hydrolysis. It reduces nitrate to nitrite and uses several carbohydrates, organic acids, and amino acids as sole carbon and energy sources.

Poseidonocella

Poseidonocella, containing the species *P. pacifica* and *P. sedimentorum*, was isolated from sediment samples taken at the Sea of Japan (Romanenko et al. 2012). The pair has *Roseivivax* spp. clade as the closest neighbor (*Fig. 20.5*) but relates to *Loktanella* spp. in other analyses. Generic features include Gram-negative cells with enlarged poles that divide by budding. They are strict aerobes, oxidase and catalase positive, chemoorganoheterotrophic, and Na^+ requiring. They do not produce Bchl *a*. PC, PG, DPG, phosphatidic acid, one aminolipid, and other lipids are present. C18:1 ω7c and its 11-methyl derivative are the major fatty acids. Q10 is the predominant quinone. The two recognized species have ovoid, nonmotile cells and produce beige or yellowish beige colonies. Their temperature range is 5–42 °C (*P. pacifica*) and 7–41 °C (*P. sedimentorum*) with optimum above 20 °C. They grow at pH from 4 to 9.5 and optimally at 7–8. Salinity ranges are 0.5–8 % for *P. pacifica* and slightly lower (up to 6 %) for *P. sedimentorum*, with optima between 2 % and 4 % NaCl. They are negative for nitrate reduction to nitrite. Both species are able to use glucose, maltose, citrate, L-serine and L-treonine, among others, as sole carbon and energy sources.

The G+C content of their DNA is 60–65.4 mol%.

Primorskyibacter

Primorskyibacter was described by Romanenko et al. (2011c) as a new genus on isolates obtained from the same source than the previous *Poseidonocella* spp. shallow sediments of the Sea of Japan. From a phylogenetic point of view, *P. sedentarius*, the type and sole species currently recognized in the genus, is a relative of *Marinovum algicola*, to which it merges in different analyses. The genus is defined as Gram negative, strictly aerobic, oxidase and catalase positive, rod shaped, and budding. It is chemoorganoheterotrophic and requires sodium ion for growth. The polar lipid profile includes PC, PE, PG, DPG, and one unidentified lipid. C18:1 ω7c and 11-methyl C18:1 ω7c are the major fatty acids and Q10 is the predominant respiratory quinone. The G+C content of the single species, *P. sedentarius*, is 60–62 mol%. The species produces nonmotile cells, is Bchl *a* negative, and is able to grow with only NaCl added to the medium. Growth occurs at salinities from 0.5 % to 8 % (optimally at 3–5 %) and at temperatures of 4–39 °C (optimum 25–30 °C). The pH range is 6.5–9.5 with its optimum at 7.5–8.5. It is unable to reduce nitrates or hydrolyze gelatin or starch. It uses glucose, maltose, L-rhamnose, L-arabinose, sucrose, fructose, cellobiose, raffinose, D-mannitol, L-ornithine, L-tyrosine, L-asparagine, citrate, acetate, fumarate, and malate as carbon and energy sources.

Profundibacterium

Profundibacterium mesophilum, the type and sole species of this recently described genus, was isolated from a deep sea sediment sample (Lai et al. 2013). The genus is described as Gram negative, strictly aerobic, chemoheterotrophic, slightly halophilic, and halotolerant. The cells are non-flagellated cocci. Oxidase and catalase tests are positive, while nitrate reduction is negative. Among the chemotaxonomic features, *Profundibacterium* shows Q10 as predominant quinone; its major fatty acids are C18:1 ω7C/ω6c, 11-methyl C18:1 ω7c, and C16:1 ω7c/ω6c. The DNA G+C content of the type species is 64 mol%. *P. mesophilum* is a slow-growing bacterium with huge spherical cells (5.0–8.0 μm diameter) that do not accumulate PHB. They require NaCl (0.5 %) and tolerate up to 20 % (optimum 2–6 %). Mesophilic and neutrophilic, they are unable to hydrolyze gelatin, casein, DNA, or starch and grow on few carbohydrates (D-galactose, D-xylose, D-glucose, glycerol), organic acids (pyruvate, succinate, citrate, propionate), and amino acids (L-glutamate, L-arginine) as sole carbon sources. Major polar lipids are PG and PE.

P. mesophilum is distantly related to *Hwanghaeicola* and *Maribius* species.

Pseudoruegeria

The genus *Pseudoruegeria* was described by Yoon et al. (2007e) with *P. aquimaris* as type species and emended recently (Jung et al. 2010a) to incorporate a second species, *P. lutimaris*. In addition to the common features shared with the vast majority of members of the *Roseobacter* group (Gram-negative, nonmotile, aerobic rods with Q10 as major quinone and C18:1 ω7c as dominant fatty acid). The G+C content is 67–73.5 mol%. The emended description of the genus states that PG plus an unidentified lipid and one aminolipid are the common polar lipids but the polar lipid profile is quite different between the two species: *P. aquimaris* shows PG, DPG, an aminolipid, and a lipid as major components while *P. lutimaris* contains PC, PG, PE, an aminolipid, a glycolipid, and a lipid (Jung et al. 2010a). The fatty acid composition is also very different, as none of the components of their profiles (see *Table 20.2* in Jung et al. 2010a) is common to both strains, with the only exception of C18:1 ω7c. DDH levels relating *P. aquimaris* and *P. lutimaris* DNAs are only 5 % (which is at the borderline of "noise"). Altogether, considering the high similarity in 16S rRNA gene sequences (96.6 %) and in *gyrB* sequences (79.4 %), Jung and col. (2010b) considered them members of the same genus.

P. aquimaris forms grayish-yellow colonies on Marine Agar, grows between 15 °C and 49 °C, and with up to 8 % NaCl (but not in the absence of this salt). Anaerobic growth with nitrate does not occur. Acid is produced from several carbohydrates, and fructose and malate are used as carbon and energy sources. *P. lutimaris* has a lower maximum temperature for growth (37 °C), requires not only sodium but also magnesium ions, and is able to grow in the absence of O_2 on Marine Agar (alone or with nitrate). Both species are positive for catalase and oxidase tests and are unable to hydrolyze gelatin, casein, starch, or Tweens.

The two species of the genus relate marginally to the subclade containing *Celeribacter*, *Vadicella*, and *Huaishuia* species.

Roseibacterium

Roseibacterium includes two species: *R. elongatum* (Suzuki et al. 2006) and *R. beibuensis* (Mao et al. 2012). The genus comprises bacteriochlorophyll-producing aerobes with a consistent phylogenetic relationship to *Dinoroseobacter shibae*, another AAP (*Fig. 20.5*). Phylogenies based on *pufM* gene sequences reveal a relationship between the photosynthetic genes of both species that is closer to *Jannaschia* sp. CCS1 than to *D. shibae* (Mao et al. 2012).

Roseibacterium species have been isolated from coastal sand and from surface marine water. The two species form pigmented colonies, pink to red, and their cells are nonmotile rods with monopolar growth and budding division. They are aerobic chemoorganotrophs and synthetize bacteriochlorophyll *a* under aerobic conditions. Oxidase and catalase are positive. C18:1 ω7c is the major cellular fatty acid and Q10 is the predominant quinone. While the type species *R. elongatum* requires sodium ion for growth (range 0.5–7.5 % NaCl), *R. beibuensis* is able to grow without NaCl, although it has its optimum at 3–4 % NaCl. Growth is optimal at 28–30 °C and pH is 7.5–8.0 for both species. They are urease positive and able to accumulate PHA but differ in the ability to reduce nitrates to nitrites and in the minor components of the fatty acid profile. The G+C content is 68–76 mol%.

A close phylogenetic neighbor of *Roseibacterium* was described recently (Csotonyi et al. 2011) and given the generic name of *Charonomicrobium*, a name without standing in nomenclature to date and lacking public available deposition of the type strain. *Charonomicrobium ambiphototrophicum* shows a 98.3 % 16S rRNA gene similarity in to *R. elongatum*. This level might be compatible with generic identity, but the species is described as unique and separated from other taxa in the vicinity due to its ability to grow as photoorganoheterotroph both in aerobic and anaerobic conditions in the light, a behavior not found in any of the phototrophic members of the family. EG17, the proposed type strain, produces LHI complex (expressed aerobically and anaerobically) and LHII complex (mostly anaerobically). It was found to yield proportionally the greatest aerobic photosynthetic biomass under oligotrophic conditions. Other features of *C. ambiphototrophicum* are oxidase negative, brown pigmentation, the requirement of yeast extract as growth factor, a salinity range of 2–16 % NaCl, and a G+C content of 65.6 mol%. Clearly, more studies are needed to assess the taxonomic relationships between this strain and members of the genus *Roseibacterium*.

Roseicyclus

Roseicyclus mahoneyensis, isolated from a meromictic saline lake from Canada, is currently the only species of the genus *Roseicyclus* and another AAP of the *Roseobacter* group (Rathgeber et al. 2005). In contrast with the majority of members of the family, cells of *Roseicyclus* are ring shaped (although rods and ovoid cells are also observed). They are similar to other AAP bacteria in the pigmentation (pink to purple), strictly aerobic character, lack of motility, and ability to accumulate PHA. They synthetize Bchl *a* and carotenoids and produce LHI and LHII complexes. They do not denitrify or ferment carbohydrates. *R. mahoneyensis* strains are able to grow with yeast extract, but also with acetate, pyruvate, butyrate, citrate, malate, succinate, lactate, fructose, glucose, or glutamate. They are mesophilic (4–37 °C) and slightly halophilic (0.5–10 % NaCl, sodium ion is required) and have a G+C content of 66 mol%.

The photosynthetic apparatus and photoinduced electron transfer of *R. mahoneyensis* strain ML6 have been analyzed recently (Rathgeber et al. 2012).

The species occupies an isolated position among the members of the *Roseobacter* group, as an independent lineage in the edge of the clade, with a distant connection to *Wenxinia marina* and the rubellimicrobia.

Roseisalinus

The hypersaline, meromictic, Antarctic Ekho Lake has been the source of several genera and species of the *Roseobacter* group, including *Roseisalinus antarcticus*, the only recognized species of the genus *Roseisalinus* (Labrenz et al. 2005). It is, as the previous genus, an AAP which synthetizes Bchl *a* and carotenoids in aerobic conditions, producing red colonies. The genus contains Gram-negative, motile rods which accumulate PHB and have complex ionic requirements. Growth in aerobic to microaerophilic conditions is positive with various carboxylic acids and sugars as carbon and energy sources. They do not grow photoautotrophically with H_2/CO_2, photoorganotrophically with glutamate or acetate or fermentatively with glucose. Oxidase, catalase, and peroxidase activities are present. Temperature range for growth is <3–33 °C, pH range is 5.5–9.5, and salinities allowing growth are 1–13 % artificial seawater. The peptidoglycan contains *m*-diaminopimelic acid. Polar lipids present are DPG, PG, and PC, but PE and phosphatidyl-momomethylethanolamine are absent. Dominant fatty acids are C18:1 ω7c and C16:0, with minor amounts of C18:0, C10:0 3OH, and C16:1 ω7c. The quinone system is Q10.

In addition to this generic profile, the species *R. antarcticus* is characterized by a strong tendency to form rosettes, monopolar growth that suggests budding division, and a DNA G+C content of 67 mol%.

The position of the species in the 16S rRNA gene tree suggests a moderate relationship to the type species of the genus *Oceanicola*, *O. granulosus*.

Roseivivax

The genus *Roseivivax* includes five species: *R. halodurans* and *R. halotolerans* (Suzuki et al. 1999b), *R. lentus* (Park et al. 2010), *R. isoporae* (Chen et al. 2012a), and *R. sediminis* (Xiao et al. 2012). Three of them (*R. halodurans*, *R. halotolerans* and *R. isoporae*) isolated from biological material (charophytes and stromatolites from a saline lake, coral tissues) synthetize Bchl *a* and form pink pigmented colonies. They form the core of the clade containing the five *Roseivivax* species (Fig. 20.5), while the other two members of the genus, *R. lentus* and *R. sediminis*, isolated from sediments from a tide flat and a salt mine crystallizer pond, respectively, do not produce Bchl *a* and develop grayish-yellow or cream-yellow colonies. The genus, as defined by Suzuki et al. (1999b) and emended by Park et al. (2010) and Chen et al. (2012a), includes Gram-negative chemoheterotrophic rods that, when motile, have subpolar flagella. Catalase and oxidase tests are positive. It may be aerobic or facultatively anaerobic (some species grow on Marine Agar under anaerobic conditions) and may synthetize Bchl *a* in aerobiosis. The quinone system is Q10. The major cellular fatty acid is C18:1 ω7c and the dominant polar lipids are PG, PE, and an unidentified phospholipid. G+C content of the DNA is 59–69 mol%. The species in the genus are mesophilic, neutrophilic, and variable in their preferred salinities: all of them tolerate salinities over 12 % (some up to 20 %) and two, including the type species *R. halodurans*, are able to grow without salt addition to the media; hence they have to be regarded as halotolerant rather than halophilic. They are able to use several sugars, organic acids, and some amino acids as sole carbon sources. Acid is produced from some carbohydrates and nitrate reduction to nitrite is variable among species. *R. isoporae* produces PC, DPG, phosphatidyl-dimethylethanolamine, and sulfo-quinovosil diacylglycerol, in addition to unidentified phospholipids, among its major polar lipids, exhibiting a more complex profile than the other *Roseivivax* species. In addition to the dominant fatty acid, C18:1 ω7c, other major components are C19:0 cyclo ω8c, 11-methyl C18:1 ω7c, C18:0, C16:0, and C12:0 3OH.

Roseivivax halodurans DSM 15395^T contains two large linear plasmids of 264 and 368 kbp and its *pufLM* genes are located in the chromosome (Pradella et al. 2004).

Roseovarius

Roseovarius is, together with *Loktanella*, the largest genus in the *Roseobacter* group, containing 13 species at present, but almost all phylogenetic analyses show that the genus is not coherent and comprises four different lineages dispersed among other roseobacters (Fig. 20.5): the true *Roseovarius*, grouped along with the type species *R. tolerans* (Labrenz et al. 1999) are *R. aestuarii* (Yoon et al. 2008), *R. mucosus* (Biebl et al. 2005b), *R. nanhaiticus* (Wang et al. 2010), and *R. nubinhibens* (González et al. 2003). Another group includes *R. crassostreae*

(Boettcher et al. 2005), causative agent of the juvenile oyster disease (JOD) or *Roseovarius* oyster disease (ROD), *R. halocynthiae* (Kim et al. 2012c), and *R. sediminilitoris* (Park and Yoon 2013). A third group is formed by *R. pacificus* (Wang et al. 2009b), *R. halotolerans* (Oh et al. 2009), *R. indicus* (Lai et al. 2011b), and *R. litoreus* (Jung et al. 2012b). This group merges with the "tolerans" group in ML analysis and both have *Pelagicola litoralis* marginally related (not shown). Finally, the species *R. marinus* (Jung et al. 2011) is kept apart from other *Roseovarius* species and very close to *Pacifibacter maritimus* (99.2 % 16S rRNA similarity), being a candidate for reclassification in this genus or even into the species (synonymy).

The genus description, not emended since the original publication, states that *Roseovarius* members are Gram-negative rods with one or both poles pointed, multiplying by monopolar growth (budding process). Motility and PHB accumulation may be positive, as well as Bchl *a* production and colony pigmentation. Temperature range enabling growth is from <3 °C to 43 °C, salinity from <1 % to 10 % NaCl, and pH from 5.3 to >9. They are heterotrophic, strict aerobes, and have no fermentative ability. They do not grow in anaerobiosis with nitrate and not able to grow photoautotrophically with H_2/CO_2 or photoorganotrophically with acetate or glutamate. Catalase, peroxidase, and cytochrome oxidase activities are positive, although the latter is weak. Cells contain the following polar lipids: DPG, PG, PC, PE, an unknown phospholipid, and an unknown lipid. The dominant fatty acid is C18:1 ω7c, accompanied by C18:2, C12:0 2OH, C12:1 3OH, C16:1, C16:0, and C18:0. Q10 is the major respiratory quinone. The G+C content of the type species is 62–64 mol%. The range of G+C content within the currently named species goes from 55.4 to 66 mol%. Some species require vitamins. Despite the absence of emendation to this description, some species of *Roseovarius* have been described as denitrifiers (*R. crassostreae*) or have different polar lipid profiles (*R. litoreus* lacks DPG). As a general rule, *Roseovarius* spp. require sodium ion for growth and some require also magnesium (*R. halocynthiae*, *R. marinus*, and *R. sediminilitoris*). The salinity range of some species reaches 20 % (*R. halotolerans*). Bchl *a* production is detected in strains of *R. tolerans* and *R. mucosus* (weakly in the later) and *pufLM* genes are present in the genome of *R. indicus* but absent for all other species except for *R. tolerans* and *R. mucosus* (Lai et al. 2011b). An outstanding activity of *R. nubinhibens* is its ability to demethylate dimethylsulfoniopropionate (DMSP), one of the key activities in the marine sulfur cycle.

DDH experiments between *Roseovarius* species have been performed for several pairs with the following results: González et al. (2003) reported 42 % between *R. nubinhibens* and *R. tolerans*; Boettcher et al. (2005) found 11 % relatedness between *R. crassostreae* and *R. tolerans* and 47 % between the former and *R. nubinhibens*; a 13 % DDH was found between *R. aestuarii* and *R. nubinhibens* by Yoon and coworkers (2008); *R. halotolerans* showed 1–5 % DDH relatedness to *R. tolerans*, *R. crassostreae*, and *R. nubinhibens* and 25 % to *R. mucosus*, according to Oh et al. (2009); the relationship between *R. indicus* and *R. halotolerans* and *R. pacificus* was 48 % and 44 %, respectively (Lai et al. 2011b); *R. halocynthiae* exhibited a 13 % relatedness to *R. crassostreae* (Kim et al. 2012c); and *R. litoreus* showed 33 % relatedness to *R. halotolerans* and 18 % to *R. pacificus* (Jung et al. 2012b). The recently described *R. sediminilitoris* was related to its neighbors *R. crassostreae* and *R. halocynthiae* by 16 and 22 % DDH, respectively (Park and Yoon 2013).

The genome of *R. nubinhibens* ISM^T has been sequenced and their general features are as follows: estimate genome size is 3.68 Mbp, estimated number of coding sequences is 3,547, and G+C content is 63.9 mol%. This strain has been the subject of several studies related to its activities on sulfur-containing organic molecules (González et al. 1999, 2003).

A "hidden" prophage has been reported in the genome of *R. nubinhibens* ISM^T (Zhao et al. 2010) that was unnoticed when employing methods aimed to classical prophage gene detection, but could be revealed through induction experiments.

Strains close to *R. tolerans* are able to produce free and organic iodine from iodide (Fuse et al. 2003), and iodide-oxidizing bacteria related to this species were isolated from iodide-rich natural gas brines and seawater (Amachi et al. 2005).

While members of the *Roseobacter* group are mostly non-pathogenic, a few exceptions could be found among *Roseovarius* species: *R. crassotreae* is involved in the juvenile oyster disease (JOD), also called *Roseovarius* oyster disease (ROD), a condition that causes seasonal mortalities among commercially produced eastern oysters (*Crassostrea virginica*) in the Northeastern coast of the USA. *R. crassotreae* is dominant among the bacterial consortia associated with the inner shell surface of JOD-affected animals. The polar attachment of the *R. crassostreae* cells to shell and conchiolin is believed to be related to polar fimbriae production and may protect the bacteria from hemocyte-mediated killing (Boardman et al. 2008). Culture-independent methods have revealed that strains similar to *R. crassostreae* are significantly associated to Australian Subtropical White Syndrome, an infectious, temperature-dependent disease of the coral *Turbinaria mesenterina*. The fact that this association is seen in a range of different types of disease lesions and on a range of different coral species suggests that these strains may be present simply as opportunists and are not directly responsible for causing the disease, but their possible role as pathogens cannot be ruled out (Godwin et al. 2012).

Rubellimicrobium

The genus *Rubellimicrobium* contains four species isolated from habitats other than the typical marine environments that inhabit most roseobacters. The first species described, *Rubellimicrobium thermophilum* (Denner et al. 2006), was isolated from colored biofilms formed in two fine-paper machines and a pulp dryer from the paper industry in Finland. The strains were slightly thermophilic, pigmented, and resistant to a common industrial biocide, 2,2-dibromo-3-nitrilopropionamide. Two other species, *R. mesophilum* (Dastager et al. 2008) and

R. roseum (Cao et al. 2010), were isolated from soil, while *R. aerolatum* was obtained from an air sample (Weon et al. 2009). In addition, field studies using culture-independent detection methods have revealed that *Rubellimicrobium* is a part of the characteristic soil communities of noncultivated soils, from which they disappear after agricultural use (Köberl et al. 2011). Along with *Acidobacteria*, they have been proposed as management indicator to discriminate between sustainable and non-sustainable agricultural practices (Figuerola et al. 2012). It has been also suggested that terrestrial hot springs might be a habitat for rubellimicrobia: isolate OSrt, enriched from Octopus Spring (Yellowstone Park, USA) and used as feed bacterium for the cultivation of the thermophilic amoeba *Echinamoeba thermarum*, is either a strain of *Rubellimicrobium thermophilum* or a closely related, unnamed species, based on 16S rRNA gene sequence similarity (>99 %) (Baumgartner et al. 2003; Denner et al. 2006).

Rubellimicrobium spp. form a well-defined subclade in the boundaries of the *Roseobacter* clade, to which *Roseicyclus mahoneyensis* and *Wenxinia marina* are loosely related (*Fig. 20.5*).

Defining features of the genus, as established by Denner et al. (2006), were as follows: Gram-negative, rod-shaped cells that produce carotenoids but not endospores, strict aerobes, positive for oxidase, and weakly positive for catalase. It is chemoorganoheterotrophic, using a large number of organic compounds for growth. Q10 is the major respiratory lipoquinone and DPG and PC are the major polar lipids, with minor amounts of PG. The predominant fatty acid is C19:0 cyclo ω8c, followed by C18:0 and C16:0. Main polyamines are spermidine, *sym*-homospermidine, and putrescine. DNA G+C content is 69–70 mol% for the type species, *R. thermophilum*, but the range for the four recognized species is 67–72 mol%. The type species differ from the rest by its moderate thermophily, having an optimum temperature for growth of 45–52 °C and a maximum of 56 °C, while the other three species are clearly mesophilic (28–30 °C optima, maxima 30–37 °C). Notable differences in polar lipid profile and cellular fatty acid composition are also found among species (probably as a consequence of the different growth conditions). In particular, the dominant fatty acids of the mesophilic rubellimicrobia are more similar to the typical major fatty acids of the rest of the roseobacters, with C18:1 ω7c as the most abundant one. Rubellimicrobia are non-halophilic, in consonance to their habitats; some of the species are sensitive to NaCl, which is not tolerated in the medium or only when lower than 1 %. All species form red colonies.

Draft genome of *R. thermophilum* DSM 16684^T, which is 3.16 Mb in size, contains 3,244 protein-coding genes.

Ruegeria

Ruegeria, one of the earliest described genera in the *Roseobacter* group, contains ten species. The history of this genus is an example of the taxonomic turmoil undergone at other parts of the group. The type species of the genus *R. atlantica* was formerly known as *Agrobacterium atlanticum*. Uchino and coworkers reclassified this and other species of the so-called marine agrobacteria (Rüger and Höfle 1992) in three new genera, *Stappia*, *Ahrensia*, and *Ruegeria* (Uchino et al. 1998). At that time, the genus comprised the species *Ruegeria atlantica* (resulting from the reclassification of *Agrobacterium atlanticum* and *Agrobacterium meteori*, both considered synonyms), *Ruegeria gelatinovora* (from *Agrobacterium gelatinovorum*), and *Ruegeria algicola* (from *Roseobacter algicola*). *R. gelatinovora* was later reclassified in the genus *Thalassobius* (Arahal et al. 2005) and *R. algicola* became *Marinovum algicola* (Martens et al. 2006), leaving the genus with only one species. Shortly after, Yi et al. (2007) proposed to transfer the two species of the genus *Silicibacter*, *S. lacuscaerulensis* (Petursdottir and Kristjansson 1999), and *S. pomeroyi* (González et al. 2003) to the genus *Ruegeria*, based on the close phylogenetic relationship between the three taxa (sequence similarities of 96.9–98.2 % on 16S rRNA gene, monophyletic clade), and emended the genus for a second time (a first emendation was proposed by Martens et al. 2006). The transfer has not been universally accepted, as some authors still support the maintenance of *Silicibacter* genus based on phenotypic differences (Muramatsu et al. 2007). Since, the following species have been described within the genus: *R. mobilis* (Muramatsu et al. 2007), *R. pelagia* (Lee et al. 2007c; found to be a later heterotypic synonym of *R. mobilis* by Lai et al. 2010a), *R. scottomollicae* (Vandecandelaere et al. 2008b), *R. marina* (Huo et al. 2011), *R. faecimaris* (Oh et al. 2011a), *R. halocynthiae* (Kim et al. 2012d), *R. arenilitoris* (Park and Yoon 2012b), and *R. conchae* (Lee et al. 2012b). All species have been isolated from marine samples, except for *R. lacuscaerulensis*, that were isolated from the water of the Blue Lagoon, a shallow geothermal lake in Iceland.

All *Ruegeria* species, including the two reclassified *Silicibacter*, merge in one clade that includes also *Sediminimonas* and *Salinihabitants* and have the clade *Phaeobacter caeruleus-Phaeobacter daeponensis-Leisingera sensu stricto-Nautella italica* as closest neighbor (*Fig. 20.5*).

After the last emendation, the genus is defined by the following traits: Gram-negative, chemoheterotrophic, strict aerobes, negative for Bchl *a* production and *puf* genes; they accumulate PHB and require sea salts for growth. Oxidase and catalase are positive. When motile, cells have polar flagella. Q10 is the dominant quinone, major fatty acids are C18:1 ω7c and 11-methyl C18:1 ω7c, and polar lipids include PC, PE, PG, and several phospholipids. The DNA G+C content is 55–68 mol%. Some exceptions to this profile can be found among currently recognized species; thus, *R. mobilis* and *R. marina* do not have a strict requirement of salt for growth, and PE is not detected among the polar lipids of several species (*R. faecimaris*, *R. halocynthiae*, *R. arenilitoris*, and *R. conchae*). Some species denitrify (*R. atlantica*, *R. lacuscaerulensis*); others are described as able to reduce nitrate or to grow with nitrate in anaerobic conditions, but denitrification ability is not confirmed (*R. faecimaris*, *R. arenilitoris*, *R. halocynthiae*). *R. lacuscaerulensis*

and *R. mobilis* produce tan or brown pigments, which may be related to antifouling/antimicrobial activities, in a way similar to that of *Phaeobacter* species (Porsby et al. 2008; Gram et al. 2010). Riclea et al. (2012) have identified five lactones in the volatile fraction of *R. pomeroyi* DSS-3. The structures of these lactones have been unambiguously assigned by comparison to synthetic standards that showed, in agar diffusion assays, specific activity against algae, but not against bacteria or fungi, suggesting that the lactones may have an ecological function in the interaction between the bacteria and the algae in fading algal blooms, similar to the recently described roseobacticides from *P. gallaeciensis*, which are active against *Emiliania huxleyi*.

Ruegeria species are relevant for the metabolism of DMSP in the marine environment, being *R. pomeroyi* one of the most studied contributors (González et al. 2003; Reisch et al. 2008, 2011; Todd et al. 2011, 2012).

The genomes of up to six *Ruegeria* strains have been sequenced (Roseobase), being *R. pomeroyi* type strain, DSS-3^T, the first of the *Roseobacter* clade that was completed and analyzed (Moran et al. 2004). It contains 4,283 coding sequences distributed between a chromosome of 4.11 Mbp and one megaplasmid of 491 Kbp. Three rRNA operons and 53 tRNA genes are present. Analysis of gene content revealed a metabolic strategy based on lithotrophic exploitation of carbon monoxide and sulfide, combined with heterotrophy. Abundant genes encoding for ABC-type transporters of peptides, amino acids, polyamines, and osmolytes (glycine betaine, DMSP) are also present, as well as multiple TRAP transporter systems. Other *Ruegeria* genomes also contain genes for carbon monoxide oxidation, namely, strain TM1040 (Moran et al. 2007) and TW15—now *R. conchae* (Lee et al. 2011b). Quorum-sensing related genes are also present in some *Ruegeria* genomes, including the one of *R. pomeroyi* DDS-3^T and *Ruegeria* sp. KLH11 (Zan et al. 2011).

An interesting approach by high-throughput proteogenomics has been conducted on *R. pomeroyi* DDS-3^T aiming at a better genomic annotation for the whole marine *Roseobacter* clade (Christie-Oleza et al. 2012). For this, a large dataset of peptides was obtained after searching over 1.1 million MS/MS spectra, from *R. pomeroyi* DDS-3^T cultivated under 30 different conditions against a six-frame translated genome database. The authors detected open reading frames that had not previously been annotated and genes that were wrongly annotated. By extending these re-annotations to the genomes of the other 36 *Roseobacter* isolates (20 different genera) available at that time, they proposed the correction of the assigned start codons of 1,082 homologous genes in the clade and reported the presence of novel genes within operons encoding determinants of the important tricarboxylic acid cycle, a feature characteristic of some *Roseobacter* genomes.

Bacteriophages infecting *R. pomeroyi* (Zhao et al. 2009) and *Ruegeria* sp. strain TM1040 (Chen et al. 2006) have been detected and characterized. The former were lytic phages isolated from seawater, while the later were obtained after induction with mitomycin C and were assumed to be attenuated phages representative of three of the five prophage-like elements detected in the genome of TM1040.

Sagittula

Sagittula stellata is the type species of the genus *Sagittula* (González et al. 1997), one of the oldest members of the *Roseobacter* group and distantly related to *Antarctobacter heliothermus*, *Ponticoccus litoralis*, and *Mameliella alba* (Fig. 20.5). The type strain E-37^T was isolated from a marine enrichment community obtained from salt marsh seawater in Georgia (USA) and grown on pulp mill effluent, rich in lignin.

The genus is defined by the following traits: cells are rod shaped and Gram negative, have a polar holdfast, and numerous vesicles on the surface, derived from the outer membrane. They form rosettes and aggregates. They are strict aerobes, oxidase and catalase positive, and grow on sugars, fatty acids, and amino acids. The type species also uses methanol and some aromatic compounds. They require sea salts for growth. In a recent emendation of the genus, Lee et al. (2013b) added to these generic properties the presence of Q10 as major respiratory quinone and a complex polar lipid profile: DPG, PC, PE, PG, and several unidentified (two ALs, one PL, and four Ls) polar lipids. These authors added a second species to the genus *S. marina*, which shows biochemical and physiological features similar to the ones of *S. stellata*: both species are mesophilic, neutrophilic, and grow up to 7 % NaCl; do not reduce nitrates; are able to use several carbohydrates, organic acids, and amino acids; and have C18:1 ω7c, C12:1 3OH, 11-methyl C18:1 ω7c, and C16:0 as major fatty acids. However, *S. marina* lacks the ability to degrade carboxymethylcellulose and to partially solubilize synthetic lignin in presence of glucose, defining properties of the genus according to González (2005), among other differences. The C+G content of the genus is 61–65 mol%.

The estimated genome size of E-37^T, type strain of *S. stellata*, is 5.26 Mbp and contains 5,067 coding sequences (Roseobase). *S. stellata* produces amphiphilic exopolymeric substances which induce self-assembly of marine dissolved organic matter and formation of microgels (Ding et al. 2008), and it is able to use dimethylsulfide oxidation as an anergy source, while growing on fructose or succinate (Boden et al. 2011).

Salinihabitans

Salinihabitans currently contains a single species, *S. flavidus* (Yoon et al. 2009b), represented by a single strain that shows optimal growth at 7 % NaCl and tolerates up to 17 % in the culture media. Thus, it is a true moderate halophile better adapted to the conditions of the isolation habitat, a marine solar saltern, than most other roseobacters. It forms pale yellow colonies on Marine Agar, is mesophilic, neutrophilic, and strictly aerobic. Chemotaxonomic features include Q10 as predominant quinone and a fatty acid composition that features C19:0 cyclo ω8c as the major fatty acid (46 % of the total), an atypical trait in the whole family (the usually dominant fatty acid is 18:1 ω7c, which represents only 18 % of the total in *S. flavidus* profile). This trait, being the only that clearly

differentiates this taxon from other related roseobacters, might be due to methodological bias (Yoon et al. 2009b).

Salinihabitans flavidus merges the *Ruegeria* clade in the complete analysis of the *Rhodobacteraceae*, along with *Sediminimonas qiaohouensis*.

Salipiger

The genus *Salipiger* was described based on an isolate from a saline soil bordering a saltern, at the Spanish Mediterranean coast (Martínez-Cánovas et al. 2004). To date, it contains a single species, *S. mucosus*, named after its ability to produce an exopolysaccharide. This polysaccharide incorporates notorious amounts of sulfates, an unusual trait in other bacterial counterparts, according to Llamas et al. (2010) who characterized its composition and properties.

The genus is related to *Tranquillimonas* and *Palleronia*, the later isolated from the same geographic area than *Salipiger mucosus* type strain.

Characters defining the genus are as follows: Gram-negative, nonmotile rods; strictly aerobic chemoorganotrophs; unable to grow in anaerobic conditions either by fermentation, nitrate or fumarate reduction, or photoheterotrophy; produce polyhydroxyalkanoates (PHA); and are oxidase and catalase positive. Strictly halophilic, they require Na ion for growth and display very low nutritional and biochemical versatility. Contain ubiquinone Q10 and present C18:1 ω7c and C16:0 as major cellular fatty acids.

The species *S. mucosus* forms encapsulated cells and mucoid colonies, growing optimally with 9–10 % sea salts added to the medium (total range 0.5–20 % NaCl), at 20–40 °C and at pH 6 to 10. Hydrolytic abilities are restricted to urea and Tween 20. It does not synthetize Bchl *a* and is unable to use any of the sole carbon and energy sources tested. The C+G molar content is 64.5 mol%. The draft genome of *S. mucosus* DSM 16094^T has 5.7 Mb.

Sediminimonas

Sediminimonas, described by Wang et al. (2009c), accommodates one species, *S. qiaohouensis*, a relative to the *Ruegeria* clade and to the genus *Salinihabitans*. The species was isolated from a salt mine sediment in China but has been also detected by DGGE in coastal seawater (South China Sea; Yang et al. 2012).

The genus contains Gram-negative, nonmotile rods that are aerobic and unable to synthetize Bchl *a*, do not produce PHB or exopolysaccharides, and are able to reduce nitrates to nitrites. It is chemoheterotrophic and strictly halophilic, require NaCl for growth, and are able to use a variety of carbon sources, including carbohydrates. Major fatty acids are C18:1 ω7c, C16:0, C18:1 ω9c, 11-methyl C18:1 ω7c, and C19:0 cyclo ω8c. Polar lipids include DPG, PG, PC, and four unknown PLs. Q10 is the only respiratory quinone and the G+C molar content of the type and only species is 63–64 mol%. The draft genome of *S. qiaohouensis* DSM 21189^T has 3.55 Mb.

S. qiaohouensis is able to grow between 0.25 % and 20 % NaCl content, having optimal growth between 1.5 % and 10 % NaCl. Colonies are faint brown to yellow colored. It is mesophilic and neutrophilic, positive for catalase and oxidase, able to use several sugars (D-glucose, D-fructose, D-galactose, maltose, D-mannose, sucrose, D-psicose, trehalose, turanose), sugar alcohols (glycerol, D-mannitol), and some amino acids (D- and L-alanine, L-glutamic acid, and L-serine).

Seohaeicola

Seohaeicola is another monospecific genus: *S. saemankumensis*, the only species recognized up to date, was described based on a single strain isolated from a tidal flat (Yoon et al. 2009c). It is close to the *Phaeobacter sensu stricto* clade. Main generic traits include the presence of Q10 as predominant quinone, C18:1 ω7c and 11-methyl C18:1 ω7c as major cellular fatty acids, and PC, PG, and PE plus an unidentified lipid as major polar lipids. The cells are Gram negative, ovoid to coccoid, and nonmotile. The species forms pale yellow colonies on Marine Agar and does not synthetize Bchl *a*. It is unable to growth without NaCl or with more than 7 %. Growth is possible between 4 °C and 40 °C. The strain grows anaerobically in Marine Agar with or without nitrate, which is reduced. Catalase and oxidase tests are positive. Acetate was used as sole carbon and energy source. The G+C molar content is 63.4 mol%.

Shimia

Shimia marina, the type species of the genus, was described by Choi and Cho (2006b) after the study of one isolate obtained from a fish farm biofilm. A second species, *S. isoporae*, was later described to accommodate novel isolates from the reef-building coral *Isopora palifera* (Chen et al. 2011b). The genus is related to the clade that contains all the *Thalassobius* species plus *Epibacterium ulvae*.

Traits defining the genus *Shimia* include presence of Q10 and C18:1 ω7c as dominant quinone and cellular fatty acid, respectively, absence of Bchl *a*, a positive response to oxidase and catalase tests, and a heterotrophic, strictly aerobic metabolism. Their cells are Gram negative, rod shaped, and do not contain cytoplasmic granules.

Shimia species are slightly halophilic (with salinity ranges of 1–5 %, *S. isoporae*, and 1–7 % NaCl, *S. marina*), neutrophilic (optimal growth at pH 7–8), and mesophilic (optimal growth at 30–35 °C, *S. marina*, or 25–30 °C, *S. isoporae*). They are motile by monopolar flagella. Both species of *Shimia* are related by DDH levels around 43 % (Chen et al. 2011b) and differ in their response to nitrate reduction, indole, arginine dihydrolase, lipase, lecithinase, and β-galactosidase activities, as well as in their ability to assimilate some sugars (glucose, maltose, and mannose).

Sulfitobacter

The genus *Sulfitobacter* is one of the oldest members of the *Roseobacter* group. It was described by Sorokin (1995) to encompass a new heterotrophic, marine species able to oxidize sulfite, *Sulfitobacter pontiacus*, isolated from the Black Sea. Currently, the genus contains nine species, including the former *Staleya guttiformis* that was reclassified as *Sulfitobacter guttiformis* (Yoon et al. 2007f). The nine species are grouped in a stable clade that also includes *Oceanibulbus indolifex* as a very close relative to the pair *S. delicatus* and *S. dubius*. Next to the clade two *Roseobacter* species appear as their closest neighbors. A deeply branching clade that contains *Sulfitobacter* (plus *Oceanibulbus*) and *Roseobacter* species is also recovered by analyzing 70 conserved, single-copy genes retrieved from 32 roseobacters genomes (Newton et al. 2010), confirming the solid nature of the *Sulfitobacter-Oceanibulbus-Roseobacter* grouping.

Members of the genus *Sulfitobacter* have rod-shaped cells, sometimes pointed for one pole and showing monopolar growth and budding division. Rosette formation is common. When motile, flagella are inserted subpolarly. Several species, including the type species, accumulate PHB granules. None of them synthetizes Bchl *a*, with the exception of some *S. guttiformis* strains, a trait that contributed to its former consideration as a different genus. Photosynthetic genes are plasmid located in *S. guttiformis* (Pradella et al. 2004), and the plasmid has an independent origin from the one in *Roseobacter denitrificans*, the other AAP with plasmid-encoded photosynthetic gene clusters (Petersen et al. 2012). Sulfite oxidation is displayed by five species, being *S. pontiacus* the one with higher tolerance to this compound (up to 60 mM). *S. pontiacus* derives energy from sulfite oxidation during growth on organic compounds as acetate. Thus, it can be considered a lithoheterotroph. It is also able to oxidize sulfur and thiosulfate. Ability to perform oxidation of sulfite to sulfate is dependent of an AMP-independent soluble sulfite dehydrogenase (Sorokin et al. 2005a). As obligate heterotrophs, sulfitobacters are able to assimilate a variety of organic acids and amino acids. They are strict aerobes and present oxidase and catalase activities. Peptidoglycan contains *m*-DAP in *S. guttiformis* and *S. brevis**. Common polar lipids of the genus are PC, PG, and PE (with low levels of DPG in some species and the occasional presence of one AL or one PL), and main cellular fatty acids correspond to C18:1 ω7c, C16:0, C10:0 3OH, and 11-methyl C18:1 ω7c. Predominant quinone is Q10 and the G+C content of their DNA is 55 to 64 mol%.

*[Gorshkova et al. (2007) reported a surprising finding in *Sulfitobacter brevis* KKM 6006: a teichoic acid containing glycerol, ribitol, and *N*-Acetyl-D-glucosamine. To the best of our knowledge, this is the first time a teichoic acid is found in Gram-negative bacteria].

The highest interspecific value of DDH between *Sulfitobacter* species relates the pair *S. pontiacus-S. mediterraneus* by 46 % (Pukall et al. 1999). Ivanova et al. (2004) determined the DDH values for the type strains of *S. dubius* and *S. delicatus* against *S. pontiacus*, *S. mediterraneus*, *S. brevis*, and *S. guttiformis*, with the higher figure relating *S. mediterraneus* and *S. dubius* (41 % DDH) and the lower *S. guttiformis* and *S. delicatus* (5 %). Yoon et al. (2007g) found values of 9–21 % DDH between *S. marinus* and the *Sulfitobacter* species previously described. *S. litoralis* and *S. pontiacus* are related by a 24 % DDH (Park et al. 2007) and *S. donghicola* and *S. guttiformis* show a 17 % DDH, according to Yoon et al. (2007f).

Sulfitobacter species are widespread in marine habitats: they have been detected/isolated from seawater, biofilms, sediments, marine animals, and algae and aquaculture environments. In addition to the original isolation from Black Sea waters (Sorokin 1995), they have been found as part of the bacterioplankton of polar seawater (Mergaert et al. 2001), deep layer oxycline at South Pacific waters (Stevens and Ulloa 2008), Adriatic seawater (Silović et al. 2012), near-shore mud in French Guiana (Madrid et al. 2001), biofilms formed in submersed glass (Kwon et al. 2002), and microbial mats in Antarctic lakes (Van Trappen et al. 2002), and they are enriched in oil-contaminated seawater (Brakstad and Lødeng 2005; Prabagaran et al. 2007; Jung et al. 2010b). As epiphytes, they have been found on diatoms (Shäfer et al. 2002; Hünken et al. 2008), marine brown and red algae (Beleneva and Zhukova 2006), or dinoflagellates—*Alexandrium fundyense* (Li et al. 2011b). Isolation from marine animals is also documented, both from wild and cultured fish and invertebrates: Griffiths et al. (2001) isolated *Sulfitobacter* strains from healthy haddock larvae; McIntosh et al. (2008) found them in cod larvae, and several invertebrates also show *Sulfitobacter* colonization—spiny lobster larvae (Payne et al. 2008), cultivated mollusks (Beleneva et al. 2007), sea anemones (Du et al. 2010), cnidarians (Schuett and Doepke 2010), and corals (Higuchi et al. 2012).

Sulfitobacter participates in the metabolism of diverse sulfur compounds in marine environments: they are among the bacteria that show elevated nucleic acid content when the water is amended with DMSP (Mou et al. 2005) and, in fact, are able to metabolize DMSP to DMS (Curson et al. 2008). Other interesting metabolic abilities of members of the genus are related to phthalate degradation (Iwaki et al. 2012b). *S. pontiacus* sulfite oxidase has been investigated with the aim of constructing a biosensor system for sulfite detection on food and beverages (Muffler and Ulber 2008).

The ethanol extract of strain P1-17B, an isolate close to *S. pontiacus* and collected from the interface of brine pools and sea water of the Red Sea, was among the most potent against tested cancer cell lines in a study of bioactive molecules with cytotoxic and apoptotic activity (Sagar et al. 2013).

Genomes of two *Sulfitobacter* strains have been sequenced: *Sulfitobacter* EE-36 has an estimated genome size of 3.55 Mbp with 3,474 coding sequences and a G+C molar content of 60.3 mol%. This strain is a host for a bacteriophage isolated from Baltimore Inner Harbor water (Zhao et al. 2009), similar to the one isolated from *Ruegeria pomeroyi* DSS-3 by the same authors. *Sulfitobacter* NAS-14.1, an isolate that was obtained

with DMSP as sole carbon source, has a genome of 4.0 Mbp in size that contains 3,962 coding sequences (Roseobase).

Tateyamaria

The genus *Tateyamaria* (Kurahashi and Yokota 2007) was raised to encompass a single species, *T. omphalii*, isolated from the shell of a molluskan collected in Japan coastal waters. Three years later, a second species was described, *T. pelophila*, on isolates obtained from tidal flat sediments from the German coast of the North Sea. The description of the second species was accompanied by an emendation of the genus (Sass et al. 2010). The pair of species is located nearby *Nereida*, *Lentibacter*, and the *Roseobacter-Sulfitobacter* clade, in the 16S rRNA-based tree (Fig. 20.5).

The emended description of the genus establishes that *Tateyamaria* members are Gram-negative coccoid or short rods that may be motile by polar flagella and require sodium chloride for growth. They are aerobic, mesophilic, and positive for oxidase, catalase, and phosphatase activities. They may produce Bchl *a*. Q10 is the predominant respiratory quinone. Cellular fatty acids comprise C18:1 ω7c, C16:0, 11-methyl C18:1 ω7c, C19:0 cyclo ω8c, C10:0 3 OH, and C12:0 3 OH. The G+C content of the two currently recognized species is 56.4–61.6 mol%.

T. omphalii and *T. pelophila* differ in several traits, as motility and the ability for Bchl *a* production, both positive in the second species. *T. pelophila* is also able to oxidize sulfite, thiosulfate, and hydrogen while growing lithoheterotrophically. Its salinity range is wider (0.3–10 % NaCl) than the one of *T. omphalii* (0.2–6 % NaCl), but the maximal temperature for growth is lower. Both species require biotin and pantothenate (plus thiamine in *T. pelophila*). Polar lipids have been determined only for *T. pelophila*, which shows PG and PE as major components and only trace amounts of PC and an unidentified lipid.

Tateyamaria is one of the genera recently isolated from soft corals and shown to display antimicrobial activities (Chen et al. 2012b).

Thalassobacter

The genus *Thalassobacter* (Macián et al. 2005b) is phylogenetically allocated in the vicinity of the genus *Jannaschia* (Fig. 20.5). It contains a single species, *T. stenotrophicus*, which includes the strain used for the genus description, along with the ones originally proposed as members of *Jannaschia cystaugens* (Adachi et al. 2004), later considered a synonym of *T. stenotrophicus* (Pujalte et al. 2005b).

The genus includes Gram-negative ovoid to irregular rods, motile by one polar flagellum. They are strict aerobes, chemoorganotrophic, and slightly halophilic, positive for catalase and oxidase. Cells show budding and binary division and accumulate PHB. Bchl *a* is produced. Seawater or a complex mixture of marine salts is required for growth. Mesophilic. No hydrolytic activities on polymeric substrates (proteins, polysaccharides, lipids, nucleic acids) are observed. Major polar lipids are PG, DPG, and PC. PE is absent. Major cellular fatty acids are C18:1 ω7c, 11-methyl C18:1 ω7c, C18:1 ω9c, and C20:1 ω7c. The G+C content of its DNA is around 59 mol%. The predominant respiratory quinone was determined by Adachi et al. (2004) to be Q10.

In 2009 a second species, *T. arenae*, was described by Kim et al., but it was later reclassified into the genus *Litoreibacter* as *L. arenae* (Kim et al. 2012b).

T. stenotrophicus grows on Marine Agar producing reddish to brown pigmentation and does not reduce nitrate to nitrite or gas. The type strain is able to use several organic acids and amino acids as sole carbon and energy source (pyruvate, acetate, 2-oxoglutarate, succinate, 3 hydroxybutyrate, L-glutamate, L-ornithine, L-citrulline, and putrescine) provided that the basal medium is supplemented with small amounts of yeast extract, a fact that suggests growth factor requirements. The preferred carbon sources of the species were confirmed in enrichment experiments by Gómez-Consarnau et al. (2012) who found that *Thalassobacter* sp. increased in relative abundance in seawater amended with pyruvate and amino acids.

The strains formerly known as *J. cystaugens* are able to inhibit cyst formation on toxic dinoflagellate *Alexandrium tamarense* (Adachi et al. 2002).

Thalassobius

The genus *Thalassobius* was established by Arahal et al. (2005) to account for two species, *T. mediterraneus* type species, isolated from seawater, and *T. gelatinovorus*, which corresponds to the reclassification of the former *Ruegeria gelatinovorans* (Uchino et al. 1998). Currently, two more species are included in the genus: *T. aestuarii* (Yi and Chun 2006) and *T. maritimus* (Park et al. 2012). The four species merge in a group that also includes *Epibacterium ulvae* (specifically related to *T. aestuarii*), both species of *Shimia*, *Thalassococcus* and *Octadecabacter* (Fig. 20.5), with *T. maritimus* being the more loosely related to the *Thalassobius* core species.

Thalassobius species present the following features: they are Gram-negative, strictly aerobic, chemoorganotrophic bacteria that divide by binary fission. Cells are coccoid to rod shaped and accumulate PHB. They require seawater or combined marine salts for growth and are slightly halophilic and mesophilic. They do not ferment carbohydrates and prefer organic acids and amino acids as carbon sources. Their G+C content ranges from 57–61 mol%. The predominant quinone is Q10. Major cellular fatty acid is C18:1 ω7c, with minor amounts of C16:0, C18:0, and 11-methyl C18:1 ω7c. Park et al. (2012) determined polar lipids for the four species, which include PE and PG and smaller amounts of PC plus an unidentified lipid.

T. aestuarii shows DDH values of 20 % and 43 % to *T. mediterraneus* and *T. gelatinovorus*, respectively (Yi and Chun 2006), while *T. maritimus* is related to *T. gelatinovorus* (its nearest species) by 17 % (Park et al. 2012). The four species could be differentiated by motility and nitrate reduction (both positive for *T. aestuarii* and *T. gelatinovorus*), a distinctive range of hydrolytic abilities on casein, gelatin, Tweens, and urea and differences on the carbon source pattern (Park et al. 2012).

Unidentified *Thalassobius* spp. have been related to epizootic shell disease (ESD) lesions on American lobster, *Homarus americanus*, and other crustacea. The lesions are colonized by a Bacteroidetes bacterium (*Aquimarina* sp.) altogether with *Thalassobius* sp. (Quinn et al. 2012; Chistoserdov et al. 2012). Algicidal activities have been also reported for strains tentatively identified as *Thalassobius* (Oh et al. 2011b; Wang et al. 2010b). A *Thalassobius* sp. strain able to utilize phthalate has been isolated from seawater (Iwaki et al. 2012b).

The genome draft of a strain of *Thalassobius* sp., R2A62, has been obtained (Roseobase) and reveals a genome size of 3.49 Mbp, 3,696 coding sequences, and a G+C content of 55 mol%, slightly below of the one of the species described so far.

Thalassococcus

Closely related to *Thalassobius* species, the genus *Thalassococcus* contained a single species *T. halodurans* (Lee et al. 2007b) until the recent proposal of *Thalassococcus lentus* (Park et al. 2013b). The type species was isolated from the surface of a marine sponge, *Halichondria panicea*, at the pacific coast of the USA.

Defining features of the genus are as follows: Gram-negative, ovoid-shaped, nonpigmented cells. They are nonmotile, oxidase and catalase positive, strictly aerobic, and halophilic. Q10 is the major respiratory quinone and C18:1 ω7c, C18:1 ω9c, C16:0, and C18:0 are the major cellular fatty acids. *T. halodurans* requires at least 2% NaCl for growth and is able to grow in the presence of up to 18 %. It is mesophilic and neutrophilic, lacks extracellular hydrolytic abilities, and reduces nitrates to nitrite, but not to N_2. It produces acid from some carbohydrates on API 50CH and is also able to use carbohydrates as carbon sources (glycerol, glucose, and galactose, among others). The G+C content of its DNA is 58.8 mol% (58 % for *T. lentus*, which is related by a 17 % DDH value to *T. halodurans*, according to Park et al. 2013b).

Tranquillimonas

This monospecific genus occupies an isolated position near *Salipiger mucosus* and *Palleronia marisminoris* in the 16S rRNA tree. Its type and single species, *Tranquillimonas alkanivorans* (Harwati et al. 2008), was isolated from seawater at the Semarang Port, Indonesia, during a search for hydrocarbon-degrading bacteria, and, as indicated by the species name, it is able to degrade alkanes ($C_{10–13}$). Its general characteristics fit the profile of the majority of roseobacters, with no other special trait: they are Gram negative, nonmotile, obligately halophilic, requiring Na ion for growth, oxidase and catalase positive, and pink pigmented on Marine Agar. Cells contain polyhydroxyalkanoates, Q10 as major respiratory quinone, and C18:1 ω7c, C19:0 cyclo ω8c, and C16:0 as major fatty acids. DNA G+C content is 69 mol%. *T. alkanivorans* is able to grow up to 13 % NaCl and from 10 °C to 50 °C, with an optimum at 43 °C. It uses a variety of carbon sources on Biolog plates, including carbohydrates, organic acids, and amino acids, and is able to reduce nitrate to nitrite.

Tropicibacter

In 2009, Harwati and colleagues proposed a new genus, *Tropicibacter*, to allocate a marine species able to degrade aromatic hydrocarbons. *Tropicibacter naphthalenivorans* (Harwati et al. 2009a) was isolated from Indonesian Semarang Port seawater and demonstrated to degrade hydrocarbons in crude oil (Harwati et al. 2007). The same study also originated the isolates later described as *Tropicimonas isoalkanivorans* (Harwati et al. 2009b). The type species of *Tropicibacter* was soon followed by new members of the genus, *T. multivorans* (Lucena et al. 2012b), *T. phthalicicus* (Iwaki et al. 2012a), *T. mediterraneus*, and *T. litoreus* (Lucena et al. 2013). *Tropicibacter* species are close to *Pelagimonas* and to the clade of *Phaeobacter sensu stricto* and relatives (*Fig. 20.5*).

The genus was described as containing Gram-negative, rod-shaped, peritrichously flagellated rods, but species described lately failed to show motility (*T. mediterraneus* and *T. litoreus*) or, when motile, displayed polarly flagellated cells (*T. multivorans*, *T. phthalicicus*). All species require sodium ion for growth and some of them also magnesium or calcium ions. The oxidase test is positive. Three of the species, including the type, are able to reduce nitrate to nitrite or to nitrogen gas. PHB accumulation is found only in *T. naphthalenivorans*. As it is usual among roseobacters, they contain Q10 as predominant quinone and their major cellular fatty acid is C18:1 ω7c, followed by C16:0 and 11-methyl C18:1 ω7c. Polar lipids have been determined in two species: they include PE, PG, PC, and AL and four PLs in *T. multivorans*, while *T. mediterraneus* contains PG, PE, and unidentified amino- and phospholipids, and lipids but lacks PC. The G+C content ranges from 58 to 64.6 mol%.

ANI values were used as alternative parameter to DDH for species delineation of *T. mediterraneus* and *T. litoreus*: the former species contains four strains that showed 97.5–99 % ANIb (98.9–99.7 ANIm) while interspecies values were 85–86 % ANIb (90 % ANIm) between both species (Lucena et al. 2013).

Tropicimonas

Harwati and coworkers described *Tropicimonas* after studying isolates obtained from the same location and samples that gave origin to the description of *Tropicibacter*, Semarang Port

seawater (Indonesia). The type species of their new genus, *Tropicimonas isoalkanivorans*, was able to degrade alkanes, branched alkanes, and alkylnaphthalenes (Harwati et al. 2009b). Two additional species have been recently added: *T. aquimaris*, which description was accompanied by an emended description of the genus (Oh et al. 2012), and *T. sediminicola* (Shin et al. 2012), both isolated from marine samples. Phylogenetic relatives of *Tropicimonas* species within the *Rhodobacteraceae sensu lato* are somewhat difficult to determine, as there is not always agreement between the trees obtained by different reconstruction methods. A relation with *Thioclava* was reported at the time of genus description and is recovered with NJ methods, but most parsimonious analyses fail to support this relation and locate the genus within *Roseobacter* group (this study, MP tree, not shown and LTP 111). In any case, all three species merge in a well-defined clade.

The genus contains members with the following features: cells are Gram negative, peritrichously flagellated (when motile), rod shaped, and oxidase positive. Catalase and PHA accumulation are variable. Halophilic, they require sodium ion for growth (but *T. sediminicola* is able to grow without NaCl). They grow optimally at 30–37 °C. Although Q10 is the most abundant respiratory quinone (57–60 %), it is accompanied by substantial amounts of others: 36 % of Q9 in *T. aquimaris* and two quinones of undetermined chain length in *T. isoalkanivorans*, accounting for 28 % and 14 % each. The major cellular fatty acids are C18:1 ω7c, C18:1 ω9c, and C16:0. Common major polar lipids are PC, PG, an unidentified aminolipid, and an unidentified lipid. G+C content of their DNA is 66.5–69.6 mol%.

All *Tropicimonas* species described so far are able to reduce nitrates to nitrites and *T. aquimaris* grows on Marine Agar incubated anaerobically, with and without nitrate supplementation. They grow at least up to 40 °C (*T. isoalkanivorans* up to 46 °C) and up to 6 % salinity. They produce acids from several carbohydrates and are able to assimilate a variety of carbon sources. *T. isoalkanivorans* is able to use decane, pristane, and methylnaphthalene for growth, but it is unable to grow on thiosulfate as energy source.

T. aquimaris shows a DNA-DNA relatedness of 12 % to the type species *T. isoalkanivorans* (Oh et al. 2012). On the other hand, *T. sediminicola* is related to *T. aquimaris* by DDH values of 46 %, while only a 6 % DDH could be found with *T. isoalkanivorans* (Shin et al. 2012).

Vadicella

The genus *Vadicella* contains a single species, *V. arenosi*, described after the study of several isolates obtained from a sandy sediment sample of the Sea of Japan (Romanenko et al. 2011d). The species has the genus *Celeribacter* as closest phylogenetic relative and belongs to the clade that also includes *Huaishuia* and *Pseudoruegeria* (Fig. 20.5). Basic properties defining the genus are as follows: they are rod shaped, Gram negative, strictly aerobic, oxidase and catalase positive, and chemoorganotrophic. They have an absolute requirement for sodium ion. They do not synthetize Bchl *a*. Their predominant quinone is Q10. Polar lipids include PC, PG, phosphatidic acid, an unknown aminolipid, and an unknown lipid; PE is present in minor amounts. Their major cellular fatty acids are C18:1 ω7c, 11-methyl C18:1 ω7c, C12:1, and C10:0 3OH. The only currently recognized species of *Vadicella* is nonmotile and nonpigmented, grows from 1 % to 7 % NaCl, from 4 °C to 37 °C, and from pH 5.5 to 9.5. It reduces nitrate and does not produces acid from carbohydrates. It uses a few sugars and organic acids as carbon and energy sources. The G+C content of its DNA is 56.7–60 mol%.

Wenxinia

Wenxinia marina represents the only recognized species so far in the genus *Wenxinia* (Ying et al. 2007). The type and single strain of this species was isolated from the sediment of an oil field (100 m depth) in the South China Sea. *Wenxinia* occupies a marginal position on the *Roseobacter* group, having a distant relationship to the genera *Rubellimicrobium* and *Roseicyclus*, at the edge of the clade.

The genus was described as containing Gram-negative, ovoid or short rods that are nonmotile, strictly aerobic, and heterotrophic. They require NaCl for growth and are slightly pigmented of pink, but do not produce Bchl *a*. Catalase and oxidase tests are positive. They reduce nitrates to nitrites. Their quinone system is Q10; they contain PG, PC, and an unidentified glycolipid as major polar lipids and have C18:1 ω7c and C16:0 as major cellular fatty acids.

W. marina is able to grow from 15 °C to 42 °C, from 0.5 % to 9 % NaCl, and at pH 6.5–8.5. It accumulates PHA and contains PE and an unidentified phospholipid as minor components of its polar lipid profile. It is able to produce acid from some carbohydrates and uses several sugars, organic acids, and amino acids. The C+G content of its DNA is 69.4 mol%. The draft genome of *W. marina* DSM 24838^T has 4.2 Mb.

Yangia

The genus *Yangia* belongs to the subclade that contains *Citreicella* and *Citreimonas* (Fig. 20.5). It was recognized as a new genus by Dai et al. (2006) and contains a single species, *Y. pacifica*, whose type and only characterized strain was isolated from a coastal sediment of the East China Sea. The genus is defined by the following properties: cells are Gram-negative, motile rods. They are aerobic, heterotrophic, and require NaCl for growth. They form slightly yellowish colonies on Marine Agar, which are positive for catalase and weakly positive for oxidase tests. Q10 is the predominant quinone, C18:1 ω7c and C16:0 are the major fatty acids, and C12:0 is also present.

Y. pacifica, the type species, does not produce Bchl *a*, accumulates PHB, is negative for nitrate reduction, and grows at 22–40 °C, with 1–10 % NaCl and with maltose, lactate,

malate, arginine, and glutamate as sole carbon sources. The DNA G+C content is 63.3 mol%.

In a survey about polyester production by halophilic and halotolerant bacterial strains obtained from mangrove soil samples located in Northern Vietnam, five strains (QN187, ND199, ND218, ND240, and QN271) phylogenetically close to *Y. pacifica* were found to accumulate PHAs in noticeable amounts. Strains QN187, ND240, and QN271 synthesized poly(3-hydroxybutyrate) (PHB) from glucose, whereas strains ND199 and ND218 synthesized poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) from this carbohydrate (Van-Thuoc et al. 2012).

Genera Not Belonging (Phylogenetically) to *Rhodobacteraceae Sensu Stricto*

The Stappia Group

The group formed by the genera *Stappia*, *Labrenzia*, *Nesiotobacter*, *Pannonibacter*, *Polymorphum*, *Pseudovibrio*, and *Roseibium* (Fig. 20.6) constitute the clearest candidate to the rank of new family in the currently heterogeneous *Rhodobacteraceae sensu lato*. These genera form a well-defined, stable, and neat clade, separated from *Rhodobacteraceae sensu stricto* by several other alphaproteobacterial branches that constitute families by themselves (*Hyphomonadaceae*, *Koordimonadaceae*, *Phyllobacterriaceae*, *Hyphomicrobiaceae*, etc., Fig. 20.1). The detachment of stappias from the rest of *Rhodobacteraceae* was already noted by Gupta and Mok (2007) in their phylogenomic study of the alphaproteobacteria, on the basis of signature proteins. These authors suggested the placement of *Labrenzia* (*Stappia*) *aggregata* among *Rhizobiaceae*, although more recent analysis based on 16S rRNA gene of the whole group indicates a separate position from this family (LTP111).

Stappias are aerobic or facultatively anaerobic and chemoorganotrophic but some species do contain *pufLM* genes (*Roseibium* spp., *Labrenzia alexandrii*, *Stappia stellulata*) and are able to synthetize Bchl *a*, although its amount may be very low in some species (Biebl et al. 2007). Members of the group display interesting activities related to the production of different bioactive compounds, carbon monoxide oxidation, aromatic ring cleavage, heavy metal detoxification, and oil degradation abilities. While several of the species are isolated from marine water and sediments, some were isolated from lake water, hot springs, or saline soil, as well as from marine invertebrates and surfaces of aquatic plants and algae. One species has been isolated from human blood samples and the other is related to an oyster disease affecting juveniles of *Crassostrea virginica* cultured in USA (Table 20.8).

Stappia

The genus *Stappia* has a history that parallels that of *Ruegeria*, in the *Roseobacter* group. As *Ruegeria*, *Stappia* was established as a new genus by Uchino et al. (1998) to accommodate species previously known as marine agrobacteria: *Agrobacterium stellulatum* and *Agrobacterium aggregatum* (Rüger and Höfle 1992). The new genus *Stappia* was phylogenetically distant to agrobacteria and was defined in terms of chemotaxonomic as well as physiological and biochemical properties (Uchino et al. 1998). After the description of two additional species, *S. alba* (Pujalte et al. 2005c) and *S. marina* (Kim et al. 2006b), Biebl and colleagues (2007) rebuilt the group by creating a new genus, *Labrenzia*, to which they transferred *S. alba* and *S. marina* as new combinations. Almost simultaneously, Weber and King (2007) proposed four new *Stappia* species which names have never been validated and thus have no standard in nomenclature (*S. meyerae*, *S. conradae*, *S. kahanamokuae*, and *S. carboxydovorans*). 16S rRNA genes of these species were analyzed by Lai et al. (2010b) who found they probably are more related to *Labrenzia* and *Pannonibacter* than to *Stappia*. The genus *Stappia* remained as monospecific until then with the description of *S. indica* (Lai et al. 2010b), and more recently *S. taiwanensis* (Kämpfer et al. 2013a) has also been proposed.

The emended description of *Stappia* by Biebl et al. (2007) defined the genus by the following properties: Gram-negative rods, motile by polar flagella, aerobic, and oxidase and catalase positive. They require seawater or Na ion for growth. Q10 is the predominant quinone. Polar lipid profile includes PG, DPG, PE, PMME, PC, and an aminolipid. The glycolipid SQDG is absent. Cellular fatty acids comprise C16:1 ω7c, C16:0, C18:1 ω7c, C18:0, 11-methyl C18:1 ω6t, C20:1 ω7c, C22:0, C22:1, and the hydroxy fatty acids C14:0 3OH (ester linked), C16:0 3OH, C18:1 3OH, C18:0 3OH, and C20:0 3OH (all amide linked). The G+C content of the type species is 59 mol% but extends up to 65.9 % in *S. indica*. *S. taiwanensis* contains spermidine and spermine as major polyamines. The draft genome of *S. stellulata* DSM 5886^T has 4.6 Mb.

DDH relatedness between *Stappia* species are reported by Lai et al. (2010b) for *S. indica* and *S. stellulata* (43 %) and Kämpfer et al. (2013a) for *S. taiwanensis* and *S. stellulata* (24–25 %) and *S. indica* (27 %).

Some *Stappia* contain *coxL* gene, being able to oxidize carbon monoxide (a common ability in members of the group) (Biebl et al. 2007), and some also present *pcaH* gene, encoding for a key ring-cleaving enzyme of the β-ketoadipate pathway (Buchan et al. 2001). *S. stellulata*-like strains have been related to a pathology of cultured oysters, JOD (juvenile oyster disease), that causes seasonal mortalities in *Crassotrea virginica* hatcheries, in the USA Northeast coast (Boettcher et al. 2000). *Stappia* spp. also participates in DMS production from DMSP, through a DMSP-lyase also present in several members of the *Roseobacter* group (Curson et al. 2008).

Labrenzia

Labrenzia was established as a new genus by Biebl et al. (2007), with the Bchl *a*-producing *L. alexandrii* as type species and the former *Stappia aggregata*, *S. marina*, and *S. alba* as new combinations. The four *Labrenzia* species were closely related according with the analysis of their 16S rRNA genes and only loosely related to *Stappia stellulata*, the type species of the genus *Stappia*, which joined to the clade only after *Roseibium spp.*,

Table 20.8
Species in the *Stappia* group and unaffiliated or isolated genera

Species (synonym)	Isolation/habitat	Metabolism[a]	Na^+ requirement, others	References
Stappia stellulata (*Agrobacterium*)	Marine sediment and water	AAP	Yes	Uchino et al. 1998
S. indica	Deep seawater, Indian Ocean	COH a	Yes	Lai et al. 2010b
S. taiwanensis	Coastal hot spring, Taiwan	COH a	Yes	Kämpfer et al. 2013a
Labrenzia alexandrii	Cultured dinoflagellate (*Alexandrium*), Germany	AAP	Yes	Biebl et al. 2007
L. aggregata (*Stappia*)	Sediment, Baltic Sea, Germany	COH a	Yes	Biebl et al. 2007; Uchino et al. 1998
L. alba (*Stappia*)	Oysters, Mediterranean coast, Spain	COH a	Yes	Biebl et al. 2007; Pujalte et al. 2005c
L. marina	Tidal flat, Yellow Sea, S. Korea	AAP	Yes	Biebl et al. 2007; Kim et al. 2006b
Nesiotobacter exalbescens	Hypersaline lake water, Hawaii, USA	COH	Yes	Donachie et al. 2006
Pannonibacter phragmitetus	Decomposing rhizomes of reed (Phragmites), from soda lake, Hungary	COH fac an	No, alkalitolerant	Biebl et al. 2007; Borsodi et al. 2003
P. indicus	Hot spring sediment, India	COH a	No	Bandyopadhyay et al. 2013
Polymorphum gilvum	Crude oil-contaminated, saline soil, China	COH fac an	No	Cai et al. 2011
Pseudovibrio denitrificans	Seawater, Taiwan	COH fac an	Yes	Shieh et al. 2004
P. ascidiaceicola	Ascidians (Polycitor and Botryllidae), Japan	COH fac an	Yes	Fukunaga et al. 2006
P. axinellae	Marine sponge, Axinella, Ireland	COH fac an	Yes	O'Halloran et al. 2013
P. japonicus	Coastal seawater, Japan	COH fac an	Yes	Hosoya and Yokota 2007b
Roseibium denhamense	Red algae (Botryocladia) surface, Australia	AAP	Yes	Biebl et al. 2007; Suzuki et al. 2000
R. hamelinense	Marine sand, Australia	AAP	No	Biebl et al. 2007; Suzuki et al. 2000
Unaffiliated				
Agaricicola taiwanensis	Mushroom (Agaricus), Taiwan	COH a	No	Chu et al. 2010
Ahrensia kielensis[b] (*Agrobacterium*)	Seawater, Baltic Sea, Germany	COH a	Yes	Uchino et al. 1998
Rhodothalassium salexigens[c] (*Rhodospirillum*)	Salterns, hypersaline lakes, Oregon, USA	PNS	Yes	Venkata Ramana et al. 2013a; Imhoff et al. 1998

[a] *AAP* aerobic anoxygenic photoheterotroph, *COH* chemoorganoheterotroph, *COH a* chemoorganoheterotroph aerobic, *COH fac an* chemoorganoheterotroph facultative anaerobic, *PNS* purple non-sulfur anoxygenic photoheterotroph
[b] *Phyllobacteriaceae*
[c] *Rhodothalassiaceae*

Pannonibacter phragmitetus, two *Pseudovibrio spp.*, and *Nesiotobacter exalbescens*. The current phylogenetic tree, including all newly described members of the *Stappia* group, maintains the same large separation between *Stappia* and *Labrenzia* species (*Fig. 20.6*).

Members of *Labrenzia* are defined as having Gram-negative, rod-shaped cells that are motile by one to several polar flagella. They may produce small amounts of Bchl *a* in the dark and a pink colony pigment in appropriate conditions. They require NaCl for growth, which occurs optimally in 1–10 % NaCl concentrations. Optimum pH is 7 to 8.5. Nitrate reduction activity (to nitrite or to N_2) is variable. They are chemoheterotrophic and non-fermentative under aerobic or anaerobic conditions. Indole production is negative. The major respiratory quinone is Q10. Polar lipids include PG, DPG, PE, PMME, PC, SQDG, and an amino lipid. Fatty acids comprise C16:0, C18:1 ω7c, C18:0, 11-methyl C18:1 ω6t, and C20:1 ω7c and the following hydroxy fatty acids: C14:0 3OH, C18:0 3OH, and C20:0 3OH (all amide linked). The G+C content ranges from 56 to 60 mol%.

The four species are mesophilic and neutrophilic and some are denitrifiers and accumulate PHB (*L. alba*). *L. marina* and

L. alexandrii produce Bchl *a*, while *L. alba* and *L. aggregata* lack this ability and the *pufLM* genes. All four species contain *coxL* genes and are capable of carbon monoxide oxidation (Biebl et al. 2007; Weber and King 2007).

Labrenzia strains have been isolated from the dinoflagellate *Alexandrium lusitanicum* (*L. alexandrii*), marine sediment (*L. aggregata*), oysters (*L. alba*), and tidal flat (*L. marina*), but isolates pertaining to the genus have also been found on estuarine microbial mats (Villanueva et al. 2010), as endophytes on siphonous green seaweeds (Hollants et al. 2011) and in soft corals (Chen et al. 2012b). A putative new *Labrenzia* species has been isolated and extensively investigated in suboxic waters of the Arabian Sea, where they contribute actively to the removal of N_2O through its N_2O-reductase activity, developing in colonies of *Trichodesmium* spp. (Wyman et al. 2013). Some *Labrenzia* spp. draw attention because of their polyhydroxyalcanoate accumulation (Koller et al. 2011; Xiao and Jiao 2011) or because of the production of particular enzymes, as a new nitrilase from *L. aggregata*, interesting to the drug manufacturing industry (Zhang et al. 2012c).

The genome of *L. alexandrii* type strain, DFL-11^T, has been sequenced, bringing attention, among other traits, in the ability to form R-bodies (Fiebig et al. 2013). The genome size is 5.46 Mbp and comprises one chromosome (5.3 Mbp) and two plasmids. It contains 3 rRNA operons and 52 tRNA genes, and its G+C content is 56.4 mol%. Homologues to the three *Caedibacter taeniospiralis* genes determining R-body production were found in the chromosome. The draft genome of *L. aggregata* IAM 12614^T has 6.56 Mb and a G+C content of 59.4 mol%.

Nesiotobacter

Nesiotobacter contains a single species, *N. exalbescens*, isolated from hypersaline lake water in Hawaii (Donachie et al. 2006). The species occupies a position next to *Pseudovibrio* in the *Stappia* clade (Fig. 20.6). Strains belonging to *Nesiotobacter* are Gram-negative, motile rods, positive for oxidase and catalase tests, which reduce nitrates to N_2. They do not synthetize Bchl *a*. Their major fatty acid is C18:1 ω7c. The DNA G+C content of the only strain is 61 mol%.

N. exalbescens grows on Tryptone Soya Agar with 0.5–13.5 % NaCl added (but not at 0 % NaCl) and in Marine Broth with 1–17.5 % NaCl added. It is able to grow up to 45 °C but not at 50º, a behavior that was described as moderately thermophilic in the species description, but corresponds probably to a mesophile with a high upper temperature limit. It produces acid from several carbohydrates and hydrolyzes gelatin. DNA of the type strain showed 14–15 % DDH levels with type strains of *Roseibium denhamense* and *Labrenzia aggregata*, their closest neighbors at the time of the description. The draft genome of *N. exalbescens* DSM 16456^T has 4.16 Mb.

Pannonibacter

The type species of the genus *Pannonibacter*, *P. phragmitetus*, was isolated from rhizomes of reed (*Phragmites australis*) in a Hungarian soda lake (Borsodi et al. 2003). A few years later, Holmes et al. (2006) reported that the new genus and species have been isolated and characterized previously (as *Achromobacter* groups B and E) from blood samples of human origin. This makes *P. phragmitetus* one of the very few *Rhodobacteraceae* bacteria isolated from human clinical specimens, along with *Paracoccus yeei* and *Haematobacter* spp. (see above). The human strains were related to a case of replacement valve endocarditis and two septicemia cases (Holmes et al. 2006).

The original genus description was emended by Biebl et al. (2007), by adding information on their chemotaxonomic traits. It includes strains with Gram-negative, rod-shaped cells, which are motile by polar flagella, contain PHA, and are chemoorganotrophic and facultative anaerobic. They ferment glucose without gas production, are positive for oxidase and catalase, and reduce nitrate to dinitrogen gas. Bchl *a* is not synthetized. The dominant quinone is Q10. Polar lipid profile includes PG, DPG, PMME, PC, an amino lipid, and an unidentified lipid. PE is not detected (although it is a precursor of PMME). Phosphatidylserine and SQDG are absent. Cellular fatty acid comprise C16:1 ω7c, C16:0, C18:1 ω7c, C18:0, 11-methyl C18:1 ω6t, C20:1 ω7c, C22:0, and the hydroxy fatty acids C14:0 3OH (ester linked), C16:0 3OH, C18:1 3OH, C18:0 3OH, and C20:1 3OH (all amide linked). The peptidoglycan contains *m*-DAP. The G+C content of the species described so far is 63–64.6 mol%. The draft genome of *P. phragmitetus* DSM 14782^T has a size of 4.8 Mb.

P. indicus, the second species in the genus, shows DDH values of 34–55 % against different strains of *P. phragmitetus* (Bandyopadhyay et al. 2013) and, unlike the type species, is unable to grow anaerobically, does not reduce nitrates, and is able to grow up to 45 °C. It resembles *P. phragmitetus* in its alkalitolerant character and in the absence of salt requirements for growth.

Pannonibacter strains isolated from wastewater treatment plants have shown remarkable abilities for degradation of some undesired compounds as tert-butyl alcohol (Reinauer et al. 2008) or 4-aminobenzene sulfonate (Wang et al. 2009d; Zhang et al. 2011b), but the bulk of the literature on applied uses of *Pannonibacter* is related to its activity as a chromium (IV) reducing agent, which makes *Pannonibacter phragmitetus* (strains BB and LSSE-09, among others) an effective resource for contaminated soil bioremediation (Chai et al. 2009; Xu et al. 2011a, b, 2012; Wang et al. 2013a). It is also remarkable the tolerance to arsenate of the newly described species *P. indicus* (Bandyopadhyay et al. 2013), which type strain is able to grow in the presence of up to 500 mM of sodium arsenate. The strain was isolated from hot spring sediment in India. Other hot spring environments also contain *Pannonibacter* strains, as recently reported by Coman et al. (2013).

Polymorphum

The genus *Polymorphum* was recently described to accommodate a new species, *P. gilvum*, isolated from crude oil-contaminated saline soils, in China (Cai et al. 2011), the same samples from which *Rubrimonas shengliensis* was isolated and described. *Polymorphum's* closest taxon corresponds to the pair

of *Pannonibacter* species (*P. phragmitetus* and *P. indicum*) (Fig. 20.6, LTP111), to which they reassemble in their facultatively anaerobic character.

Cells of *Polymorphum* exhibit different morphologies, as suggested by the generic name, as short rods or dumb-shaped cells with different branches that occasionally aggregate in star-shaped groups. They are Gram negative, facultatively anaerobic, and motile (one polar flagellum is present in the cells of the type species). The predominant quinone is Q10; major fatty acids correspond to C18:1 ω7c, 11-methyl C18:1 ω7c, C18:0, C16:0, and C20:1 ω7c. The polar lipid profile includes DPG, PMME, PG, PC, SQDG, and unidentified aminolipids and phospholipids. The type species has a DNA G+C content of 65.6 mol%.

P. gilvum is able to grow from 4 °C to 50 °C, at pH 5–9, and with 0–6 % NaCl. Optimum growth occurs at 37 °C, pH 6.0, and 1 % NaCl. The species is positive for catalase activity but negative for oxidase, an atypical behavior in the group. It does not reduce nitrates. Bchl *a* is not synthetized and *pufLM* genes are absent. Acid is produced from several carbohydrates (glycerol, L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, and D-cellobiose, among others). The type strain SL003B-26A1^T is able to grow on crude oil as sole carbon source, an ability that justifies the interest in its complete genome sequence: Li et al. (2011c) found that the genome of this strain consists of a circular chromosome 4.65 Mbp in size and a plasmid of 69.6 kbp with 67 and 61 mol% G+C content, respectively. The chromosome contains 50 tRNA and two rRNA encoding operons. The genes for membrane-bound alkane monooxygenase and for cytochrome P450 (related to oil degradative capabilities in other bacteria) are not found, but *ladA*, coding for long chain-alkane hydroxylation, is present. This gene codes for an extracellular enzyme that can hydroxylate C_{15} to C_{36} alkanes. The authors suggest it might have been acquired by horizontal gene transfer.

Pseudovibrio

Four species have been described in the genus *Pseudovibrio* since Shieh et al. (2004) named the new genus: *P. denitrificans*, the type species, *P. ascidiaceicola* (Fukunaga et al. 2006), *P. japonicus* (Hosoya and Yokota 2007b), and *P. axinellae* (O'Halloran et al. 2013). All four contain strains of Gram-negative, straight, or curved rods, which are motile by one to several lateral/subpolar flagella. *P. ascidiaceicola* forms conspicuous rosettes. They are able to grow in anaerobic conditions, either by carbohydrate fermentation (glucose and mannose are fermented by the four species) or through denitrification, as all of them reduce nitrates to nitrogen gas. They are oxidase and catalase positive, mesophilic and halophilic, requiring NaCl for growth. Their fatty acid profile includes C18:1 ω7c as major component, accompanied by varying amounts of C16:0, C18:0, C19:0 cyclo ω8c, and the hydroxylated C14:0 3OH (as SF 2), C16:0 3OH, and C18:0 3OH (O'Halloran et al. 2013). The G+C content of their DNA is 50–52 mol%. The species could be differentiated by their fermentative and assimilative patterns as well as for the relative amounts of minor fatty acids. DDH values between *P. ascidiaeceicola* and *P. denitrificans* are lower than 30 % (Fukunaga et al. 2006); *P. japonicus* shows values of 35 % against *P. denitrificans* and 14–32 % against *P. ascidiaceicola* strains (Hosoya and Yokota 2007b). Finally, *P. axinellae* shows DDH values of 26 % with *P. ascidiaceicola*, 18 % with *P. denitrificans*, and 16 % with *P. japonicus* (O'Halloran et al. 2013).

Pseudovibrio species form a well-defined, tight clade, which closest relative is *Nesiotobacter exalbescens* (Fig. 20.6).

Although two of the species were isolated from seawater, most of the reports on isolation of pseudovibrios are associated to microbiota of marine invertebrates, with a significative dominance of sponges of very different geographic sites. Several reports document the production of bioactive metabolites from these sponge-, tunicate-, ascidian-, coral- or polychaetide-associated bacteria (Radjasa et al. 2007; Muscholl-Silberhorn et al. 2008; Riesenfeld et al. 2008; Kennedy et al. 2009; Santos et al. 2010; O'Halloran et al. 2011; Penesyan et al. 2011; Flemer et al. 2012; Margassery et al. 2012; Chen et al. 2012b; Rizzo et al. 2013). The genomes of two *Pseudovibrio* strains, isolated from a coral and a sponge, revealed a number of ORFs and gene clusters that seem to be involved in symbiont-host interactions: attachment and interaction with eukaryotic cell machinery, production of secondary metabolites, and supply of the host with cofactors (Bondarev et al. 2013). The size of these genomes, 5.73 and 5.92 Mb, are among the largest reported within the *Rhodobacteraceae*.

Roseibium

The genus *Roseibium* was described by Suzuki et al. (2000) to accommodate two new species of APPs isolated from algal surfaces and sand from coastal areas of Australia. *R. denhamense* and *R. hamelinense* synthetized Bchl *a* in aerobiosis and produced pink colonies. Their cells are Gram-negative rods, motile by means of peritrichous flagella. They are aerobic, chemoorganotrophic, and positive for catalase, oxidase, phosphatase, and nitrate reductase. Q10 is the predominant quinone and C18:1 ω7c is the major fatty acid. The G+C molar content of their DNA expands from 57.6 to 63.4 mol%. Biebl et al. (2007) emended the genus description, adding the polar lipid profile, which comprises PG, DPG, PE, PMME, PC, SQDG, and an amino lipid. They also added a more detailed fatty acid profile that includes C16:0, C18:1 ω7c, C18:0, 11-methyl C18:1 ω6t, and C20:1 ω7c and the hydroxy fatty acids C14:0 3OH (ester linked), C18:0 3OH, C20:0 3OH, and C20:1 3OH (amide linked).

Roseibium species are mesophilic and neutrophilic (optimal growth at 27–30 °C and pH 7.5–8.0). *R. hamelinense* does not require NaCl for growth (range: 0–10 % NaCl), in contrast to *R. denhamense* (range: 0.5–7.5 % NaCl). They are chemoorganotrophic and aerobic and unable to grow phototrophically under anaerobic conditions in the light. Their carbon sources include butyrate, L-glutamate, L-aspartate, and pyruvate. Acid is produced from some carbohydrates: D-glucose, D-fructose, D-ribose, and maltose. They produce indole and hydrolyze gelatin. DDH determinations related both species by values of 12–17 % (Suzuki et al. 2000).

Roseibium species have *Labrenzia* spp. as their closest phylogenetic relatives (Fig. 20.6). The draft genome of strain *Roseibium* sp. TrichSKD4 has 5.7 Mb and a G+C content of 53.9 mol%.

Agaricicola

The description of the genus *Agaricicola* and its only species, *A. taiwanensis*, was based on the study of a single strain, CC-SBAB117^T, isolated from the stipe of the edible mushroom *Agaricus blazei* in Taiwan. After analysis of the 16S rRNA gene sequence, a distant relationship was found to some members of the *Stappia* clade (*Pannonibacter phragmitetus*, 92.5 % sequence similarity; *Nesiotobacter exalbescens*, 91.5 %) and to *Prosthecomicrobium pneumaticum* (92.3 %) (Chu et al. 2010). Although some early analysis might suggest *Agaricicola* being a peripheral member of the clade *Stappia*, the more recent version of LTP, LTP111, shows *Agaricicola* paired with *Prosthecomicrobium pneumaticum* and clearly unrelated to the stappias. Thus, *Agaricicola* should be removed from *Rhodobacteraceae sensu lato*, as currently considered.

The genus is defined by the following traits: Gram-negative, strictly aerobic, nonspore-forming, motile, club-shaped cells that accumulate PHB granules as polar inclusions. Oxidase test is positive but catalase is negative. It contains the following polar lipids: DPG, PG, PC, and PE. SQDG and PMME are not detected. The major respiratory quinone is Q10. The predominant fatty acids are C18:1 ω7c, C19:0 cyclo ω8c, C16:0, and C18:0 whereas C10:0 3OH is absent.

The type and only species of the genus, *A. taiwanensis*, grows in Nutrient Agar forming circular, shiny, beige pigmented colonies. Growth is optimal at 30–35 °C and occurs between 20 °C and 40 °C, at pH 6.0–9.0 and with 1–4 % NaCl. NaCl is not required for growth. Cells have polar flagella. Nitrate reduction to nitrite or N_2 is negative and no growth is observed with nitrate in anaerobic conditions. Bchl *a* is not synthetized. No acid is produced from carbohydrates. It uses a few sugars and several organic acids as carbon sources, including acetate, pyruvate, propionate, and β-hydroxybutyric acid. The G+C content of its DNA is 62.7 mol%.

Ahrensia

The type species of the genus *Ahrensia*, *A. kieliensis*, was formerly known as one of the "marine agrobacteria," but its relation to the main group was considered ungranted by Rüger and Höfle (1992). Years later, Uchino et al. (1997) revised the phylogenetic position of the whole group of marine agrobacteria, including the non-validated species names, based on 16S rRNA sequence analysis: they found that *Agrobacterium kieliense* IAM 12618 sequence was positioned in the α-2 branch of *Proteobacteria* and recognized it should be classified as a species in new genera. Shortly after that, the proposal of *Ahrensia kieliense* (corrected *kielensis*) gen. nov., sp. nov., nom. rev. was published (Uchino et al. 1998), and since then *Ahrensia* has remained as a monospecific genus, distantly related to the other reclassified marine agrobacteria (*Stappia stellulata*, *Labrenzia aggregata*, *Ruegeria atlantica*, *Thalassobius gelatinovorus*). On recent times, the affiliation of *Ahrensia* to the family *Phyllobacteriaceae* has become evident: *A. kieliensis* merges with species of the genus *Hoeflea*, in particular, with *H. phototrophica* (LTP 111), to which it shows a 95.7 % 16S rRNA gene sequence similarity (Kim et al. 2012f). It should be noted that the type species of the genus *Hoeflea*, *H. marina*, is itself a reclassified marine agrobacterium (*A. ferrugineum* LMG 128).

Ahrensia displays the following generic features: Gram-negative rods, motile by polar flagella, aerobic, and they have a strictly respiratory type of metabolism with oxygen as terminal electron acceptor. Catalase and oxidase are positive. Nitrate is not reduced; gelatin, starch, chitin, and alginate are not hydrolyzed; and indole is not produced. Sodium ion is required for growth. Optimal temperatures are 20–30 °C (range: 5 °C to less than 37 °C). The major fatty acid is C18:1 ω7c and hydroxyl fatty acid is C12:0 3OH, while 2OH fatty acids are absent. Major respiratory quinone is Q10. The G+C content is 48 mol% (Uchino et al. 1998; Krieg 2005). *A. kieliensis* was isolated from seawater from the Baltic Sea.

The draft genome of *A. kieliensis* DSM 5890^T has 3.36 Mb and a G+C content of 48.1 mol%, whereas *Ahrensia* sp. R2A130 has similar size (3.73 Mb) but a considerably higher G+C content (56.9 mol%).

Rhodothalassium

The position of *Rhodothalassium salexigens* (Imhoff et al. 1998; formerly, *Rhodospirillum salexigens*) as a separate phylogenetic branch amid alphaproteobacterial families in the vicinity of *Rhodobacteraceae* was already stressed in the second edition of *Bergey's Manual of Systematic Bacteriology*, where it is classified as Genus *Incertae Sedis*. This unique position in the 16S rRNA tree is accompanied by a combination of chemotaxonomic features unlike other purple non-sulfur phototrophs in *Rhodobacteraceae*: presence of menaquinones (MK10), ornithine lipids, or carotenoids of the spirilloxanthine series. Only the lack of additional strains had prevented an earlier reclassification of the genus, but after the study of several *R. salexigens*-like strains obtained from a solar saltern in India, Venkata Ramana et al. (2013) had emended the description of *Rhodothalassium* and proposed *Rhodothalassiaceae* fam. nov. and *Rhodothalassiales* ord. nov. At the time of writing of this chapter, these names have not been yet validated.

The closest relatives to *Rhodothalassium*, according to the more recent 16S rRNA phylogenetic analyses, are *Kordiimonas* species (fam. *Kordiimonadaceae*, order *Kordiimonadales*), as can be seen in *Fig. 20.1* and LTP111 tree.

According to the emended description, the genus *Rhodothalassium* is defined by the following traits: cells are Gram negative, vibrioid to spiral shaped, motile by polar flagella, and multiply by binary fission. The intracytoplasmic photosynthetic membranes are lamellar stacks. Bchl *a* and carotenoids of the spirilloxanthin series are the major photosynthetic pigments. The quinone system is composed of ubiquinone Q10 and menaquinone MK10. Halophilic, they require NaCl or sea salts for growth. Optimum salinity is above the seawater salt content and tolerate up to 20 % total salts. Growth occurs

preferably photo-organotrophically under anoxic conditions in the light, but is generally possible under microoxic to oxic conditions in the dark (except in some strains). They may require amino acids or niacin and thiamine. Major polar lipids are DPG, PG, ornithine lipid (OL), an unidentified phospholipid, and an amino lipid. Major fatty acids are C18:1 ω7c, 11-methyl C18:1 ω7c, and C16:0, with minor amounts of C14:0, C18:0, C18:1 ω5c, and C16:1 ω7c/C16:1 ω6c. The G+C content of the DNA is 60–62.8 mol%. Hamana et al. (2001) reported aminopropylhomospermidine as the major polyamine in *R. salexigens* cells.

The only species currently recognized is *R. salexigens*. This species is able to grow with N_2 as nitrogen source, requires glutamate, and is unable to grow photoautotrophically with H_2, sulfide, or thiosulfate as electron donors. The temperature range is 20–45 °C (optimum 40 °C) and the salinity range is 5–20 % NaCl (optimum: 4–8 %). Their habitats include anoxic zones of hypersaline environments, such as salterns and partially evaporated seawater pools.

Addendum

The following new genera and species have been effectively published after completion of the manuscript and are not included in the phylogenetic trees and the main text, but their names might be eventually included in validation lists.

Genera:

- *Albirhodobacter marinus* gen. nov., sp. nov. (Nupur et al. 2013), affiliated to the *Rhodobacter* group.
- *Falsirhodobacter halotolerans* gen. nov., sp. nov. (Subhash et al. 2013), affiliated to the *Rhodobacter* group.
- *Litorisediminicola beolgyonensis* gen. nov., sp. nov. (Yoon et al. 2013c), affiliated to the *Roseobacter* group.
- *Paenirhodobacter enshiensis* gen. nov., sp. nov. (Wang et al. 2013b), affiliated to the *Rhodobacter* group.
- *Planktomarina temperata* gen. nov., sp. nov. (Giebel et al. 2013), affiliated to the *Roseobacter* group.
- *Pleomorphobacterium xiamenensis* gen. nov., sp. nov. (Yin et al. 2013), affiliated to the *Amaricoccus* group.
- *Sinorhodobacter ferrireducens* gen. nov., sp. nov. (Yang et al. 2013), affiliated to the *Rhodobacter* group.

Species:

- *Albimonas pacifica* (Li et al. 2013).
- *Defluviimonas aestuarii* (Math et al. 2013).
- *Gemmobacter lanyuensis* (Sheu et al. 2013b).
- *Gemmobacter megaterium* (Liu et al. 2013)
- *Litoreibacter halocynthiae* (Kim et al. 2013).
- *Loktanella sediminilitoris* (Park et al. 2013c).
- *Loktanella soesokkakensis* (Park et al. 2013d).
- *Pelagicola litorisediminis* (Park et al. 2013e).
- *Phaeobacter leonis* (Gaboyer et al. 2013).
- *Roseivivax pacificus* (Wu et al. 2013).
- *Roseovarius lutimaris* (Choi et al. 2013).
- *Roseovarius marisflavi* (Li et al. 2013).
- *Ruegeria intermedia* (Kämpfer et al. 2013b).
- *Shimia biformata* (Hameed et al. 2013).
- *Shimia haliotis* (Hyun et al. 2013).
- *Sulfitobacter porphyrae* (Fukui et al. 2013).
- *Thioclava dalianensis* (Zhang et al. 2013), with emended description of the genus.

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21 The Family *Rhodobiaceae*

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Abstract
Rhodobiaceae is a family within the order *Rhizobiales* of the class *Alphaproteobacteria*. This family embraces the genera *Afifella*, *Anderseniella*, *Lutibaculum*, *Parvibaculum*, *Rhodobium*, *Rhodoligotrophos*, *Tepidamorphus*, *and Bauldia*. With the exception of slightly thermophilic *Tepidamorphus gemmatus*, the other members of this family are mesophilic. The species of two genera of this family are photoheterotrophic when grown under anaerobic conditions in the light, while the species of the other genera are strictly chemoorganotrophic. The major fatty acids in all mesophilic species are straight chain, namely, $C_{18:1}\omega 7c$, $C_{16:0}$, and $C_{18:1}\omega 9c$, but in the slightly thermophilic *Tepidamorphus gemmatus*, the major fatty acid is $C_{19:0\ cyclo}\omega 8c$. The morphology of the species of the family is variable ranging from coccoid forms to rods, diving by budding asymmetric division; some possess prosthecae and complex structures. The members of this family have been isolated from a variety of environments, such as seawater, deep sea, hydrothermal vent, salterns, freshwater, hot springs, a mud volcano, activated sludge, and soil.

Taxonomy, Historical and Current

Short Description of the Family

The family *Rhodobiaceae* was described by Garrity et al. (2005b) based on the species of the genus *Rhodobium*. This family, as currently understood, is a rather diverse group of organisms, despite representing a monophyletic clade that comprises organisms that share mainly characteristics commonly encountered in many other *Alphaproteobacteria*, namely, fatty acids, polar lipids, and the respiratory quinones. Four of the species of this family possess bacteriochlorophyll *a*, are facultative photoheterotrophs, and may even be capable of anaerobic photolithotrophic growth. Other species are strictly chemoorganotrophic. The morphology is also diverse ranging from rod-shaped cells to cocci that divide by budding or asymmetric cell division. Some species produce prosthecae or form irregular appendages and hyphal-like budding structures.

The genus *Rhodobium* was initially described to include a species previously named *Rhodopseudomonas marina* (Imhoff 1983), as *Rhodobium marinum*, and the newly described species named *Rhodobium orientis* (Hiraishi et al. 1995). Later, Caumette et al. (2007) described a species that was named *Rhodobium pfennigii*. That same year the species *Rhodobium gokarnense* was described by Srinivas et al. (2007). These organisms have a marine origin, possess bacteriochlorophyll *a*, are phototrophic, and share morphological, physiological, and biochemical characteristics but actually belong to two clades. The taxonomy of the genus *Rhodobium* was later questioned because 16S rRNA gene sequence analysis showed that the validly named species of the genus *Rhodobium* did not form a monophyletic clade (Urdiain et al. 2008). These authors, therefore, proposed the genus *Afifella*, not only based on phylogenetic analysis but also based on the phenotypic and chemotaxonomic characteristics, for the species formerly named *R. marinum* and *R. pfennigii*, and now as *Afifella marina* and *Afifella pfennigii*.

The species now classified as *Bauldia consociata* and *B. litoralis* (Yee et al. 2010) were originally placed in the genus *Prosthecomicrobium*, as *P. consociatum* and *P. litoralum* by Bauld et al. (1983), and Vasil'eva et al. (1991). The classification of these organisms in the *Prosthecomicrobium* was based primarily on the presence of prosthecae and budding.

E. Rosenberg et al. (eds.), *The Prokaryotes – Alphaproteobacteria and Betaproteobacteria*, DOI 10.1007/978-3-642-30197-1_252,

The description of species of the genus *Parvibaculum* begins with the description of *P. lavamentivorans* by Schleheck et al. (2004) who were isolating organisms that degraded surfactants such as alkylbenzenesulfonate. The description of this organism was followed years later by the isolation of *P. indicum* and *P. hydrocarboniclasticum* (Lai et al. 2011; Rosario-Passapera et al. 2012). Two species, namely, *P. lavamentivorans* and *P. hydrocarboniclasticum*, are known to degrade and grow on surfactants and alkanes, respectively. These characteristics were not examined in *P. indicum*. It is noteworthy that *P. indicum* was considered by the authors to be a member of the family *Phyllobacteriaceae* (Lai et al. 2011).

The species *Anderseniella baltica* is a red-pigmented aerobic and chemoorganotrophic organism isolated from an anoxic marine sediment that forms pleomorphic rod-shaped cells and complex structures and short filaments with bulges (Brettar et al. 2007). *Tepidamorphus gemmat*us is the only extant slightly thermophic member of the family *Rhodobiaceae*. This organism, unlike most other species of the family, forms complex structures that in some cases resemble short hyphae.

The species *Rhodoligotrophos appendicifer* is a coccoid, red-pigmented, non-bacteriochlorophyll *a*-producing, aerobic, chemoorganotrophic organism that forms appendages that connect cells to each other (Fukuda et al. 2012). These authors show this species to be phylogenetically most closely related to the species of the genus *Parvibaculum* and belong to the family *Rhodobiaceae*.

The genus *Lutibaculum* comprises only one species named *L. baratangense* (Kumar et al. 2012). This rather nondescript, pale orange organism that forms coccoid- to rod-shaped cells is a mesophilic, aerobic, and chemoorganotrophic organism isolated from a marine mud volcano.

Rhodobiaceae Garrity et al. 2006

Rhodobiaceae (Rho.do.bi.a'ce.ae. N.L. neut. n. *Rhodobium*, type genus of the family; suff. *-aceae*, ending to denote family; N.L. fem. pl. n. *Rhodobiaceae*, the *Rhodobium* family).

The members of the family *Rhodobiaceae* of the order *Rhizobiales* stain Gram-negative and form rod-shaped cells, although more complex cellular structures appear in some genera such as *Tepidamorphus*. These organisms do not form endospores. Some species are strictly aerobic; others grow under aerobic conditions in the dark and under anaerobic conditions in the light. The species of the genera have ubiquinone 9, 10, 11 (U-9, U-10, U-11), and/or menaquinone 10 (MK-10) as the major respiratory lipoquinone. Unsaturated straight-chain fatty acids are the predominant acyl groups of the family; among these are $C_{18:1}\omega7c$, $C_{18:1}\omega9c$, $C_{19:0\ cyclo}\omega8c$, and $C_{18:1}\omega7c$ 11-methyl. $C_{16:0}$ is also a major fatty acid in *Rhodoligotrophos appendicifer*. Hydroxy fatty acids are also present in members of this family. The type genus of the family is *Rhodobium* (Hiraishi et al. 1995; Garrity et al. 2005b). *Afifella*, *Anderseniella*, *Lutibaculum*, *Parvibaculum*, *Rhodoligotrophos*, *Tepidamorphus*, and *Bauldia* are additional genera of the family (Albuquerque et al. 2010a; Brettar et al. 2007; Caumette et al. 2007; Fukuda et al. 2012; Schleheck et al. 2004; Kumar et al. 2012; Urdiain et al. 2008; Yee et al. 2010).

Phylogenetic Structure of the Family and Its Genera

The phylogenetic reconstruction of the family *Rhodobiaceae* shows a paraphyletic affiliation that is not supported by phenotypic characteristics. There are, in fact, pronounced differences in the public databases, on the genera that belong to this family, which do not agree entirely with the genera we consider to belong to the family *Rhodobiaceae* (*Fig. 21.1*). In the one hand, the species *Roseospirillum parvum* (Glaeser and Overmann 1999, 2001), which was later proposed to belong to the family *Rhodospirillaceae* (Garrity et al. 2005a), is classified in the family *Rhodobiaceae* in the List of Prokaryotic Names with Standing in Nomenclature (www.bacterio.cict.fr) and the NCBI Taxonomy Browser (http://www.ncbi.nlm.nih.gov/taxonomy). Despite the fact that some phenotypic characteristics are shared with the species of the genera *Rhodobium* and *Afifella*, the phylogenetic affiliation of this species clearly positions it within the family *Rhodospirillaceae*. On the other hand, the species of the genus *Bauldia* are not included in the family *Rhodobiaceae* by the List of Prokaryotic Names with Standing in Nomenclature and the NCBI Taxonomy Browser, and this genus is considered to be an unclassified member of the order *Rhizobiales*. However, our phylogenetic reconstruction affiliates the species of the genus *Bauldia* with a fairly high (about 93 %) 16S rRNA sequence similarity with *Rhodobium* spp.

Regarding the 16S rRNA identity, some lineages of the family *Rhodobiaceae* appear as closely related to genera included in other families as they are to genera of this family. Actually, only the neighbor-joining algorithm using the out-group-supporting sequences was the only analysis that showed all genera as monophyletic (*Fig. 21.1*). Alternative reconstructions using RAxML algorithm always gave paraphyletic topologies (data not shown). Four genera of this family are monospecific, namely, *Lutibaculum*, *Tepidamorphus*, *Rhodoligotrophos*, and *Anderseniella*; two genera comprise two species; the genus *Afifella* includes *A. pfennigii* and *A. marina*, while the genus *Rhodobium* comprises *R. orientis* and *R. gokarnense*. The genus *Parvibaculum* comprises three species, namely, *P. lavamentivorans*, *P. indicum*, and *P. hydrocarboniclasticum*. *Lutibaculum baratangense* is most closely related, based on 16S rRNA gene sequence analysis, to *Tepidamorphus gemmatus* (95 % similarity), *Bauldia consociata* (94.5 % similarity), and *Afifella pfennigii* (94.4 % similarity). The 16S rRNA gene sequence of *Anderseniella baltica* is most similar to the species of *Rhodobium* (92.6 %) and *Tepidamorphus gemmatus* (92.1 %). The two species of *Bauldia* share 96 % 16S rRNA gene sequence similarity and 95 % sequence similarity with *Lutibaculum baratangense*. The type strain of *Rhodobium*

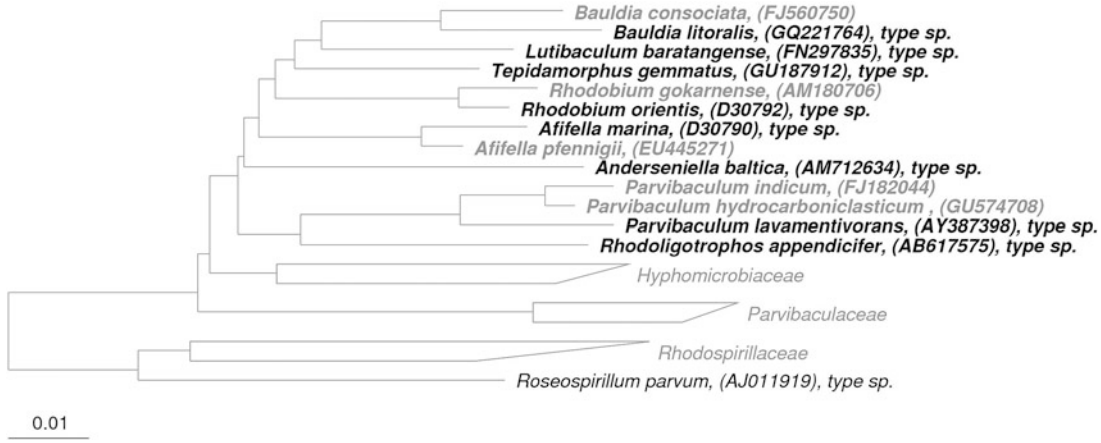

Fig. 21.1

Phylogenetic reconstruction of the family *Rhodobiaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. The scale bar indicates estimated sequence divergence

orientis (the type strain of the family) shares almost 99 % sequence similarity with the type strain of *Rhodobium gokarnense*. Despite the very similar 16S rRNA sequence between both type strains, the DNA: DNA hybridization is reported to have a relatedness of only 35.1 % (Srinivas et al. 2007), which would indicate that these organisms belong to two different species. The two species of the genus *Afifella* share about 98.3 % 16S rRNA sequence similarity with each other. The species *Rhodoligotrophos appendicifer* appears to be the most distantly related organism in the family *Rhodobiaceae* sharing only 92.7 % 16S rRNA sequence similarity with *Lutibaculum baratangense*.

Molecular Analyses

Genome Comparison

The only member of the family *Rhodobiaceae* to have the whole genome sequenced is *Parvibaculum lavamentivorans* strain DS-1^T, because it completely degrades laundry surfactants. In this respect the organism is potentially important for bioremediation (Schleheck et al. 2011). The genome is large with 3,914,745 bp and a total of 3,714 genes. The inability of the organism to assimilate several carbon sources examined is confirmed by the lack of candidate genes for ATP-dependent sugar uptake systems. There are no known homologues for amino acid or di-/oligopeptide transport systems. The organism has the genes necessary for the synthesis of all amino acids and essential cofactors. Most interestingly, the genome of *P. lavamentivorans* possesses a multitude of genes for alkyl-chain degradation.

Phenotypic Analyses

The main features of members of the family *Rhodobiaceae* are listed in *Table 21.1*. The main features of members of the genera *Afifella*, *Anderseniella*, *Lutibaculum*, *Parvibaculum*, *Rhodobium*, *Rhodoligotrophos*, *Tepidamorphus*, and *Bauldia* are listed in *Tables 21.2*, *21.3*, and *21.4*.

Afifella Urdian et al. 2009b

Afifella (A.fi.fel'la. N.L. fem. dim. n. *Afifella*, named after S. Afif, a British philosopher and painter in recognition of his expertise and guidance in the subject of the philosophical tendency of structuralism, essential for the development and understanding of taxonomy as a science).

Afifella forms motile rod-shaped cells that divide by budding and asymmetric cell division. Mesophilic, marine bacteria that require NaCl for growth and grow at seawater and environments with higher salinities. Chemoorganotrophic growth in the dark can occur aerobically or microaerobically and in some cases fermentatively. The organisms grow anaerobically in the light by photoheterotrophic metabolism and may be photolithotrophic on thiosulfate. Phototrophic grown cells contain intracytoplasmic membranes of the lamellar type together with bacteriochlorophyll *a* and carotenoids belonging to the spirilloxanthin series. The absorption maxima of living cells for *A. marina* and *A. pfennigii* are, respectively, 375, 483, 516, 533, 590, 803, and 883 nm and 395, 483, 512, 548, 594, and 909 nm. The major fatty acid is $C_{18:1}\omega7c$. When grown aerobically in the dark, species of this genus exhibit a quinone system with ubiquinone 10 (U-10) predominating and significant

Table 21.1
Phenotypic and chemotaxonomic characteristics of genera of *Rhodobiaceae*

	Afifella[1,2,3]	*Anderseniella*[4]	*Parvibaculum*[5,6,7]	*Lutibaculum*[8]	*Rhodobium*[1,2,9]	*Rhodoligotrophos*[10]	*Tepidamorphus*[11]	*Bauldia*[12]
Morphology	Rods	Rod-shaped to pleomorphic	Rods	Rods	Rods	Irregular coccoids	Irregular rods	Rods
Gram stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Cell division by budding	+	–	–	–	+	–	+	+
Motility	+	–	+	+	Variable	–	+	–
Metabolism	Aero- or microaerobically in the dark and anaerobically in the light	Aerobic	Aerobic	Aerobic	Aerobically in the dark and anaerobically in the light	Aero- or microaerobically	Aerobic	Aerobic
Phototrophic growth	+	–	–	–	+	–	–	nd
Pigmentation under aerobic growth	Nonpigmented	Orange red	Nonpigmented	Pale orange	Nonpigmented	Red	Nonpigmented	Nonpigmented
Pigmentation of photosynthetic cultures	Pink to red	–	–	–	Pink to red	–	–	nd
Presence of bacteriochlorophyll *a*	+	–	nd	–	+	–	–	nd
Nitrate reduction	Variable	–	–	+	Variable	–	+	nd
Presence of								
Oxidase	nd	+	Variable	+	nd	+	+	+
Catalase	+	+	+	+	nd	+	+	+

Temperature for growth (°C)								
Range	4–30	1,037	10–42	25–37	nd	5–35	30–52.5	5–39
Optimum	25–35	25–30	25–37	30–37	30–35	30	45–50	26–35
pH for growth								
Range	6.3–8.5	nd	4.0–8.5	7.0–9.0	5.0–9.0	6.0–9.0	6.5–9.5	nd
Optimum	6.9–7.5	7.0–7.5	7.5	8.0	6.5–7.5	7.0	7.5–8.5	nd
NaCl requirement	+	+	+	nd		−	−	+
NaCl concentration for growth (%)								
Range	0.5–16	0.8–6.0	0.5–8	nd–4	0.5–16	0–5	0–3	0.3–nd
Optimum	1–5	1.5–3.0	1–5	2	2–5	0–0.5	0–1	1–3
Major fatty acids	$C_{18:1}\omega 7c$	$C_{18:1}\omega 7c$	$C_{18:1}\omega 7c$	$C_{18:1}\omega 7c$	$C_{18:1}\omega 7c$	$C_{16:0}$	$C_{19:0\ cyclo}\omega 8c$	nd
Major polar lipids[a]	DPG, PG, PC, PE, AL, GL	nd	DPG, PG, PC, PE, AL	DPG, PG, PE	DPG, PG, PC, PE, PME, AL	PG, PE, PL	DPG, PG, PC, PE, PME, AL	nd
Major polyamine[b]	HSPD	nd	nd	nd	SPD and SPM	nd	nd	nd
Major respiratory lipoquinone[c]	U-10 and MK-10	nd	U-10 or U-11	U-10	U-10 and MK-10	U-9 and U-10	U-10	nd
G+C content (mol%)	62.2–67.5	61.2	62.1–64.0	70.5	65.2–65.8	61.1	65.6–66.9	66–69
Habitat	Seawater	Surface layer of anoxic sediment in sea	Deep sea, hydrothermal vent, activated sludge	Mud volcano	Saltern, seawater	Freshwater	Hot spring	Seawater, soil

[1]Urdiain et al. 2008; [2]Hiraishi et al. 1995; [3]Caumette et al. 2007; [4]Brettar et al. 2007; [5]Lai et al. 2011; [6]Rosario-Passapera et al. 2012; [7]Schleheck et al. 2004; [8]Kumar et al. 2012; [9]Srinivas et al. 2007; [10]Fukuda et al. 2012; [11]Albuquerque et al. 2010a; [12]Yee et al. 2010

Symbols: +positive, –negative

[a]*DGP* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PC* phosphatidylcholine, *PME* phosphatidylmonoethanolamine, *GL* glycolipid, *AL* aminolipid

[b]*HSPD sym*-homospermidine, *SPD* spermidine, *SPM* spermine

[c]*U* ubiquinone, *MK* menaquinone

Table 21.2

Comparison of selected characteristics of members of the genera of *Afifella*, *Anderseniella*, *Parvibaculum*, *Lutibaculum*, *Rhodobium*, *Rhodoligotrophos*, *Tepidamorphus*, and *Bauldia*. Some characteristics were examined in some species but not others as follows; in the Api ZYM tests, *Anderseniella baltica*, *Parvibaculum indicum*, and *P. lavamentivorans* are positive for alkaline phosphatase, esterase (C 4), esterase lipase (C 8), and leucine arylamidase and negative for α-*chymotrypsin*, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. *Afifella marina* assimilates caproate, ascorbate, and galactose. *Afifella pfennigii* assimilates glutamate, aspartate, cyclohexanecarboxylate, crotonate, 2-oxoglutamate, and cysteine, but does not assimilate caprylate, pelargonate, palmitate, gluconate, glycolate, gallate, nicotinate, catechol, *N*-acetylglucosamine, methionine, glycine betaine, thioacetamide, trehalose, and butanol. *A. marina* and *A. pfennigii* assimilate acetate, butyrate, pyruvate, succinate, casamino acids, and yeast extract, but does not assimilate benzoate and methanol. *A. baltica* does not assimilate D-arabinose, mannose, maltose, *N*-acetylglucosamine, caprate, phenylacetate, and gluconate; does not hydrolyse DNA, urea, chitin, starch, and casein; and does not produce acid from any carbon source in the Api 50CH. *P. indicum* and *P. lavamentivorans* assimilate L-arabinose and does not assimilate mannose, maltose, *N*-acetylglucosamine, caprate, phenylacetate, potassium gluconate, and trisodium citrate. In the biolog GN2 test, *A. baltica*, *P. indicum*, and *P. lavamentivorans* are negative for α-cyclodextrin, dextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, D-galactonic acid lactone, D-glucosaminic acid, D-glucoronic acid, *p*-hydroxybutyric, malonic acid, quinic acid, D-sacharic, bromosuccinic acid, D-alanine, L-alanine, L-asparagine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, uronic acid, inosine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, α-D-glucose 1-phosphate, and D-glucose 6-phosphate. *P. indicum* and *P. lavamentivorans* are sensitive to erythromycin, gentamicin, kanamycin, carbenicillin, cefazolin, chloromycetin, cefobid, rifampicin, minomycin, piperacillin, and vibramycin and resistant to lincomycin, oxacillin, ofloxacin, cephradine, cefalexin, ciprofloxacin, and metronidazole

	A. marina[1,2]	*A. pfennigii*[1,3]	*A. baltica*[4]	*P. hydrocarboniclasticum*[5]	*P. indicum*[6]	*P. lavamentivorans*[6,7,8]
	DSM 2898^T	AR2102^T	BA141^T	EPR92^T	P31^T	DS-1^T
Morphology	Ovoid rods	Ovoid rods	Rod to pleomorphic	Rods	Rods	Short rods
Cell size (μm)	0.7–0.9 × 1.0–2.5	0.6 × 1.0–2.0	0.5–1.0 × 1.0–5.0	0.4 × 1.4	0.4–0.5 × 1.3–1.5	0.2 × 0.8
Pigmentation						
Aerobic cultures	Nonpigmented	Nonpigmented	Orange red	nd	Nonpigmented	Nonpigmented
Photosynthetic cultures	Pink to red	Pink to red	–	–	–	–
Cell division by budding	+	+	–	nd	–	–
Motility	+ (polar flagella)	+ (polar flagella)	–	+ (polar flagellum)	+ (polar flagellum)	+ (when growing with acetate, octane or in complex medium)
Presence of bacteriochlorophyll a	+	+	–	nd	nd	nd
Presence of carotenoids	+	+	+	nd	nd	nd
Phototrophic growth	+	+	–	–	–	–
Anaerobic growth with nitrate	+	–	–	nd	nd	nd
Temperature for growth (°C)						
Range	4–30	nd	10–37	20–40	10–42	nd
Optimum	25–30	30–35	25–30	35	25–37	nd
pH for growth						
Range	nd	6.3–8.5	nd	4.0–8.5	nd	nd
Optimum	6.9–7.1	7.2–7.5	7.0–7.5	7.5	nd	nd

NaCl concentration for growth (%)						
Range	1–16	0.5–16	0.8–6	1–5	0.5–8	nd
Optimum	1–5	1–2	1.5–3	2.5	1–5	nd
Nitrate reduction	$+^{1},-^{2}$	–	–	nd	–	–
Production of						
Poly-β-hydroxybutyrate	nd	nd	+	nd	–	–
Indole	nd	nd	–	nd	–	–
Presence of						
Oxidase	nd	nd	+	+	–	+
Catalase	nd	+	+	+	+	+
Enzymes (Api Zym)						
Lipase (C14)	nd	nd	–	nd	w	+
Valine arylamidase	nd	nd	–	nd	+	+
Cystine arylamidase	nd	nd	–	nd	+	+
Trypsin	nd	nd	–	nd	+	–
Acid phosphatase	nd	nd	–	nd	+	+
Naphthol-AS-BI-phosphohydrolase	nd	nd	–	nd	+	+
β-galactosidase	nd	nd	–	nd	+	–
α-glucosidase	nd	nd	–	nd	+	–
β-glucosidase	nd	nd	–	nd	+	–
Hydrolysis of						
Gelatin	nd	nd	–	nd	–	+
Esculin	nd	nd	–	nd	–	+
Fermentation of glucose	nd	nd	–	nd	–	–
Assimilation of						
Propionate	$w^{1},+^{2,3}$	+	nd	nd	nd	nd
Valerate	$+^{2},w^{1}$	+	nd	nd	nd	nd
Lactate	$-^{1},+^{2}$	+	nd	nd	nd	nd
Fumarate	+	$+^{3},-^{1}$	nd	nd	nd	nd
Malate	$-^{1},+^{2}$	+	–	nd	+	+
Formate	$-^{1},w^{2},+^{3}$	–	nd	nd	nd	nd
Tartrate	$-^{2},w^{1}$	–	nd	nd	nd	nd
Citrate	+	–	–	nd	nd	nd
Malonate	+	–	nd	nd	nd	nd
Adipate	nd	nd	–	nd	+	+

Table 21.2 (continued)

	A. marina[1,2]	*A. pfennigii*[1,3]	*A. baltica*[4]	*P. hydrocarboniclasticum*[5]	*P. indicum*[6]	*P. lavamentivorans*[6,7,8]
	DSM 2898^T	AR2102^T	BA141^T	EPR92^T	P31^T	DS-1^T
Fructose	$-^1$,$+^2$	–	nd	nd	nd	nd
Glucose	$-^1$,$+^{2,3}$	–	–	nd	–	–
Sucrose	+	–	nd	nd	nd	nd
Mannitol	+	–	–	nd	–	–
Sorbitol	+	–	nd	nd	nd	nd
Glycerol	w	–	nd	nd	nd	nd
Ethanol	w^{1,3},$+^2$	–	nd	nd	nd	nd
Propanol	+	–	nd	nd	nd	nd
Electron donors						
Thiosulfate	–	$+^3$,$-^1$	nd	nd	nd	nd
Sulfur	–	–	nd	nd	nd	nd
Sulfide	$+^2$,$-^3$	–	nd	nd	nd	nd
Sulfite	–	–	nd	nd	nd	nd
Hydrogen	nd	–	nd	nd	nd	nd
Biolog GP2 microplate						
Glycogen	nd	nd	–	+	–	–
Tween 40	nd	nd	–	+	+	$+^6$,$-^5$
Tween 80	nd	nd	–	nd	+	+
L-arabinose	nd	nd	+	nd	–	–
D-arabitol	nd	nd	–	+	–	–
D-cellobiose	nd	nd	–	+	–	–
i-erytritol	nd	nd	–	+	–	–
m-inositol	nd	nd	–	–	–	$-^6$,$+^5$
β-methyl-D-glucoside	nd	nd	–	+	$-^6$,$+^5$	–
Pyruvic acid methyl ester	nd	nd	–	nd	+	+
Succinic acid mono-methyl-ester	nd	nd	+	+	$+^6$,$-^5$	$-^6$,$+^5$
Acetic acid	nd	nd	w	nd	+	+
cis-aconitic acid	nd	nd	–	+	–	$-^6$,$+^5$
Citric acid	nd	nd	–	–	–	$-^6$,$+^5$
Formic acid	nd	nd	+	nd	–	–
D-galacturonic acid	nd	nd	–	–	–	$-^6$,$+^5$
D-gluconic acid	nd	nd	–	–	–	$-^6$,$+^5$

α-hydroxybutyric acid	nd	nd	+	+	−	$+^{6},-^{5}$
β-hydroxybutyric acid	nd	nd	+	+	+	$+^{6},-^{5}$
γ-hydroxybutyric acid	nd	nd	−	+	+	$+^{6},-^{5}$
Itaconic acid	nd	nd	−	+	$-^{6},+^{5}$	$-^{6},+^{5}$
α-ketobutyric acid	nd	nd	+	nd	+	+
α-ketoglutaric acid	nd	nd	−	+	−	$-^{6},+^{5}$
α-ketovaleric acid	nd	nd	−	+	$+^{6},-^{5}$	$+^{6},-^{5}$
DL-lactic acid	nd	nd	+	+	−	$-^{6},+^{5}$
Propionic acid	nd	nd	−	+	$+^{6},-^{5}$	$+^{6},-^{5}$
Sebacic acid	nd	nd	−	+	−	$-^{6},+^{5}$
Succinic acid	nd	nd	−	nd	+	−
Succinamic acid	nd	nd	+	nd	−	−
L-alaninamide	nd	nd	+	−	−	$-^{6},+^{5}$
L-alanyl-glycine	nd	nd	−	nd	+	−
L-aspartic acid	nd	nd	−	nd	+	−
L-glutamic acid	nd	nd	−	+	−	−
Hydroxy-L-proline	nd	nd	−	−	−	−
γ-aminobutyric	nd	nd	−	+	−	$-^{6},+^{5}$
Uridine	nd	nd	−	−	−	$-^{6},+^{5}$
Glycerol	nd	nd	−	+	$-^{6},+^{5}$	−
DL-α-glycerol phosphate	nd	nd	−	+	$-^{6},+^{5}$	−
Sensitivity to						
Streptomycin	nd	nd	nd	nd	+	−
Ampicillin	nd	nd	nd	nd	−	+
Tetracycline	nd	nd	nd	nd	+	−
Vancomycin	nd	nd	nd	nd	−	+
Co-trimoxazole	nd	nd	nd	nd	−	+
Clindamycin	nd	nd	nd	nd	−	+
Polymycin B	nd	nd	nd	nd	−	+
Norfloxacin	nd	nd	nd	nd	−	+
Penicillin G	nd	nd	nd	nd	−	+
Neomycin	nd	nd	nd	nd	−	+
Furazolidone	nd	nd	nd	nd	−	+
Rocephin	nd	nd	nd	nd	−	+

Table 21.2 (continued)

	A. marina[1,2]	*A. pfennigii*[1,3]	*A. baltica*[4]	*P. hydrocarboniclasticum*[5]	*P. indicum*[6]	*P. lavamentivorans*[6,7,8]
	DSM 2898^T	AR2102^T	BA141^T	EPR92^T	P31^T	DS-1^T
Predominant fatty acids	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$, $C_{16:0}$, $C_{19:0\ cyclo}\omega8c$	$C_{18:1}\omega7c$, $C_{19:0\ cyclo}\omega8c$, $C_{18:1}\omega7c$ 11-methyl	$C_{18:1}\omega7c$, $C_{18:1}\omega7c$ 11-methyl, $C_{19:0\ cyclo}\omega8c$	($C_{18:1}\omega7c$, $C_{18:1}\omega7c$ 11-methyl, $C_{19:0\ cyclo}\omega8c$)[6] ($C_{18:1}\omega7c$, $C_{19:0\ cyclo}\omega8c$)[7]
Polar lipids[a]	nd	DPG, PG, PC, PE, PME, AL(s), PL(s), GL(s)	nd	DPG, PG, PC, PE, AL(s)	nd	DPG, PG, PC, PE, AL(s)
Major respiratory lipoquinone	U-10 and MK-10	U-10 and MK-10	nd	U-10	U-11	U-11
G+C content (mol%)	62.2[1]/63.4[2]	65.6[1]/67.5[3]	61.2	60.7	62.1	64.0
Habitat	Marine environments and brackish ponds	Seawater	Seawater	Hydrothermal vent	Deep sea	Activated sludge

[1]Urdiain et al. 2008; [2]Hiraishi et al. 1995; [3]Caumette et al. 2007; [4]Brettar et al. 2007; [5]Rosario-Passapera et al. 2012; [6]Lai et al. 2011; [7]Schleheck et al. 2004; [8]Schleheck et al. 2011

For Symbols see *Table 21.1*

[a]*OH-PE* hydroxy-phosphatidylethanolamine, *AL(s)* unknown aminolipid(s), *PL(s)* unknown phospholipid(s), *GL(s)* unknown glycolipid(s), *UL(s)* unknown lipid(s)

Table 21.3

Comparison of selected characteristics of members of the genera of *Afifella*, *Anderseniella*, *Parvibaculum*, *Lutibaculum*, *Rhodobium*, *Rhodoligotrophos*, *Tepidamorphus*, and *Bauldia*. Some characteristics were examined in some species but not others as follows; in the Api ZYM tests, *Rhodoligotrophos appendicifer* and *Tepidamorphus gemmatus* are positive for esterase (C 4), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase and negative for lipase (C 14), cystine arilamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. *T. gemmatus* hydrolyzes hippurate, but does not hydrolyze esculin, xylan, arbutin, and elastin. *T. gemmatus* and *Lutibaculum baratangense* hydrolyze casein, but do not hydrolyze DNA and starch. *L. baratangense* assimilates methionine, phenylalanine, and threonine, but does not assimilate cysteine, leucine, isoleucine, tyrosine, tryptophan, melezitose, inulin, salicin, methyl α-D-glucoside, methyl α-D-mannoside, adonitol, and dulcitol. *R. appendicifer* assimilates adipate and gluconate, but does not assimilate caprate, phenylacetate, and *N*-acetylglucosamine. *T. gemmatus* assimilates α-ketoglutarate, gluconate, lysine, and asparagine, but does not assimilate glucuronate, erytrithol, D-arabitol, L-arabitol, ribitol, and L-fucose. *L. baratangense* and *T. gemmatus* assimilate glutamine, serine, valine, L-arabinose, and ribose, but does not assimilate aspartate, arginine, glycine, sucrose, trehalose, maltose, raffinose, L-sorbose, *myo*-inositol, and xylitol. *T. gemmatus* produced acid from D-turanose, D-lyxose, D-tagatose, and potassium 5-ketogluconate but not from erythritol, methyl-βD-xylopyranoside, methyl-α-D-mannopyranoside, methyl-αD-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, starch, glycogen, gentiobiose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, and potassium 2-ketogluconate. *T. gemmatus* and *L. baratangense* produced acid from D-glucose, D-fructose, L-sorbose, and D-ribose but not from D-adonitol, D-mannose, dulcitol, inositol, D-mannitol, D-sorbitol, salicin, D-maltose, D-melibiose, D-sucrose, D-trehalose, inulin, D-melezitose, and xylitol. *L. baratangense* and *R. appendicifer* are sensitive to streptomycin, gentamicin, kanamycin, chloramphenicol, and rifampicin and resistant to cefoperazone and penicillin G. *L. baratangense* is sensitive to cefotaxime, cefazolin, cefuroxime, ciprofloxacin, tobramycin, nalidixic acid, novobiocin, norfloxacin, amikacin, lomefloxacin, spectinomycin, and roxithromycin and resistant to ampicillin, vancomycin, bacitracin, carbenicillin, polymycin B, and oleandomycin. *R. appendicifer* is sensitive to amoxicillin/clavulanic acid, enrofloxacin, spectinomycin, apramycin, oxolinic acid, and flumequine and resistant to oxacillin, amoxicillin, cephalothin, fusidic acid, metronidazole, pristinamycin, sulfamethizole, and tylosin

	L. baratangense[1]	*R. gokarnense*[2,3]	*R. orientis*[2,4]	*R. appendicifer*[5]	*T. gemmatus*[6]
	AMV1^T	JA173^T	MB312^T	120-1^T	CB-27A^T
Morphology	Ovoid rods	Rods	Ovoid rods	Irregular coccoids with appendages	Irregular rods with ovoid- and hyphal-like budding structures
Cell size (μm)	0.9–1.2 × 1.5–2.0	0.5–0.6 × 1.0–2.0	0.7–0.9 × 1.5–3.2	0.7 in diameter	0.5–2.0 × 1.0–1.5
Pigmentation					
Aerobic cultures	Pale orange	Nonpigmented	Nonpigmented	Red	Nonpigmented
Photosynthetic cultures	–	Pink to red	Pink to red	–	–
Cell division by budding	–	+	+	–	+
Motility	+ (two monopolar flagella)	–	+	–	+
Presence of bacteriochlorophyll a	–	+	+	–	–
Presence of carotenoids	nd	+	+	+	–
Phototrophic growth	–	+	+	–	–
Anaerobic growth with nitrate	–	–	+	nd	–
Temperature for growth (°C)					
Range	25–37	nd	nd	5–35	30–52.5
Optimum	30–37	30	30–35	30	45–50
pH for growth					
Range	7.0–9.0	5.0–9.0	6.0–8.5	6.0–9.0	6.5–9.5
Optimum	8.0	6.5–8.0	7.0–7.5	7.0	7.5–8.0
NaCl concentration for growth (%)					
Range	nd–4	0.5–14	2–8[2] 2–16[1]	0–5	0–3
Optimum	2	2–6	4–5	0.5–10	0–1
Nitrate reduction	+	–	+	–	+

Table 21.3 (continued)

	L. baratangense[1] AMV1^T	*R. gokarnense*[2,3] JA173^T	*R. orientis*[2,4] MB312^T	*R. appendicifer*[5] 120-1^T	*T. gemmatus*[6] CB-27A^T
Presence of					
Oxidase	+	nd	nd	+	+
Catalase	+	nd	nd	+	+
Enzymes (Api Zym)					
Alkaline phosphatase	nd	nd	nd	−	+
Esterase lipase (C 8)	nd	nd	nd	−	+
Trypsin	nd	nd	nd	−	+
Hydrolysis of					
Gelatin	−	nd	nd	nd	+
Fermentation of fructose in the dark	nd	−	−	nd	nd
Assimilation of					
Acetate	nd	+	+	nd	+
Propionate	nd	−	−	nd	nd
Butyrate	nd	+[4],−[1]	+	nd	nd
Valerate	nd	−	+[2],−[1]	nd	nd
Caproate	nd	−	+	nd	nd
Lactate	nd	−	+	nd	+
Pyruvate	nd	+	+	nd	+
Succinate	nd	+	+	nd	+
Fumarate	nd	+	+	nd	−
Malate	nd	+	+	+	−
Formate	nd	−	−	nd	nd
Benzoate	nd	−	−	nd	−
Tartrate	nd	−	−	nd	nd
Citrate	−	w	−	−	−
Malonate	−	+	w	nd	nd
Glutamate	+	−	−	nd	+
Caprylate	nd	−	−	nd	nd
Alanine	−	nd	nd	nd	+
Histidine	−	nd	nd	nd	+
Fructose	−	−	+[2],−[1]	nd	+
Glucose	+	+[4],−[1]	+[2],w[1]	+	+
Galactose	+	w	−	nd	+
D-arabinose	+	nd	−	+	−
Mannose	−	nd	−	nd	−
Xylose	+	nd	+	nd	+
Cellobiose	+	nd	nd	nd	−
Melibiose	+	nd	nd	nd	−
Lactose	+	nd	nd	nd	−
L-rhamnose	−	nd	nd	nd	+
Mannitol	−	+	+[2],w[1]	nd	−
Sorbitol	−	+	+[2],−[1]	nd	−
Glycerol	−	−	−	nd	+
Ethanol	nd	−	−	nd	nd
Propanol	nd	−	−	nd	nd

Table 21.3 (continued)

	L. baratangense[1] AMV1^T	*R. gokarnense*[2,3] JA173^T	*R. orientis*[2,4] MB312^T	*R. appendicifer*[5] 120-1^T	*T. gemmatus*[6] CB-27A^T
Methanol	nd	–	–	nd	nd
Casamino acids	nd	+	–	nd	nd
Yeast extract	nd	–[4],+[1]	+	nd	nd
Peptone	nd	–	nd	nd	nd
Electron donors					
Thiosulfate	nd	–	+	nd	nd
Sulfur	nd	nd	–	nd	nd
Sulfide	nd	–	–	nd	nd
Hydrogen	nd	–	nd	nd	nd
Acid production from					
Glycerol	–	nd	nd	nd	+
D-arabinose	+	nd	nd	nd	–
L-arabinose	+	nd	nd	nd	–
D-xylose	–	nd	nd	nd	+
L-xylose	–	nd	nd	nd	+
D-galactose	+	nd	nd	nd	–
L-rhamnose	+	nd	nd	nd	–
D-cellobiose	+	nd	nd	nd	–
D-lactose	+	nd	nd	nd	–
D-raffinose	+	nd	nd	nd	–
Sensitivity to					
Erythromycin	+	nd	nd	–	nd
Lincomycin	+	nd	nd	–	nd
Tetracycline	–	nd	nd	+	nd
Colistin	–	nd	nd	+	nd
Co-trimoxazole	+	nd	nd	–	nd
Nitrofurantoin	–	nd	nd	+	nd
Doxycycline	–	nd	nd	+	nd
Predominant fatty acids	$C_{18:1}\omega7c$	$C_{18:1}\omega7c$, $C_{19:0\ cyclo}\omega8c$	$C_{18:1}\omega7c$, $C_{19:0\ cyclo}\omega8c$	$C_{16:0}$, $C_{18:1}\omega9c$, $C_{18:1}\omega7c$	$C_{19:0\ cyclo}\omega8c$, $C_{18:0}$
Polar lipids[a]	DPG, PG, PE, PL, AL, UL(s)	DPG, PG, PE, PC, PME, AL(s), PL(s)	DPG, PG, PE, PC, PME, AL(s), PL(s)	PG, PE, OH-PE, PL(s)	DPG, PG, PC PE, PME, PL, GL(s), AL(s)
Major respiratory lipoquinone	U-10	U-10 and MK-10	U-10 and MK-10	U-9 and U-10	U-10
G+C content (mol%)	70.5	72.4[4] 65.8[1]	65.3	61.1	66.9
Habitat	Mud volcano	Saltern	Seawater	Freshwater	Hot spring

[1]Kumar et al. 2012; [2]Urdiain et al. 2008; [3]Srinivas et al. 2007; [4]Hiraishi et al. 1995; [5]Fukuda et al. 2012; [6]Albuquerque et al. 2010a

For Symbols see *Table 21.1*

[a]*OH-PE* hydroxy-phosphatidylethanolamine, *AL(s)* unknown aminolipid(s), *PL(s)* unknown phospholipid(s), *GL(s)* unknown glycolipid(s), *UL(s)* unknown lipid(s)

amounts of menaquinone 10 (MK-10). S*ym*-homospermidine (HSPD) is the predominate polyamine. The major polar lipids are diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), an unknown aminolipid (AL), and an unknown glycolipid (GL); phosphatidylmonomethylethanolamine (PME) is also present. The DNA G+C content varies between 62.2 and 67.5 mol%. The species of this genus have been isolated from saline environments. The type species is *Afifella marina*. The type strain is DSM 2698^T (=BN 126^T =ATCC 35675^T =CIP 104405^T =NBRC 100343^T) (Imhoff 1983; Urdiain et al. 2008). *Afifella pfennigii* is the second species of this genus; the type strain is AR2102^T (=DSM 17143^T =ATCC BAA-1145^T), isolated from a benthic microbial mat in a brackish pond located on the rim of the Rangiroa atoll (French Polynesia) (Caumette et al. 2007; Urdiain et al. 2008).

Table 21.4

Comparison of selected characteristics of members of the genera of *Afifella*, *Anderseniella*, *Parvibaculum*, *Lutibaculum*, *Rhodobium*, *Rhodoligotrophos*, *Tepidamorphus*, and *Bauldia*. *Bauldia consociata* is oxidase and catalase positive, but does not hydrolyze cellulose. *Bauldia consociata* assimilates methanol and methylamine. *Bauldia litoralis* assimilates acetate, lactate, pyruvate, succinate, malate, β-hydroxybutyrate, fructose, glucose, sucrose, galactose, trehalose, arabinose, mannose, maltose, xylose, cellobiose, lactose, ribose, rhamnose, fucose, furanose, mannitol, sorbitol, glycerol, adonitol, dulcitol, erythritol, arabitol, and agar and does not assimilate propionate, fumarate, benzoate, glucoronate, glycine. melibiose, raffinose, ethanol, gelatin, pectin, and urea

	B. consociata[a]	*B. litoralis*[a,b]
	11^T	524-16^T
Morphology	Rods	Rods
Cell size (μm)	0.5–1.0 × 0.8–1.35	1.0–1.5
Prosthecae (μm)	0.2–0.25	< 1
Cell division by budding	+	+
Motility	–	–
Temperature for growth (°C)		
Range	nd	5–39
Optimum	28–30	26–35
NaCl concentration for growth (%)		
Range	nd	0.3–nd
Optimum	nd	1–3
G+C content (mol%)	66–68.5	66–67
Habitat	Soil	Seawater

[a]Yee et al. 2010; [b]Bauld et al. 1983

For Symbols see *Table 21.1*

Anderseniella Brettar et al. 2007

Anderseniella (An.der.sen.i.el'la. N.L. fem. dim. n. *Anderseniella*, named in honor of the late marine scientist Valérie Andersen, Observatoire Océanologique de Villefranche sur Mer, France, in recognition of her valuable work on marine ecosystems).

Anderseniella forms rod-shaped to pleomorphic cells and is nonmotile. Strictly aerobic and chemoorganotrophic. Catalase and oxidase positive. Cells contain carotenoids but not bacteriochlorophyll *a*. Mesophilic requiring NaCl for growth. Predominant fatty acid is $C_{18:1}\omega7c$, and other major fatty acids are $C_{16:0}$ and $C_{19:0\ cyclo}\omega8c$. The organism does not produce acid from carbohydrates with the API 50CH and metabolizes few organic acids and L-arabinose on the Biolog GN2. The DNA G+C content of the type strain is 61.2 mol%. The type species is *Anderseniella baltica*. The type strain BA141^T (=CIP 109499^T =LMG 24028^T) was isolated from the surface layer of anoxic sediment in central Baltic Sea (Brettar et al. 2007).

Lutibaculum Kumar et al. 2012

Lutibaculum (Lu.ti.ba'cu.lum. L. n. *lutum*, mud; L. neut. n. *baculum*, rod; N.L. neut. n. *Lutibaculum*, rod from mud).

Lutibaculum forms oval to rod-shaped cells, motile by means of two monopolar flagella. Strictly aerobic and chemoorganotrophic. Catalase and oxidase positive. Cells contain carotenoids but not bacteriochlorophyll *a*. Mesophilic. Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE) are the major polar lipids. Major fatty acid is $C_{18:1}\omega7c$. Ubiquinone-10 (U-10) is the major respiratory lipoquinone. The organism produces acid from several carbohydrates and assimilates several sugars and amino acids. The DNA G+C content of the type strain is 70.5 mol%. The type species is *Lutibaculum baratangense*. The type strain AMV1^T (=KCTC 22669^T =NBRC 105799^T) was isolated from a soil sample collected from a mud volcano of Baratang Island, Middle Andaman, India (Kumar et al. 2012).

Parvibaculum Schleheck et al. 2004

Parvibaculum (Par.vi.ba'cu.lum. L. adj. *parvus*, small; L. neut. n. *baculum*, stick; N.L. neut. n. *Parvibaculum*, small stick).

Parvibaculum forms motile rod-shaped cells. Strictly aerobic and chemoorganotrophic. Catalase and oxidase variable. The members of this genus assimilate a few carbon sources such as acetate and L-arabinose but seem to be capable of assimilating long-chain alkanes and derivatives like alkylbenzenesulfonate (LAS). Mesophilic and require NaCl for growth. Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) are the major polar lipids. According to Schleheck et al. (2004), the predominant fatty acid of *P. lavamentivorans* is $C_{18:1}\omega7c$ and $C_{19:0\ cyclo}\omega8c$, while $C_{18:1}\omega7c$ 11-methyl was not detected; however, according to Lai et al. (2011), the predominant fatty acids of the same species are $C_{18:1}\omega7c$, $C_{18:1}\omega7c$ 11-methyl, and $C_{19:0\ cyclo}\omega8c$. Ubiquinone-11 (U-11) is the major respiratory lipoquinone. The DNA G+C content varies between 62.1 and 64.0 mol%. The type species is *Parvibaculum lavamentivorans*. The type strain is DS-1^T (=DSM 13023^T =NCIMB 13966^T) (Schleheck et al. 2004). *Parvibaculum indicum* is another species of this genus, and the type strain is P31^T (=LMG 24712^T =CCTCC AB 208230^T =MCCC 1A01132^T), isolated from deep-sea water of the Indian Ocean (Lai et al. 2011). More recently, the species *Parvibaculum hydrocarboniclasticum* was described based on strain EPR92^T (=DSM 23209^T =JCM 16666^T), which was isolated from hydrothermal vent fluids collected from the East Pacific Rise (Rosario-Passapera et al. 2012).

Rhodobium Hiraishi et al. 1995; Emend. Urdian et al. 2008

Rhodobium (Rho.do'bi.um. Gr. n. *rhodos*, rose; Gr. n. *bios*, life; N.L. neut. n. *Rhodobium*, red life).

Rhodobium forms rod-shaped cells. Cells divide by budding and asymmetric cell division and grow aerobically in the dark and anaerobically in the light; under these conditions the organisms are photoorganotrophic. Cells contain carotenoids and bacteriochlorophyll *a*. The absorption maxima of living cells for *R. orientis* and *R. gokarnense* are, respectively, 377, 468, 500, 530, 591, 802, and 870 nm and 370, 402, 488, 530, 590, 803, and 872 nm. Chemoorganotrophic in the dark depending on several single-carbon sources for growth such as organic acids, short-chain fatty acids, and a few sugars. Energy can be derived from reduced sulfur compounds. Mesophilic and require NaCl for growth. Phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylmonoethanolamine (PME) are the major polar lipids. Predominant fatty acid is $C_{18:1}\omega7c$. U-10 and MK-10 are the major respiratory lipoquinone. Spermidine (SPD) and spermine (SPM) are the predominate polyamines. The DNA G+C content varies between 65.2 and 65.8 mol%. The type species is *Rhodobium orientis*. The type strain MB312^T (=JCM 9337^T) was isolated from seawater samples with sediment mud (Hiraishi et al. 1995; Urdiain et al. 2008, 2009a, b). *Rhodobium gokarnense* is the second species of this genus; the type strain is JA173^T (=ATCC BAA-1215^T =DSM 17935^T =JCM 13532^T), isolated from a saltern in Gokarna, India (Srinivas et al. 2007; Urdiain et al. 2008, 2009a, b).

Rhodoligotrophos Fukuda et al. 2012

Rhodoligotrophos (Rho.do.li.go.tro'phos. Gr. n. *rhodon*, the rose; Gr. adj. *oligos*, little, few; Gr. masc. or fem. n. *trophos*, feeder, rearer, that which nourishes; N.L. masc. n. *Rhodoligotrophos*, red utilizer of few substrates).

Rhodoligotrophos forms nonmotile irregular coccoids cells. Chemoorganotrophic. Grows aerobically or microaerobically. Catalase and oxidase positive. Mesophilic. The organism assimilates a few organic acids and a few sugars. Predominant fatty acid is $C_{16:0}$, and other major fatty acids are $C_{18:1}\omega9c$ and $C_{18:1}\omega7c$. Ubiquinone 9 (U-9) and ubiquinone 10 (U-10) are the major respiratory lipoquinone. The DNA G+C content of the type strain is 61.1 mol%. The type species is *Rhodoligotrophos appendicifer*. The type strain 120-1^T (=JCM 16873^T =ATCC BAA-2115^T) was isolated from a freshwater lake in Skarvsnes, Antarctica (Fukuda et al. 2012).

Tepidamorphus Albuquerque et al. 2010b

Tepidamorphus (Te.pi.da.mor'phus. L. adj. *tepidus* -a -um, moderately warm, lukewarm, tepid; Gr. masc. adj. *amorphous*, without form, shapeless; N.L. masc. n. *Tepidamorphus*, an organism without a distinctive morphology that grows at warm temperatures).

Tepidamorphus forms motile irregular rod-shaped cells with hyphal-like structures; multiplies by budding. Strictly aerobic and chemoorganotrophic. Catalase and oxidase positive. Slightly thermophilic. Bacteriochlorophyll *a* is not present. Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylmonoethanolamine (PME) are the major polar lipids. Major fatty acid is $C_{19:0\ cyclo}\omega8c$. Ubiquinone 10 (U-10) is the major respiratory lipoquinone. The organism produces acid from several carbohydrates and assimilates sugars, organic acids, and amino acids. The DNA G+C content varies between 65.6 and 66.9 mol%. The type species is *Tepidamorphus gemmatus*. The type strain CB-27A^T (=DSM 19345^T =LMG 24113^T) was isolated from a runoff of the hot spring known as Caldeira da Barrela, Furnas, on the Island of São Miguel in the Azores, Portugal. Strain CB-26A (=DSM 19344 =LMG 24114) is an additional strain of this species (Albuquerque et al. 2010a).

Bauldia Yee et al. 2010

Bauldia (Baul' di.a. N.L. fem. n. *Bauldia*, of Bauld, named in honor of John Bauld, an Australian microbiologist who isolated, investigated, and named members of the genus *Prosthecomicrobium* and *Planctomyces maris*).

Bauldia forms nonmotile budding prosthecate cells with short appendages. Strictly aerobic and chemoorganotrophic. Catalase and oxidase positive. Mesophilic. Several carbon sources can be used for growth including sugars, polyols, and organic acids. The DNA G+C content varies between 66 and 69 mol%. The type species is *Bauldia litoralis*. The type strain 524-16^T (=NCIB 2233^T = ATCC 35022^T) was isolated from littoral marine waters from the Washington coastlines, USA (Bauld et al. 1983; Yee et al. 2010). *Bauldia consociata* is the second species of this genus; the type strain is 11^T (=VKM B-2498^T =CCM 7594^T) and was isolated from the soil (Vasiléva et al. 1991, 2009; Yee et al. 2010).

Isolation, Enrichment, and Maintenance Procedures

Afifella marina was isolated from marine and moderately hypersaline waters and marine coastal sediments using DSM medium 27 http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium27.pdf with 3 % NaCl (Hiraishi et al. 1995; Imhoff 1983).

Afifella pfennigii was isolated from a red layer of a microbial mat in a brackish water pond on the rim of Rangiroa atoll, French Polynesia (Caumette et al. 2007). The salinity was 7 g L^{-1} and the temperature of the water above the mat was 34 °C (Mao Che et al. 2001). The organism was isolated from

deep-agar dilution series prepared from the red layer of the microbial mat. The medium, prepared according to Pfennig and Trüper (1992), contained the following (per liter deionized water): 0.35 g KH_2PO_4, 0.05 g $CaCl_2 \cdot 2H_2O$, 0.5 g NH_4Cl, 10 g NaCl, 0.7 g $MgCl_2 \cdot 6H_2O$, 0.35 g $MgSO_4 \cdot 7H_2O$, 1.5 g $NaHCO_3$, 1 mL vitamin solution V7 (Pfennig and Trüper 1981), 1 mL trace element solution SL12B containing (per liter of deionized water) 3 g $Na_2EDTA \cdot 2H_2O$, 1.1 g $FeSO_4 \cdot 7H_2O$, 0.3 g H_3BO_3, 0.19 g $CoCl_2 \cdot 6H_2O$, 0.05 g $MnCl_2 \cdot 4H_2O$, 0.042 g $ZnCl_2$, 0.024 g $NiCl_2 \cdot 2H_2O$, 0.018 g $NaMoO_4 \cdot 2H_2O$, 0.002 g $CuCl_2 \cdot 2H_2O$ (Overmann et al. 1992), 0.5 g yeast extract, 1.35 g (5 mM) disodium succinate, 0.68 g (5 mM) sodium acetate, and pH 6.8. For enrichments and to obtain better growth of pure cultures, the growth medium was supplemented with sodium ascorbate (0.5 g L^{-1}) to maintain reducing conditions. The cultures were incubated at 30 °C with a light intensity of 50 µmol quanta m^{-2} s^{-1} measured within the photosynthetically active radiation (16 h light, 8 h dark). Purity of cultures was checked by microscopic observations as well as through growth tests in oxygen gradients established in deep-agar AC medium (Difco), supplemented with thiosulfate (5 mM), and incubated in the dark. Pure cultures in liquid medium were stored in screw-capped bottles at 4 °C in the dark.

Anderseniella baltica was isolated from the black anoxic surface of sediment collected at a sampling site in the central Baltic Sea, station TEILI1, at a depth of 165 m (Brettar et al. 2007). The sediment sample was serially diluted with sterile seawater, spread on agar plates, and incubated in the dark for 3 weeks. Half-strength Zobell agar was used for enrichments (5 g Bacto peptone, 1 g yeast extract, 15 g Bacto agar, 250 mL aged seawater, 750 mL deionized water, pH 7.2) (Oppenheimer and ZoBell 1952). The strain grew well on half-strength Zobell agar, in half-strength marine broth 2216 (Difco), and on marine agar 2216 (Difco).

Lutibaculum baratangense was isolated from a soil sample collected from a mud volcano of Baratang Island, Middle Andaman, India (Kumar et al. 2012). The solid sample (100 mg) was suspended in water containing 1 % NaCl and plated on Zobell's marine agar medium containing (per liter of deionized water) 5 g Bacto peptone, 1 g yeast extract, 19.4 g NaCl, 8.8 g $MgCl_2$, 3.24 Na_2SO_4, 1.8 $CaCl_2$, 0.55 g KCl, 0.16 g $NaHCO_3$, 0.1 g $C_6H_5FeO_7$, 0.08 g KBr, 0.034 g $SrCl_2$, 0.022 g H_3BO_3, 0.008 g Na_2HPO_4, 0.004 g Na_2SiO_3, 0.0024 g NaF, 0.0016 g NH_4NO_3, and 15 g Bacto agar (Zobell 1941) and incubated at room temperature for 15 days.

Parvibaculum hydrocarboniclasticum was isolated from the Tica vent on East Pacific Rise at a depth of 2,513 m (Rosario-Passapera et al. 2012). Hydrothermal fluids were collected with titanium samplers. A sample (1 mL) was inoculated into 10 mL of artificial seawater minimal medium (ASW MM) containing (per liter of deionized water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2 \cdot 6H_2O$, 5.94 g $MgSO_4 \cdot 7H_2O$, 1.3 g $CaCl_2 \cdot 2H_2O$, 43 mg $Na_2HPO_4 \cdot 7H_2O$, 0.22 g $NaNO_3$, and 0.65 g NH_4Cl (Crespo-Medina et al. 2009) and serially diluted in the same medium. Each tube was then supplemented with dodecane, as a single-carbon and energy source, and incubated at 28 °C. A pure culture was isolated by successive transfers on ASW MM solidified with 1.5 % (w/v) Noble agar (Sigma) with dodecane. The organism can be cultured in artificial seawater (ASW) medium containing (per liter of deionized water) 24 g NaCl, 0.7 KCl, 7 g $MgCl_2$, 3 g yeast extract, and 2.5 g peptone and in low-stretch ASW (LS ASW) medium (modified ASW) containing 0.01 % (w/v) yeast extract and 0.05 % (w/v) peptone at 35 °C.

Parvibaculum indicum was isolated from deep-sea water sample of the Southwest Indian Ridge (Lai et al. 2011). The sample was collected using Niskin bottles from a depth of 1,068 m. The water sample was enriched with a PAH mixture (final concentrations 200 p.p.m. each) of naphthalene, phenanthrene, anthracene, and pyrene dissolved in crude oil. 1 mL of the enrichment culture was transferred into 100 mL medium containing (per liter of water) 1 g NH_4NO_3, 0.5 g KH_2PO_4, and 2.8 mg $FeSO_4 \cdot 7H_2O$, with the PAH mixture as the only carbon and energy source. PAH stock solution in chloroform was added to the flasks to the same concentrations as above, and the flasks were then shaken at 160 r.p.m. for 2 days to evaporate the chloroform before inoculation. After 3 weeks of incubation at 28 °C with shaking at 160 r.p.m., 1 mL of the culture was transferred to the same medium for further enrichment (Lai et al. 2009). One strain was isolated on a 216-L marine agar containing (per liter of seawater) 1 g sodium acetate, 10 g tryptone, 2 g yeast extract, 0.5 g sodium citrate, 0.2 g NH_4NO_3, 15 g agar, and pH 7.5. The organism is routinely cultured on a 216-L medium.

Parvibaculum lavamentivorans was isolated from activated sludge and from American forest soil (Schleheck et al. 2000). Activated sludge as an inoculum for experiments was obtained from the urban sewage treatment plants in Konstanz and Radolfzell, Germany, and from the industrial plant in Ludwigshafen, Germany. Activated sludge obtained from urban sewage treatment plants and American forest soil was used as inoculums of phosphate-buffered minimal-salts medium. Enrichment cultures on LAS or LADPEDS as the sole source of carbon and energy for growth were performed with a 5 % (v/v) inoculum in 3-mL cultures screw cap tubes aerated at 30 °C on a roller. Cultures were deemed positive when growth occurred and substrate decreases as assessed by HPLC. Anaerobic mineral salts medium was supplemented with 1.0 mM LAS, 20 mM succinate, and 20 mM acetate, and cultures were incubated at 25 °C about 25 cm from a 40-W lamp. Enrichment cultures were streaked on selective agarose plates prepared by solidifying 1 mM LADPEDS-salts medium with 1 % agarose. Pinpoint colonies developed after 3 weeks at 30 °C in the dark. Single colonies were picked into selective liquid medium (1 mM LADPEDS-salts), e.g., 3-mL cultures with 10 mg of glass wool in 30-mL tubes aerated on a roller. The culture was subjected to two further rounds of plating and picking, and microscopic purity was confirmed by the absence of colonies on nutrient agar within 1 week.

Rhodobium gokarnense was isolated from a soil sample with a pH of 6.8 and a temperature 30 °C in a saltern in Gokarna, India (Srinivas et al. 2007). One strain was isolated from photoheterotrophic enrichments of the soil sample. Pfennig's

medium was used (Pfennig and Trüper 1992)—0.34 g KH_2PO_4, 0.34 g NH_4Cl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.05 g $CaCl_2 \cdot 2H_2O$, and 0.34 g KCl—supplemented with NaCl (2 % w/v) and pyruvate (0.3 % w/v) as single-carbon source and ammonium chloride (0.12 % w/v) as nitrogen source used for photoheterotrophic growth under light (2,400 lx) at 30 °C. Purification of the culture was done by using the repeated agar shake dilution method (Pfennig and Trüper 1992). Purified cultures were grown in completely filled screw cap test tubes (Srinivas et al. 2006).

Rhodobium orientis was isolated from seawater samples with sediment mud obtained from two different areas in Japan (Hiraishi et al. 1995). One of the samples was collected from the bottom of a tidal pool (30–50 cm) in Goto Island, Nagasaki, and the other was obtained from the bottom of a tidal pool (50–70 cm) in Makurazaki, Kagoshima. Enrichments were performed in screw cap 20-mL test tubes which contained 10 mL of mineral base (Hiraishi and Kitamura 1984) supplemented with 10 mM acetate, 10 mM succinate, 2 mM thiosulfate, 2 mM sodium ascorbate, 2.5 % (w/v) NaCl, 0.01 % (w/v) yeast extract, and 1 mL L^{-1} vitamin solution VA (10 mg biotin, 35 mg niacin, 30 mg thiamine HCl, 20 mg *p*-aminobenzoic acid, 10 mg pyridoxine HCl, 10 mg calcium pantothenate, 5 mg vitamin B_{12}, 100 ml deionized water) (Imhoff and Trüper 1992) and were inoculated with 0.1 or 1 mL samples of seawater samples with mud, completely filled with the same medium and incubated at 28 °C under light (2,000 lx). The agar shake technique (Imhoff and Trüper 1992) was used to isolate colonies from the enrichment cultures on the medium described above containing 0.8 % agar. Single colonies that were pink to red were picked from the tubes and then purified using agar plates and the GasPak anaerobic jars. The organism that was routinely cultured in MMYS-III medium contained mineral base RM2, 20 mM sodium DL-malate as the carbon source, 2 mM thiosulfate, 5 % (w/v) NaCl, and 0.1 % (w/v) yeast extract as the growth factor, anaerobically in the light in screw cap test tubes or bottles filled with medium.

Rhodoligotrophos appendicifer was isolated from freshwater samples collected at the bottom of a freshwater lake, Naga-ike, in Skarvsnes region, Antarctica (Fukuda et al. 2012). The sample was added to 0.1× Luria-Bertani (LB) medium containing (per liter of tap water) 1 g tryptone, 0.5 g yeast extract, and 5 g NaCl and incubated at 25 °C. The resulting culture was transferred to 0.1× LB medium solidified with 2 % agar (w/v). Red colonies were found on the plate medium after incubation. The isolate grew well in 0.25× LB medium; however, no growth was observed in medium containing organic compounds at high concentration (per liter of tap water) 7.5 g tryptone, 3.8 g yeast extract, and 5 g NaCl. The strain was able to grow in NZCYM medium containing (per liter of tap water) 10 g NZ amine, 5 g yeast extract, 1 g NaCl, 2 g MgSO4·7H_2O, and 1 g casamino acids or in half-strength marine broth 2216 medium (Difco) at 30 °C.

Tepidamorphus gemmatus was isolated from a hot spring at Caldeira da Barrela, Furnas, on the Island of São Miguel in the Azores, Portugal (Albuquerque et al. 2010a). Water samples were maintained without temperature control for 6 days, and then 10–100 mL were filtered through membrane filters (Gelman type GN-6; pore size 0.45 μm; diameter 47 mm). The filters were placed on the surface of solidified *Thermus* medium (Williams and da Costa 1992) containing (per liter of media) 1 g yeast extract (Difco), 1 g tryptone (Difco), 20 g agar (Difco), 100 mL of a macronutrients solution 10x concentrated, 10 mL of a trace elements solution 100x concentrated, and 10 mL of 0.17 mM $FeCl_3 \cdot 6H_2O$. The 10× concentrated macronutrients solution contained per liter 1 g nitrilotriacetic acid, 0.6 g $CaSO_4 \times 2H_2O$, 1 g $MgSO_4 \cdot 7H_2O$, 0.08 g NaCl, 1.03 g KNO_3, 6.89 g $NaNO_3$, and 1.11 g $NaHPO_4$. The 100x concentrated trace elements solution contained per liter 0.22 g $MnSO_4 \cdot H_2O$, 0.05 g $ZnSO_4 \cdot 7H_2O$, 0.05 g H_3BO_3, 0.0025 g $CuSO_4 \cdot 5H_2O$, 0.0025 g $Na_2MoO_4 \cdot 2H_2O$, and 0.0046 g $CoCl_2 \cdot 6H_2O$. The plates were wrapped in plastic to prevent evaporation and incubated at 50 °C for up to 4 days. Cultures were purified by subculturing on solidified *Thermus* medium at 50 °C. The strain was able to grow on solidified Degryse medium 162 (Degryse et al. 1978), containing 0.25 % (w/v) yeast extract and 0.25 % (w/v) tryptone. Degryse medium contained the following components per liter of media: 2.5 g yeast extract (Difco), 2.5 g tryptone (Difco), 20 g agar (Difco), 100 mL of a macronutrients solution 10× concentrated, 5 mL of a trace elements solution 100x concentrated, 0.5 mL of 0.01 M $C_6H_5O_7Fe \cdot 5H_2O$, 10 mL of 0.2 M KH_2PO_4, and 15 mL of 0.2 M $Na_2HPO_4 \cdot 12H_2O$. The 10x concentrated macronutrient solution contained per liter 1 g nitrilotriacetic acid, 0.4 g $CaSO_4 \cdot 2H_2O$, and 2 g $MgSO_4 \cdot 7H_2O$. The 100× concentrated trace elements solution contained per liter 0.22 g $MnSO_4 \cdot H_2O$, 0.05 g $ZnSO_4 \cdot 7H_2O$, 0.05 g H_3BO_3, 0.0025 g $CuSO_4 \cdot 5H_2O$, 0.0025 g $Na_2MoO_4 \cdot 2H_2O$, and 0.0046 g $CoCl_2 6H_2O$. The organism is routinely grown in *Thermus* or Degryse liquid medium or on solidified *Thermus* or Degryse medium at 45–50 °C.

Bauldia consociata was isolated from soil habitats and compost with high cellulolytic activity (Vasiléva et al. 1991; Yee et al. 2010).

Bauldia litoralis was isolated from littoral marine waters from the Washington State (USA) coastline (Bauld et al. 1983). The isolation of organism was achieved when small pieces of *Ulva* spp. fronds, located on rocks in the sampling area, were incubated in dilute peptone enrichment medium [0.01 % peptone (w/v)]. The incubation temperature for enrichments is not mentioned in the original paper.

Members of this family do not require special procedures for maintenance and long-term storage. Generally strains are maintained on the isolation medium at 4 °C for a few days and can be stored frozen at –70 °C in medium containing 15 % glycerol without loss of viability. Long-term preservation is by lyophilization.

Ecology

Habitat

Most of the species of the family *Rhodobiaceae* are of marine origin; only the strains of the species *Rhodoligotrophos*

appendicifer and *Tepidamorphus gemmatus* were isolated from freshwater: the first from a small freshwater lake in Antarctica and the latter from a hot spring in the Azorean Island of São Miguel venting freshwater.

Environmental clones with 16S RNA similar to cultured strains can add to our knowledge of the habitats colonized by phylogenetic relatives. For example, the most closely related 16S rRNA genes to *Rhodoligotrophos appendicifer* (JN391616, 94 % sequence similarity) have been retrieved from biofilm-activated sludge system for nitrogen and phosphorus removal. Sequences very closely related to *Tepidamorphus gemmatus* have been retrieved from (DQ23097, 99 % sequence similarity) the deep aquifer of the Kalahari Shield in South Africa (Gihring et al. 2006), which is low in salinity alkaline and has a temperature of 45 °C, and white plague disease-associated community of the Caribbean coral *Montastraea faveolata* (FJ202923, 98 % sequence similarity). The latter clone implies that organisms closely related to *Tepidamorphus gemmatus* also inhabit marine environments.

The species of the genus *Rhodobium* have been isolated from marine ecosystems; the species *R. gokarnense* was isolated from salterns in India. Environmental 16S rRNA clone sequences related to the genus *Rhodobium* have not been deposited in the databases; the most closely related being sequences JN391616 and FJ623370, both of which share 94 % sequence similarity with *R. gokarnense* and were isolated from activated sludge. The same is true of uncultured organisms related to *R. orientis* such as DQ230971 (94 % sequence similarity) from the subsurface water of the Kalahari Shield in South Africa, JN391616 (94 % sequence similarity) from an activated sludge and AB630707 (94 % sequence similarity) from biofilms of a freshwater Antarctic lake. There are only a few environmental sequences that have low similarity with the two *Afifella* spp. These are EU528243 retrieved from what appears to be a tributary of a Kentucky Reservoir (USA) with sequence similarities of 98 % to *A. marina* and 96 % to *A. pfennigii*. Sequence JF344261 with 94 % sequence similarity to both *Afifella* spp. was retrieved from marine sediment after an oil spill in Galicia (Spain).

In contrast to the paucity of 16S rRNA gene sequences to the above mentioned organisms, there are several clones that are closely related to *Anderseniella baltica*, many of which were obtained from mangrove environments or marine sediments, namely, AM176883, EF061946, GU180151, and EU700185, all with 98 % sequence similarity. Two other clones (EU700185 and DQ028258) were obtained from marine plankton and aquaculture ponds that also shared 98 % sequence similarity with this species.

Many environmental 16S rRNA sequences have been reported with similarities of 99 % with the type strain of the species *Parvibaculum lavamentivorans*. Many of these are of marine origin (GU474930, HM598180, HM598131, and FM242452); others are saline alkaline soils (HQ697798 and JF727690) or deep aquifers (AB478001), natural asphalt from the La Brea Tar Pits in California (EF157176), livestock manure (AB594043), and chromium-contaminated tannery sludge (HM468043). This wide distribution of organisms closely related to *Parvibaculum lavamentivorans* contrasts with the rarity of environmental sequences that are similar to the species *P. indicum* and *P. hydrocarboniclasticum*. Only one sequence with a similarity of 99 % (HQ326336) to both species was reported from seawater reverse osmosis (SWRO) filters and are deposited in the databases.

The species *Bauldia consociata* were isolated from the soil, while the species *Bauldia litoralis* was from leaves of the macroalga *Ulva* sp. Sequences with high similarity (97–98 %) with *Bauldia consociata* were retrieved from a freshwater lake in Antarctica (AB630694), highly alkaline saline soil of the former lake Texcoco in Mexico (FJ152803), soil (JQ978641, HQ864104), rock biofilms from an ancient gold mine (HE614829, FM253576), and perhaps more surprisingly inflammatory bowel disease (HQ755623). Sequences very closely related to *B. litoralis* have been retrieved from a freshwater lake in Antarctica (AB630712, 99 % sequence similarity) and sediment in the northern Bering Sea (EU925889, 97 % sequence similarity). There are no environmental clones that can be considered to be closely related to *Lutibaculum baratangense* deposited in the public databases.

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22 The Family *Rhodospirillaceae*

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Abstract

Rhodospirillaceae Pfennig and Trüper 1971, 17[AL]

Rhodospirillaceae are a family within the order *Rhodospirillales* in the subclass of *Alphaproteobacteria*. The family *Rhodospirillaceae*, the so-called purple non-sulfur bacteria, have the type genus *Rhodospirillum* and embrace a total of 34 genera: *Azospirillum*, *Caenispirillum*, *Constrictibacter*, *Defluviicoccus*, *Desertibacter*, *Dongia*, *Elstera*, *Ferrovibrio*, *Fodinicurvata*, *Inquilinus*, *Insolitispirillum*, *Limimonas*, *Magnetospira*, *Magnetospirillum*, *Magnetovibrio*, *Marispirillum*, *Nisaea*, *Novispirillum*, *Oceanibaculum*, *Pelagibius*, *Phaeospirillum*, *Phaeovibrio*, *Rhodocista*, *Rhodospira*, *Rhodospirillum*, *Pararhodospirillum*, *Rhodovibrio*, *Roseospira*, *Skermanella*, *Telmatospirillum*, *Thalassobaculum*, *Thalassospira*, *Tistlia*, and *Tistrella*. According to 16S rRNA gene sequence similarities, the genera within the *Rhodospirillaceae* can be grouped into three big clusters: *Azospirillum–Skermanella–Desertibacter–Rhodocista–Dongia–Elstera–Inquilinus, Magnetospirillum–Nisaea–Thalassobaculum–Oceanibaculum–Fodinicurvata–Pelagibius –Tistlia–Phaeospirillum–Telmatospirillum–Defluviicoccus–Tistrella–Constrictibacter–Rhodovibrio–Limimonas*, and *Rhodospirillum–Pararhodospirillum–Roseospira–Rhodospira–Phaeovibrio–Novispirillum–Marispirillum–Insolitispirillum–Caenispirillum–Thalassospira–Magnetospira–Magnetovibrio–Ferrovibrio*. Some genera in the family *Rhodospirillaceae* grow photoheterotrophically under anoxic conditions in the light and chemoheterotrophically in the dark, while others grow heterotrophically under aerobic/microaerobic conditions. The members of the *Rhodospirillaceae* stain Gram negative and form rod shaped to spirillum-formed cells. The chemoheterotrophs include the facultative anaerobic genera *Skermanella*, *Telmatospirillum*, *Caenispirillum*, *Thalassobaculum*, and *Nisaea* and the strictly aerobic and microoxic genera *Azospirillum*, *Conglomeromonas*, *Magnetospirillum*, *Thalassospira*, *Tistrella*, and *Inquilinus*. The genus *Azospirillum* contains several diazotrophic, plant-associated bacteria having plant growth-promoting potential with agricultural application. Other genera include strains with interesting biotechnological potentials. Some genera also harbor opportunistic pathogenic bacteria, whose risk potential is not yet clear.

Taxonomy, Historical and Current

Short Description of the Family

Rhodospirillaceae (*Rho.do.spi.ril.la´ce.ae*: M.L. neut. n. *Rhodospirillum*, type genus of the family; L. suff. -aceae, ending

to denote a family; N.L. fem. pl. n. *Rhodospirillaceae*, the *Rhodospirillum* family; Pfennig and Trüper 1971a).

Phylogenetically, the family of *Rhodospirillaceae* is a member of the order *Rhodospirillales*, subclass *Alphaproteobacteria* in the phylum Proteobacteria (Stackebrandt et al. 1988). According to the 16S rRNA gene sequence comparison of the type species, the next related family within the the order *Rhodospirillales* is the family *Acetobacteraceae* (Gillis and De Ley 1980). The family *Rhodospirillaceae* contain the genera *Azospirillum*, *Caenispirillum*, *Constrictibacter*, *Defluviicoccus*, *Desertibacter*, *Dongia*, *Elstera*, *Ferrovibrio*, *Fodinicurvata*, *Inquilinus*, *Insolitispirillum*, *Limimonas*, *Magnetospira*, *Magnetospirillum*, *Magnetovibrio*, *Marispirillum*, *Nisaea*, *Novispirillum*, *Oceanibaculum*, *Pelagibius*, *Phaeospirillum*, *Phaeovibrio*, *Rhodocista*, *Rhodospira*, *Rhodospirillum*, *Rhodovibrio*, *Roseospira*, *Skermanella*, *Telmatospirillum*, *Thalassobaculum*, *Thalassospira*, *Tistlia*, and *Tistrella*. Some genera of the family *Rhodospirillaceae* grow photoheterotrophically under anoxic conditions in the light and chemotrophically in the dark (Pfennig and Trüper 1971a), while others grow chemoheterotrophically under aerobic conditions. They stain Gram negative and form rod shaped to spirillum-formed cells. Members of *Rhodospirillaceae* have varying metabolic and nutritional properties, which include photoheterotrophs, photoautotrophs, and chemoheterotrophs. The major respiratory lipochinones are ubiquinones 9, 10, and 11 and/or menaquinone 10 (MK-10). Unsaturated straight chain fatty acids are the predominant acyl groups of the family; among these are summed feature 8 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω6*c*), summed feature 3 ($C_{16:1}$ ω7c and/or $C_{168:1}\omega$6c), and summed feature 2 (consisting of $C_{14:0}$ 3 OH and or iso-$C_{16:0}$ 3-OH). The polar lipids consist mainly of phosphatidylglycerol, phosphatidylcholine, and other lipids which differ from species to species level. The type genus of the family is *Rhodospirillum*.

Azospirillum–Skermanella–Desertibacter–Rhodocista–Dongia–Elstera–Inquilinus

The genus *Azospirillum* (Tarand et al. 1979) forms a subcluster within the family *Rhodospirillaceae* together with the genera *Skermanella, Rhodocista, Desertibacter, Dongia*, *Elstera*, and *Inquilinus* (*Fig. 22.1*). These bacteria belong to the large group of "hydrobacteria," a clade of prokaryotes that originated in marine environments (Battistuzzi and Hedges 2009). Nearly all known representatives of the family *Rhodospirillaceae* are found in aquatic habitats, suggesting that *Azospirillum* represents a lineage which might have transitioned to terrestrial environments much later than the Precambrian split of "hydrobacteria" and "terrabacteria" (Wisniewski-Dyé et al. 2011). *Azospirillum* spp. are members of the α-subclass of *Proteobacteria*, and this genus was initially described by Krieg and Döbereiner (1984) to include a species previously named as *Spirillum lipoferum* (Beijerinck 1925). The growth of a spirillum-like bacterium in nitrogen-deficient malate- or lactate-based media, which had been inoculated heavily with garden soil, was first observed by Beijerinck in 1925. When this new bacterium was cultivated in malate medium, the nitrogen content increased, which led to the original species name *Azotobacter spirillum*. Three years later, it was renamed into *Spirillum* genus. In 1978 a group of isolates was utilized in a detailed taxonomic study by Tarrand et al. (1978). Based on the DNA homology group II bacteria *Azospirillum lipoferum* genus and species were described. This group of isolates seemed to correspond in several ways to Beijerinck's original description of *Spirillum lipoferum*, particularly with regard to growth with glucose or mannitol and to the formation of spirillum-shaped cells under certain conditions (Krieg and Döbereiner 1984).

These bacteria are spiral or slightly curved rod-shaped non-spore-forming cells with polyhydroxybutyrate (PHB) granules, which can form cysts. The Gram staining is negative and the cells are very motile with a single polar flagellum and several lateral flagella, shorter in length. Cells are polymorphic and positive for oxidase as well as catalase reaction (Tarrand et al. 1978). *Azospirillum* has a large amount of $C_{18:1}$ ω7*c* lipids (55·3 %) and contains also 16:1ω7*c*, 16:0 as a major component; the major hydroxy fatty acids are 3-OH $C_{14:0}$ and 3-OH $C_{16:0}$. When grown aerobically, species of this genus exhibit a quinone system with ubiquinone 10 (Q-10). The polar lipids consist mainly of phosphatidylglycerol, phosphatidylcholine, and one unidentified phospholipid. The DNA G+C content varies between 64 and 71 mol%.

The occurrence of *Azospirillum* spp. is widespread in the environment and has significant agricultural importance specifically as aerobic nitrogen-fixing species with considerable plant growth-promoting abilities. *A. brasilense* and *A. lipoferum* are known to associate with, and stimulate the growth of, numerous grasses and cereals. Most of the species were described from plant roots and soil samples. These organisms have a plant root tissue origin, especially in soils of tropical and subtropical regions, but also in temperate regions (Lavrinenko et al. 2010). *Azospirillum lipoferum* and *A. brasilense* are the two species which were described at first. Later, Magalhães et al. (1983) described the third species, *A. amazonense*. Four years later, another species was described, *A. halopraeferens* (Reinhold et al. 1987) from Kallar grass (*Leptochloa fusca*) in Pakistan. In 1989, Khammas isolated the 5th species of this genus, *A. irakense*, using root samples of rice. The taxonomy of the species *Conglomeromonas largimobilis* subsp. *largomobilis hodobium* was questioned as its similarity of the species *A. lipoferum*. The strains of this species were renamed to a new *Azospirillum* species called *A. largomobilis* and then corrected to *A. largimobile* (Sly and Stackebrandt 1999). In 2001, a new species was described and received the name of the famous scientist Johanna Döbereiner, calling *A. doebereinerae* (Eckert et al. 2001). In 2005, another species was described in China, also from rice samples, *A. oryzae* (Xie and Yokota 2005). Again a new species was described using plant tissue collected in China, *A. melinis* (Peng et al. 2006), using roots and stem of a plant called *Melinis*

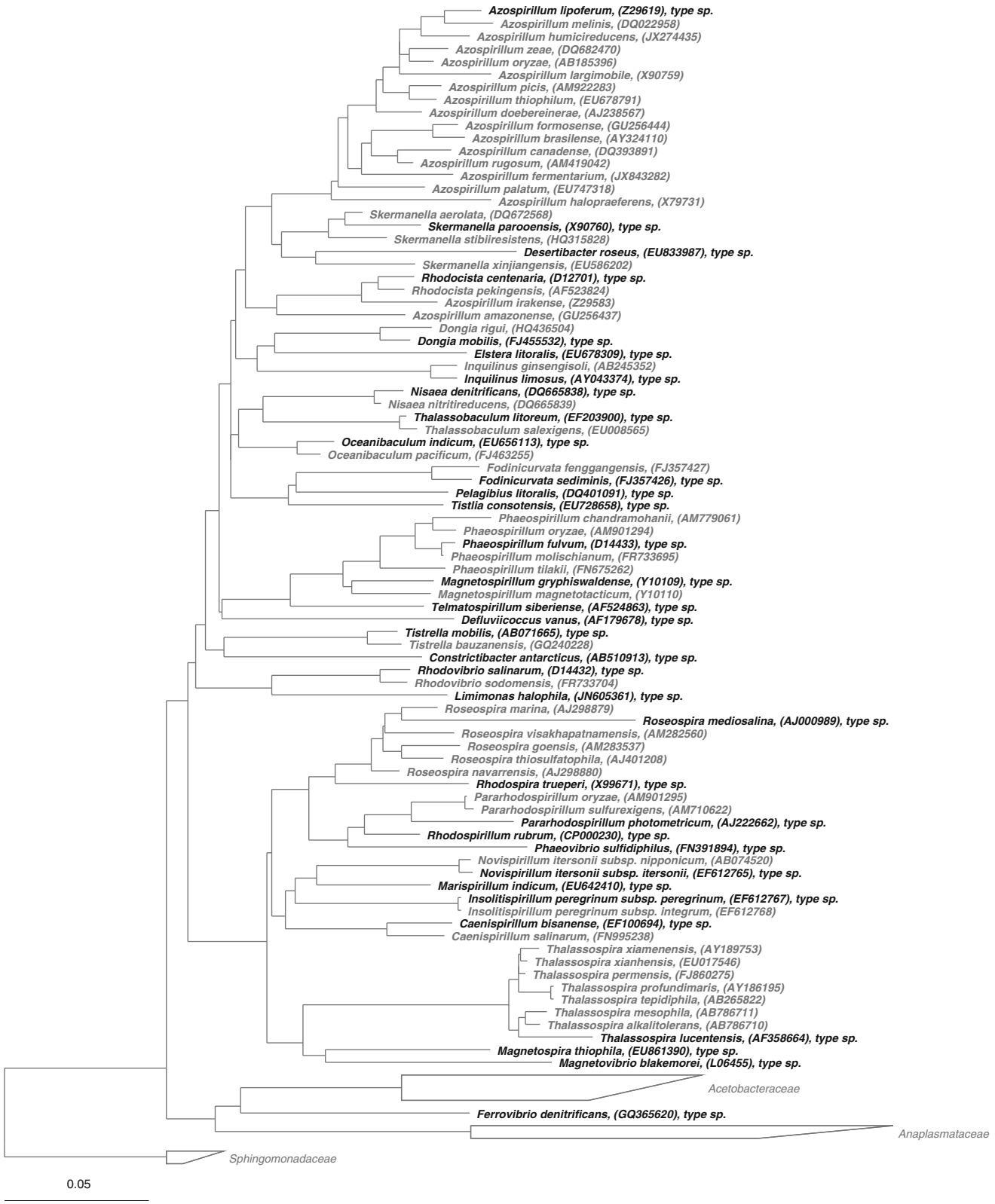

Fig. 22.1

Phylogenetic reconstruction of the family *Rhodospirillaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40% maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

minutiflora. In this case, a modification of the culture medium to a high pH contributed to discover not only this species but also two new ones: *A. canadense* (Mehnaz et al. 2007a) and *A. zeae* (Mehnaz et al. 2007b). In 2008, a new species was described using contaminated soils collected in Taiwan. This species was named *A. rugosum* as its colony morphology was different from the closely related ones (Young et al. 2008). This species was isolated from discarded road tar soil. In 2009, two new species were described: *A. palatum* (Zhou et al. 2009) and *A. picis* (Lin et al. 2009). *A. picis* was isolated from oil-contaminated soil samples in Taiwan, and it fixes nitrogen and possesses nitrate reduction activity, differing from *A. palatum*. Both species do not have indole production. *A. thiophilum* was isolated from sulfide spring collected in Russia (Lavrinenko et al. 2010). *A. formosense* was isolated from agricultural soil collected in Taiwan (Lin et al. 2012), and the last species, until to date (February 2014), is *A. humicireducens* that was isolated from microbial fuel cell and also fixes nitrogen. *A. fermentarium* was isolated from a fermentative tank in Taiwan.

More recently, the relatedness of *Roseomonas fauriae* and R. genomospecies 6 (originally members of the *Roseomonas* genus in the *Acetobacteraceae*) and *Azospirillum* spp. became apparent in comparative studies using phenotypic methods and molecular techniques. Conventional biochemical tests could not differentiate between the two taxa, and 16S rRNA and DNA–DNA hybridization experiments revealed rather high values for relatedness between *R. fauriae* with several type strains of *Azospirillum*. It was suggested that strains previously identified as *R. fauriae* and R. genomospecies 6 should be reclassified as *A. brasilense*, with the name *Roseomonas fauriae* as a later heterotypic synonym of *Azospirillum brasilense* (Helsel et al. 2006). Thus, *R. fauriae* and R. genomospecies 6, which possess a pink pigment like *A. brasilense*, were included into the genus *Azospirillum*; however, clinical isolates are also listed as *R. fauriae*.

Skermanella parooensis was first described as *Conglomeromonas largomobilis* subsp. *parooensis* together with *Conglomeromonas largomobilis* subsp. *largomobilis*. However, 16S rRNA comparison and nucleic acid hybridization (Falk et al. 1986; Ben Dekhil et al. 1997) showed that the latter was closely related to the genus *Azospirillum* and was therefore transferred as *Azospirillum largimobile* (Ben Dekhil et al. 1997) and *Conglomeromonas largomobilis* subsp. *parooensis* was elevated to *Conglomeromonas parooensis* (Ben Dekhil et al. 1997) since it was more distant from *Azospirillum* species. However, according to Rule 37a (1) of the International Code of Nomenclature of Bacteria, it should be classified in a different genus; thus, it was transferred to *Skermanella* gen. nov. as *Skermanella parooensis* gen. nov. (Sly and Stackebrandt 1999). New isolates from air (Weon et al. 2007) and soil from coal mine (Luo et al. 2012) made amendments to the genus description necessary as strictly aerobic (Weon et al. 2007), variable ability of ferment glucose and other phenotypic characteristics (Luo et al. 2012). The fourth species of genus was isolated from desert sand (An et al. 2009).

Recently, a bacterial isolate from sandy soil of the Taklimakan desert in Xinjiang, China, was described as a new genus, *Desertibacter roseus*. Based on 16S rRNA sequence comparison, it is more closely related to *Skermanella* than to *Azospirillum* (Liu et al. 2011).

Description of the genus *Rhodocista* was proposed by Kawasaki et al. (1992), to include a previously named *Rhodospirillum centenum* strain isolated from water of a hot spring (Favinger et al. 1989) as *Rhodocista centenaria* (Kawasaki et al. 1992). The presence of clearly distinct phenotypic, biochemical, and genetic properties from *Rhodospirillum rubrum* (type species of *Rhodospirillum* genus) supported the reclassification of this organism. The second species of this genus was described later by Zhang et al. (2003) that was named *Rhodocista pekingensis*, isolated from wastewater treatment plant. Rhodocista cells are vibrioid to spiral-shaped, anaerobic phototrophs or aerobic chemoorganotrophs; they are mesophilic and possess bacteriochlorophyll *a*. Colonies are pink pigmented, differentiating into dormant thermotolerant cysts when growing aerobically. *Rhodocista* type species form a close cluster with two species of the genus *Azospirillum* (*A. irakense* and *A. amazonense*, sharing 94.1 % and 91 % 16S rRNA gene sequence similarity). It encompasses 89.9 % 16S rRNA gene sequence similarity with the *Rhodospirillum rubrum* type species.

The bacterial genera *Dongia*, *Elstera*, and *Inquilinus* form a subcluster of strictly aerobic chemoheterotrophic bacteria. *Dongia mobillis* was isolated from freshwater wetland in Korea (Liu et al. 2010), while *Elstera litoralis* from biofilms on stones in the littoral zone of Lake Constance in Germany (Rahalkar et al. 2012). *Inquilinus* isolates are derived from clinical samples of, e.g., a cystic fibrosis patient (Coenye et al. 2001).

Magnetospirillum–Phaeospirillum–Nisaea–Thalassobaculum–Oceanibaculum–Fodinicurvata–Pelagibius–Tistlia–Telmatospirillum–Defluviicoccus–Tistrella–Constrictibacter–Rhodovibrio–Limimonas

According to the 16S rRNA gene sequence analysis (neighbor joining and maximum likelihood), a second branch of bacterial genera around *Magnetospirillum* and *Phaeospirillum* is evident (◉ *Fig. 22.1*).

Magnetotactic bacteria (MTB) is a general name used to group microorganisms capable of showing magnetotaxis and synthesizing intracellular organelles filled with crystals of a variety of mineral sources, named magnetosome. They are phylogenetically distributed into Alpha-, Gamma-, and Deltaproteobacteria classes of the Proteobacteria phylum, the Nitrospirae phylum, and the candidate division OP3, part of the Planctomycetes–Verrucomicrobia–Chlamydiae (PVC) bacterial superphylum (Bazylinski and Frankel 2004; Kolinko et al. 2012; Lefèvre et al. 2012a). Among the *Alphaproteobacteria* families, the *Rhodospirillaceae* comprises 3 genera representative of MTB: *Magnetospirillum*, *Magnetospira*, and *Magnetovibrio*. Characterization of the second isolated MTB that could be axenically cultivated, named MSR-1, and other

proteobacteria showed that it was closely related to *Aquaspirillum magnetotacticum* (94.1 %) than to *A. serpens* or to other reference organisms of the alpha subclass of *Proteobacteria* (84.1–88.9 %). Based on these results, Scheifer et al. (1991) proposed a new genus description, *Magnetospirillum*, in which both *A. magnetotacticum* and strain MSR-1 could be placed.

The genus *Phaeospirillum* was originally described as part of the study of Imhoff et al. (1998) which reclassified the *Rhodospirillum* species known so far into different genera mainly based on 16S rRNA gene sequence analysis. At this work *Rhodospirillum fulvum* (van Niel 1944) and *Rhodospirillum molischianum* (Giesberger 1947) were transferred to *Phaeospirillum fulvum* and *Phaeospirillum molischianum*, respectively. Reclassification of *Phaeospirillum* species was followed by the description of *P. chandramohanii*, *P. oryzae*, and *P. tilakii* (Kumar et al. 2009; Lakshmi et al. 2011a; Raj et al. 2012). These organisms were found in freshwater-rich environments such as mud and rhizosphere soil; are spiral shaped, mesophilic, and photoheterotrophic with photosynthetically grown cell suspension showing brown to brown-orange/brown-red color; present bacteriochlorophyll *a*, and major quinones are Q-9 and MK-9. *Phaeospirillum* type species share 94.4 % 16S rRNA gene sequence similarity with *Magnetospirillum* type species and 89.1 % 16S rRNA gene sequence similarity with *Rhodospirillum* type species.

A strain named P24 was shown to form a deep branch within the family *Rhodospirillaceae* based on comparative 16S rRNA gene sequence analysis (Lai et al. 2009a). The strain clustered closely to *Thalassobaculum litoreum* CL-GR58T (92.7 %), but the highest 16S rRNA gene sequence similarity was shared with strain SL3.14 (99 %), a bacterium isolated from the Silver Lake throughflow playa (Navarro et al. 2009; Lai et al. 2009a). Based on many peculiar phenotypic, biochemical, and molecular traits, a new genus, *Oceanibaculum* (Lai et al. 2009b emend. Dong et al. 2010) was proposed within the family *Rhodospirillaceae*. The type species is *Oceanibaculum indicum* P24^T (Lai et al. 2009b).

The genus *Pelagibius* was described by Choi et al. (2009) to include a marine bacterial strain isolated from seawater of the east coast of Korea. *Pelagibius* is a monospecific genus affiliated with the *Rhodospirillaceae* family according to 16S rRNA sequence analysis. The type species *Pelagibius litoralis* forms slightly curved or straight rods, is a mesophilic non-fermentative heterotroph, and is strictly aerobic forming circular, convex, and creamy colonies when grown on marine agar. The type species of *Pelagibius* share 92.9 % 16S rRNA gene sequence similarity with *Fodinicurvata* type species.

Tistlia consotensis is the only species decribed for this genus. This aerobic, slightly halophilic bacterium was recently isolated from a saline spring in Colombia (Diaz-Cárdenas et al. 2010).

Telmatospirillum siberiense was the first and only species described in the genus. Three isolates of this species were recovered from acidic wetland in Northern Russia. Based on 16S rRNA comparison, these isolates were allocated to *Telmatospirillum* nov. gen. as *Telmatospirillum siberiense*.

The genus *Rhodovibrio* comprises two species named *Rhodovibrio salinarum*, which is the type species, and *Rhodovibrio sodomensis* (Imhoff et al. 1998), formerly classified as *Rhodospirillum salinarum* and *Rhodospirillum sodomense*, respectively.

Rhodospirillum–Pararhodospirillum–Roseospira–Rhodospira–Phaeovibrio–Novispirillum–Marispirillum–Insolitispirillum–Caenispirillum–Thalassospira–Magnetospira–Magnetovibrio–Ferrovibrio

The genus *Rhodospirillum* is the type genus, and at present it consists of only one species, *Rhodospirillum rubrum* (Skerman et al. 1980; Molisch 1907; Imhoff et al. 1998), as the type species. The genus has ever contained other 11 species, including *Rhodospirillum photometricum*, *Rhodospirillum sulfurexigens*, *Rhodospirillum oryzae* (now *Pararhodospirillum photometricum*, *Pararhodospirillum sulfurexigens*, and *Pararhodospirillum oryzae*, respectively (Lakshmi et al. 2014)), *Rhodospirillum tenue* (now *Rhodocyclus tenuis* after (Imhoff et al. 1984)), *Rhodospirillum centenum* (presently *Rhodocista centenaria* after (Kawasaki et al. 1992)), and *Rhodospirillum fulvum*, *Rhodospirillum molischianum*, *Rhodospirillum salinarum*, *Rhodospirillum sodomense*, *Rhodospirillum salexigens*, and *Rhodospirillum mediosalinum* (these have been transferred respectively to *Phaeospirillum fulvum*, *Phaeospirillum molischianum*, *Rhodovibrio salinarum*, *Rhodovibrio sodomensis*, *Rhodothalassium salexigens*, and *Roseospira mediosalina* (Imhoff et al. 1998). The genus *Pararhodospirillum* consists of three species named *Pararhodospirillum photometricum*, *Pararhodospirillum sulfurexigens*, and *Pararhodospirillum oryzae* (Lakshmi et al. 2014), which have been classified previously as *Rhodospirillum photometricum*, *Rhodospirillum sulfurexigens*, and *Rhodospirillum oryzae*, respectively. *Pararhodospirillum photometricum* is the type species.

Roseospira mediosalina was first described as *Rhodospirillum mediosalinum* by Kompantseva and Gorlenko (1984). In 1998 Imhoff et al. based on comparison of the 16S rRNA sequence proposed to transfer it to *Roseospira* gen. nov. as *Roseospira mediosalina* comb. nov. Later Guyoneaud et al. (2002) classified 3 new isolates as new species of the genus (*R. marina* sp. nov., *R. navarrensis* sp. nov., and *R. thiosultatophila* sp. nov.) based on 16S rRNA gene sequence, DNA–DNA hybridization, and phenotypic characteristics. These authors also emended the genus description to take into account the characteristics of the new species. Chakravarthy et al. (2007) isolated from water of the fishing harbor at Visakhapatnam (India) strain JA131, later classified as *Roseospira visakhapatnamensis* sp. nov., and strain JA135 from sediment of Kurka saltern, Goa (India), which was classified as *Roseospira goensis* sp. nov.

Reallocation of *Aquaspirillum itersonii* and *Aquaspirillum peregrinum* (Hylemon et al. 1973) to the family *Rhodospirillaceae* leads to their reclassification into 2 new genera, *Novispirillum* and *Insolitispirillum* (Ding and Yokota 2002; Yoon et al. 2007b).

The species *Phaeovibrio sulfidiphilus*, the only species of this genus, was isolated from brackish water (Lakshmi et al. 2011b). Cells are vibrioid, mesophilic, strictly anaerobic, photoheterotrophic, and able to grow in a limited number of carbon substrates (acetate, pyruvate, and succinate). Chimeric internal membranes of lamellar stacks and vesicles are present in a single cell, and photosynthetically grown cultures are light brown. *Phaeovibrio* type species share 91.5 % 16S rRNA gene sequence similarity with *Rhodospirillum* type species and 80–91 % sequence similarity to *Rhodocista*, *Phaeospirillum*, *Rhodovibrio*, *Rhodospira*, and *Roseospira* type species.

The genus *Rhodospira* comprehends only one species named *Rhodospira trueperi* (Pfennig et al. 1997). This genus was assigned to describe a marine photosynthetic non-sulfur bacteria strain isolated from salt marsh that forms vibrioid- to spirilloid-shaped cells, and mesophilic, peach-colored photoheterotrophic cultures were observed under anoxic conditions. *R. trueperi* presents bacteriochlorophyll *b* and forms elemental sulfur globules outside the cells in the presence of sulfide, with Q-7 and MK-7 as major quinones. *Rhodospira trueperi* shares 93.9 % 16S rRNA gene sequence similarity with *Roseospira mediosalina* (type species) and 92.8 % 16S rRNA gene sequence similarity with *Rhodospirillum* type species.

Phylogenetic Structure of the Family and Its Genera

According to the phylogenetic relationship based on 16S rRNA gene sequence analyses within the order *Burkholderiales*, the family *Rhodospirillaceae* (Pfennig and Trüper 1971a) is moderately affiliated to the family *Acetobacteraceae* (Gillis and De Ley 1980). Extensive 16S rRNA gene sequence analyses of type species and strains constitute the phylogenetic structure within the family *Rhodospirillaceae* (Fig. 22.1).

Azospirillum–Skermanella–Desertibacter–Rhodocista–Dongia–Elstera–Inquilinus

The 16S rRNA gene sequence analysis within the genus *Azospirillum* reveals that *A. lipoferum, A. largimobile, A. brasilense, and A. halopraeferens* have 96.6 %, 96.6 %, 95.9 %, and 93.6 % similarity, respecticvely, with *A. doebereinerae* (Eckert et al. 2001). *A. formosense* is closely related to *A. brasilense* (98 % 16S rRNA similarity). *A. canadense* and *A. rugosum* are 96 % similar, while *A. thiophilum* and *A. picis* present a lower level of similarity (72 %). *A. halopraeferens* formed another cluster with 86 % of similarity to the above species described. *Skermanella aerolata* and *Skermanella parooensis* are included into the 16S rRNA tree as the closely related genus of *Azospirillum*. *A. amazonense* is the species with lower level of similarity together with *A. irakense* and forms a branch with *Rhodocista centenaria* and *Rhodocista pekingensis* (Lin et al. 2012). *A. fermentarium* strain CC-LY743^T revealed a high similarity level to *A. picis* DSM 19922^T (96.1 %), *A. oryzae* JCM 21588^T (96.0 %), and *A. rugosum* DSM 19657^T (96.0 %), while these values were lower (<96.0 %) for other species (Lin et al. 2013). The recently described *A. humicireducens* is closely related to *A. lipoferum* forming a subclade with 98 % similarity and also presents high levels to *A. thiophilum* (97.6 %) and *A. oryzae* (97.1 %) (Zhou et al. 2013).

Skermanella parooensis was originally classified as *Conglomeromonas largomobilis* subsp. *paraooensis*. The transfer of *Conglomeromonas largomobilis* subsp. *largomobilis* to the genus *Azospirillum* and the low 16S rRNA similarity (93 %) (Ben Dekhil et al. 1997) and DNA–DNA hybridization (5–12 %) between the two subspecies led to the elevation of the former to a new species *Skermanella parooensis* (Falk et al. 1986). Phylogenetic analyses of 16S rRNA gene sequences showed that species of the *Skermanella* genus form a cluster with *Desertibacter roseus* and are related phylogenetically to members of the genera *Azospirillum* and *Rhodocista* within the *Alphaproteobacteria*. Comparison of the 16S rRNA gene sequence of *Skermanella aerolata* 5416 T-32 showed highest level of similarity (96.2 %) with *S. parooensis* DSM 9527 but has lower levels of sequence similarity (<92 %) with respect to other species (Weon et al. 2007). *Skermanella xinjiangensis* strain 10-1-101 has the highest degree of similarity to *S. aerolata* 5416 T-32 (94.07 %) and *S. parooensis* DSM 9527 (92.74 %) (An et al. 2009). Partial 16S rRNA gene sequence (1,420 bp) of *Skermanella stibiiresistens* strain SB22T showed the highest degree of similarity to *S. aerolata* 5416 T-32 (97.3 % similarity), *S. parooensis* ACM 2042^T (95.8 %), and *S. xinjiangensis* 10-1 (92.9 %) (Luo et al. 2012). The phylogenetic analysis revealed that strain SB22T was closely related to the members of the genus *Skermanella* and grouped in the same cluster with *S. aerolata* 5416 T-32, *S. parooensis* ACM 2042^T, and *S. xinjiangensis* 10-1-101.

Phylogenetic analysis of the 16S rRNA gene sequence showed that the nearest phylogenetic neighbors of *Desertibacter roseus* 2622^T are species of the genus *Skermanella*. It shared 91.7 % and 90.1 % similarities to the type strains of *S. xinjiangensis* and *S. aerolata*, respectively. These values were lower (89.8–88.1 %) when compared to the type strains of the genus *Azospirillum* (Liu et al. 2011).

The 16S rRNA gene sequence analysis of the genus *Rhodocista* indicated that it forms a distinct phylogenetic branch within the *Rhodospirillaceae* family. In fact, the work of Kawasaki et al. (1992) clarified the phylogenetic positioning of spiral-shaped purple non-sulfur bacteria on the basis of 16S rRNA gene sequences, highlighting the heterogeneity of *Rhodospirillum* genus. The phylogenetic positioning of the *Rhodocista* species indicates a close cluster with *Azospirillum irakense* but quite distant from the other *Azospirillum* species. This very close relationship was also shown by Zhang et al. (2003), who described the second species *Rhodocista pekingensis*. *Rhodocista* species and *Azospirillum irakense* share about 96–97 % sequence similarity, although the latter is not phototrophic while the former did not grow using malate as carbon source.

Dongia mobilis LM22^T exhibited the highest 16S rRNA sequence similarity with *Inquilinus limosus* AU0476^T (90.4 %) and less than 90 % similarity with other members of the family *Rhodospirillaceae* such as *Skermanella*, *Azospirillum*, and *Rhodocista* (Liu et al. 2011). *Inquilinus ginsengisoli* Gsoil 080^T (Jung et al. 2011) was most closely related to *I. limosus* strains AU0476^T and AU1979 (Wayne et al. 1987) with 98.9 % 16S rRNA sequence similarity level. Recently, Baik et al. (2013) described a bacterium enrichment culture clone 04SU4-P as *Dongia rigui* 04SU4-P^T. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain 04SU4-P^T forms an evolutionary lineage within the genus *Dongia* and its nearest neighbor is *Dongia mobilis* LM22^T (98.0 %). The 16S rRNA gene sequence analysis indicated that *Elstera litoralis* Dia-1^T was closely related to representatives of the genera *Azospirillum* (90–91 %), *Skermanella* (88–89 %), *Rhodocista* (87–88 %), and *Dongia* (88–89 %) (Rahalkar et al. 2012).

Magnetospirillum–Phaeospirillum–Telmatospirillum–Thalassobaculum–Nisaea–Oceanibaculum–Fodinicurvata–Pelagibius–Tistlia–Defluviicoccus–Tistrella–Constrictibacter–Rhodovibrio–Limimonas

Magnetotactic bacteria (MTB) is a general name used to group microorganisms capable of showing magnetotaxis and synthesizing intracellular organelles filled with crystals of a variety of mineral sources, named magnetosome. They are phylogenetically distributed into Alpha-, Gamma-, and Deltaproteobacteria classes of the Proteobacteria phylum, the Nitrospirae phylum, and the candidate division OP3, part of the Planctomycetes–Verrucomicrobia–Chlamydiae (PVC) bacterial superphylum (Bazylinski and Frankel 2004; Kolinko et al. 2012; Lefèvre et al. 2012a). Among the *Alphaproteobacteria* families, the *Rhodospirillaceae* comprises 3 genera representative of MTB: *Magnetospirillum, Magnetospira, and Magnetovibrio.* Based on 16S rRNA gene identity, it was shown that the closest organism of *Magnetospirillum bellicus* was *Magnetospirillum gryphiswaldense* MSR-1, with 96 % sequence similarity to strain VDY^T. The species *Dechlorospirillum anomalous* strain WD was shown to be closely related *to the* magnetotactic *Magnetospirillum species* (Michaelidou et al. 2000), but at the time, none of the *Magnetospirillum* species tested (*M. gryphiswaldense, M. magnetotacticum*, and *Magnetospirillum* strain AMB-1) could couple growth to the reduction of perchlorate or chlorate, but later on, the presence of a homolog of the cld gene in strains VDY, WD, and MS-1 was reported by Bender et al. (2004). Meanwhile, according to Lefèvre et al. (2013a), the magnetospirilla are a large group that appears to phylogenetically span a number of genera. Current evidences suggest that a detailed study considering the phylogenetic relationship between *Phaeospirillum* and *Magnetospirillum* would be necessary. The phylogenetic positioning of the *Phaeospirillum* type species is distributed in three sister clades. *P. fulvum* and *P. molischianum* form one clade with 99.1 % 16S rRNA gene sequence similarity. Another clade encompasses *P. chandramohanii* and *P. oryzae*, which shares 98.2 % similarity. The third clade harbors *P. tilakii* type strain sharing approximately 97 % similarity with *P. chandramohanii*/*P.oryzae* clade and around 96.7 % similarity with *P. fulvum*/*P. molischianum* clade. The clades *P. fulvum*/*P. molischianum* and *P. chandramohanii*/*P.oryzae* share approximately 97 % similarity. The 16S rRNA gene sequence similarities between *P. tilakii* JA492^T and the other *Phaeospirillum* type strains ranged from 96.5 % to 97.4 %. Besides the neighbors, strains *P. fulvum* DSM 113^T and *P. molischianum* ATCC 14031^T share the same branch with a 99.1 % 16S rRNA gene sequence similarity. Phylogenetic tree based on 16S rRNA gene sequences of strains of *Telmatospirillum siberiense* formed a separate cluster with purple non-sulfur bacteria of the genera *Phaeospirillum* and *Magnetospirillum*, within the family *Rhodospirillaceae.* Sequence similarity of *Telmatospirillum siberiense* strains between each other was 98.3–98.9 %, but only 90.9–92.5 % when compared to *Phaeospirillum* and *Magnetospirillum.* Comparison of partial amino acid sequence obtained from amplified *nifH* gene (148 residues) also showed that 2 strains (26-4b1 and K-1) of *Telmatospirillum siberiense* formed a separate branch with higher similarity to each other (97.0 % of amino acid sequence identity) than to their closest *Azospirillum* relatives (92.0–94.1 %). In addition, comparison of partial sequences of the "red-like" *cbbL* gene encoding large (catalytic) subunits of RuBisCO, corresponding to 231 amino acid residues, also showed close relationship of *Telmatospirillum siberiense* strains to each other (96.9 %), forming a separate branch in the phylogenetic tree. Their similarity with *Azospirillum lipoferum* (88.1–91.3 %) "red-like" *cbb*L gene was similar to other *Alphaproteobacteria* (77.9–94.2 %).

The 16S rRNA gene sequence of *Thalassobaculum litoreum* strain CL-GR58^T showed 90.9 % similarity to the type strain of *Azospirillum lipoferum*, 89.8 % to *Azospirillum oryzae*, 89.7 % to *Azospirillum canadense*, 89.5 % to *A. doebereinerae*, and 79.3–89.5 % to the other type species of the family *Rhodospirillaceae* (Zhang et al. 2008). The 16S rRNA sequence analysis of strain *Thalassobaculum salexigens* CZ41-10a^T showed that it was phylogenetically affiliated to the family *Rhodospirillaceae* (Urios et al. 2010) and presented variable similarity relatedness values to the relatives *Thalassobaculum litoreum* CL-GR58^T (99 %), *Nisaea nitritireducens* DSM 19540^T (94 %), and *Nisaea denitrificans* DSM 18348^T (93 %). *Oceanibaculum* is closely related to *Thalassobaculum litoreum* CLGR58 and *Nisaea*, but each of them forms a separate clade, as independent monophyletic cluster in the family *Rhodospirillaceae* (Urios et al. 2008; Lai et al. 2009a, b).

Phylogenetic analysis of almost-complete 16S rRNA gene sequences of *Fodinicurvata sediminis* strain YIM D82^T and *Fodinicurvata fenggangensis* YIM D812^T revealed that they formed a distinct lineage within the family *Rhodospirillaceae* (Wang et al. 2009). The similarity between the 16S rRNA gene sequences of the two strains was 98.2 %. The levels of 16S rRNA

gene sequence similarities between strain YIM D82^T and the type strains of *Rhodovibrio sodomensis* and *Rhodovibrio salinarum* were 90.6 % and 90.5 %, respectively, while the sequence similarity levels were 90.2 % and 90.1 %, respectively, against *Fodinicurvata fenggangensis* YIM D812^T. The single species of the *Pelagibius* genus formed a branch closely related to *Tistlia* type species (Díaz-Cárdenas et al. 2010) showing about 91 % of 16S rRNA gene sequence similarity (Choi et al. 2009). The 16S rRNA gene sequence analysis of strain *Tistlia consotensis* USBA 355^T indicated that it formed a distant phylogenetic line of descent with members of the genus *Thalassobaculumn* (90 % gene sequence similarity). This level was much lower when strain USBA 355T was compared to all other members of the family *Rhodospirillaceae* (Díaz-Cárdenas et al. 2010).

The species *Defluvicoccus vanus* showed 16S rRNA gene sequence similarity to the species *Rhodospirillum rubrum* (87.5 %), *P. fulvum* (88.5 %), *Magnetospirillum gryphiswaldense* (88.2 %), *Magnetospirillum magnetotacticum* (88.5 %), and *Rhodocista centenaria* (89 %). A comparison of the inferred 16S rRNA gene sequence nucleotide signature between members of *Alphaproteobacteria* supports the view that type strain Ben 114^T is not closely related to any of them (Maszenan et al. 2005).

The almost complete 16S rDNA sequence of *Tistrella mobilis* strain IAM 14872^T (Shi et al. 2002) showed sequence similarity values of 86.0 % to *Craurococcus roseus* JCM 9933 T and 90.1 % to *Phaeospirillum molischianum* ATCC 14031^T. Phylogenetic analysis using the neighbor-joining method showed that strain BZ78*T* (1,493 bp) formed a distinct cluster with *T. mobilis* IAM 14872 *T*, supported by a relatively high bootstrap value (98.3 % 16S rRNA gene sequence similarity) within the family *Rhodospirillaceae* (Zhang et al. 2011). A similar tree topology was also found in the tree generated using the maximum-likelihood method. Levels of 16S rRNA gene sequence similarities between strain BZ78*T* and the type strains of other species in the family *Rhodospirillaceae* were 90.1 %.

The 16S rRNA gene sequence of *Constrictibacter antarcticus* (strain 262-8^T) indicated high sequence similarities (99–90 %) with sequences of uncultured bacteria found in environmental samples worldwide. In comparative analysis with type strains, the most closely related neighbors of strain 262-8^T were *Stella vacuolata* DSM 5901 T (90.2 %), *Stella humosa* DSM5900T (90.2), and *Tistrella mobilis* IAM 14872 T (89.7 %). In agreement with Yamada et al. (2011), the low similarity values suggested that it would be difficult to analyze the phylogenetic position of strain 262-8^T by DNA–DNA hybridization.

The genus *Rhodovibrio* comprises two species named *Rhodovibrio salinarum*, which is the type species, and *Rhodovibrio sodomensis* (Imhoff et al. 1998), formerly classified as *Rhodospirillum salinarum* and *Rhodospirillum sodomense*, respectively. *Limimonas* 16S rRNA sequence showed similarity with *Rhodovibrio sodomensis* DSM 9895^T (91.6 %) and *Rhodovibrio salinarum* NCIMB 2243^T (91.2 %) forming an independent cluster with the halophilic members of the family *Rhodospirillaceae* although in a separate clade (Amoozegar et al. 2013).

Rhodospirillum–Pararhodospirillum–Phaeovibrio–Roseospira–Rhodospira–Novispirillum–Marispirillum–Insolitispirillum–Caenispirillum–Thalassospira–Magnetospira–Magnetovibrio–Ferrovibrio

Analysis of the 16S rRNA sequence of *Rhodospirillum rubrum* ATCC11170^T, *Pararhodospirillum photometricum* (formerly *Rhodospirillum photometricum*) strains DSM122^T and E11, *Rhodovibrio sodomensis* (formerly *Rhodospirillum sodomense*) strain ATCC51195^T, and *Rhodovibrio salinarum* (previously *Rhodospirillum salinarum*) strain ATCC35394^T resulted in the separation of these two later into a phylogenetic clade and the proposal of the new genus *Rhodovibrio* (Imhoff et al. 1998).

The 16S rRNA sequence analysis between *Pararhodospirillum sulfurexigens* (formerly *Rhodospirillum sulfurexigens*) strain JA143^T with *Rhodospirillum rubrum* ATCC11170^T and *Pararhodospirillum photometricum* (formerly *Rhodospirillum photometricum*) strain DSM122^T showed sequence similarity of 95.72 % and 95.58 %, respectively, which justified the description of the former as the type strain of the novel species (Kumar et al. 2008). In the same analysis, *Rhodovibrio sodomensis* (previously *Rhodospirillum sodomense*) strain DSI^T and *Rhodovibrio salinarum* (originally *Rhodospirillum salinarum*) strain ATCC35394^T were grouped into a clade apart. Later on, phylogenetic relationships based on the 16S rRNA gene sequence analysis of *Pararhodospirillum oryzae* (formerly *Rhodospirillum oryzae*) strain JA318^T with *Pararhodospirillum sulfurexigens* (formerly *Rhodospirillum sulfurexigens*) strain JA143^T, *Pararhodospirillum photometricum* (formerly *Rhodospirillum photometricum*) strain DSM122^T, and *Rhodospirillum rubrum* ATCC 11170^T indicated that the *P.oryzae* clustered with type strains of the genus *Rhodospirillum* (which was then included in the *Pararhodospirillum*) (Lakshmi et al. 2014). The highest sequence similarity for *Pararhodospirillum oryzae* strain JA318^T was found with the type strain of *Pararhodospirillum sulfurexigens* (99.9 %).

Comparative 16S rDNA sequence analyses of *Roseospira marina* CE2105, *Roseospira navarrensis* SE3104, *Roseospira thiosulfatophila* AT2115 (AJ401208), *Roseospira mediosalina*, *Roseospira visakhapatnamensis* JA131, and *Roseospira goensis* JA135 showed that these strains form a subgroup together with *Rhodospira trueperi* within the *Rhodospirillaceae* family of the *Alphaproteobacteria*, well separated from *Rhodospirillum* genus, their closest relatives. *Roseospira marina* CE2105, *Roseospira navarrensis* SE3104, and *Roseospira thiosulfatophila* AT2115 (AJ401208) have similar salt requirements that are phylogenetically closely related, with 16S rRNA similarity ranging from 97.6 % to 96.5 %, whereas *Roseospira mediosalina* requires a higher optimal salt concentration salinity and similarity ranging from 95.6 % to 94.7 % in comparison with the former three species (Guyoneaud et al. 2002). Comparison of 16S rRNA sequences of *Roseospira visakhapatnamensis* JA131 and *Roseospira goensis* JA135 showed that they are closest to *Roseospira navarrensis* (95.9 %), *Roseospira marina* (95.5 %), *Roseospira mediosalina* (94.2 %), and *Roseospira thiosulfatophila* (96.1 %).

Sequence similarity between *Roseospira visakhapatnamensis* JA131 and *Roseospira goensis* JA135T is 96.6 % (Chakravarthy et al. 2007).

Analysis of the phylogenetic positioning of the genus *Rhodospira* indicates its close relationship with the genus *Roseospira*, with 16S rRNA gene sequence similarity of about 93–94 %. However, the presence of bacteriochlorophyll *b* and tetrahydrospirilloxanthin as main pigments in *Rhodospira* differentiates this genus from *Roseospira* which contains Bchl *a* and carotenoids of the normal spirilloxanthin series as main pigments.

Phaeovibrio genus formed by the species *Phaeovibrio sulphidiphilus* branches separately from other *Rhodospirillaceae* genera. The highest similarities of the 16S rRNA gene sequences are observed with representatives from the *Rhodospirillum* genus, sharing approximately 91–92 % similarity.

Phylogenetic analysis based on 16S rRNA gene revealed that species previously named as *Aquaspirillum itersonii* and *Aquaspirillum peregrinum* (Hylemon et al. 1973) were more closely related to the *Alphaproteobacteria* than to the *Betaproteobacteria* group and formed distinct phylogenetic lineages leading to their reallocation to the family *Rhodospirillaceae* and creation of the genera *Novispirillum* and *Insolitispirillum* (Ding and Yokota 2002; Yoon et al. 2007b).

Representatives of the genera *Marispirillum*, *Insolitispirillum*, and *Novispirillum* cluster independently in the same branch of *Caenispirillum*, but apart from the *Rhodospirillum* and *Rhodospira*, representatives of the photosynthetic group of *Rhodospirillaceae*. Comparisons of 16S rRNA gene sequences showed that *Marispirillum indicum* type strain was most closely related to the type strains of two *Insolitispirillum peregrinum* subspecies (93.0–93.1 % sequence similarity), two *Novispirillum itersonii* subspecies (92.8–92.9 %), and *Caenispirillum bisanense* (91.7 %); sequence similarities with respect to other taxa were below 90.5 % (Lai et al. 2009a). The phylogenetic analysis based on 16S rRNA and the neighbor-joining algorithm showed that strains K92T and K93 of *Caenispirillum bisanense* joined a phylogenetic clade comprising *Novispirillum itersonii* (formerly *Aquaspirillum itersonii*) and *Insolitispirillum peregrinum* (formerly *Aquaspirillum peregrinum*) exhibiting the highest 16S rRNA gene sequence similarity values (91.3–91.5 %). Similar analysis indicated that *Caenispirillum salinarum* strain AK4^T was most closely related to *Caenispirillum bisanense* (96.6 %). In contrast, it shared less than 93.2 % sequence similarity with other members of the family.

Phylogenetic analysis based on the partial 16S rRNA gene sequencing of the type strain *Thalassospira lucentensis* QMT2^T indicated a high sequence identity (89 %) to the well-characterized species *Rhodospirillum rubrum*, *Novispirillum itersonii* (formerly *Aquaspirillum itersonii*), and *Terasakiella pusilla* (formerly *Oceanospirillum pusillum*) microorganisms, which are representatives of the α-subclass of the *Proteobacteria* (López-López et al. 2002). Analysis of 16S rRNA gene sequences of *Thalassospira xiamenensis* M-5^T and *Thalassospira profundimaris* WP0211^T indicated that both species were closely related to *Thalassospira lucentensis* (96.1 % and 96.2 % gene sequence similarities, respectively). The 16S rRNA gene sequence analysis of *Thalassospira tepidiphila* 1-1B^T showed a very high level of similarity to *Thalassospira profundimaris* WP0211^T (99.8 %), *Thalassospira xiamenensis* M-5^T (98.2 %), and *Thalassospira lucentensis* DSM 14000^T (98.1 %). However, the levels of DNA–DNA relatedness between strain 1-1B^T and these type strains were 50.7 ± 17.2, 35.7 ± 17.8, and 32.0 ± 21.1 %, respectively. Very high level of similarity was also observed between *Thalassospira alkalitolerans* MBE#61^T and *T. mesophila* MBE#74^T (98.9 % similarity), and these strains shared the highest levels of similarity with *T. lucentensis* QMT2T (99.0 % and 98.5 %, respectively). High levels of similarity were also detected when these strains were compared to *T. xianhensis* P-4^T (97.9 % and 97.7 %, respectively), *T. profundimaris* WP0211^T (97.7 % and 97.2 %, respectively), *T. xiamenensis* M-5^T (97.5 % and 97.2 %, respectively), and *T. tepidiphila* 1-1B^T (97.5 % and 96.9 %, respectively) (Tsubouchi et al. 2014). Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences reveals that *Magnetospira thiophila* and *Magnetovibrio blakemorei* are closely related to *Thalassospira* spp. while other MTB representatives cluster closely to *Phaeospirillum* spp. within the second main group of *Rhodospirillaceae*. Lefèvre et al. (2012a) observed the same distribution when comparing their phylogenetic relationship based on 16S rRNA or Mam proteins cluster analysis and concluded that the evolution of MTB and magnetosomes is congruent, indicating that they were acquired by a common ancestor of the *Magnetospirillum* clade, except in one case. The congruency of the evolutionary path of MTB and magnetotaxis is also confirmed when composition and morphological properties of magnetosome minerals are taken into account (Lefèvre et al. 2013a; Pósfai et al. 2013).

Phylogenetic analysis, based on 16S rRNA gene sequence, showed that *Ferrovibrio denitrificans* Sp-1^T formed a cluster with members from two different orders: *Sneathiellales* and *Rhodospirillales* within the class *Alphaproteobacteria* (Sorokina et al. 2012). 16S rRNA gene sequence of strain Sp-1^T showed similarities with *Sneathiellales chinensis* (89.4 %) followed by *Inquilinus limosus* (89.0 %) and 88.9 % with both *S. glossodoripedis* and *I. ginsengisoli*.

Molecular Analyses

DNA–DNA Hybridization Studies

Almost all descriptions of the *Azospirillum* species include results of DNA–DNA hybridization (DDH) studies within the genus possessing approximately 64–71 mol% (Ben Dekhil et al. 1997). Only for the species *A. rugosum* the data are not available. In 1987, Reinhold et al. detected the generic relationship of *A. halopraeferens* and also confirmed the generic status of *A. amazonense* using the DNAs of five representative strains of the genus. The authors hybridized labeled rRNA from *A. brasilense* ATCC 29145^T. The strains Au 4^T and Au 5 of *A. halopraeferens* as well as the strains YI^T, Y9, and Y13 of *A. amazonense* are located at the same level on the *Azospirillum* rRNA branch, which forms

a trinity together with the rRNA branches of *Rhodospirillum rubrum* and some *Aquaspirillum* species obtained previously by De Smedt et al. (1980). Each of these branches deserved at least a separate generic rank. With Tm(e) values ranging from 73.4 °C to 75.3 °C, both *A. halopraeferens* strains are quite distinct from the *A. brasilense–Azospirillum lipoferum* cluster. One year earlier Falk et al. (1986) described the results of DNA–DNA hybridization of *Conglomeromonas largomobilis* subsp. *largomobilis* that proved the similarity to the species *A. lipoferum* althought the 47 % of this similarity was considered lower to the criteria of 70 % recommended by Wayne et al. (1987). Ben Dekhil et al. (1997) compared the binary sequence similarity values; corrected dissimilarity values indicate that *C. largomobilis* subsp. *largomobilis* is most closely related to *A. lipoferum* and *A. brasilense* with 97.1 % and 95.2 % similarities, respectively. Based on this comparison and also other features, they transfer the species into *Azospirillum largomobilis* comb. nov., and subsequently the name was corrected to *Azospirillum largimobile* by Sly and Stackebrandt (1999). Other study involving *Azospirillum* species was reported by (Peng et al. 2006). The DNA–DNA hybridization among strains of *A. melinis* isolated from molasses grass varied from 81 % to 95 %, with a mean of 88.7 %, indicating that they represented the same genomic species. As expected, the DNA–DNA relatedness was 54–57 % for *Azospirillum lipoferum* DSM 1691^T and 30–34 % for *Azospirillum brasilense* Sp 7^T hybridized against the three strains of *A. melinis*. The DNA–DNA hybridization value between *Skermanella stibiiresistens* SB22^T and *S. aerolata* KACC 11604^T (= 5416 T-32^T) was 43.3 %. DNA–DNA hybridization studies of *Roseospira marina* CE2105, *Roseospira navarrensis* SE3104, and *Roseospira thiosulfatophila* AT2115 (AJ401208) showed low homologies between them and supported the separation into three species (Guyoneaud et al. 2002). No data are available for the other species or genera. DDH analysis showed that *Inquilinus ginsengisoli* Gsoil 080^T exhibited 12 ± 3.2 % DNA–DNA relatedness with *Inquilinus limosus* AU0476^T, whereas reciprocal hybridization resulted in a higher value of 15 ± 2.7 %. DDH studies for the other genera are missing, and in many cases, the 16S rRNA has been used to create new species.

DNA–DNA hybridization study showed that a dissimilatory perchlorate-reducing bacteria (DPRB) strain VDY^T, described as *Magnetospirillum bellicus*, has only 46.2 % similarity with *Dechlorospirillum anomalous*, although 99 % similarity was observed in 16S rRNA gene sequence analysis (Thrash et al. 2007). No data about DNA–DNA hybridization is available for *Magnetospirillum* (*M. gryphiswaldense* and *M. magnetotacticum*). Studies of DNA–DNA hybridization (DDH) on *Phaeospirillum* type strains are available for *P. oryzae* and *P. tilakii* between the other type strains of the genus. The neighbors strain *P. oryzae* JA317T and *P. chandramohanii* JA145T which share 98.2 % 16S rRNA gene sequence similarity present 55 % DDH similarity, which is lower than the level generally accepted to distinguish species. In addition, values of DDH for *P. oryzae* and the type strains *P. fulvum* DSM 113 T and *P. molischianum* ATCC 14031 T are 42 % and 38 % similarities, while 16S rRNA gene sequence similarities are 97.1 % and 97.4 %, respectively (Lakshmi et al. 2011b). Studies perfomed by Raj et al. (2012) presented very low DDH values for *P. tilakii* JA492T and the other *Phaeospirillum* type strains: 10–12 %, 10.2–14.6 %, 35.3–39.5 %, and 35.3–38 % DDH similarities between DSM 113 T, ATCC 14031 T, JA317T, and JA145T, respectively. DDH analysis indicated DNA–DNA relatedness value of 66 ± 1 % between *Thalassobaculum litoreum* DSM 18839^T and *Thalassobaculum salexigens* strain CZ41-10a^T (Urios et al. 2010). DNA–DNA hybridization assays indicated that *Thalassospira lucentensis* QMT2^T was the closest phylogenetic neighbor, and *T. xiamenensis* M-5^T and *T. tepidiphila* 1-1B^T were distantly related neighbors. The species *T. alkalitolerans* MBE#61^T and *T. mesophila* MBE#74^T showed relatively high levels of DNA–DNA relatedness (%); however, they exhibited low levels of hybridization value with *T. lucentensis* QMT2^T (12.5–16.0 % and 7.1–11.0 %, respectively), with *T. xiamenensis* M-5^T (24.1–25.0 % and 8.0–15.8 %, respectively), and with *T. tepidiphila* 1-1B^T (11.3–19.4 % and 9.0–10.4 %, respectively). The DNA–DNA relatedness between *T. alkalitolerans* MBE#61^T and *T. mesophila* MBE#74^T is 7.3–15.1 %. Studies involving species of genus *Tristella* showed that the level of DNA–DNA relatedness between strains *T. bauzanensis* BZ78^T and *T. mobilis* JCM 21370^T was 37.3 %, which was well below the threshold value of 70 % recommended for the delineation of bacterial species. In the case of the genus *Tistlia*, the DNA **GC%** of strain USBA 355^T was calculated to be 71 ± 1 mol% (Díaz-Cárdenas et al. 2010). This GC% value is closer to the genera *Inquilinus* and *Caenispirillum* and in the same range of the genera *Azospirillum* and *Magnetospirillum*. In contrast, it is quite distant from the genera *Thalassospira* and *Nisaea*. DNA–DNA hybridization (DDH) performed between species *Fodinicurvata sediminis* YIM D82T and *Fodinicurvata fenggangensis* YIM D812T showed a DNA–DNA relatedness of 27.5 %.

DDH experiments carried out between strains JA318^T of *Pararhodospirillum oryzae* and JA143^T of *Pararhodospirillum sulfurexigens* and strains JA318^T of *Pararhodospirillum oryzae* and DSM122^T of *Pararhodospirillum photometricum* resulted in reassociation values of 52 ± 2 % and 45.1 ± 1 % (n = 5, including reciprocal analyses), respectively, and these hybridization values supported the classification of strain JA318^T as a distinct species (Lakshmi et al. 2014). There are no reports of DNA–DNA hybridization studies involving neither *Rhodospirillum rubrum* nor *Rhodovibrio* sp. DNA–DNA relatedness of 93 % was reported, when *Caenispirillum bisanense* strain K92T was hybridized with strain K93, suggesting that the two strains represent the same genomic species (Yoon et al. 2007a).

Riboprinting and Ribotyping

The use of the riboprinting and ribotyping methods for clustering and characterization of members of the *Rhodospirillaceae* family is scarce. So far, the methods have been used for clustering several strains from few *Azospirillum* species and *Phaeospirillum*. The intraspecific diversity of *Azospirillum amazonense* isolates was studied by Azevedo et al. (2005) and

Reis Junior et al. (2006). Both authors used the intergenic space of 16–23S rDNA as target region, where the applied restriction enzymes allowed a highly resolving diversity analysis. Azevedo et al. (2005) observed a genetic diversity within the *A. amazonense* species and divided the isolates into four clusters with 78 % of similarity using *Hae*III, *Alu*I, *Rsa*I, *Cfo*I, *Msp*I, and *Eco*RI restriction enzymes. Reis Junior et al. (2006) obtained two groups defined at 56 % of similarity using *Alu*I, *Rsa*I, and *Cfo*I for other strains from the same species. Peng et al. (2006) used the IS-PCR fingerprinting to discover a new species of *Azospirillum*, *A. melinis*.

Oda et al. (2002) studied the bacterial community of aquatic sediments using BOX-PCR, RFLP, and 16S rRNA gene sequencing and reported two *Phaeospirillum fulvum* isolates.

MALDI-TOF

The use of the MALDI-TOF method for genotypic characterization of members of the *Rhodospirillaceae* family is scarce. Recently the method was used to differentiate few species from the genus *Azospirillum* (Stets et al. 2013). The authors compared the *A. brasilense*, *A. amazonense*, and *A. lipoferum* species commonly found associated to grasses to validate the discriminatory and identification efficiency of the method; MALDI-TOF MS was proposed to classify also other bacteria isolated from wheat roots. Six strains (Sp7, Sp245, FP2, HM210, SF0, and SF5) of *A. brasilense*, two strains (Y2 and Y6) of *A. amazonense*, and one strain DSM 1691 of *A. lipoferum* were grown in DYGS medium and analyzed in biological triplicates following the same procedures used for wheat isolates. The three *Azospirillum* species were grouped into separated clusters, and the four derivative strains of *A. brasilense* Sp7 and Sp245 also clustered according to their parent strains. It is noteworthy that the replicates always clustered together, but different strains formed distinct branches, distinguishing parent and derivative strains of *A. brasilense*. The technique is still under development; therefore, new data are not available yet.

Rudney et al. (2010) identified peptides assigned to *Phaeospirillum molischianum* when studying the metaproteome of the salivary microbiota by tandem mass spectrometry (MS/MS) followed by a cation exchange step-gradient chromatography linked to a microcapillary reverse-phase liquid chromatography.

Genome Comparison

Azospirillum–Skermanella–Rhodocista–Inquilinus

The most studied strains of the *Azospirillum* genus belong to the *A. brasilense*, *A. lipoferum*, and *A. amazonense* species that have complete or draft genome sequences available. The first data on genome structure of *Azospirillum* was described by Martin-Didonet et al. (2000), and at that time only six species were described. The authors used 10 strains of five *Azospirillum* species: *A. brasilense*, strains Sp7 (ATCC 29145), Cd (ATCC 29710), FP2, and Sp245; *A. lipoferum*, strains Sp59b (ATCC 29707) and JA25; *A. amazonense*, strains Y2 (ATCC 35120) and Y6 (ATCC 35121); *A. irakense*; and *A. halopraeferens*. The results showed the presence of several megareplicons with molecular sizes ranging from 0.2 to 2.7 Mbp as determined by pulsed-field gel electrophoresis (PFGE). The PFGE DNA patterns differed within the same species, which indicates that they are strain specific. In all strains tested, the presence of 16S rDNA was detected in more than one replicon, suggesting that *Azospirillum* contains multiple chromosomes. This assumption was confirmed later on with the genomes of three members of the *Azospirillum–R. centenaria* group available: *Azospirillum* sp. B510 (Kaneko et al. 2010), *A. brasilense* Sp245 (http://genome.ornl.gov/microbial/abra/19sep08/), and *R. centenaria* SW (Lu et al. 2010) detailed below. *Azospirillum* is usually compared to *Rhodocista centenaria* (formerly *Rhodospirillum centenum*), since the latter species possesses multiple chemotaxis operons and is used as a model organism to study chemotaxis (Xie et al. 2010).

Plasmids are present in *A. lipoferum* and *A. brasilense* strains tested over several years. Some of the strains contain as many as six plasmids ranging in size from 4 MDa to over 300 MDa (Elmerich 1983, 1986). A plasmid with a size of 90 Mda is present in all strains of *A. brasilense* and in some of *A. lipoferum* (p90) and shares conserved regions and carries several genes involved in the *A. brasilense*–plant root interaction (Croes et al. 1991; Alexandre and Bally 1999). Another plasmid – also described in detail – is pRhico found in *A. brasilense* Sp7, responsible for the interaction with roots. The *A. amazonense* strain Y2 presents four replicons with the following estimated sizes: 2.7 Mb, 2.2 Mb, 1.7 Mb, and 0.75 Mb (Martin-Didonet et al. 2000).

The *A. brasilense* Sp245 genome carries seven replicons of 3, 1.76, 0.912, 0.778, 0.690, 0.191, and 0.167 Mbp (Wisniewski-Dyé et al. 2011). These genomes encode genes related to nitrogen/carbon metabolism, energy production, phytohormone production, quorum sensing, antibiotic resistance, chemotaxis/motility, and bacteriophytochrome biosynthesis, as well as those involved in nitrogen and carbon fixation.

The genome of *Azospirillum* spp. strain B510, isolated from surface-sterilized stems of rice plants (*Oryza sativa* cv. Nipponbare) in Japan (Xie and Yokota 2005), consists of a single chromosome and six circular plasmids (pAB510a (1, 455, 109 bp), pAB510b (723, 779 bp), pAB510c (681, 723 bp), pAB510d (628, 837 bp), pAB510e (537, 299 bp), and pAB510f (261, 596 bp)) with the total size of 7,599,738 bp with no linear plasmids that are present in *A. brasilense* and *A. lipoferum*. Also *A. lipoferum* has the largest number of chromids (intermediates between chromosomes and plasmids) among all prokaryotes sequenced, indicating a potential for genome plasticity (Wisniewski-Dyé et al. 2011).

One of the most surprising features of the *A. amazonense* Y2 genome sequenced recently (Sant'Anna et al. 2011) is the presence of a gene cluster implicated in carbon fixation (the Calvin–Benson–Bassham cycle). The main genes of this cluster are the genes *cbbL* and *cbbS*, and they encode, respectively, the large and

small subunits of ribulose-1,5-bisphosphate carboxylase (RuBisCO). At least *R. centenaria* (formerly *R. centenum*) and *A. lipoferum* are known to be capable of growing autotrophically by means of RuBisCO in contrast to *Azospirillum* sp. B510 and *A. brasilense* Sp245, which do not contain Form I or II of RuBisCOs ("true" RuBisCOs) encoded in their genomes (Sant'Anna et al. 2011).

In *A. brasilense*, at least three pathways for IAA biosynthesis exist, two tryptophan-dependent pathways (indole-3-acetamide (IAM) pathway and indole-3-pyruvate (IPyA) pathway) and one tryptophan-independent pathway (Steenhoudt and Vanderleyden 2000; Spaepen et al. 2007). Similarly, the genome of *Azospirillum* sp. B510 contains genes responsible for the IAM pathway and three putative plant hormone-related genes encoding tryptophan 2-monooxytenase (*iaaM*) and indole-3-acetaldehyde hydrolase (*iaaH*), which are involved in IAA biosynthesis (Kaneko et al. 2010). However, the *iaaM*, *iaaH*, and *ipdC* genes, related to the IAM or IPyA pathways, were not located in the *A. amazonense* genome, and no *ipdC* homologue and *iaaC* was found in the B510 genome (Kaneko et al. 2010; Sant'Anna et al. 2011), suggesting that another pathway is present in these bacteria. Comparison among genome sequences of *A. brasilense* strains Sp 245 (origin: Brazil), Az39 (origin: Argentina), and CBG497 (origin: Mexico); *A. lipoferum* 4 B (origin: France); *Azospirillum* sp. B510 (origin: Japan); and *A. amazonense* Y2 (origin: Brazil), using BLAST for putative genes involved in IAA biosynthesis, showed that ipdC is present in *A. brasilense*, aromatic aminotransferase hisC1 is absent only in *A. amazonense*, aldehyde dehydrogenase is present only in Sp245 and Az39 genomes and nitrilase is present in Sp245, Az39, and Y2 genomes (Cassán et al. 2013). Further analysis of the genome sequence of *A. amazonense* revealed a gene encoding a protein with about 70 % similarity to nitrilases from plant species, like *Arabidopsis thaliana* and *Zea mays*, which catalyze the conversion of indole 3-acetonitrile to IAA (Kriechbaumer et al. 2007; Vorwerk et al. 2001). Future studies may indicate if this gene is envolved in IAA biosynthesis in *A. amazonense*.

Other features are also described to the *Azospirillum* genus such as chemotaxis/motility (Bible et al. 2008) including flagellum gene distributions (Chang et al. 2007; Sant'Anna et al. 2011), type IV secretion system (Kaneko et al. 2010), quorum sensing (Lerner et al. 2009), transport (TonB-dependent transport), antibiotic resistance (multidrug resistance (MDR) transporters), and several others (Sant'Anna et al. 2011). All three *Azospirillum* species possess three chemotaxis operons that are orthologous to those in *R. centenum*; however, they also have additional chemotaxis operons that are absent from their close aquatic relative (Wisniewski-Dyé et al. 2011).

Two species of the genus *Skermanella*, namely, *Skermanella aerolata* KACC 11604 and *Skermanella stibiiresistens* SB22, are being sequenced, but no sequence is available to date (http://www.ncbi.nlm.nih.gov/bioproject/).

The genome of *Rhodocista centenaria* ATCC 43720 T (formerly *Rhodospirillum centenum* strain SW = ATCC 51521) was sequenced (INSDC ID CP000613.2) (Lu et al. 2010). The genome presents 4,355,548 bp, 4,003 proteins, and 4,102 genes with a DNA G+C content of 70.5 %. The G+C content determined by thermal denaturation method on purified DNA is 69.9 mol% (Kawasaki et al. 1992). Extrachromosomal elements were absent. Genome assembly presents a single circular chromosome containing 35 pseudogenes, 4,003 protein coding genes, and 64 genes coding for RNA (11 rRNA genes, 52 tRNA genes, and 1 miscellaneous RNA gene). Nitrogenase reductase genes include *nif*, *mod*, and *fix* clusters for nitrogenase biosynthesis, molybdenium transport, and electron transport respectively. The absence of nitrogenase enzymes DRAT and DRAG, which mediated posttranslational regulation of nitrogenase Fe protein, suggests different environmental requirements for nitrogen fixation by *R. centenaria*. Two forms of RuBisCO (subtypes IAq and IC) and a gene encoding PEPC reveal superior ability for carbon fixation. Two genes encoding bacteriophytochrome are present (*ppr* and RC1_3803), and two genes coding for flavin-binding photoreceptors (RC1_2193 and RC1_0351) are suggested to play a role in controlling the bacterial metabolism in response of light. The sequences of the four 16S rRNA gene copies in the genome of *R. centenaria* SW are identical. A total of 72 flagella genes distributed in five gene clusters were identified to accomplish for the dual flagella system of *R. centenaria*: a constitutive polar flagellum and an inducible lateral flagellum.

The first draft assembly genome sequence of *Inquilinus limosus* (INSDC AUHM00000000) was reported by Pino et al. (2012). This genome was 7,413,714 bp long with a 69.87 % GC content and contained 7,081 predicted genes with 6,998 protein coding genes and 83 RNA genes. Besides genes with unknown function (610; 9.34 %), the highest number of genes are involved in amino acid transport and metabolism (857; 13.12 %), followed by genes coding for general function prediction only (848; 12.98 %), transcription (627, 9.60 %), carbohydrate transport and metabolism (705, 10.79 %), and inorganic ion transport and metabolism (408; 6.25 %). Other 199 genes (3.05 %) were found to code for secondary metabolite biosynthesis, transport, and catabolism, and 85 (1.30 %) were found related to defense mechanisms. According to Pino et al. (2012) 89 genes are likely involved in susceptibility or resistance to antibiotics and toxic compounds. Up to 19 could be coded for multidrug resistance efflux pumps while 21 for different classes of β-lactam recognizing proteins as penicillin-binding proteins. From them, four are homologous to β-lactamases deposited in databases. No transposable elements or pathogenicity islands could be detected.

Magnetospirillum–Phaeospirillum–Oceanibaculum–Thalassobaculum–Nisaea–Fodinicurvata–Tistlia–Tistrella–Rhodovibrio

Complete genome sequences of magnetotactic spirilla, *Magnetospira* sp. QH-2 and *Magnetospirillum* sp. (*Magnetospirillum magneticum*) AMB-1, from marine and freshwater environment, respectively, raised very important data

Table 22.1

Morphological, physiological and molecular differentiating characteristics among the genera of the family *Rhodospirillaceae*

	Azospirillum	*Desertibacter*	*Dongia*
Morphology	Straight or slightly curved rods	Rods	Slightly curved to straight rods
Gram-stain	Negative	Negative	Negative
Motility	+	+	+
Metabolism	Aero- and microaerobic	Strictly aerobic	Strictly aerobic and heterotrophic
Nitrogen fixation	+	Negative	Negative
Nitrate reduction	+	+	+
Range for growth			
Temperature (°C)	5–42 optimum 30	47–40	30–35
pH	5–9 optimum 7.0	7–10	7.0–7.5
Major fatty acids	18:1ω7c, 16:1ω7c, 16:0	C18:1 ω7c, C16:0, C18:0	C18:1v7c, C19:0 cyclo v8c, C16:0 and C16:0 2-OH
Ubiquinone type	Q-10	Q-10	Q-10
Genome size	6.8–7.6		
G+C content	64–69.6	71.4	65.6
Habitat	Mainly rhizosphere soil of several plants specially members of *Poaceae* family	Sand soil	Reactor
Additional characteristics	Denitrification variable		

	Constrictibacter	*Defluviicoccus*	*Fodinicurvata*	*Limimonas*	*Magnetospirillum*	*Nisaea*
Morphology	Ovoid to rods	Cocci/coccobacilli in tetrads and clusters	Vibrioid and rod-shape	Rod	Helical (clockwise) spirilla	Rod
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+	–	–	–	+	+
Metabolism	Aerobically or micro-aerobically	Chemoheterotrophic	Facultative anaerobic	Strictly aerobic	Microaerobic	Facultative anaerobic, microaerophilic
Nitrogen fixation	Negative		Negative	nd	+, variable	nd
Nitrate reduction	+	+	+	–	+	Variable
Range for growth						
Temperature (°C)	5.0–30.0	20–30	15–42	30–50	20–45	15–44
pH	6.0–8.0	5.0–8.5	6.5–8.5	6.0–8.0	6.5–6.9	5.0–9.0
Major fatty acids	C18:1, C16:0, C18:0	nd	C18:1v7c, C18:1 2-OH and C16:0	$C_{19:0}$ cyclo ω7*c* and $C_{18:0}$	$C_{18:1}\omega7$, $C_{16:0}$	$C_{18:1}\omega7$, $C_{16:1}\omega7$
Ubiquinone type	Q-10	nd	Q-10	Q-10	Q-10	Q-10
Genome size				nd	4.1–9.21	4.63
G+C content	69.8	66	61.5–62.3	67.0	62.0–71.0	60.0
Habitat	A gap of white rock	Sludge	Salt mine	Saline muds	Chemically stratified water and sediments of freshwater, brackish, marine, and hypersaline habitats	Marine
Other important characteristics			Accumulation of Fe(III) oxides on the cell surface	Extremely halophilic. Growth occurs at 2.5–5.2 M NaCl (optimum 3.4 M = 23.8 %)	Magnetotaxis, Magnetosome production variable	Tolerates salinity up to 6 % (optimum growth at 2 %)

Elstera	*Inquilinus*	*Rhodocista*	*Skermanella*
Rods	Rods	Vibrioid to spiral	Rod-shaped, with rounded or tapered end, and a straight or slightly curved axis
Negative	Negative	Negative	Negative
–	Variable	+	+
Chemotrophic	Strictly aerobic; chemo-organotrophic	Anaerobic/aerobic	Aerobic, chemo-organotroph
No	Negative	+	–
–	–		Variable
20–25	15–30	25–44	5–35
6.5–7.0		6.5–8	4–9
18:1ω7c, 18:1 2-OH and 16:0	18:1W7c, 18:1-2OH, 18:0-3OH		C18:1 ω7c and C16:0
Q-10	Q-10	Q-9	Q-10
		4.36	nd
58.5–62.5	70.3–70.9	68.8–69.9	65–68.8
Biofilm on stone	Respiratory Secretions	Water sample at the edge of a thermophilic hot spring/wastewater treatment plant	Fresh water, soil, air
Accumulation of Fe(III) oxides on the cell surface		Cells are converted to cysts under aerobic incubation. Anaerobically grown colonies are pink	NaCl concentration 0–5 %

Oceanibaculum	*Pelagibius*	*Phaeospirillum*	*Rhodovibrio*	*Telmatospirillum*	*Thalassobaculum*	*Tistlia*	*Tistrella*
Rod	Slightly curved or straight rods	Vibrioid to spiral-shaped	Vibrioid to spiral shaped cells	Vibrioid to spiral shaped	Slightly curved and rod	Slightly curved to straight rods	Rods
Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
+	+	+	+	+	+	+	+
Aerobic	Strictly aerobic	Anaerobic/ microaerobic	Photoheterotrophic and anaerobic, chemotrophic and microarobic to aerobic	Chemo-organotroph under anoxic or microoxic conditions/ chemolithotrophic (H_2 oxidation) under microoxic conditions	Aero- and facultative anaerobic	Aerobic	Aerobic
nd	–	+	–(?)	+	–	+	–
Variable	+		?	nd	Variable	+	+
10–42	15–33	25–35	20–47	4–30	15–37	20.0–40.0	20.0–40.0
6.0–11.0	6–11	5–9	7–8	4–7	5.0–10.0	5.0–8.0	5.0–9.0
$C_{16:1}$ω7c, $C_{16:0}$	C18:1ω7c, C18:0 3-OH, C19:0 cyclo ω8c	C18:1ω7c, C16:0, C16:1 ω6c/C16:1 ω7c	$C_{18:1}$, $C_{18:0}$, $C_{16:0}$	C18:1 ω7c, 17:0 cyclopropane and C16:0	C18:1ω7c; C16:0	C19:0ω8c; C18:1ω7c; C18:0	C18:1ω7c
nd	Q-10	Q-9, MK-9	Q-10, MK-10	nd	Q-10	Q-10	Q-10
3.95		3.79–3.81	nd	nd			
64.8–67.7	66.3	60.5–64.8	66–67	61.6–64	65.0–68.0	71.0	65.8–67.5
Marine, Sediments of hydrothermal field	Coastal seawater	Freshwater, mud and rhizosphere soil	Anoxic zones of saline or hypersaline environments such as sea water, brines and salt lakes that are exposed to the light	Marsh water	Seawater	Water	Wastewater and soil
Moderately halophilic. Tolerates salinity up to 9 % (optimum varies among species)	Non-fermentative heterotroph; Salt required for growth	Non-fermentative. Fully pigmented under photosynthetic conditions; photoautotrophic	Cells contain internal photosynthetic membranes as vesicles. Photosynthetic pigments are bacteriochlorophyll a (esterified with phytol) and carotenoids of the spirilloxanthin series with spirilloxanthin as the major component. Photoheterotrophs under anaerobiosis in the light, or chemotrophs under microoxic to oxic conditions in the dark; complex nutrients are required	NaCl concentration up to 0.58 %	Halophilic	Slightly halophilic	

Table 22.1 (continued)

	Caenispirillum	*Ferrovibrio*	*Insolitispirillum*	*Magnetospira*	*Magnetovibrio*	*Marispirillum*
Morphology	Helical	Short and thin vibrios	Helical	Variable, truncated or fully helical spirillum	Vibrioid to helical	Helical
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+	+	+	+	+	+
Metabolism	Chemoheterotrophic; aerobic	Facultative anaerobes	Aerobic	Obligately microaerophilic	Facultative anaerobic, microaerophilic	Facultative anaerobic
Nitrogen fixation	Negative		+	+	+	nd
Nitrate reduction	+	+	−	+	+	+
Range for growth						
Temperature for growth (°C)	15–47	5–45	12–39	5–37	4–31	10–41
pH	6.5–10	5.5–8.0	5.0–8.0	6.9–7.1	7.0–7.5	5.0–10.0
Major fatty acids	$C_{18:1}$ ω7c	18:1x7c, 19:0 cyc and 16:0	$C_{18:1}\omega7c$, $C_{16:0}/C_{18:1}$ 2-OH	$C_{18:1}\omega7$, $C_{16:1}\omega7$	$C_{18:1}\omega7$, $C_{16:1}\omega7$	$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH
Ubiquinone type	Q-10	Q-10	Q-9	nd	nd	nd
Genome size			nd	nd	3.7	nd
G+C content	70	64.2	62.0–66.0	47.2	52.9–53.5	
Habitat	Sludge	Iron–sulfide mineral spring	Freshwater, Oxidation ponds	Marine	Sediments	Marine
Other important characteristics		Accumulation of Fe(III) oxides on the cell surface	Coccoid non-motile organisms and microcysts forms differentiate subspecies	Polar magnetotaxis	Axial and polar magnetotaxis	Moderately halophilic. Tolerates salinity up to 12 % (optimally at 2–8 %)

Novispirillum	*Phaeovibrio*	*Rhodospira*	*Rhodospirillum*	*Pararhodospirillum*	*Roseospira*	*Thalassospira*
Spirilla, helical and coccoid	Vibrioid	Vibrioid to spirilloid	Vibrioid to spiral shaped cells	Spiral shaped cells	Vibrioid to spiral shaped	Vibrioid to spiral
Negative	Negative	Negative	Negative	Negative	Negative	Negative
+	+	+	+	+	Variable	Variable
Aerobic	Anaerobic	Anaerobic/microaerobic	Photoheterotrophic and anaerobic, photoautotrophic, chemotrophic and microaerobic to aerobic, fermentative	Phototrophic anaerobes	Photo-organotroph in anoxic conditions	Aero- and anaerobic
+	−		+	+/−	+ or −	−
+			−	nd	−	+
15–43	20–40	20–35	30–35	25–40	30–35	4.0–40.0
5.0–9.0	7–8	7–7.8	6.8–7.0	6–8	5.3–8.5	4.5–11.0
$C_{18:1}\omega7c$, $C_{16:0}$/$C_{18:1}$ 2-OH	$C_{18:1}\omega7c$ and $C_{16:0}$	$C_{18:1}$, $C_{16:0}$ and $C_{14:0}$	$C_{18:1}\omega7c$/C18:1ω6c, C16:0, C16:1ω7c/ C16:1ω6c, and also three hydroxyl acids of C14 and C16	C16:0, $C_{18:1}\omega7c$/ C18:1ω6c, and three hydroxy acids of C14, C15 and C16	nd	C16:0
Q-10		Q-7, MK-7 and RQ-7	Q-10, RQ-10	Q-8, RQ-8	nd	Q-10
4.29			4.35	3.8	nd	
63.0–65.0	67.8–68.8 mol%	65.7	63.8–65.8	60–65.8	66.6–72.3	47.0–54.7
Freshwater	Brackish water	Salt marsh	Freshwaters	Freshwaters, exceptionally in rhizosphere	Anoxic marine sediments; microbial mats in coastal waters; marine environments	Saline soil, marine environment, plant
Sensitive to 3.0 % NaCl, produces water-soluble yellowish green fluorescent or brown pigment, the last in presence of tyrosine and tryptophan	Non-fermentative. Brown pigmented under photoautotrophic growth	Anaerobically grown colonies are beige to peach-colored	Cells contain internal photosynthetic membranes as vesicles. Photosynthetic pigments are bacteriochlorophyll a (esterified with phytol or geranylgeraniol) and carotenoids of the spirilloxanthin series. Photoheterotrophs under anaerobiosis in the light, or photoautotrophs with molecular hydrogen or sulfide as electron donors, or chemotrophs under microoxic to oxic conditions in the dark, or fermentatives and oxidant-dependent	Cells contain internal photosynthetic membranes as lamellar stacks forming a sharp angle to the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll a and carotenoids lycopene and rhodopin. Strict anaerobes and obligate phototrophs with preference for neutral pH	NaCl concentration −0.2–15 %	Halophilic; growth up to 10 % NaCl

about peculiarities associated with their gene structural organization, ecosystem origin, and adaptative evolution (Matsunaga et al. 2005; Richter et al. 2007; Ji et al. 2013). A 130 kb region representing a putative genomic "magnetosome island" (MAI) of *M. gryphiswaldense* was shown to undergo frequent transposition and subsequent deletion under physiological stress conditions (Ullrich et al. 2005). In this region a great abundance of multiple copies of transposase genes that belongs to different families of IS elements was observed, suggesting that these mobile genetic elements play a major role in driving the hypervariability shown by the organizational differences in spontaneous magnetosome mutants obtained upon subculture in the laboratory. Comparison of magnetosome gene organization among some MTB of *Rhodospirillaceae* revealed that distinct variations in gene order and sequence similarity, as well as copy numbers, are present in the MAI of the MTB. Based on these data and on a detailed comparison study including MTB of other classes, it has been considered that at least a set of *mam* genes (*mamH, I, E, K, M, O, P, A, Q, L, B, S, T, C, D, Z*, and *X*), *mms6* and *mmsF* are universally shared by the MAI on MTB of the *Rhodospirillaceae* family (Richter et al. 2007; Lefèvre et al. 2013b). Genes for nitrogen fixation and assimilatory nitrate respiration are well conserved among freshwater magnetospirilla, but absent from the *Magnetospira* sp. QH-2 genome. As observed in the QH-2 genome by gene cluster synteny and gene correlation analyses, the insertion of the magnetosome island probably occurred after divergence between freshwater and marine magnetospirilla. The presence of a sodium-quinone reductase, sodium transporters, and other functional genes is evidence of the adaptive evolution of *Magnetospira* sp. QH-2 to the marine ecosystem. In contrast, marine *Magnetospira* sp. QH-2 neither has TonB and TonB-dependent receptors nor does it grow on trace amounts of iron (Ji et al. 2013). Further draft genome comparison of other *Magnetospirillum* spp. revealed that the genome size varies from 4,2 Mbp to 4,9 Mbp and annotated ORFs from 3,878 to 4,925 (Dzyuba et al. 2012). The presence of plasmid is indicated to *M. gryphiswaldense* type strain, and a 3.7-kb cryptic plasmid designated pMGT was found in *M. magneticum* MGT-1 (Bertani et al. 2001; Okamura et al. 2003).

The genome of *Phaeospirillum molischianum* strain DSM 120^T was sequenced (INSDC ID CAHP00000000.1) (Duquesne et al. 2012). The genome presents 3,805,617 bp, 3,803 protein sequences, and 3,888 genes with a G+C content of 61.5 %. The G+C content determined as buoyant density and by thermal denaturation was in this range (60.5–64.8 mol%) (Imhoff et al. 1998). Extrachromosomal elements were absent. The genome assembly presents 61 contigs (sizes of 522–416,194 bp), 11 pseudogenes, 3,803 protein coding genes, and 62 genes coding for RNA (5 rRNA genes, 49 tRNA genes, and 8 other miscellaneous RNA genes). Proteins involved in signal transduction include 60 histidine kinase-type sensors and 65 response regulators (*LuxR, Fis, CheY*, and *OmpR* families), which are believed to play important roles in the adaptability of *P. molischianum* to environmental changes. Nitrogenase reductase genes include two types of nitrogenases, a Mo-Fe-dependent nitrogenase and an alternative Fe-Fe nitrogenase. In addition, 5.89 % of the overall genome corresponds to repetitive sequences, and a total of 81 transposases were predicted. The genome of the *Phaeospirillum fulvum* strain MGU-K5, isolated from a lake mud in Khabarovsk (Russia), was fully sequenced, and the automatic annotation is available (AQPH00000000.1 at NCBI). The genome presents 3,789,403 bp, 3,462 proteins, and 3,510 genes with a DNA G+C content of 63.9 %. Nitrogenase reductase genes, including molybdenium–iron and vanadium–iron nitrogenase subunits, are present in the genome. A high number of proteins involved in signal transduction (including ~49 histidine kinase-type sensors), response regulators (including approximately 2 *LuxR*, 1 *Fis*, and 21 *CheY* families), and cell detoxification (including approximately 12 multidrug efflux pumps, 4 heavy metal efflux pumps, and 8 RND-related efflux transporters) are present.

Oceanibaculum indicum P24^T draft genome contains 3,952,792 bp corresponding to a total of 3,755 protein-coding and 45 tRNA genes. According to Lai and Shao (2012a), the proteins associated with amino acid transport and metabolism (COG initial, E) were the most abundant COG group (415 open reading frames (ORFs), 14.4 %), followed by the ones associated with inorganic ion transport and metabolism (P; 244 ORFs, 8.5 %) and transcription (K;216 ORFs, 7.4 %).

One ongoing draft genome sequencing project on *Thalassobaculum salexigens* strain DSM 19539 is available at the public GenBank (NCBI) with a total length of 5.08 Mb and a GC content of 67.4 %. No more information about the proteins and ribosomal genes is available.

The *Nisaea denitrificans* DR41_21^T genome sequenced (GenBank accession number AUFM00000000) by DOE Joint Genome Institute is represented by 20 scaffolds covering 4626718 bases; of this total 91.53 % are coding DNA (98.62 % of protein coding, 1.38 % of RNA, and 0.67 % of pseudogenes coding, that is not additive under total gene count since it could be counted as protein coding or RNA genes). Another draft genome related to this genus is that of *Nisaea* sp. BAL199 (GenBank accession number ABHC00000000), by J. Craig Venter Institute, that consists of a total of 69 DNA scaffolds covering 6102701 bases that represent 90.10 % of total DNA coding bases, and a total of 6182 genes (99.13 % protein coding and 0.87 % RNA coding). Up to date, although publically available at NCBI, no further details about these genomes have been published elsewhere.

Fodinicurvata sediminis DSM 21159 (YIM D82T) genome (INSDC ATVH00000000.1), analyzed in the course of the Genomic Encyclopedia of Bacteria and Archaea, showed 3,690,548 bp long with 60.63 % GC content. This value is similar to those determined by T_m and nuclease method performed on purified DNA (61 mol%, ❯ *Table 22.1*). Besides 28 pseudogenes, 3,551 genes have been predicted, of which 3,490 were protein-coding genes and 61 were RNA genes. The distribution of genes into clusters of orthologous groups (COGs) functional categories indicates that the highest number of genes is involved in amino acid transport and metabolism (435; 13.42 %), followed by genes coding for general function prediction only (403; 12.43 %), transcription (215, 6.63 %), energy production and conversion (176; 5.43 %),

and carbohydrate transport and metabolism (175, 5.4 %). A total of 141 genes (4.35 %) were found to code for secondary metabolite biosynthesis, transport, and catabolism, and 35 (1.08 %) were found related to defense mechanisms.

The genome of *Tistlia consotensis* was sequenced using the Roche GS FLX + System allowing for long sequence reads (Rubiano-Labrador et al. 2014). After assembling the 171,055 reads, 2,377 contigs were obtained comprising a total of 5,701,113 bp sequence. The high sequence coverage obtained (10.4×) indicates that the genome size should be slightly above 5.7 Mbp. Based on the nucleotide sequences, the G+C mole percentage was estimated at 70.4 %. Remarkably, this value is among the highest G+C ratio known for *Alphaproteobacteria*, but the GC content between 60 and 70 mol% is consistent with that reported to halophilic and halotolerant microorganisms in *Rhodospirillaceae* family (Rubiano-Labrador et al. 2014). A high GC content (above 60 %), as found in *T. consotensis*, could be a common feature in halophilic microorganisms, since it is correlated with the abundance of acidic residues, especially Asp, in halophilic proteins. The acidic nature of the proteins contributes to adaptation at high salt concentrations.

The complete genome sequence of *Tistrella mobilis* strain KA081020-065 totaling 6,513,401 bp was established by 454 pyrosequencing to reveal five replicons comprising a 3,919,492-bp circular chromosome and four circular plasmids ranging in size from 83,885 bp (pTM4) to 1,126,962 bp (pTM3); it has also a high average G+C content of 68 % (Xu et al. 2012). Its genome organization with three megaplasmids greater than 600 kb each is reminiscent of several other α-proteobacteria members such as the rice plant endophyte *Azospirillum* sp. B510 that harbors six plasmids, five of which are in excess of 500 kb.

One ongoing draft genome sequencing project on *Rhodovibrio salinarum* strain DSM 9154 is available at the public GenBank (NCBI) with a total length of 4.18 Mb and a GC content of 64 %. No more information about the proteins and ribosomal genes is available.

Rhodospirillum–Pararhodospirillum–Novispirillum–Caenispirillum–Thalassospira

The *Rhodospirillum rubrum* genome consists of a 4,352,825-bp-long chromosome with 65 % G+C content and a 53,732-bp plasmid with 60 % G+C content (Munk et al. 2011). A total of 3,850 protein-coding genes and 83 RNA genes were predicted, including four copies of rrn operon. The *Pararhodospirillum photometricum* genome consists of a 3,876,289-bp chromosome, which encodes 3,281 proteins and 3 rRNAs (Duquesne and Sturgis 2012).

Novispirillum itersonii subsp. *itersonii* ATCC 12639 draft genome (GenBank accession number ARMX00000000) corresponds to 34 scaffolds, including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), covering a total of 4290651 number of bases; of this total only 88.58 % was assigned as base coding for proteins (98.13 %) and RNA (1.87 %) genes.

A draft genome sequence of *Caenispirillum salinarum* AK4T (INSDC ANHY00000000) is available and consisted of 61 contigs of 4,952,365 bp. A total of 4,574 coding regions were found in the genome, where 3,092 (67.8 %) were functionally annotated. The number of genes transcribed from positive strands was 2,276 and from negative strands were 2,327. Besides genes with unknown function (401; 10.11 %), the distribution of genes into clusters of orthologous groups (COGs) functional categories indicates that the highest number of genes is involved only in general function prediction (458; 11.55 %), followed by genes coding for amino acid transport and metabolism (353; 8.9 %) and energy production and conversion and signal transduction mechanisms (both 295; 7.44 %). A total of 124 genes (3.13 %) were found to code for secondary metabolite biosynthesis, transport, and catabolism, and 47 (1.19 %) were found related to defense mechanisms. In agreement with Khatri et al. (2013), the functional comparison of genome sequences available on the RAST server revealed the closest neighbors of *Caenispirillum salinarum* to be *Rhodospirillum rubrum* ATCC 11170 (score 533) followed by *Magnetospirillum magneticum* AMB-1 (score 520), *Rhodospirillum rubrum* (score 490), *Magnetospirillum gryphiswaldense* MSR-1 (score 472), and *Alphaproteobacterium* BAL199 (score 318).

The genome of *Thalassospira profundimaris* WP0211^T was sequenced and contains 4,040 candidate protein-encoding genes (with an average size of 958 bp), giving a coding intensity of 88.4 % (Lai and Shao 2012b). A total of 3,157 proteins could be assigned to clusters of orthologous groups (COG) families. Forty-three tRNA genes for 18 amino acids (lacking Asn and Lys) were identified. The proteins associated with amino acid transport and metabolism (COG initial, E) were the most abundant group among the COGs (412 open reading frames (ORFs), 13.1 %), followed by the proteins associated with transcription (K; 360 ORFs, 11.4 %) and inorganic ion transport and metabolism (P; 263 ORFs, 8.3 %).

Phenotypic Analyses

The main features of the members of *Rhodospirillaceae* are listed in ❯ *Table 22.1*.

Azospirillum Tarrand et al. 1979 (1980), Emend. Falk et al. 1985

A.zo.spi.ril'lum. French noun azote, nitrogen; Greek noun spira, a spiral; spillum, a small spiral; *Azospirillum*, a small nitrogen spiral.

Bacteria belonging to the genus *Azospirillum* are Gram negative, aerobic, curved, or slightly curved rods, spiral with diameter varying from 0.6 to 1.5 μm, and lengths vary with the species and culture medium from 1.0 to 7.0 μm. Motile by a single polar flagellum and lateral several smaller ones, shorter in length. Generally contains granules of poly-β-hydroxybutyrate (PHB). Oxidase, catalase, and urease activity is present in some species. Also nitrate–nitrite and denitrification is present in some species but not all three pathways together and a single species,

Table 22.2

Morphological, physiological and molecular characteristics differentiating species within genus *Azospirillum*

	Azospirillum lipoferum Sp. 59b^T = ATCC 29707^T	*Azospirillum brasilense* Sp7^T = ATCC 29145^T	*Azospirillum amazonense* Am 14 (=Y1^T) = ATCC 35119	*Azospirillum halopraeferens* Au 4 = ATCC 43709^T	*Azospirillum irakense* KBC1^T = ATCC 51182^T	*Azospirillum largimobile* ACM 2041^T	*Azospirillum doebereinerae* GSF71^T = DSM 13131^T	*Azospirillum oryzae* COC8^T = CCTCC AB204051^T
Morphology	Rod, sometimes spiral	Rod, sometimes spiral	Rod, sometimes spiral	Vibroid, S-shape	Rod, sometimes spiral	Rod, curved axis	Rod or S-shape	Spiral or vibroid
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Cell size (μm)	1.0 × 1.5	1.0 × 1.2	0.8 × 1.0	0.7–1.4 × 2.4–5.0	0.6–0.9	0.7–1.5 × 1.5–4.5	1.0–1.5 × 2.0–3.0	1.0–1.0 × 5.0–5.0
Pleomorphic cells	+	–	+	+	+	+	+	nd
Flagellum	1, polar	1, polar	1, polar	1, polar	1, polar	nd	1, polar	1, polar
Lateral flagella	+	+	–	–	+	–	–	–
Motility	+	+	+	+	+	+	+	+
Metobolism	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
N_2 fixation	+	+	+	+	+	+	+	+
Detection *nifD* and/or *nifH*	+	+	+	+	+	+	+	+
Nitrate reductase	+	+	v	+	v	+	+	+
Temperature for growth (°C):								
Range	nd	nd	nd	nd	30–33	10–37	nd	nd
Optimum	37	37	35	41	33	28	30	30
pH for growth:								
Range	5.7–6.8	6.0–8.0	nd	6.0–8.0	5.5–8.5	nd	6.0–7.0	6.0–7.0
Optimum	nd	nd	nd	7.2	nd	nd	nd	nd
PHB accumulation	+	+	+	+	+	+	+	+
Oxidase	–	nd	nd	+	+	+	+	+
Catalase	–	nd	nd	+	nd	+	+	nd
Urease	nd	nd	nd	nd	+	+	+	nd
Nitrite reduction	nd	nd	nd	nd	nd	nd	nd	nd
Growth with 3% NaCl	–	v	–	+	+	–	–	–
Denitrification	+	+	–	+	v	–	+	–
Biotin requirement	+	–	–	+	–	–	–	+
N-acetylglucosamine	+	+	v	nd	+	+	–	nd
D-galactose	+	v	+	–	+	+	v	+
myo-inositol	v (+)	–	+	nd	–	–	nd	–
L-rhamnose	–	–	v	nd	+	–	nd	+
L-arabinose	+	v	+	v	+	+	+	+
Malate	+	+	+	+	+	+	+	+
Sucrose	–	–	+	–	+	nd	–	–
D-glucose	+	v	+	–	–	+	v	+
D-fructose	+	+	+	+	v	+	+	+
D-mannitol	+	–	–	–	–	+	+	–
Glicerol	+	+	–	+	nd	+	+	nd
D-sorbitol	+	–	–	–	–	+	+	–
Indol production	+	+	+	+	+	+	+	nd

Azospirillum melinis TMCY 0552^T	*Azospirillum canadense* DS2^T = LMG 23617^T	*Azospirillum zeae* N7^T = LMG 23989^T	*Azospirillum rugosum* IMMIB AFH-6^T = CCUG 53966^T	*Azospirillum picis* IMMIB TAR-3^T = CCUG 55431^T	*Azospirillum palatum* ww 10^T = LMG 24444^T	*Azospirillum thiophilum* BV-S^T = DSM 21654	*Azospirillum formosense* CC-Nfb-7^T = BCRC 80273^T	*Azospirillum humicireducens* SgZ-5T = CCTCC AB 2012021^T	*Azospirillum fermentarium* CC-LY723^T = BCRC 80505^T
Rod, sometimes spiral	Short rods	Rod, sometimes spiral	Rods, slighty curved	Rods, slighty curved	Rod	Spiral, curved rods	Rods, slighty curved	Spiral, curved rods	Spiral, curved rods
Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
0.7–0.8 × 1.0–1.5	0.9–1.8 × 2.5	0.9–1.5 × 1.9–6.8	nd	nd	0.6–1.0 × 2.0–2.6	1.1–2.0 × 3.6–7.0	0.8 × 2.5	1.2–1.8 × 2.5–5.5	1.0–1.2 × 2.4–2.6
–	nd	nd		nd	nd	nd	nd	nd	nd
–	1, polar	1, polar	1, polar	1, polar	Peritrichous	nd	1, polar	1, polar	1, polar
–	–		–	–	+	nd	nd	nd	nd
–	+	+	+	+	+	+	+	+	+
Facultative anaerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Mixotrophic–anaerobic growth with nitrate	Aerobic	Facultative anaerobic	Aerobic
+	+	+	+	+	–	+	+	+	+
+	*nif H*	+		*nif H*	–	nd	*nif H*	*nif H*	
+	+	+	+	+	–	+	+	+	+
5–37	20–37	20–41	22–37	22–37	15–42	15–40	20–37	25–37	20–37
20–33	25–30	30	30	37	30–37	37	30	30	30
4.0–8.0	nd	5.0–7.0	nd	6.5–9.0	5.0–9.0	6.5–8.5	5.0–9.0	5.5–8.5	6.0–8.0
nd	5.0–7.0	5.3–8.0	nd	nd	6.0–8.0	7.5	7.0	7.2	7.0
+	+	+	+	+	+	+	+	+	+
nd	+	nd	+	+	w	+	–	+	+
nd	+	+	+	+	+	+		+	+
nd	nd	+	nd	+	+	–		nd	nd
nd	nd	–	nd		–	nd	nd	–	+
+	–	–	–	–	–	+	–	+	+
nd	nd	+	nd	nd	nd	–	nd	–	nd
–	–	nd	nd		nd	nd	–	–	–
+	–	v	–	+	v	–	–	+	+
+	–	+	–	+	–	+	+	–	+
+	–	–	–	nd	–	–	–	+	+
+	–	–	–	nd	–	–	–	+	+
+	–	+	–	+	v	+	+	+	–
+	+	+	+	+	+	+	+	+	+
–	–	–	–	–	+	+	–	+	+
+	–	v	+	+	+	+	+	+	–
+	–	+	–	nd	+	+	+	+	–
+	–	+	–	+	+	+	–	+	–
+	–	+	+	nd	–	+	+	+	–
+	–	+	–	+	–	+	+	+	+
nd	–	+	–	–	–	–			+

Table 22.2 (continued)

	Azospirillum lipoferum Sp. 59b^T = ATCC 29707^T	*Azospirillum brasilense* Sp7^T = ATCC 29145^T	*Azospirillum amazonense* Am 14 (=Y1^T) = ATCC 35119	*Azospirillum halopraeferens* Au 4 = ATCC 43709^T	*Azospirillum irakense* KBC1^T = ATCC 51182^T	*Azospirillum largimobile* ACM 2041^T	*Azospirillum doebereinerae* GSF71^T = DSM 13131^T	*Azospirillum oryzae* COC8^T = CCTCC AB204051^T
Predominant cellular fatty acids – Genus *Azospirillum* has 18:1ω7c, 16:1ω7c, 16:0 as a major component.	C18:1 ω7c or C18:1 ω6c (60.71%), C16:1 ω7c or C16:1 ω6c (4.61%), C14:0 3–OH and/or iso-16:1 (3.62%), C 16:0 3-OH (2.07%) C18:1 2-OH (0.30%)	C18:1 ω7c or C18:1 ω6c (60.2%), C16:1 ω7c or C16:1 ω6c (13.1%), C14:0 3-OH and/or iso-16:1 (4.8%), C 16:0 (4.1%). C 16:0 3-OH (3.7%) C18:1 2-OH (5.9%)	nd	nd	nd	nd	nd	C18:1 ω7c or C18:1 ω6c (64.04%), C16:1 ω7c or C16:1 ω6c (8.54%), C14:0 3-OH and/or iso-16:1 (4.44%), C 16:0 (7.32%), C 16:0 3-OH (2.64%) C18:1 2-OH (5.35%)
Predominant ubiquinone	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10
Genome size	Genome sequenced of strain 4B (isolated from rice–France)– Genome size 6.8 Mbp and seven replicons	Genome sequenced of strain Sp 245 (isolated from wheat–Brazil). Genome size of 7.5 Mbp and seven replicons. Strain CBG497 genome size of 6.5 Mbp and six replicons	Draft genome of Y2T 0f 7.0 Mbp and four replicons	nd	nd	nd	nd	* strain close to type strain COC8– Genome size of 7.6 Mbp and six replicons
DNA G+C (mol%)	69–70	69–71	66–68	70	64–67	70	69	66.8
Habitat	Roots, stem and leaves-several plants	Roots, stem and leaves-several plants	Roots, stem and leaves-several plants	Saline soils and rhizosphereof Kallar grass	Rhizosphere	Water	Roots, stem and leaves-several plants	Rice (*Oryza sativa*) roots

Azospirillum melinis TMCY 0552^T	*Azospirillum canadense* DS2^T = LMG 23617^T	*Azospirillum zeae* N7^T = LMG 23989^T	*Azospirillum rugosum* IMMIB AFH-6^T = CCUG 53966^T	*Azospirillum picis* IMMIB TAR-3^T = CCUG 55431^T	*Azospirillum palatum* ww 10^T = LMG 24444^T	*Azospirillum thiophilum* BV-S^T = DSM 21654	*Azospirillum formosense* CC-Nfb-7^T = BCRC 80273^T	*Azospirillum humicireducens* SgZ-5T = CCTCC AB 2012021^T	*Azospirillum fermentarium* CC-LY723^T = BCRC 80505^T
nd	18:1ω7c (54.9%), 16:0 (12.3%), Summed feature 3 (12%).	C18:1 ω7c (54.4%), C 16:0 (5.6%), 16:0 3-OH (4.3%), Summed feature 3 (14.4%) and summed feature 2 (5.6%)	C18:1ω7c (39.61%), C16:0 (12.40%), C14:0 3-OH (5.6%), C16:0 3-OH (3.2%)	C18:1ω7c (57.86 %), C16:1ω7c (6.94 %), C16:0 (19.48 %), C14:0 3-OH (4.21 %), C16 3-OH (47.42 %) and C18:0 3-OH (14.07 %), C14:0 3-OH (4.21 %), C16 3-OH (47.42 %) and C18:0 3-OH (14.07 %)	C18:1ω7c (35.9%), C16:0 (13.0%), cyclo-C19:0 ω8c (32.1%)	C18:1 ω7c or C18:1 ω6c (60.65%), C16:1 ω7c or C16:1 ω6c (14.79%), C 16:0 (7.61%), C14:0 3-OH and/or iso-16:1 (3.76), C 16:0 3-OH (1.71%) C 18:0 (1.33%), C18:1 2-OH (3.66%)	C18:1 ω7c or C18:1 ω6c (56.4%), C16:1 ω7c or C16:1 ω6c (15.1%), C14:0 3-OH and/or iso-16:1 (5.8%), 16:0 (4.47%), C 16:0 3-OH (4.2%) C18:1 2-OH (6.9%)	C18:1 ω7c or C18:1 ω6c (62.37%), C16:1 ω7c or C16:1 ω6c (7.75%), C14:0 3-OH and/or iso-16:1 (4.61%), 16:0 (9.47%), C 16:0 3-OH (2.23%) C18:1 2-OH (4.84%)	C18:1 ω7c or C18:1 ω6c (46.7%), C16:1 ω7c or C16:1 ω6c (12.5%), C14:0 3-OH and/or iso-16:1 (5.5%), 16:0 (13.6%), C 16:0 3-OH (4.2%) C18:1 2-OH (1.4%)
Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10
nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
68.7	67.6	64–65	nd	68.7	67.3	67	65.5	67.7	69.6
Molasses grass	Rhizosphere soil of corn (*Zea mays*)	Rhizosphere soil of corn (*Zea mays*)	Oil contaminated soil	Dicarded road tar	Forest soil	Mat of the sulfide spring	Soil	Microbial fuel cell	Microbial fuel cell

A. palatum, described as a non-nitrogen-fixing bacterium. Most bacteria of the genus *Azospirillum* grow aerobically, but three species were classified as facultative anaerobic such as *A. melinis, A. thiophilum*, and *A. humicireducens*. Sugars are oxidized but not fermented. It is considered a mesophilic genus with growth at 20–37 °C, optimum temperature at 30 °C. Some species can grow at 5 ° C and a maximum temperature of 41 °C was found for *A. halopraeferens*. The pH range can vary from 4.0 to 8.0, but neutral pH is the optimum for most of the species. Growth in 3 % NaCl is also variable, but it is a special feature for *A. halopraeferens*. Bacteria of this genus also utilize several carbon sources such as organic acids, sugars, amino acids, and sugar alcohols; the pattern of carbon utilization has been used for discriminatory purpose between the species of the genus. The use of the nitrogen-free NFb semisolid medium allows the enrichment and isolation of several species although new media can also be used. Several new isolates obtained from soil and other environments confirm that this genus is widespread in nature and in different regions of the world. *Azospirillum* lipids have a large amount of $C_{18:1}\omega7c$ (55.3 %) and also contain $C_{16:1}\omega7c$ and $C_{16:0}$ as major components; the major hydroxy fatty acids are $C_{14:0}$ 3-OH and $C_{16:0}$ 3-OH. When grown aerobically, the species of this genus exhibit a quinone system with ubiquinone 10 (Q-10). The polar lipids consist mainly of phosphatidylglycerol, phosphatidylcholine, and one unidentified phospholipid. The DNA G+C content varies between 64 and 71 mol%. This genus is closely related to *Rhodocista* and *Skermanella* based on the 16S rRNA gene sequence comparison. The type species of the genus is *A. lipoferum* and the type strain is 59b (= ATCC 29707 = CIP 106280 = DSM 1691 = JCM 1247 = LMG 13128 = NBRC 102290 = NCAIM B.01801 = NRRL B-14654 = VKM B-1519). Besides the type species, the genus embraces 16 other species, isolated either from the rhizosphere or from endophytic plant tissues, agricultural or contaminated soil samples, water, and fermented tank, as follows: *A.brasilense* (Tarrand et al. 1978), *A. amazonense* (Magalhães et al. 1983), *A. halopraeferens* (Reinhold et al. 1987), *A. irakense* (Khammas et al. 1989), *A. largimobile* (Ben Dekhil et al. 1997), *A. doebereinerae* (Eckert et al. 2001), *A. oryzae* (Xie and Yokota 2005), *A. melinis* (Peng et al. 2006), *A. canadense* (Mehnaz et al. 2007a), *A. zeae* (Mehnaz et al. 2007b), *A. rugosum* (Young et al. 2008), *A. picis* (Lin et al. 2009), *A. palatum* (Zhou et al. 2009), *A. thiophilum* (Lavrinenko et al. 2010), *A. formosense* (Lin et al. 2012), *A. humicireducens* (Zhou et al. 2013), and *A. fermentarium* (Lin et al. 2013). The type strains and additional characteristics for these species are listed in *Table 22.2*.

Conglomeromonas Skerman et al. 1983

Con. glom. e. ro. monas, L. pp. conglomeratus to form in a [rounded] mass; Gr. fem. n. monas a unit, monad; M. L. fem. n. *Conglomeromonas* monad forming in a [rounded] mass.

The genus *Conglomeromonas* has one species, *Conglomeromonas largomobilis*, and two subspecies, *Conglomeromonas largomobilis* subsp. *largomobilis* and *Conglomeromonas largomobilis* subsp. *parooensis*, as was proposed by Skerman et al. (1983). This genus was proposed to incorporate the strains described as follows: Gram-negative, non-spore-forming organisms which exhibit unicellular and multicellular phases of growth. Unicellular phase cells are rod shaped, with rounded or tapered ends and a straight or slightly curved axis; cells are arranged singly or in pairs. Motile cells have mixed flagellation, with a single polar flagellum and one or more distinctive lateral flagella of different thickness and length. The DNA G+C content was 67.0 mol%. Although these organisms were isolated from freshwater sources rather than from soil or plant roots and are not able to fix nitrogen under aerobic conditions, other characteristics suggested that they may be related to the genus *Azospirillum* (Skerman et al. 1983). Ben Dekhil et al. (1997) transferred the type species of the genus *Conglomeromonas largomobilis* subsp. *largomobilis* to the genus *Azospirillum* as *A. largomobile* on the basis of phylogenetic evidence based on16S rRNA gene sequence comparisons and earlier nucleic acid hybridization studies (Falk et al. 1986). Furthermore, the genus *Conglomeromonas* became invalid, and consequently, a new genus was required to accommodate *C. largomobilis* subsp. *parooensis*. Thus, Sly and Stackebrandt (1999) created a new genus *Skermanella* and transferred the subspecies of *C. largomobilis* subsp. *parooensis* as *Skermanella parooensis*.

Skermanella Sly and Stackebrandt (1999), Emended Weon et al. (2007) and Luo et al. 2012

Sker.ma.nel'la. M.L. dim. ending -ella; M.L. fem. dim. n. *Skermanella* named after V. B. D. Skerman who first isolated this bacterium, and in honor of his contribution to bacterial systematics.

Bacteria of this genus are Gram negative and non-spore forming, which exhibits unicellular and multicellular phases of growth. Unicellular-phase cells are rod shaped, with rounded or tapered ends and a straight or slightly curved axis, arranged singularly or in pairs. Motile cells have mixed flagellation, with a single polar flagellum and one or more distinct lateral flagella of different lengths. Multicellular conglomerates arise from single cells, which lose motility, become optically refractile, and reproduce by multi-planar septation. Under suitable conditions conglomerates dissociate into single motile cells, which produce water-clear colonies in which the sparse number of cells move in a sluggish manner. No filamentous structures are formed and no buds are produced. The bacterium is an obligate chemoorganotroph and strictly aerobic. All members of the genus are positive for catalase, oxidase, alkaline phosphatase, acid phosphatase, esterase (C4), naphthol-AS-BI-phosphohydrolase, and leucine arylamidase, but negative for α-galactosidase, β-galactosidase, α-mannosidase, β-fucosidase, N-acetyl-β-glucosaminidase, α-chymotrypsin, and trypsin. Carbohydrate metabolism is fermentative and glucose fermentation is variable. Cells do not fix dinitrogen under microaerophilic

conditions. Strains of this genus have a high DNA G+C content (65.0–69.6 mol%), and the major respiratory quinone is Q-10. The major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and an unknown aminolipid. The nearest phylogenetic relative is *Azospirillum largimobile* (93 % 16S rRNA gene sequence similarity). The type species is *Skermanella parooensis* and the type strain is ACM 2042 (= CIP 106994 = DSM 9527 = UQM 2042). The other species of the genus are *S. xinjiangensis* (An et al. 2009), *S. stibiiresistens* (Luo et al. 2012), and *S. aerolata* (Weon et al. 2007). The *S. stibiiresistens* strain SB22^T is highly resistant to antimony, growing in the presence of 4 mM Sb(III) in R_2A broth. The type strains and additional characteristics for these species are listed in ● *Table 22.3*.

Desertibacter Liu et al. 2011

De.ser.ti.bac'ter. L. n. *desertum* desert; N.L. masc. n. *bacter* rod; N.L. masc. n. *Desertibacter* a desert bacterium.

The cells are Gram-negative rods, motile by means of a single polar flagellum and strictly aerobic. They are catalase and oxidase positive and are able to reduce nitrate to nitrite. *Desertibacter* is not able to fix nitrogen. Colonies of the type strain 2622^T are pink, circular, and convex with regular margins. Cells contain PHB. Growth occurs at 12–42 °C (optimum 37–40 °C), at pH 7–10 (optimum pH 8), and at NaCl concentrations of up to 1.5 % (optimum 0.5 % NaCl). Hydrolyse aesculin and gelatin but not casein, tyrosine, or starch. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase/lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase (weakly), and α-glucosidase (weakly) and negative for lipase (C14), trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, N-acetyl-b-glucosaminidase, β-glucosidase, α-mannosidase, and α-fucosidase. API 20NE tests show positive reactions for nitrate reduction, aesculin hydrolysis, gelatin hydrolysis, urease, and β-galactosidase and negative reactions for arginine dihydrolase, indole production, and glucose fermentation. It does not assimilate D-glucose, L-arabinose, maltose, D-mannose, D-mannitol, N-acetylglucosamine, adipic acid, capric acid, malic acid, potassium gluconate, trisodium citrate, or phenylacetic acid. The DNA G+C content of strain 2622^T is 71.4 mol%. The type species is *Desertibacter roseus* and type strain 2622^T (= CCTCC AB 20812 T = KCTC 22436 T), isolated from a gamma-irradiated sand sample from the Taklimakan desert in Xinjiang, China. The type strains and additional characteristics for these species are listed in ● *Table 22.4*.

Rhodocista Kawasaki et al. 1994

Rho.do.ci'sta. L. fem. n. *rhodos* the rose; L. fem.n. *cista* a basket; M.L. fem.n., *Rhodocista* red basket.

Cells have vibrioid to spiral cell form with a size of 0.6–2 µm; they are motile by means of a polar flagellum. Growth is mesophilic. Photosynthetic membranes are present as lamellae lying parallel to cytoplasmatic membrane when cells are grown phototrophically. Growth occurs phototrophically under anaerobic conditions in the light and chemoheterotrophically under aerobic conditions in the dark. Anaerobically grown colonies are pink. Bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series are present. Cells are converted to cysts under aerobic incubation, becoming resistant to desiccation and heat. The major cellular ubiquinone is ubiquinone Q-9. The DNA G+C content is 68.8–69.9 mol%. The type species is *Rhodocista centenaria* ATCC 43720^T (= DSM 9894^T = IAM 14193^T = NRBC 16667^T = JCM 21060^T) isolated from a water sample at the edge of a thermophilic hot spring at Wyoming, USA (Kawasaki et al. 1994). This species is a homotypic synonym of *Rhodospirillum centenum* (Favinger et al. 1989). *Rhodocista pekingensis* is the second species of the genus, and the type strain 3-p^T (= AS 1.2194^T = JCM 11669^T) was isolated from a municipal wastewater treatment plant at Beijing, China (Zhang et al. 2003). The type strains and additional characteristics for these species are listed in ● *Table 22.5*.

Dongia Liu et al. 2010

Don'gi.a. N.L. fem. n. *Dongia* after Professor Xiu-Zhu Dong, a bacteriologist and bacterial taxonomist in China.

The cells are Gram negative, 0.3–0.5 µm wide and 0.6–1.0 µm long, non-spore forming, motile, and slightly curved to straight rods. They have a strictly aerobic metabolism and are heterotrophic – never phototrophic. Internal membrane systems and bacteriochlorophyll a are absent. Cells reduce nitrate to nitrite and oxidase is variable. Dongia cells are negative for ß-galactosidase, urease, catalase, and production of indole and H_2S. They hydrolyze Tweens 20 and 80 weakly, but starch, L-tyrosine, casein, arginine, gelatin, and aesculin are not hydrolyzed. Using the standard mineral base according to Dong and Cai (2001), weak growth on L-arabinose, cellobiose, glucose, lactose, maltose, raffinose, sucrose, D-xylose,erythritol, glycerol, and D-glucitol was observed; no growth occurred with sodium acetate, casein, citrate, citric acid, inositol, malic acid, methanol, L-rhamnose, sorbitol, and succinic acid. API ZYM tests are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, phosphatase acid, and naphthol-AS-BI-phosphohydrolase and weakly positive for lipase (C14), valine arylamidase, and cystine arylamidase. The type species of the genus is *Dongia mobilis*. Type strain is LM22^T (5CGMCC 1.7660 T 5JCM15798T), isolated from a sequencing batch reactor for the treatment of malachite green effluent. A second species, *Dongia rigui*, type strain 04SU4-P^T (KCTC 23341 = JCM 17521), was isolated from freshwater collected from the Woopo wetland, Republic of Korea (Baik et al. 2013). Both species have as majors fatty acids (>10 % of the total) $C_{19:0}$ω8c cyclo, $C_{16:0}$, and $C_{18:0}$ω7c. The major ubiquinone is Q-10. The DNA G+C content varies from 65.6 to 71.5 mol%. The type strains and additional characteristics for these species are listed in ● *Table 22.6*.

Table 22.3

Morphological, physiological and molecular characteristics differentiating species within genus *Skermanella*

	Skermanella aerolata 5416T-32^T (kit API 20Ne; API ID 32 GN API 50 CH)	*Skermanella parooensis* ACM 2042^T (kit API)	*Skermanella stibiiresistens* SB22^T (kit API 20Ne; API ID 32 GN)	*Skermanella xinjiangensis* 10–1-101^T (kit API 20Ne; API ID 32 GN API 50 CH)
Morphology	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped
Cell size (mm)	0.6–1.2 × 1.5–3.5	1.0–1.5 × 1.5–3	nd	0.8–0.9 × 1.1–1.6
Flagellation	Single polar or subpolar flagellum	Single polar and one or more lateral flagella	nd	Single polar or sub-polar flagellum
Motility	+	+	+	+
N_2 fixation	–	–	–	–
PHB accumulation or other characteristics	+	+	nd	+
Detection *nifD* and/or *nifH*	nd	nd	nd	nd
Main carotenoids	nd	nd	nd	nd
Photolithoautotrophic growth (electron donor)	nd	nd	nd	nd
Temperature for growth (°C):				
Range	5–35	10–37	4–37	17–37
Optimum	25–35	28	28	28–37
pH for growth:				
Range	4.0–9.0	6.0–9.0	5–9	6–9
Optimum	6.0–7.0		7	7–8
Oxidase	+	+	+	+
Catalase	+	+	+	+
Color of cell suspension	nd	nd	nd	nd
Urea hydrolise	+	+	–	+
Nitrate reduction	+	+	–	–
Nitrite reduction	nd	nd	nd	nd
NaCl concentration for growth (%)	0–5%	0–2%	0–4%	0–4%
Utilization of:				
Acetate	–	+	–	+
Aspartate	nd	nd	nd	nd
Benzoate	nd	nd	nd	nd
Butyrate	nd	nd	nd	nd
Citrate	–	+	–	–
Crotonate	nd	nd	nd	nd
N-acetyl-D-glucosamine	–	–	–	–
meso-inositol	–	–	+	–
L-rhamnose	–	+	+	+
meso-erythritol	–	–	nd	–
Adonitol	–	–	nd	–
L-arabinose	+	+	–	+
D-arabinose	+	nd	–	+
Malate	+	+	+	+
Sucrose	–	–	–	–
D-glucose	+	+	+	+
D-fructose	–	+	–	–

Table 22.3 (continued)

	Skermanella aerolata 5416T-32^T (kit API 20Ne; API ID 32 GN API 50 CH)	*Skermanella parooensis* ACM 2042^T (kit API)	*Skermanella stibiiresistens* SB22^T (kit API 20Ne; API ID 32 GN)	*Skermanella xinjiangensis* 10–1-101^T (kit API 20Ne; API ID 32 GN API 50 CH)
Mannitol	+	+	–	+
Glycerol	–	–	nd	–
Adipate/adipic acid	–	–	nd	–
Azelaic acid	nd	nd	nd	nd
Sebacic acid	nd	nd	nd	nd
Phenol	nd	nd	nd	nd
4-Chlorophenol	nd	nd	nd	nd
Predominant ubiquinone	Q-10 and Q-8	Q-10	Q-10	Q-10
Predominant cellular fatty acids	C18:1 ω7c and C16:0	C18:1 ω7c and C16:0	C18:1 ω7c (63.5%), summed feature 2 (C14:0 3-OH and/or iso-C16:1 I (10.8%)) and C16:0 (9.9%)	C18:1 ω7c and C16:0
Indol production	–	–	–	–
DNA G+C (mol%)	65.0	67 (66.4–68.0)	69.6	68.8
Genome size	nd	nd	nd	nd
Habitat	Air	Fresh water	Soil of a coal mine	Soil

nd, not determined

Elstera Rahalkar et al. 2012

Els'te.ra. N.L. fem. n. *Elstera* named after Hans-Joachim Elster, a German limnologist working on Lake Constance who was one of the first to establish the importance of the littoral zone for the lake ecosystem.

Cells are Gram-negative rods; they are catalase and oxidase positive. They grow chemoheterotrophically and use sugars, some organic acids, and alcohols as preferred substrates. Ubiquinone Q-10 is the dominant quinone and putrescine is the dominant polyamine. Cells are slightly curved rods, 1.0 × 2.0–5.0 μm in size, and nonmotile, with a minimum doubling time of approximately 40 h. Based on API 20NE and Biolog PM1 tests, cells are negative for the reduction of nitrate, sulfate, and iron (III); nitrogen fixation; and indole production from tryptophan. The cells grow in the presence of many carbon sources such as D-glucose, L-rhamnose, D-fructose, D-galactose, L-arabinose, D-xylose, D-mannitol, D-sorbitol, D-glucuronic acid, glycerol, ethanol, and L-malate. Weak or no growth was observed in the presence of trehalose, D-maltose, dulcitol, adonitol, D-saccharic acid, N-acetyl-β-*o*-mannosamine, glucuronamide, D-glucosaminic acid, formate, acetate, propionate, pyruvate, L-lactate, D-malate, fumarate, succinate, glycolate, glyoxylate, citric acid, m-tartrate, 2-oxoglutarate, and Tweens 20, 40, and 80. The DNA G+C content of strain Dia-1 is 61.0 ± 1.5 mol%. The type species is *Elstera litoralis* and type strain is Dia-1^T (= DSM 19532 T = LMG 24234 T). It was isolated from biofilms on stones in the littoral zone of Lake Constance, Germany. The type strains and additional characteristics for this species are listed in *Table 22.7*.

Inquilinus Coenye et al. 2002

In.qui'li.nus. L. masc. n., *Inquilinus* an inhabitant of a place that is not its own.

Cells are Gram negative, strictly aerobic, chemoorganotrophic, nonmotile, nonsporulating, and rod shaped; *I. ginsengisoli* has a cell size of 0.6–0.8 × 2.5–4.0 μm when grown for 2 days at 30 °C on R_2A agar. Catalase activity is present. Growth is observed at 25–42 °C and in 1 % NaCl. *I. ginsengisoli* cannot grow with 3 % (w/v) NaCl and at 37 °C. Growth is observed on BCSA at 32 °C. There are no denitrification and indole production. Based on RapID NF Plus (Remel) and API 20E (bioMerieux, Hazelwood, MO), lysine decarboxylase, ornithine decarboxylase, or arginine dihydrolase activities are negative. There are no pigment production, lipase, phosphatase, N-acetylglucosaminidase, β-glucosidase, proline aminopeptidase, pyrrolidonyl aminopeptidase, and tryptophan aminopeptidase; N-benzyl-arginine aminopeptidase activity may be present. Utilization of carbon sources (API 20NE and API ID 32 GN kits) is variable between species (Jung et al. 2011). The predominant ubiquinone is Q-10, and the majority of cellular fatty acids are $C_{18:0}$ω7c, $C_{18:1}$2-OH, and $C_{18:0}$3-OH. The G+C content of the genome is between 69.9 and 70.9 mol%. The genus *Inquilinus* was originally described by Coenye et al. (2002).

Table 22.4

Morphological, physiological and molecular characteristics differentiating species within genus *Desertibacter*

	Desertibacter roseus CCTCC AB 208152^T
Morphology	Rod
Cell size (μm)	nd
Flagellation	Single polar flagellum
Motility	+
N_2 fixation	–
PHB accumulation or other characteristics	+ / Gamma radiation-resistant bacteria
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	12.0–42.0
Optimum	37.0–40.0
pH for growth:	
Range	7.0–10.0
Optimum	8.0
Oxidase	+
Catalase	+
Urease hydrolise	+
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	0–1.5
Utilization of (less than 20):	
4-Chlorophenol	nd
Adipate adipic acid	–
Adonitol	nd
Azelaic acid	nd
citrate	nd
D-arabinose	–
D-fructose	nd
D-glucose	–
Glycerol	nd
L-rhamnose	–
Malate	–
Mannitol	–
meso-erythritol	nd
meso-inositol	nd
N-acetyl-D-glucosamine	–
Phenol	nd
Sebacic acid	nd
Sucrose	–
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	C18:1v7c
Indol production	–
DNA G+C (mol%)	71.4
Genome size	nd
Habitat	Sand
Biochemical characteristics kit	API ZYM, API 20E, API 20NE

+, positive; –, negative; nd, not determined; v, variable; na, not available

Table 22.5

Morphological, physiological and molecular characteristics differentiating species within genus *Rhodocista*

	Rhodocista centenaria ATCC 43720^T	*Rhodocista pekingensis* 3-p^T
Morphology	Vibrioid to spiral	Vibrioid to spiral
Cell size (μm)	1–2 × 3	0.6–0.8 × 0.8–1.5
Flagellation	1, polar	1, polar
Motility	+	+
N_2 fixation	+	nd
PHB accumulation or other characteristics	+	nd
Detection *nifD* and/or *nifH*	+	nd
Temperature for growth (°C):		
Range	39–47	25–44
Optimum	39–45	31–42
pH for growth:		
Range	5.7–7	6.5–8
Optimum	6.8	7
Oxidase	nd	nd
Catalase	nd	nd
Urease hydrolise	nd	nd
Nitrate reduction	nd	nd
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	0	0
Utilization of:		
N-acetyl-D-glucosamine	nd	nd
meso-inositol	nd	nd
L-rhamnose	nd	nd
meso-erythritol	nd	nd
Adonitol	nd	nd
D-arabinose	nd	nd
Malate	–	–
Sucrose	nd	–
D-glucose	–	–
D-fructose	–	nd
Mannitol	nd	–
Glicerol	nd	–
Adipate adipic acid	nd	nd
Azelaic acid	nd	nd
Sebacic acid	nd	nd
Phenol	nd	nd
4-Chlorophenol	nd	nd
D-galactose	nd	nd
D-xylose	nd	nd
Trehalose	nd	nd
Predominant ubiquinone	Q-9	Q-9
Predominant cellular fatty acids	nd	nd

Table 22.5 (continued)

	Rhodocista centenaria ATCC 43720^T	*Rhodocista pekingensis* 3-p^T
Indol production	nd	nd
carotenoids	Spirilloxanthin	Spirilloxanthin
DNA G+C (mol%)	68.8	69.9
Genome size	4.36	na
Habitat	Water sample at the edge of a themophilic hot spring	Wastewater treatment plant

nd, not determined

Table 22.6
Morphological, physiological and molecular characteristics differentiating species within genus *Dongia*

	Dongia mobilis CGMCC 17660	*Dongia rigui* 04SU4-P^T
Morphology	Slightly curved to straight rods	Curved to twisted rod-shaped
Cell size (μm)	0.3–0.5 × 0.6–1.0	0.3 × 0.7–1.7
Flagellation	Single polar flagellum	Single polar flagellum
Motility	+	+
N_2 fixation	–	–
PHB accumulation or other characteristics	nd	nd
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	20.0–40.0	15–37
Optimum	30.0–35.0	25–30
pH for growth:		
Range	6.0–10.0	5.0–11.0
Optimum	7.0–7.5	7
Oxidase	–	+
Catalase	–	–
Urease hydrolise	–	+
Nitrate reduction	+	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	0.5	0
Utilization of (less than 20):		
4-Chlorophenol	nd	nd
Adipate adipic acid	nd	nd
Adonitol	nd	nd
Azelaic acid	nd	nd
Citrate	–	nd
D-arabinose	+	nd

Table 22.6 (continued)

	Dongia mobilis CGMCC 17660	*Dongia rigui* 04SU4-P^T
D-fructose	nd	nd
D-glucose	+	nd
Glycerol	nd	nd
L-rhamnose	–	nd
Malate	–	nd
Mannitol	nd	nd
meso-erythritol	nd	nd
meso-inositol	–	nd
N-acetyl-D-glucosamine	nd	nd
Phenol	nd	nd
Sebacic acid	nd	nd
Sucrose	+	nd
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1v7c, C19:0 cyclo v8c, C16:0	C19:0 ω8c cyclo, C16:0 and C18:1 ω7c
Indol production	–	–
DNA G+C (mol%)	65.6	71.5
Genome size	nd	nd
Habitat	Reactor	Freshwater
Biochemical characteristics kit	API ZYM and API 20NE	API ZYM

+, positive; –, negative; nd, not determined; v, variable; na, not available

It comprises the species *Inquilinus limosus*, isolated from respiratory secretions of a cystic fibrosis patient in the USA (Coenye et al. 2002), and *Inquilinus ginsengisoli*, isolated from ginseng field soil (Jung et al. 2011). The *Inquilinus limosus* AU0476^T (= LMG 20952 T = CCUG45653T) is the type species. The type strains and additional characteristics for these species are listed in *Table 22.8*.

Magnetospirillum Scheifer et al. 1992

Mag.ne'to.spir.il'lum, Gr.n.magnes, magnet, comb. form magneto-, Gr.n.*spira* a spiral; M.L.dim.neut.n.*spirillum* a small spiral; *Magnetospirillum* a small magnetic spiral.

Bacteria are characterized by a helical (clockwise) spirillum cell shape; cells are 0.2–0.7 μm wide and 1.0–20.0 μm long. The cells have a Gram-negative cell wall and are motile by means of a single flagellum at each pole. Each magnetotactic cell contains membrane enveloped crystals, named magnetosomes, which are arranged in a chain within the cytoplasm. Mobility and magnetic behavior can be diminished or lost after several subcultivations. The cells are microaerophilic and chemoorganotrophic. Catalase and oxidase can be present or absent. Growth occurs on various organic acids; carbohydrates

Table 22.7
Morphological, physiological and molecular characteristics differentiating species within genus *Elstera*

	Elstera litoralis DSM 19532^T
Morphology	Slightly curved rods
Cell size (μm)	1.06 × 2.0–5.0
Flagellation	nd
Motility	–
N_2 fixation	–
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	10.0–25.0
Optimum	20.0–25.0
pH for growth:	
Range	5.5–8.0
Optimum	6.5–7.0
Oxidase	+
Catalase	+
Urease hydrolise	nd
Nitrate reduction	–
Nitrite reduction	nd
NaCl concentration for growth (%)	nd
Utilization of (less than 20):	
4-Chlorophenol	nd
Adipate adipic acid	nd
Adonitol	–
Azelaic acid	nd
Citrate	–
D-arabinose	+
D-fructose	+
D-glucose	+
Glycerol	+
L-rhamnose	+
Malate	+
Mannitol	+
meso-erythritol	nd
meso-inositol	nd
N-acetyl-D-glucosamine	+
Phenol	nd
Sebacic acid	nd
Sucrose	–
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	18:1v7c, 18:1 2-OH and 16:0.
Indol production	–
DNA G+C (mol%)	61.0± 1.5
Genome size	nd
Habitat	Biofilms on stones
Biochemical characteristics kit	API 20NE and Biolog (PM1)

+, positive; –, negative; *nd*, not determined; *v*, variable; *na*, not available

are utilized only occasionally. *Magnetospirillum* is nitrogen fixation positive as indicated by acetylene reduction assay, *nif*HDK hybridization, and growth in N-free media (Bazylinski et al. 2000). The G+C content of DNA is 64–71 mol%. The type species is *Magnetospirillum gryphiswaldense* and the type strain is MSR-1 (= DSM 6361 = IFO – now NBRC 15271 = JCM 21280). The other species of the genus is *Magnetospirillum magnetotacticum*, type strain MS-1 (= ATCC 31632 = DSM 3856 = IFO – now NBRC 15272 = JCM 21281 = LMG 10894). This species is the basonym of *Aquaspirillum magnetotacticum* (Maratea and Blakemore 1981 emended by Scheifer et al. 1992). The type strains and additional characteristics for these species are listed in ➤ *Table 22.9*.

Phaeospirillum Imhoff et al. 1998

Phae.o.spi.ril´lum. Gr. adj. *phaeos*, brown; M.L. neut. n. *Spirillum*, a bacterial genus; M.L. neut. n., *Phaeospirillum*, brown *Spirillum*.

Phaeospirillum forms vibrioid- to spiral-shaped cells, 0.5–1.2 μm in size. The cells are motile by means of polar flagella. Carotenoid glycosides and bacteriochlorophyll *a* are present on intracytoplasmatic photosynthetic membranes as lamellar stacks. Growth is mesophilic. The major fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, and $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$. Growth is preferably photo-organotrophically under anaerobic conditions in the light or in the dark under microaerobic conditions. Cell suspensions are dark brown to brown-orange/brown-red colored. Cells harbors ubiquinone Q-9 and menaquinone MK-9 as major components. The DNA G+C content is 60.5–64.8 mol%. This genus name was created by Imhoff et al. (1998) after reclassification of brown-colored spiral-shaped phototrophic purple non-sulfur bacteria formerly classified originally as *Rhodospirillum*, based on genetic and phenotypic characteristics. The species of this genus have been isolated from freshwater, mud, and rhizosphere soil and show no salt requirement for growth. The type species is *Phaeospirillum fulvum* and the type strain ATCC 15798^T (= ATCC 53113^T = DSM 113^T) was isolated from sewage pond (van Niel 1944; Imhoff et al. 1998). *P. molischianum* was described as the second species, and the type strain ATCC 14031^T (= DSM 120^T = LMG 4354^T) was isolated from mud from a ditch (Giesberger 1947; Imhoff et al. 1998). The third species is *P. chandramohanii*, type strain JA145^T (= JCM 14933^T = KCTC5703^T = NBRC 104961^T), isolated

Table 22.8
Morphological, physiological and molecular characteristics differentiating species within genus *Inquilinus*

	Inquilinus limosus LMG 20952^T	*Inquilinus ginsengisoli* Gsoil 080^T
Morphology	Rods	Rod-shaped
Cell size (μm)	nd	0.6–0.8×2.5–4.0
Flagellation	nd	nd
Motility	–	–
N_2 fixation	nd	nd
PHB accumulation or other characteristics	nd	nd
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	25–42	15–30
Optimum	32	nd
pH for growth:	nd	nd
Range	nd	nd
Optimum	nd	nd
Oxidase	+	+
Catalase	+	+
Urease hydrolise	–	–
Nitrate reduction	–	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	1.0	2
Utilization of (less than 20):		
4-Chlorophenol	nd	nd
Adipate adipic acid	Weakly positive	–
Adonitol	nd	nd
Azelaic acid	nd	nd
Citrate	–	+
D-arabinose	+	+
D-fructose	nd	nd
D-glucose	+	+
Glycerol	nd	nd
L-rhamnose	–	nd
Malate	+	–
Mannitol	+	+
meso-erythritol	nd	nd
meso-inositol	+	+
N-acetyl-D-glucosamine	+	+
Phenol	nd	nd
Sebacic acid	nd	nd
Sucrose	+	+
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	18:1W7c, 18:1–2OH, 18:0–3OH	C18:1v9c/v12t/v7c and C19:0 cyclo v8c
Indol production	nd	–
DNA G+C (mol%)	70.3	69.9
Genome size	nd	nd
Habitat	Respiratory secretions	Soil
Biochemical characteristics kit	RapIDNF Plus (Remel) and API20E bioMerieux, Hazelwood, Mo.)	API 20 NE and API ID32GN kits

+, positive; –, negative; nd, not determined, v, variable; na, not available

from freshwater reservoir at Mudasarlova, India (Kumar et al. 2009). The fourth species described is *P. oryzae*, and the type strain JA317^T (= KCTC 5704^T = NBRC 104938^T) was isolated from rhizosphere soil of a paddy at Nadergul, India (Lakshmi et al. 2011); the fifth species of the genus is *P. tilakii*, and the type strain JA492^T (= KCTC 15012^T = NBRC 107650^T) was isolated from a water/mud from Nelapattu Bird Sanctuary, India (Raj et al. 2012). The type strains and additional characteristics for these species are listed in *Table 22.10*.

Nisaea Urios et al. 2008

Nis´ae.a. L. fem. n., *Nisaea* nymph of the sea (1 of the 50 daughters of Nereus and Doris), referring to the marine origin.

Cells are motile, Gram-negative rods growing optimally at 30 °C, pH 6.0, and 20 g l^{-1} NaCl. The major fatty acids are $C_{18:1}\omega7c$ (69.1 %), $C_{16:1}\omega7c$ (13.9 %), and $C_{16:0}$ (11.3 %). The cells harbor Q-10 and the polar lipid phosphatidylglycerol. On Biolog GN2 plates positive reactions are obtained for fructose, glucose, raffinose, acetate, γ-hydroxybutyrate, and propionate. Positive reactions with API ZYM are obtained for alkaline phosphatase, acid phosphatase, and leucine arylamidase. Oxidase and catalase are positive. The genus *Nisaea* was created after characterization of strains isolated from one of the major sites of water-column denitrification among the world's oceans (Urios et al. 2008). It comprises two species, the type species *Nisaea denitrificans* (type strain DR41_21 = OOB 129 = CIP 109265 = DSM 18348) and *N. nitritireducens* (type strain DR41_18 = OOB 128 = CIP 109601 = DSM 19540). The DNA G+C content is around 60.1–60.2 mol%. The type strains and additional characteristics for these species are listed in *Table 22.11*.

Table 22.9

Morphological, physiological and molecular characteristics differentiating species within genus *Magnetospirillum*

	Magnetospirillum gryphiswaldense MSR-1 = DSM 6361	*Magnetospirillum magnetotacticum* MS-1= ATCC 31632= DSM 3856	*Magnetospirillum magneticum* AMB-1 = ATCC 700264	*Magnetospirillum bellicus* VDY= DSM 21662 = ATCC BAA-1730	*Magnetospirillum aberrantis* SpK = VKPM B-11049
Morphology	Helical (clockwise) spirilla	Helical (clockwise) spirilla	Helical	Spirillum-shaped	Spirilla
Cell size (μm)	0.7 × 1–20. Younger cells are usually shorter (3 to 4 mm)	0.2–0.4 × 4.0–6.0. Tendency to form long chains and coccoid bodies in older cultures	0.4–0.6 × > 3.0	0.5 × 3	0.3–0.4 × 1.5–5.0
Magnetosome number and structure	Variable, enveloped particles arranged in a chain	Variable, enveloped particles arranged in a chain	Arranged in a chain of over 15 magnetosomes	–	Sparse magnetic particles (30–40 nm), single or in small cluster
Flagellation	Single flagellum at each pole	Single flagellum at each pole	Single flagellum at each pole	–	Polar flagella
Motility	+	+	+	+	+
N_2 fixation	+	+	+	nd	Growth on semisolid forming subsuperficial pelicle
PHB accumulation or other characteristics	+	+	nd	nd	+
Detection *nifD* and/or *nifH*	+	+	+	nd	nd
Temperature for growth (°C):					
Range	nd	15–37	nd	<10–42	20–45
Optimum	28–34	30	28–34	42	31
pH for growth:					
Range	nd	nd	5.8–8.2	6.0–7.5	nd
Optimum	7.0–7.5	7.0–7.5	7.0–7.5	6.8	6.5–6.9
Oxidase	+	– or faintly positive with toluene treated cells	nd	–	+
Catalase	+	–	–	–	–
Urease hydrolise	nd	–	nd	nd	nd
Nitrate reduction	+	+	+	+	+
Nitrite reduction	+	+	+	+	+
NaCl concentration for growth (%)	nd	< 1	nd	1.5	nd
Utilization of:					
Lactate	+	+	+	+	+
Acetate	+	+	+	+	+
Fumarate	nd	+	+	+	+
Malate	+	nd	+	–	nd
Peptone	+	nd	nd	nd	nd
Pyruvate	+	+	+	+	+
Succinate	+	+	+	+	+
Sucrose	–	nd	nd	–	–
Aspartic acid	–	nd	nd	nd	nd

Table 22.9 (continued)

	Magnetospirillum gryphiswaldense MSR-1 = DSM 6361	*Magnetospirillum magnetotacticum* MS-1= ATCC 31632= DSM 3856	*Magnetospirillum magneticum* AMB-1 = ATCC 700264	*Magnetospirillum bellicus* VDY= DSM 21662 = ATCC BAA-1730	*Magnetospirillum aberrantis* SpK = VKPM B-11049
Galactose	–	nd	nd	nd	nd
α-ketoglutarate	–	nd	+	nd	nd
Lactose	–	nd	nd	nd	nd
Maltose	–	–	nd	+	nd
D-sorbose	–	nd	nd	nd	nd
Tartrate	–	nd	nd	nd	+
N-butyrate	nd	–	+	+	+
β-hydroxybutyrate	nd	nd	+	nd	nd
Propionate	nd	–	+	+	+
Predominant ubiquinone	na	na	na	na	Q-10
Predominant cellular fatty acids	$C_{14:0}$ 3OH, $C_{18:1}$, $C_{16:1}$	$C_{14:0}$ 3OH, $C_{18:1}$, $C_{16:0}$ 3OH	na	C18:1ω7, C16:0	C18:1ω7, C16:0
Pigment production	nd	–	nd	nd	nd
Indol production	nd	nd	nd	nd	nd
DNA G+C (mol%)	71.0	64.5–66.4	65.1	64.8	62.6
Genome size (Mb)	4.26	4.50	4.97	nd	4.15
Habitat	Fresh water sediments	Microaerobic zones from freshwater sediments	Freshwater	Bioelectrical reactor (BER) inoculated from creek water	Freshwater sediments

+, positive; –, negative; *nd*, not determined; *v*, variable; *na*, not available

Thalassobaculum Zhang et al. 2008, Emend. Urios et al. 2010

Tha.las´so.ba.cu.lum. Gr. n. *Thalassa*, the sea; L. neut. n. *baculum*, stick; N.L. neut. n. *Thalassobaculum* rod-shaped bacterium from the sea.

Cells are Gram negative, slightly curved, and straight rod shaped; they are motile by means of a polar flagellum. Growth is heterotrophic and some of the strains are facultative anaerobes. Cells are positive for oxidase and catalase. Bacteriochlorophyll a is not present. Cells do not fix atmospheric N_2 under anoxic conditions. Optimal growth occurs at 30 °C, at pH 8.0, and at high salinity ranging from 34 to 40 g l^{-1}. Cells are tested positive for leucine arylamidase and valine arylamidase activities with the API ZYM kit. Carbon source utilization is variable according to the kit applied. The major fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, $C_{17:0}$, and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The isoprenoid quinone is Q-10. The G+C content of the DNA is 65–68 mol%. The type species of the genus is *Thalassobaculum litoreum* and type strain CL-GR58^T (= KCCM 42674^T = DSM 18839^T) that was isolated from coastal seawater, Korea (Zhang et al. 2008). The second species is *T. salexigens*, type strain CZ41-10a^T (= DSM 19539^T = CIP 109064^T = MOLA 84^T), isolated from the water column in the bay of Banyuls-sur-Mer, France (Urios et al. 2010). The type strains and additional characteristics for these species are listed in Table 22.12.

Oceanibaculum Lai et al. 2009, Emend. Dong et al. 2010

O.ce.a'ni.ba'cu.lum. Gr. n. *oceanus*, ocean; L. neut. n. *baculum*, stick; N.L. neut. n. *Oceanibaculum*, rod-shaped bacterium from the ocean.

The genus is characterized by rod-shaped cells, motile by means of a single polar flagellum. The cells stain Gram negative and are oxidase positive. Catalase activity and nitrate reduction are variable. The type species share similar characteristics of growth on 216 L agar plates, forming smooth, gray colonies with regular edges, 1–2 mm in diameter after 72 h incubation at 28 °C, and are nonpigmented and slightly raised in the center. The strains are unable to ferment glucose; they are moderately halophilic, but optimum NaCl concentration varies among them. The dominant fatty acids are $C_{16:1}\omega7c$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}\omega7c$, $C_{18:1}$2-OH, and $C_{19:0}\omega8c$ cyclo. Bacteriochlorophyll *a* is not present. The DNA G+C content is 64.8–67.7 mol%. The type species is *Oceanibaculum indicum* and the type strain is P24 (= CCTCC AB 208226 = LMG 24626 = MCCC 1A02083).

Table 22.10

Morphological, physiological and molecular characteristics differentiating species within genus *Phaeospirillum*

	Phaeospirillum fulvum ATCC 15798^T	*Phaeospirillum molischianum* ATCC 14031^T	*Phaeospirillum chandramohanii* JA145^T	*Phaeospirillum oryzae* JA317^T	*Phaeospirillum tilakii* JA492^T
Morphology	Vibrioid to spiral-shaped	Vibrioid to spiral-shaped	Spiral-shaped	Spiral-shaped	Spiral-shaped
Cell size (μm)	0.5–0.7 × 3.5	0.7–1.0 × 4–6	0.8–1 × 4–8	0.8–1.2 × 2–6	0.5–0.8 × 2–6
Flagellation	1, polar	1, polar	1, polar	1, polar	1, polar
Motility	+	+	+	+	+
N_2 fixation	+	+	+	+	nd
PHB accumulation or other characteristics	nd	nd	nd	nd	nd
Detection *nifD* and/or *nifH*	nd	+	nd	nd	nd
Temperature for growth (°C):					
Range	25–30	25–30	25–35	25–35	25–35
Optimum	25	25	30	30	30
pH for growth:					
Range	6–8.5	6–8.5	6.5–8	6–8	5–9
Optimum	7.3	7.3	7	7	6–6.5
Oxidase	nd	nd	nd	nd	nd
Catalase	nd	nd	nd	nd	nd
Urease hydrolise	nd	nd	–	+	nd
Nitrate reduction	nd	nd	–	nd	nd
Nitrite reduction	nd	nd	–	nd	nd
NaCl concentration for growth (%)	0	0	0	0	0
Utilization of:					
N-acetyl-D-glucosamine	nd	nd	nd	nd	nd
meso-inositol	nd	nd	nd	nd	nd
L-rhamnose	nd	nd	nd	nd	nd
meso-erythritol	nd	nd	nd	nd	nd
Adonitol	nd	nd	nd	nd	nd
D-arabinose	nd	nd	nd	nd	nd
Malate	+	+	+	–	(+)/–
Sucrose	nd	nd	nd	–	nd
D-glucose	+	–	–	–	–
D-fructose	–	–	–	–	–
Mannitol	–	–	(+)	–	–
Glicerol	–	–	–	–	–
Adipate adipic acid	nd	nd	nd	nd	nd
Azelaic acid	nd	nd	nd	nd	nd
Sebacic acid	nd	nd	nd	nd	nd
Phenol	nd	nd	nd	nd	nd
4-Chlorophenol	nd	nd	nd	nd	nd
D-galactose	nd	nd	nd	nd	nd
D-xylose	nd	nd	nd	nd	nd
trehalose	nd	nd	nd	nd	nd
Predominant ubiquinone	Q-9:MK-9 (8:2)	Q-9:MK-9 (8:2)	Q-9:MK-9 (9:1)	Q-9:MK-9 (8:2)	Q-9:MK-9 (7:3)

Table 22.10 (continued)

	Phaeospirillum fulvum ATCC 15798^T	*Phaeospirillum molischianum* ATCC 14031^T	*Phaeospirillum chandramohanii* JA145^T	*Phaeospirillum oryzae* JA317^T	*Phaeospirillum tilakii* JA492^T
Predominant cellular fatty acids	$C_{18:1}\omega7c$, $C_{16:0}$, and $C_{16:1}\omega6c$/$C_{16:1}\omega7c$	$C_{18:1}\omega7c$, $C_{16:1}\omega6c$/$C_{16:1}\omega7c$ and $C_{16:0}$	$C_{16:1}\omega7c$/$C_{16:1}\omega6c$, $C_{16:0}$ and $C_{18:1}\omega7c$	$C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega6c$/$C_{16:1}\omega7c$	$C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega6c$/$C_{16:1}\omega7c$
Indol production	nd	nd	nd	nd	nd
Carotenoids[a]	LY, RP, HLG	LY, RP, HLG, DHLYDG	LY, RP, HLG, DHLYDG	LY, RP, HLG	LY, RP, HLG, DHLYDG
DNA G+C (mol%)	64.8	60.5–64.8	60.5	63.3	62.7
Genome size	3.79 Mb	3.81 Mb	nd	nd	nd
Habitat	Sewage pond	Mud	Freshwater	Rhizosphere soil	Salt marsh

nd, not determined

[a]DHLYDG, Dihydroxylycopene diglucoside; HLG, hydroxylycopene glucoside; LY, lycopene; RP, rhodopin; SP, spirilloxanthin

The second species of the genus is *Oceanibaculum pacificum*, type strain LMC2up-L3 (= MC2UP-L3 = CCTCC AB 209059 = LMG 24859 = MCCC 1A02656), and was isolated from a hydrothermal field sediment of the southwest Pacific Ocean (Dong et al. 2010). The type strains and additional characteristics for these species are listed in *Table 22.13*.

Fodinicurvata Wang et al. 2009

Fo.di.ni.cur.va'ta. L. fem. n. *fodina*, mine; L. adj. curvatus -a -um curved; N.L. fem. n. *Fodinicurvata*, curved-shaped bacterium isolated from a mine.

Cells have a Gram-negative cell wall structure and a size of 0.2–0.5 × 0.5–1.5 μm. They are facultatively anaerobic, vibrioid, and rod shaped. Neither flagella nor endospores are present. Catalase and oxidase are positive. Colonies are cream–white, circular, convex, and opaque with irregular margins after growth on NA supplemented with 5 % at 28 °C for 5 days. Growth occurs under anaerobic conditions. The temperature range for growth is 15–42 °C (optimum, 28 °C), and the pH range for growth is 6.5–8.5 (optimum, 7.5). The carbon sources L-arabinose, D-mannitol, and sucrose are used by *F. sediminis*, while *F. fenggangensis* utilizes myoinositol. Growth occurs at NaCl concentrations of 1.5–20 % (w/v) (optimum, 5 %). Bacteriochlorophyll *a* is not present. Cells accumulate PHB granules and are able to reduce nitrate. The two species strains do not produce H_2S or L-phenylalanine deaminase. Biochemical tests for nitrate reduction, arginine dihydrolase, and urease are positive. Hydrolysis of aesculin and gelatin, indole production, glucose acidification, and phenylalanine deaminase and b-galactosidase are negative. The predominant polar lipids consist of diphosphatidylglycerol, phosphatidylmethylethanolamine, and phosphatidylcholine. Phosphatidylinositol is variable. The DNA G+C content varies from 61.5 to 62.3 mol%. The type species of the genus is *F. sediminis* and the type strains is YIM D82^T (= DSM 21159 T = KCTC22351T). The second species is *F. fenggangensis* strain YIM D812^T (= CCTCC AA 208037 T = DSM 21160 T). Both species were isolated from a salt mine of Fenggang in Yunnan, southwest China. The type strains and additional characteristics for these species are listed in *Table 22.14*.

Pelagibius Choi et al. 2009

Pe.la.gi.bi'us. L. n. *pelagus*, the sea; N.L. masc. n. *bius* from Gr. N. *bios* life; N.L. masc. N., *Pelagibius*, sea life.

Pelagibius forms slightly curved or straight rods, motile by means of a polar flagellum. Cells are strictly aerobic, non-fermentative heterotrophs; they require salt for growth. Cells are oxidase and catalase positive. Growth is mesophilic. Poly-β-hydroxybutyrate granules are formed. Dominant fatty acids are $C_{18:1}\omega7c$, $C_{18:0}$ 3-OH, and $C_{19:0}$ cyclo $\omega8c$. Ubiquinone 10 (Q-10) is the major isoprenoid quinone. Chemotactic and phenotypic characteristics differentiate *Pelagibius* from other related genera in the family *Rhodospirillaceae*, such as temperature range for growth (15–33 °C), pH 6–11, salt tolerance range (2–6 %), and absence of bacteriochlorophyll *a*. Amylase and gelatinase are not produced. Cells grow on L-tyrosine, but casein, hypoxanthine, Tween 80, and xanthine are not hydrolyzed. Cells reduce nitrate to nitrite. According to API ZYM substrate panel, the type strain produces alkaline and acid phosphatases, esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and β-galactosidase. Aesculin is hydrolyzed (API 20NE). Cells utilize L-arabinose, D-galactose, D-glucose, inositol, inulin, D-mannitol, D-mannose, pyruvic acid, succinate, tartrate, and D-xylose as sole carbon sources. The DNA G+C content of the type species is 66.3 mol%. The type species is *Pelagibius litoralis*, and the type strain CL-UU02^T (= KCCM 42323^T = JCM 15426^T) was isolated from seawater of the east coast of Korea (Choi et al. 2009). The type strains and additional characteristics for this species are listed in *Table 22.15*.

Table 22.11
Morphological, physiological and molecular characteristics differentiating species within genus *Nisaea*

	Nisaea denitrificans DR41_21 =DSM 18348=CIP 109265=OOB 129	*Nisaea nitritireducens* DR41_18 = DSM 19540=CIP 109601=OOB 128
Morphology	Rod	Rod
Cell size (μm)	0.9±0.2×2.5±0.6	0.9±0.2×2.5±0.6
Flagellation	Single polar flagellum	Single polar flagellum
Motility	+	+
N_2 fixation	nd	nd
PHB accumulation or other characteristics	nd	nd
Temperature for growth (°C):		
Range	15–44	15–44
Optimum	30	30
pH for growth:		
Range	5.0–9.0	5.0–9.0
Optimum	6.0	6.0
Oxidase	+	+
Catalase	+	+
Urease	nd	nd
Nitrate reduction	+	–
Nitrite reduction	+	+
NaCl concentration for growth (%)	0–6.0	0–6.0
Utilization of:		
Citrate	–	–
Glycogen	–	–
D-fructose	+	+
Glycerol	–	–
Malate	nd	nd
Aesculin	nd	nd
Pyruvate	nd	nd
Succinate	–	–
Sucrose	+	+
Glucose	+	+
Aspartic acid	–	–
Galactose	–	–
L-rhamnose	–	–
α-ketoglutarate	–	–
Lactose	–	–
Maltose	–	–
D-sorbose	nd	nd
Tartrate	nd	nd
N-butyrate	nd	nd
γ-hydroxybutyrate	+	+
Propionate	+	+
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1w7c, C16:1w7c/iso-15 2-OH	C18:1w7c, C16:1w7c/iso-15 2-OH, C19:0w8c cyclo
Indol production	nd	nd
DNA G+C (mol%)	60.1–60.5	60.1–60.5
Genome size	4.63	nd
Habitat	water column, Marine	water column, Marine

+, positive; – negative; nd, not determined; v, variable; na, not available

Tistlia Díaz-Cárdenas et al. 2010

Tistlia Tist'li.a. N.L. fem. n. *Tistlia* named after Tistl, honoring Michael Tistl, a geologist, for his rediscovery of the Salado de Consotá saline spring.

Cells of *Tistlia* are strictly aerobic, slightly curved to straight rods which do not possess pili or form spores. Gram reaction is negative. In *Tistlia consotensis* (Díaz-Cárdenas et al. 2010) cells reveal a Gram-positive cell-wall ultrastructure. Cell sizes are 0.6–0.7 × 3.0–3.5 μm. Cells multiply by binary fission and show tumbling motility. Growth is mesophilic and slightly halophilic with optimum growth occurring at 30 °C, pH 6.5–6.7, and a salinity of 0.5 % (w/v). Growth is chemoheterotrophic; cells grow on glucose or peptone as a sole carbon source. Yeast extract is not required for growth but increases the biomass yields. Growth occurs with pyruvate, butyrate, succinate, glucose, mannose, xylose, galactose, arabinose, trehalose, cellobiose, lactose, sucrose, rhamnose, fructose, maltose, peptone, casamino acids, tryptone, pepticase, gelatin, arginine, alanine, leucine, isoleucine, valine, glutamate, glycerol, inositol, and starch, but formate, acetate, methanol, lactate, citrate, α-ketoglutarate, ribose, raffinose, methionine, threonine, lysine, glycine, histidine, Tween 80, ethyl oleate, olive oil, benzoate, and cinnamate cannot be used as substrates. Cells are able to fix dinitrogen, showing very high acetylene reduction activity, and were found to possess the *nifH* gene. Urea, nitrate, and glutamate can serve as sole nitrogen sources. Q-10 is the predominant ubiquinone and $C_{19:0}\omega8c$ cyclo, $C_{18:1}\omega7c$, and $C_{18:0}$ are the dominant fatty acids. The DNA G+C content is 71 ± 1 mol%. The type species is *T. consotensis*, strain USBA 355 T (= JCM 15529 T = KCTC 22406 T), isolated from the Salado de Consotá saline spring, Colombia (Díaz-Cárdenas et al. 2010). The type strains and additional characteristics for this species are listed in *Table 22.16*.

Table 22.12
Morphological, physiological and molecular characteristics differentiating species within genus *Thalassobaculum*

	Thalassobaculum litoreum DSM 18839^T	*Thalassobaculum salexigens* DSM 19539^T
Morphology	Curve-straight rods	Rods
Cell size (μm)	1.3–1.5	1.6+-0.3
Flagellation	Polar	Single, polar
Motility	+	+
N_2 fixation	–	–
PHB accumulation or other characteristics	+	nd
Detection *nifD* and/or *nifH*	–	–
Temperature for growth (°C):		
Range	10.0–35.0	15–37
Optimum	30–35	30
pH for growth:		
Range	7.0–9.0	5.0–10.0
Optimum	8	8
Oxidase	+	+
Catalase	+	+
Urease hydrolise	nd	nd
Nitrate reduction	+	nd
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	2.0–4.0	3.4–4.0
Utilization of:		
N-acetyl-D-glucosamine	–	–
meso-inositol	–	nd
L-rhamnose	–	nd
meso-erythritol	nd	+
Adonitol	nd	nd
D-arabinose	+	–
Malate	nd	nd
Sucrose	+	–
D-glucose	–	–
D-fructose	–	+
Mannitol	–	+
Glicerol	nd	–
Adipate adipic acid	nd	nd
Azelaic acid	nd	nd
Sebacic acid	nd	nd
Phenol	nd	nd
4-Chlorophenol	nd	nd
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1w7c; C16:0; C17:0	C18:1w7c; C17:0; C16:0; C16:1w7c
Indol production	nd	nd
DNA G+C (mol%)	68.0	65.0
Genome size	nd	nd
Habitat	Water	Water

+, positive; –, negative; nd, not determined

Telmatospirillum Sizova et al. 2007

Tel.ma.to.spi.ril'lum Gr.n. *telma -atos*, marsh, swamp, fen; N.L. dim neut. n. *Spirillum*, a bacterial genus; N.L. neut. n. *Telmatospirillum*, a fen *Spirillum*.

Cells are Gram negative, vibrioid to spiral shaped, and motile by means of polar or subpolar flagella. Major cellular fatty acids are $C_{18:1}\omega7c$, $C_{17:0}$ cyclopropane, and $C_{16:0}$. Cells grow chemoorganotrophically under anoxic conditions or at low oxygen pressures in the dark as well autotrophically on $H_2 + CO_2$ at low oxygen pressure, being tolerant up to 5 kPa of oxygen. Cells are catalase and oxidase negative. The growth temperature range between 4 °C and 30 °C and the pH range is 4–7. Growth is supported by several organic acids and glucose. Cells can fix atmospheric N_2. Liquid medium is superior to solid agar medium. The G+C content of the DNA is 61.6–64 mol%. The type species is *Telmatospirillum siberiense*, and the type strain 26-4b1 (= ATCC BAA-1305 = KACC 11899) was isolated from northern acidic wetlands under *Sphagnum*. The type strains and additional characteristics for this species are listed in Table 22.17.

Defluvicoccus Maszenan et al. 2005

De.flu.vi.coc'cus. L. neut. n. *defluvium*, sewage; N.L. (Gr. derived) masc. n. *coccus*, berry (spherical microbe); N.L. masc. n. *Defluvicoccus*, a coccus from sewage.

Cells are Gram-negative, non-spore-forming, and nonmotile cocci; they grow chemoheterotrophically under aerobic conditions with a mean cell size of 1.5–4.0 μm. Cells are usually arranged in clusters or tetrads, stain very faintly, and appear empty after staining. Oxidase is negative and catalase is positive. It grew optimally at 25–30 °C and at a pH of 7.5–8.0. Urease and gelatin liquefaction are weakly positive. Many carbon sources (Biolog GN and GP systems), including adonitol, malate, and D-arabinose, are utilized by strain Ben 114^T as presented in description of species and summarized in Table 22.1. Cells are positive for the following enzyme activities as detected with the API ZYM system: alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid

Table 22.13
Morphological, physiological and molecular characteristics differentiating species within genus *Oceanibaculum*

	Oceanibaculum indicum P24=CCTCC AB 208226=LMG 24626=MCCC 1A02083	*Oceanibaculum pacificum* LMC2up-L3=CCTCC AB 209059=LMG 24859 =MCCC 1A02656
Morphology	Rod	Rod
Cell size (μm)	0.6–1.5 × 2.3–2.5	0.5–0.7 × 1.7–2.1
Flagellation	Single polar flagellum	Single polar flagellum
Motility	+	+
N_2 fixation	nd	nd
PHB accumulation or other characteristics	nd	nd
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	10–42	10–45
Optimum	25–37	28–37
pH for growth:		
Range	6.0–11.0	6.0–11.0
Optimum	7.0–9.0	7.0–9.0
Oxidase	+	+
Catalase	+	+
Urease	–	+
Nitrate reduction	+	–
Nitrite reduction	–	–
NaCl concentration for growth (%)	0–9.0	0–9.0
Utilization of:		
Citrate	–	–
Glycogen	–	–
D-fructose	–	+
Glycerol	–	–
Malate	nd	nd
Aesculin	nd	nd
Pyruvate	nd	nd
Succinate	–	–
Sucrose	–	–
Glucose	–	–
Aspartic acid	–	–
Galactose	+	–
L-rhamnose	+	–
α-ketoglutarate	v	–
Lactose	–	–
Maltose	–	–
D-sorbose	nd	nd
Tartrate	nd	nd
N-butyrate	nd	nd
γ-hydroxybutyrate	–	–
Propionate	–	–
Predominant ubiquinone	nd	nd
Predominant cellular fatty acids	$C_{16:0}$, $C_{18:0}$, $C_{18:1}$w7c, $C_{18:1}$ 2-OH and $C_{19:0}$w8c cyclo/$C_{18:0}$ 3–OH	$C_{16:1}$, $C_{16:0}$, $C_{18:1}$w7c
Pigment production	–	–
Indol production	–	–
DNA G+C (mol%)	64.8–65.5	67.7
Genome size	3.95	nd
Habitat	Deep water, Marine	Sediments of hydrothermal field

+, positive; –, negative; *nd*, not determined; *v*, variable; *na*, not available
Utilization of carbon source was tested using API 20NE and Biolog GN2

phosphatase, and naphthol-AS-BI-phosphohydrolase. The DNA G+C content is 66 mol%. The type species is *Defluvicoccus vanus* and the type strain is Ben 114 T (= NCIMB 13612 T = CIP107350T), isolated from a sample of biomass from an enhanced biological phosphorus removal (EBPR) activated sludge plant in the Czech Republic (Maszenan et al. 2005). The type strains and additional characteristics for this species are listed in *Table 22.18*.

Tistrella Shi et al. 2002

Tistr.el'la. M. L. dim. fem. ending -ella; N. L. fem. n. *Tistrella*, arbitrary name formed from the acronym of Thailand Institute of Scientific and Technological Research, TISTR, where the isolation of strain IAM 14872^T was performed.

Cells are Gram negative and rod shaped, with variable sizes (0.7–1.2 × 1.5–1.2 μm). They often occur in chains with a length of approximately 12 mm and are highly motile by means of a single polar flagellum. The cells show binary fission. Bacteria are strictly aerobic and chemoorganotrophic. They are non-photosynthetic and the cells lack intracytoplasmic membrane systems and bacteriochlorophyll a. Cells accumulate polyhydroxyalkanoates. Optimal growth temperatures and pH depend on the species and are between 25 °C and 30 °C and pH 7–7.4, respectively. The salt requirement is variable. Malic acid is readily used as carbon source. The use of other carbon sources such as

Table 22.14
Morphological, physiological and molecular characteristics differentiating species within genus *Fodinicurvata*

	Fodinicurvata sediminis DSM 21159^T	*Fodinicurvata fenggangensis YIM D812^T*
Morphology	Rod and vibroid	Rod and vibroid
Cell size (μm)	0.3–0.5 × 0.7–1.5	0.2–0.5 × 0.5–1.3
Flagellation	Absent	Absent
Motility	–	–
N_2 fixation	–	–
PHB accumulation or other characteristics	+	+
Detection *nifD* and/or *nifH*	–	–
Temperature for growth (°C):		
Range	15.0–42.0	15.0–42.0
Optimum	28.0	28.0
pH for growth:		
Range	6.5–8.5	6.5–8.5
Optimum	7.5	7.5
Oxidase	+	+
Catalase	+	+
Urease hydrolise	+	+
Nitrate reduction	+	+
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	1.5–20	1.5–20
Utilization of (less than 20):		
4-Chlorophenol	nd	nd
Adipate adipic acid	nd	nd
Adonitol	–	–
Azelaic acid	nd	nd
Citrate	+	+
D-arabinose	+	–
D-fructose	–	–
D-glucose	+	+
Glycerol	+	+
L-rhamnose	–	–
Malate	nd	nd
Mannitol	+	+
meso-erythritol	nd	nd
meso-inositol	–	+
N-acetyl-D-glucosamine	nd	nd
Phenol	nd	nd
Sebacic acid	nd	nd
Sucrose	+	–
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1v7c, C18:1 2-OH and C16:0.	C18:1v7c, C18:1 2-OH and C16:0.
Indol production	nd	nd
DNA G+C (mol%)	61.5	62.3

Table 22.14 (continued)

	Fodinicurvata sediminis DSM 21159^T	*Fodinicurvata fenggangensis YIM D812^T*
Genome size	nd	nd
Habitat	Salt mine	Salt mine
Biochemical characteristics kit	Carbon utilization was tested using artificial seawater medium	Carbon utilization was tested using artificial seawater medium

+, positive; –, negative; *nd*, not determined, v, variable; na, not available

L-arabinose, D-mannitol, N-acetylglucosamine, and adipic acid is variable. The cells produce indole, reduce nitrate to nitrite, but do not fix dinitrogen. Aesculin, gelatin, and arginine are hydrolyzed. Cells are positive for catalase and oxidase. The major ubiquinone is Q-10. The major cellular fatty acid is $C_{18:1}\omega7c$. Both 2-hydroxy and 3-hydroxy fatty acids are present, and the major hydroxy fatty acids are $C_{18:0}$ 2-OH and $C_{14:0}$ 3-OH. The $C_{19:0}$ ω8c cyclo may be present. The G–C content of DNA is 65.8–67.5 mol%. The type species is *T. mobilis* and the type strain is IAM 14872 T (= TISTR 1108 T), isolated from wastewater in Thailand (Shi et al. 2002). A second species, named *T. bauzanensis*, type strain BZ78T (= DSM 22817 T = CGMCC 1.10188 T = LMG 26047 T), was isolated from hydrocarbon-contaminated soil in Bozen, South Tyrol, Italy, and was described by Zhang et al. (2011). The type strains and additional characteristics for these species are listed in ❯ *Table 22.19*.

Constrictibacter Yamada et al. 2011

Cons.tric.ti.bac'ter. L. adj. *constrictus*, compressed, contracted; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Constrictibacter*, rod with compressed parts.

Cells of *Constrictibacter antarcticus* are ovoid to rod shaped and often occur in pairs or chains. Cells are motile and do not form spores and grow aerobically or micro-aerobically; they have a diameter of 0.8–1.0 μm and a length of 1.5–2.0 μm. Colonies are white and circular, 0.2 mm in diameter on 0.256LB/MA agar. Biochemical characteristics, analyzed using the API 20NE and API ZYM, indicate that catalase is produced, but oxidase is not produced. Produces acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8),b-glucosidase, leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase. *Constrictibacter* cells reduce nitrate and complex nutrients (tryptone or yeast extract) are essential for growth. The respiratory quinones are Q-10 and Q-8. The major cellular fatty acids are $C_{18:1}$, $C_{16:0}$, and $C_{18:0}$. The DNA G+C content of the type strain/species is 69.8 mol%. The type species is *Constrictibacter antarcticus* 262-8^T (= JCM, ATCC16422T, BAA1906T) that was isolated from a cavity within white rock collected in the Skallen region of Antarctica. The type strains and additional characteristics for this species are listed in ❯ *Table 22.20*.

Table 22.15
Morphological, physiological and molecular characteristicas differentiating species within genus *Pelagibius*

	Pelagibius litoralis KCCM 42323^T
Morphology	Slightly curved or straight rods
Cell size (um)	0.5–1.0 × 1.2–2.5
Flagellation	1, polar
Motility	+
N_2 fixation	nd
PHB accumulation or other characteristics	+
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	15–33
Optimum	28–30
pH for growth:	
Range	6–11
Optimum	7–8
Oxidase	+
Catalase	+
Urease hydrolise	–
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	2–6%
Utilization of:	According to the API 20NE system
N-acetyl-D-glucosamine	–
meso-inositol	+
L-rhamnose	–
meso-erythritol	nd
Adonitol	nd
L-arabinose	+
Malate	nd
Sucrose	–
D-glucose	+
D-fructose	–
Mannitol	+
Glicerol	–
Adipate adipic acid	nd
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
D-galactose	+
D-xylose	+
Trehalose	–
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	C18:1ω7c, C18:0 3-OH, C19:0 cyclo ω8c

Table 22.15 (continued)

	Pelagibius litoralis KCCM 42323^T
Indol production	–
Carotenoids[a]	nd
DNA G+C (mol%)	66
Genome size	nd
Habitat	Coastal seawater

[a]*DHLYDG* Dihydroxylycopene diglucoside, *HLG* hydroxylycopene glucoside, *LY* lycopene, *RP* rhodopin, *SP* spirilloxanthin

Rhodovibrio Imhoff et al. 1998

Rho.do.vi'bri.o. Gr. n. *rhodon*, the rose; M.L. masc. n. *Vibrio*, a bacterial genus; M.L. masc. n., *Rhodovibrio*, the rose *Vibrio*.

Cells of the genus *Rhodovibrio* are vibrioid to spiral shaped, 0.6–0.9 µm in size. They are motile by means of polar flagella and multiply by binary fission (Imhoff et al. 1998; Imhoff 2005a). Cell staining is Gram negative. Internal photosynthetic membranes are present as vesicles. They contain bacteriochlorophyll a as well as carotenoids of the spirilloxanthin series. Cells harbor ubiquinones and menaquinones with 10 isoprene units (Q-10 and MK-10). Major cellular fatty acids are $C_{18:1}$ and $C_{18:0}$. The polyamines putrescine and spermidine may be present (Haitiana et al. 2001). Bacteria of this genus grow preferably photoheterotrophically under anoxic conditions in the light, but it is also possible to grow the cells chemotrophically under microoxic to oxic conditions in the dark (Imhoff et al. 1998; Imhoff 2005a). Complex nutrients are required, as no growth is observed in the complete absence of yeast extract or peptone. Under low concentration of yeast extract, lactate or casamino acids increase growth markedly in case of *R. salinarum*, while acetate, malate, succinate, or pyruvate has a similar effect in *R. sodomensis* (Nissen and Dundas 1984; Mack et al. 1993). These species are halophiles, require NaCl or sea salt for growth, and have salt optima above seawater salinity. Cell growth is mesophilic with the preference for neutral pH. Both species show best growth in the presence of 0.1 M Mg^{2+} (Mack et al. 1993). Their DNAs have G+C contents between 65 and 69 mol%. *R. salinarum* is the genus type species and the type strain is ATCC 35394^T (= DSM 9154). The type strain of the species *R. sodomensis* is DSI (= ATCC 51195 = DSM 9895). The type strains and additional characteristics for these species are listed in *Table 22.21*.

Limimonas Amoozegar et al. 2013

Li.mi.mo'nas. L. n. *limus*, mud; L. fem. n. *monas*, a unit, monad; N.L. fem. n. *Limimonas*, a unit (bacterium) isolated from mud.

Cells are Gram negative, strictly aerobic, nonmotile, and rod shaped. They are catalase and oxidase positive and extremely halophilic. Optimal growth occurs with 3.4 M NaCl, at pH 7.0 and 40 °C. The polar lipid pattern consists of phosphatidylglycerol,

Table 22.16
Morphological, physiological and molecular characteristics differentiating species within genus *Tistlia*

	Tistlia consotensis JCM 15529^T
Morphology	Curved-straight rods
Cell size (μm)	3.0–3.5
Flagellation	−
Motility	−
N_2 fixation	+
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	+
Temperature for growth (°C):	
Range	20–40
Optimum	30
pH for growth:	
Range	5.0–8.0
Optimum	6.5–6.7
Oxidase	nd
Catalase	+
Urease hydrolise	+
Nitrate reduction	+
Nitrite reduction	+
NaCl concentration for growth (%)	0.5
Utilization of:	
N-acetyl-D-glucosamine	nd
meso-inositol	+
L-rhamnose	+
meso-erythritol	nd
Adonitol	nd
D-arabinose	+
Malate	nd
Sucrose	+
D-glucose	+
D-fructose	+
Mannitol	nd
Glicerol	+
Adipate adipic acid	nd
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	C19:0 cyclo w8c; C18:1w7c; C18:0
Indol production	nd
DNA G+C (mol %)	71.0
Genome size	nd
Habitat	Water

+, positive; −, negative; nd, not determined

Table 22.17
Morphological, physiological and molecular characteristics differentiating species within genus *Telmatospirillum*

	Telmatospirillum siberiense 26–4b1^T (BAA-1305^T)
Morphology	vibriod to spiral shaped
Cell size (mm)	0.2–0.6 (mm) diameter
Flagellation	1–2 polar or subpolar flagella
Motility	+
N_2 fixation	+
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	+
Main carotenoids	nd
Photolithoautotrophic growth (electron donor)	nd
Temperature for growth (°C):	
Range	4–30
Optimum	25–28
pH for growth:	
Range	4–7
Optimum	5.7–6.5
Oxidase	–
Catalase	–
Color of cell suspension	nd
Urease hydrolise	nd
Nitrate reduction	nd
Nitrite reduction	nd
NaCl concentration for growth (%)	0–0.58%
Utilization of:	
Acetate	+ (microaerophilic)
Aspartate	nd
Benzoate	nd
Butyrate	nd
Citrate	nd
Crotonate	nd
N-acetyl-D-glucosamine	nd
meso-inositol	nd
L-rhamnose	nd
meso-erythritol	nd
Adonitol	nd
L-arabinose	nd
D-arabinose	nd
Malate	nd
Sucrose	nd
D-glucose	nd
D-fructose	nd
Mannitol	nd
Glicerol	nd
Adipate adipic acid	nd

Table 22.17 (continued)

	Telmatospirillum siberiense 26–4b1^T (BAA-1305^T)
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
Predominant ubiquinone	nd
Predominant cellular fatty acids	C18:1 ω7c, 17:0 cyclopropane and C16:0
Indol production	
DNA G+C (mol%)	64
Genome size	nd
Habitat	Acidic wetlands under Sphagnum (bogs, fens)

nd, not determined

Table 22.18
Morphological, physiological and molecular characteristics differentiating species within genus *Defluvicoccus*

	Defluvicoccus vanus NCIMB 13612^T
Morphology	Cocci/coccobacill
Cell size (μm)	1.5–4.0
Flagellation	Absent
Motility	−
N_2 fixation	nd
PHB accumulation or other characteristics	+
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	20.0–30.0
Optimum	25.0–30.0
pH for growth:	
Range	5.0–8.0
Optimum	7.5–8.0
Oxidase	−
Catalase	+
Urease hydrolise	+
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	nd
Utilization of (less than 20):	
4-Chlorophenol	nd
Adipate adipic acid	nd
Adonitol	+
Azelaic acid	nd
Citrate	nd
D-arabinose	+
D-fructose	nd

Table 22.18 (continued)

	Defluvicoccus vanus NCIMB 13612^T
D-glucose	nd
Glycerol	nd
L-rhamnose	nd
Malate	+
Mannitol	nd
meso-erythritol	nd
meso-inositol	nd
N-acetyl-D-glucosamine	+
Phenol	nd
Sebacic acid	nd
Sucrose	nd
Predominant ubiquinone	nd
Predominant cellular fatty acids	nd
Indol production	−
DNA G+C (mol%)	66
Genome size	nd
Habitat	Activated sludge biomass
Biochemical characteristics kit	Biolog GN and GP systems; Microbact 24E system tests (Oxoid)

+, positive; − negative; nd, not determined, v, variable; na, not available

diphosphatidylglycerol, four unidentified phospholipids, three unidentified amino lipids, and two other unidentified lipids. Ubiquinone Q-10 is the major isoprenoid quinone. The predominant fatty acids are $C_{19:0}$ω7c cyclo and $C_{18:0}$. The DNA G+C content of the type strain is 67.0 mol%. The type species is *Limimonas halophila* and the type strain is IA16^T (= IBRC-M 10018^T = DSM 25584^T). The type strains and additional characteristics for this species are listed in *Table 22.22*.

Rhodospirillum Molisch 1907, Emend. Lakshmi et al. 2013

Rho.do.spi.ril'lum. Gr. n. *rhodon*, the rose; M.L. neut. n. *Spirillum*, a bacterial genus; M.L. neut. n., *Rhodospirillum*, the rose Spirillum.

Cells of the genus *Rhodospirillum* are vibrioid to spiral shaped and motile by means of bipolar flagella and multiply by binary fission. They are Gram negative and mesophilic and prefer neutral pH. Cells contain internal photosynthetic membranes as vesicles. Photosynthetic pigments are bacteriochlorophyll *a* (esterified with phytol or geranylgeraniol) and carotenoids of the spirilloxanthin series, such as spirilloxanthin itself and rhodovibrin. Ubiquinones and rhodoquinones with 10 isoprene units are present. Main cellular fatty acids include $C_{18:1}$ω7c/$C_{18:1}$ω6c, $C_{16:1}$ω7c/$C_{16:1}$ω6c, $C_{16:0}$, $C_{14:0}$3-OH, and $C_{16:0}$3-OH. They grow generally well using fatty acids as carbon sources, except formate and propionate. No appreciable development occurs with tartrate, gluconate, or citrate (Van Niel 1944).

Table 22.19
Morphological, physiological and molecular characteristics differentiating species within genus *Tistrella*

	Tistrella mobilis TISTR 1108^T	*Tistrella bauzanensis* DSM 22817^T
Morphology	Rods	Rods
Cell size (μm)	0.7–1.0	1.0–1.2
Flagellation	Single, polar	Single, polar
Motility	+	+
N_2 fixation	nd	nd
PHB accumulation or other characteristics	+	+
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	20–40	1.0–25.0
Optimum	30	20
pH for growth:		
Range	5.0–9.0	nd
Optimum	7.4	7.0
Oxidase	+	+
Catalase	+	+
Urease hydrolise	–	–
Nitrate reduction	+	+
Nitrite reduction	–	nd
NaCl concentration for growth (%)	nd	nd
Utilization of:		
N-acetyl-D-glucosamine	+	–
meso-inositol	nd	–
L-rhamnose	nd	–
meso-erythritol	nd	nd
Adonitol	nd	nd
D-arabinose	+	+
Malate	+	+
Sucrose	nd	–
D-glucose	nd	–
D-fructose	nd	–
Mannitol	+	–
Glicerol	nd	–
Adipate adipic acid	+	nd
Azelaic acid	nd	nd
Sebacic acid	nd	nd
Phenol	nd	nd
4-Chlorophenol	nd	nd
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1w7c	C18:1w7c; C19:0w8c cyclo
Indol production	+	+
DNA G+C (mol%)	67.5	65.8
Genome size	nd	nd
Habitat	Water	Soil

+, positive; –, negative; nd, not determined

Ethanol is a good substrate, whereas carbohydrates and their corresponding polyalcohols are not utilized. Alanine, asparagine, and aspartic and glutamic acids result in satisfactory growth; glycine and leucine give rise, at best, to slight development. Bacteria of this genus grow preferably photoheterotrophically under anaerobic conditions in the light, but they can also grow photoautotrophically with molecular hydrogen and sulfide, but not with thiosulfate as photosynthetic electron donor. They also can grow chemotrophically under microoxic to oxic conditions in the dark. Fermentation and oxidant-dependent growth may occur. Their DNA has a G+C content between 63 and 66 mol%. *Rhodospirillum rubrum* is the type species of the genus and the type strain is S1^T (= ATCC 11 170^T = NCIB 8355^T) (Skerman et al. 1980, Pfennig and Trüper 1971b; Lakshmi et al. 2014). The type strains and additional characteristics for this species are listed in ❯ *Table 22.23*.

Pararhodospirillum Lakshmi et al. 2014

Pa.ra.rho.do.spi.ril'lum. Gr. prep. *para*, beside, alongside of, near, like; N.L. neut. n. *Rhodospirillum*, a bacterial generic name; N.L. neut. n. *Pararhodospirillum*, resembling *Rhodospirillum*.

Cells of the genus *Pararhodospirillum* are spiral shaped and motile by means of bipolar flagella and multiply by binary fission. They are Gram negative and grow under mesophilic conditions with preference for neutral pH. Cells contain internal photosynthetic membranes as stacks of lamellae that form a sharp angle to the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll *a* (esterified with phytol or geranylgeraniol) and carotenoids of the spirilloxanthin series, which include lycopene and rhodopin, although spirilloxanthin itself may be absent in some *P. photometricum* strains. Ubiquinones and rhodoquinones with 8 isoprene units are present. Main cellular fatty acids include $C_{18:1}\omega7c/C_{18:1}\omega6c$, $C_{16:0}$, $C_{14:0}$3-OH, $C_{15:0}$3-OH, and $C_{16:0}$3-OH. Bacteria of this genus are strictly anaerobes and obligate phototrophs. Growth factors are required for growth. Their DNA has a G+C content between 60.0 and 65.8 mol%. *Pararhodospirillum photometricum* is the type species of the genus (DSM 122^T = ATCC 49918^T). Other species are *Pararhodospirillum sulfurexigens* (JA143^T = DSM 19785^T = JCM 14885^T = NBRC 104433^T) and *Pararhodospirillum oryzae* (JA318^T = KCTC 5960^T = NBRC 107573^T). The type strains and additional characteristics for these species are listed in ❯ *Table 22.24*.

Table 22.20

Morphological, physiological and molecular characteristics differentiating species within genus *Constrictibacter*

	Constrictibacter antarcticus JCM 16422^T
Morphology	Ovoid to rods
Cell size (μm)	0.8–1.0 × 1.5–2.0
Flagellation	Few polar flagellum
Motility	+
N_2 fixation	–
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	5.0–30.0
Optimum	25
pH for growth:	
Range	6.0–8.1
Optimum	7.0
Oxidase	−
Catalase	+
Urease hydrolise	nd
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	0.5
Utilization of:	nd
4-Chlorophenol	−
Adipate adipic acid	−
Adonitol	nd
Azelaic acid	nd
citrate	nd
D-arabinose	−
D-fructose	+
D-glucose	+
Glycerol	+
L-rhamnose	nd
Malate	−
Mannitol	−
meso-erythritol	−
meso-inositol	−
N-acetyl-D-glucosamine	nd
Phenol	nd
Sebacic acid	nd
Sucrose	+
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	C18:1, C16:0, C18:0
Indol production	nd
DNA G+C (mol%)	69.8
Genome size	nd
Habitat	White rock
Biochemical characteristics kit	API 20 NE and API ZYM

+, positive; −, negative; nd, not determined, v, variable; na, not available

Table 22.21

Morphological, physiological and molecular characteristics differentiating species within genus *Rhodovibrio*

	Rhodovibrio salinarum ATCC 35394^T	*Rhodovibrio sodomense* DSI^T
Morphology	Rod to spiral-shaped cells	Vibrio-shaped cells
Cell size (μm)	0.3 × 1–3	0.6–0.7 × 1.6–2.5
Flagellation	A pair at one end of the cell	Presumably polar flagella
Motility	+	Weakly motile
N_2 fixation	nd	−
PHB accumulation or other characteristics	−	+
Detection *nifD* and/or *nifH*	nd	−
Temperature for growth (°C):		
Range	20–45	25–47
Optimum	42	35–40
pH for growth:		
Range	nd	nd
Optimum	7.5–8.0	7.0
Oxidase	+	+
Catalase	nd	nd
Urease hydrolise	nd	nd
Nitrate reduction	nd	nd
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	4 (3–24)	12 (6–20)
Utilization of:		
Malate	ǂ	†
Sucrose	ǂ	
Mannitol	ǂ	
Glicerol	ǂ	
Acetate	ǂ	†
Butyrate		ǂ
Citrate	ǂ	ǂ
Fructose	ǂ	
Fumarate	ǂ	
Glutamate	ǂ	
Lactate	†	
Propionate	ǂ	ǂ
Pyruvate	ǂ	†
Succinate	ǂ	†
Predominant ubiquinone	Q-10, MK-10	nd
Predominant cellular fatty acids	$C_{18:1}$, $C_{18:0}$, $C_{16:0}$	nd
Indol production	nd	nd

Table 22.21 (continued)

	Rhodovibrio salinarum ATCC 35394^T	*Rhodovibrio sodomense* DSI^T
DNA G+C (mol%)	67.4	66.2–66.6
Genome size	nd	nd
Habitat	Saltern	Salt lakes

+, positive; −, negative; nd, not determined
ǂ = Does not stimulate growth significantly in the presence of reduced complex nutrients (required)
† = Stimulates growth in the presence of reduced complex nutrients (required)

Table 22.22
Morphological, physiological and molecular characteristics differentiating species within genus *Limimonas*

	Limimonas halophila IA16 =IBRC-M 10018 =DSM 25584
Morphology	Rod
Cell size (μm)	0.1–0.2 × 1.5– 2.0
Flagellation	−
Motility	−
N_2 fixation	na
PHB accumulation or other characteristics	na
Detection *nifD* and/or *nifH*	na
Temperature for growth (°C):	
Range	30–50
Optimum	40
pH for growth:	
Range	6.0–8.0
Optimum	7.0
Oxidase	+
Catalase	+
Urease	−
Nitrate reduction	−
Nitrite reduction	nd
NaCl concentration for growth (%)	15–30
Utilization of:	
Citrate	nd
Glycogen	nd
D-fructose	nd
Glycerol	+
Malate	nd
Aesculin	nd
Pyruvate	nd
Succinate	−
Sucrose	+
Glucose	+

Table 22.22 (continued)

	Limimonas halophila IA16 =IBRC-M 10018 =DSM 25584
Aspartic acid	+
Galactose	+
L-rhamnose	nd
α-ketoglutarate	nd
Lactose	+
Maltose	nd
D-sorbose	nd
Tartrate	nd
N-butyrate	nd
γ-hydroxybutyrate	nd
Propionate	nd
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	$C_{19:0}$ cyclo ω7c and $C_{18:0}$
Pigment production	−
Indol production	−
DNA G+C (mol%)	67.0
Genome size	nd
Habitat	Mud of hypersaline Lake

+, positive; −, negative; nd, not determined, v, variable; na, not available
Utilization of carbon source were (1%, w/v) was performed using salts of MGM broth, without peptone and containing 0, 1 g l^{-1} of yeast extract, as indicated by Amoozegar et al. (2013)

Roseospira Imhoff et al. 1998, Emend. Guyoneaud et al. 2003

Ro.se.o.spi'ra. L. adj. *roseus*, rosy; Gr. n. *spira*, the spiral; M.L. fem. n., *Roseospira*, the rosy spiral.

The cells of this genus are vibrioid or spiral shaped, 0.4–1.0 μm in size, and motile by means of polar or bipolar flagella and divide by binary fission. Cells are staining Gram negative. Intracytoplasmic photosynthetic membranes are present as vesicles and contain bacteriochlorophyll *a* as well as various carotenoids as photosynthetic pigments. *Roseospira* are slightly halophilic bacteria requiring NaCl or sea salt for growth. Optimum NaCl concentrations are between 0.5 % and 7 % (w/w). Growth occurs preferably photo-organotrophically under anoxic conditions in the light, but cells can also grow under microoxic conditions in the dark. Phototrophic grown cells contain intracytoplasmic membranes of the lamellar type together with bacteriochlorophyll *a* as well as carotenoids. Growth factors niacin, thiamine, *p*-aminobenzoic acid, and yeast extract are required. Their DNAs have G+C contents between 65.0 and 72.3 mol%. The type species of the genus is *Roseospira mediosalina* and type strain is strain BN 280. The genus embraces four other species: *R. marina*, *R. visakhapatnamensis*, *R. goensis*, and *R. navarrensis*. A new species, *R. thiosulfatophila*, has been proposed (Guyoneaud et al. 2002) but, to date,

Table 22.23
Morphological, physiological and molecular characteristics differentiating species within genus *Rhodospirillum*

	Rhodospirillum rubrum S1^T
Morphology	Vibrioid to spiral-shaped cells
Cell size (μm)	0.8–1.0 × 7–10
Flagellation	Bipolar
Motility	+
N_2 fixation	+
PHB accumulation or other characteristics	+
Detection *nifD* and/or *nifH*	+
Temperature for growth (°C):	
Range	nd
Optimum	30–35
pH for growth:	
Range	6.0–8.5
Optimum	6.8–7.0
Oxidase	+
Catalase	+
Urease hydrolise	nd
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	None
Utilization of:	
Malate	+
Mannitol	–
Glicerol	–
Acetate	+
Arginine	+
Aspartate	+
Benzoate	–
Butyrate	+
Caproate	+
Citrate	–
Crotonate	–
Ethanol	+
Formate	–
Fructose	–
Fumarate	+
Glucose	–
Glutamate	+
Lactate	+
Methanol	–
Propionate	+
Pyruvate	+
Succinate	+
Tartrate	–

Table 22.23 (continued)

	Rhodospirillum rubrum S1^T
Valerate	+
Predominant ubiquinone	Q-10, RQ-10
Predominant cellular fatty acids	$C_{18:1}$, $C_{16:1}$, $C_{16:0}$
Indol production	nd (it produces 4-hydroxyphenethyl alcohol – a cytokinin-like substance)
DNA G+C (mol%)	65
Genome size	4, 352, 825 bp
Habitat	Fresh water

+, positive; –, negative; nd, not determined

the name has not been validated. The type strains and additional characteristics for these species are listed in *Table 22.25*.

Rhodospira Pfennig et al. 1997

Rho.do.spi'ra. Gr.n. *rhodos*, the rose; Gr.n. *spira*, the spiral; M.L. fem.n., *Rhodospira*, the rose spiral.

Cells are vibrioid to spirilloid with a size of 0.6–0.8 μm; they are motile by means of flagella. Growth is mesophilic. The major fatty acids are $C_{18:1}$, $C_{16:0}$, and $C_{14:0}$. Cells harbor photosynthetic membranes of the vesicular type. The cells grow preferably photoorganotrophically under anaerobic conditions in the light and microaerobically in the dark. Anaerobically grown colonies are beige to peach colored. Bacteriochlorophyll *b* and the carotenoid tetrahydrospirilloxanthin are present. The absorption maxima of living cells for *R. trueperi* type strain are 397, 458, 490, 600, 689, 801, 889, and 986 nm. Major quinone components are Q-7, MK-7, and RQ-7. Cell growth requires reduced sulfur compounds and extracellular sulfur depositions are produced. Biotin, thiamine, and pantothenate are required as growth factors. Cells do not grow in the absence of NaCl. The DNA G+C content is 65.7 mol%. The type species is *Rhodospira trueperi* 8316^T (= ATCC 700224^T), isolated from a peach-colored layer of a laminated microbial mat in a salt marsh at Massachusetts, USA (Pfennig et al. 1997). The type strains and additional characteristics for this species are listed in *Table 22.26*.

Phaeovibrio Lakshmi et al. 2011

Phae.o.vib'ri.o. Gr. adj. *phaeos*, brown; L. v. *vibro*, to set in tremulous motion, move to and fro, vibrate; N.L. masc. n. *vibrio*, that which vibrates, and also a genus name of bacteria possessing a curved rod shape; N.L. masc. n., *Phaeovibrio*, brown vibrio.

Table 22.24

Morphological, physiological and molecular characteristics differentiating species within genus *Pararhodospirillum*

	Pararhodospirillum photometricum 132^T	*Pararhodospirillum sulfurexigens JA143*^{T}	*Pararhodospirillum oryzae* JA318^T
Morphology	Spiral-shaped cells	Spiral-shaped cells	Spiral-shaped cells
Cell size (μm)	1.1–1.5 × 4–7	1.0–1.3 × up to 30	1–3 × 4–16
Flagellation	Polar tufts	Bipolar tufts	Amphitrichous
Motility	+	+	+
N_2 fixation	–	–	–
PHB accumulation or other characteristics	nd	nd	nd
Detection *nifD* and/or *nifH*	+	nd	nd
Temperature for growth (°C):			
Range			25–40
Optimum	25–30	30	30
pH for growth:			
Range			6–8
Optimum	6.5–7.5	7.0	7.0
Oxidase	nd	nd	nd
Catalase	nd	nd	nd
Urease hydrolise	nd	nd	nd
Nitrate reduction	nd	nd	nd
Nitrite reduction	nd	nd	nd
NaCl concentration for growth (%)	None	None	None
Utilization of:			
Malate	+	+	+
Mannitol	+	–	+
Glicerol	+	–	–
Acetate	+	+	+
Arginine	–	–	–
Aspartate	–	–	–
Benzoate	–	–	–
Butyrate	+	+	+
Caproate	–	+	–
Citrate	–	–	–
Crotonate	–	–	+
Ethanol	+	–	+
Formate	–	–	+
Fructose	+	(+)	–
Fumarate	+	+	+
Glucose	+	(+)	(+)
Glutamate	–	(+)	(+)
Lactate	+	(+)	+
Methanol	–	–	(+)
Propionate	+	–	–
Pyruvate	+	+	+
Succinate	+	+	+
Tartrate	–	–	+

Table 22.24 (continued)

	Pararhodospirillum photometricum 132^T	*Pararhodospirillum sulfurexigens JA143*^{T}	*Pararhodospirillum oryzae* JA318^T
Valerate	+	+	+
Predominant ubiquinone	Q-8, RQ-8	Q-8, RQ-8	Q-8, RQ-8
Predominant cellular fatty acids	$C_{18:1}$, $C_{16:1}$, $C_{16:0}$	nd	$C_{16:0}$, $C_{18:1}\omega7c$
Indol production	nd	nd	nd
DNA G+C (mol%)	64.74	64.7	60.2
Genome size	3, 876, 289 bp	nd	nd
Habitat	Fresh water	Fresh water	Soil

+, positive; −, negative; (+), weak growth or microaerobic growth only; nd, not determined

Cells are vibrioid, 0.3–0.5 μm in size. They are motile by polar flagella and multiply by binary fission. They grow obligately phototrophic and strictly anaerobic. Growth is mesophilic. Bacteriochlorophyll a as well as carotenoids of rodopinal series are present in chimeric internal membranes of lamellar stacks and vesicles. The absorption maxima of living cells for *P. sulfidiphilus* type strain are 377, 488, 524, 593, 794, and 863 nm. Cells require biotin and *p*-aminobenzoic acid as growth factors; a limited number of organic substrates can be photoassimilated. Sulfide is required as sulfur source. Major fatty acids are $C_{18:1}\omega7c$ and $C_{16:0}$. The DNA G+C content of the type strain is 67.8–68.8 mol%. Phylogenetic information from 16S rRNA gene sequences differentiates *Phaeovibrio* from other related genera in the family *Rhodospirillaceae*. The type species is *Phaeovibrio sulfidiphilus* and the type strain JA480^T (= KCTC 5825^T = NBRC 106163^T = DSM 23193^T) was isolated from brackish water at Nagapattinam, India (Lakshmi et al. 2011b). The type strains and additional characteristics for this species are listed in *Table 22.27*.

Novispirillum Yoon et al. 2007b

No'vi.spi.ril'lum. L. adj. *novus*, new; N.L. dim. neut. n. *spirillum*, a small spiral; N.L. neut. n., *Novispirillum*, a new small spiral.

Cells are Gram negative and have spirillum, helical, and coccoid forms. The cell size ranges from 0.4 to 0.6 × 2.0 to 7.0 μm (diameter × length). Cells are motile by means of bipolar and fascicles flagella. Positive growth occurs on EMB, MacConkey, TSI, and Seller agars and in MR-VP broth; a predominance of coccoid bodies was observed in older cultures. Colonies are white, circular, and convex with smooth edges. The cells produce water-soluble yellowish green fluorescent or brown pigment, the last in presence of tyrosine and tryptophan. All strains are sensitive to 3.0 % NaCl, negative for hydrolysis of aesculin. They contain as predominant ubiquinone type Q-10. Cells contain esterase (C4) and esterase lipase (C8) activity and hydrolysis of tyrosine; they reduce nitrate if grown anaerobically with KNO_3. The strains grow on malate but only weakly on glycerol. DNA G+C content is 63–65 mol%. The genus *Novispirillum* comprises one species *Novispirillum itersonii* (previously *Aquaspirillum itersonii*) classified into two subspecies, subsp. *nipponicum* and subsp. *itersonii* (Yoon et al. 2007b). The type species of the genus is *Novispirillum itersonii* and the type strain is ATCC 12639 (= CCUG 49447 = CIP 105798 = JCM 21278 = JCM 21494 = LMG 4337 = NBRC 15648). The type strains and additional characteristics for this species are listed in *Table 22.28*.

Marispirillum Lai et al. 2009a

Ma.ri.spi.ril'lum. L. neut. n. *mare*, the sea; N.L. dim. neut. n. *spirillum*, a small spiral; N.L. neut. n., *Marispirillum*, a small spiral of the sea.

Cells are Gram negative, oxidase negative, catalase positive, and helical in shape. Cells are motile by means of polar flagella (three per cell) and moderately halophilic. Growth occurs at salinities of 0.5–12 % and at temperatures of 10–41 °C. The bacteria are capable of denitrification, but they are unable to degrade Tween 80 or gelatin. Major fatty acids are $C_{16:1}\omega7c$, iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$, $C_{18:0}$, and $C_{19:0}\omega8c$ cyclo. The G+C content of DNA is 67.3 mol%. The type species is *Marispirillum indicum* and the type strain is B142 (= CCTCC AB 208225 = LMG 24627 = MCCC 1A01235). The type strains and additional characteristics for this species are listed in *Table 22.29*.

Insolitispirillum Yoon et al. 2007b

In.so.li'ti.spi.ril'lum. L. adj. *insolitus*, unaccustomed; N.L. dim. neut. n. *spirillum*, a small spiral; N.L. neut. n., *Insolitispirillum*, an unaccustomed small spiral.

The cell form is helical, but in older cultures, coccoid, nonmotile organisms and microcysts predominate. The cell diameter is 0.5–0.7 μm and cell length varies from 5 to 22 μm. Cells are motile by means of bipolar fascicles of flagella that persist even in nonmotile forms. The predominant ubiquinone is Q-9. The bacteria are positive for hydrolysis of urea,

Table 22.25

Morphological, physiological and molecular characteristics differentiating species within genus *Roseospira*

	Roseospira marina CE2105	*Roseospira thiosulfatophila* AT2115	*Roseospira navarrensis* SE3104	*Roseospira mediosalina*	*Roseospira visakhapatnamensis* JA131^T	*Roseospira goensis* JA135^T
Morphology	Spiral to vibriod-shaped	Spiral to vibriod-shaped	Vibriod-rod, often in pairs	Vibriod- to spiral-shaped	Vibriod	Vibriod to crescent-shaped
Cell size (mm)	0.4–0.8 × 1.5–6.0	0.4–0.8 × 2.5–6.5	0.6–0.9 × 3.5–6.5	0.8–1.0 × 2.2–6.0	0.5–0.96 × 2–6	0.8–1.0 × 3.0–8.0
Flagellation	Bipolar tufts of flagella	Polar tufts of flagella	Bipolar tufts of flagella	Polar tufts of flagella	Non-motile	Pair of monopolar flagella
Motility	+	+	+	+	−	+
N_2 fixation	+	+	+	+	−	−
PHB accumulation or other characteristics	nd	nd	nd	nd	nd	nd
Detection *nifD* and/or *nifH*	nd	nd	nd	nd	nd	nd
Main carotenoids	Rhodovibrine, rhodopine	Rhodovibrine, spirilloxanthin	Rhodopine, lycopene	Rhodopine, lycopene	Rhodovibrine	Rhodovibrine
Photolithoautotrophic growth (electron donor)	−	(H_2S, S2O3)	(H_2S)	(H_2S)	–	–
Temperature for growth (°C):						
Range						
Optimum	30−35	30−35	30−35	30−35	30	30
pH for growth:						
Range	5.3–8.4	5.6−8.6	6.0−8.5			
Optimum	6.7–6.8	6.8–7.0	6.8–7.0	7.0	7.0	7.5
Oxidase	nd	nd	nd	nd	nd	nd
Catalase	+	+	+	+	nd	nd
Color of cell suspension	Red	Red	Brown-red	Brown-red	Red-brown	Red-brown
Urease hydrolise	nd	nd	nd	nd	nd	nd
Nitrate reduction	nd	nd	nd	nd	−	−
Nitrite reduction	nd	nd	nd	nd	−	−
NaCl concentration for growth (%)	2–4 (0.5–10)	0.5 (0.2–5)	3–4 (1–10)	4–7 (0.5–15	2 (1–5)	1–3(1–5)
Utilization of:						
Acetate	+	+	+	+	−	weak
Aspartate	+	+	+	+	+	−
Benzoate	−	−	+	−	−	−
Butyrate	+	+	+	+	−	+
Citrate	−	−	+	−	−	−
Crotonate	+	+	+	nd	−	−
N-acetyl-D-glucosamine	nd	nd	nd	nd	nd	nd
meso-inositol	nd	nd	nd	nd	nd	nd
L-rhamnose	nd	nd	nd	nd	nd	nd
meso-erythritol	nd	nd	nd	nd	nd	nd
Adonitol	nd	nd	nd	nd	nd	nd

Table 22.25 (continued)

	Roseospira marina CE2105	*Roseospira thiosulfatophila* AT2115	*Roseospira navarrensis* SE3104	*Roseospira mediosalina*	*Roseospira visakhapatnamensis* JA131^T	*Roseospira goensis* JA135^T
L-arabinose	nd	nd	nd	nd	nd	nd
D-arabinose	nd	nd	nd	nd	nd	nd
Malate	+	–	+	+	–	weak
Sucrose	–	nd	–	nd	nd	nd
D-glucose	–	+	–	–	–	weak
D-fructose	+	–	–	–	–	–
Mannitol	+	–	+	–	+	–
Glycerol	+	+	+	+	–	+
Adipate adipic acid	nd	nd	nd	nd	nd	nd
Azelaic acid	nd	nd	nd	nd	nd	nd
Sebacic acid	nd	nd	nd	nd	nd	nd
Phenol	nd	nd	nd	nd	nd	nd
4-Chlorophenol	nd	nd	nd	nd	nd	nd
Predominant ubiquinone	nd	nd	nd	nd	nd	nd
Predominant cellular fatty acids	nd	nd	nd	nd	nd	nd
Indol production	nd	nd	nd	nd	nd	nd
DNA G+C (mol%)	68.8–69.4	71.9–72.3	66.8	66.6	67	71
Genome size	nd	nd	nd	nd	nd	nd
Habitat	Anoxic sediments; water from coastal and marine environments	Microbial mats in coastal and marine environments	Anoxic sediments exposed to light in inland saline springs	Marine	Marine waters	Marine salterns
Assimilatory SO_4 reduction	+	–	–	+	–	–

utilization of aesculin, and β-glucosidase activity; they are negative for utilization of malate, nitrate reduction, and anaerobic growth with KNO_3. A yellow, water-soluble pigment is formed from phenylalanine, but no pigments are formed from tryptophan or tyrosine. Growth in the presence of 1 % bile was achieved, but not in 1 % glycine. Growth occurs on EMB, TSI, and Seller agars, but not on MacConkey agar or in MR-VP broth. Maximum growth temperature is 39 °C. The pH conducive for growth ranges from pH 5.0 to 8.0. Colonies are finely granular, round, and 2–3 mm in diameter; the color on nutrient agar and potato glucose agar differs from grayish to yellowish, respectively. The G+C content of the DNA is 62–66 mol%. The type species is *Insolitispirillum peregrinum* (Pretorius 1963) and the type strain is ATCC 15387 (= CCUG 13795 = DSM 1839 = JCM 21450 = LMG 4340 = NBRC 14922). This species comprises 2 subspecies based on slightly higher G+C values and the development of cell coccoid forms in older cultures. The subsp. *integrum* (Terasaki 1973 emended Yoon et al. 2007b), basonym *Aquaspirillum peregrinum* subsp. *integrum* (Terasaki 1973; Terasaki 1979), type strain ATCC 33334 (= CCUG 49449 = DSM 11589 = JCM 21428 = LMG 5407 = NBRC 13617), was isolated from oxidation pond water. The type strain for the subsp. *peregrinum* (Pretorius 1963 emend Yoon et al. 2007b), basonym *Aquaspirillum peregrinum* subsp. *peregrinum* (Pretorius 1963; Hylemon et al. 1973), is ATCC 15387 (= CCUG 13795 = DSM 1839 = JCM 21450 = LMG 4340 = NBRC 14922). The type strains and additional characteristics for these species are listed in *Table 22.30*.

Caenispirillum Yoon et al. 2007a

Cae.ni.spi.ril'lum. L. n. *caenum*, sludge, mud; Gr. n. *spira*, a spiral; N.L. dim. neut. n. *spirillum*, a small spiral; N.L. neut. n. *Caenispirillum*, a small spiral isolated from sludge.

Cells are Gram negative, non-spore forming, and motile by means of a single polar flagellum. The cells are helical shaped with a size of 0.5–0.7 × 0.7–7.0 µm. The utilization of various substrates, activities of various enzymes, and other physiological and biochemical properties were tested by using the API 20E, API 20NE, and API 50 CH systems (bioMérieux). In assays with

Table 22.26
Morphological, physiological and molecular characteristics differentiating species within genus *Rhodospira*

	Rhodospira trueperi 8316^T
Morphology	Vibroid to spirilloid
Cell size (μm)	0.6–0.8 × 1.5–3
Flagellation	Bipolar tufts of flagella (two to five fibrils at both ends)
Motility	+
N_2 fixation	nd
PHB accumulation or other characteristics	+
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	20–35
Optimum	25–30
pH for growth:	
Range	7–7.8
Optimum	7.3–7.5
Oxidase	nd
Catalase	nd
Urease hydrolise	nd
Nitrate reduction	nd
Nitrite reduction	nd
NaCl concentration for growth (%)	0.5–5
Utilization of:	
N-acetyl-D-glucosamine	nd
meso-inositol	nd
L-rhamnose	nd
meso-erythritol	nd
Adonitol	nd
D-arabinose	nd
Malate	+
Sucrose	nd
D-glucose	nd
D-fructose	nd
Mannitol	nd
Glicerol	nd
Adipate adipic acid	nd
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
D-galactose	nd
D-xylose	nd
Trehalose	nd
Predominant ubiquinone	Q7, MK7
Predominant cellular fatty acids	C18:1, C16:0, C14:0
Indol production	nd
carotenoids	Tetrahydrospirilloxanthin
DNA G+C (mol%)	65.7
Genome size	nd
Habitat	Salt marsh

Table 22.26 (continued)

nd, not determined

the API ZYM system, alkaline phosphatase, esterase (C4), and esterase lipase (C8) are present and naphthol-AS-BI-phosphohydrolase is weakly present. The type species K92^T is positive for catalase, oxidase, and aesculin hydrolysis and negative for Gram staining, indole production, and hydrolysis of casein and gelatin, while strain AK4^T was positive for oxidase, urease, and DNase activities but negative for gelatinase, catalase, ornithine decarboxylase, lysine decarboxylase, nitrate reduction, indole, and lipase activities. The predominant ubiquinone is Q-10. The major fatty acid is $C_{18:1}\omega7c$. The major respiratory quinone contains Q-10. Phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylcholine are the major polar lipids. The DNA G+C content is 70.0–71.0 mol%. The type species is *Caenispirillum bisanense*, type strain K92^T (= KCTC 12839^T = JCM 14346^T). The second species is *C. salinarum*, type strain AK4^T (= JCM 17360 = MTCC 10963), and was isolated from a solar saltern lake (Ritika et al. 2012). The type strains and additional characteristics for these species are listed in *Table 22.31*.

Thalassospira López-López et al. 2002, Emend. Liu et al. 2007

Tha.las'so.spi.ra. Gr. fem. n. *thalassa*, the sea; Gr. fem. n. *spira*, a spire; N.L. fem. n. *Thalassospira*, spiral-shaped organism from the sea.

Bacteria are Gram negative and vibrioid to spiral shapedwith a cell size of 3–5 μm length and 0.6 μm width. Cells are nonmotile and nonflagellated or motile by a single polar flagellum. Some species can grow under anaerobic conditions by reducing nitrate. The bacteria are halophilic, require Na^+ ions for growth, and are able to grow in the presence of up to 12 % NaCl. No requirement exists for organic growth factors. Carbohydrates are used as sole carbon sources and both nitrate and ammonium are used as sole nitrogen sources. Principal fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, and $C_{18:0}$, while $C_{16:1}\omega7c$, $C_{14:0}$, $C_{16:1}\omega7c$, $C_{14:0}$, $C_{17:0}$, $C_{17:0}$, and $C_{19:0}$ cyclo are variable among species. The G+C content of the genomic DNA ranges from 47 to 61.2 mol%. The following additional species have been described: *T. lucentensis* (López-López et al. 2002), *T. xiamenensis* (Liu et al. 2007), *T. profundimaris* (Liu et al. 2007),

Table 22.27

Morphological, physiological and molecular characteristics differentiating species within genus *Phaeovibrio*

	Phaeovibrio sulfidiphilus JA480^T
Morphology	Vibrioid
Cell size (μm)	0.3–0.5 × 1.2–2.5
Flagellation	0 to 1, polar
Motility	+
N_2 fixation	–
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	20–40
Optimum	25–30
pH for growth:	
Range	7–8
Optimum	7
Oxidase	nd
Catalase	nd
Urease hydrolise	–
Nitrate reduction	–
Nitrite reduction	–
NaCl concentration for growth (%)	0
Utilization of:	
N-acetyl-D-glucosamine	nd
meso-inositol	nd
L-rhamnose	nd
meso-erythritol	nd
Adonitol	nd
D-arabinose	nd
Malate	–
Sucrose	nd
D-glucose	–
D-fructose	–
Mannitol	–
Glicerol	–
Adipate adipic acid	nd
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
D-galactose	nd
D-xylose	nd
Trehalose	nd
Predominant ubiquinone	nd
Predominant cellular fatty acids	$C_{18:1}\omega7c$ and $C_{16:0}$
Indol production	nd

Table 22.27 (continued)

	Phaeovibrio sulfidiphilus JA480^T
carotenoids[a]	RP
DNA G+C (mol%)	67.8
Genome size	nd
Habitat	Brackish water

nd, not determined

[a]DHLYDG, Dihydroxylycopene diglucoside; HLG, hydroxylycopene glucoside; LY, lycopene; RP, rhodopin; SP, spirilloxanthin

Table 22.28

Morphological, physiological and molecular characteristics differentiating species within genus *Novispirillum*

	Novispirillum itersonii subsp. *itersonii* ATCC 12639=LMG 4337=CCUG 49447	*Novispirillum itersonii* subsp. *nipponicum* KF8=ATCC 33333=LMG 7370=CCUG 49448
Morphology	Spirilla, helical and coccoid forms	Spirilla, helical and coccoid forms
Cell size (μm)	0.4–0.6 × 2.0–7.0	0.4–0.6 × 2.0–7.0
Flagellation	Bipolar and fascicles flagella	Bipolar and fascicles flagella
Motility	+	+
PHB accumulation or other characteristics	+	+
Detection *nifD* and/or *nifH*	na	na
Temperature for growth (°C):	na	na
Range	43	12–40
Optimum	nd	35
pH for growth:		
Range	5.5–9.0	5.5–9.0
Optimum	nd	nd
Oxidase	+	+
Catalase	+	+
Urease	–	–
Nitrate reduction	+	+
Nitrite reduction	+	+
NaCl concentration for growth (%)	<2.0	<2.0
Utilization of:		
Citrate	–	–
Glycogen	–	–
D-fructose	+	+
Glycerol	–	+

Table 22.28 (continued)

	Novispirillum itersonii subsp. *itersonii* ATCC 12639=LMG 4337=CCUG 49447	*Novispirillum itersonii* subsp. *nipponicum* KF8=ATCC 33333=LMG 7370=CCUG 49448
Malate	+	+
Aesculin	–	–
Pyruvate	–	+
Succinate	+	+
Sucrose	–	–
Glucose	–	–
Aspartic acid	+	nd
Galactose	–	–
L-rhamnose	–	–
α-ketoglutarate	+	nd
Lactose	–	–
Maltose	–	–
D-sorbose	–	–
Tartrate	–	–
N-butyrate	nd	+
γ-hydroxybutyrate	nd	nd
Propionate	+	+
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1ω7c, C16:0	C18:1ω7c, C16:0/ C18:1 2-OH
Indol production	–	–
DNA G+C (mol%)	63.1	64.7
Genome size	4.29	nd
Habitat	Freshwater	Freshwater

+, positive; –, negative; nd, not determined, v, variable; na, not available
Utilization of carbon source was tested using defined basal medium (DBM) vitamine-free containing NH_4Cl as the nitrogen source (0.1%, wt/vol) or using API 20E, API 20NE and API 50CH

T. tepidiphila (Kodama et al. 2008), *T. xianhensis* (Zhao et al. 2010), *T. alkalitolerans* (Tsubouchi et al. 2014), and *T. mesophila* (Tsubouchi et al. 2014). In 2011, the species *T. permensis* was described (Plotnikova et al. 2011). It was isolated from a naphthalene-utilizing bacterial consortium obtained from primitive technogeneous soil in Russia and proposed as a new species within the genus *Thalassospira*. The type strains and additional characteristics for these species are listed in *Table 22.32*.

Magnetospira Williams et al. 2012

Mag.net.o.spi'ra. L. n. *magnes*, fr. Gr. n. Magnes [lithos], "Magnesian stone"[=magnet]; Gr. n. *spira*, the spiral; M.L. fem. n.

Table 22.29
Morphological, physiological and molecular characteristics differentiating species within genus *Marispirillum*

	Marispirillum indicum B142=CCTCC AB 208225=LMG 24627=MCCC 1A01235
Morphology	Helical
Cell size (μm)	0.6–0.7 × 1.9–2.7
Flagellation	1–3, bipolar flagella
Motility	+
N_2 fixation	nd
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	10–41
Optimum	25–37
pH for growth:	
Range	5.0–10.0
Optimum	8.0
Oxidase	–
Catalase	+
Urease	+
Nitrate reduction	+
Nitrite reduction	+
NaCl concentration for growth (%)	0.5–12
Utilization of:	
Citrate	–
Glycogen	+
D-fructose	–
Glycerol	–
Malate	+
Aesculin	na
Pyruvate	+
Succinate	+
Sucrose	–
Glucose	–
Aspartic acid	–
Galactose	–
L-rhamnose	–
α-ketoglutarate	–
Lactose	–
Maltose	–
D-sorbose	nd
Tartrate	nd
N-butyrate	nd
Hydroxybutyrate (α, β and γ)	+
Propionate	+
Predominant ubiquinone	nd

Table 22.29 (continued)

	Marispirillum indicum B142=CCTCC AB 208225=LMG 24627=MCCC 1A01235
Predominant cellular fatty acids	$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH
Pigment production	nd
Indol production	+
DNA G+C (mol%)	67.3
Genome size	nd
Habitat	Deep water, Marine

+, positive; −, negative; nd, Not determined, v, variable; na, not available
Carbon source test were performed using API 20NE and Biolog GN2 system

Table 22.30
Morphological, physiological and molecular characteristics differentiating species within genus *Insolitispirillum*

	Insolitispirillum peregrinum subsp. *peregrinum* ATCC 15387=LMG 4340=CCUG 13795=DSM 1839	*Insolitispirillum peregrinum* subsp. *Integrum* MF19 = ATCC 33334=LMG 5407=CCUG 49449=DSM 11589
Morphology	Spirilla	Spirilla
Cell size (μm)	0.7–8.5 × 6.0–3.7	0.7–8.5 × 6.0–3.7
Flagellation	single polar flagellum	single polar flagellum
Motility	+	+
N_2 fixation	na	na
PHB accumulation or other characteristics	+	+
Detection *nifD* and/or *nifH*	na	na
Temperature for growth (°C):		
Range	12–39	12–39
Optimum	32	32
pH for growth:		
Range	5.0–8.0	5.0–8.0
Optimum	nd	nd
Oxidase	+	+
Catalase	+	+
Urease	+	+
Nitrate reduction	−	−
Nitrite reduction	−	−

Table 22.30 (continued)

	Insolitispirillum peregrinum subsp. *peregrinum* ATCC 15387=LMG 4340=CCUG 13795=DSM 1839	*Insolitispirillum peregrinum* subsp. *Integrum* MF19 = ATCC 33334=LMG 5407=CCUG 49449=DSM 11589
NaCl concentration for growth (%)	<2.0	<1.5
Utilization of:		
Citrate	−	−
Glycogen	−	−
D-fructose	+	+
Glycerol	−	−
Malate	−	−
Aesculin	+	+
Pyruvate	+	+
Succinate	+	+
Sucrose	−	−
Glucose	−	−
Aspartic acid	nd	nd
Galactose	−	−
L-rhamnose	−	−
α-ketoglutarate	nd	nd
Lactose	−	−
Maltose	−	−
D-sorbose	−	−
Tartrate	−	−
N-butyrate	+	+
γ-hydroxybutyrate	nd	nd
Propionate	+	+
Predominant ubiquinone	Q-9	Q-9
Predominant cellular fatty acids	C18:1ω7c, C16:0/ C18:1 2-OH	C18:1ω7c, C16:0
Pigment production	Yellow in presence of phenilalanine	Brown in presence of tyrosine and tryptophan
Indol production	−	−
DNA G+C (mol%)	62.4–65.6	62.3–63.7
Genome size	nd	nd
Habitat	Oxidation ponds	Freshwater

+, positive; −, negative; nd, not determined, v, variable; na, not available

Magnetospira, the magnetic spiral, which references the spiral morphology and magnetotactic behavior of this bacterium.

Cells are Gram negative and present variable morphology, ranging from truncated spirillum (lima bean shaped) to fully

Table 22.31
Morphological, physiological and molecular characteristics differentiating species within genus *Caenispirillum*

	Caenispirillum bisanense KCTC 12839^T	*Caenispirillum salinarum* AK4^T
Morphology	Helical	Vibrio
Cell size (μm)	0.5–0.7 × 0.7–7.0	0.8–1.2 × 4.0–6.0
Flagellation	1 polar flagellum	Single monopolar
Motility	+	+
N_2 fixation	–	–
PHB accumulation or other characteristics	nd	nd
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	15–47	15–45
Optimum	37	30–37
pH for growth:	nd	nd
Range	6.5–10.0	6-Oct
Optimum	7.0–8.0	7.5–8.5
Oxidase	+	+
Catalase	+	–
Urease hydrolise	–	+
Nitrate reduction	–	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	0.5	2.0–4.0
Utilization of (less than 20):		
4-Chlorophenol	–	nd
Adipate adipic acid	–	nd
Adonitol	–	–
Azelaic acid	–	nd
Citrate	+	+
D-arabinose	–	nd
D-fructose	–	nd
D-glucose	+	nd
Glycerol	–	nd
L-rhamnose	–	nd
Malate	–	–
Mannitol	–	nd
meso-erythritol	+	nd
meso-inositol	+	nd
N-acetyl-D-glucosamine	–	nd
Phenol	–	nd
Sebacic acid	–	nd
Sucrose	–	–
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1v7c	C18:1ω7c and/or C18:103C96c
Indol production	nd	nd
DNA G+C (mol%)	70.0	71
Genome size	nd	nd
Habitat	Sludge	Solar saltern
Biochemical characteristics kit	nd	VITEK 2 GN

+, positive; –, negative; nd, not determined, v, variable; na, not available

helical forms (Meldrum et al. 1993). Cells assimilate inorganic carbon (as CO_2) and grow chemolithoautotrophically with S_2O_3- as the electron donor, using the CBB cycle. The cells harbor form II RuBisCO (CbbM). They are motile by means of bipolar flagella (amphitrichous), with a single flagellum at each pole. *Magnetospira* cells exhibit only polar magnetotaxis and biomineralize a single chain of magnetosomes that contain elongated cuboctahedral magnetite crystals positioned along the long axis of the cell. The G+C content of the DNA was 47.2 mol%. The type species for genus *Magnetospira* is *M. thiophila*, type strain MMS-1 (= ATCC BAA-1438 = JCM 17960). The type strains and additional characteristics for this species are listed in *Table 22.33*.

Magnetovibrio Bazylinski et al. 2013

Ma.gne.to.vi'bri.o. Gr. n. *magnês -êtos*, a magnet; N.L. pref. magneto-, pertaining to a magnet; N.L. masc. n. *vibrio*, a vibrio; N.L. masc. n. *Magnetovibrio*, the magnetic vibrio, which references the vibrioid morphology and magnetotactic behavior of this bacterium.

Cells are Gram negative and vibrioid to helicoid in morphology; they are motile by means of a single polar flagellum. Cells assimilate inorganic carbon (as CO_2) and grow chemolithoautotrophically with thiosulfate and sulfide as the electron donors, using a form II ribulose-1,5-bisphosphate carboxylase/oxygenase (CbbM) and the CBB cycle. Cells of strain MV-1^T exhibit characteristics of both axial and polar magnetotaxis and biomineralize a single chain of magnetosomes that contain magnetite crystals of truncated hexa-octahedral habit, positioned along the long axis of the cell. Major polar lipids identified include phosphatidylethanolamine and phosphatidylglycerol. The G+C content of the DNA is 52.9–53.5 mol%. The type species is *M. blakemorei*, strain MV-1^T. The type strains and additional characteristics for this species are listed in *Table 22.34*.

Table 22.32

Morphological, physiological and molecular characteristics differentiating species within genus Thalassospira

	Thalassospira lucentensis DSM 14000^T	*Thalassospira xiamenensis* DSM 17429^T	*Thalassospira profundimaris* DSM 17530^T	*Thalassospira tepidiphila* DSM 18888^T	*Thalassospira xianhensis* JCM 14850^T	*Thalassospira permensis* NBRC 106175^T	*Thalassospira alkalitolerans* JCM 18968^T	*Thalassospira mesophila* JCM 18969^T
Morphology	Curved rods; spirilla	Curved rods	Curved rods	Vibrioid, spirilla	Curved rods, spirilla	Curved rods	Spirilla	Curved rods
Cell size (μm)	3.0–5.0	0.8–2.3	0.8–2.3	0.9–2.0	1.0–1.6	1.23–1.98	1.3–3.0	1.5–2.9
Flagellation	Single, monopolar	Single, monopolar	–	Single, monopolar	–	Single, monopolar	Single, monopolar	Single, monopolar
Motility	+	+	–	+	–	+	+	+
N_2 fixation	nd	nd	nd	nd	nd	nd	nd	nd
PHB accumulation or other characteristics	nd	nd	nd	nd	nd	nd	nd	nd
Detection *nifD* and/or *nifH*	nd	nd	nd	nd	nd	nd	nd	nd
Temperature for growth (°C):								
Range	4.0–40.0	4.0–40.0	10.0–37.0	8.0–45.0	10.0–42.0	10.0–40.0	4.0–38.0	4.0–36.0
Optimum	20	22	22	43	30	28–30	32	30
pH for growth:								
Range	nd	nd	nd	5.0–10.0	5.5–9.0	nd	4.5–11	4.0–10.5
Optimum	7.2	nd	nd	7	7.2	nd	7.0–8.0	7.0–8.0
Oxidase	+	+	+	+	+	+	–	–
Catalase	+	+	+	+	+	+	–	+
Urease hydrolise	nd	nd	nd	nd	+	–	nd	nd
Nitrate reduction	–	+	+	+	+	+	–	–
Nitrite reduction	–	+	+	–	+	+	–	–
NaCl concentration for growth (%)	2	2.0–4.0	3.0–4.0	3	3.0–6.0	2.0–5.0	2	4
Utilization of:								
N-acetyl-D-glucosamine	+	+	–	–	+	+	–	w
meso-inositol	+	+	–	+	+	+	–	w
L-rhamnose	+	–	–	–	–	–	+	+
meso-erythritol	nd	nd	–	–	+	–	nd	nd
Adonitol	nd	nd	–	–	+	+	nd	nd
D-arabinose	+	+	+	+	+	+	–	–

Table 22.32 (continued)

	Thalassospira lucentensis DSM 14000^T	*Thalassospira xiamenensis* DSM 17429^T	*Thalassospira profundimaris* DSM 17530^T	*Thalassospira tepidiphila* DSM 18888^T	*Thalassospira xianhensis* JCM 14850^T	*Thalassospira permensis* NBRC 106175^T	*Thalassospira alkalitolerans* JCM 18968^T	*Thalassospira mesophila* JCM 18969^T
Malate	+	nd	nd	−	nd	nd	nd	nd
Sucrose	−	+	−	−	+	+	−	+
D-glucose	+	+	+	+	+	+	−	+
D-fructose	+	+	+	+	+	+	−	+
Mannitol	w	+	+	+	+	+	−	+
Glicerol	+	nd	+	+	+	−	nd	nd
Predominant ubiquinone	Q-10	nd	nd	nd	Q-9	Q-10	Q-10	Q-10
Predominant cellular fatty acids	C18:1 w7c; C16:0; C16:1 w7c	C18:1w7c; C16:0; C18:0; C16:1w7c; C14:0	C18:1w7c; C16:0; C18:0; C19:0	C18:1w7c; C16:0; C17:0	C18:1w7c:C16:0; C16:1w7c; C14:0	C18:1w7c; C16:0; C18:0	C18:1 ω7c; C16:0	C10:0; C17:0 cyclo: C18:1 ω7c; C19:0 cyclo ω8c; C10:0
Indol production	nd	nd	nd	nd	nd	nd	−	−
DNA G+C (mol%)	54.7	52.6	47.0	55.1	61.2	53.7	54.4	55.9
Genome size	nd	nd	nd	nd	nd	nd	nd	nd
Habitat	Water	Water	Water	Water	Saline soil	Soil	Plant	Plant

+, positive; −, negative; nd, not determined; w, weak

Table 22.33
Morphological, physiological and molecular characteristics differentiating species within genus *Magnetospira*

	Magnetospira thiophila MMS-1 = ATCC BAA-1438 = JCM 17960
Morphology	Spirillum or lima bean-shaped
	Mostly singly, but also in pairs, chains, and clumps
Cell size (μm)	0.2–0.5 × 1.0–3.0
magnetosome number and structure	Cells produce internal sulfur globules when grown on $S_2O_3^{2-}$
Flagellation	Bipolar flagella (amphitrichous)
Motility	+
N_2 fixation	+
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	5–37
Optimum	25
pH for growth:	
Range	6.9–7.1
Optimum	nd
Oxidase	–
Catalase	–
Urease hydrolise	nd
Nitrate reduction	–
Nitrite reduction	–
NaCl concentration for growth (%)	nd
Utilization of:	
Lactate	–
Acetate	+
Fumarate	+
Malate	+
Peptone	–
Pyruvate	+
Succinate	+
Sucrose	–
Aspartic acid	–
Galactose	–
α-ketoglutarate	–
Lactose	–
Maltose	–
D-sorbose	–
Tartrate	–
N-butyrate	nd
β-hydroxybutyrate	nd
Propionate	–
Predominant ubiquinone	nd
Predominant cellular fatty acids	$C_{16:1}\omega7c$, $C_{16:1}\omega7t$, $C_{16:0}$, $C_{20:0}$
Pigment production	na
Indol production	na
DNA G+C (mol%)	47.2
Genome size (Mb)	nd
Habitat	Marine

+, positive; –, negative; nd, not determined, v, variable; na, not available
Artificial seawater semi-solid agar medium was used for Carbon sources test (final concentration of 0.1%–wt/vol or vol/vol)

Ferrovibrio Sorokina et al. 2012

Fer.ro.vi'bri.o L. n. *ferrum*, iron; L. v. *vibrio*, move to and fro; N. L. masc. n. *vibrio*, which vibrates; N. L. masc. n. *Ferrovibrio*, an iron-oxidizing organism of vibrioid shape.

The cells are vibrioid and motile with one polar flagellum and 0.3 × 0.8–1.3 μm size. Division occurs by binary fission. The cell wall is of Gram-negative type. The cells have a facultative anaerobic metabolism. Growth occurs within the ranges of 5–45 °C and pH 5.5–8.0. Oxidase activity and low catalase activity are present. Organotrophic, mixotrophic, or lithoheterotrophic growth is possible owing to oxidation of Fe(II) coupled to reduction of NO_3^- or N_2O, with accumulation of Fe(III) oxides on the cell surface. Fe(II) may be used as an electron donor for anaerobic mixotrophic or lithoheterotrophic growth. Aerobic organotrophic growth occurs with acetate, butyrate, citrate, fumarate, glycerol, lactate, malate, propanol, propionate, pyruvate, succinate, peptone, and yeast extract as carbon and energy sources. Weak growth occurs on amino acids alanine, histidine, aspartate, and glutamate. Sugars, asparagine, benzoate, butanol, ethanol, formate, glutamine, leucine, oxalate, phenylalanine, proline, tryptophan, and casein hydrolysate are not utilized. Ammonium salts, NO_3^-, N_2O, urea, yeast extract, and peptone may be used as nitrogen sources. NO_2, histidine, aspartate, and casein hydrolysate are not used. Anaerobic growth does not occur with ClO_4^-, SO_4^{2-}, $S_2O_3^{2-}$, or $Fe(OH)_3$ as electron acceptor. In mineral medium with nitrate, H_2 is not used as an electron donor. The DNA G+C content is 64.2 mol%. The type species is *F. denitrificans* and the type strain is Sp-1^T (= LMG 25817 ^{T} = VKMB-2673 ^{T}) – isolated from a moderately thermal, iron-sulfide mineral spring of the Psekups mineral water deposit (Northern Caucasus, Russia). The type strains and additional characteristics for this species are listed in *Table 22.35*.

Table 22.34
Morphological, physiological and molecular characteristics differentiating species within genus *Magnetovibrio*

	Magnetovibrio blakemorei MV-1 = ATCC BAA-1436 = DSM 18854
Morphology	Vibroid to helical
Cell size (μm)	0.2–0.4 × 1.0–3.0
magnetosome number and structure	Single chain of magnetosomes that contain magnetite crystals of truncated hexa-octahedral habit. Produce internal sulfur globules when grown on $S_2O_3^{2-}$
Flagellation	Single polar flagellum
Motility	+
N_2 fixation	+
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	4–31
Optimum	27
pH for growth:	
Range	7.0–7.5
Optimum	7.0
Oxidase	+
Catalase	−
Urease hydrolise	nd
Nitrate reduction	+
Nitrite reduction	+
NaCl concentration for growth (%)	nd
Utilization of:	
Lactate	+
Acetate	+
Fumarate	+
Malate	+
Peptone	+
Pyruvate	+
Succinate	+
Sucrose	−
Aspartic acid	+
Galactose	−
α-ketoglutarate	+
Lactose	−
Maltose	−
D-sorbose	−
Tartrate	−
N-butyrate	−
β-hydroxybutyrate	nd
Propionate	+
Predominant ubiquinone	nd
Predominant cellular fatty acids	$C_{18:1}\omega7$, $C_{16:1}\omega7$, $C_{16:0}$, and $C_{16:1}\omega5$
Pigment production	na
Indol production	na
DNA G+C (mol%)	52.9–53.5
Genome size (Mb)	3.7
Habitat	Marine

+, positive; −, negative; nd, not determined, v, variable; na, not available
Carbon sources at a concentration of 0.1% (w/v) were tested for microaerobic growth in semi-solid media using N_2O as the terminal electron acceptor

Isolation, Enrichment, and Maintenance Procedures

Azospirillum

The isolation of *A. lipoferum* and *A. brasilense* is based on the use of N-free semisolid medium, containing agar (1.75 g L^{-1}). The recipe contains (g L^{-1}) the following: malic acid, 5.0; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $CaCl_2.2H_2O$, 0.02; micronutrient solution A, 2 mL; bromothymol blue (0.5 % in 0.2 N KOH), 2 mL; Fe-EDTA (solution 1.64 %), 4 mL; vitamin solution B, 1 mL; and KOH, 4.5 g; complete volume to 1,000 mL and adjust pH to 6.5–6.8. To semisolid medium, add 1.75–1.80 g agar L^{-1}. To solid medium, add 15 g agar L^{-1}. Micronutrient solution (g L^{-1}): $CuSO_4.5H_2O$, 0.04; $ZnSO_4.7H_2O$, 0.12; H_3BO_3, 1.40; $Na_2MoO_4.2H_2O$, 1.0; $MnSO_4.H_2O$, 1.175. Complete volume to 1,000 mL with distilled water. Store the solution in the refrigerator. Vitamin solution: biotin, 10 mg; pyridoxal HCl, 20 mg. Dissolve in hot-water bath. Complete to 100 mL adding distilled water. Store the solution in refrigerator.

These microaerobically nitrogen-fixing (diazotrophic) bacteria are selectively enriched because they can grow with N_2 as nitrogen source. Because azospirilla do not harbor powerful oxygen-protective mechanisms for the oxygen-sensitive nitrogen-fixing system, they are unable to grow on N_2 as sole N-source in N-free agar plates or liquid media because of too high oxygen levels in air. Microaerobic diazotrophs are aerotactic, and as a result, the nitrogen-fixing population collects in zones of reduced oxygen concentration. There, they form a thin pellicle or veil that moves upward as it becomes thicker (Döbereiner and Pedrosa 1987). After characteristic pellicles have formed, N_2 fixation can be checked by acetylene reduction activity, and active cultures are transferred to new vials

Table 22.35
Morphological, physiological and molecular characteristicas differentiating species within genus *Ferrovibrio*

	Ferrovibrio denitrificans LMG 25817^T
Morphology	Vibrioid
Cell size (μm)	0.3 × 0.8–1.3
Flagellation	Single polar
Motility	+
N_2 fixation	nd
PHB accumulation or other characteristics	Accumulation of Fe(III) oxides on the cell surface.
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	5.0–45.0
Optimum	35.0
pH for growth:	
Range	5.5–8
Optimum	6.2
Oxidase	+
Catalase	+
Urease hydrolise	nd
Nitrate reduction	+
Nitrite reduction	–
NaCl concentration for growth (%)	0–2.5
Utilization of (less than 20):	
4-Chlorophenol	nd
Adipate adipic acid	nd
Adonitol	–
Azelaic acid	nd
Citrate	+
D-arabinose	nd
D-fructose	nd
D-glucose	nd
Glycerol	+
L-rhamnose	–
Malate	nd
Mannitol	nd
meso-erythritol	nd
meso-inositol	nd
N-acetyl-D-glucosamine	nd
Phenol	nd
Sebacic acid	nd
Sucrose	nd
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	18:1w7c, 19:0 cyc, 16:0
Indol production	nd
DNA G+C (mol%)	64.2
Genome size	nd
Habitat	Iron–sulfide mineral spring
Biochemical characteristics kit	na

+, positive; –, negative; nd, Not determined, v, variable; na, not available

containing the same medium. As soon as a new pellicle is visible, the cultures are streaked out on agar plates containing the same medium with yeast extract (20 mg L^{-1}) added. The small amount of yeast extract permits the growth of small colonies on the surface of plates. Characteristic individual colonies are then transferred again to N-free semisolid media, and those that grow well are streaked out on potato agar for final purification.

The species *A. irakense* (Khammas et al. 1989) can also be isolated using the semisolid NFb medium containing up to 0.3 % NaCl, pH adjusted to 7.0–8.5 and incubation at 33 °C. Similarly, *A. doebereinerae* (Eckert et al. 2001) can be isolated using the NFb semisolid medium after incubation for 3–5 days at 30 °C. Further purification is done on NFb (supplemented with 50 mg yeast extract L^{-1}).

The species *A. oryzae* can be isolated using the M (malate) medium with the following composition in g L^{-1}: sodium malate, 5.0; $CaCl_2.2H_2O$, 0.02; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 0.1; KH_2PO_4, 0.4; NaCl, 0.1; $FeCl_3$,0.010, $Na_2MoO_4.2H_2O$, 0.002; yeast extract, 0.1; and biotin, 2 μg. Complete to 1,000 mL with distilled water and adjust the pH to 6.8. The NFG medium can also be used with the composition (g L^{-1}): glucose, 10.0; $CaCl_2.2H_2O$, 0.020; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 1.0; $CaCO_3$, 5.0; $FeSO_4.7H_2O$, 0.050; and $Na_2MoO_4.2H_2O$, 0.001. Complete to 1,000 mL with distilled water and adjust the pH to 7.3. Similarly, *A. zeae* and *A. canadense* can be isolated using the M medium by omitting the addition of biotin, and the pH of the medium is adjusted to 7.2–7.4. (Xie and Yokota 2005; Mehnaz et al. 2007a, b). Sub-cultivation is done on the same medium at 30 °C for 48–72 h.

The species *A. amazonense* (Magalhães et al. 1983) is isolated in a semisolid sucrose medium (LGI or Fam). Composition of LGI medium (g L^{-1}): sucrose, 5.0; K_2HPO_4, 0.2; KH_2PO_4, 0.6; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.02; $Na_2MoO_4.2H_2O$, 0.002; bromothymol blue (0.5 % in 0.2 N KOH), 5 mL; Fe-EDTA (solution 1.64 %), 4 mL; vitamin solution (see above), 1 mL. Complete volume to 1,000 mL with distilled water. Adjust pH to 6.0–6.2 with H_2SO_4. For semisolid medium, add 1.75–1.80 agar L^{-1} and 15 g agar L^{-1} for solid medium. FAM medium has the composition (g L^{-1}): sucrose, 5.0; KH_2PO_4, 0.12; K_2HPO_4, 0.03; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.02; Fe-EDTA, 0.066; NaCl, 0.1; $Na_2MoO_4.2H_2O$, 0.002; $MnSO_4$, 0.00235; H_3BO_3, 0.0028; $CuSO_4.5H_2O$, 0.00008; $ZnSO_4.7H_2O$, 0.00024; biotin, 0.0001; and pyridoxine-HCl, 0.0002 g. Complete volume to 1,000 mL with distilled water. For semisolid medium, add 1.75 g agar L^{-1} and adjust pH to 6.0.

The species *A. melinis* (Peng et al. 2006) can also be isolated in a semisolid LGI medium or a semisolid NFB medium after incubation at 28 °C for 3–5 days. Purification can be done by repeatedly streaking the isolates on plates of solid LGI or NFb medium.

The species *A. halopraeferans* (Reinhold et al. 1987) can be isolated in the semisolid SM medium supplemented with 1.5 % NaCl, pH adjusted to 8.5 and vials incubated at 41oC. Composition (g L^{-1}): DL-malic acid, 5.0; KOH, 4.8; NaCl, 1.2; $NaSO_4$, 2.4; $NaHCO_3$, 0.5; $CaCl_2$, 0.22; $MgSO_4.7H_2O$, 0.25; K_2SO_4, 0.17;

Na_2CO_3, 0.09; Fe-EDTA, 0.077; K_2HPO_4, 0.13; biotin, 0.0001; $MnCl_2.4H_2O$, 0.0002; H_3BO_3, 0.0002; $ZnCl_2$, 0.00015; $CuCl_2.2H_2O$, 0.000002; $Na_2MoO_4.2H_2O$, 0.002; distilled water, completed to 1,000 mL. The final pH of the medium is 8.5. Cells may grow to 1.2 µm length and 0.7–1.4 µm thick if the pH turns alkaline.

The non-nitrogen-fixing *A. palatum* (Zhou et al. 2009) can be isolated using a TYB medium containing 0.3 % yeast extract, 0.2 % beef extract, 0.6 % tryptone, 0.3 % NaCl, and 001 % $FeCl_3$, pH 7.0.

For rapid multiplication, many *Azospirillum* species can be grown in liquid media to which a combined nitrogen source has been added (NH_4Cl (1 g/l), KNO_3 (1 g/l), or yeast extract (0.4 g L^{-1})). Alternatively, complex media such as nutrient broth (NB) or 1/2 DYGS medium (D,L-malate (1 g L^{-1}), yeast extract (2 g L^{-1}), glucose (1 g L^{-1}), glutamate (1.5 g L^{-1}), peptone (1.5 g L^{-1}), $MgSO_4.7H_2O$ (0.5 g L^{-1})) can be applied (Rodrigues Neto et al. 1986). In such media, with rapid stirring or shaking, cell concentrations of 10^8 per ml are reached after 24–48 h. To stabilize the pH at the desired value upon prolonged growth, the addition of 50 mM MOPS (3-(N-morpholino)propanesulfonic acid) buffer (pH 6.8) or MES (2-(N-morpholino)ethanesulfonic acid) buffer (pH 6.0; for *A. amazonense*) is recommended. Alternatively, the *Azospirillum* minimal medium of Okon et al. (1977), which also contains high phosphate levels, can be used.

Storage of the cultures for many years at −80 °C or in liquid N_2 is also possible after adding 50 % glycerin or dimethyl sulfoxide (DMSO) to an exponentially growing culture. The cells can also be preserved by lyophilization according to the following protocol (Döbereiner et al. 1995). The cultures are grown to late log phase in the following medium: K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; K-DL-malate (*A. brasilense*) or glucose (*A. lipoferum*), 5 g; yeast extract, 0.4 g; and 1 l of distilled water. The cells must then be collected by centrifugation and resuspended to a dense cell suspension with 10 % sucrose solution containing 5 % peptone. Then 0.1 ml portions are transferred into lyophilization ampoules, which are frozen and lyophilized according to the procedures recommended for *Rhizobium* spp. (Vincent 1970).

Caenispirillum, *Conglomeromonas*, *Constrictibacter*, *Defluviicoccus*, *Desertibacter*, *Dongia*, *Elstera*, *Ferrovibrio*, *Fodinicurvata*, and *Inquilinus*

Caenispirillum bisanense (Yoon et al. 2007a) was isolated from a sludge sample collected from the wastewater treatment plant of a dye works at Daegu, Korea. The type strains K92 and K93 were isolated on nutrient agar (Difco) and trypticase soy agar (TSA; Difco) at 30 °C, using standard dilution plating technique. Colonies on TSA are circular, raised, smooth, glistening, grayish yellow in color, and 1.5–2.5 mm in diameter after incubation for 2 days at 37 °C. *Caenispirillum salinarum* AK4T was isolated from a solar saltern at Kakinada, Andhra Pradesh, India (Ritika et al. 2012) using the same medium.

Constrictibacter antarcticus (Yamada et al. 2011), strain 262-8^T, was obtained from the white rock sample from the Skallen region in Antarctica. The white rock collected by the summer party of the 46th Japanese Antarctic Research Expedition in 2004–2005 was stored at 4 °C for 6 months. To screen for autotrophic bacteria, rock samples were crushed, added to BG-11 liquid medium (ATCC medium 616), and incubated at 25 °C in the light. Strain 262-8^T was able to grow in 0.25 × LB/MA, 0.25 × LB/ASW and marine broth 2216. After growth on 0.25 × LB/MA medium for 2 weeks, colonies were white and circular, with a diameter of 0.2 mm. Strain 262-8^T was able to form colonies microaerobically, but anaerobic growth could not be observed after 2 weeks in either light or dark conditions. No growth was observed in synthetic media. The type strain could be stored as a 20 % (v/v) glycerol suspension at −80 °C for at least 2 years.

Defluvicoccus vanus (Maszenan et al. 2005), a tetrad-forming organism, was isolated by micromanipulation from a sample of activated sludge biomass from an enhanced biological phosphorus removal (EBPR) plant in Pilsen, Czech Republic, in 1997 (Maszenan et al. 1997). For growth, freshly prepared GS medium (Williams and Unz 1985) was the most successful in supporting growth of this organism from activated sludge. Strain Ben 114^T grew very slowly on GS agar, taking 2–3 weeks to produce visible mucoid beige colonies of < 5 mm diameter. In GS broth dispersed growth was seen. Strain Ben 114^T was stored at −80 °C for 8 years.

Desertibacter roseus (Liu et al. 2011), gamma radiation-resistant bacterium, was isolated from the Taklimakan desert, Xinjiang, China. Sand was sampled from the Taklimakan desert, and 1-g samples were exposed to 10-kGy radiation at a dose of 300 Gy min at room temperature. After exposure, the samples were serially diluted in water (0.85 %, w/v, NaCl) and plated on different media TSA (Difco), nutrient agar (Difco), and R_2A agar (Difco). After incubation at 30 °C for 20 days, the type strain 2622^T was isolated on R_2A agar. Colonies were pink, circular, and convex with regular margins after growth on R_2A agar at 37 °C for 4 days at pH 8. Strain 2622^T was stored by lyophilization.

Dongia mobilis, strain LM22^T, was isolated during an investigation of the culturable microbial diversity in the activated sludge of a sequencing batch reactor for the treatment of malachite green effluent. A sludge sample was suspended in normal saline by vigorous vortexing, and 0.1 ml suspension was spread onto 1/10-diluted trypticase soy agar (TSA; Difco) and incubated at 30 °C for 1 week. A pure culture of strain LM22T was obtained after subcultivation on YP agar (Difco). Colonies on YP agar are white, transparent, smooth, circular, convex, and 0.5–1 mm in diameter after incubation at 30 °C for 3 days. Abundant growth was observed on R2A. No growth was seen on LB agar, NA, or TSA (Liu et al. 2010). Strain LM22^T was maintained on YP agar and stored in 15 % (w/v) glycerol at −80 °C.

Elstera litoralis (Rahalkar et al. 2012), a biofilm-associated bacterium, was isolated from the stones of the littoral zone (20–30-cm water depth) of Lake Constance, Germany, on 2006. The biofilm material was diluted and vortexed vigorously to disperse the bacteria. Strain Dia-1^T was isolated from the final plated dilution of the biofilm sample where EPS was used as the carbon source. It also grew well in 1:2-diluted nutrient broth supplemented with 10 mM glucose and in VM medium (pH 6.5–7.0) with ethanol as sole carbon source at room temperature (20–23 °C), both in liquid medium and on solid medium plates. In liquid VM media without shaking, the strain initially formed small aggregates or white flocks. On agar plates, milky white to cream-colored colonies were formed within 3–4 days, which turned light yellow at the periphery after extended incubations. Strain Dia-1^T grew well in VM-ethanol medium and did not grow in nutrient broth only, i.e., a sugar or ethanol was required for growth.

Ferrovibrio denitrificans (Sorokina et al. 2012) was isolated from freshly precipitated sediments from the redox zone at the FeS–Fe(OH)3 boundary in the bottom sediments of the Marka low-salinity iron-rich spring at its confluence with a sulfide spring located at the groundwater discharge zone of the Psekups mineral water deposit, Northern Caucasus (Krasnodar Krai, Russia). The cultivation medium was described by Sorokina et al. (2012) and contained 0.2 mL of a freshly prepared FeS suspension (Hanert 1981) that was added to each tube per 10 mL of the medium. The incubation time was 2–3 weeks. In agar medium, the bacteria formed small (2–3 mm in diameter), loose spherical colonies. The colonies are orange colored because of the presence of iron oxides. In liquid medium of the same composition, an ochreous precipitate is formed at the bottom of the vials. FeS, $FeSO_4$, and $FeCO_3$ are used as Fe(II) sources for lithotrophic growth.

Fodinicurvata sediminis (Wang et al. 2009) was isolated during the course of a study of the microbial diversity of the Fenggang salt mine in Yunnan, southwest China. The type strains YIM D82^T and YIM D812^T were isolated from a sediment sample collected from the salt mine by using a standard dilution-plating technique at 28 °C on Difco marine agar 2216 (MA; pH 7.2), supplemented with 3 % (w/v) NaCl. Pure cultures are maintained on nutrient agar (NA; Difco) supplemented with 5 % NaCl. Colonies are cream–white, circular, convex, and opaque with irregular margins after growth on NA supplemented with 5 % at 28 °C for 5 days. Growth occurred under anaerobic conditions. Pure cultures are maintained on nutrient agar (NA; Difco) supplemented with 5 % NaCl and stored as 20 % (v/v) glycerol suspensions at −80 °C.

Inquilinus limosus was isolated from respiratory secretions of cystic fibrosis patients in the USA in 1995 (Pitulle et al. 1999) and described by Coenye et al. (2002). The type strain AU0476^T grows on BCSA at 32 °C. During the course of a study on the culturable aerobic and facultatively anaerobic bacterial community of ginseng field soil in Pocheon Province, South Korea, a large number of bacteria were isolated (Im et al. 2005). One of these isolates, Gsoil 080^T, was identified as *I. limosus*. It was one of the several isolates that appeared on modified R_2A agar plates under aerobic conditions and was routinely cultured on R_2A agar (Difco) at 30 °C. After 2 days of incubation on R_2A agar, colonies are creamy white, round to slightly irregular, and 1.0–5.0 mm in diameter. It was routinely cultured on R_2A agar (Difco) at 30 °C and maintained as a glycerol suspension (20 %, w/v) at −70 °C.

Insolitispirillum–Limimonas–Magnetospira–Magnetospirillum–Magnetovibrio–Marispirillum–Nisaea–Novispirillum–Oceanibaculum

Magnetospirillum gryphiswaldense was isolated from water and the muddy upper layers of sediment collected from the eutrophic river Ryck near Greifswald, Germany, after magnetotactic enrichment collected from jars. Jars were filled with 100 ml mud and 200 ml water. Magnetotactic bacteria found near the magnetic pole were collected with a pipette over several weeks. After centrifugation, a drop of the cell concentrate was placed on one edge of a 5-cm-long and 1-mm-wide strip of sterile soft agar (2 g agar/I tap water) processed as describe by Scheifer et al. (1991) and used as inocula for the isolation medium consisting of 50 ml mud, 100 ml water, and 1.5 mg disodium succinate. It was filled into 200-ml bottles and sterilized at 121 °C for 20 min. The bottles were tightly sealed by rubber stoppers. The inoculated medium was incubated at 30 °C for 10 days.

Magnetospirillum magnetotacticum strain MS-1 was isolated from sediments collected in Cedar Swamp (Woods Hole, MA, USA) after enrichment of sampled material by application of steady, nonuniform magnetic fields as described by (Blakemore et al. 1979). The semisolid isolation medium consisted of (per 90 ml of distilled water) 10 ml of filtered swamp or bog water, 1 ml of vitamin elixir (23), 1 ml of mineral elixir (Wolin et al. 1963), and 0.5 mM potassium phosphate buffer (pH 6.7). To this mixture were added 5 g of vitamin B12, 25 mg of NH_4Cl, 10 mg of sodium acetate (anhydrous), 0.2 mg of resazurin, and 90 mg of Ionagar no. 2 (Oxoid). The pH was adjusted to 6.7 with NaOH. A well-isolated area of growth was homogenized, and cells were cloned by serial dilution into tubes containing molten, prereduced isolation medium containing 0.85 % (wt/vol) Ionagar no. 2. Well-isolated colonies which appeared in these tubes after 1 week at 30 °C were homogeneous as evidenced by microscopy. Strain MS-1 was maintained at 30 °C with weekly transfers in screw-capped culture tubes containing a semisolid growth medium consisting of (per 98 ml of distilled water) 1 ml of vitamin elixir, 1 ml of mineral elixir, 5 mM KH_2PO_4, 25 MuM ferric quinate, and 0.2 mg of resazurin. To this mixture were added (per 100 ml) 0.1 g of succinic acid, 20 mg of sodium acetate (anhydrous), 10 mg of $NaNO_3$, 5 mg of sodium thioglycolate, and 130 mg of agar (GIBCO Laboratories). The ferric quinate solution was prepared by combining 2.7 g of $FeCI_3$ and 1.9 g of quinic acid with 1 l of distilled water. Before adding the agar, the pH of the medium was adjusted to 6.7 with NaOH. The medium was boiled, and 12 ml was added to each

screw-capped tube (16 by 125 mm) containing approximately 0.1 ml of 5 % (wt/vol) sodium thioglycolate in distilled water. After autoclaving, the medium stands overnight for the establishment of O_2 gradients.

M. magneticum AMB-1^T is a magnetic bacterium capable of growing aerobically, isolated from freshwater sludges and sediments obtained from ponds at Koganei in Tokyo (Matsunaga et al. 1991). Separation of magnetic bacteria from sediment and water samples was achieved using an apparatus adapted from Matsunaga and Kamiya (1987). This apparatus allowed that magnetic bacteria migrated through the cotton plug toward the south pole of a samarium–cobalt (Sin-Co) magnet (produced by TDK, Tokyo, Japan) placed on the side of the sterile solution. Dark gray suspension around the magnet was sampled with a pipette and inoculated into the isolation medium. The isolation medium contained (per liter of distilled water) 2 ml Wolfe's mineral solution (Wolin et al. 1963), 0.2 g potassium dihydrogen phosphate, 0.12 g sodium nitrate, 0.02 g yeast extract, 0.02 g malt extract, and 0.05 g L-cysteine HC1. H_2O, 10 M ferric gallate (prepared in 100 ml distilled water containing 0.27 g $FeCI_3$ and 0.19 g gallic acid.), and 0.5 mg biotin. The medium was adjusted to pH 7.0, and after sterilization, 0.6 ml of 10 % glucose filter sterilized (pore size 0.45 m) solution was added to the medium.

The *Magnetospirillum* strains can be routinely grown microaerobically in semisolid (1.5 g l^{-1} agar Noble; Difco Laboratories) revised Magnetic Spirillum Growth Medium (MSGM) (American Type Culture Collection, 1989 – available at http://www.atcc.org). Bazilinski et al. (2000) proposed the following modifications to MSGM during a study of nitrogen fixation in *Magnetospirillum* strains: tartaric acid was omitted and the concentration of succinic acid was raised to 5 mM; ascorbic acid was replaced by 0.1 g l^{-1} sodium thioglycolate; the Wolfe's mineral solution added at 5 ml l^{-1} was modified by increases in the amounts of $Na_2MoO_4.2H_2O$ (from 0.01 g to 0.4 g l^{-1}) and $CuSO_4.5H_2O$ (from 0.01 g to 0.02 g l^{-1}) and by the addition of 0.01 g l^{-1} $NiCl_2.6H_2O$. They grow in the presence of NH_4Cl (4 mM), $NaNO_3$ (8 mM), and N_2 as sole nitrogen sources.

Magnetospirillum bellicus, the second dissimilatory perchlorate-reducing bacteria (DPRB), was isolated from the surface of a working electrode in an active perchlorate-reducing bioelectrical reactor (BER) that was inoculated with water from Strawberry Creek on the University of California, Berkeley, campus (Thrash et al. 2007). Perchlorate-reducing enrichments were established by transferring 1 g of electrode surface scrapings into 9 mL of prepared anoxic medium as indicated by Miller and Wolin (1974) under a gas stream of N_2-CO_2 (80:20; v/v). Acetate (590 mg L^{-1}) was the electron donor and perchlorate (990 mg L^{-1}) was the electron acceptor. Incubations were done at 30 °C in the dark. Positive enrichments were identified by visual increase in optical density and by microscopic examination. Once a positive enrichment was established, the perchlorate-reducing culture was transferred (10 % inoculum) into 9 mL of fresh anoxic medium. Isolated colonies were obtained from transfers of positive enrichments by the standard agar shake-tube technique with acetate (590 g L^{-1}) as the sole electron donor and perchlorate (990 mg L^{-1}) as the sole electron acceptor.

Magnetospirillum aberrantis was isolated from the coastal bottom sediment of the Ol'khovka River in the city of Kislovodsk (Gorlenko et al. 2011). Enrichment cultures were obtained by microaerobic incubation of the medium inoculated with the bottom sediments. The medium contained the following (g/l): KH_2PO_4, 0.4; NH_4Cl, 0.33; KCl, 0.33; $MgCl_2$, 0.33; Na_2SO_4, 0.25; $Na_2S_2O_3$, 0.25; $NaNO_3$, 0.33; $NaHCO_3$, 0.25; sodium acetate, 1.0; and yeast extract, 0.1, as well as Fe(III) citrate, 30 μM; resazurin, 0.5 mg/l; sodium thioglycolate, 50 mg/l; vitamin B12, 15 μg/l; and trace elements, 1 ml/l. The optimal oxygen concentration determined by cultivation in sealed Hungate tubes was from 1 % to 20 %. Magnetic separation was used to obtain pure bacterial cultures as described by Gorlenko et al. (2011). Bacteria were grown in 5-ml syringes under microaerobic conditions with a small air bubble at 30°C, pH 6.7. The same medium was used for the subsequent cultivation of the isolates.

Magnetospira thiophila strain MMS-1 was obtained from mud and water samples using the capillary magnetic racetrack technique (Wolfe et al. 1987). Concentrated magnetotactic cells were inoculated into ASW medium containing 5 ml of modified Wolfe's mineral elixir (Frankel et al. 1997), 0.25 g of NH_4Cl, and 100 μL of 0.2 % (wt/vol) aqueous resazurin. To produce an oxygen gradient, the medium was modified into semisolid by addition of 2.0 g of Agar Noble. Cultures were incubated at 25–28 °C and cells grew as a microaerophilic band at the oxic–anoxic transition zone of the tubes (pink/colorless interface). Cells also grew in this same medium when 3.7 mM sodium succinate replaced the thiosulfate. Separate colonies were obtained in a serial dilution of a culture in ASW solid medium shake tubes with succinate as the electron donor. Colonies were removed aseptically and the process was repeated three times and the purity of the cultures was determined using light microscopy as described in Williams et al. (2012). To achieve a sufficient yield of biomass cells, these authors grew MMS-1 chemolithoautotrophically in 2L glass bottles containing 850 ml using the same medium modified by the addition of thiosulfate ($S_2O_3^{2-}$) as the electron donor and O_2 as the terminal electron acceptor. After sterilization, the medium was cooled to room temperature, and the following solutions were injected (per liter) into the medium bottles, in order, from oxygen-free stocks (except for the cysteine, which was made fresh and filter sterilized directly into the medium): 1.5 ml of 0.5 M Potassium phosphate buffer, pH 6.9, neutralized cysteine HCl.H_2O to give a final concentration of 0.04 g l1, 10 ml of 25 % (wt/vol) $Na_2S_2O_3.5H_2O$, and 0.5 ml of vitamin solution (Frankel et al. 1997). The medium was allowed to become reduced (= colorless), after which 2.5 ml of 0.01 M $FeSO_4$ dissolved in 0.2 N HCl was injected. The medium was inoculated with several bands of cells from semisolid medium, after which sterile O_2 was introduced (0.4 % of the final headspace), and carefully placed at 25 °C for O_2 gradient establishment, indicated by pink color at the surface while the remaining medium

remained colorless. Growth initiated at the oxic–anoxic interface near the surface, and as growth increased, O_2 in the headspace was replenished up to a maximum of 4 % of the headspace every 24–48 h during 7–10 days.

Magnetovibrio blakemorei was isolated from shallow, brackish, salt-marsh pools near the Neponset River estuary in Milton, MA, USA. Samples were placed under dim light at room temperature, and after several days, formation of a horizontal "plate" of microorganisms in the water column was observed in one of the bottles. The characterization of the environmental conditions that favored the enrichment of this bacterium suggested that the gradient of sulfide was in the presence of an opposing gradient of oxygen diffusing from the surface to the bottom and thus the plate probably formed at the oxic–anoxic interface within the bottle at pH 7.5. Cells removed from the plate were used to inoculate sulfide–O_2 concentration gradient medium, prepared following the recipe of Nelson and Jannasch (1983) but modified by using diluted artificial seawater (ASW) solution rather than natural seawater and by the addition of 25 mM ferric quinate (Blakemore et al. 1979) and 200 µl 0.2 % aqueous resazurin per liter. The ASW was adjusted to approximately 23 % and consisted of (g l^{-1}) NaCl, 16.4; $MgCl_2.6H_2O$, 3.5; Na_2SO_4, 2.7; KCl, 0.47; and $CaCl_2.2H_2O$, 0.39. After enrichment into ASW modified medium, small amounts of magnetotactic cells were observed in a low percentage of the cultures forming microaerophilic bands of cells. For isolation of the strain, cells from these enrichment gradient cultures were inoculated in a dilution series of solid agar (13 g Agar Noble l^{-1}; Difco Laboratories) shake tubes of ASW [O_2]-gradient medium containing 5 ml modified Wolfe's mineral elixir containing 0.5 ml vitamin solution as described in Bazylinski et al. (2013). Anoxic conditions and the use of nitrous oxide (N_2O) at a pressure of 2 atm (202.7 kPa) were necessary to avoid contamination by nonmagnetic bacteria. After 2–3 weeks, shake tube black, lens-shaped colonies consisting of the magnetotactic vibrio individual colonies were removed and used as inocula for a second series of shake tubes, and the process was repeated once more to ensure purity of the culture. Since then, cells of *M. blakemorei* are routinely grown in oxygen-free liquid cultures of ASW modified medium containing 5 ml modified Wolfe's mineral elixir and N_2O at 1 atm as the terminal electron acceptor.

Species of the genera *Novispirillum* and *Insolitispirillum* are routinely grown on LMG medium no. 8 (composition per liter: 1 g succinic acid, 10 g peptone, 1 g $(NH_4)_2SO_4$, 1 g $MgSO_4.7H_2O$, 2 mg $FeCl_3.6H_2O$, 2 mg $MnSO_4.H_2O$, and 15 g agar, pH 7.0).

Nisaea spp. were isolated from one of the major sites of water-column denitrification among the world's oceans using filter-sterilized seawater from the isolation site for the preparation of media and dilution to extinction as described by Schut et al. (1993). After 1 month at 20 °C, positive cultures were plated on seawater R2A agar (Difco) and incubated at 20 °C for 1 week. After subculturing, two isolates forming cream-colored colonies on Marine Broth 2216 medium (MB; Difco) were obtained and designated as *N. denitrificans* DR41_21^T and *N. nitritireducens* DR41_18^T. According to genus description (Urios et al. 2008), growth occurs at 15–44 °C (optimum, 30 °C), at pH 5.0–9.0 (optimum, pH 6.0), and at salinities in the range 0–60 g l^{-1} (optimum, 20 g l^{-1}).

Marispirillum indicum was isolated from the seawater of the Southwest Indian Ridge, Indian Ocean (Lai et al. 2009a). Seawater sample was added with crude oil, as carbon and energy source, for enrichment of oil-degrading bacteria. After 2 months, 1 ml enrichment culture was transferred into 100 ml fresh MM medium as described in Lai et al. (2009b). Sequential transfers were performed three times at intervals of 2 weeks and incubation at 28 °C with shaking at 160 r.p.m. Bacteria were isolated using the plate screening method on 216 L medium (containing, per liter seawater: CH_3COONa, 1.0 g; tryptone, 10.0 g; yeast extract, 2.0 g; sodium citrate, 0.5 g; NH_4NO_3, 0.2 g; pH 7.5). The 216 L medium was used for all studies of strain B142^T.

Oceanibaculum species were isolated from deep seawater during a survey for PAH-degrading bacteria (Lai et al. 2009b; Dong et al. 2010). PAH-degrading bacteria media containing 1 % (v/v) sterilized crude oil and two different PAH mixtures were used for enrichment. *O. indicum* P24^{T}was isolated from the Southwest Indian Ridge, using PAH mixture containing naphthalene, phenanthrene, anthracene, and pyrene, at 200 p.p.m each, dissolved in crude oil as the carbon and energy source. *O. pacificum* strain LMC2up-L3^T was isolated from a hydrothermal field of the southwest Pacific Ocean, using PAH mixture containing naphthalene and phenanthrene at a final concentration of 100 p.p.m. and 20 p.p.m. of pyrene. After 2 months, 1 mL of each enrichment culture was transferred into 100 ml fresh seawater medium containing per liter 1.0 g NH_4NO_3, 0.5 g KH_2PO_4, and 2.8 mg $FeSO_4.7H_2O$, using the PAH mixture respective to each species, as the sole carbon and energy source. After 3 weeks of incubation at 28 °C with shaking at 160 r.p.m., each culture was transferred repeatedly to the same medium for further enrichment every 4 weeks three times. Bacteria were isolated by using the plate screening method on 216 L medium (per liter seawater: 1.0 g CH_3COONa, 10.0 g tryptone, 2.0 g yeast extract, 0.5 g sodium citrate, and 0.2 g NH_4NO_3; pH 7.5). For further studies of these species, the same medium has been used as described by Lai et al. (2009b) and Dong et al. (2010).

Limimonas halophila (Amoozegar et al. 2013) designated strain IA16^T was isolated using the modified growth medium (MGM) with 24 % (w/v) total salt concentration as described (Dyall-Smith 2008): 5 g peptone (Oxoid), 1 g yeast extract, and 200 ml pure water with 767 ml of a stock salt solution that contained (L^{-1}) 240 g NaCl, 35 g $MgSO_4.7H_2O$, 30 g $MgCl_2.6H_2O$, 7 g KCl, and 1 g $CaCl_2$. The pH of the medium was adjusted to pH 7.2–7.4 with Tris base, and agar was added to the medium to give a final concentration of 1.5 % (w/v). The isolation procedure consisted of spreading mud sample serial dilution in sterile 20 % (w/v) on plates of MGM agar. After growth development, achieved at 40 °C after 2 months under aerobic conditions, successive cultivation leads to pure isolate of IA16^T.

Pelagibius–Phaeospirillum–Phaeovibrio–Rhodocista–Rhodospira

Pelagibius litoralis was isolated from coastal seawater off the east coast of Korea. Autoclaved seawater (500 ml) supplemented with urea (100 mM) was inoculated with seawater (100 µl) and incubated at 20 °C in the dark for about 8 months. Incubated material was spread on a Marine Agar 2216 (Difco) plate (https://www.bd.com/europe/regulatory/Assets/IFU/Difco_BBL/212185.pdf) following incubation aerobically at 30 °C for 2 weeks. Purified cultures were obtained by subsequently streaking the isolated strain onto fresh MA plates at 30 °C under aerobic conditions. Pure cultures were stored in Marine Agar 2216 at 30 + C and in Marine Broth 2216 (Difco) supplemented with 30 % (v/v) glycerol at −80 °C (Choi et al. 2009).

In general, media and growth conditions for *Phaeospirillum* species can be the same applied for other freshwater photosynthetic non-sulfur bacteria such as *Rhodospirillum*, considering the need to establish and maintain reduced oxygen partial pressure. Cultures can be cryopreserved in liquid nitrogen or at −80 °C through standard techniques.

Phaeospirillum fulvum and *Phaeospirillum molischianum* were both isolated from an enrichment culture using mud or surface water as inoculum in a glass stoppered bottle. Carprylate or pelargonate (up to 0.04 % at pH 7.5) were added to the mineral salt media used to isolate *P. fulvum* (van Niel 1944; Imhoff et al. 1998) and can provide selective growth conditions for *P. fulvum* and *P. molischianum*. Hay was used as organic substrate to isolate *P. molischianum* (Giesberger 1947; Imhoff et al. 1998). Pure cultures were obtained by using successive dilutions of enrichment culture in agar medium under anaerobic conditions according to van Niel (1944).

Phaeospirillum chandramohanii was isolated from a photolithoheterotrophic enrichment of a water sample from a freshwater reservoir sampled at Mudasarlova (India), using mineral medium (Biebl and Pfennig 1981) supplemented with $Na_2S.9H_2O$ (1 mM) plus 0.3 % pyruvate (w/v), in anaerobiose (Kumar et al. 2009). Pure cultures were obtained by using the repeated agar shake dilution method (Pfennig and Truper 1992; Imhoff 1988) using the medium described by Pfennig and Truper (1974) supplemented with $Na_2S_2O_3$ (4 mM).

Phaeospirillum oryzae was isolated from an enrichment culture of the rhizosphere soil of a paddy (Nadergul, India), using a photoheterotrophic medium (Biebl and Pfennig 1981), pH 7.0, incubated at 2,400 lx, 28–30 °C for 7 days in fully filled screw-capped bottles (Lakshmi et al. 2011a). Pure cultures were obtained by repeated streaking on agar slants in test tubes (25 × 150 mm) sealed with butyl rubber corks and replacing the gas phase with argon to achieve anaerobic conditions. Purification media contained (g l^{-1}) KH_2PO_4 (0.5), $MgSO_4.7H_2O$ (0.2), NaCl (0.4), NH_4Cl (0.6), $CaCl_2.2H_2O$ (0.05), sodium pyruvate (0.5), sodium succinate (0.5), sodium acetate (0.5), yeast extract (0.3), ferric citrate (5 ml l^{-1} forms a 0.1 % w/v stock solution), and trace element solution SL 7 (1 ml l^{-1}; Biebl and Pfennig 1981).

Phaeospirillum tilakii was isolated from an enrichment culture of aquatic sediment (Nelapattu, India) using photoheterotrophic medium prepared according to Lakshmi et al. (2011b) containing the following: NH_4Cl (18 mM), $MgSO_4.7H_2O$ (1.2 mM), $CaCl_2.2H_2O$ (1.3 mM), KH_2PO_4 (3.6 mM), NaCl (17 mM), sodium succinate (7.4 mM), yeast extract (2.0 g l^{-1}), and Na_2HPO_4 (2 mM), pH 7.0. Enrichment cultures were incubated at 2,400 lx, 28–30 °C for 7 days in fully filled screw-capped bottles (Raj et al. 2012). Pure cultures were obtained by repeated streaking on agar slants in test tubes (25 × 150 mm) sealed with butyl rubber corks and replacing the gas phase with argon to achieve anaerobic conditions. Purification media contained (g l^{-1}) KH_2PO_4 (0.5), $MgSO_4.7H_2O$ (0.2), NaCl (0.4), NH_4Cl (0.6), $CaCl_2.2H_2O$ (0.05), sodium pyruvate (0.5), sodium succinate (0.5), sodium acetate (0.5), yeast extract (0.3), ferric citrate (5 ml l^{-1} forms a 0.1 % w/v stock solution), and trace element solution SL 7 (1 ml l^{-1}; Biebl and Pfennig 1981).

Phaeovibrio sulfidiphilus was isolated from sediment of a brackish shrimp pond (pH 8.2) at Vadkku Poigainallur (India). Enrichment culture was obtained in a photoheterotrophic medium containing the following: NH_4Cl (18 mM), $MgSO_4.7H_2O$ (1.2 mM), $CaCl_2.2H_2O$ (1.3 mM), KH_2PO_4 (3.6 mM), NaCl (17 mM), sodium succinate (7.4 mM), yeast extract (2.0 g l^{-1}), and Na_2HPO_4 (2 mM), pH 8.2. Cultures were incubated at 2,400 lx, 28–30 °C for 7 days in fully filled screw-capped bottles (Lakshmi et al. 2011b). Pure cultures were obtained by repeated streaking on agar slants in test tubes (25 × 150 mm) sealed with butyl rubber corks and replacing the gas phase with argon to achieve anaerobic conditions. Purification media contained KH_2PO_4 (3.6 mM), $MgSO_4.7H_2O$ (0.8 mM), NaCl (6.8 mM), NH_4Cl (11 mM), $CaCl_2.2H_2O$ (0.34 mM), sodium pyruvate (4.5 mM), sodium succinate (1.8 mM), sodium acetate (3.6 mM), yeast extract (0.3 g l^{-1}), Na_2S (1 mM), $NaHCO_3$ (100 mM), ferric citrate (0.2 mM), and trace element solution SL 7 (1 ml l^{-1}; Biebl and Pfennig 1981).

Rhodocista centenaria was isolated from a water sample collected at the edge of a hot spring (55 °C) at Wyoming (USA) (Favinger et al. 1989). Enrichment culture was at 40 °C using a procedure selective for anoxygenic N_2-fixing photosynthetic bacteria according to Guest et al. (1985). Maintenance of pure cultures can be achieved in SA agar medium kept at 10 °C in the dark (Kawasaki et al. 1992).

Rhodocista pekingensis originates from activated sludge from a municipal wastewater treatment plant in Beijing (China). Dilluted samples were inoculated in soft-agar (0.7 % agar) tubes using the following media modified from the AT medium (Imhoff and Trüper 1992): ATB medium with butyrate as sole carbon source, ATY medium with 0.05 % w/v yeast extract and removal of sodium hydrogen carbonate, and ATYP medium with addition of 0.03 % w/v peptone to ATY medium. Inoculated tubes were incubated anaerobically at 34–41 °C under incandescent illumination of 1,000–2,000 lx for 1 week. After incubation period, pink-reddish colonies were picked and streak onto agar plates (1.5 % agar) with the same medium and incubation conditions (Zhang et al. 2003).

Rhodospira trueperi was isolated from the peach-colored layer of a laminated microbial mat in Massachusetts (USA). Material was suspended in sterile seawater and inoculated in a deep-agar dilution series. Cultures were grown phototrophically in 100-ml screw-capped bottles with rubber seals, at 20–22 °C and a light intensity of 300–500 lx, using basal medium containing (g l^{-1}) KH_2PO_4 (0.25), NH_4Cl (0.4), KCl (0.35), NaCl (20.0), $MgSO_4.7H_2O$ (2.8), $CaCl_2.2H_2O$ (0.25), $NaHCO_3$ (1.5), $Na_2S.9H_2O$ (0.3), 1 ml vitamin solution (Pfennig and Trüper 1981), 1 ml trace element solution SL 12 (Overmann et al. 1992), 3 mM acetate, and 1 % w/v washed agar. Pure cultures were obtained after repeated deep-agar dilution series (Pfennig et al. 1997). Pure cultures were stored at 4 °C in the dark.

Rhodospirillum–Pararhodospirillum–Rhodovibrio

A number of media have been used for the isolation and enrichment of *Rhodospirillaceae* species (Biebl and Pfennig 1981; Imhoff and Trüper 1992). Among these, a mineral medium has been used for culturing the majority of "purple non-sulfur bacteria" (J. F. Imhoff 2005b): AT medium contains 1.0 g·L^{-1} KH_2PO_4, 0.5 g·L^{-1} $MgCl_2{\cdot}6H_2O$, 0.1 g·L^{-1} $CaCl_2{\cdot}2H_2O$, 1.0 g·L^{-1} NH_4Cl, 3.0 g·L^{-1} $NaHCO_3$, 0.7 g·L^{-1} Na_2SO_4, 1.0 g·L^{-1} NaCl, 1 mL of sulfate-free trace element solution SLA (Imhoff and Trüper 1977; Imhoff 1992), and 1 mL of vitamin solution VA (Imhoff and Trüper 1977; Imhoff 1992). Organic carbon sources include (routinely 10 mM) sodium malate, sodium succinate, sodium pyruvate, or sodium acetate and also, for oxygen-sensitive strains, 0.5 g·L^{-1} of sodium ascorbate or 0.25 g·L^{-1} thioglycolate, added separately. The initial pH is adjusted to 6.9. Vitamin solution VA, prepared in double distilled water, contains 0.01 % biotin, 0.035 % niacinamide, 0.03 % thiamine dichloride, 0.02 % p-aminobenzoic acid, 0.01 % pyridoxal hydrochloride, 0.01 % calcium pantothenate, and 0.005 % vitamin B12. The trace element solution SLA has the following composition: 1.8 g·L^{-1} $FeCl_2{\cdot}4H_2O$, 250 mg·L^{-1} $CoCl_2{\cdot}6H_2O$, 10 mg·L^{-1} $NiCl_2{\cdot}6H_2O$, 10 mg·L^{-1} $CuCl_2{\cdot}5H_2O$, 70 mg·L^{-1} $MnCl_2{\cdot}4H_2O$, 100 mg·L^{-1} $ZnCl_2$, 500 mg·L^{-1} H_3BO_3, 30 mg·L^{-1} $Na_2MoO_4{\cdot}2H_2O$, and 10 mg·L^{-1} $Na_2SeO_3{\cdot}5H_2O$; the pH of the solution is adjusted with HCl to 2–3.

Bacteria of the *Rhodospirillum* species can be isolated through standard techniques for anaerobes in agar dilution series and on agar plates, keeping oxygen-free conditions, especially for oxygen-sensitive species (Biebl and Pfennig 1981; Imhoff and Trüper 1992). This can be accomplished by adding 0.5 g·L^{-1} of sodium ascorbate or 0.25 g·L^{-1} thioglycolate to the growth medium in completely filled screw-capped bottles. Cell cultures can be maintained by standard techniques in liquid nitrogen or at −80°C (Imhoff 2005b).

Bacteria of the *Pararhodospirillum* species can be isolated through standard techniques for anaerobes in agar dilution series and on agar plates, keeping oxygen-free conditions, as these are oxygen sensitive (Biebl and Pfennig 1981; Imhoff and Trüper 1992). This can be accomplished by adding 0.5 g·L^{-1} of sodium ascorbate or 0.25 g·L^{-1} thioglycolate to the growth medium in completely filled screw-capped bottles. Cell cultures can be maintained by standard techniques in liquid nitrogen or at −80°C (Imhoff 2005b).

Rhodovibrio species require high salt concentrations and complex nutrients for growth (Imhoff 2005a). Thus, complex media with salt concentrations of ∼10 % and anaerobic incubation in the light constitute selective conditions for the enrichment of *Rhodovibrio* species. A suitable medium for both *Rhodovibrio* species, named DSIC$^-$ or SAL (Mack et al. 1993), contains per liter: 1 g yeast extract, 1 g sodium acetate, 125 g NaCl, 10 g $MgCl_2{\cdot}6H_2O$, 0.2 g $CaCl_2{\cdot}2H_2O$, 0.5 g NH_4Cl, 0.6 g KH_2PO_4, 2.5 g K_2SO_4, 1 g $NaHCO_3$, 0.1 g $Na_2S_2O_3{\cdot}5H_2O$, 2.1 g MOPS buffer, 20 µg vitamin B12, 1 ml trace element solution SLA (see above), and pH 7. To avoid precipitation, the magnesium and calcium salts, as well as the $NaHCO_3$, are autoclaved as separate solutions. *Rhodovibrio* species can be isolated through standard techniques for anaerobes in agar dilution series and on agar plates, keeping oxygen-free conditions (J. F. Imhoff and Trüper 1992; Imhoff 2005a). This can be achieved by photosynthetic growth, in completely filled screw-capped illuminated tubes, at 37°C. Cell cultures can be maintained by standard techniques in liquid nitrogen, by lyophilization, or storage at −80°C (Imhoff 2005a).

Roseospira–Skermanella–Telmatospirillum

Roseospira marina strain CE2105 was isolated from brackish Certes Fishponds (Arcachon Bay, French Atlantic coast), which are periodically flooded with seawater. Liquid enrichment cultures were prepared from the upper layer of the anoxic sediments (Guyoneaud et al. 2002). Enrichment and isolation of strain CE2105 were obtained by using a basal medium containing filtered (0.2-µm pore size) seawater, 750 ml; distilled water, 250 ml; NH_4Cl, 0.035 % (w/v); yeast extract, 0.04 % (w/v); and Fe citrate, 0.001 % (w/v). The medium was autoclaved and cooled under a gas mixture of N_2/CO_2 (90/10, v/v). Vitamin V7 solution (Pfennig and Truper 1992; 1 ml.l^{-1}), phosphate buffer (0.1 M, pH 6.8, 36 ml.l^{-1}), and Na ascorbate/cysteine HCl (0.25 % (w/v)/0.5 % (w/v) solution at pH 7.0, 0.2 ml.l^{-1}) were then aseptically added to the medium.

Roseospira navarrensis strain SE3104 was isolated from the surface of sulfide-rich sediment from a small saline pond in the Spanish Pyrenees, formed from the outflow of a saline spring (Salinas de Oro, Navarra, Spain) with salinity varying from 2 % to 10 % (total salinity) (Guyoneaud et al. 2002). This spring water is rich in chloride (46 % w/v), sodium (28 % w/v), sulfate (15 % w/v), calcium (5 % w/v), and potassium (4 % w/v). For enrichment and isolation of strain SE3104, the culture medium was prepared according to the method of Pfennig and Trüper (1992) which contained (per liter water) 0.35 g KH_2PO_4, 0.05 g $CaCl_2.2H_2O$, 0.5 g NH_4Cl, 10 g NaCl, 0.7 g $MgCl_2.6H_2O$, 0.35 g $MgSO_4.7H_2O$, 1.5 g $NaHCO_3$, 1 ml vitamin solution V7 (Pfennig and Trüper 1981), 1 ml trace element solution SL12B containing (per liter of deionized water) 3 g $Na_2EDTA.2H_2O$,

1.1 g $FeSO_4.7H_2O$, 0.3 g H_3BO_3, 0.19 g $CoCl_2.6H_2O$, 0.05 g $MnCl_2.4H_2O$, 0.042 g $ZnCl_2$, 0.024 g $NiCl_2.2H_2O$, 0.018 g $NaMoO_4.2H_2O$, 0.002 g $CuCl_2.2H_2O$ (Overmann et al. 1992), 0.5 g yeast extract, 1.35 g (5 mM) disodium succinate, 0.68 g (5 mM) sodium acetate, and pH 6.8 and supplemented with 5 % (w/v) NaCl and 1 % (w/v) $MgCl_2.6H_2O$.

Roseospira thiosulfatophila strain AT2115 was isolated from microbial mats in French Polynesia (Tetiaroa Atoll, Society Islands) (Guyoneaud et al. 2002). The culture medium used for enrichment and isolation of strain AT2115 contained filtered (0.2-µm pore size) seawater, 1,000 ml; NH_4Cl, 0.05 % (w/v); KH_2PO_4, 0.02 % (w/v); and yeast extract, 0.05 % (w/v). The medium was autoclaved and cooled under N_2/CO_2 (90/10, v/v). Vitamin V7 solution (1 ml.l–1), $NaHCO_3$ (0.15 % w/v), and $Na_2S.9H_2O$ (0.02 % w/v) were then aseptically added to the medium. The final pH for all media was adjusted to 6.8. The media were dispensed into sterile 50-ml screw-capped bottles. Organic substrates (5 mM sodium acetate and 5 mM disodium succinate) were added just before utilization. Pure cultures were obtained by repeated application of the deep-agar dilution method (Pfennig 1978). Deep-agar tubes were incubated at 25 °C under a light/dark cycle (16 h light/8 h dark) using tungsten lamps.

The pure cultures of *Roseospira marina, Roseospira navarrensis*, and *Roseospira thiosulfatophila* are cultivated, characterized and maintained in the synthetic media with the composition (per liter of distilled water): KH_2PO_4, 0.03 % (w/v); NH_4Cl, 0.05 % (w/v); $CaCl_2.2H_2O$, 0.005 % (w/v); $MgCl_2.6H_20$, 0.1 % (w/v) (0.3 % w/v for strain SE3104); $MgSO_4.7H_2O$, 0.05 % (w/v) (0.2 % w/v for strain SE3104); NaCl, 2 % (w/v) (5 % w/v for strain SE3104); trace element solution SL12 (Overmann et al. 1992), 1 ml; and yeast extract, 0.05 % (w/v). Media were autoclaved and cooled under N_2/CO_2 (90/10, v/v). Vitamin V7 solution (1 ml.l–1), Na ascorbate (0.05 % w/v), and $NaHCO_3$ (0.15 % w/v) were then aseptically added to the medium. The final pH was adjusted to 6.8–7.0 and the medium was dispensed into sterile 50-ml screw-capped bottles. Organic substrates (5 mM Na acetate and/or 5 mM di-Na succinate) were added as substrates before use. In addition, for strains SE3104 and AT2115, $Na_2S.9H2O$ (0.02 % w/v) was also added to the medium prior to utilization. Pure cultures were grown in 50-ml screw-capped bottles and stored at +4 °C in the dark for preservation.

Roseospira visakhapatnamensis strain JA131 was isolated from a water sample (pH ~6.8, 30°C, 2–3 % (w/v) salinity) collected on 25 March 2004 from the fishing harbor at Visakhapatnam, India (17° 41′ N 83° 18 E). *Roseospira goensis* strain JA135 was isolated from a sediment sample (pH ~6.8; 30°C, 6–7 % (w/v) salinity) collected on 12 February 2005 from Kurka saltern, Goa, India (15° 29′ N 73° 49 E). Original enrichments of both strains were from photolithoheterotrophic media (anaerobic, 1 mM $Na_2S.9H_2O$ + 0.3 % (w/v) pyruvate/malate). Strain JA131 was isolated from an enriched culture containing 2 % NaCl, and strain JA135 was isolated from an enrichment containing 8 % NaCl. Subsequent culturing, purification, and characterization were as described by Biebl and Pfennig (1981) medium with the following modifications (g per liter): 1 g $MgSO_4.7H_2O$, 0.15 g $CaCl_2.2H_2O$, and 20 g NaCl and supplemented with $Na_2S_2O_3.5H_2O$ (2 mM) (Chakravarthy et al. 2007).

Skermanella parooensis was isolated from the water of the Paroo Channel in southwest Queensland, Australia (Skerman et al. 1983). A drop of water was inoculated on the surface of lake water agar (LWA) (Franzmann and Skerman 1981) plates, air-dried, and incubated for periods of up to 3 weeks. Multicellular bodies developed on some plates were transferred by micromanipulation to fresh LWA, in which each conglomerate transformed after periods of 4–8 h to actively motile cells containing highly refractive granules. Further incubation led these cells to produce water-clear colonies. Single cells selected and cultured on LWA produced this colony form. After prolonged incubation, a few of the multicellular forms appeared among the dense population.

Skermanella aerolata was isolated from air samples (20–1,000 ml) collected with a MAS-100 air sampler (a single-stage, multiple-hole impactor; Merck) on the roof of Taean Lily Experimental Station (Chungnam Provincial Agricultural Research and Extension Services in the Taean district of Korea) on 16 April 2005 (Weon et al. 2007). The sampler contained Petri dishes with R2A agar (BBL) supplemented with 200 micrograms/milliliter cycloheximide and incubated in the dark at 28 ° C for 5 days.

Skermanella xinjiangensis was isolated from sand soil sample collected from Xinjiang (An et al. 2009). Strain 10-1-101^T was isolated after dilution and plating on 0.1 × trypticase soy broth (TSB) agar plates (Difco) at 28 °C. The isolate could also grow on R2A (Difco).

Skermanella stibiiresistens was isolated from soil collected from Jixi coal mine (45° 18′ N 130° 57′ E) of Jixi City, Heilongjiang Province, Northeast China (Luo et al. 2012). The soil texture was sandy with a pH of 7.2, and total As, Sb, Fe, and Cu concentrations were 0.04, 0.01, 18.0, and 0.09 $g.kg^{-1}$, respectively. Total C, N, P, S, and nitrate concentrations were 303.0, 3.8, 0.6, 0.2, and 0.04 g kg^{-1}, respectively. Sb-resistant bacteria were isolated using CDM medium (per liter): $MgSO_4.7H_2O$, 2.0 g; NH_4Cl, 1.0 g; Na_2SO_4, 1.0 g; K_2HPO_4, 0.013 g; $CaCl_2.2H_2O$, 0.067 g; sodium lactate, 5.0 g; $Fe_2SO_4.7H_2O$, 0.033 g; $NaHCO_3$, 0.798 g; and 15.0 g agar, pH 7.2 (Weeger et al. 1999) containing 0.1 mM $C_8H_4K_2O_{12}Sb_2.3H_2O$ (potassium antimony tartrate trihydrate).

Telmatospirillum siberiense was isolated by plating of acidotolerant methanogenic consortia from northern acidic peatlands on 1 % agarose N-free mineral medium (g per liter): K_2HPO_4, 0.25; KH_2PO_4, 1.0; $CaCl_2$, 0.1; $MgSO_4$, 0.4; Na-EDTA, 0.01; $FeCl_3 \cdot 6H_2O$, 1×10^{-3}; KI, 2×10^{-4}; $CoCl \cdot 6H_2O$, 2×10^{-4}; $MnCl_2 \cdot 4H_2O$, 8×10^{-4}; $ZnSO_4$, 8×10^{-4}; H_3BO_2, 1×10^{-4}; $Na_2MoO_4 \cdot 2H_2O$, 1×10^{-4}; $CuCl_2$, 1×10^{-4}; and $NiCl_2 \cdot 6H_2O$, 2×10^{-4}, with 0.6 mM Ti(III) citrate as reducing agent and H_2:CO_2:N_2 in the headspace. Brown colonies of non-methanogenic microorganisms formed after 5–7 months of anaerobic incubation. Intensive growth occurred in liquid Na citrate medium after complete utilization of citrate in 4–7 days.

Small brown, beige, and pink colonies formed on citrate-agar medium in 10–14 days. Colonies were subcultured at 28 °C on the same liquid medium supplemented with $(NH_4)_2SO_4$ and Na citrate instead of Ti(III) citrate under N_2 (Sizova et al. 2007).

Thalassobaculum

For isolating *Thalassobaculum litoreum* CL-GR58^T, coastal seawater and sediment samples were incubated in a 150-mm-diameter glass Petri dish for around 15 months at room temperature. Without disturbing the sediment, a 100-ml sample of seawater was removed from the surface and spread on a Marine Agar 2216 (MA; Difco) plate, which was then incubated at 30 °C for 1 week. Strain CL-GR58^T was isolated and subsequently purified on MA at 30 °C four times. The strain was maintained both on MA at 30 °C and in Marine Broth 2216 (MB; Difco) supplemented with 30% (v/v) glycerol at −80 °C (Zhang et al. 2008).

Thalassobaculum salexigens CZ41-10a^T was isolated from seawater samples. Subsamples were spread on nutrient agar plates (Bio-Rad) prepared with filtered seawater and incubated at 25 °C for 2 weeks. Colonies were picked and purified by three subcultures. Among these colonies, an isolate forming cream-colored colonies was obtained and designated strain CZ41-10a^T (Urios et al. 2010).

Thalassospira

For isolating *Thalassospira lucentensis*, the culture medium consisted of autoclaved and filtered seawater supplemented with cocktail FRV (at 0.01 g l^{-1}), which contains *Spirulina* (Sigma), fish meal, and *Artemia salina* (1 :1: 1). The pH of the medium was adjusted to 7.2. A 2-l bioreactor was completely filled up with the sample and incubated at 13 °C (in situ temperature) and with slow magnetic stirring. After 24 h, a flow rate was established to obtain a dilution rate of 0.0004 h^{-1}. The setup was maintained for three months. Weekly, 100 µl of enrichment was plated onto solidified FRV medium and incubated at 13 °C. Initially, bacteria grow as very small colonies on the complex oligotrophic FRV medium. After subculturing, they are able to grow in media containing a higher nutrient content than that of the medium used for initial isolation. In fact, the peptone-yeast extract-based media, marine agar, or YEA routinely used to culture fast-growing, copiotrophic marine bacteria allow fairly good growth.

For isolating *Thalassospira xiamenensis* M-5^T and *Thalassospira profundimaris* WP0211^T, bacteria were enriched by culturing in artificial seawater medium (ASM; Liu and Shao 2005), supplemented with 10 g diesel fuel l^{-1} (strain M-5^T) or 5 g pyrene l^{-1} (strain WP0211^T) as the sole carbon source. HLB medium (modified from Luria–Bertani medium by increasing the NaCl concentration to 30 g l^{-1}; Liu and Shao 2005) was used for routine cultivation of the isolates and for most of the phenotypic tests. All cultures were incubated at 28 °C with rotation at 200 r.p.m. unless noted otherwise. As was previously found for *Thalassospira lucentensis* QMT2^T (López-López et al. 2002), both strains formed very small colonies in the oligotrophic medium, but showed fairly good growth in the HLB media (Liu et al. 2007).

Thalassospira tepidiphila 1-1B^T was isolated from petroleum-contaminated seawater during a bioremediation experiment (Kodama et al. 2008). Seawater was collected from a bioremediation tank, serially diluted, and spread onto 1 % (w/v) Gelrite plates containing the artificial seawater medium ONR7a (Dyksterhouse et al. 1995). The plates were then coated with heat-treated Arabian light crude oil (0.2 %, w/v) (Kasai et al. 2002) and incubated at 20 °C. After incubation for 3 weeks, small colonies appearing on these plates were picked and streaked onto solid plates containing Marine Broth 2216 (MB; Difco) and 1 % (w/v) Gelrite for purification. MB was used for routine cultivation. Cells of strain 1-1B^T were stored at −80 °C in MB supplemented with 15 % (v/v) glycerol.

To isolate *Thalassospira xianhensis* P-4^T (Zhao et al. 2010), 5 % sea-salt defined medium (5 % SSDM; Zhao et al. 2009) and 5 % SSDM with 0.5 % yeast extract (5 % SSDMY) were used. Solid 5 % SSDMY medium was prepared with 1.5 % agar. A sample of oil-polluted saline soil (1 g) was added to 100 ml 5 % SSDM medium supplemented with phenanthrene (100 mg ml^{-1}) in a 300-ml Erlenmeyer flask. The culture was aerobically incubated at 30 °C in darkness on a rotary shaker operating at 200 r.p.m. After 2 weeks, 10 ml culture was transferred to 100 ml 5 % SSDM medium and incubated under the conditions described above. The enrichment was performed five or six times. Next, a culture broth dilution series was spread on 5 % SSDMY agar. After incubation for 2 days, single colonies were picked and cultivated in 5 ml 5 % SSDM using phenanthrene as the sole source of carbon and energy. These isolates developed a yellowish-orange or reddish-brown color, which is an indication of ring cleavage of polycyclic aromatic hydrocarbons (Guerin and Jones 1988) and, thus, phenanthrene-degrading activity.

For isolating *Thalassospira permensis* SMB34^T (Plotnikova et al. 2011), the enrichment culture was incubated aerobically at 28 °C with shaking in Raymond's mineral medium (RMM), containing (g l^{-1}) NH_4NO_3 (2.0), $MgSO_4{\cdot}7H_2O$ (0.2), KH_2PO_4 (2), Na_2HPO_4 (3), $CaCl_2{\cdot}6H_2O$ (0.01), Na_2CO_3 (0.1), 2 ml of 1 % $MnSO_4{\cdot}5H_2O$, and 2 ml of 1 % $FeSO_4{\cdot}7H_2O$ that was supplemented with naphthalene (0.1 %, w/v) and NaCl (6 %, w/v). Strain SMB34^T was isolated by plating the enrichment onto RMM agar supplemented with 0.5 % (w/v) tryptone, 0.25 % (w/v) yeast extract, and 3 % (w/v) NaCl (designated complete Raymond's medium, CRM). The strain was routinely cultured on CRM agar and Marine Agar 2216 (MA; Difco) at 28 or 30 °C (Plotnikova et al. 2011).

Thalassospira alkalitolerans MBE#61^T and *Thalassospira mesophila* MBE#74^T were isolated from a piece of sunken bamboo in the coastal area of Japan (Tsubouchi et al. 2014). Bamboo is a fast-growing plant and significant bioresource in the east and south area of Asia. A portion (approximately 1 g) of sinker was soaked in 2 ml of sterile artificial seawater (ASW; Nihon Pharmaceuticals, Japan) and shaken briefly on

a vortex at room temperature. The immersion fluid was incubated at 25 °C for 1 day and then spread on 1.5 % (w/v) agar containing milled Japanese timber bamboo (MJTB; 2 % (w/v) milled Japanese timber bamboo and 0.5 × ASW). Both strains were isolated after incubation at 25 °C for 10 days. After incubation, small colonies appearing on MJTB plates were picked and streaked onto solid plate containing Marine Broth (MB; BD Difco) for purification. For routine cultivation, MB was used.

Tistrella

Tistrella mobilis was isolated from samples of wastewater in mineral salt medium prepared with 0.81 mM $MgSO_4$, 0.58 mM $CaSO_4$, 18 mM $FeSO_4$, 1.0 mM $NaMoO_4$ in 5 mM potassium phosphate, 50 mM ferric citrate, 3 % glucose, and 15 mM ammonium acetate (pH 7.1) (Shi et al. 2002). The polyhydroxyalkanoate (PHA) content in bacterial colonies can be determined qualitatively by observing the presence of visible, intracellular granules using a phase-contrast microscope. To recognize PHA-rich colonies, colonies grown on nitrogen-deficient agar after 5-day incubation at 30 °C are stained with Sudan Black B (0.02 % in 96 % ethanol). The dye is removed after 20 min, and the plates are then treated for 1 min with 10 ml of 96 % ethanol. The colonies of PHA-rich cells retain the dye and appear dark blue, whereas those of PHA-deficient cells decolorize and appear light gray.

Tristella bauzanensis BZ78^T was isolated from soil containing high levels of heavy oil and heavy metals (Zhang et al. 2011). For that, 10-g soil was shaken with 90 ml of sterile 1 % sodium pyrophosphate for 20 min at 150 r.p.m. Appropriate dilutions, prepared with sterile saline solution (0.9 % NaCl), were plated (0.1 ml) on R2A agar (0.05 % yeast extract, 0.05 % peptone, 0.05 % casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K_2HPO_4, 0.005 % $MgSO_4$, 1.5 % agar; pH 7; Reasoner and Geldreich 1985) and incubated at 20 °C. Strain BZ78^T was routinely cultured in R2A liquid medium at 20 °C and maintained as a suspension in skimmed milk (10 %, w/v) at −80 °C.

Tistlia

For isolating *Tistlia consotensis*, water samples were collected aseptically from the Salado de Consotá spring in 2006 by filling sterile glass containers to the brim (Díaz-Cárdenas et al. 2010). Enrichments were initiated by inoculating a 2-ml water sample in 10-ml filter-sterilized saline spring water which had been amended with 0.1 % (w/v) starch (Sigma) and 0.02 % (w/v) yeast extract (Sigma). Turbidity was observed after 10 days incubation at 37 °C. Subsequent phase-contrast microscopy (Eclipse 50i; Nikon) revealed the presence of curved and rod-shaped cells. Several colonies developed from serial dilutions of the enrichment culture streaked onto the same medium fortified with 2 % (w/v) Noble Agar (Sigma) after 3 days of incubation at 37 °C. A beige colour and slightly raised, circular, mucoid colony (1-mm diameter) was selected and the culture derived from this, designated strain USBA 355^T. Then it was routinely cultured in a basal medium (BM) supplemented with 20 mM D-glucose and 0.1 % (v/v) yeast extract. BM contained (l^{-1} deionized water) 1 g NH_4Cl, 0.3 g K_2HPO_4, 0.3 g KH_2PO_4, 3 g $MgCl_2.6H_2O$, 0.1 g $CaCl_2.2H_2O$, 0.1 g KCl, 23 g NaCl, and 1 ml Zeikus' trace element solution (Zeikus et al. 1979); the pH of the medium was adjusted to 7.1 with 1 M NaOH. Cells were preserved at −20 °C in BM supplemented with 20 % (v/v) glycerol.

Ecology

The species belonging to the family *Rhodospirillaceae* present wide range of habitats. For example, *Azospirillum* genus was first described as bacterial colonizing plant tissues, but more recently has been reported in broad range of niches, such as oil-contaminated soil and discarded road and fermentative tank. Species from the other genera have the aquatic environment (freshwater, stagnant, anoxic, acid, or saline, petroleum-contamined seawater), ocean, and saline soil as the common habitats although the species *I. limosus* was isolated from respiratory secretions of cystic fibrosis patients and from ginseng field soil.

Azospirillum–Skermanella–Desertibacter–Rhodocista–Dongia–Elstera–Inquilinus

The nitrogen-fixing genera are widespread in agricultural soils, where they are frequently associated with grasses, cereals, and crops (Bally et al. 1983; Day and Dobereiner 1976; Kirchhof et al. 1997; Ladha et al. 1987; Patriquin et al. 1983; Baldani and Baldani 2005) grown especially in soils of tropical and subtropical and temperate regions (Lavrinenko et al. 2010). More detailed, *A. lipoferum* and *A. brasilense* followed by *A. amazonense* were the first three species described and found associated with many cereals and other grasses grown in different regions of Brazil (Magalhães et al. 1983; Baldani and Baldani 2005), while the species named *A. halopraeferens* was found exclusively associated with kallar grass (*Leptochloa fusca*) grown in saline soils in Pakistan (Reinhold et al. 1987). In 1989, Khammas and collaborators isolated the species *A. irakense* using root samples of rice grown in Iraq. Many other species were also found associated with plants: *A. doebereinerae* with washed roots and rhizosphere soil of *Miscanthus sinensis* cv. Giganteus and *Miscanthus sacchariflorus* grown in Germany (Eckert et al. 2001), *A. oryzae* (Xie and Yokota 2005) with rice roots, and *A. melinis* (Peng et al. 2006) with subtropical molasses grass plants collected in China. *A. canadense* (Mehnaz et al. 2007a) and *A. zeae* (Mehnaz et al. 2007b) had its origin in the rhizosphere of corn (Zea mays) plants grown in Canada. In contrast, *A. rugosum* (Young et al. 2008) and *A. picis* (Lin et al. 2009) were enriched from contaminated soils and discarded road tar collected in Taiwan. *A. palatum* (Zhou et al. 2009) was isolated from forest soil in Zhejiang province, China, while *A. thiophilum* had its origin from a sulfide spring in Russia

(Lavrinenko et al. 2010). *A. formosense* was isolated from agricultural soil collected in Taiwan (Lin et al. 2012) and the species *A. humicireducens* from microbial fuel cell in Guangdong, China. On the other hand, the *A. largomobile* (formerly *Conglomeromonas largomobilis* subsp. *largomobilis*) was enriched from a freshwater sample collected in Australia (Sly and Stackebrandt 1999), while *A. fermentarium* was isolated from a fermentative tank in Taiwan (Lin et al. 2013). All species of the *Azospirillum* genus fix nitrogen, except *A. palatum*, a nonvalidated species. The species described more recently are less studied, and the knowledge about their ecological distributions is restricted to the original description of the type species. Therefore, more new data on the ecology of *Azospirillum* species are needed.

The genus *Azospirillum* is widely known as containing free-living nitrogen-fixing plant-growth-promoting rhizobacteria (Okon and Labandera-Gonzalez 1994, Okon and Itzigsohn 1992, and many others), and the carbon source and N-metabolism in this genus make it well adapted to several conditions of the soil and competent to colonize the rhizosphere and in some cases the inner part of the plant tissue (Döbereiner 1992; Steenhoudt and Vanderleyden 2000). They predominantly colonize the root surface, and only a few strains are able to infect plants (Patriquin et al. 1983; Döbereiner et al. 1995). Some *Azospirillum* strains have specific mechanisms to interact with roots and colonize even the root interior, while others colonize the mucigel layer or injured root cortical cell (Baldani et al. 1986). The physiological basis for the observed invasiveness of *A. brasilense* and others is not known. Even species are known which possess enzymes degrading carbon polymer structures of plant host cells, such as in the case of *A. irakense*; a conclusive model of invasiveness is not established (Khammas et al. 1989). Usually, bacteria enter in inner part of the plant using opportunities such as disrupted cortical tissue at lateral root junction, lysed root hairs, or natural cracks on the plant tissue (Steenhoudt and Vanderleyden 2000). Mainly the data on *Azospirillum* interaction with plants are based on a single species and the most studied one: *A. brasilense*. Chemotaxis is the basis of attraction and primary interaction with the host and flagella and frimbriae are involved in the adhesion, anchoring phase and irreversible adsorption that involves polar flagellum (Croes et al. (1993) and extracellular polysaccharide production (Michiels et al. 1991; Skvortsov and Ignatov 1998).

Skermanella species seems to have a large range of habits, being isolated from air, freshwater, and soil. *S. aerolata* was isolated from air samples in Korea (Weon et al. 2007), whereas *S. parooensis* was isolated in a microbial survey of waters of the Paroo Channel, Queensland, Australia. The two other species, *S. xinjiangensis* and *S. stibiiresistens*, were isolated from soil. The former was isolated from desert soil sample from Xinjiang, China (An et al. 2009), while the latter was from coal mine soil and exhibits resistance up to 4 mM Sb(III) in R2A broth (Liu et al. 2011).

Desertibacter roseus was isolated from gamma-irradiated sand sample from the Taklimakan desert, Xinjiang, China. The high resistance of this organism against the lethal actions of DNA-damaging agents including ionizing radiation and ultraviolet light (UV) has been widely reported (Zhang et al. 2007). Radiation-resistant bacteria can survive severe damage from gamma radiation, which implies that they have high DNA repair efficiency (Sghaier et al. 2008) and are adept at detoxifying reactive oxygen species (ROS) (Zhang et al. 2007).

The species of the genus *Rhodocista* have been mainly found in freshwater ecosystems; *Rhodocista* sp. T4 (tentatively *Rhodocista hanoiensis*) was isolated from an enrichment culture from a wastewater pond in Vietnam (Do et al. 2007a). An additional record at NCBI taxonomy browser assigned *Rhodocista* to include *R. xerospirillum* (no standing in the taxonomy) isolated from an upland paddy soil in India (accession no. AM072288, 98.7 % sequence similarity to *R. centenaria*). *Rhodocista* sp. strain JA353 (AM999778, 99.3 % sequence similarity to *R. peckingensis*) was indeed isolated from rhizosphere soil in India. *Rhodocista* sp. strains AR2107 and AT2107 (AJ401217 and AJ401204, respectively) were isolated from microbial mats in Rangiroa Atoll at French Polynesia and share 99.6 % and 99.3 % sequence similarities to *R. centenaria*, respectively. *Rhodocista* sp. strain W38 (KC248056, 96.3 % and 95.7 % sequence similarities to *Azospirillum irakense* and *R. peckingensis*, respectively) was obtained from a water sample, although no further information about this isolate is available. Isolation of *Rhodocista* sp. strain CAJ2-2 from the digestive organ of the Asian lady beetle *Harmonia axyridis* (HQ876734, 92.6 % and 90.7 % sequence similarities to *R. peckingensis* and *Azospirillum irakense*, respectively) is intriguing, although this genus is phylogenetically close to some *Azospirillum*, which are found in association with several plant species. In addition, sequences assigned as *Rhodocista* isolates from saline/marine environments (EU374900, EF650482, and DQ658977) are rather related to other species. In this same sense, the isolate *Rhodocista* sp. M71 (KC464867) originating from rhizosphere soil is more closely related to *Azospirillum*/*Skermanella* genus than to *Rhodocista*. Environmental 16S rRNA clone sequences related to *Rhodocista* are scarce, nevertheless reinforce the preference of this organism to inhabit freshwater environments. Few environmental sequences have >97 % 16S rRNA gene sequence similarity to *R. centenaria*; these are JF739669 (97.5 % sequence similarity) retrieved from soil from peatlands at Indonesia and JF278043 (97.8 % sequence similarity) retrieved from a biofilm grown in flow cell of an urban water canal at Singapore. Environmental clones with 16S rRNA sequences that have low similarity to *Rhodocista* reference sequences include FJ572031 (95.5 % similarity) retrieved from lake water in China. The clone sequences JF412910, JF412944, JF413199, JF413867, JF413890, JF413920, and JQ700914 (88.7–92.1 % 16S rRNA gene sequence similarities to *R. pekingensis*) were retrieved from the aquatic microbial community from the enclosed Cuatro Cienegas Basin in Mexico. Clone sequences retrieved from the vaginal microbiota of HIV-infected women were unusually assigned as *Rhodocista* sp. (JF475184, JF478845, JF487499, JF487508, JF487548, JF487561, JF487568, JF487584, JF487604, JF487608, JF487611, JF487613); nevertheless, such sequences present ~50 % coberture and 81.6–93.3 % similarity

to *R. pekingensis* that has been more closely related to *Rhodovulum*, *Ancalomicrobium*, and *Yangia* species.

The species *Dongia mobilis* LM22^T was found associated with a batch reactor for the treatment of malachite green effluent. In addition, sequences of 16S rRNA with high similarity (94–99 %) with *D. mobilis* were retrieved from a marine hot spring from Kalianda Island, Indonesia (JX047098), in soils, and in the associated Fe–Mn nodules of four regions in China (JX493260, JX493549, JX493661, JX493873, JX494044) and marine coastal sediment in India (KF465352, KF465359).

Elstera litoralis Dia-1^T was isolated from biofilms on stones in the littoral zone of Lake Constance, Germany (Rahalkar et al. 2012). *E. litoralis* was found living associated with diatoms in photic biofilms. It is possible that the extracellular polysaccharides (EPS) produced by the diatoms are an important source of organic carbon for heterotrophic bacteria in such biofilms (Bruckner et al. 2008). So far, there are no environmental clones known closely related to *Elstera.*

The genus *Inquilinus* comprises the species *I. limosus* (Coenye et al. 2002) and *I. ginsengisoli* (Jung et al. 2011). *I. limosus* was isolated from respiratory secretions of cystic fibrosis patients in the USA while *I. ginsengisoli* from ginseng field soil in Pocheon Province, South Korea. Sequences very closely related to *Inquilinus* have been retrieved from polluted soil contaminated by Zn and Cd (Gomez-Balderas et al. 2014), tufa core sample formation (FM177000) water from long-term experimental oligotrophic mesocosms in Cuatro Cienegas in Mexico (JQ701641), soil (JN64589, EF662791, GU300421), and bronchoalveolar lavage fluid from human with cystic fibrosis (DQ188295).

Magnetospirillum–Nisaea–Thalassobaculum–Oceanibaculum–Fodinicurvata–Pelagibius–Tistlia–Phaeospirillum–Telmatospirillum–Defluviicoccus–Tistrella–Constrictibacter–Rhodovibrio–Limimonas

The occurrence of MTB, surprisingly, appears to not be dependent on particularly high concentrations of iron in the environment but on the presence of an oxygen/anoxic interface that represents, in most environments, opposing gradients of oxygen from the surface and reduced compounds (usually reduced sulfur species) in sediments or water columns (reviewed by Lefévre et al. 2013a). Siderophore production was not detected in the culture supernatants of *Magnetospirillum gryphiswaldense* (Schüler and Baeuerlein 1996). However, during the growth of *M. magnetotacticum* MS-1 and *M. magneticum* AMB-1 under iron-rich condition, iron is rapidly assimilated from the medium, and the initial high concentration is reduced to levels comparable to iron-deficient cultures, thereby triggering siderophore production and excretion (Paoletti and Blakemore 1986; Calugay et al. 2003; Calugay et al. 2006). Based on this characteristic and their participation in other biogeochemical processes, Simmons and Edwards (2007) suggested that representatives of this group show a great potential for iron, nitrogen, sulfur, and carbon cycling in natural environments. Since the discovery of MTB, several morphologically and metabolically diverse types of magnetotactic bacteria are detected worldwide and ubiquitously in sediments of freshwater, brackish, marine, and hypersaline habitats as well as in chemically stratified water columns of these environments (reviewed by Frankel 2009; Lefèvre and Bazylinski 2013). Although widely spread in different ecosystems worldwide, only few axenic cultures mostly isolated from aquatic environment are available.

The chemoheterotrophic bacterium *Thalassobaculum litoreum* CL-GR58^T was isolated from coastal seawater in Gori, Korea (Zhang et al. 2008). *Thalassobaculum salexigens* CZ41-10a^T was obtained from seawater samples collected at the SOLA station located in the bay of Banyuls-sur-Mer, France, at a depth of 3 m (Urios et al. 2010). *Nisaea* species were detected in marine denitrification sites (Urios et al. 2008). No further data about their distribution and ecological importance is available.

Both *Oceanibaculum* species were isolated from a PAH-degrading consortium from deep-seawater sample collected from the Indian Ocean and Pacific Ocean. A study to determine the compositions of the bacterial community associated with oil and water phases in a mesothermic oil field showed that sequences representative of *Oceanibaculum* associated to the pooled microbial community are associated with the aqueous phase (Kryachko et al. 2012). Noteworthy, *Oceanibaculum* strains are reported to consume both water-insoluble aromatic hydrocarbons and water-soluble substances, but their primary metabolic substrate(s) must be water soluble, as suggested for other microorganisms found associated with the aqueous phase. However, further studies are necessary to elucidate the ecology of this microorganism under environmental conditions.

Two *Fodinicurvata* strains, designated YIM D82^T and YIM D812^T, were isolated from a Fenggang salt mine in Yunnan, southwest China. No environmental 16S rRNA gene sequences resembling the genus *Fodinicurvata* were deposited in the public databases.

Species of *Pelagibius* genus have been cultured exclusively from marine environment, such as coastal seawater (Korea) or in association with the coral reef *Eunicea fusca* in the USA (accession no. KC545308, 93 % sequence similarity). Environmental clones with 16S rRNA gene sequences assigned as *Pelagibius* have been identified associated to the coral reef *Montastraea faveolata* in Puerto Rico (JQ516378, JQ515728, JQ515699, JQ515532), sediments of the Baltic Sea in Finland (FR820363), groundwater contaminated with chlorinated aliphatic hydrocarbon in the southwest of North China Plain (JQ279035), microbial fuel cell (JF522342), and oil sands tailings pond in Canada (HQ043938). Indeed, DGGE bands of 16S rRNA gene sequences retrieved from a study of diet-induced lesions in the shell of lobsters (JF297201, JF297191) were also assigned as *Pelagibius.* Although most of clone sequences indicate marine environment as the natural niche of *Pelagibius*, the sequences closely related retrieved from groundwater and from a microbial fuel cell suggest that this organism can also inhabit nonmarine

environments. Further information on ecological function of members of this genus is needed.

The aerobic, chemoheterotrophic, nitrogen-fixing bacterium *Tistlia consotensis* USBA 355^T was isolated from samples collected from the saline spring, Salado de Consotá, located in the Colombian Andes (64° 40′ 43.1″ N, 75° 31′ 34.3″ W) (Díaz-Cárdenas et al. 2010). Salado de Consotá is a neutral pH spring with a salt content of 4.5 % (w/v), close to seawater, and the dominant ions are Na^+, Ca^{2+}, and Cl^-.

The habitat of the genus *Phaeospirillum* appears to be freshwater-rich environments, since all type strains were isolated from mud and rhizosphere soil. Additionally, *P. fulvum* was isolated from lake mud in Russia and from the top layer of aquatic sediments in the Netherlands. Information regarding the occurrence of additional isolation of *P. molischianum* strains is scarce, although Paterek and Paynter (1988) identified the presence of *P. molischianum* – by using morphological and physiological approaches – in the anaerobic photosynthetic bacterial community of salt-marsh sediment in the USA. Despite the need for further evidence, Paterek and Paynter (1988) findings suggest the possibility that the bacteria of this genus can also colonize saline environments. The species *P. molischianum* has an additional record at NCBI taxonomy browser assigned as *Phaeospirillum* sp. strain AK-42, isolated from sediment from a lake in India (unpublished results, accession number HF562217, 98.2 % 16S rRNA gene sequence similarity). A single record of a *Phaeospirillum* sp. strain closely related to *P. oryzae* is available at NCBI taxonomy browser, in addition to the isolated strains obtained by Lakshmi et al. (2011b) from paddy soil. *Phaeospirillum* sp. strain MPA1 was obtained in a survey to characterize spiral-shaped purple non-sulfur bacteria from New Zealand thermal environments (unpublished results, accession number AF487433, 98.8 % 16S rRNA gene sequence similarity to *P. oryzae* type strain). Additional records of *Phaeospirillum* strains related to *P. tilakii* at NCBI taxonomy browser were presented by Hisada et al. (2007), who isolated *Phaeospirillum* sp. strain TUT3101 from the microbial community associated to *Chloroflexus* and cyanobacterial mats developing at 50–65 °C from a hot spring in Japan (accession number AB250624, 98.7 % sequence similarity to *P. tilakii* type strain). Environmental 16S rRNA clone sequences listed in the NCBI taxonomy browser – as well as some isolates such as *Phaeospirillum* sp. JA795 (accession no. HF559003) – related to *Phaeospirillum* include ambiguous assignments, as their relateness to *Phaeospirillum* reference sequences is lower than to other bacterial species. In this sense, although low identity to *Phaeospirillum* type species is mostly observed, few environmental sequences will be presented as putative members of this genus. These include KC994874 (identical to KC994669), retrieved from a metagenome survey of biofilms associated with the microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* in a photobioreactor in Germany (Krohn-Molt et al. 2013), with a sequence similarity of 89.2 % to *P. chandramohanii*. Sequences KF523288 and JF340073 were obtained from agricultural soil in China and share 94.7 % sequence similarity to *P. chandramohanii* and 100 % sequence similarity to *P. fulvum*, respectively, even though the similarity to other few reference sequences was the same, probably due to the short size of these sequences (169 and 172 bases, respectively). The sequences JF278044 and HM371256, with 90.3 % and 90.7 % sequence similarities to *P. tilakii*, respectively, were retrieved from biofilm grown on polyethylene terephthalate water canal in Singapore and from the estuarine belt of Narmada River (India), respectively. Sequences DQ252395 and EU682492 with 94.5 % and 91.0 % sequence similarities to *P. fulvum*, respectively, were cloned from the indigenous microflora associated with the decomposition of jute stalks in India and from the surface of coastal marine sediments in Hong Kong, respectively. Sequence GQ257682 with 98.8 % sequence similarity to *P. oryzae* was retrieved from groundwater contaminated with the explosive compound RDX in the USA. In addition, Rudney et al. (2010) performed a metaproteomic survey from the human salivary microbiota and assigned one peptide to *P. molischianum*. Lenchi et al. (2013) used pyrosequencing analysis to assign 16S rRNA gene sequences to *Phaeospirillum* as part of the bacterial community associated with production waters from flooded wells and in injection waters used for flooding Algerian oilfields (temperature range 51–96 °C, salinity range 0.58–21.18 g NaCl l^{-1}). It is clear from the above data that *Phaeospirillum* species have a preference for freshwater environments and that its inhabitation in marine environments needs further experimental evidence.

Telmatospirillum siberiense was isolated from northern acidic waters from peatlands under *Sphagnum* moss mats (Sizova et al. 2007).

Defluvicoccus genus with only one species, *D. vanus* Ben 114^T, was isolated from a sample of biomass from an enhanced biological phosphorus removal activated sludge plant in the Czech Republic. Several clones closely related to *Defluvicoccus* were detected in marine samples (JQ516397, JQ793263, FN687095, and JN210804); others are from activated sludge (KC797679) or contaminated soils (HE974846) and gypsum-treated oil sands tailings pond (HQ044218, HQ041215, HQ042479, HQ042046); some are also related to human diseases as cystic fibrosis sinuses (JQ794646) and cutaneous microbiome (KF509289).

The type strain *Tistrella mobilis* IAM 14872^T was isolated from wastewater in Thailand and showed a good ability in producing polyhydroxyalkanoates (PHA) efficiently (Shi et al. 2002). *Tistrella bauzanensis* BZ78^T was isolated from soil containing high levels of heavy oil and heavy metals located in Bozen, South Tyrol, Italy (Zhang et al. 2011).

The unique species of *Constrictibacter*, *C. antarcticus* 262-8^T, was isolated from a cavity within white rock collected in the Skallen region of Antarctica. Environmental 16S rRNA clone sequences related to the genus *Constrictibacter* have not been deposited.

Limimonas is a genus of extremely halophilic microorganism in this family isolated from a saline mud sample collected from the hypersaline Lake Aran-Bidgol in Iran, and its characterization led to the description of the species

Limimonas halophila (Amoozegar et al. 2013). This microorganism is able to tolerate salt concentrations in the range of 15–30 % (w/v).

Rhodospirillum–Pararhodospirillum–Phaeovibrio–Roseospira–Rhodospira–Novispirillum–Marispirillum–Insolitispirillum–Caenispirillum–Thalassospira–Magnetospira–Magnetovibrio–Ferrovibrio

Stagnant and anoxic freshwater that is exposed to the light is the habitat of *Rhodospirillum* and most *Pararhodospirillum* species (Imhoff 2005b; Kumar et al. 2008; Lakshmi et al. 2014). A number of culture-dependent and culture-independent studies have shown that anoxic zones of saline or hypersaline environments such as seawater, brines, and salt lakes that are exposed to the light are the habitat of *Rhodovibrio* species (Nissen and Dundas 1984; Mack et al. 1993; Sørensen et al. 2005; Blazejak et al. 2006; Maturrano et al. 2006; Boutaiba et al. 2011; Atanasova et al. 2012; Makhdoumi-Kakhki et al. 2012; Liu and Liu 2013; Ntougias 2014; Schneider et al. 2013). Additional information on strains of *Phaeovibrio* is lacking, and the description of the species is the only source of ecological distribution (Lakshmi et al. 2011b). Environmental 16S rRNA sequences related to *Phaeovibrio* genus have not been deposited in the databases. Most closely related sequences are from *Rhodospirillum* (~93 % 16S sequence similarity).

Roseospira species are spiral-shaped purple nonsulfur bacteria which are slightly to moderately halophilic. The main habitat seems to be anoxic sediments in coastal waters such as brackish lagoons and saline springs. *R. navarrensis* was isolated from a microbial mat in Tetiaroa Atoll, Society Islands (Guyoneaud et al. 2002).

The description of the type species of *Rhodospira mediosalina* is the only source of ecological distribution (Pfennig et al. 1997), and information on additional strains of *Rhodospira* is lacking since environmental 16S rRNA sequences related closely to the genus *Rhodospira* have not been deposited in the databases. The available 16S rRNA gene sequences are HE797786 (94.5 % similarity), retrieved from anoxic photosynthetic biofilm from brackish water in France, and AM691104 (94.3 % similarity), retrieved from a hypersaline spring in Canada.

Marispirillum indicum strain B142^T is a marine bacterium isolated after enrichment from crude oil-contaminated seawater (Lai et al. 2009a). At the time of characterization, it showed the highest 16S rRNA gene sequence similarity (97.1 %) with an uncultured proteobacterial clone isolated from subsurface water from the Kalahari Shield in South Africa, but no further data about species distribution is available.

Species of *Novispirillum* and *Insolitispirillum* were isolated from primary oxidation pond water (Pretorius 1963; Hylemon et al. 1973).

The species of the genus *Caenispirillum* have been isolated from aquatic ecosystems; the species *C. bisanense* was isolated from sludge sample collected from the wastewater treatment plant of a dye works at Daegu, Korea, while *C. salinarum* from a sediment sample collected from a solar saltern at Kakinada, Andhra Pradesh, India. Uncultured 16S rRNA clone sequences related to *C. bisanense* K93 have been deposited in the NCBI databases. KF500423 (96 % sequence similarity) was obtained from the shrimp culture pond sediment in India and JF421153 (98 % sequence similarity) from a petroleum-contaminated saline–alkali soil with phytoremediation in China (unpublished).

Thalassospira lucentensis was isolated from offshore seawater samples obtained at 38° 06′87″ N, 0° 05′ 23″ W (21 nautical miles off Alicante, Spain) from a depth of 100 m by means of a Niskin bottle. For its isolation, enrichments in a continuous culture reactor designed to maintain extremely oligotrophic conditions such as those assumed to be found in the open ocean were carried out (López-López et al. 2002). *Thalassospira xiamenensis* M-5^T was isolated from surface water collected from a waste-oil pool at an oil storage dock in the city of Xiamen, Fujian Province, on the eastern coast of China (Liu et al. 2007). This seawater-based waste-oil pool had suffered long-term pollution with crude oil. *Thalassospira profundimaris* WP0211^T was retrieved from a deep-sea sediment sample, which was collected by a multi-core sampler from the West Pacific (region 973, station WP02-3; 147° 58′ 55″ E, 12° 59′ 54″ N; water depth 4,480 m) (Liu et al. 2007). *Thalassospira tepidiphila* 1-1B^T was isolated from petroleum-contaminated seawater in a beach-simulation tank during a bioremediation experiment (Kodama et al. 2008). *Thalassospira xianhensis* P-4^T originated from a saline soil contaminated by crude oil, collected from Xianhe, Shandong Province, China (Zhao et al. 2010). In 2011, Hütz et al. reported that bacteria affiliated with the genus *Thalassospira* were found to constitute a regular, low-abundance member of the bacterioplankton that can be detected throughout the water column of the Eastern Mediterranean Sea. A representative (strain EM) was isolated in pure culture and exposed to a strong positive chemotaxis toward inorganic phosphate that was induced exclusively in phosphate-starved cultures. Although *Thalassospira* sp. represents only up to 1.2 % of the total bacterioplankton community in the water column of the Eastern Mediterranean Sea, its chemotactic behavior potentially leads to an acceleration of nutrient cycling and may also explain the persistence of marine copiotrophs in this extremely nutrient-limited environment (Hütz et al. 2011). The halotolerant bacterium *Thalassospira permensis* SMB34^T was isolated from a naphthalene-utilizing bacterial consortium obtained from primitive technogeneous soil formed on salt-mine spoils at the Verchnekamsk deposit of potassium–magnesium salts (Berezniki, Perm region, Russia); this was located at the place of the ancient Permian sea about 280 Ma ago. In contrast to the majority of organisms of the genus *Thalassospira* which are marine inhabitants, it might be speculated that *Thalassospira permensis* or its ancestor also originally inhabited the ocean and then survived, being trapped within salt crystals, subsequently evolving as a terrestrial bacterium, together with other members of the local microbial community (Plotnikova et al. 2011). Recently, two new species

of marine bacteria were isolated from a piece of sunken bamboo in the marine environment in Japan (Tsubouchi et al. 2014): *Thalassospira alkalitolerans* MBE#61^T and *Thalassospira mesophila* MBE#74^T. These isolations were the result of searching for microbes that show high metabolizing activity against lignin-related compounds; indeed, they metabolize effectively lignin-related aromatic compounds.

For example, *Magnetovibrio blakemorei* strain MV-1 was isolated from sulfide-rich sediments in a salt marsh near Boston, MA (Bazylinski et al. 1988). Like many marine bacteria, strain MV-1 is euryhaline but has a growth requirement for salts, as it will not grow at very low concentrations of ASW or in freshwater media (Kaye and Baross 2004; Bazylinski et al. 2013). Magnetospira type strain "MV-4" (magnetic vibrio number 4) was isolated from a salt marsh in Woods Hole (Meldrum et al. 1993; Williams et al. 2012), but in contrast to their closest characterized magnetotactic relative, *M. blakemorei* and *M. thiophila* can use only a relatively small number of organic acids as carbon and energy sources.

The species *Ferrovibrio denitrificans* is able to use FeS, $FeSO_4$, and $FeCO_3$ as Fe(II) sources for lithotrophic growth and unable to use NO_2, ClO_4^-, S^0, $S_2O_3^{-2}$, and $Fe(OH)_3$ as electron acceptors for anaerobic growth. The incrustation phenomenon, which occurs not only at laboratory conditions but also in the natural habitats, indicates that this is a natural way of living under anaerobic conditions (Sorokina et al. 2012). No environmental 16S rRNA gene sequences resembling the genus *Ferrovibrio* are available yet.

Pathogenicity and Clinical Relevance

Analysis of the species belonging to the family *Rhodospirillaceae* indicates the presence of species that have been detected as opportunistic human pathogens. On the other hand, no plant related pathogenicity has been reported for the genera. Despite the large spectrum of antibiotic resistance among the species, the majority of them are nonpathogenic to human or no information is available.

Opportunistic Human Pathogen

Azospirillum – Recent analyses of 16S rRNA gene sequences and phenotypic characteristics suggested that *R. fauriae* was closely related to *Azospirillum brasilense* (Cohen et al. 2004; Han et al. 2003; Weyant and Whitney 2005). *Roseomonas* species are opportunistic pathogens and have been isolated from a range of human infections including septicemia, occurring primarily in patients with underlying medical conditions (Dé et al. 2004; Struthers et al. 1996). One single report was based on four phenotypically similar bacterial strains isolated from fungal, plant, and human sources that were identified as *Azospirillum* species (Cohen et al. 2004). Strains RC1 and LOD4 were isolated from the mycelium of the apple root pathogen *Rhizoctonia solani* AG 5 and from the rhizosphere of wheat grown in apple orchard soil, respectively. Strains C610 and F4626 isolated from human wounds were previously misclassified as *Roseomonas* genomospecies 3 and 6. All four strains demonstrated close similarities in 16S rRNA gene sequences, having greater than or equal to 97 % identity to *A. brasilense* type strain ATCC 29145 and <90 % identity to *Roseomonas gilardii*, the *Roseomonas* type strain. Authors also report that their results indicate a wide range of potential sources for *Azospirillum* spp. with the isolation of *Azospirillum* spp. from human wounds warranting further investigation. In 2013 another case was described related to *Azospirillum* infection in an immunocompetent middle-aged female manifesting as granulomatous tenosynovitis on the right hand (Serelis et al. 2013). A reclassification of *Roseomonas fauriae* and R. genomospecies 6 into the *A. brasilense* species has been suggested, mostly based on very close 16S rRNA gene similarities (Helsel et al. 2006). A case of peritonitis in a 65-year-old woman with ESRD was published in 2000 (Bibashi et al. 2000). McLean et al. (2006) described a 3-month-old girl suffering from stage III neuroblastoma who had experienced a 2-day history of fever, vomiting, and diarrhea. Blood cultures drawn through her catheter recovered *R. fauriae*. These cases may represent the first reported opportunistic human infections caused by the *Azospirillum*-related bacteria. Hogue et al. (2007) described that the overall mortality rate associated with these pink-pigmented bacteria (similar to *A. brasilense*) is essentially negligible and although a few deaths have been recorded (most notably in association with *Roseomonas*), these bacteria have not been conclusively demonstrated to be involved in the patient's demise. The relatedness of *R. fauriae* and *A. brasilense* is still a matter of debate, and whole genome comparisons are in progress to clarify this issue (A. Hartmann, unpublished results).

Inquilinus – *I. limosus* has been documented mainly in CF patients and was sometimes accompanied with exacerbation or respiratory decline. The pathogenic potential, the impact on respiratory function, and the risk of patient-to-patient transmission of *I. limosus* are still unclear, and the environmental habitat of this bacterium is unknown. It has been isolated in the USA, Germany, France, the UK, and Spain (Pitulle et al. 1999; Coenye et al. 2002; Schmoldt et al. 2006; Cooke et al. 2007; Wellinghausen et al. 2005; Salvador-García et al. 2013). *I. limosus* may represent a new threat to CF patients, as it has a mucoid phenotype (i.e., production of EPS), multiresistance to a wide number of antibiotics, and the ability to persist in the respiratory tract. In agreement with Kuttel et al. (2012), *I. limosus* secretes two uniques exopolysaccharides (EPS), α-(1→2)-linked mannan and β-(1→3)-linked glucan. They demonstrated that the mixture of these two EPS is able to inhibit the lytic action of antimicrobial peptides of the innate immune system and it is possible that the coexistence of the two different secondary structures could enhance this biological activity. Additionally, these EPS form an effective barrier to penetration of chemically reactive biocides, antibiotics, and antimicrobial agents.

Antibiotic Sensitivity

Azospirillum – Wild-type strains of *Azospirillum lipoferum* and *Azospirillum brasilense* were found to be naturally resistant to penicillin, a resistance that was attributed to β-lactamase activity (Franche and Elmerich 1981). Antibiotic resistance is also studied in *Azospirillum*, for example, the β-lactam antibiotics that cause cell envelope stress by inhibiting peptidoglycan biosynthesis, and nalidixic acid that inhibits DNA gyrase activity is present in *A. brasilense*. *Azospirillum lipoferum* RG20 was found to be naturally resistant to penicillins and cephalosporins (Boggio et al. 1989). This strain showed high resistance to benzylpenicillin, ampicillin, carbenicillin, cephalosporin C, cephaloridine, cephalothin, and cefotaxime (MIC 1,000 μg ml^{-1}), whereas it was more susceptible to oxacillin and cloxacillin (MIC = 200 μg ml^{-1}) (Boggio and Roveri 2003). *A. amazonense* strains showed similar resistance to *A. lipoferum* and *A. brasilense* such as penicillin and relative tolerance to chloramphenicol and tetracycline (Magalhães et al. 1983).

Inquilinus – *I. limosus* is sensitive to imipenem, ciprofloxacin, and meropenem, but resistant to aminoglycosides, piperacillin–tazobactam, cefotaxime, ceftazidime, cefepime, aztreonam, kanamycin, gentamicin, amikacin, fosfomycin, doxycycline, and colistin (Bittar et al. 2008; Chiron et al. 2005). This bacterium is multiresistant to several antimicrobial drugs, particularly colistin, which is widely used for treatment for *P. aeruginosa* colonization (as was the case for our four patients). Bittar et al. (2008) hypothesize that this bacterium is selected during the evolution of the disease.

Constrictibacter – According to ATB VET system (bioMérieux), the type strain of the genus is resistant to erythromycin, lincomycin, pristinamycin, tylosin, co-trimoxazole, sulfamethizole, nitrofurantoin, fusidic acid, metronidazole, penicillin, amoxicillin, oxacillin, cephalothin, cefoperazone, chloramphenicol, and tetracycline, but sensitive to colistin, flumequine, oxolinic acid, enrofloxacin, rifampicin, amoxicillin/clavulanic acid, streptomycin, spectinomycin, kanamycin, gentamicin, apramycin, and doxycycline.

Desertibacter–Dongia–Ferrovibrio–Desertibacter roseus is susceptible to erythromycin, vancomycin, streptomycin, acheomycin, and penicillin. *Dongia mobilis* is susceptible to vancomycin, gentamicin, carbenicillin, polymyxin B, streptomycin, kanamycin, ampicillin, neomycin, chloramphenicol, and penicillin and weakly sensitive to tetracycline, erythromycin, novobiocin and rifampicin, while the species *Ferrovibrio denitrificans* is sensitive to amikacin, lincomycin, neomycin, polymyxin, streptomycin, rifampicin, and nalidixic acid. The last species is resistant to ampicillin, bacitracin, vancomycin, gentamicin, kanamycin, mycostatin, novobiocin, penicillin, and tetracycline.

Novispirillum and *Insolitispirillum* – Strains from these species show susceptibility to streptomycin, chloramphenicol, gentamicin, tetracycline, kanamycin, and neomycin, but not to penicillin G, ampicillin, cephalothin, and oleandomycin (Ding and Yokota 2002; Yoon et al. 2007b). In addition, representatives of the genus *Novispirillum* are susceptible to novobiocin, only weakly susceptible to polymyxin B, and resistant to carbenicillin, but each subspecies can be differentiated based on their characteristic lincomycin susceptibility. Both species of the genus *Insolitispirillum* present susceptibility to polymyxin B and carbenicillin.

Marispirillum – *M. indicum* is susceptible to carbenicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, minocycline, neomycin, norfloxacin, ofloxacin, rifampicin, ceftriaxone, streptomycin, and doxycycline and resistant to ampicillin, cefalexin, cefazolin, cefoperazone, cefradine, clindamycin, co-trimoxazole, furazolidone, lincomycin, metronidazole, oxacillin, penicillin G, piperacillin, polymyxin B, tetracycline, and vancomycin (Lai et al. 2009a).

Oceanibaculum – The *Oceanibaculum* species differ from each other according to their characteristic sensitivity or resistance to several antibiotics (Lai et al. 2009b; Dong et al. 2010). The species are resistant to cefalexin, cefazolin, cefradine, clindamycin, erythromycin, furazolidone, lincomycin, metronidazole, norfloxacin, ofloxacin, oxacillin, and vancomycin. *Oceanibaculum indicum* is susceptible to ciprofloxacin, co-trimoxazole, kanamycin, neomycin, polymyxin B, and streptomycin, while *O. pacificum* is susceptible to ampicillin, carbenicillin, chloromycetin, ciprofloxacin, gentamicin, kanamycin, neomycin, penicillin G, polymyxin B, rifampicin, rocephin, streptomycin, tetracycline, and vibramycin.

Limimonas – *L. halophila* is susceptible to nitrofurantoin, novobiocin, and rifampicin, but resistant to amikacin, amoxicillin, bacitracin, carbenicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, polymyxin B, streptomycin, tetracycline, cephalothin, nalidixic acid, tobramycin, and penicillin G (Amoozegar et al. 2013).

Rhodospirillum–Rhodovibrio–Pararhodospirillum – *R. rubrum* is resistant to penicillin, ampicillin, carbenicillin, and nalidixic acid, while it is sensitive to triclosan, chloramphenicol, tetracycline, kanamycin, streptomycin, and gentamicin (Weaver et al. 1975; Pycke et al. 2010), while *Rhodovibrio salinarum* is sensitive to chloramphenicol, tetracycline, kanamycin, streptomycin, rifampicin, and spectinomycin (Borghese et al. 2001). *Rhodovibrio* sp. (isolates GV-2 and GV-3) are sensitive to antimicrobial substances produced by the halophilic archaea *Haloferax* (Atanasova et al. 2013). *Pararhodospirillum photometricum* is sensitive to penicillin (Imhoff 2005b).

Thalassospira – The antibiotic sensitivity varies among the *Thalassospira* species. *T. xiamenensis* is sensitive to chloramphenicol, norfloxacin, furazolidone, co-trimoxazole, ofloxacin, midecamycin, ceftriaxone, polymyxin B, doxycycline, tetracycline, neomycin, kanamycin, gentamicin, amikacin, erythromycin, minocycline, carbenicillin, cefalexin, cefradine, ciprofloxacin, and cefuroxime, but resistant to cefoperazone, clindamycin, vancomycin, ceftazidime, cefazolin, penicillin, oxacillin, ampicillin, and piperacillin (Liu et al. 2007). *T. profundimaris* is sensitive to chloramphenicol, norfloxacin, furazolidone, co-trimoxazole, ofloxacin, midecamycin, ceftriaxone, polymyxin B, doxycycline, tetracycline, neomycin, kanamycin, gentamicin, amikacin, erythromycin, minocycline, carbenicillin, cefalexin, ciprofloxacin, cefoperazone, vancomycin, ceftazidime, cefazolin, penicillin, oxacillin, ampicillin, and piperacillin but

resistant to clindamycin, cefuroxime, and cefradine (Liu et al. 2007). *T. xianhensis* is sensitive to ampicillin, cephalothin, clarithromycin, clindamycin, erythromycin, penicillin, and vancomycin, but resistant to ceftriaxone, cefotaxime, ciprofloxacin, gentamicin, streptomycin, and tetracycline (Zhao et al. 2010). *T. alkalitolerans* is sensitive to kanamycin, gentamicin, chloramphenicol, penicillin G, polymyxin B, and carbenicillin, slightly sensitive to tetracycline, streptomycin, novobiocin, rifampicin, and erythromycin, but resistant to bacteriocin, vancomycin, neomycin, ampicillin, and lincomycin (Tsubouchi et al. 2014). *T. mesophila is* sensitive to kanamycin, neomycin, novobiocin, gentamicin, chloramphenicol, ampicillin, penicillin G, and carbenicillin, slightly sensitive to tetracycline, streptomycin, rifampicin, erythromycin, and polymyxin B, and resistant to bacteriocin, vancomycin, and lincomycin (Tsubouchi et al. 2014).

Thalassobaculum–Tistrella–Tistlia – Thalassobaculum litoreum is sensitive to streptomycin, gentamicin, vancomycin, kanamycin, penicillin, erythromycin, tetracycline, chloramphenicol, ciprofloxacin, and ampicillin (Zhang et al. 2008). *Tistrella bauzanensis* is sensitive to kanamycin, amikacin, rifampicin, and neomycin but resistant to chloramphenicol, tetracycline, and erythromycin (Zhang et al. 2011), while *Tistlia consotensis* is sensitive to ampicillin, streptomycin, chloramphenicol, tetracycline, and penicillin (Díaz-Cárdenas et al. 2010).

Application

The *Rhodospirillaceae* family is formed by bacterial species with very diverse metabolic functions, but so far, only *Azospirillum* species have already been applied as biofertilizer in the agriculture, while other genera have been exploited for industry and environmental application. Many species of other genera have shown biotechnological potential as suggested by the genome sequencing analyses, while others have not been exploited yet.

Agricultural Application

Members of the genus *Azospirillum* are commonly used in the field as biofertilizers and other field tests in many countries such as (alphabetical order): Argentina, Brazil, Colombia, Egypt, France, Israel, Turkey, South Africa, and many others (Okon and Labandera-Gonzalez 1994; Dobbelaere et al. 2001; Turan et al. 2012). Most of these applications are linked to the oldest species described, especially the commercial inoculants based on *A. brasilense* strains. At the beginning, only cereals were tested but recently its application is spread in co-inoculation of rhizobia in legumes such as soybean (Juge et al. 2012), *Vicia sativa* (Star et al. 2012), and beans. Also its single application was extended to other cultures such as lettuce (Fasciglione et al. 2012), cactus (Lopez et al. 2012) trees (Leyva and Bashan 2008), and even in microalgae (Choix et al. 2012).

Species from the *Azospirillum* genus has the capacity to produce several plant growth regulators such as abscisic acid, ethylene, gibberellic acid, indole 3-acetic acid, zeatin (Tien et al. 1979; Bashan et al. 2004), nitric oxide (NO) (Steenhoudt et al. 2001), polyamides (Thuler et al. 2003), and siderophores (Saxena et al. 1986). However, the bacteria also produce a wide variety of these signaling molecules and influence plant growth. *Azospirillum* is a well known bacterium that produces high amounts of auxins "in vitro," and different pathways are involved in this production but not in all species the indole production was determined such as *A. humicireducens* (Zhou et al. 2013). The best characterized pathway in *Azospirillum* auxin production is via indole-3-acetamide (IAM) and indole-3-pyruvate (IPyA) intermediates, both well described by Spaepen et al. (2007) to generate osmotic stress response in plants (Aziz et al. 1997) and solubilize phosphates (Seshadri et al. 2000). Several modifications of plant architecture and physiology of the cells were recorded: increased respiration rates of root cell in plants inoculated with *Azospirillum* such as maize and sorghum (Okon and Kapulnik 1986; Sarig et al. 1992); membrane proton efflux in wheat; increased mineral uptake (Bashan et al. 1989); and hydrolysis of conjugated phytohormones and flavonoids (Volpin et al. 1996). The main effect visually observed after inoculation is the root proliferation. This effect causes enhancement of the root surface activity to the plant that increases mineral nutrients and water (Spaepen et al. 2007). *Azospirillum* can contribute some nitrogen in cellulose-decomposing mixed cultures with Cellulomonas gelida (Halsall and Gibson 1985) or through the ability of straw decomposition by some specific N_2-fixing *Azospirillum* sp. isolates (Halsall et al. 1985). Another feature of *Azospirillum* is related to the accumulation of polybetahydroxyalkanoates (PHA) in the cells. It appears to be an important trait for root colonization and plant growth promotion, especially for A. brasilense inoculants where cells with high amounts of PHA play a better capacity of plant growth in field experiments carried out in South America with maize and wheat. These assays revealed that increased crop yields were consistently obtained using inoculants prepared with PHA-rich *Azospirillum* cells (Dobbelaere et al. 2001; Helman et al. 2011).

In the last years, *Azospirillum* spp. have been applied in consortium with other PGPR bacteria such as *Pseudomonas* in maize (Salamone et al. 2012), other nitrogen-fixing bacteria in sugarcane (Oliveira et al. 2006), or with mycorrhizal fungi (Couillerot et al. 2013). It has also been used as a biocontrol component in cotton inoculation (Marimuthu et al. 2013), or reported its activity in stressed conditions such as drought (Abbasi and Zahedi 2013; Bano et al. 2013) and saline conditions (Nakade 2013).

Two recently reported new species of the genus *Thalassospira*, *T. alkalitolerans* and *T. mesophila*, metabolize effectively lignin-related aromatic compounds, and therefore, their use is expected by the biochemical industries in the degradation of plant biomass (Tsubouchi et al. 2014).

Industrial Application

The species *Rhodospirillum rubrum* provides several potential biotechnological applications for the industry. It produces

polyhydroxyalkanoates (PHAs) composed of both short- and medium-chain-length monomers, and it can produce up to 50 % (dry weight) of the cell in PHAs (Brandl et al. 1989; Liebergesell et al. 1991; Ulmer et al. 1994). Because of its metabolic versatility, R. rubrum offers the potential for converting many different carbon sources to PHA (Do et al. 2007b; Smith et al. 2008). *R. rubrum* also provides the potential for hydrogen fuel production. It may produce H_2 growing photoheterotrophically using, for example, malate as carbon source and electron donor; growing anaerobically using fructose, lactate, acetate, or succinate as carbon source, and dimethyl sulfoxide (DMSO) or trimethylamine-N-oxide (TMAO) as electron donor; or fermenting fructose or pyruvate when an external electron acceptor is absent (Gest and Kamen 1949; Uffen 1973; Schultz and Weaver 1982). *R. rubrum* has been genetically modified in order to increase its capacity to produce hydrogen (Kars and Gündüz 2010); in this way, a Hup plus (uptake hydrogenase) mutant has been generated, and this showed a significant increase in H_2 production (Kern et al. 1994). Through an applied approach, *R. rubrum* has been grown on synthesis gas in order to produce both H_2 and PHA (Do et al. 2007b). *R. rubrum* offers the potential for production of the food additive coenzyme Q10 (CoQ10) and the carotenoid lycopene, which is also a natural colorant (Tiana et al. 2010; Wang et al. 2012). R. rubrum has been used as a heterologous expression system of membrane proteins (Butzin et al. 2010). No data are available for application of neither *Pararhodospirillum* nor *Rhodovibrio* species.

The biologically derived magneto-functional inorganic nanocrystals of magnetite have been used as carriers for enzymes and in immunoassay methods including those involving nucleic acids, antibodies, and targeted delivery of anticancer drugs (Naresh et al. 2012). The process of magnetosome synthesis has been used to develop novel tools for ligand–receptor interaction analysis, such as those applied for TRAb immunoassay in Graves' disease patients (Sugamata et al. 2013).

Ghosh et al. (2012) reported on lactose hydrolysis of milk by crude enzyme extracted from deep marine psychrophilic strain *Thalassospira* sp. 3SC-21. They showed that 80.18 % of lactose was hydrolyzed after 43 min of incubation at 20 °C, within a pH range of 6.5–7.5. This activity was also observed at 10 °C (65 %), indicating that this enzyme is useful at refrigerated temperature. From these findings, they conclude that *Thalassospira* sp. 3SC-21 is a source for the production of cold active β-galactosidase enzyme that can be applicable at cold temperature and might be considered in food industry including dairy industry on a larger scale.

Tistrella mobilis and *Tistrella bauzanensis* produce didemnins (antineoplastic agents) via unique post-assembly line maturation process (Xu et al. 2012). Complete genome sequence analysis of *T. mobilis* strain KA081020-065 revealed the putative didemnin biosynthetic gene cluster (*did*) on the megaplasmid pTM3. The *did* locus encodes a 13-module hybrid non-ribosomal peptide synthetase–polyketide synthase enzyme complex organized in a collinear arrangement for the synthesis of the fatty acylglutamine ester derivatives didemnins X and Y rather than didemnin B. Imaging mass spectrometry of *T. mobilis* bacterial colonies captured the time-dependent extracellular conversion of the didemnin X and Y precursors to didemnin B, in support of an unusual post-synthetase activation mechanism. Significantly, the discovery of the didemnin biosynthetic gene cluster may provide a long-term solution to the supply problem that presently hinders this group of natural products and pave the way for the genetic engineering of new didemnin congeners.

The bacterium *Tistlia consotensis*, isolated from a saline spring in the Colombian Andes, represents an interesting environmental model to be compared with extremophiles or other moderate organisms (Díaz-Cárdenas et al. 2010). To explore the halotolerance molecular mechanisms of the *T. consotensis*, through a proteogenomic approach, a large number of proteins were found to be produced in greater amounts when cells were cultivated in either hypo-osmotic or hyper-osmotic conditions (Rubiano-Labrador et al. 2014).

Other genera present biotechnological potential such as the *Phaeospirillum* species that have the photosynthetic apparatus of phototrophic bacteria (e.g., Mascle-Allemand et al. 2010) and evolutionary importance due its high amount of genes related to signal transduction (Duquesne et al. 2012). The close phylogenetic relationship of *Rhodocista centenaria* and *Rhodocista pekingensis* to *Azospirillum irakense/Azospirillum amazonense* also indicates scientific importance to this genus due to cyst-forming ability (Lu et al. 2010).

Environmental Application

New features are arising on the environmental application for the genus *Azospirillum*. *A. brasilense* strains Sp 7 and Sp245 are able to reduce selenium (IV) to elementary selenium (Tugarova et al. 2013), while *A. thiophilum* has the capacity for lithotrophic growth coupled oxidation for reduced sulfur compounds (Frolov et al. 2013).

Defluvicoccus vanus has been identified as glycogen-accumulating organisms (GAOs) (Meyer et al. 2006; Burow et al. 2007). According to Burow et al. (2007), the activity of glycogen-accumulating organisms (GAOs) in enhanced biological phosphorus removal (EBPR) wastewater treatment plants has been proposed as one cause of deterioration of EBPR. GAOs possess the ability to take up volatile fatty acids (VFA) under anaerobic conditions and convert them to polyhydroxyalkanoates (PHA), which are stored until the following aerobic period and then oxidized to CO_2 or transformed to glycogen.

Magnetotaxis and magnetosome productions are special traits shared by some magnetotactic and magnetosome bacteria that have been extensively employed for the development of new technology in the nanotechnology field. Besides the important role of nanobiotechnology to medicine, its application has already become a matter of study into many other applied sciences, such as environmental engineering and agricultural science. Ginet et al. (2011) demonstrated that functionalized magnetic nanoparticles efficient as a reusable nanobiocatalyst

for pesticide bioremediation in contaminated effluents can be produced by genetically modified magnetotactic bacteria.

The strains *Thalassospira xiamenensis* M-5^T and *Thalassospira profundimaris* WP0211^T were isolated separately from bacterial consortia that used hydrocarbons as their sole carbon sources; neither strain could degrade any of the hydrocarbons tested in their characterization tests (Liu et al. 2007). However, the analysis of 16S rRNA gene sequences obtained from various samples from marine environments has revealed that these two species and their close relatives were frequently detected in petrol-oil-degrading consortia. This suggests they may play some role in the degradation of petroleum hydrocarbons (Liu et al. 2007). However, no further study was done.

The strain T. profundimaris WP0211^T cannot use pyrene as the sole carbon source for growth, though it was isolated from a pyrene-degrading consortium. Its genome sequence analysis revealed a gene encoding a ring hydroxylating dioxygenase and therefore supports further characterization (Lai and Shao 2012b).

Thalassospira tepidiphila is considered to play important roles in marine spilled-oil bioremediation (Kodama et al. 2008). Polycyclic aromatic hydrocarbons (PAHs), hydrocarbons containing two or more fused aromatic rings, are released into the marine environment as a result of various anthropogenic activities such as marine seepage and accidental discharges during the transport and disposal of petroleum products and the use of fossil fuels (Sohn et al. 2004). Some PAHs are highly carcinogenic, genotoxic, and cytotoxic to marine organisms and may be transferred to humans through seafood consumption (Menzie et al. 1992). Therefore, removal of PAHs from contaminated marine environments is of considerable importance, hence the importance of isolating PAH-degrading bacteria such as *Thalassospira xianhensis* (Zhao et al. 2010). Recently, Um et al. (2013) reported that chemical investigation on the marine unicellular bacterium *Thalassospira* sp. led to the discovery of a new peptide, thalassospiramide G, along with thalassospiramides A and D. The peptides are structurally unique, with unusual γ-amino acids, such as 4-amino-5-hydroxy-penta-2-enoic acid (AHPEA) and 4-amino-3,5-dihydroxy-pentanoic acid (ADPA). In addition, thalassospiramide G bears a 2-amino-1-(1*H*-indol-3-yl) ethanone (AIEN) moiety, which is quite rare in a natural product. In the LPS-induced NO production assay, thalassospiramide D displayed more significant inhibition of NO production than thalassospiramide A, indicating its potential as an anti-inflammatory agent. The structural novelty and the biological activity of the secondary metabolites isolated from this marine α-proteobacterial taxonomic group suggest that marine unicellular bacteria, particularly α-proteobacteria, which have been overlooked in the search for new bioactive compounds, could potentially provide a rich source of chemically and pharmaceutically interesting natural products (Um et al. 2013).

Tistrella mobilis IAM 14872^T produces polyhydroxyalkanoates (PHAs) as intracellular granules (Shi et al. 2002). PHA is a biodegradable, biocompatible, and thermoplastic material, which has a potential role as a so-called biomass transducer, i.e., it can be used for the microbial transformation of carbohydrate feedstock via PHA into chiral depolymerization products (Seebach and Zuger 1984) or small-molecule organic chemicals by pyrolysis (Anderson and Dawes 1990). So far, these biodegradable PHAs, however, are not priced competitively mainly because of the high cost, which lies in both the use of glucose as a fermentation feedstock and the low product yield.

Tistrella sp. strain ZP5, isolated from soil samples contaminated with polycyclic aromatic hydrocarbon (PAH)-containing waste (Zhao et al. 2008), cannot degrade phenanthrene individually, but it can increase the speed of phenanthrene degradation together with *Sphingomonas* sp. ZP1. Such two strains may be useful for bioremediation applications.

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23 The Family *Rickettsiaceae*

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Abstract
The *Rickettsiaceae* family is composed of two genera, *Rickettsia* and *Orientia*, which are obligate intracellular bacteria that belong to the order *Rickettsiales*. The species of these genera are divided into two groups based on antigenic, molecular, and ecological patterns: (1) the typhus group, composed of the species *Rickettsia prowazekii, Rickettsia typhi*, and *Orientia tsutsugamushi*, which are transmitted by lice, fleas, and mites, respectively; and (2) the spotted fever group (SFG), which is composed of more than 23 valid species. The transmission of great majority of species in SFG is associated with ticks, with the exception of *Rickettsia felis* and *Rickettsia akari*, which are associated with fleas and mites, respectively. Other *Rickettsia* species, such as *Rickettsia bellii* and *Rickettsia canadensis*, are not included in either of the two groups. In SFG, at least 12 species of *Rickettsia* cause infections in humans (*Rickettsia rickettsii, Rickettsia conorii, Rickettsia africae, Rickettsia parkeri, Rickettsia australis, Rickettsia honei, Rickettsia sibirica, Rickettsia japonica, Rickettsia massiliae, Rickettsia aeschlimannii, R. akari*, and *R. felis*). However, species of nonpathogenic rickettsiae or of unknown pathogenicity might have a key role in the natural history of the pathogenic species; ticks infected by a kind of rickettsia that is nonpathogenic to humans (e.g.: *Rickettsia montana, Rickettsia peacockii*) may become unable to maintain (via transovarial transmission) infection by other pathogenic species (e.g., *R. rickettsii*). This fact is of great practical importance because there are populations of ticks infected with nonpathogenic rickettsiae for which the infection rate is often higher compared to pathogenic rickettsiae.

Taxonomy, Historical and Current

Short Description of the Family and Their Genera

Rickettsiaceae (Pinkerton, 1936)

Members of this family are Gram-negative bacteria that grow only inside living cells and therefore are characterized as obligate intracellular parasites. They have high polymorphism and reside in the cytoplasm or nucleus in the host, where they divide by binary fission (Raoult and Roux 1997). They are classified as α-proteobacteria and belong to the order *Rickettsiales*. Within the *Rickettsiaceae* family, they currently have two genera: *Rickettsia* and *Orientia*, following Dumler et al. (2001). Based on their 16S rRNA and *groEL* genes, *Rickettsia* and *Orientia* represent very closely related evolutions of microorganisms in *Alphaproteobacteria* (Lee et al. 2003, Batra and Bakshi 2011).

The organisms identified as *Rickettsia* can be divided in two main groups by phylogenetic characteristics and clinical characteristics of disease: the typhus group (TG), composed of *Rickettsia typhi* and *Rickettsia prowazekii*, and the spotted fever group (SFG). SFG consists of more than 25 species, including *Rickettsia rickettsii* and *Rickettsia parkeri* in the American continent, *Rickettsia conorii* and *Rickettsia africae* in Europe and Africa, and *Rickettsia japonica* and *Rickettsia israeli* in Asia and the Middle East. However, new studies have shown that some species showed marked phenotypic differences and proposed two groups: the ancestral group (AG) composed of *Rickettsia bellii* and *Rickettsia canadensis* (of unknown pathogenicity in humans), and the transitional group (TRG), consisting of *Rickettsia akari*, *Rickettsia australis*, and *Rickettsia felis* (Gillespie et al. 2007). This newly proposed organization of rickettsial species was heavily based on a comparison of major conserved gene sequences (e.g. *ompB*, *gltA*) within the genus. In addition, because the best intrinsic aspects of bacterial growth temperature have a G + C content related to the genome of the bacterium, the ability to polymerize actin in the host cell (Heinzen et al. 1993; Teysseire et al. 1992), the cross-reaction of sera from a patient with rickettsial infection, the distribution of strains, and hemolytic activity are used to differentiate between different rickettsiae groups (Fournier and Raoult 2007).

The genus *Orientia* is classified in a single group, the scrub typhus group, represented by the species *O. tsutsugamuchi*.

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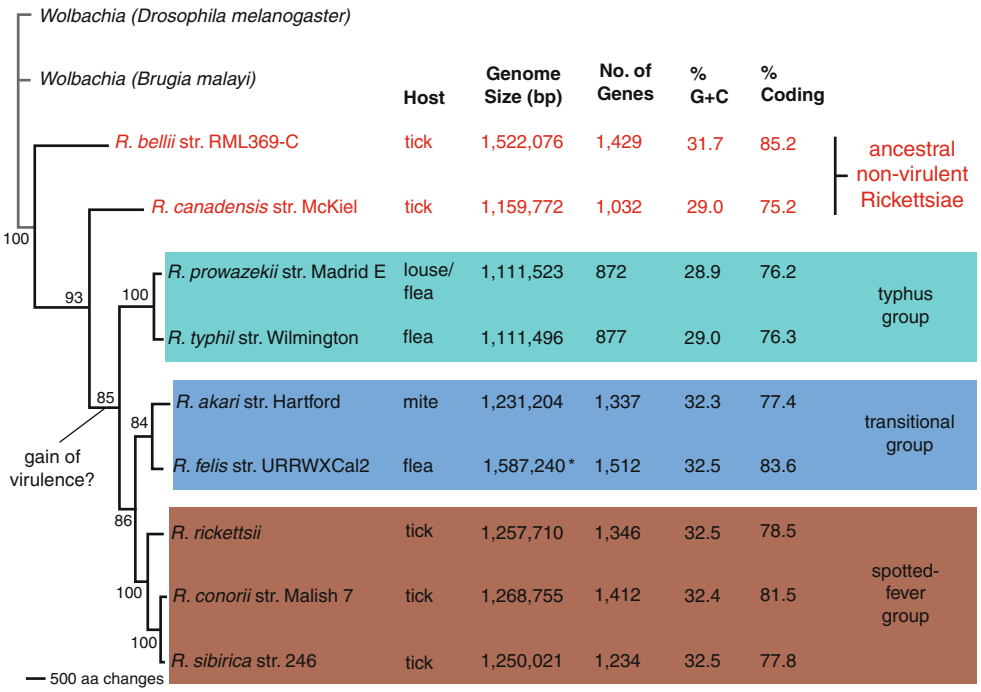

Fig. 23.1
Phylogeny estimation describing the four groups of the *Rickettsiaceae* family (Gillespie et al. 2007)

This organism was given its own genus designation because it is phylogenetically distinct from the other rickettsiae (Tamura et al. 1995).

This group of diseases affects humans through parasitism by blood-sucking arthropods, including ticks, fleas, lice, and mites (Hoogstraal 1967; Friedhoff 1990). These microorganisms thrive in nucleated cells from vertebrate and invertebrate, preferably a vertebrate host and endothelial cells (Weiss and Moulder 1984), and invertebrates, intestinal cells, salivary glands, and ovaries (Parola et al. 2005).

Regarding rickettsiosis, the genus *Rickettsia* is the most important due to the clinical and epidemiological aspects associated with human infections. The distribution of rickettsiae around the world is directly linked to their arthropod vectors, which includes a variety of species, described in regions of different countries from all continents (Parola et al. 2005).

Phylogenetic Structure of the Family and Its Genera

Previously, the original classification and phylogenic arrangement of the genus *Rickettsia* was determined by serological studies. The genus was subdivided into the conventionally well-defined TG and SFG, based mainly on phenotypic and serological features (Vitorino et al. 2007). Since the original organization, conserved genera-specific genes have been sequenced, compared among available *Rickettsia* species, and used to construct phylogenies based upon gene differences and their likely evolution (Reif 2009).

Many types of studies are realized throughout analysis of phylogenetic relation. According to the endosymbiont hypothesis, the mitochondria may be derived from an ancestral Alphaproteobacterium. Phylogenetic studies indicate that the mitochondrial ancestor is most closely related to the *Rickettsiales* (Brindefalk et al. 2011; Andersson et al. 1998). The most ancestral species of the genus appear to be *Rickettsia bellii* and *Rickettsia canadensis* (Stothard et al. 1994); because of their characteristics, these species have been recognized as belonging to ancestral group of *Rickettsiaceae*.

A reorganization of *Rickettsia* species within the genus *Rickettsia* has been proposed, based on phylogenetic analysis of a number of conserved genus-specific genes and the presence of a plasmid in *R. felis* (Gillespie et al. 2007). In this new arrangement, rickettsiae are organized into one of four groups: the ancestral group (AG; e.g. *R. bellii* and *R. canadensis*), the typhus group (TG; e.g. *Rickettsia typhi* and *Rickettsia prowazekii*), the transitional group (TRG; e.g. *Rickettsia felis* and *Rickettsia akari*), and the spotted-fever group (SFG; e.g. *Rickettsia rickettsii*, *Rickettsia sibirica*) (*Fig. 23.1*).

On account of phylogenetic studies based on the sequence of the 16S rRNA gene of members of the family *Rickettsiaceae*, it was initially observed that *Rickettsia tsutsugamushi*, the agent of scrub typhus, was found to be distinct enough by 16S rRNA gene sequence comparison to warrant transfer into the genus *Orientia*, which includes a single species, *Orientia tsutsugamushi* (Tamura et al. 1995) (*Fig. 23.2*). In addition, polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) applied to the *gltA* and *ompA* genes showed

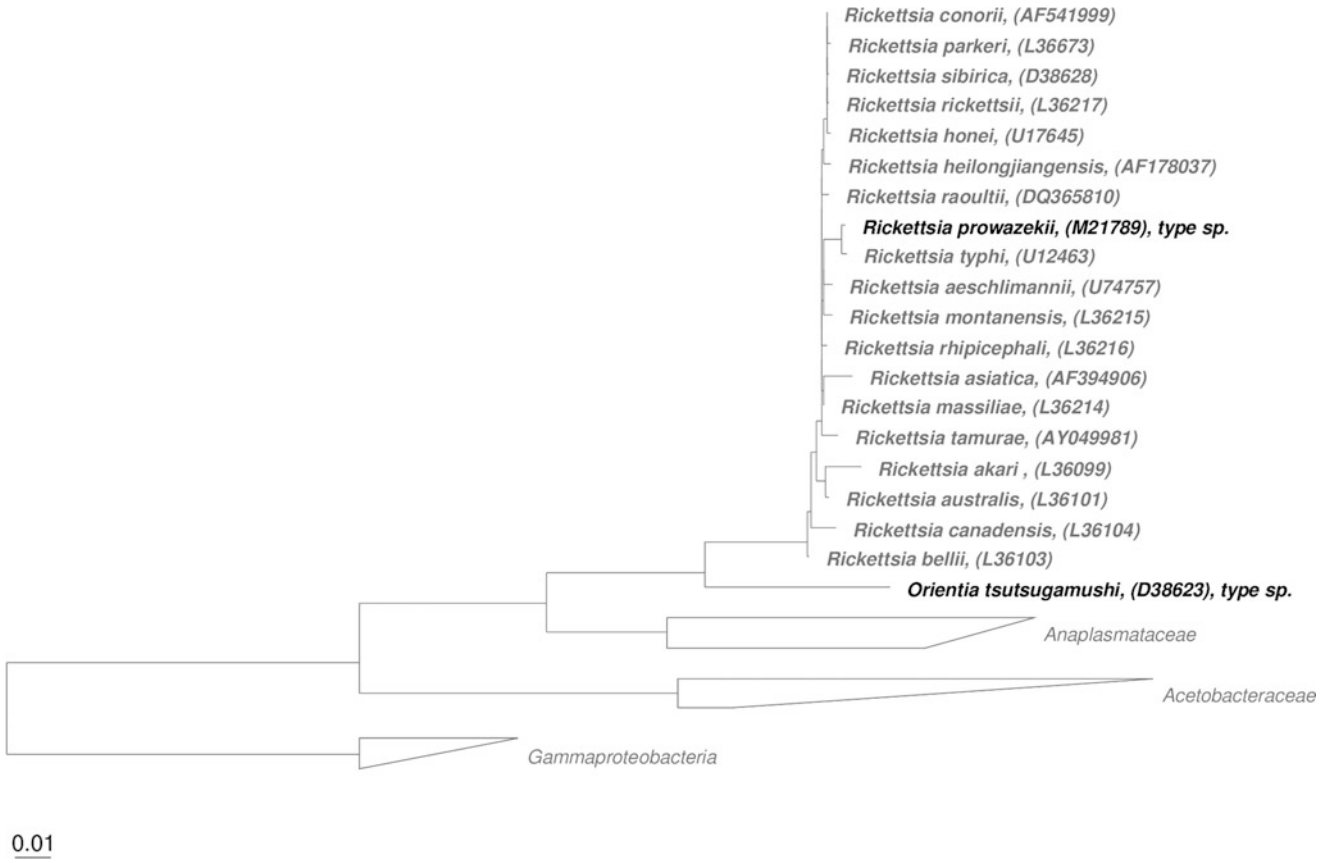

Fig. 23.2

Phylogenetic relationships of the organisms belonging to the *Rickettsiaceae* family in relation to the *Anaplasmataceae* family and others classes beyond *Alphaproteobacteria*, based on DNA sequences. The GenBank numbers are provided to the *right* of the species names

that *R. canadensis* and *R. bellii* occupied an intermediate position between the typhus and SFGs (*Table 23.1*).

- Phylogenetic reconstruction of the family *Rickettsiaceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, a 40% maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence.

In the *Rickettsiales* order are the *Anaplasmataceae* and *Rickettsiaceae* families, which are the most commonly studied. However, the *Holosporaceae* family was recently described by Görtz and Schmidt (2005); entered in that order were two new families, *Pelagibacteraceae* (Thrash et al. 2011) and *Midichloriaceae* (Montagna et al. 2013) (*Fig. 23.3*). According to Montagna et al. (2013), phylogenetic studies provided evidence for the deep branching of a lineage of the *Rickettsiaceae* that infects aquatic protista (Vaninni et al. 2005). Considering that current evidence, this places the family of ciliate-infecting bacteria *Holosporaceae* as the sister group of the lineage leading to *Rickettsiaceae*, *Anaplasmataceae*, and *Midichloriaceae*. There is evidence indicating that intracellular *Rickettsiales* were originally associated with aquatic/environmental protista that served (and potentially still serve) as an ecological and evolutionary reservoir for *Rickettsiales*-infecting animals (Montagna et al. 2013), based on phylogenetic studies.

Molecular Analyses

According to the genome project database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi), 21 genomes of *Rickettsia* and one of *Orientia* species have been sequenced completely: *Rickettsia prowazekii* str. Breinl, *R. typhi* str. B9991CWPP, *R. conori* str. Malish 7, *R. rickettsii* str. Brazil, *R. parkeri* str. Portsmouth, *R. felis* URRWXCal2, *R. akari* str. Hartford, *R. bellii* OSU 85-389, *R. sibirica* 246, *R. africae* ESF-5, *R. monacensis* IrR/Munich, *R. peacockii* str. Rustic, *R. australis* str. Cutlack, *R. rhipicephali* str. 3-7-female6-CWPP, *R. slovaca* 13-B, *R. canadensis* str. CA410, *R. massiliae* MTU5, *Orientia tsutsugamushi* str. Boryong, *R. montanensis* str. OSU 85-930, *R. philipii* str. 364D, *R. japonica* YH, *R. heilongjiangensis* 054 (*Table 23.2*). The availability of the complete sequences of

Table 23.1
Brief description of the set of commonly sequenced genes used for phylogenetic studies in the *Rickettsiaceae* family

Gene	Characteristic of the gene	References
Citrate synthase gene (*gltA*)	The citrate synthase gene is one of the most common genes used to differentiate *Rickettsia* species from other bacterial species.	Bouyer et al. 2001, Regnery et al. 1991
16S rRNA gene	The 16S rRNA gene has stability as a housekeeping gene, which makes it less affected by host immune responses and other environmental stresses.	Reif 2009, Higgins et al. 1996
17-kDa protein gene (*htrA*)	The 17-kDa common antigen gene (*htrA*): The 17-kDa antigen found in all species of *Rickettsia* is an immunologically important surface protein and is one of the most characterized loci in any rickettsial genome. Only bacteria in the genus *Rickettsia* are known to possess the 17-kDa antigen gene; areas of divergence present in this gene between rickettsial species aided in the formation of the SFG and TG.	Anderson et al. 1987, 1988, Anderson and Tzianabos 1989, Tzianabos et al. 1989
OmpA protein gene (*ompA*)	The 190-kDa outer membrane antigen protein gene (*ompA*) is the major gene that defines the SFG. The immunodominant OmpA protein is believed to have a critical role in SFG rickettsial pathogenesis (e.g. cell adhesion and invasion) (Li and Walker 1998; Crocquet-Valdes et al. 2001). Antigenic variation in the conserved regions of this gene that flank tandemly repeated sequences are useful in differentiating *Rickettsia* species and strains within the SFG.	Regnery et al. 1991, Gilmore 1993, Zavala-Castro et al. 2005
OmpB protein gene (*OmpB)*	The 120-kDa protein outer-membrane protein B.	Eremeeva et al. 1994, Roux and Raoult 2000
groEL genes	The *groEL* genes, which encode the 60-kDa heat shock protein GroEL, are ubiquitous in both prokaryotes and eukaryotes and encode highly conserved housekeeping proteins that are essential for the survival of these cells. The *groEL* genes provide the defining evolutionary relationships among the members of the eubacterial lineage. *groEL* gene analysis is useful for the differentiation of STG strains and strains of the genus *Rickettsia.*	Lee et al. 2003
Gene D (*SCA4*)	'Gene D' is the PS120-protein-encoding gene, first described in *Rickettsia conorii* and *Rickettsia japonica.* 'Gene D' is considered as a complementary approach in phylogenetic studies of rickettsiae because it presents significant bootstrap values to the most of the nodes.	Sekeyova et al. 2001

different species belonging to a single genus enables comparative genomics to identify differences and commonalities among them.

The size, architecture, and composition of bacterial genomes vary incredibly. A common characteristic of bacteria of the family *Rickettsiaceae* is the small genome, ranging from 1.11 (*Rickettsia* sp.) to 2.13 Mb (*Orientia tsutsugamuschi*) (*Table 23.1*). One factor that clearly contributes to the genome reduction is the habitat. The genome reduction is probably related to adaptations to a parasitic and pathogenic lifestyle, in which certain functions are no longer required (Moran 1996), resulting in total dependence on host cells. In a study on the evolution of the *Rickettsiales*, it was demonstrated that evolution from a free-living lifestyle to an obligate intracellular one was associated with the loss of 2,135 genes (Georgiades et al. 2011a). Furthermore, the 12 deadliest epidemic species for humankind have significantly smaller genomes, with fewer open reading frames (ORFs), than less dangerous species (Georgiades et al. 2011b). One of the best examples of genomic reduction of epidemic bacteria is *R. prowazekii*, the agent of epidemic typhus. No virulence genes have been identified in its genome, and 24 % of its small genome is composed of pseudogenes and non-coding DNA (Andersson et al. 1998; Bechah et al. 2010). In fact, the genome of *R. prowazekii* (834 ORFs) represents a subset of *R. conorii* (1374 ORFs); it possesses almost no genes that are not present in *R. conorii*, with the exception of four TG-specific genes that also are present in *R. typhi* (RP624, RP338, RP164 and RP174) and absent from, or split in, other rickettsiae (Gillespie et al. 2008; Ammerman et al. 2009).

In *Listeria monocytogenes* and *Shigella* (Goldberg and Theriot 1995; Moliner et al. 2010), intracellular motility is considered a virulence factor. In *R. prowazekii*, this characteristic was not observed because the bacteria have no motility within cytoplasm (Andersson et al. 1998). In a study of comparative genomics between *R. rickettsii* and *R. africae*, pathogenicity development was associated with the loss of essential genes (Fournier et al. 2009). Studies of rickettsial genomics challenge traditional concepts of pathogenesis, which were based principally on the acquisition of virulence factors.

Another intriguing phenomenon about the reduced rickettsial genomes concerns the large fraction of non-coding DNA and

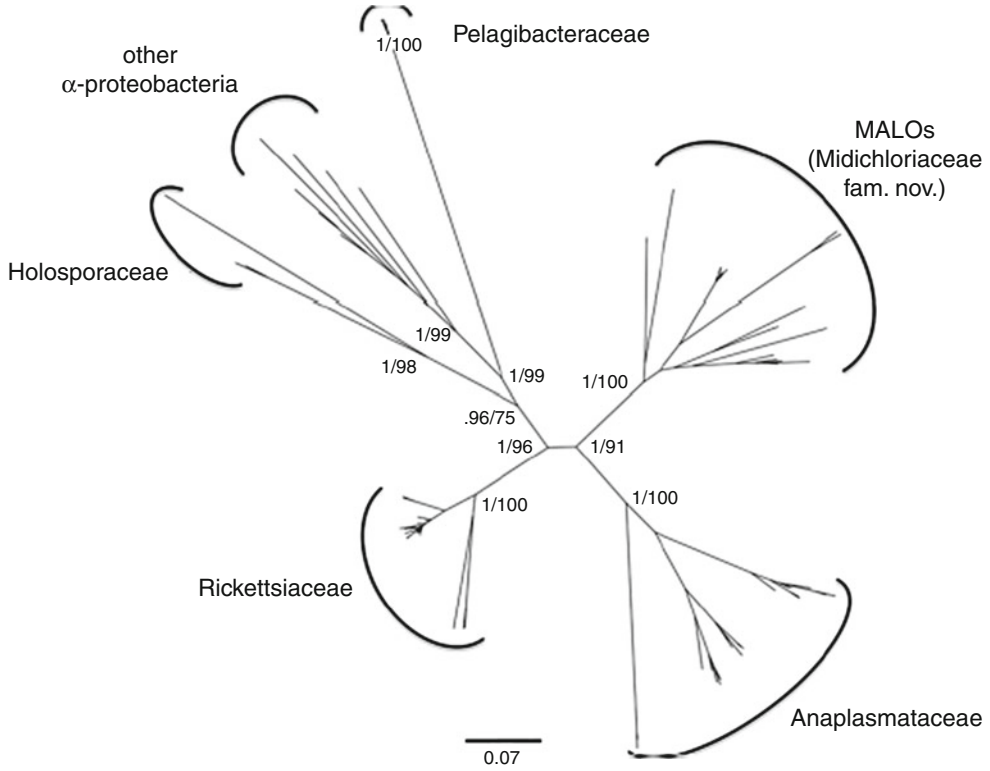

Fig. 23.3

An unrooted Bayesian phylogram of the *Rickettsiales* based on the 16S rRNA gene sequence. Bayesian posterior probability and ML (Maximum Likelihood) bootstrap values are reported for the main lineages (Montagna et al. 2013)

possible functionality of these "non-coding" sequences because of the high conservation of these regions. "Pathogenicity islands" (a set of genes coding for virulence traits) are present in many bacteria; however, they are not present in species of *Rickettsia* spp. vertebrate-pathogenic (Hacker and Kaper 2000). In many studies, the presence of plasmids in *Rickettsia* species does not appear to be directly associated with pathogenicity (Fournier et al. 2009; Ogata et al. 2005). However, it has been suggested that the genes encoding the proteins responsible for recognition, invasion, and pathogenicity are located in plasmids of some *Rickettsia* spp. species. The acquisition of new genes does not necessarily imply pathogenicity change, as has been shown in several studies on comparative genomics of *Rickettsia* sp. Furthermore, the emergence of virulent traits has occurred with the loss of gene function. In *Shigella* spp. and *Yersinia* spp., such events may provide a selective advantage, as noted by a series of genetic events that contribute to the emergence of virulence (Maurelli et al. 1998; Sun et al. 2008).

Compared with other bacteria species with sequenced genomes, the percentage of non-coding DNA in *Rickettsia* species not decreased, suggesting that the reduction of the genome is associated with the loss of genes (Rogozin et al. 2002). Genome analysis of various rickettsial genomes shows that the genome reduction process described for alpha-proteobacteria (Boussau et al. 2004) has occurred independently in different rickettsial lineages, leading to the existing species assemblage (Blanc et al. 2007b).

The changes in genome size may occur by gene duplication and the emergence of new sequences. In rickettsia, genomes have been found with repeating elements without defined cellular function (Ogata et al. 2000). In bacterial genomes, repeated elements are located in intergenic regions (Van Belkum et al. 1998). In some of these, variable numbers of tandem repeats (VNTR) represent an interindividual variability in the sequence length; some have been used for genetic typing (Eremeeva et al. 2006; Fournier et al. 2004).

In *Rickettsia* spp., elements palindromic repeat (EPR) of approximately 100–150 bp were found. These repetitions can invade coding and non-coding regions of the genome (Amiri et al. 2002; Claverie and Ogata 2003; Ogata et al. 2000). When inserted within genes encoding proteins, these repeats generated a new reading frame as a part of a preexisting gene. Therefore, the final gene product has an additional peptide segment (30–50 amino acids). Over evolutionary time, multiple random insertions of such elements within genes, followed by selection on the resulting peptide sequences within the context of different host proteins, may have contributed to the emergence of new protein sequences, domains, and functions. Thus, EPR enhance sequence diversity in coding regions of *Rickettsia* spp.

Mobile elements have been identified in the rickettsia genome by genome comparative studies. These elements mediate the DNA movement within and between genomes, by means of transposable elements, plasmids, bacteriophages, and genes associated with horizontal mobility. These mobile elements

Table 23.2
Sequenced bacterial genomes of *Rickettsiaceae*

Bacteria	Genome	G+C %	Proteins	Genes	Reference/GenBank
Rickettsia prowazekii str. Breinl	One chromosome (1.11 Mb)	29	920	956	CP004889.1
R. typhi str. B9991CWPP	One chromosome (1.11 Mb)	28.9	839	875	CP003398.1
R. conori str. Malish 7	One chromosome (1.27 Mb)	32.4	1,374	1,414	NC_003103.1
R. rickettsii str. Brazil	One chromosome (1.26 Mb)	32.5	1,332	1,369	NC_016913.1
R. parkeri str. Portsmouth	One chromosome (1.30 Mb)	32.4	1,318	1,355	NC_017044.1
R. felis URRWXCal2	One chromosome (1.59 Mb) and two plasmids (pRF-62.9 Kb and pRF delta-39,8 Kb)	32.6	1,512	1,551	NC_007109.1 (chromosome), NC_007110.1 (pRF), NC_007111.1 (pRF delta)
R. akari str. Hartford	One chromosome (1.23 Mb)	32.3	1,257	1,292	NC_009881.1
R. bellii OSU 85-389	One chromosome (1.53 Mb)	31.6	1,429	1,511	NC_009883.1
R. sibirica 246	One chromosome (1.25 Mb)	32.4	–	–	NZ_AABW00000000.1
R. africae ESF-5	One chromosome (1.29 Mb) and one plasmid (pRAF-12.36 Kb)	32.4	1,041	1,167	NC_012633.1 (chromosome), NC_012634.1 (pRAF)
R. monacensis IrR/ Munich	One chromosome (1.27 Mb)	32.3	1,460	1,503	NZ_CBUA000000000.1
R. peacockii str. Rustic	One chromosome (1.31 Mb) and one plasmid pRPR-26,42 Kb)	32.6	947	984	NC_012730.1 (chromosome), NC_012732.1 (pRPR)
R. australis str. Cutlack	One chromosome (1.32 Mb) and one plasmid (p-MC5-1 26.66 Kb)	32.3	1,261	1,297	NC_017058.1 (chromosome), NC_017041.1 (pMC5-1)
R. rhipicephali str. 3-7-female6-CWPP	One chromosome (1.31 Mb) and one plasmid (pMCC-1 15. 09 Kb)	32.4	1,266	1,302	NC_017042.1 (chromosome), NC_017055.1 (pMCC-1)
R. slovaca 13-B	One chromosome (1.28 Mb)	32.5	1,112	1,323	NC_016639.1
R. canadensis str. CA410	One chromosome (1.15 Mb)	31	1,016	1,052	NC_016929.1
R. massiliae MTU5	One chromosome (1.38 Mb) and one Plasmid (pRMA-15.28 Kb)	32.5	980	1,436	NC_009900.1 (chromosome), NC_009897.1 (pRMA)
Orientia tsutsugamushi str. Boryong	One chromosome (2.13 Mb)	30.5	1,182	2,216	NC_009488.1
R. montanensis str. OSU 85-930	One chromosome (1.28 Mb)	32.6	1,217	1,254	NC_017043.1
R. philipii str. 364D	One chromosome (1.29 Mb)	32.5	1,344	1,380	NC_016930.1
R. japonica YH	One chromosome (1.28 Mb)	32.4	971	1,010	NC_016050.1
R. heilongjiangensis 054	One chromosome (1.28 Mb)	32.3	1,297	1,338	NC_015866.1

Source: http://www.ncbi.nlm.nih.gov/genome/?term=Rickettsia

constitute "the mobilome" (Frost et al. 2005; Koonin and Wolf 2008). Some *Rickettsia* spp. contain transposases (Blanc et al. 2007a; Ogata et al. 2005), phage-related genes (Andersson et al. 1998; McLeod et al. 2004; Ogata et al. 2001, 2005, 2006), plasmids (Baldridge et al. 2007, 2008; Fournier et al. 2008; Ogata et al. 2005), and an apparatus for conjugation (*tra* genes) (Blanc et al. 2007a; Fournier et al. 2008; Ogata et al. 2005, 2006). In intracellular bacteria, the lateral gene transfer (LGT) phenomenon has long been considered rare (Audic et al. 2007); in rickettsiae, the discovery of the mobilome is possible. Ogata et al. (2006) described various mechanisms in rickettsial genomes for the influx of foreign DNA sequences. Further analysis of the rickettsial genome can identify candidates for LGT between *Rickettsiae* species.

The transfer of genes between chromosomes and plasmids was observed after analysis of *R. felis* genome. Eleven genes

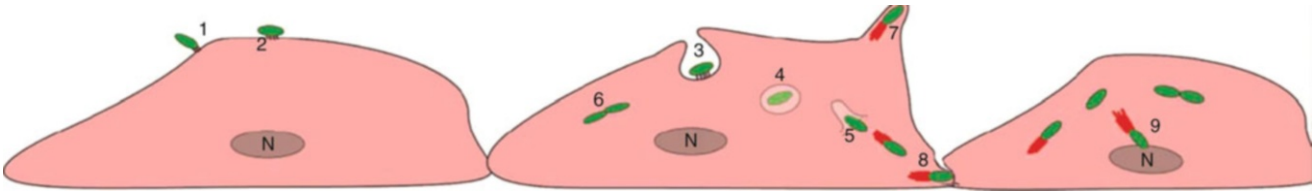

Fig. 23.4

Spotted fever group rickettsia–endothelial cell interaction. (*1*) Adhesion of bacteria to the cell membrane via adhesins; (*2*) Recruitment of more receptors; (*3*) Engulfment of the bacteria by cell extensions; (*4*) Inclusion of bacteria in phagocytic vacuoles; (*5*) Rickettsial enzymes lyse vacuolar membrane; (*6*) Replication by binary fission; (*7*) Host actin-based mobility; (*8*) Cell-to-cell spread; and (*9*) Invagination into the endothelial cell nucleus (*N*) (Walker 2007)

located on plasmid PRF exhibiting homologous chromosome of *R. felis* were observed. These two genes encoded heat shock proteins, thymidylate kinase, and patatin-like phospholipase transposase seven. All sequenced *Rickettsia* genomes exhibit patatin-like phospholipase-coded chromosome (pat1). In plasmid PRF, *R. felis* has a paralog additional pat2. Phylogenetic reconstruction among species of *Rickettsia* shows a close relationship for pat1 of *R. felis* and *R. Akari* pat2 with *R. felis*. This provides a clear case of transfer of genes from plasmid to chromosome; the chromosome was replaced by pat1 pat2 plasmid-encoded in the lineage, leading to the ancestor of *R. felis* and *R. akari* (Ogata et al. 2005).

The development of technologies for large-scale genome sequencing may modify the way we view microbiology, thus starting a postgenomic era in which the development of computational tools for the analysis of biological data is a priority. The genome comparative analysis of the family *Rickettsiaceae* with other bacteria has provided information about the bacteria-host relationship, pathogenicity, and evolutionary history. The ultimate understanding of the molecular mechanisms related to adaptation to different conditions requires the application of global approaches, including differential transcriptome analysis using platforms such as HiSeq 2000 (Illumina). Such a methodology has not yet been extensively applied for *Rickettsiaceae*, but we believe that experimental progress will make the technology more accessible in a few years. A better knowledge of a microorganism can also be gained by proteomic analysis.

Phenotypic Analyses

All the microorganisms of *Riackettsiaceae* are similar in the following aspects: they are obligate intracellular parasites, morphologically similar to gram-negative bacteria, and survive in vertebrates and arthropod hosts. When human infection occurs, this is mediated by arthropod vectors.

Bacteria of the genus *Rickettsia* and *Orientia* are small rods. *Rickettsia* measure 0.3–0.5 μm^2 in length and 0.8–2.0 μm^2 in width, whereas *Orientia* measure 0.5–0.8 μm^2 in length and 1.2–3 μm^2 in width, respectively. Generally, SFG members have an optimal growth temperature of 32 °C, whereas TG members have an optimum growth temperature of 35 °C. SFG members have 32–33 % GC content and TG members have nearly 29 % GC. In addition, only SFG members can enter the nucleus of parasitized cells (Heinzen et al. 1993; Teysseire et al. 1992).

Pathogenic *Rickettsiaceae* do not have flagella, although *Rickettsiae* of the SFG members move by a peculiar mechanism. They use the host cell actin to form a tail and move freely in the cytoplasm of the cell; however, this mechanism only occurs after escaping the phagocytic vacuole. The scientists suppose that the polymerization of actin and the tail formation play a fundamental role in the invasion movement from cell to cell in host infection; this could explain why *Rickettsiae* spreads rapidly in host organisms (Heinzen et al. 1993, 1999; Gouin et al. 1999) (*Fig. 23.4*). Concerning the polymerization of actin in the host cell, SFG members are capable and TG members are not capable.

The membrane structure of *Rickettsiaceae* is similar to those observed in gram-negative bacteria, in general. It has a trilamellar structure, as follows: a lipopolysaccharide outer membrane and inner membrane composed of a phospholipid bilayer, separated by a peptidoglycan layer in a periplasmic space. Furthermore, SFG members are surrounded by an electron-lucent slime layer when they are infecting a host cell (Raoult and Parola 2007; Quinn et al. 2011). One exception is *O. tsutsugamushi*, which lacks muramic acid, glucosamine, heptose, 3-deoxy D-mannooctulosonic acid (KDO), and hydroxy fatty acids—the basic components of peptidoglycan and LPS, so it is deficient of both in the cell wall (Amano et al. 1987). This fact explains why *O. tsutsugamushi* is resistant to beta-lactam antibiotics (Miyamura et al. 1989).

The major antigens of *Rickettsiae* are lipopolysaccharides, lipoproteins, outer membrane proteins of the surface cell antigen (SCA) family, and heat shock proteins. Other antigens have been characterized in *Rickettsiae* species, including a 17-kDa lipoprotein and members of the autotransporter protein family SCA, which includes the 120 kDa protein S-layer (OmpB or SCA5), OmpA (present only in SFG *Rickettsiae*), and Sca4 (Anderson et al. 1990; Blanc et al. 2005; Fournier et al. 1998b).

With light microscopy, the majority of *Rickettsiaceae* stains well with Giménez, Macchiavello, and Giemsa stains. *Rickettsia akari* does not stain with Giemsa or eosin-azure-based techniques, just with Macchiavelo, Giménez, and carbol basic fuchsin (Giménez 1964). In addition, *O. tsutsugamushi* does not stain with Macchiavello, and a modification of the Gimenez stain is

required (Timoney et al. 1992). Concerning microscopy fluorescence, it is possible to use acridine orange stain in cell culture or smears. Also, materials such as host tissue stains could be evaluated by immunohistochemistry or fluorescein-conjugated polyclonal antibodies (Raoult and Parola 2007).

Due to the strictly intracellular nature of *Rickettsiaceae*, traditional identification methods used in bacteriology cannot be applied. It must be cultivated in tissue culture or the yolk sac of developing chicken embryos (Teysseire et al. 1992). *Rickettsiaceae* can be isolated from decanted plasma, blood collected on heparin or citrate anticoagulant (because EDTA anticoagulant is harmful to the cell monolayers used for recovery of rickettsiae), skin biopsy, necropsy tissue, and arthropod samples (Brouqui et al. 2004). The material can be inoculated into experimental animals, in primary cultures of chicken embryo, HEL, MRC5, WI-38, LLC-MK2, BSC-1 or Hep-2 cells, further VERO cells, and L929 cells, which are used more often (Cox 1941; Cory et al. 1974; Brouqui et al. 2004). Today, cell culture systems have replaced the yolk sacs of developing chicken embryos and experimental animal inoculation, which were widely used in the past (La Scola and Raoult 1997). All of these procedures must be performed only in Biosafety Level 3 laboratories. The cultivation of *Rickettsiae* in cell monolayers is observed by the disruption of massively infected cells. SFG members form a plaque with a diameter of 2–3 mm in 5–8 days, while TG members form a plaque smaller than 1 mm in 8–10 days (Raoult and Parola 2007).

Concerning the metabolism of *Rickettsiaceae*, they do not use glucose as an energy source; rather, they use glutamate. Likewise, they do not synthesize or degrade nucleoside monophosphates (Raoult and Parola 2007).

Isolation, Enrichment and Maintenance Procedures

Isolation and Enrichment

Because of their intracellular binding, bacterial culture is one of the necessary procedures for cells' isolation. Another method for their isolation is the inoculation of biological material containing the bacteria in guinea pigs and embryonated eggs. However, cell culture is currently the most widely used system for primary isolation. Tissue culture requires prior disaggregation of the original tissue where the cells are grown and an adhesive layer on a solid substrate or suspended in culture medium.

For the isolation and cultivation of the parasite, samples used can be obtained from minced clot plasma, skin biopsy, and autopsy tissue samples (La Scola and Raoult 1997); arthropod material is collected and inoculated into cell lines (Paddock et al. 2010). It is important to note that performing routine laboratory cell isolation and cultivation of many of these rickettsial agents requires biosafety level 3 (BSL3) (Champman et al. 2006), which becomes impractical for implementation in many laboratories.

The cell cultivation may be performed in different lines, including Vero cells, MRC5 cells, L-929 cells, HEL cells, LLC-MK2, BSC-1, or Hep-2 (Cox 1941; Cory et al. 1974; Jonhson and Pedersen 1978; Dumler and Walker 2005). Vero or L929 cells have been shown to allow better and faster isolation of rickettsiae, especially from heavily infected samples, than HEL or MRC5 cells (Kelly et al. 1991; La Scola and Raoult 1997). However, these types of cell lines are established when an inhibition of cell division due to close contact occurs in the monolayer and may subsequently be used in a prolonged incubation.

The adaptation of the system shell vial employed for the detection of cytomegalovirus (Paya et al. 1987) has been successful for growing rickettsiae in VERO cells (La Scola and Raoult 1997), which may show multiplication of rickettsiae by the cytopathic effect (CPE). However, the cultivation of this technique has limitations because getting material from symptomatic patients is not always possible. Another important aspect of the isolation and cultivation of rickettsiae is the use of embryonic cell lineages of the tick, particularly for species struggling for multiplication in mammalian cell lines (Bell-Sakyi et al. 2007). Thus, using different strains of ticks, it is possible cultivation of different species of rickettsiae that have medical and veterinary importance (*Table 23.3*) (Bell-Sakyi et al. 2007).

Ecology

Rocky Mountain Spotted Fever (RMSF) is endemic to regions of North, Central, and South America. Cases have been reported from Canada to Argentina; however, some countries in these areas have not yet reported any cases. The greatest numbers of fatalities have been reported in the United States and Brazil. The distribution of RMSF reflects the distribution and abundance of the tick vectors *D. variabilis* (the American dog tick) and *D. andersoni* (*Table 23.4*).

The transmission of RMSF almost always results from rickettsiae infection in a human host by a tick as it obtains a blood meal from that host (McCalla 1908). Tick vectors of *R. rickettsii* include at least four species: *D. andersoni, D. variabilis, R. sanguineus*, and *Amblyomma cajenennse* (Parker 1933; Burgdorfer 1975; Demma et al. 2005). Because *R. rickettsii* is passaged transovarially and transstadially, all hematophagous stages of these ticks are potentially capable of transmitting rickettsiae to a susceptible host. Rare routes of transmission of *R. rickettsii* to human hosts include blood transfusion (Wells et al. 1978) and inoculation of rickettsiae through mucous membranes following contact with fingers contaminated during the crushing of infected ticks removed manually from a human or animal (Price 1954; Spencer and Parker 1930; Gordon et al. 1984).

Most of reported cases of RMSF occur during the months of April through September in the temperate United States (Kirkland et al. 1995; Gordon et al. 1984; Treadwell et al. 2000), because this period coincides with the greatest host-seeking activity of the *Dermacentor* spp. ticks (Clark et al. 1998;

Table 23.3
***Rickettsia* species propagated in tick cell lines since 1995 (Adapted by Bell-Sakyi et al. 2007)**

Rickettsia species	Mammalian host (disease caused)	Tick cell line (s) used
Rickettsia rickettsii	Human (Rocky mountain spotted fever)	IDE2, DALBE3, ISE6, IDE8,
Rickettsia peacockii	–	DAE 100, ISE6, BME26, DVE1, DAE3, DAE15, IDE12, IDE2, IDE8, IRE11, CCE3
Rickettsia monacensis	?	ISE6, IRE11, DAE100, IDE8
Rickettsia helvetica	Humans (fever, perimyocarditis?)	IRE11
Rickettsia montanensis	Various small mammals	IDE2, DALBE3
Rickettsia sp. (spotted fever group)	?	RAE25, IDE2, IDE8
Rickettsia felis	Humans (flea-associated spotted fever)	ISE6

Eads and Smith 1983). In Brazil, tick surveys in the county of Pedreira documented peak numbers of larvae and nymphs of *A. cajennense* between June and October, the months coinciding with the most reports of RMSF in Brazil (de Lemos et al. 1997).

African Tick-Bite Fever is transmitted by ixodid ticks of the genus *Amblyomma*: *Amblyomma variegatum* in West, Central, and East Africa and the eastern Caribbean, and *Amblyomma hebraeum* in southern Africa (Kelly and Mason 1991; Kelly et al. 1994; Parola et al. 2001). In contrast to most other ticks of human importance, *Amblyomma* ticks are hunter ticks and exhibit a notoriously aggressive behavior. Both tick species prefer semihumid habitats with tall grass or bush. Cattle, wild game, and other ungulates are the principal hosts, although young stages may also parasitize birds and rodents. *A. hebraeum* and *A. variegatum* are active all year, but their number peaks during and after the rainy season, from January to May (Norval 1983). Both species act as vectors of *R. africae* and are also reservoirs in which the infection is maintained through transstadial transmissions (Kelly and Mason 1991).

Mediterranean Spotted Fever (MSF) is caused by *R. conorrii* and transmitted by *R. sanguineus*, which probably has the most widespread distribution of all Ixodid ticks. Although *R. sanguineus* has a worldwide distribution, *R. conorii* is confined to particular regions of the world. *R. sanguineus* lives in peridomestic environments shared with dogs but has relatively low affinity for humans. Because of these circumstances, cases of MSF are sporadic and typically encountered in urban areas.

MSF is endemic in northern Africa, southern Europe, and the Mediterranean area. It is a reportable disease in Portugal (Bacellar et al. 2003), where there is an annual incidence rate of 9.8 cases per 100,000 persons (De Sousa et al. 2003). Overall, in endemic countries, fluctuation in incidence has been variable; this may be due to variations in climatic conditions, such as an increase in temperature and the lack of rainfall (Espejo-Arenas et al. 1986; Segura-Porta et al. 1989; Raoult et al. 1992). Sporadic cases in nonendemic countries are also frequently observed as a consequence of tourism (Rolain et al. 2004; Jensenius et al. 2004). Notably, although *R. sanguineus* is prevalent in North America, no cases of MSF were described until recently on that continent. In Europe, cases are encountered in late summer; this period is associated with the peak of activity of immature ticks, which are difficult to observe even when attached to the body (Raoult and Roux 1997).

Louse-Borne Epidemic Typhus has the most serious epidemic potential of all rickettsiae. It should be considered a serious threat, even in developed countries (Drancourt et al. 1995; Brouqui et al. 1996; Van Der Laan and Smit 1996; Brouqui et al. 1999). Epidemic typhus is currently considered as a potential bioterrorism agent (category B, Centers for Disease Control and Prevention). The human body louse is the only established vector for *R. prowazekii.* Lice die of their infection and do not transmit their infection to their progeny; because of this, the main reservoir appears to be humans. Bacteremia may occur and be prolonged to contaminate lice and allow transmission. Humans who contract typhus retain some rickettsiae for the rest of their lives, leading immunocompromised patients to relapse with Brill-Zinsser disease, a milder but bacteremic form of typhus (Green et al. 1990).

In the louse, rickettsiae only infect the epithelial cells of the first part of the louse's digestive tract (Weigl 1924; Houhamdi et al. 2002), where they multiply. As a result of its excessive growth, infected epithelial cells release the rickettsiae into the gut lumen (Houhamdi et al. 2002). Massive quantities of rickettsiae are discharged in the feces (Houhamdi et al. 2002). The rupture of digestive epithelium allows the ingested blood to diffuse through the intestine to the hemolymph and the louse becomes red (Burgess 1995); because of this, typhus has also been named "red louse disease". Because the ruptured epithelial cells are not replaced, infection with *R. prowazekii* leads to the death of the louse shortly thereafter (Houhamdi et al. 2002). *R. prowazekii* is the only *Rickettsia* species unable to be transmitted transovarially to its progeny in its vector.

Lice live in clothing, and their prevalence is determined by the weather, humidity, poverty, and lack of hygiene. They are more prevalent during the colder months; therefore, epidemic typhus is more frequently reported during winter and the early

Table 23.4

Species classified in SFG rickettsiae pathogenic for humans, SFG rickettsia never isolated from humans, the typhus group, and the genus *Orientia* (La Scola and Raoult 1997)

Group	Species	Disease	Associated arthropod	Distribution
SFG rickettsiae (human pathogens)	*Rickettsia conorii sensu stricto*	Mediterranean spotted fever	*Rhipicephalus sanguineus*	Mediterranean countries, Europe, Africa, Asia
	Rickettsia conorii complex	Israeli spotted fever	*Rhipicephalus sanguineus*	Israel
	Rickettsia conorii complex	Astrakhan spotted fever	*Rhipicephalus pumilo*	Russia
	Rickettsia rickettsii	Rocky Mountain spotted fever	*Dermacentor variabilis, D. andersoni, Rhipicephalus sanguineus, Amblyomma cajennense*	North and South America
	Rickettsia sibirica	Siberian tick typhus	*Dermacentor nuttalli, Dermacentor marginatus, Haemophysalis concinna*	Northern China, Pakistan (Asia Siberia)
	Rickettsia akari	Rickettsial pox	*Allodermanyssus sanguineus*	USA, Ukraine, Croatia, Korea
	Rickettsia africae	African tick bite fever	*Amblyomma hebraeum*	Southern Africa
	Rickettsia australis	Queensland tick typhus	*Ixodes hlocyclus*	Australia
	Rickettsia japônica	Japanese tick typhus	*Haemophysalis longicornis*	Japan
	Rickettsia honei	Finders Island tick typhus	*Aponomma hydrosauri*	Finders Islands
SFG rickettsiae (never isolated from humans)	*Rickettsia massiliae*		*Rhipicephalus turanicus, Rhipicephalus sanguineus, other Rhipicephalus spp.*	France, Greece, Spain, Portugal, central Africa.
	Rickettsia rhipicephali		*Rhipicephalus sanguineus*	USA, France, Portugal, central Africa
	Rickettsia parkeri		*Amblyomma maculatum*	USA
	Rickettsia Montana		*Dermacentor variabilis*	USA
	Rickettsia belli		*Dermacentor spp.*	USA
Typhus group	*Rickettsia prowazekkii*	Epidemic typhus	*Pediculus humanus corporis*	Worldwide (most in highlands areas of South America, Asia, Africa)
	Rickettsia typhi	Murine typhus	*Xenopsylla cheopis*	Worldwide
Scrub typhus	*Orientia tsutsugamushi*	Scrub typhus	*Leptotrombidium deliense*	Eastern Asia, northern Australia, western Pacific Islands

spring (Patterson 1993). Infestation with body lice and louse-transmitted diseases is also being increasingly reported in the inner cities of developed countries (Koehler et al. 1992; Van Der Laan and Smit 1996; Jackson and Spach 1996; Rydkina et al. 1999).

Murine Typhus is mainly transmitted by rat flea *Xenopsylla cheopis* (Azad 1990; Chaniotis et al. 1994); other flea species and arthropod vectors also have been reported to transmit *R. typhi*, including the cat flea *Ctenocephalides felis*, lice, mites, and ticks (Azad 1990; Sexton 2005; Raoult and Roux 1997). The flea remains infected for life after a blood meal from an infected rat (Azad 1990). The primary reservoirs are rats belonging to the subgenus *Rattus*, mainly *Rattus norvegicus* and *Rattus rattus* (Azad 1990). However, various rodents and other wild and domestic animals have also been occasionally seen to act as hosts. Rats serve not only as simple hosts but also as amplifying hosts (Azad 1990).

Rickettsia enters the midgut epithelial cells of the flea, where it multiplies without causing any damage and is excreted with the feces. The bacterium is additionally maintained in fleas by

transovarial and transstadial transmission (Raoult and Roux 1997) and is then transmitted back to a susceptible vertebrate host upon subsequent feeding (Azad 1990).

The role of humans in the natural cycle of *R. typhi* is secondary, as they are only accidental hosts. While feeding on a human host, the flea defecates; the irritation caused by the bite causes the host to scratch and thus inoculate the rickettsiae into the flea-bite site or skin abrasions. *Rickettsiae* are also thought to infect humans via inhalation or contamination of the conjunctiva (Azad 1990; Raoult and Roux 1997).

Many reports illustrate that murine typhus is an emerging disease with worldwide distribution, but it is most prevalent in warmer countries (Raoult and Roux 1997). Most cases occur in late summer or early autumn; this seasonal variation directly correlates with the abundance of the vector fleas (Azad 1990). A decline in the incidence of murine typhus occurs when rat and flea control programs are initiated by public health services (Azad 1990; Sexton 2005).

Scrub typhus Transmission occurs within an area bounded by the Asiatic and Australian continents, to the north by Siberia and the Kamchatka Peninsula, to the south by Queensland, and to the west by Afghanistan. The public health impact of *O. tsutsugamushi* infection is greatest for agricultural laborers in rural areas (Duffy et al. 1990), although disease transmission also has been reported in suburban areas (Fan et al. 1987). Travelers to endemic areas can become infected (Woodruff et al. 1988), but it is not common. The majority of scrub typhus cases go undiagnosed because commercial diagnostic tests are generally unavailable in the rural tropics. *O. tsutsugamushi* was documented to be the most common cause of acute fever in Malaysia and Thailand (Lorandos 1934; Binford and Ecker 1947).

Transmission occurs by mites in zones where the primary forest has been cleared and replaced. Humans are accidental hosts, acquiring *O. tsutsugamushi* during feeding of a larval trombiculid mite of the genus *Leptotrombidium*. These chiggers only feed on mammalian tissue fluid once in their lifetime (McLeod et al. 2004); the reservoir of infection is through transovarial transmission. Chigger activity is determined by temperature and humidity. Mites are normally maintained in nature by feeding on a variety of wild rodents, but rodents are not a reservoir of *O. tsutsugamushi*.

Leptotrombidium deliense is the most important vector species in Southeast Asia and southern China, whereas *L. akamushi, L. scutellare*, and *L. pallidum* are the main vectors in Korea and Japan (Duffy et al. 1990; McLeod et al. 2004). *L. chiangraiensis* is a newly described vector found in cultivated rice fields in Thailand (Schmiela and Millera 1999).

Pathogenicity and Clinical Significance

Rickettsiae are obligately intracellular organisms that do not replicate extracellularly. Organisms in this family generally target macrophages, leukocytes, and endothelial cells. The main target cells of rickettsiosis are endothelial cells, which are most likely the result of vascular location and hematogenous dissemination. The lungs and brain are the critical target organs determining the lethality of rickettsioses. The events are visible in the skin: vasodilatation, perivascular edema, and disruption of vascular integrity (Raoult and Parola 2007). The pathogenesis of diseases caused by *Rickettsia* and *Orientia* genera differ greatly, according to the species of bacteria:

Rocky Mountain Spotted Fever (RMSF), the most severe of all the spotted fever group (SFG), is caused by *Rickettsia rickettsii*. It is a disease of the capillary circulation, being the only organism in the genus that invades beyond the blood vessel lining of the endothelium; it also invades adjacent vascular smooth muscle cells, particularly in arterioles. This bacteria is transmissible from the blood of infected patients to healthy hosts. These bacteria have the capacity to infect and replicate in the cytosol, and occasionally in the nucleus of vertebrate cells (e.g., endothelium, vascular smooth muscle, and macrophages) and invertebrate cells (e.g., hemocytes and salivary gland epithelium) (Raoult and Parola 2007).

In vertebrate hosts, these organisms are found in apparently uninjured endothelium of normal vessels, in areas of proliferated endothelium of the intima of vessels, in hyaline necrosed intima of more advanced lesions, in apparently normal and necrosed smooth muscle fibers of vessels with lesions, and in endothelial cells in the perivascular zones of proliferation. The largest masses are seen in smooth muscle cells of affected arteries and veins (Wolbach 1916).

Infection by *R. rickettsii* causes a significant reduction in key enzymes involved in the protection of endothelial cells from oxidative injury (Devamanoharan et al. 1994), resulting in increased levels of intracellular peroxides accompanied by ultrastructural indications of cell injury (Hong et al. 1998). Endothelial cell infection by *R. rickettsii* activates the nuclear transcription factor, which exerts an antiapoptotic effect (Joshi et al. 2003) by inhibiting proteins in the caspase family, which mediate apoptosis (Joshi et al. 2004). Inhibition of apoptosis is essential for host cell survival and site persistence of active infection (Clifton et al. 1998).

RMSF is a systemic illness that can involve endothelial cells of capillaries of all tissues and organs; however, the first signs and symptoms of disease resemble many other infectious syndromes. Following the bite of an infected tick, the disease begins with abrupt onset of fever accompanied by headache, nausea, vomiting, anorexia, and generalized myalgia. Other findings were recorded but with low frequencies. The rash begins small, typically on the wrists, ankles, and forearms, then evolves. It spreads centrally, and the entire body may be involved in few hours (Ong and Raffeto 1940; Harrell 1949). Characteristics of the rash considered to be classic for RMSF—that is, petechial lesions and a distribution that includes the palms and soles—occur in most of patients (Hazard et al. 1969; Kaplowitz et al. 1981; Ong and Raffeto 1940; Harrell 1949). Children are less likely than adults to present without a rash (Helmick et al. 1984). The life-threatening pathophysiologic consequences of infection and inflammation are microvascular damage and increased vascular permeability, which results in edema, localized hemorrhage, and hypoperfusion of one or more organ systems.

The invertebrate hosts for *R. rickettsii* include several species of ticks from several genera, including *Dermacentor*, *Rhipicephalus*, and *Amblyomma* (Burgdorfer 1988; Guedes et al. 2005). In these hosts, rickettsiae infect and replicate in several cell types, such as ovaries, salivary glands, midgut epithelium, and hemocytes (Sonenshine 1993). Vertebrates host that these bacteria has been isolated include domestic dogs, field voles (*Microtus pennsylvanicus*) (Badger 1933; Price 1954), pine voles (*M. pinetorum*), white-footed mice (*Peromyscus leucopus*), cotton rats (*Sigmodon hispidus*), cottontail rabbits (*Sylvilagus floridanus*), Rocky Mountain cottontail rabbits (*S. nuttallii*), snowshoe hares (*Lepus americanus*), opossums (*Didelphis virginiana*), chipmunks (*Tamias amoenus*), and golden-mantled ground squirrels (*Spermophilus lateralis*) (Burgdorfer 1988; McDade and Newhouse 1986; Schriefer and Azad 1994). The importance of birds as reservoir hosts for *R. rickettsii* remains unproven (Burgdorfer 1975).

Most cases of severe rickettsial disease are confirmed by serologic testing and on this basis are attributed to RMSF caused by *R. rickettsii*. However, the recent description of *R. parkeri* as a cause of rash-associated febrile illness (Paddock et al. 2004) in the United States highlights the low reliability of the results of serologic tests.

Despite the current availability of effective treatment and advances in medical care, many patients still die from rickettsial diseases. The majority of deaths are attributable to delayed diagnosis and failure to initiate specific antibiotic treatment within the first several days of the illness (Dalton et al. 1995; Kirkland et al. 1995). The recommended therapy for RMSF is doxycycline (CDC 2006).

African Tick-Bite Fever is probably an ancient disease in sub-Saharan Africa caused by *Rckettsia africae*. This disease may be one of the most common causes of acute febrile disease in rural sub-Saharan Africa, particularly in visitors from abroad. *R. africae* is a spotted-fever group rickettsia closely related to *R. parkeri* in North America and *R. sibirica* in Northeast Asia. These bacteria are mainly transmitted by *Amblyomma variegatum* and *Amblyomma hebraeum* ticks (Kelly and Mason 1991; Kelly et al. 1994; Parola et al. 2001). Humans are usually attacked by ticks on the legs, typically on moist skin behind the knee, in the groin, the perineum, or the axilla.

The pathophysiological hallmark of African tick-bite fever is the formation of focal or disseminated vasculitis (Toutous-Trellu et al. 2003). *R. africae* primarily invades the endothelial cells of smaller blood vessels (Jensenius et al. 2003a) and results in intramural and perivascular inflammation composed mainly of polymorphonuclear leukocytes, T-cells, and macrophages (Lepidi et al. 2006). The clearance of *R. africae* from endothelial cells is characterized by increased circulating levels of cytokines and chemokines (Jensenius et al. 2003b).

Rickettsia africae infection is symptomatic in few cases (Jensenius et al. 2002). The clinical course typically includes an abrupt onset of fever, nausea, headache, and neck myalgia commencing days after a tick bite (Raoult et al. 2001; Jensenius et al. 2003b). Most patients develop a painless large black crust surrounded by a red halo at the site of the tick bite. A painful regional lymphangitis (Brouqui et al. 1997) is detected in half of the cases. The majority of patients with African tick-bite fever develop mild to moderately severe illness that either resolves spontaneously within 10 days or responds promptly to anti-rickettsial treatment (Raoult et al. 2001; Jensenius et al. 2003b; Fournier et al. 1998a; Smoak et al. 1996). Complications are rare and no fatalities have ever been reported.

R. africae is susceptible in vitro to tetracycline, chloramphenicol, rifampin, fluoroquinolones, newer macrolides, and ketolides (Rolain et al. 1998, 2000). Most patients respond quickly to doxycycline. Patients with mild symptoms may not require any treatment at all (Jensenius et al. 2003a).

Mediterranean Spotted Fever (MSF) was described in Tunisia and was soon reported in the Black Sea littoral, India, the Middle East, and southern Africa. The causative agent was named as *Rickettsia conorii*. It was thereafter also known as "boutonneuse fever" because of a papular rather than macular rash. The brown dog tick *Rhipicephalus sanguineus* was recognized as a vector in Europe (Brumpt 1932); this vector is found throughout the world, but *R. conorii* is found only in some regions.

This bacterium does not normally infect humans during its natural cycle between its arthropod and vertebrate hosts, dogs. After an asymptomatic incubation of 6 days (Raoult et al. 1986; Martin Farfan et al. 1985), the onset of MSF is abrupt. Typical cases present with high fever and flu-like symptoms such as headache, chills, arthromyalgia, and a black eschar at the tick-bite site (Raoult and Roux 1997; Anton et al. 2003). The eschar is usually localized on the trunk, legs, and arms and rarely occurs in multiples. The rash often involves the palms and soles, but not the face. Generally, patients will recover within 10 days without any sequelae.

R. conorii multiply in the endothelial cells of small to medium vessels in human hosts, causing a vasculitis that is responsible for the clinical and laboratory abnormalities occurring in MSF (Parola and Raoult 2001). After phagocytosis and internalization, the phagocytic vacuole is lysed and rickettsiae escape the phagocytic digestion to multiply freely in the host cell cytoplasm and nucleus (Raoult and Roux 1997).

Before confirmation of the diagnosis, early empirical antibiotic therapy should be prescribed in any suspected cases. Doxycycline remains the treatment of choice for MSF (Raoult and Roux 1997). In patients with severe hypersensitivity to tetracyclines, chloramphenicol can be considered as an alternate therapy (Shaked et al. 1989).

Murine typhus, also known as endemic typhus, is a flea-borne infectious disease caused by *Rickettsia typhi*. The disease occurs in environments where rats and humans live in close proximity and typically in temperate and subtropical seaboard regions. The illness is less commonly diagnosed in developed countries than in the developing part of the world due to improved hygiene and rat control efforts. It is difficult to establish the true incidence because of the difficulty in distinguishing murine typhus from other causes of rash and fever.

R. typhi is a member of the typhus group of rickettsiae that also includes the agent responsible for epidemic typhus, *R. prowazekii*. *R. typhi* (or *R. mooseri*) is carried by the rat flea *Xenopsylla cheopis* and typically infects humans in markets, grain stores, breweries, and garbage depots. It usually causes a mild form of illness, but severe forms of the disease can also occur.

Scrub typhus is a chigger-borne zoonosis that is of greatest public health importance in tropical Asia; the causative agent is *Orientia tsutsugamushi*. The chigger bite can occur on any part of the body, is painless, and is not usually remembered by the patient (Sayen et al. 1946). Humans are accidental hosts. It is not known whether organisms deposited in the skin spread to internal organs via the bloodstream, the lymphatics, or by another mechanism. The disease begins as a small papule, enlarges, undergoes central necrosis, and acquires a blackened crust to form a lesion resembling a cigarette burn. Atypical eschar is pathognomonic when viewed by a clinician experienced in scrub typhus diagnosis. The rash appears on the trunk and spreads peripherally; however, it is often difficult to detect on dark-skinned persons.

O. tsutsugamushi infection appears to be a vasculitis, but the host cell in human scrub typhus is not known with certainty. The basic histopathological lesions suggest that macrophages are a more important target cell than the endothelium (Park and Hart 1946). Scrub typhus bacteria have been demonstrated in a variety of cells in humans, including monocytes, macrophages, Kuppfer cells, cardiac myocytes, hepatocytes, and endothelial cells (Settle et al. 1945; Allen and Spitz 1945; Walsh et al. 2001; Moron et al. 2001). In fatal cases, histopathology showed chiefly disseminated focal vasculitis and perivasculitis in vessels of the skin, lungs, heart, and brain. Endovasculitis and focal hemorrhage may be present but are less prominent than in Rocky Mountain spotted fever and epidemic typhus (Settle et al. 1945). The most important lesions are interstitial pneumonia with alveolar edema, hemorrhage, and meningoencephalitis (Settle et al. 1945; Allen and Spitz 1945; Park and Hart 1946).

Fever and headache begin abruptly and are frequently accompanied by myalgia and malaise. Cough, sometimes accompanied by infiltrates on the chest radiograph, is one of the most common presentations of scrub typhus infection (Chaykul et al. 1988). In severe cases, tachypnea progresses to dyspnea, the patient becomes cyanotic, and full-blown acute respiratory distress syndrome (ARDS) may develop. ARDS is associated with older age and preceding infiltrates on chest radiographs (Tsay and Chang 2002). Respiratory failure is the most common cause of death in severe scrub typhus infection, but survivors recover without sequelae.

O. tsutsugamushi infection can be cured and prevented by chloramphenicol and safer tetracyclines (Smadel et al. 1950). The response of mild scrub typhus to treatment with doxycycline or chloramphenicol is typically so rapid that resolution of fever is used as a diagnostic test; if the temperature has not returned to normal within 48 h after beginning doxycycline treatment, then the infection is not due to *O. tsutsugamushi* (Watt et al. 1996).

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24 The Family *Sneathiellaceae*

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Abstract
Sneathiellaceae, a family in the order *Sneathiellales*, comprises the genera *Oceanibacterium* and *Sneathiella*. The cultures are Gram-negative asporogenous rods, which have been recovered from marine samples. The dominant fatty acids include $C_{18:1}\omega7c$ and $C_{16:0.}$ The G + C content of the DNA is ~57 mol%. 16S rRNA gene sequencing evidence was used principally to delineate the new order, family, and genera.

Taxonomy, Historical and Current

Short Description of the Family

Sneathiellaceae (Snea.thi.el.la'ceae. N.L. fem. N. *Sneathiella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. N. *Sneathiellaceae* the family of *Sneathiella*).

LMG 23452^T, which became the type and so far only strain of *S. chinensis*, was recovered from coastal sediment in an aquacultural site located near Qingdao, China, in 2000. 16S rRNA gene sequencing pointed to its unique position with ~89 % sequence homology to *Devosia*, *Hyphomonas*, *Ensifer*, and *Chelatococcus*; phylogenetic examination of which showed that the culture formed a separate branch in the order *Rhizobiales*, recovered between the genera *Devosia* and *Ensifer* in the families *Hyphomicrobiaceae* and *Rhizobiaceae*, respectively. A second species, *S. glossodoripedis*, was described by Kurahashi et al. (2008) for a single isolate MKT133^T (= IAM 15419^T = KCTC 12842^T) from the nudibranch *Glossodoris cincta*, which was recovered from the sea of Aka island, Okinawa, Japan. However, these workers considered that *Sneathiella* formed a distinct phyletic lineage separate from all eight orders of the class *Alphaproteobacteria* and proposed a new family, i.e., *Sneathiellaceae*, in a new order *Sneathiellales*. A second genus, *Oceanibacterium* with a single species *O. hippocampi*, was described and added by Balcázar et al. (2012) for a single isolate BFLP-8^T (=CECT 7691^T = DSM 23444^T) obtained from the cutaneous mucus of wild long-snouted seahorse (*Hippocampus guttulatus*). The three cultures comprising the three species in the two genera of the family *Sneathiellaceae* are Gram-negative aerobic, asporogenous rods, which have been obtained from marine samples. The dominant fatty acids include $C_{18:1}\omega7c$ and $C_{16:0.}$ The G + C content of the DNA is ~57 mol%.

Phylogenetic Structure of the Family and Its Genera

Sequence similarity analyses involving the neighbor-joining algorithm revealed that *O. hippocampi* clustered most closely with uncultured bacterial clones (GenBank accession no. FJ202709 and FJ205319). The nearest culturable neighbors were *Oceanibaculum pacificum* MC2UP-L3^T, *S. glossodoripedis* IAM 15419^T, *S. chinensis* LMG 23452^T, and *Oceanibaculum indicum* P24^T with homologies of 91.5 %, 91.1 %, 90.9 %, and 90.5 %, respectively. The phylogenetic tree revealed that the *Sneathiella*, *Oceanibacterium*, and the uncultured bacterial clones form a distinct clade within the family *Sneathiellaceae* (*Fig. 24.1*).

Molecular Studies

The BLASTN search with the almost-complete (1,412 bp) 16S rRNA gene sequence of *S. chinensis* LMG 23452^T indicated that *Sneathiella* is phylogenetically related to members of the α-subgroup of the class *Proteobacteria*. The closest phylogenetic neighbors with similarities of 89 % were *Chelatococcus asaccharovorans* DSM 6462^T, *Devosia riboflavina* DSM 7230^T, and *Hyphomonas polymorpha* DSM 2665^T. The neighbor-joining tree showed that LMG 23452^T clustered most closely with an uncultured bacterial clone D101 that was found in deep-sea sediment of a western Pacific warm pool in China. LMG 23452^T was initially considered to form a separate branch in the order *Rhizobiales*, with <90 % 16S rRNA sequence homology with its neighbors (Jordan et al. 2007). However, Kurahashi et al. (2008) regarded that *Sneathiella* formed a separate and new phyletic lineage distinct from all eight orders, i.e., *Caulobacterales*, *Kordiimonadales*, *Parvularculales*, *Rhizobiales*, *Rhodobacterales*, *Rhodospirillales*, *Rickettsiales*, and

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Fig. 24.1

Phylogenetic reconstruction of the family *Sneathiellaceae* based on the neighbor-joining algorithm with the Jukes-Cantor correction. Sequence dataset and alignments, according to the All-Species Living Tree Project, release LTPs108 (Yarza et al. 2010). The tree topology was stabilized with the use of a representative set of 767 high quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied to remove hypervariable positions. The bar indicates 1 % sequence divergence

Sphingomonadales, of the class *Alphaproteobacteria*, segregating on a distinct and separate monophyletic 16SrRNA gene tree branch (Garrity et al. 2005; Kwon et al. 2005). Thus, the family *Sneathiellaceae* was proposed on the basis of phylogenetic analysis of 16S rRNA gene sequences (Kurahashi et al. 2008). *S. glossodoripedis* MKT133^T shared 97.2 % 16S rRNA sequence similarity with *S. chinensis* (Kurahashi et al. 2008). Moreover, the phylogenetic analysis revealed that the clade formed a distinct lineage with <90 % 15S rRNA sequence similarity to other orders in the *Alphaproteobacteria*. Therefore, it was proposed to elevate *Sneathiella* into a new order (*Sneathiellales*) and family (*Sneathiellaceae*). A second genus *Oceanibacterium* with *O. hippocampi* as the type and only species was added by Balcázar et al. (2012) after sequence similarities revealed 90.9 % and 91.1 % homology with *S. chinensis* and *S. glossodoripedis*, respectively.

DNA-DNA Hybridization Studies

The DNA-DNA relatedness between *S. chinensis* and *S. glossodoripedis* was ~22 % (Kurahashi et al. 2008).

Phenotypic Analyses

The main differential features of members of *Sneathiellaceae* are included in *Table 24.1*.

Table 24.1

Diagnostic properties in which the type strains of the species in the family *Sneathiellaceae* may be differentiated from each other

Taxon	Growth in/at:		Arginine dihydrolase	Urease production
	0 % (w/v) NaCl	45 °C		
O. hippocampi	–	–	+	+
S. chinensis	+	–	–	+
S. glossodoripedis	–	+	+	–

Oceanibacterium Balcázar et al. 2012

O.ce.an.i.bac.te'ri.um M.L. n. *oceanus* ocean; Gr. neut. Dim. N. *bakterium* a rod; N.L. n. *Oceanibacterium* rod-shaped bacterium from the ocean.

The single culture examined to date of the sole species *O. hippocampi* comprises slightly curved salt-requiring (1.0–6.0 % w/v) Gram-negative, aerobic, asporogenous rods of 0.4 × 1.0–16 μm in size that grow at 10–15 °C, and optimally at 25 °C on marine agar, and at pH 5.0–9.0, but not below pH 4.5 or above pH 9.5. Arginine dihydrolase, catalase, oxidase, and urease but not β-galactosidase or indole are produced. By means of API ZYM, acid, and alkaline phosphatases, esterase (C4), esterase lipase (C8), lipase (C14), cystine, leucine and valine arylamidases, and naphthol-AS-BI-phosphohydrolase were produced, but not α-chymotrypsin, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, α-mannosidase, or trypsin. Adipate and malate are assimilated, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, caprate, and citrate of phenyl acetate. Neither Aesculin nor gelatin is hydrolyzed. Nitrates are reduced to nitrite (Balcázar et al. 2012).

The ubiquinone system is Q-10; $C_{18:1}\omega7c$, $C_{19:0}$ cyclo $\omega8c$, $C_{16:0}$, $C_{18:1}$ 2-OH, and $C_{16:1}$ $\omega11c$ are the dominant fatty acids with smaller amounts of $C_{18:0}$, $C_{18:1}$ ω 9*c*, 11-methyl $C_{18:1}$ ω 7*c*, and $C_{18:0}$ 3-OH (Balcázar et al. 2012).

The G + C ratio of the DNA of the type strain is 57.8 mol% (T_m).

Sneathiella Jordan et al. 2007

Sneath.i.el'la N.L. fem. dim. n. *Sneathiella* honoring the British microbiologist P.H.A. Sneath for his contributions to bacterial taxonomy.

Cultures of the single isolate examined to date comprise aerobic, Gram-negative motile asporogenous rods. Growth occurs at 37 °C and in 1–3 % (w/v) NaCl. Catalase and oxidase are produced.

The major cellular fatty acids are $C_{18:1}$ ω7*c* and $C_{16:0}$.

After 48 h incubation, cultures of *S. chinensis* are butyrous and beige and 0.5–2.0 mm in diameter on marine agar, and comprise aerobic, Gram-negative motile asporogenous rods. *S. chinensis* grows in 0–3 % (w/v) NaCl, at 4–37 °C but not at 45 °C; reduces nitrate; hydrolyzes aesculin, casein, starch, tween 20, 40, 60, and 80; and deaminates tyrosine, but does not hemolyze horse blood. Alkaline phosphatase, leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase are produced, but not *N*-acetyl-β-glucosaminidase, α-chymotrypsin, esterase (C4), esterase lipase (C8), α-fucosidase, α- or β-galactosidase, β-glucuronidase, α-glucosidase, lipase (C14), α-mannosidase, cysteine or valine arylamidase, or trypsin. Neither glucose is assimilated nor arginine dihydrolase or urease produced; *p*-nitrophenyl-β-D-galactopyranoside, arabinan, cellulose, galactan, gelatin, pullulan, or xylan hydrolyzed; or *N*-acetylglucosamine, adipate, arabinose, caprate, citrate, gluconate, glucose, malate, maltose, mannitol, mannose, or phenylacetate assimilated. The major cellular fatty acids are $C_{18:1}$ ω7*c* and $C_{16:0}$, $C_{19:0}$ cyclo ω8*c*, $C_{16:1}$ ω7*c*, and $C_{17:1}$ ω6*c*. The G + C content of the type strain LMG 23452^T = CBMAI 737^T is 57.1 mol% (Jordan et al. 2007).

The single culture of *S. glossodoripedis* produces <1 mm diameter colorless, circular, convex, smooth colonies after 4–5 days incubation aerobically at 30 °C on marine agar and contains slightly curved rods of 0.2–0.3 × 0.7–1.0 μm in size with intracellular granules. Motility is by means of single polar flagella. Growth occurs at 17–45 °C [optimally at 40 °C], but not at 10 °C or 50 °C, and in 4 % (w/v) NaCl. Growth does not occur in 0 % (w/v) NaCl or anaerobically. Acid and alkaline phosphatase, arginine dihydrolase, esterase (C4) [weakly], esterase lipase (C8) [weakly], α-glucosidase [weakly], leucine arylamidase, *N*-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, ornithine decarboxylase, trypsin [weakly], and tryptophan deaminase are produced but not α-chymotrypsin, α-fucosidase, α-or β-galactosidase, gelatinase, β-glucosidase, β-glucuronidase, H_2S, indole, lipase (C4), α-mannosidase, urease or cysteine, or valine arylamidase; the Voges-Proskauer reaction is positive, citrate is utilized, and nitrates are reduced to nitrite. Neither amygdalin, arabinose, D-glucose, inositol, D-mannose, melibiose, rhamnose, sorbitol nor sucrose is assimilated. The ubiquinone system is Q10. The dominant cellular fatty acids are $C_{18:1}$ω7*c*, $C_{16:0}$, $C_{17:1}$, and $C_{14:0}$ 3-OH. The G + C content of the type strain MKT13^T [IAM 15419^T = KCTC 12842^T) is 56.9 mol% (Kurahashi et al. 2008).

Isolation, Enrichment, and Maintenance Procedures

Isolation was achieved on marine agar with incubation at 20 °C [for *O. hippocampi* and *S. glossodoripedis*) or 28 °C [for *S. chinensis*] for 3–7 days. Subculturing was achieved on the same medium with incubation at room temperature to 30 °C for up to 7 days. Long-term preservation was at −70 or −80 °C in marine broth supplemented with 20 % or 30 % (v/v) glycerol (Kurahashi and Yokota 2002; Jordan et al. 2007; Kurahashi et al. 2008; Balcázar et al. 2012).

Ecology

Habitat

Isolates have been recovered from the marine environment. Thus, the single culture of *O. hippocampi* BFLP-8^T = CECT 7691 T = DSM 23444^T was obtained from cutaneous mucus of wild long-snouted seahorse (*Hippocampus guttulatus*) (Balcázar et al. 2012). *S. chinensis* LMG 23452^T = CBMAI 737^T was isolated from sediment within a coastal aquaculture site in China (Jordan et al. 2007). *S. glossodoripedis* was recovered from the foot epidermis of a nudibranch (*Glossodoris cincta*) from seawater at a depth of 4 m collected off the coast of Japan (Kurahashi et al. 2008).

Pathogenicity and Clinical Relevance

To date, there has not been any involvement with human or animal disease.

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25 The Family *Sphingomonadaceae*

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Abstract
Besides the *Erythrobacteraceae*, the family *Sphingomonadaceae* represents the order *Sphingomonadales*, which currently

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includes the genera *Sphingomonas, Sandaracinobacter, Blastomonas, Novosphingobium, Sphingobium, Sphingopyxis, Sandarakinorhabdus, Sphingosinicella, Stakelama, Sphingomicrobium, Sphingorhabdus, Parasphingopyxis*, and *Zymomonas*. The genus *Sphingomonas* is the type genus. The family was proposed based on the 16S rRNA gene sequence phylogeny and the presence of 2′hydroxymyristol dihydrosphingosine 1-glucuronic acid (SGL-1) as major sphingoglycolipid in the cellular lipids (Kosako et al. 2000). Originally the family embraced six genera, *Sphingomonas*, *Erythrobacter*, *Erythromicrobium*, *Prophyrobacter*, *Zymomonas*, and *Sandaracinobacter*. Because the genera *Erythrobacter*, *Erythromicrobium*, and *Prophyrobacter* formed a distinct monophyletic 16S rRNA gene sequence cluster, they were transferred to the family *Erythrobacteraceae*, supported by differences in the sphingoglycolipid composition. The genera *Blastomonas* (Sly and Cahill 1997) and *Erythromonas* (Yurkov et al. 1997) were first transferred to the genus *Sphingomonas*, then both to the genus *Blastomonas*. The type genus *Sphingomonas* was dissected into four genera based on clustering in the 16S rRNA gene sequence phylogeny and differences in the major polyamine and the 2-hydroxy fatty acid patterns; the genus *Sphingomonas sensu stricto* and the three new genera *Novosphingobium*, *Sphingobium*, and *Sphingopyxis*. The genus *Sphingopyxis* was further dissected by the proposal of the genus *Parasphingopyxis*.

Members of the family are defined by the presence of SGL-1 in their cellular lipids, ubiquinone-Q10 as the major respiratory quinone system, and the octadecenoic fatty acid (C18:1) as the major fatty acid in total extractable lipids. The presence of 2-OH fatty acids of different chain length (C14 to C16) and the absence of 3-OH fatty acids are characteristic. Either *sym*-homospermidine or spermidine are the predominant polyamines. Most of the *Sphingomonadaceae* are chemoorganotrophic. The genera *Blastomonas, Sandaracinobacter*,and *Sandarakinorhabdus* are facultative photoeterotrophic due to the bacteriochlorophyll a (BChl *a*) content. One species of the genus *Sphingomonas*, *Sphingomonas kaistensis*, also contains BChl *a*. *Sphingomonadaceae* are commonly isolated from soils, freshwater and marine habitats, activated sludge, or the plant phyllosphere or rhizosphere. Only a few *Sphingomonadaceae* are known to cause human infections (*Sphingomonas paucimobilis*) or to be plant pathogens (*S. suberifaciens*); some are antagonistic against plant pathogens and induce plant growth promotion. Several species can degrade xenobiotic and recalcitrant (poly)aromatic compounds of natural or anthropogenic origin. Sphingomonads are very interesting with respect to their applications for bioremediation. *Sphingomonas* species can produce exopolysaccharides (sphingans), which are gelling agents that are used for food, pharmaceutical, or industrial applications. The genus *Zymomonas* differs from other members of the *Sphingomonadaceae* because of its fermentative, obligate, or facultative anaerobic metabolism. *Zymomonas*, which can produce biofuels, is used in natural fermentation processes of cider or plan fruits but is also the causative agent of the secondary fermentation products in biotechnological processes.

Taxonomy; Historical and Current

Short Description of the Family

Sphingomonadaceae Kosako, Yabuuchi, Naka, Fijiwara and Kobayashi 2000b, 1953^{VP} (Effective Publication: Kosako, Yabuuchi, Naka, Fijiwara and Kobayashi 2000a, 563)

Sphing.o.mon.a.da'ceae. M.L. fem. n. *Sphingomonas* type genus of the family; *-aceae* ending to demote a family; M.L. fem. pl. n. *Sphingomonadaceae* the family of *Sphingomonas* (modified from *Bergey's Manual*). The description is an emended version of the description given in the *Bergey's Manual*, 2nd edition (Yabuuchi and Kosako 2005).

Sphingomonadaceae are phylogenetically assigned to the order *Sphingomonadales* (Yabuuchi and Kosako 2005), representing group-4 *Alphaproteobacteria*. The family currently contains 13 validated genera, the type genus *Sphingomonas* (Yabuuchi et al. 1990; emend. by Yabuuchi et al. 1999, 2002; Takeuchi et al. 2001; Busse et al. 2003; Chen et al. 2012a), and the genera *Zymomonas* (Kluyver and van Niel 1936), *Sandaracinobacter* (Yurkov et al. 1997), *Blastomonas* (Sly and Cahill 1997; emend. by Hiraishi et al. 2000), *Novosphingobium* (Takeuchi et al. 2001), *Sphingobium* (Takeuchi et al. 2001; emend. by Li et al. 2013), *Sphingopyxis* (Takeuchi et al. 2001; emend. by Baik et al. 2013), *Sandarakinorhabdus* (Gich and Overmann 2006), *Sphingosinicella* (Maruyama et al. 2006; emend. by Geueke et al. 2007; Yasir et al. 2010.), *Stakelama* (Chen et al. 2010), *Sphingomicrobium* (Kämpfer et al. 2012), *Parasphingopyxis* (Uchida et al. 2012), and *Sphingorhabdus* (Jogler et al. 2013).

The family was originally proposed by Kosako et al. (2000) based on the 16S rRNA gene sequence phylogeny and the presence of 2′hydroxymyristol dihydrosphingosine 1-glucuronic acid (SGL-1) as major sphingoglycolipid in the cellular lipids (Kosako et al. 2000). The family originally consisted of six genera, *Sphingomonas* (Yabuuchi et al. 1990), *Erythrobacter* (Shiba and Simidu 1982), *Erythromicrobium* (Yurkov et al. 1994), *Porphyrobacter* (Fuerst et al. 1993), *Zymomonas* (Kluyver and van Niel 1936), and *Sandaracinobacter* (Yurkov et al. 1997). The type genus is *Sphingomonas* (Yabuuchi et al. 1990).

Blastomonas, *Erythromonas*, and *Rhizomonas* were single species genera; the three respective species *Blastomonas natatoria* (Sly 1985), *Erythromonas ursincola* (Yurkov et al. 1997), and "*Rhizomonas*" *suberifaciens* (van Bruggen et al. 1990) were transferred to the genus *Sphingomonas* as "*Sphingomonas natatoria*", "*Sphingomonas ursincola*", and "*Sphingomonas suberifaciens*" by Yabuuchi et al. (1999). Later, the genus *Blastomonas* was revived and "*Sphingomonas natatoria*" and "*Sphingomonas ursincola*" were reclassified as *Blastomonas natatoria* and *Blastomonas ursincola* (Hiraishi et al. 2000). Based on the fact that "*Sphingomonas suberifaciens*" contained spermidine as the major polyamine (Takeuchi et al. 1995) and clustered in the 16S rRNA gene sequence phylogeny closest to *Sphingobium*

boeckii, the species was recently transferred from "*Sphingomonas suberifaciens*" to *Sphingobium suberifaciens* (Chen et al. 2013).

Rejection of the Genus "*Rhizomonas*"

The genus "*Rhizomonas*" was proposed by van Bruggen et al. (1990). Based on comparative 16S rRNA gene sequences analysis and the cellular content of sphingoglycolipids (SGLs), it was suggested that the genus *Sphingomonas* (Yabuuchi et al. 1990) is synonymous with the genus *Rhizomonas*. Based on Rule 24b of the bacteriological code, the genus *Rhizomonas* has nomenclatural priority but the name *Rhizomonas* is illegitimate (Laplace et al. 1992) and placed into the "Rejected names of genera and subgenera of bacteria (*nomina generum et subgenerum bacteriorum rejicienda*)" by the Judicial Opinion 14. The reason for the rejection was that it was figured out to be a later homonym of a name of a taxon of protozoa (*Rhizomonas*; Kent 1880). The type species of this single species genus "*Rhizomonas*" was transferred to the genus *Sphingomonas*, as "*Sphingomonas suberifaciens*" recently reclassified as *Sphingobium suberifaciens* (Chen et al. 2013).

The genera *Erythrobacter* (Shiba and Simidu 1982), *Erythromicrobium* (Yurkov et al. 1994), and *Porphyrobacter* (Fuerst et al. 1993) formed a distanced monophyletic cluster in phylogenetic trees based on 16S rRNA gene sequences. The genera were therefore transferred to a separate family, the *Erythrobacteraceae* (Lee et al. 2005), which is now the second family harboring Group-4 *Alphaproteobacteria* beside the *Sphingomonadaceae*. The dissection of the *Sphingomonadaceae* was supported by phenotypic and chemotaxonomic features including differences in the SGL composition, pigmentation, and content of BChl *a*. *Erythromonas*, *Erythromicrobium*,and *Prophyrobacter* are mainly orange to red in color and contain BChl *a*.

Members of the *Sphingomonadaceae* are Gram-negative, nonsporulating, rod-shaped bacteria differing in cell size. They are nonmotile or motile; if motile, it is most often by means of a polar flagella. Subpolar flagella or peritrichous flagella were also detected. Several species were motile only in the early exponential growth phase. *Sphingomonadaceae* are aerobic or partially facultative anaerobic chemoorganotrophs. Strains of the three genera *Blastomonas, Sandaracinobacter*, and *Sandarakinorhabdus* contain BChl *a* and are facultative prototrophs. BChl *a* was also detected in some strains of the species *Sphingomonas kaistensis* (Kim et al. 2007). Colonies of *Sphingomonadaceae* are most often yellow, but they may also be creamy, faint, or deep yellow; orange or brown-orange; red colored; or, rarely, uncolored. They contain carotenoids.

Sphingomonadaceae lack lipopolysaccharides in their outer membrane which are instead replaced by SGLs (Kawahara et al. 1991; Kawasaki et al. 1994). Beside the 16S rRNA gene sequence phylogeny, the presence of N-2′-hydroxymyristyl dihydrosphingosine 1-glucuronic acid (SGL-1) is the main commonly shared feature of all members of the family *Sphingomonadaceae*. Kosako et al. (2000) first determined SGL-1 in the alkaline stable lipid fraction using an acidic solvent system by type species of the genera *Sphingomonas*, *Erythrobacter*, *Erythromicrobium*, *Porphyrobacter*, and *Zymomonas* (Kosako et al. 2000). Two district SGL types were determined. The genera *Sphingomonas*, *Sphingobium*, *Novosphingobium*, *Sphingopyxis*, and *Zymomonas* contain monosaccharide type SGLs and one or more oligosaccharide-type SGLs, whereas the genera *Erythrobacter*, *Erythromicrobium*, and *Porphyrobacter* contain only monosaccharide-type GSLs. Those differences clearly supported the dissection of the *Sphingomonadaceae* and the proposal of the family *Erythrobacteraceae*.

Octadecenoic acid (C18:1) is the major fatty acid, 2-OH fatty acids of different chain lengths (mainly C14 to C16) occur, with genus specific differences in proportions; 3-hydroxy fatty acids are absence (the genus *Zymomonas* as exception). Further exceptions occur where minor to trace amounts of 3-OH fatty acid are detected. For example, minor amounts of 3-OH C13:0 were found in the fatty acid patterns of the type strains of *Novosphingobium stygium* and *Novosphingobium aromaticivorans* (Busse et al. 1999).

Members of the *Sphingomonadaceae* share ubiquinone-10 (Q-10) as the major respiratory quinone. Minor amounts of Q-9 and/or Q-8 were also detected in several type strains. The major polyamine is either *sym*-homospermidine or spermidine dependent of the investigated genus. The genera *Sphingomonas*, *Sphingosinicella*, and *Sphingomicrobium* contain *sym*-homospermidine as major polyamines; all other genera contain spermidine as the predominant polyamine. Only for the genus *Parasphingopyxis* are polyamines not detectable (Uchida et al. 2012). Polar lipid profiles are characterized by the presence of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and SGL in all members of the family. The majority of the *Sphingomonadaceae* also contain phosphatidylcholine (PC), phosphatidylmonomethylethanolamine (PME), and phosphatidyldimethylethanolamine (PDE). Several unidentified glycolipids (GL) and phospholipids (PL) are also present, which contribute significantly to the uniqueness of polar lipid profiles of different species (Busse et al. 1999). Quantitative and qualitative differences occurring between polar lipid profiles of different species can support the differentiation of species within the family.

The 16S rRNA gene signature pattern for the family, originally given by Kosako et al. (2000), is 105–116 (C-C), 433–422 (G-C), 750–754 (T-C), 855–858 (G-G), 1,334–1,336 (G-C) and 1,385 (G) (numbering according to *E. coli* numbering, Brosius et al. 1978). The G+C content of the genomic DNA is 59–68.5 mol% (expect: *Zymomonas mobilis*, 49.1 mol%).

Sphingoglycolipids

A characteristic feature for members of the *Sphingomonadaceae* is the content of SGLs. The presence of sphingolipids in eukaryotic and prokaryotic cells is well known; two main forms occur, sphingophospholipids and sphingoglycolipids. The central structure of sphingolipids is ceramide, which is composed of

sphingosine, a C18 amino alcohol with an unsaturated hydrocarbon chain, and a fatty acid. In SGLs, carbohydrates are linked to the alcoholic hydroxy base of sphingosine by a glycosidic bond. Sphingoglycolipids can be differentiated by the number (1–4) and kinds of linked carbohydrates (glucoronic acid, glucosamine, galactose, and mannose). Two novel SGLs were detected in *Sphingomonadaceae*, glucuronosyl-ceramide (SGL-1) (Yamamoto et al. 1978) and galacuturonosyl-ceramide (SGL-1′) (Naka et al. 2000). In *Sphingomonadaceae*, SGLs have an important physiological function because they replace lipopolysaccharides in the Gram-negative cell walls (Kawahara et al. 1991; Kawasaki et al. 1994). The localization of SGL-4 in the outer membrane of the type strain *Sphingomonas paucimobilis* was visualization by Gold-labeled immunoelectron microscopy. It was reported that SGLs can effect humans by stimulating phagocytosis and phagosome-lysosome fusion in human neutrophils and lead to the induction and release of monokine by SGL (reported for the type strain of *Sphingomonas paucimobilis*; Tahara and Kawazu 1994; Krziwon et al. 1995).

Detailed analysis of the GSL of the type strain of *Sphingomonas paucimobilis*, the type species of the type genus *Sphingomonas*, showed that *Sphingomonas paucimobilis* contains the monosaccharide-type SGL (SGL-1) commonly occurring in *Sphingomonadaceae* and additionally an oligosaccharide-type SGL, SGL-4A. SGL-4A was detected in the cell membranes of *Sphingomonas paucimobilis* IAM 12576^T, *Sphingomonas parapaucimobilis* JCM 7510^T, *Sphingomonas sanguinis* IFO 13937^T, and *Novosphingobium capuslatum*. Detailed analysis of the SGL composition was performed by Yabuuchi et al. (2002) for 22 *Sphingomonas* species, which were partially reclassified to the genera *Novosphingobium*, *Sphingobium*, and *Sphingopyxis*. Among those, only the type strains of *Sphingopyxis terrae*, *Sphingopyxis macrogoltabida*, *Sphingobium yanoikuyae*, and *Sphingomonas wittichii* contained SGL-1 (galacturonosyl ceramide) in their cellular lipids. A distinct phylogenetic relationship of those species, however, was not observed.

Carotenoids

Most of the *Sphingomonadaceae* are yellow or orange pigmented with several variations in color intensity, which is due to the relatively high content or carotenoids. The abundance and characteristics of carotenoids are often analyzed by absorption spectra measurements of acetone-extracted pigments resulting in comparable absorption peaks (λmax), which are specific for different carotenoids. More detailed analysis of the carotenoid composition by high-performance liquid chromatography analysis of extracted carotenoids was only done for few sphingomonads. Several carotenoids were thereby identified. The main found carotenoid in *Sphingomonadaceae* is nostoxanthin (Jenkins et al. 1979; Wu et al. 2011; Glaeser et al. 2009). Other carotenoids were found to be rather abundant in some species such as zeaxanthin in *Sphingomonas jaspsi* (Asker et al. 2007a) or astaxanthin in *Sphingomonas astaxanthinifaciens* (Asker et al. 2007b). Astaxanthin is a red carotenoid with a high antioxidative activity (Asker et al. 2009). Busse et al. (2003) investigated seven strains of orange-pigmented *Sphingomonas* species representing the genera *Sphingomonas aurantiaca*, *Sphingomonas aerolata*, and *Sphingomonas faeni*. The obtained absorption spectra were identical to those of several yellow pigmented species including *Sphingomonas paucimobilis*, the type species of the genus *Sphingomonas* (Jenkins et al. 1979). Polar lipid analysis, however, indicated the presence of several orange pigments present in the total lipid extract.

It was indicated recently that carotenoids may play an important role during the biodegradation of heterocyclic compounds (Liu et al. 2012), which is a metabolic feature shared by several species among the *Sphingomonadaceae*. Carotenoids can act as membrane-integrated antioxidants, changing the fluidity of membranes and protecting cells for oxidative stress. Oxidative stress occurs during the oxidative degradation of heterocyclic compounds because reactive oxygen species are formed within this process in the presence of molecular oxygen. Liu et al. (2012) showed that *Sphingobium yanoikuyae* XLDN2-5 enhances the synthesis of zeaxanthin if growing in the presence of heterocyclic compounds. In a similar manner, it is hypothesized that carotenoids may play an important role for the adaptation of *Novosphingobium acidiphilum* to the surface water of a humic lake because it is frequently exposed to reactive oxygen species, mainly singlet oxygen, during the photolysis of humic substances in the water column during sunlight exposure (Glaeser et al. 2010).

Dissection of Genus *Sphingomonas Sensu Lato* into Four Genera

The type genus *Sphingomonas* was proposed by Yabuuchi et al. (1990) based on five species (*S. adhaesiva*, *S. capsulatum*, *S. parapaucimobilis*, *S. paucimobilis* (type species), and *S. yanoikuyae*) as a genus of Gram-negative, rod-shaped bacteria that from yellow-pigmented colonies, harbor a strictly aerobic and chemoheterotrophic metabolism, and contain SGL-1. With the increasing number of described species and the transfer of *Blastomonas natatoria*, "*Erythromonas ursincola*", and "*Rhizomonas suberifaciens*" to the genus *Sphingomonas* (Yabuuchi et al. 1999) an increasing chemotaxonomic and phylogenetic heterogeneity was described. For example, *sym*-Homospermidine was given as a main polyamine only for a subgroup of *Sphingomonas* species, including *S. paucimonas*, *S. mali*, *S. pruni*, and *S. trueperi* (Kämpfer et al. 1997). Several authors described the formation of four specific subclusters by phylogenetic analysis based on 16S rRNA gene sequences (Stackebrandt et al. 1988; Takeuchi et al. 1994; Yurkov et al. 1997). Based on the formation of the four monophyletic clusters by 16S rRNA gene phylogeny, distinct polyamine pattern, differences in the content of 2-hydroxy fatty acids, and specific 16S rRNA gene sequence signature nucleotides, Takeuchi et al. (2001) proposed the subdivision of the genus *Sphingomonas*

Yabuuchi et al. (1990) into the genus *Sphingomonas sensu stricto*, including nine species (16S rRNA gene sequence cluster I) and three new genera—the genera *Sphingobium* (four species; cluster II), *Novosphingobium* (six species; cluster III), and *Sphingopyxis* (two species; cluster IV). The four clusters showed intracluster phylogenetic similarities of more than 95.8 % 16S rRNA gene sequence similarities but only 92.6–96.5 % intercluster sequence similarities. The differentiation of the clusters was supported by distinct pattern of signature nucleotides of the 16S rRNA gene sequences of the species within the separated cluster. All species shared C18:1 as major fatty acid followed by C16:0. Some considerable differences were obtained with respect to nonpolar fatty acids, but the differences could not be correlated with the distinction of the four clusters. However, distinct differences between the clusters were obtained with respect to the content of 2-hydroxyl fatty acids. The predominant 2-hydroxy fatty acids of members of the genus *Sphingomonas sensu stricto* (cluster I) were 2-OH C14:0 and/or 2-OH C15:0; for the genus *Sphingopyxis* (cluster IV), 2-OH C15:0 and/or 2-OH C16:0 were the predominant 2-OH fatty acids in addition to 2-OH C14:0. Species of the genera *Sphingobium* (cluster II) and *Novosphingobium* (cluster III) contained only 2-OH C14:0 as major 2-hydroxy fatty acids.

The species *Sphingomonas sensu stricto* could be clearly differentiated from the other three genera based on the polyamine pattern, with *sym*-homospermidine as the predominant polyamine for the genus *Sphingomonas sensu stricto* (cluster I), and spermidine for the other three genera. The dissection of the genus was supported by different physiological features. Later, Yabuuchi et al. (2002) proposed an emendation of the genus *Sphingomonas* (Yabuuchi et al. 1990) based on the argument that the dissection cannot be confirmed by clear physiological and biochemical characteristics, including cellular lipids and fatty acids. This proposal for emendation was not generally accepted. In several species descriptions, clear statements were given supporting the dissection of the genus *Sphingomonas sensu lato* into four genera. Busse et al. (2003) argued that the presence of *sym*-homospermidine as a clear distinctive chemotaxonomic feature was not considered by Yabuuchi et al. (1990, 2002), although it clearly supported the phylogenetic dissection of the genus *Sphingomonas sensu lato*. Therefore, these and several other authors proposed to retain the genus name *Sphingomonas* only for the members of cluster I as defined by Takeuchi et al. (2001). The overall acceptance of the dissection of the genus *Sphingomonas sensu lato* is illustrated by the proposal of 15 new *Sphingopyxis*, 16 *Novosphingobium*, and 28 *Sphingobium* species since 2002. The genera are also separately listed in the List of Prokaryotic Names with Standing in Nomenclature (LPSN, http://www.bacterio.net/; June 2013). Only the species of cluster I defined by Yabuuchi et al. (2001) remained in the species *Sphingomonas*, with 46 new species proposed since 2002. In this chapter, all species assigned to the genus *Sphingomonas sensu lato* (based on cluster I defined by Yabuuchi et al. 2001) will be treated as *Sphingomonas* species. All other species were treated as species of the genera *Novosphingobium*, *Sphingopyxis*, and *Sphingobium*, respectively.

Dissection of the Genus *Sphingopyxis* and Proposal of the Genus *Sphingorhabdus*

Just recently, the genus *Sphingopyxis* was further dissected by the proposal of the *genus Sphingorhabdus* (Jogler et al. 2013). Three species of the genus *Sphingopyxis*, *Sphingopyxis marina*, *Sphingopyxis litoris* (both Kim et al. 2008), and *Sphingopyxis flavimaris* (Yoon and Oh 2005) were thereby transferred to the genus *Sphingorhabdus* (Jogler et al. 2013) as *Sphingorhabdus marina*, *Sphingorhabdus litoris*, and *Sphingorhabdus flavimaris* (Jogler et al. 2013). The type species of the new genus is *Sphingorhabdus planktonica*. The new genus can be differentiated from the genus *Sphingopyxis sensu lato* by a clear separation based on the 16S rRNA gene sequence phylogeny (two monophyletic groups were formed) and differences in the G+C content, which was much lower for species of the genus *Sphingorhabdus* (52.6–57.8 mol%) compared to the species remaining in the genus *Sphingopyxis* (62.3–69.2 mol%). Furthermore, specific 16S rRNA gene sequence signature nucleotides were determined, which can distinguish the species of the two genera (Jogler et al. 2013).

History of the Proposal of the Species *Sandaracinobacter sibiricus*

Sandaracinobacter sibiricus was proposed by the reorganization of the genus *Erythromicrobium* (Yurkov et al. 1997). The type strain was originally described as the first obligately aerobic Bchl *a*-containing freshwater bacterium isolated from thin microbial mats near underwater hydrothermal vents of a river. The strain was assigned to the marine genus *Erythrobacter* (Shiba and Simidu 1982) as "*Erythrobacter sibiricus*" (Yurkov and Gorlenko 1990) based on the Bchl *a* content and the strict aerobiosis. Later on "*E. sibiricus*" was reclassified into the new genus *Erythromicrobium* that contained obligately aerobic anoxygenic photosynthetic bacteria from freshwater habitats (Yurkov and Gorlenko 1990; Yurkov et al. 1997). Beside differences in the 16S rRNA gene sequence phylogeny, differences in the carotenoid content, cell morphology and cell division, the photosynthetic reaction center and antenna complexes, differences in the ubiquinone content, and heterogeneity in DNA-DNA hybridization (DDH) and 5S rRNA sequences, "*Erythromicrobium sibiricus*" was dissected together with "*Erythromicrobium ursincola*" from the genus *Erythromicrobium*. Because the two species could be clearly differentiated from each other based on the 16S rRNA gene sequence phylogeny, two new genera were proposed and the two species were reclassified as *Sandaracinobacter sibiricus* and *Erythromonas ursincola*.

Comments on the Genera *Blastomonas* and *Erythromonas*

The genera *Blastomonas* (Sly and Cahill 1997) and *Erythromonas* (Yurkov et al. 1997) were proposed as single-species

genera. Despite the close phylogenetic relationship of the two type strains, Yurkov et al. (1997) proposed two distinct genera because of the differences in the photosynthetic properties between "*Erythromonas ursincola*" (formerly "*Erythromicrobium ursincola*") and *Blastomonas natatoria* (Sly 1985; Sly and Cahill 1997), "*Erythrobacter ursincola*" contains Bchl *a* incorporated in the photochemically active reaction center (RC) and one light harvesting complex. In *Blastomonas natatoria*,only carotenoids were detected but not BChl *a* (Yurkov et al. 1997). Based on the 16S rRNA gene sequence phylogeny and the presence of SGL in the cellular lipids, the two species *Blastomonas natatoria* and "*Erythromonas ursincola*" were then both transferred to the genus *Sphingomonas* as "*Sphingomonas natatoria*" (Sly 1985) and "*Sphingomonas ursincola*" (Yurkov et al. 1997) by Yabuuchi et al. (1999). Hiraishi et al. (2000) proposed to retain the reclassification by providing the evidence of photosynthetic properties in *Blastomonas natatoria*. The production of BChl *a* and the presence of structural genes of the type II photosynthetic reaction center (*pufM* and *pufL* genes) were proofed for both species, "*Sphingomonas natatoria*" and "*Sphingomonas ursincola*". At this time, no further photosynthetic active *Sphingomonas* species were described.

The two type strains also differ in other physiological properties, including colony color (orange- to red-colored colonies) and cell division properties (budding or asymmetric division as shown by electron microscopy). Because of the high 16S rRNA gene sequence similarity, the obtained common features regarding photosynthesis, and the fact that at that time photosynthesis genes were not determined in other *Sphingomonas* species, Hiraishi et al. (2000) emended the description of *Blastomonas natatoria* (Sly 1985) Yurkov et al. (1997) as an aerobic photosynthetic bacterium and thereby proposed the reclassification of "*Erythromonas ursincola*" to *Blastomonas ursincola*. Although both species shared a close phylogenetic relationship based on 16S rRNA gene sequence analysis and the same properties regarding photosynthesis, the species could be clearly differentiated by DDH and distinctive physiological properties. The name *Erythromonas* was changed to *Blastomonas* according to Rule 37a of the bacteriological code and the transfer of *Blastomonas natatoria* and "*Erythromonas ursincola*" (Yurkov et al. 1997) to the genus *Sphingomonas* proposed by Yabuuchi et al. (1999) was turned down (notes at LPSN; http://www.bacterio.net/b/blastomonas.html).

In the course of the recommendation to retain the genus *Sphingomonas sensu lato*, Yabuuchi et al. (2002) also suggested that the *Blastomonas* species should remain assigned to the genus *Sphingomonas*. The assignment of the two species is treated differently in the literature; for example, Kim et al. (2007) described the first additional *Sphingomonas* species, *Sphingomonas kaistensis*, containing pufML genes (amplified by polymerase chain reaction [PCR] using degenerated primers) and strain-dependent BChl *a* (not detected in the type strain). Phylogenetic analysis based on full-length 16S rRNA gene sequences provided in this chapter clearly shows that *Blastomonas natatoria* and *Blastomonas ursincola* form a separate branch distinct to species of the genus *Sphingomonas*. Therefore, the two species will be treated as *Blastomonas natatoria* and *Blastomonas ursincola*, as also listed in the LPSN database (http://www.bacterio.net/).

Molecular Analyses

Phylogeny of Type Strains of the Species

According to the 16S rRNA-based All-Species Living Tree (Release LTPs111, which contains all type strains of all species with validly published names up to August 2012), the family *Sphingomonadaceae* formed a monophyletic cluster within the Alphaproteobacteria. The *Sphingomonadaceae* branched together with the family *Erythrobacteraceae*, each forming two distinct monophyletic clusters. Based on this phylogenetic distinction, the dissection of the family *Sphingomonadaceae* and the proposal of the family *Erythrobacteraceae* were suggested previously by Lee et al. (2005), with the transfer of the three genera *Erythrobacter*, *Prophyrobacter*, and *Erythromicrobium* to the family *Erythrobacteraceae*. A phylogenetic tree including all type strains assigned to genera of the *Sphingomonadaceae* is provided in ❯ *Fig. 25.1*. The phylogenetic tree in ❯ *Fig. 25.1* clearly shows the distinction of species assigned to the four genera *Sphingomonas*, *Sphingobium*, *Sphingopyxis*, and *Novosphingobium*, which originate from the dissection of the genus *Sphingomonas sensu lato* based on the formation of four monophyletic clusters (clusters I to IV) proposed by Takeuchi et al. (2001).

Including all species assigned to the four genera since 2001, distinct monophyletic clusters were obtained for the genera *Sphingopyxis* and *Sphingobium*, harboring all of the species currently within the genera. Two species previously assigned to the genus *Sphingomonas*, "*Sphingomonas xenophaga*" (Stolz et al. 2000) and "*Sphingomonas taejonensis*" (Lee et al. 2001), were clustered either in the genus *Sphingopyxis* or *Sphingobium*. Their phylogenetic affiliation to the genera *Sphingopyxis* and *Sphingobium* was furthermore supported by spermidine as the major polyamine instead of *sym*-homospermidine. Therefore, the species were transferred to *Sphingobium xenophagum* and *Sphingopyxis taejonensis* by Pal et al. (2006). In a similar manner, the species *Sphingomonas suberfaciens* (Yabuuchi et al. 1999) (originally proposed as *Rhizomonas suberifaciens* van Bruggen et al. 1990; for more details, see above) was transferred to the genus *Sphingobium suberifaciens* by Chen et al. (2013). This species clusters together with the type strain of the newly proposed species *Sphingobium boeckii* (Chen et al. 2013) and shares spermidine as the predominant polyamine with species of the genus *Sphingobium* (Chen et al. 2013).

Within the monophyletic cluster of the genus *Sphingopyxis*, three separate branches or subclusters were formed. The first subcluster contained most of the *Sphingopyxis* species among those the type species *Sphingopyxis macrogoltabida*. Three species—*Sphingopyxis flavimaris* (Yoon and Oh 2005), *Sphingopyxis litoris*, and *Sphingopyxis marina* (both Kim et al. 2008)—formed

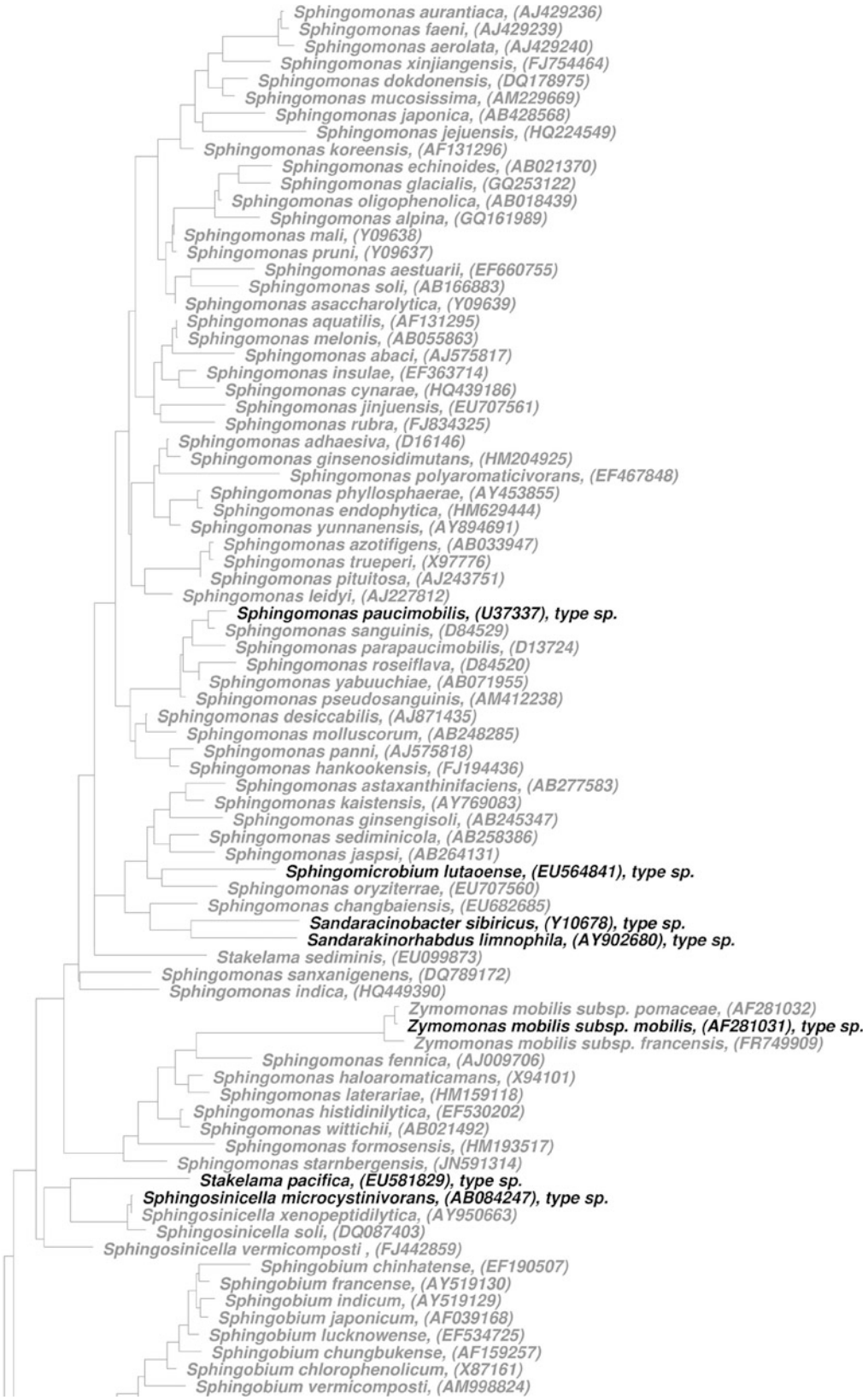

Fig. 25.1 (Continued)

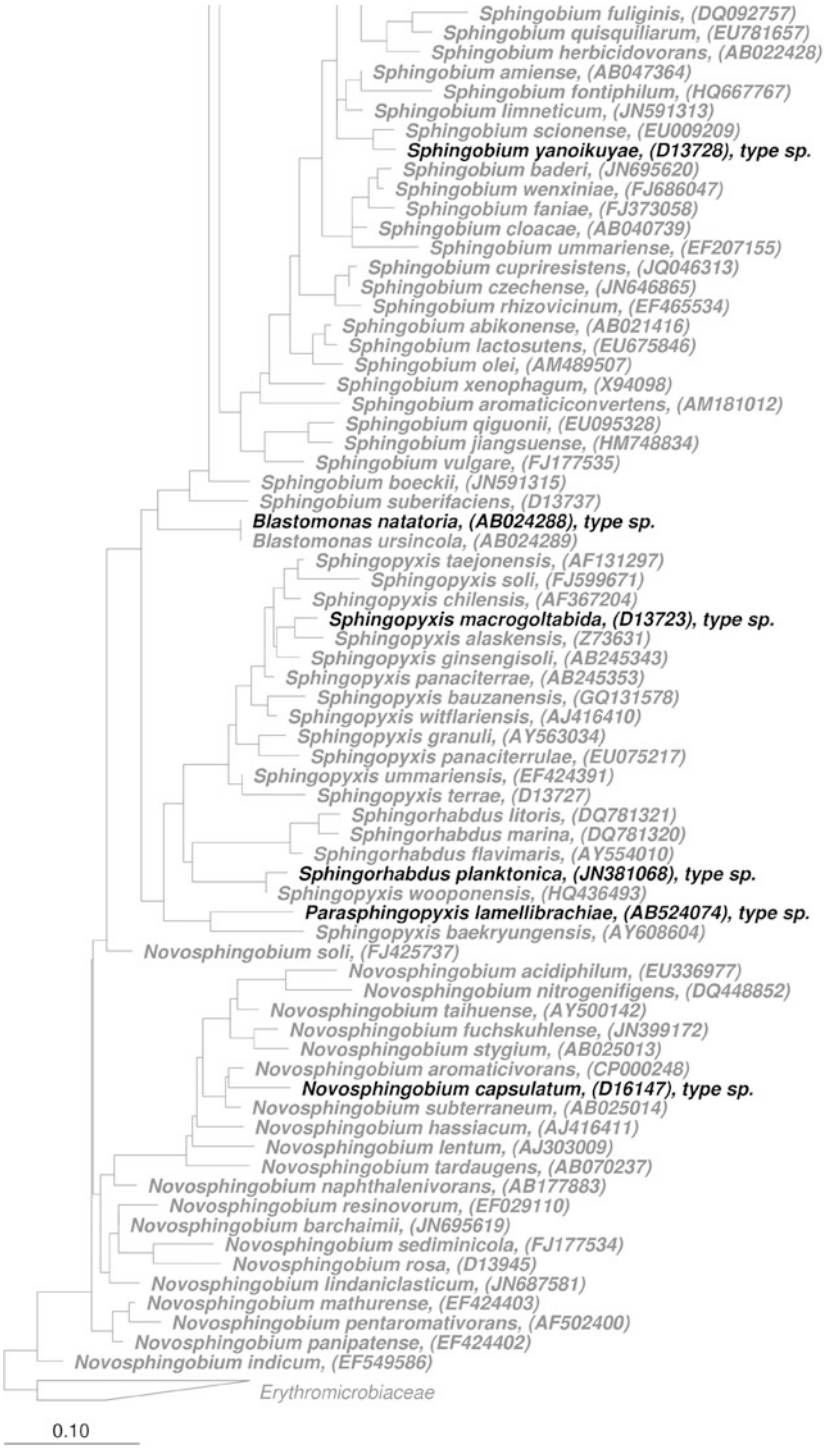

Fig. 25.1

Phylogenetic reconstruction of the family *Sphingomonadaceae* based on 16S rRNA gene sequences and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project database (Yarza et al. 2008; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. Scale bar indicates estimated sequence divergence. In all, the tree includes 61 *Sphingomonas*, 34 *Sphingobium*, 22 *Novosphingobium*, 15 *Sphingopyxis*, 4 *Sphingosinicella*, 4 *Sphingorhabdus* species, 2 *Blastomonas*, 2 Stakelama, 1 *Sandaracinobacter*, 1 *Sandarakinorhabdus*, 1 *Sphingomicrobium*, and 1 *Parasphingopyxis* species. Furthermore, three subspecies of the genus *Zymomonas* are included. The three new species of the genus *Sphingomicrobium*—*Sphingomicrobium astaxanthinifaciens* (Shahina et al. 2013a), *Sphingomicrobium flavum*, and *Sphingomicrobium marinum* (Shahina et al. 2013b)—were not yet considered in the tree

the second subcluster. Just recently, Jogler et al. (2013) investigated a new strain, which clustered into the second subcluster. Base on the phylogenetic distinction of this subcluster and distinct differences in the G+C content of type strains assigned to the different subclusters, Jogler et al. (2013) suggested the dissection of the genus *Sphingopyxis* and proposed the genus *Sphingorhabdus* with the type species *Sphingorhabdus planktonica*. The three *Sphingopyxis* species of the second subcluster were reclassified as *Sphingorhabdus flavimaris*, *Sphingorhabdus marina*, and *Sphingorhabdus litoris*. The G+C content of species remaining in the genus *Sphingopyxis* ranged from 62.3 mol% to 69.2 mol%, whereas the G+C content of species assigned to the genus *Sphingorhabdus* were lower, ranging from 52.6 mol% to 57.8 mol%. The dissection of the genus *Sphingopyxis* was furthermore supported by distinct signature nucleotides of the 16S rRNA gene, which were shared by either the *Sphingopyxis* or *Sphingorhabdus* species. At the same time as the genera were dissected, two new *Sphingopyxis* species were proposed, *Sphingopyxis wooponensis* and *Sphingopyxis rigui* (Baik et al. 2013), which also showed a low G+C content (53.3 mol% and 53.7 mol%, respectively), as the *Sphingopyxis* species transferred to the genus *Sphingorhabdus*. The phylogenetic analysis provided in ❯ *Fig. 25.1* showed that the two species were placed phylogenetically within the new genus *Sphingorhabdus*. Together with the fact that those two species also shared the low G+C content with the species of the genus *Sphingorhabdus*, a reclassification of the two species is necessary.

One species of the genus *Sphingopyxis*, *Sphingopyxis baekryungensis*, branched separately from the two subclusters formed by other species of the genus *Sphingopyxis* and the genus *Sphingorhabdus*. The species was originally proposed as a *Sphingopyxis* species because the phylogenetic analysis in the proposal indicated its closest relationship to *Sphingorhabdus flavimaris* (formerly "*Sphingopyxis flavimaris*") (Yoon et al. 2005). The phylogenetic tree provided here (❯ *Fig. 25.1*) showed that *Sphingopyxis baekryungensis* formed a third subcluster within the original monophyletic *Sphingopyxis* cluster, together with the type strain of *Parasphingopyxis lamellibrachiae* (Uchida et al. 2012). A distinctive feature of the genus *Parasphingopyxis lamellibrachiae* compared to other *Sphingomonadaceae* is that no polyamines could be detected (Uchida et al. 2012). Polyamines were not analyzed for *Sphingopyxis baekryungensis* so far (Yoon et al. 2005). The genomic G+C content of both species is in the same range with 63 % and 60.1 % for *Sphingopyxis baekryungensis* and *Parasphingopyxis lamellibrachiae*, respectively (Yoon et al. 2005; Uchida et al. 2012). Further studies are necessary to clarify the phylogenetic position of *Sphingopyxis baekryungensis*.

At the time of writing, the genus *Novosphingobium* contained 23 species with validly published names. In contrast to the genera *Sphingopyxis* and *Sphingobium*, species of the genus *Novosphingobium* are not placed into a single monophyletic cluster. The original cluster of the genus *Novosphingobium* (cluster III; Takeuchi et al. 2001) only contains the type species *Novosphingobium capsulatum* and the originally proposed *Novosphingobium* species, *N. subarcticum*, *N. stygium*, *N. subterraneum*, and *N. aromaticivorans*,and some of the *Novosphingbium* species proposed after 2001, including *N. acidiphilum*, *N. hassiacum*, *N. tardaugens*, *N. nitrogenifigens*, and *N. lentum*. A few *Novosphingobium* species are only loosely associated with this original cluster III (Takeuchi et al. 2001). *Novosphingobium rosa* (originally proposed as "*Sphingomonas rosa*") and *N. sediminicola* are clustered separately from the other *Novosphingobium* species; in the same manner, the species *N. naphtalenivorans*, *N. resinovorum*, *N. panipatense*, *N. mathurense*, and *N. pentaromaticivorans* are also clustered separately.

The type genus *Sphingomonas* represents the largest genus of the *Sphingomonadaceae*, currently including 61 species (without considering "*S. natatoria*" and "*S. ursinicola*"). All species are clustered together, expanding the original cluster as defined by Takeuchi et al. (2001). The genus is paraphyletic, forming different subclusters between those branches where the genera *Sphingomicrobium*, *Sandaracinobacter*,and *Sandarakinorhabdus* are placed (❯ *Fig. 25.1*). The type strain of *Sphingomicrobium lutaoense* (Kämpfer et al. 2012) is clustered closest to *Sphingomonas oryziterra* (Chung et al. 2011) in a cluster separate from the main *Sphingomonas* cluster, together with *Sphingomonas astaraxanthinifaciens*, *Sphingomonas kaistensis*, *Sphingomonas gingengisoli*, *Sphingomonas sediminicola*, and *Sphingomonas jaspi*. In the original proposal of *Sphingomicrobium lutaoense*, a neighbor joining tree that only included species which shared more than 93.5 % 16S rRNA gene sequence similarities to the new species. The tree indicated a separate branching of *Sphingomicrobium lutaoense*. Phylogenetic analysis including all currently proposed *Sphingomonadaceae* and the phylogenetic analysis based on the maximum likelihood methods using the RAxML algorithm (❯ *Fig. 25.1*) did not support this distinct branching, but placed the species in a separate branch into the *Sphingomonas* cluster. Furthermore, the genus *Sphingomicrobium* shared *sym*-homospermidine as major polyamine as common chemotaxonomic feature with the genus *Sphingomonas*. However, the type strain of *Sphingomicrobium lutaoense* showed only a low 16S rRNA gene sequence similarity to species of the genera *Sphingomonas*, *Sphingopyxis*, and *Sphingosinicella* (<95.5 %; Kämpfer et al. 2012) and showed clear differences regarding the cellular fatty acid pattern with respect to 2-hydroxy fatty acids. High amounts of 2-OH C18:1 were detected, unlike in other Sphingomonadaceae. Furthermore, C14 to C16 2-OH fatty acids were not present in *Sphingomicrobium lutaoense*.

Recently, three new *Sphingomicrobium* species were proposed—*Sphingomicrobium astaxanthinifaciens* (Shahina et al. 2013a), *Sphingomicrobium marinum*, and *Sphingomicrobium flavum* (Shahina et al. 2013b), which form a distinct cluster with *Sphingomicrobium lutaoense* (Shahina et al. 2013b). They share the specific pattern of 2-OH fatty acids and the absence of phosphatidylmonomethylethanolamine (PME) and phosphatidyldimethylethanolamine (PDE) in the polar lipid profiles (see below) with *Sphingomicrobium lutaoense*, supporting the differentiation of the genus *Sphingomicrobium* from other genera of

the *Sphingomonadaceae*, including the genus *Sphingomonas*. The two single species genera *Sandarakinobacter* and *Sandarakinorhabdus* are clustered together with *Sphingomonas changbaiensis* (Zhang et al. 2010b) as a separate branch in the cluster containing *Sphingomicrobium lutaoense* and the six above-listed *Sphingomonas* species (Fig. 25.1).

An additional species that was first assigned to another family but phylogenetically placed within the *Sphingomonas* cluster was *Caulobacter leidyi*. The species was originally assigned to the genus *Caulobacter* (*Caulobacteraceae*) and was described as "*Caulobacter leidyi*" (Poindexter 1964) because of its morphological properties (dimorphic prosthecate cell structures) that are common for *Caulobacter* species. Subsequent 16S rRNA gene sequence analysis revealed that this strain is phylogenetically placed within the *Sphingomonadaceae* (Stahl et al. 1992; Garrity et al. 2005). As a consequence, "*Caulobacter leidyi*" was excluded from the genus *Caulobacter*, omitted from *Bergey's Manual of Systematic Bacteriology*, and moved to limbo (Garrity et al. 2005).

Chen et al. (2012a) performed a detailed genotypic and phenotypic analysis of the type strain of *Caulobacter leidyi* and a new freshwater strain that shared high 16S rRNA gene sequence similarity (99.6 %) and DDH relatedness (82.9 %). The two strains shared high 16S rRNA gene sequence similarity with the type strain of *Sphingomonas aquatilis*—which, however, represents another separate species (DDH values <70 %). "*Caulobacter leidyi*" and the new freshwater strain shared *sym*-homospermidine as the major polyamine, main fatty acids, and predominant polar lipids with the genus *Sphingomonas* (Jogler et al. 2013). As a consequence, the species "*Caulobacter leidyi*" was transferred to the genus *Sphingomonas* as *Sphingomonas leidyi* (Chen et al. 2012a). After the reclassification of "*Caulobacter leidyi*," another prosthecate *Sphingomonas* species was proposed, *Sphingomonas canadensis* (Abraham et al. 2013). This species shared 97.2 % with *Sphingomonas oligophenolica* S213^T (=DSM 17107^T) and 97.0 % with *Sphingomonas leidyi* DSM 4733^T. Low DDH values (10.3 % and 23.3 %) to *Sphingomonas oligophenolica* and *Sphingomonas leidyi* clearly indicate that the new strain represents a separate species.

The two species *Blastomonas natatoria* and *Blastomonas ursincola*, representing the genus *Blastomonas*, form a separate branch distinct to other genera of the *Sphingomonadaceae* (Fig. 25.1). This clearly supports the emendation of the genus *Blastomonas* and the transfer of "*Erythrobacter ursincola*" to the genus *Blastomonas* proposed by Hiraishi et al. (2000), which overturned the reclassification of the two species to the genus *Sphingomonas* (Yabuuchi et al. 1999), as previously discussed. A clearly separated monophyletic branch within the *Sphingomonadaceae* is formed by the genus *Sphingosinicella*, including all type strains of the four proposed species (Fig. 25.1).

The genus *Stakelama* contains two species, *Stakelama pacifica* (Chen et al. 2010) and *Stakelama sediminis* (Thawng et al. 2013). The type strains of the two species shared only 95.7 % 16S rRNA gene sequence similarity but formed a distinct cluster in the 16S rRNA gene sequence-based phylogenetic analysis provided by Thawng et al. (2013). This clustering was one of the main criteria for the assignment of the second species to the genus *Stakelama* (Thawng et al. 2013). 16S rRNA gene sequence analysis based on the neighbor-joining methods performed here confirmed the clustering of the two species if all *Sphingomonadaceae* type strains were considered (analogous to the analysis in Fig. 25.1; data not shown). However, if the maximum likelihood method was applied (Fig. 25.1), the two species would be distinctly-branched from each other. Tindall et al. (2010) recommended that the neighbor-joining method should not be applied for final phylogenetic analysis because this case is not an exception where a new strain was assigned to a specific genus or where a new genus was proposed based on a phylogenetic analysis performed with the neighbor-joining method.

The genus *Zymomonas* was originally assigned to *Sphingomonadaceae* based on the 16S rRNA gene sequences phylogeny (Balkwill et al. 1997), but it shows distinct differences in G+C content (with much lower values of 47.5–49.5 mol%) and different metabolic properties (anaerobic metabolism) compared to all other members of the family. The genus *Zymomonas* is represented by only one species, *Zymomonas mobilis*, which is divided into three subspecies: *Zymomonas mobilis* subsp. *mobilis* (De Ley and Swings 1976), *Zymomonas mobilis* subsp. *pomaceae* (De Ley and Swings 1976), and *Zymomonas mobilis* subsp. *francensis* (Coton et al. 2006). The three subspecies form a separate branch in the 16S rRNA gene sequence phylogeny (see Fig. 25.1), which is loosely associated with a cluster of *Sphingomonas* species separated from the main *Sphingomonas* cluster, including *Sphingomonas fennica, Sphingomonas haloaromaticamans* (both Wittich et al. 2007), *Sphingomonas laterariae* (Kaur et al. 2012), *Sphingomonas histidinilytica* (Nigam et al. 2010), *Sphingomonas wittichii* (Yabuuchi et al. 2001), *Sphingomonas formosensis* (Lin et al. 2012), and *Sphingomonas starnbergensis* (Chen et al. 2012b). The 16S rRNA gene sequence of *Zymomonas mobilis* subsp. *francensis* was just recently published within the framework of the Sequencing Orphan Species project (Yarza et al. 2013); it is included in phylogenetic tree shown in Fig. 25.1

DNA-DNA Hybridization Studies

Nearly all descriptions of *Sphingomonas*, *Novosphingobium*, *Sphingopyxis*, and *Sphingobium* species included DDH analysis of the investigated strains and closest related type stains—mainly those that shared more than 97 % 16S rRNA gene sequence similarity.

Within the genus *Sphingomonas*, most of the species were rather distantly related, as indicated by low DDH values that were most often between 10 % and 30 %, such as between *Sphingomonas dokdonensis* and six related species (9–17 % similarity; Yoon et al. 2006), between *Sphingomonas hankookensis* and four related species (15–28 %; Yoon et al. 2009),

Sphingomonas ginsenosidimutans and four related species (8–28 %, Choi et al. 2010a), *Sphingomonas alpina* and two related species (Margesin et al. 2012), and *Sphingomonas panni* and three related species (18–24 %; Busse et al. 2005). Only a few species showed a closer relationship (>40 % DDH similarity). Five *Sphingomonas* species—*S. paucimobilis*, *S. sanguinis*, *S. pseudosanguinis*, *S. parapaucimobilis*, and *S. yabuuchiae*—form a distinct cluster based on 16S rRNA gene sequence analysis and share DDH similarities between 39.6 % and 51.9 % (Kämpfer et al. 2007; Li et al. 2004). An exception is *Sphingomonas roseiflava*, which also clusters with those species based on 16S rRNA gene sequence phylogeny but shows much lower similarity values based on the genomic level (e.g., 24.4 % DDH similarity to *S. yabuuchiae*; Li et al. 2004). In a similar manner, the species *Sphingomonas endophytica*, *S. phyllosphaerae*, *S. yunnanensis*,and *S. rubra* cluster together based on 16S rRNA gene sequence phylogeny and show high DDH relatedness (37.9–59 %, Huo et al. 2011; Huang et al. 2012). As exception, *Sphingomonas abaci* shared only 12–19 % and 17 % DDH similarity with *Sphingomonas melonis* and *Sphingomonas aquatilis* (Busse et al. 2005); however, the species also clusters together with those species, showing the high genomic relatedness.

Genomic relationships among species of the genus *Sphingopyxis* varied from very low relationships with DDH values below 10 % (e.g. for *Sphingopyxis panaciterrulae* and *Sphingophyxis macrogoltabida*; Srinivasan et al. 2010) to high relationships with values of up to 50 % (e.g. for *Sphingopyxis chilensis* and *Sphingopyxis alaskensis*; Godoy et al. 2003). Most of the genomic relatedness values between *Sphingopyxis* species (obtained by DDH analysis) were in the range of 30–39 %. In a similar manner, DDH values for the genus *Novosphingobium* ranged from 8.5 % between *Novosphingobium maturense* and *Novosphingobium panipatense* (Gupta et al. 2009) to 55.2 % for *Novosphingobium hassiacum* and *Novosphingobium capsulatum* (Kämpfer et al. 2002a). Approximately the same range of DDH values was obtained for the genus *Sphingobium*, in which several closely related species shared a relatively low DDH relatedness (7–20 %), but most of the species shared a higher genomic relatedness between 30 % and 59 %. *Sphingobium* species with relative high DNA-DNA relatedness include *Sphingobium cupriresistens* and *Sphingobium rhizovicinum* (57.2 ± 0.6 %; Li et al. 2013) and *Sphingobium francense* and *Sphingobium chinhatense* (54 %; Dadhwal et al. 2009).

Only a few species were proposed based on more than one representative strain. The DDH relatedness among strains within a species ranged from only 67.7 to >100 %. The two strains of *Sphingomonas aurantiaca* shared 81–118 % DNA relatedness (DDH analysis); the two strain of *Sphingomonas aerolata* (Busse et al. 2003) shared 71–107 %; and the strains of the species *Sphingomonas azotifigens* (Xie and Yokota 2006) shared 78.9 –80.6 %. Intraspecies DDH values were lower for three strains of *Sphingomonas kaistensis* (67.7–73.1 %; Kim et al. 2007). DDH values of five *Sphingopyxis alaskensis* strains were between 89 % and 100 % (Godoy et al. 2003); between two strains of *Sphingomonas leidyi* 82.9 % (Chen et al. 2012a); between two strains of *Novosphingobium hassiacum* 86 % (Kämpfer et al. 2002a); and between two strains of *Sphingobium xenophagum* were 70.7 % (Stolz et al. 2000). In parallel-performed genomic fingerprint analyses, such as repetitive extragenic palindromic (rep)-PCRs as BOX-PCRs (derived from the boxA element), clearly showed genomic differences indicating that the investigated strains are different strains assigned to the same species. Clonality of the investigated strains was thereby excluded when different genomic fingerprint patterns were obtained.

A DDH value of 100 % between *Novosphingobium resinovorum* NCIMB 8767^T and *Novosphingobium subarcticum* KCTC 2890^T indicated that both type strains represent the same species. Therefore, *Novosphingobium subarcticum* (Nohynek et al. 1996; Takeuchi et al. 2001) was assigned as a later heterotypic synonym of *Novosphingobium resinovorum* ("*Flavobacterium resinovorum*" Delaporte and Daste 1956) (Lim et al. 2007).

DDH analysis clearly demonstrated the high relationship but also distinction of the type strains of *Blastomonas natatoria* and *Blastomonas ursincola*. The values were 54–68 % or 31–47 %, depending on the used hybridization temperature—42 °C and 44 °C, respectively (Hiraishi et al. 2000). In contrast, the two species showed very low DDH relatedness (2–5 %) to type species in the closest related genera, including *Sphingomonas paucimobilis*, *Erythromicrobium ramosum*, and *Prophysobacter neustonensis*, supporting the distinction of the genus *Blastomonas*. However, further genera and further species of the genus *Sphingomonas* were not included in the analysis.

DDH analysis was not applied for the single species genera *Sandaracinobacter*, *Sandarakinorhabdus*, and *Parasphingopyxis*, because 16S rRNA gene sequence similarities were clearly below 97 % for the closest related type strains of the neighboring genera.

For the genus *Sphingorhabdus* (Jogler et al. 2013), DDH values are available for the reclassified former *Sphingopyxis* species, *Sphingorhabdus marina*, *Sphingorhabdus litoralis*, and *Sphingorhabdus flavimaris*, which ranged from 20.7 % to 36.6 % (Kim et al. 2008).

For species of the genus *Sphingosinicella*, DDH analysis for *Sphingosinicella xenopeptidilytica* and *Sphingosinicella microcystinivorans* (Geueke et al. 2007) showed a relatively high genomic relatedness of 40.6 % (reciprocal, 51.9 %). *Sphingosinicella soli* showed much lower DNA relatedness to those two species, of only 27 % and 25 %, respectively (Yoon et al. 2008). Geueke et al. (2007) showed that DDH data are strongly affected by the applied method, with the microtiter plate method of Ziemke et al. (1998) resulting in much higher values (40.6, 51.9 %) compared to other standard methods used for DDH (33 %).

MLSA

Phylogenetic analyses provided for members of the family *Sphingomonadaceae* is thus far only based on 16S rRNA gene sequence data. Single or multilocus sequence analysis (MLSA) based on protein coding (housekeeping) genes has not been

applied to investigate the phylogenetic resolution between species and genera within the *Sphingomonadaceae* for taxonomic purposes.

Only *Zymomonas mobilis* subspecies were comparatively analyzed based on additional genes beside the 16S rRNA gene sequence analysis, including the 16S–23S rRNA gene intergenic spacer region (ISR) and two housekeeping genes, *hsp60*, encoding the 60-kDa heatshock protein (Hsp60) and *gyrB*, encoding the B subunit protein of the DNA gyrase (Coton et al. 2006). A total of three, four, and six strains were investigated for *Z. mobilis* subsp. *mobilis*, Z. *mobilis* subsp. *pomaceae*, and *Z. mobilis* subsp. *francensis*, respectively. Phylogenetic trees calculated based on ISR sequences (604–617 bp) and partial sequences of *hsp60* (592 bp) and *gyrB* (1,044 bp) clearly showed the formation of three distinct groups formed by the investigated strains of each subspecies. Groups representing strains of subspecies *Z. mobilis* subsp. *mobilis* and *Z. mobilis* subsp. *francensis* always clustered closer together, and the group of *Z. mobilis* subsp. *pomaceae* strains was more distantly related to those (Coton et al. 2006). This relationship was confirmed by the respective sequence similarities. Strains of *Z. mobilis* subsp. *francensis* shared 94 % ISR sequence similarity with strains of *Z. mobilis* subsp. *mobilis* but only 87 % with strains of *Z. mobilis* subsp. *pomaceae*. In a similar manner, 90 % similarity was obtained for strains of *Z. mobilis* subsp. *mobilis*, but only 84 % for strains of *Z. mobilis* subsp. *pomaceae* if partial *hsp60* sequences were investigated and 86 % similarity versus 80 % similarity if partial *gryB* sequences were investigated. Whole-cell protein analysis using sodium dodecyl sulfate polyacrylamide gel electrophoresis protein profiling, random amplified polymorphic DNA (RAPD)-PCR and rep-PCR genotyping confirmed the formation of distinct groups separating the three subspecies (Coton et al. 2006).

In an environmental study, Vaz-Moreira et al. (2011) analysed in addition to the 16S rRNA gene sequence-based phylogeny partial nucleotide sequence of the housekeeping gene *atpD* (encoding the beta subunit of membrane ATP synthase) to assign environmental isolates more precisely into the genera *Sphingomonas*, *Sphingopyxis*, *Novosphingobium*, *Sphingobium*, and *Blastomonas*. Thereby, the *atpD*-based phylogeny supported the 16S rRNA gene sequence-based phylogeny.

DNA Pattern, Ribotyping

Nucleic acid fingerprinting methods provide a resolution at the subspecies and/or strain level (Tindall et al. 2010). Techniques generating genomic DNA patterns were successfully applied for members of the *Sphingomonadaceae*, including RAPD-PCR and different repetitive element primed PCR techniques, directed to highly conserved, repetitive DNA sequences distributed in multiple copies over a genome. Successfully applied PCR methods were rep-PCR, enterobacterial repetitive intergenic consensus sequences (ERIC)-PCR, BOX-PCR, $(GTG)_5$-PCR, and Salmonella Enteritidis Repetitive Element (SERE-PCR; Louws et al. 1994; Versalovic et al. 1994; Rademaker et al. 1998; Rajashekara et al. 1998). The application of those methods in different studies of *Sphingomonadaceae* species proposals showed diverse fingerprint patterns, which allowed a good differentiation of strains within a single species and closely related species. For example, Busse et al. (2003) successfully applied two rep-PCR fingerprint methods, ERIC and BOX-PCRs, to differentiate three *Sphingomonas aurantiaca* strains, three *Sphingomonas aerolata* strains, and the type strain of *Sphingomonas faeni*,which all shared 99.3–100.0 % 16S rRNA gene sequence similarity (Busse et al. 2003). In the same manner, ERIC-PCR (Busse et al. 2005) and BOX-PCR (Balkwill et al. 1997; Yuan et al. 2009) were applied for other *Sphingomonas* and *Novosphingobium* species, respectively. Rivas et al. (2004) applied a two-primer RAPD fingerprinting for the differentiation of *Sphingomonas phyllosphaerae* strains and five rep-PCR methods, including BOX-, ERIC-, REP-, $(GTG)_5$, and SERE-PCR, were applied for the genus *Sphingosinicella* to support the differentiation of type strains of *Sphingosinicella* species (Geueke et al. 2007; Yoon et al. 2008).

RAPD-PCR and rep-PCRs including ERIC- and BOX-PCRs were also used to differentiate strains belonging to the three subspecies of the genus *Zymomonas*. Strains representing the three subspecies were clearly separated in three different groups by subsequent cluster analysis (Coton et al. 2006).

Riboprinting

Riboprinting was established as a tool for a rapid preliminary identification of sphingomonads by Busse et al. (2000). A riboprinting pattern of a strain was obtained after genomic DNA was restricted with the restriction enzyme EcoRI; the DNA fragments were separated on an agarose gel, transferred to a nylon membrane, and hybridized with phosphorescently tagged *E. coli* rrnB operon. In the study by Busse et al. (2000), a total of 43 sphingomonads were analysed. Most of the investigated type strains showed characteristic complex banding patterns. Bands appeared in the range of 1.1–48 kb and a strong band in the area of 3.5–3.9 kb. A single strong band in the range of 2.4–2.7 kb was obtained for *Sphingobium yanoikuyae* DSM 6900, *Sphingopyxis macrogoltabidus* IFO 15033^T, and *Sphingopyxis terrae* IFO 15098^T. For *Z. mobilis*,a strong band was obtained in the area of 1.1 kb. No bands were obtained for *Sphingobium suberifaciens* DSM 7465^T and *Sphingomonas witiichii* RW1^T except in the area of 48 kb. This suggests that the DNA of those two strains was not digested by EcoR1.

Ribotype patterns enabled all 40 species to be distinguished in the study by Busse et al. (2000). A high degree of riboprinting similarities were only observed among strains of the same species, such as *Sphingobium yanoikuyae*, *Novosphingobium aromaticivorans*, *Novosphingobium subarcticum*, and *Sphingomonas chlorophenolica*. However, strains of very closely

related species could clearly be differentiated, such as type strains of the species *Sphingomonas asaccharolytica*, *Sphingomonas mali*, and *Sphingomonas pruni*. Differentiation was possible by computer-based cluster analysis but also by visual analysis. Riboprinting was applied for the comparative analysis of the three closely related *Sphingomonas* species, *Sphingomonas aurantiaca* (3 strains), *Sphingomonas aerolata* (3 strains), and *Sphingomonas faeni* (1 strain), which shared 99.3–100.0 % 16S rRNA gene sequence similarity (Busse et al. 2003). A high degree of similarity in the riboprints was only observed for strains that represent the same species (>63 % and 76 %) but no significant similarities (<54 %) were detected between riboprints of strains from different species (supported by DDH analysis). Ribotyping was also applied by Koskinen et al. (2000) to characterize 38 sphingomonad strains isolated from biofilms in drinking water distribution systems. Two different restriction endonucleases, EcoRI and PvuII, were used for the analysis in this study. Riboprints obtained for isolated strains were compared with those of reference strains to assign the respective isolates to the species.

MALDI-TOF Analysis

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is generally not applied for the differentiation of species within the *Sphingomonadaceae*. Hotta et al. (2012) established a MALDI-TOF MS method using ribosomal subunit proteins coded in the S10-spc-alpha operon as biomarkers for the classification of environmental Sphingomonadaceae (S10GERM method). A total of nine ribosomal subunit proteins coded in the S10 and spc operons, L18, L22, L24, L29, L30, S08, S14, S17, and S19, were commonly detectable subunits by MALDI-TOF MS analysis of *Sphingomonadaceae* references. Sequence analysis of the S10 and spc operons from type strains of the genera *Sphingomonas*, *Sphingopyxis*, *Sphingobium*, and *Novosphingobium* confirmed the localization of the respective ribosomal proteins in this operon. A phylogenetic tree based on respective amino acids showed mainly the same phylogenetic relationships as what was obtained by 16S rRNA gene sequence analysis. However, a higher resolution was obtained by the S10Germ method among closely related strains.

Genomic and Phenetic Properties of the Taxa

Genome Structure, Genome Architecture, Number of Chromosomes, Genes, Plasmids, Integrons, Genomic Islands

In the integrated microbial genome database of the Joint Genome Institute or the National Center for Biotechnology Information (NCBI) genome database, currently 30 genomes represent strains assigned to the family *Sphingomonadaceae*. These genomes include several type strains, including three *Sphingomonas* species (*Sphingomonas melonis* DAPP-PG 224^T, *Sphingomonas phyllosphaerae* FA2^T, and *Sphingomonas wittichii* RW1^T), four *Novosphingobium* species (*Novosphingobium acidiphilum* DSM 19966^T, *Novosphingobium aromaticivorans* DSM 12444^T, *Novosphingobium nitrogenifigens* DSM 19370^T, and *Novosphingobium pentaromativorans* US6-1, DSM 17173^T), two *Sphingobium* species (*Sphingobium chlorophenolicum* L-1^T and *Sphingobium japonicum* UT26S^T), two *Sphingophyxis* species (*Sphingopyxis alaskensis* RB2256^T and *Sphingopyxis baekryungensis* DSM 16222^T), and *Sandarakinorhabdus limnophila* DSM 17366^T, as well as two *Zymomonas* subspecies (*Zymomonas mobilis* subspecies *mobilis* T.H.Delft 1, ATCC 10988^T and *Zymomonas mobilis* subspecies *pomaceae* Barker 1, ATCC 29192^T). The size of the genomes including draft genome data range from 2.6 to 5.9 Mbp, with a G+C content between 57 % and 68 % (Table 25.1). Exceptions are the genomes of *Zymomonas mobilis* strains, with a G+C content between 46 % and 51 %. The genomes contain one or two chromosomes and one to five plasmids of different sizes (Table 25.1). Many sphingomonads are able to degrade xenobiotic and recalcitrant (in particular, aromatic) compounds.

Genes involved in degradation pathways are often encoded on large plasmids with a size of 50–500 kb (Basta et al. 2004), but partially respective genes are also encoded on chromosomes. Evidence for horizontal gene transfer is given for several genomes (see below). Currently sequenced sphingomonads were mainly selected to investigate their specific metabolic features (e.g. degradation pathways) or were sequenced within the framework of the Genomic Encyclopedia of Bacteria and Achaea (GEBA) project (Wu et al. 2009).

The draft genome of ***Sphingomonas echinoides* ATCC 14820^T** had a size of 4.2 Mb, with a G+C content of 64.7 % (Shin et al. 2012). A total of 4,047 protein-encoding genes, 45 tRNA-encoding genes, and one rRNA operon have been predicted in the draft genome so far. A total of 2,781 (68.7 %) of the predicted protein-coding open reading frames (ORFs) were annotated to known proteins. Comparative analyses were performed with genome sequences available for *Sphingomonas* sp. strain SKA58 (score, 514), *Sphingobium japonicum* UT26S^T (score, 513), and *Sphingomonas wittichii* RW1^T (score, 476) as the closest related genome-sequenced sphingomonads.

***Sphingomonas elodea* ATCC 31461^T** was genome sequenced because it is able to produce gellan gum—a heteropolysaccharide used as a commercial gelling agent in several industries, including the food and pharmaceutical industries. The aim of the genome project was to identify genes and characterize metabolic pathways involved in the biosynthesis, regulation, and modification of gellan gum (Gai et al. 2011). The draft genome had a size of 4.1 Mb and a G+C content of 67.4 %. A total of 3,813 coding ORFs and 49 structural RNAs were predicted. Comparison with the genome sequences available on the RAST server suggested that the closest genome-sequenced neighbors

Table 25.1

Overview of fully genome sequenced *Sphingomonadaceae* (Data taken from NCBI; http://www.ncbi.nlm.nih.gov/genome/)

Strain	Type	Name	Size (Mb)	G+C content (%)	Protein	rRNA	tRNA	Other RNA	Gene	RefSeq
Sphingomonas wittichii RW1^T	Chr	—	5.38	68.4	4,850	6	48	2	4,941	NC_009511.1
	Plsm	pSWIT01	0.310228	64.1	285	—	—	—	288	NC_009507.1
	Plsm	pSWIT02	0.222757	61.2	210	—	—	—	226	NC_009508.1
Sphingopyxis alaskensis RB2256^T	Chr	—	3.35	65.5	3,165	3	45	4	3,230	NC_008048.1
	Plsm	F plasmid	0.028543	60.4	30	—	—	—	30	NC_008036.1
Sphingobium chlorophenolicum L-1^T	Chr	1	3.08	63.9	2,846	3	47	3	2,970	NC_015593.1
	Chr	2	1.37	63.6	1,104	6	8	—	1,171	NC_015594.1
	Plsm	pSPHCH01	0.123733	64.8	122	—	—	—	124	NC_015595.1
Sphingobium japonicum UT26S^T	Chr	1	3.51	64.8	3,529	3	51	3	3,586	NC_014006.1
	Chr	2	0.681892	65.9	589	6	4	—	599	NC_014013.1
	Plsm	pCHQ1	0.190974	63	224	—	—	—	224	NC_014007.1
	Plsm	pUT1	0.031776	63.7	44	—	—	—	44	NC_014005.1
	Plsm	pUT2	0.005398	61	8	—	—	—	8	NC_014009.1
Novosphingobium pentaromativorans US6-1^T		master WGS	5.34	63.1	5,234	3	46	5,283		NZ_AGFM00000000.1
	Plsm	pLA1	0.188476	62.6	199	—	—	199		NZ_AGFM01000122.1
	Plsm	pLA2	0.060085	60.2	87	—	—	87		NZ_AGFM01000123.1
	Un	—	5.1	—	4,948	3	46	4,997		—
Novosphingobium aromaticivorans DSM 12444^T	Chr	—	3.56	65.2	3,324	9	57	8	3,411	NC_007794.1
	Plsm	pNL1	0.184462	62.3	182	—	—	—	187	NC_009426.1
	Plsm	pNL2	0.487268	65.9	431	—	—	—	432	NC_009427.1
Zymomonas mobilis subsp. *mobilis* ZM4 = ATCC 31821	Chr	—	2.06	46.3	1,736	9	51	1,807	6	NC_006526.2
Zymomonas mobilis subsp. *mobilis* NCIMB 11163	Chr	—	2.12	46.8	1,800	9	51	3	1,883	NC_013355.1
	Plsm	pZA1001	0.05338	42.3	46	—	—	—	54	NC_013356.1
	Plsm	pZA1002	0.040818	43.8	32	—	—	—	35	NC_013357.1
	Plsm	pZA1003	0.004551	36.4	6	—	—	—	6	NC_013358.1
Zymomonas mobilis subsp. *mobilis* ATCC 10988^T	Chr	—	2.02	46.2	1,695	6	48	3	1,797	NC_017262.1
	Plsm	pZMOB01	0.032479	43.5	28	—	—	—	38	NC_017180.1
	Plsm	pZMOB02	0.032283	45.4	24	—	—	—	32	NC_017183.1
	Plsm	pZMOB03	0.031692	43.2	24	—	—	—	27	NC_017181.1
	Plsm	pZMOB04	0.018461	41.8	27	—	—	—	32	NC_017184.1
	Plsm	pZMOB05	0.004023	37.6	3	—	—	—	3	NC_017182.1
	Plsm	pZMOBP6	0.00275	41.3	2	—	—	—	2	NC_017185.1
Zymomonas mobilis subsp. *mobilis* ATCC 29191	Chr	—	1.96	46.2	1,664	9	51	4	1,774	NC_018145.1
	Plsm	pZZ6.01	0.01835	41	16	—	—	—	22	NC_018146.1
	Plsm	pZZ6.02	0.014947	42.2	19	—	—	—	19	NC_018147.1
	Plsm	pZZ6.03	0.013742	44.2	10	—	—	—	11	NC_018148.1
Zymomonas mobilis subsp. *pomaceae* ATCC 29192^T	Chr	—	1.99	44.1	1,683	9	51	4	1,763	NC_015709.1
	Plsm	pZYMOP01	0.037387	41	33	—	—	—	36	NC_015715.1
	Plsm	pZYMOP02	0.034161	44	32	—	—	—	41	NC_015716.1

Chr Chromosome, *Plsm* Plasmid

are *Novosphingobium aromaticivorans* (score, 521), followed by *Sphingopyxis alaskensis* RB2256^T (score, 503), and *Sphingomonas wittichii* RW1^T (score, 475). A first insight into the genome showed that several genes (*pgmG*, *ugpG*, and *ugdG*) encoding enzymes involved in the synthesis of the nucleotide sugars (UDP-Glc and UDP-GlcA) from Glc-1-phosphate were found dispersed over the genome. A glycosyltransferase (involved in the synthesis of dTDP-l-Rha) and the proteins required for gellan polymerization and export were found to be located together in a gel cluster. At least 42 enzymes related to the metabolism of monosaccharides (such as mannose, D-galactonate, D-gluconate, ketogluconates, D-galacturonate, and D-glucuronate) were found, indicating the high metabolic versatility of the strain, which may be linked to the ability to synthesize heteropolysacchsarides (Gai et al. 2011).

The type strain of ***Sphingopyxis alaskensis* RB2256^T** was genome sequenced as a model for the investigation of oligotrophic (i.e., adapted to low nutrient concentrations) small marine bacteria (Lauro et al. 2009). The strain was isolated from Resurrection Bay in the Gulf of Alaska. The genome had a size of 3.9 Mb consisting of one chromosome (3.4 Mb, G+C 65.5 %, 3,165 proteins, one rRNA operon and 45 tRNAs) and one plasmid (28 kb, G+C 60.4 %, 30 proteins). The genome showed a high number of genes assigned to metabolic pathways for the degradation of aromatic compounds and a high number of genes assigned to detoxification reactions. The degradation of xenobiotic compounds and the catabolism of recalcitrant compounds are important for the use of carbon and energy in the habitat of this oligotrophic species. For example, genes involved in the degradation of beta-lactames, tyrosine, and catechol were determined. The cytochrome P450 gene was found to have six copies in the genome. Proteins of the P450 family are involved in a variety of oxidative reactions of exogenous and endogenous substrates, including fatty acid oxidation, bioconversion of recalcitrant organic compounds, and the synthesis of macromolecules.

***Sphingomonas wittichii* RW1^T** can degrade toxic dioxin pollutants and completely mineralize dibenzo-p-dioxin. The genome of this strain was sequenced to provide information on the production and regulation of enzymes involved in the degradation of dibenzo-p-dioxins and related compounds (Miller et al. 2010). The genome of *Sphingomonas wittichii* contained one chromosome (5.4 Mb, 68.4 % G+C, 4,941 ORFs, including 6 rRNAs and 48 tRNAs) and two megaplasmids, designated as pSWIT01 (310 kb, 64.1 % G+C, 288 ORFs) and pSWIT02 (222.8 kb, 61.2 % G+C, 210 ORFs). Parts of the larger plasmid, pSWIT01, showed high similarity to the plasmid pNL1 from *Novosphingobium aromaticivorans*. Specifically, a 17-kb regions shares highly similar proteins (50 % similarity cutoff), including a reverse transcriptase and a type IV pilus. All genes that were known to be required for dioxin degradation were located on the smaller megaplasmid, pSWIT02.

Three genomes of γ-hexachlorocyclohexane (HCH) degrading **Sphingobium species** were genome sequenced to investigate the genetic background of γ-HCH degradation: two *Sphingobium* type strains, *Sphingobium japonicum* UT26^T (Nagata et al. 2010) and *Sphingobium indicum* B90A^T (Anand et al. 2012) and a further *Sphingomonas* sp. strain MM-1 (Tabata et al. 2013). Differences in the genomic structures, including number of chromosomes and plasmids, were obtained. Clear differences were also obtained with respect to the localization of genes assigned to the γ-HCH degradation pathway.

The genome of ***Sphingobium japonicum* UT26^T** consists of two circular chromosomes and three circular plasmids (Nagata et al. 2010). Chromosome 1 had a size of 3.5 Mb, 64.8 % G+C, and 3,529 ORFs; the second chromosome was smaller in size (0.68 Mb) with a similar DNA G+C content (65.9 % G+C) and 589 ORFs. All three rRNA operons were determined, one at chromosome 1 and two at chromosome 2. Respectively, 51 and 4 tRNA genes were located on chromosomes 1 and 2. The genome contains one larger plasmid pCHQ1 (190 kb, 63.0 % G+C, 224 ORFs) and two smaller plasmids pUT1 (31 kb, 63.7 % G+C, 44 ORFs) and pUT2 (5.3 kb, 61.0 % G+C, 8 ORFs). Genes (*lin* genes) encoding for enzymes or transporters involved in the degradation pathway of γ-HCH (Nagata et al. 2011) were specifically analyzed. A total of 15 *lin* genes were found in the genome of strain UT26^T located either on chromosome 1 or 2 or on plasmid pCHQ1: Chr1 (*linA*, *linB*, *linC*, and *linKLMN*), Chr2 (*linF* and *linGHIJ*), and pCHQ1 (linRED). Comparative genome analysis of the genome of strain UT26^T and other sphingomonads (TM1) strains showed that the *lin* genes (*linA*, *linB*, *linC*, *linRED*, and *linF*) necessary for the conversion of γ-HCH to beta-ketoadipate were located in a DNA region that is unique for the genome of strain UT26^T. In contrast, *lin* genes necessary for the beta-ketoadipate pathway (*linGHIJ*, Nagata et al. 2007) or the ABC transporter system (*linKLMN*, Endo et al. 2005) are located in genome regions that are conserved in the compared *Sphingomonadaceae* (*Sphingomonas* sp. SKA58, *Sphingobium* sp. SYK-6, *Sphingomonas wittichii* RW1^T, *Sphingopyxis alaskensis* RB2256^T, and *Novosphingobium aromaticivorans* DSM 12444^T). The insertion sequence IS6100 was mainly found in close association with several of the *lin* genes (*linA*, *linC*, *linRED*, and *linF*) in the genome of UT26^T as it was also determined in other γ-HCH degraders. IS6100 has a size of 880 bp and carries the *tnp* gene encoding a transposase and 14-bp terminal inverted repeats (IR) at both ends (Mahillon and Chandler 1998). The insertion sequence IS6100 was often found in close proximity to *lin* genes and other HCH-degrading sphingomonads; it may be an important factor responsible for horizontal gene transfer (HGT) of *lin* genes (Böltner et al. 2005; Fuchu et al. 2008). Analysis of spontaneous *linA*-, *linC*-, and *linRED*-deletion mutants of strain UT26^T involved the role of IS6100 in deduced genome rearrangements, suggesting the importance of IS6100 in the dissemination of the *lin* genes and genome rearrangement (Nagata et al. 2011).

The draft genome of ***Sphingobium indicum* B90A^T** had a size of 4.08 Mb with a G+C content of 65 % and a total of 3,319 ORFs (Anand et al. 2012). Two rRNA gene operons were found; one of them had a doubled 23S rRNA gene sequence (arrangement: 16S-23S-23S-5S). The genome of stain B90A^T harbors nearly identical *lin* genes, as found in *Sphingobium japonicum* UT26^T. *Lin* genes were not integrated into a single operon that is constituently regulated but were dispersed over the genome. Seven copies of IS6100 were found, which were located in close proximity to the *lin* genes. In the same manner as *S. japonicum* UT26^T, the genome of strain B90A^T harbored a gene cluster for phenol- and pentachlorophenol-degradation pathways, but no homogentisate and anthranilate degradation pathways. For each *lin* gene—*linX*, *linA*, *linG*, *linH*, *linI*, and *linJ*—two copies were determined in the genome.

The genome of the third γ-HCH degrader ***Sphingomonas* sp. MM1** consists of one circular chromosome and five circular plasmids (Tabata et al. 2013). The chromosome is composed of 4.05 Mb with a G+C content of 67.2 % and 3,801 predicted ORFs. The chromosome carried 2 rRNA operons and 55 tRNAs. The five plasmids are pISP0 (275.8 kb, 63.5 % G+C, 251 ORFs), pISP1 (172 kb, 62.5 % G+C, 174 ORFs), pISP2 (53.8 kb, 62.9 % G+C, 51 ORFs), pISP3 (43.8 kb, 63.3 % GC, 44 ORFs), and pISP4 (33.2 kb, 63.0 % G+C, 39 ORFs). The *lin* genes determined in this genome were almost identical to those determined in *Sphingobium japonicum* UT26^T. However, in contrast to strain UT26^T, the *lin* genes were located on four out of the five plasmids and not on the chromosome. The *lin* gene distribution was as follows: pISP0 (*linF*), pISP1 (*linA*, *linC*, and truncated *linF*), pISP3 (*linRED*); and pISP4 (*linB*, *linC*, and truncated *linF*). Strain MM-1 harbored 15 copies of the IS6100 insertion sequence again associated with *lin* genes.

***Sphingobium chlorophenolicum* strain L-1** was genome sequenced because of its property to mineralize the toxic pesticide pentachlorophenol (PCP) (Copley et al. 2012). The genome consists of two chromosomes (Chr 1: 3.1 Mb, G+C content of 64 mol%, 2,940 ORFs including 1 rRNA operon and Chr 2: 1.4 Mb, G+C content 64 mol%; 1,159 ORFSs including 2 rRNA operons) and a small plasmid pSPHCH01 (123.7 kb; G+C content of 65 mol%, 125 ORFs). Most genes assigned to metabolic core processes are located at chromosome 1, whereas the smaller secondary chromosome encodes mainly genes associated with environmental adaptation processes. All genes of the PCP degradation pathway were located on the secondary chromosome. Some of the genes—*pcpB* (encoding a PCP hydroxylase) and *pcpD* (TCBQ reductase)—were located in one operon together with a further gene of the PCP degradation pathway, *pcpR*. The latter one encodes a LysR–transcriptional regulator involved in induction of the expression of *pcpB*, *pcpA*, and *pcpE* (Cai and Xun 2002). Further genes involved in the degradation pathway were not located in an operon but in close proximity to each other.

Different results were given on genes for PCP degradation as obtained by HGT. For example, *pcpB* and *pcpD* were located in a region of low GC content and PcpB shared a high amino acid sequence similarity (72–98 %) to PcpBs expressed by *Novosphingobium lentum* (Tiirola et al. 2002a) and other polychlorophenol-degrading sphingomonads (Tiirola et al. 2002b; Cassidy et al. 1999). The genome of strain L-1 showed a high structural similarity to the genome of *S. japonicum;* both had approximately the same size (4.57 and 4.46 Mb, respectively) with a similar number of ORFs (4,159 and 4,460, respectively) distributed on two chromosomes. Genes encoding for metabolic core processes (including the glycolysis, the tricarboxylic acid cycle, amino acid and nucleotide biosynthesis, fatty acid oxidation, DNA replication, transcription, and translation) were located in both genomes on the primary chromosome. The number of plasmids differed: a single plasmid in *Sphingobium chlorophenolicum* and three plasmids in *Sphingobium japonicum.* Homologues were determined for 1,931 genes on chromosome 1, 285 genes on the secondary chromosome, and 108 genes on plasmid pSphCh01 in the genome of *Sphingobium japonicum.* The comparative genome analysis furthermore indicated that the first three genes of the PCP degradation pathway, *pcpB*, *pcpA*, and *pcpC*, were acquired by HGT, whereas *pcpA* and *pcpE* seemed to originate from a common ancestor of *Sphingomonas chlorophenolicum* and *Sphingomonas japonicum.*

The genome of strain L-1 contained an integrated prophage which was indicated by a cluster of phage-related genes detected on chromosome 1. The cluster contained genes encoding a major capsid protein, a major tail protein, a phage portal protein, a pro-head peptidase, and some further conserved phage proteins of unknown function. Several distantly relate genes of the "phage integrase family" were determined to be distributed over both chromosomes. All shared a low GC content (between 48 mol% and 58 mol%). One of the phage integrase genes was located in the vicinity of a prophage gene (a CP4-57 regulatory protein), and two were adjacent to transposase genes. A total of 27 sequences annotated as "transposase," "transposase/integrase core domain," or "transposase and inactivated derivatives" were determined: 16 located on chromosome 1, nine on chromosome 2, and two on the plasmid. The transponases were assigned to different families of insertion elements and Tn3 family transposons, indicating a continuous gene exchange in the genome of strain L-1. PCP degradation genes, however, were not directly association with those genetic elements.

***Novosphingobium pentaromativorans* US6-1^T** was the first genome sequenced degrader of benzo(a)pyrene, a carcinogenic recalcitrant compound (Sohn et al. 2004). The draft genome comprises one chromosome (5.1 Mb; G+C content of 63.1 % with 4,948 ORFs, including one rRNA operon and 51 tRNAs) and two plasmids, pLA1 (188.5 kb, G+C content of 62.6 %, 199 ORFs) and pLA2 (60.1 kb, G+C content of 60.2 %, 87 ORFs) (Luo et al. 2012). The chromosome also contains an integrated phage. A total of 38 transposases and 13 phage integrases were found distributed all over the genome, indicating the ability of genetic rearrangement. A total of eight transponases and three phage interase copies were located on pLA1, with two transposases at pLA2. Those motile genetic elements indicate

the ability of genetic rearrangement in the genome of US6-1. The genome of strain US6-1^T showed a homology to the genome of *Novosphingobium aromaticivorans* DSM12444^T. The plasmid pLA1 of strain US6-1^T shared high similarity to the plasmid pCAR3 of *Sphingomonas* sp. strain KA1 and pNL1 of *Novosphingobium aromaticivorans* DSM 12444^T, with respect to the conjugative regions. Genes encoding proteins of the polycyclic aromatic hydrocarbon (PAH) degradation pathway were partially located on plasmid pLA1 and partially scattered on the chromosome. The genome harbors several genes encoding enzymes that commonly exist in aromatic-compound degrading bacteria as three sets of genes coding for aromatic-ring-hydroxylating dioxygenases: *bphA1a*, *bphA2a*, *bphA1b*, *bphA2b*, *bphA1e*, and *bphA2e* in *Sphingobium yanoikuyae* B1 (Chadhain et al. 2007); respective genes found on plasmid pNL1 of *Novosphingobium aromaticivorans* DSM12444^T (Romine et al. 1999); and *ahdA1a*, *ahdA2a*, *ahdA1b*, *ahdA2b*, *ahdA1e*, and *ahdA2e* in *Sphingomonas* sp. P2 (Pinyakong et al. 2003).

The type strain of ***Novosphingobium nitrogenifigens* Y88^T** was genome sequenced because of its specific metabolic properties linked to the ability of nitrogen fixation, production of poly-3-hydroxybutyrate (PHB), and the effective removal of Mn and Zn from model biorefinery effluents (Strabala et al. 2012). The strain was originally isolated from nickel-enriched pulp and paper mill wastewater (Addison et al. 2007). The draft genome had a size of 4.1 Mb, with a G+C content of 63 % and 3,801 ORFs, including 49 tRNAs. The genome harbors two small plasmids, py8 (5.9 kb) and py157 (5.7 kb), which both have a G+C content of 58 %. The plasmids encode genes for plasmid conjugation and replication and coding sequences (CDSs), which shared a high homology to CDSs on the plasmids pUT2 of *Sphingobium japonicum* UT26S^T. The genome contained all genes necessary for PHB synthesis (*phbA*, *phbB*, and *phaC*) and all genes encoding proteins involved in nitrogen fixation (*nifA*, *–D*, *–E*, *–H*, *–K*, *–N*, *–Q*, *–U*, *–W*, and *–X*, *fixA* and *fixU*, and various cofactor biosynthesis proteins). Thirteen CDSs were assigned to metal resistance, including putative metal resistance/efflux proteins for Ni, Cu, Hg, Co, and As resistance.

Sandarakinorhabdus

The first genome-sequenced strain of the genus *Sandarakinorhabdus* was ***Sandarakinorhabdus* sp. strain AAP62**, which shared 99.4 % 16S rRNA gene sequence similarity with the type strain of *Sandarakinorhabdus limnophila* (Zeng et al. 2013a). The orange-pigmented strain was isolated from surface waters of Lake Shahu (China) and was capable of producing BChl *a* under aerobic conditions (i.e., freshwater aerobic anoxygenic phototroph, AAP). The aim of the study was to characterize the metabolic potential and the composition of photosynthetic genes in freshwater AAPs.

The draft genome consisted of 22 contigs. A total genome length of 3.13 Mb with a G+C content of 65.5 %, containing 3,036 ORFs, and 46 tRNAs was thereby obtained. The strain harbored a photosynthesis gene cluster (PGC) with a total size of 44,237 bp, which was located at a 549,987-bp-long contig. The gene organization was as followed: *crtK-bchP-pucC-bchGFNBHLM-puhABC-lhaA-ORF-acsF-puhE-6xORFs-bchIDO-5xORFs-crtCDF-bchCXYZ-pufBALMC*. Genes of the pathway for autotrophic CO_2 fixation were not detected. Genes encoding enzymes of different catabolic pathways were detected, such as those of the pentose phosphate pathway, the Entner-Doudoroff pathway, and the tricarboxylic acid cycle. The first insight into the genome indicated a large metabolic versatility. Detailed studies of the genome should provide new insight into the metabolism and the ecology of AAPs. The genome of *Sandarakinorhabdus limnophila* DSM 17366^T is also being genome-sequenced as part of the GEBA project (Wu et al. 2009).

Blastomonas

For the genus *Blastomonas*, one genome sequence of an AAP strain, ***Blastomonas* sp. strain AAP53**, is available (Zeng et al. 2013b). The strain was genome sequenced to assess the metabolic potential and the photosynthesis gene composition of the *Blastomonas* species, which synthesizes BChl *a* under aerobic conditions. The yellow-pigmented strain AAP53 was isolated from surface waters of Lake Swan, a freshwater desert lake in Inner Mongolia, China, and shared 98.6 % 16S rRNA gene sequence identity with the type strain of *Blastomonas natatoria* DSM 3183^T. The draft genome was 3.6 Mb long with a G+C content of 63.6 % and 3,369 ORFs, including one rRNA operon and 42 tRNAs.

Genes of the PGC were located on two separate contigs. One contig contained the gene cluster *bchIDO-crtF-bchCXYZ-pufBALMC*. The other contig contained the other PGC genes in the following arrangement: *puhE-acsF-ORF-puhCBAlhaA-bchMLHBNF-ORF-ppsR-ORF-bchG-pucC-bchP-tspO*. *PufBLMC* genes showed a high sequence similarity (91 and 97 %) to the respective genes identified in the type strains of *B. natatoria* and *B. ursincola*. Genes for the autotrophic CO_2 fixation pathway were not found. The aerobic lifestyle of the *Blastomonas* strain was confirmed by the detection of several genes of the aerobic respiratory electron transport chain such as the NADH dehydrogenase, cytochrome c oxidase, and the succinate dehydrogenase, as well as a catalase.

Zymomonas

Strains of the species ***Zymomonas mobilis*** shared the metabolic capacity to ferment sugar to ethanol, which makes this species interesting for the industrial production of biofuel. Genomes of four *Zymomonas mobilis* subsp. *mobilis* strains, including the type strain *Zymomonas mobilis* subsp. *mobilis* T.H.Delft 1^T, ATCC 10988^T and the type strain of *Zymomonas mobilis* subsp. *pomaceae* Barker 1^T, ATCC 29192^T, are already available.

A comparative analysis of several further strains are under way at the U.S. Department of Energy Joint Genome Institute, in collaboration with the University of Athens. All genomes are relatively small in size, 2.1–2.2 Mb, with a G+C content between 44 % and 46.6 % and 1,807–1,978 ORFs. With exception of *Zymomonas mobilis* subsp. *mobilis* ZM4 (=ATCC 31821), all *Zymomonas* genomes contain several (2, 3, or 6) plasmids per genome. Evidence of plasmid DNA, however, was also given for strain ZM4 (Yang et al. 2009).

The first *Zymomonas* genome, the genome of *Zymomonas mobilis* ZM4 (ATCC 31821), was published in 2005 (Seo et al. 2005). Corrections to the genome annotation were given later (Yang et al. 2009). The genome consisted of a single circular chromosome (2.1 Mb, G+C content 46.3 %, and 1,998 predicted ORFs). Genes encoding for hexose-metabolizing enzymes were identified, such as invertases, a levansucrase, a glucokinase, glucose-6-phosphate isomerase, and glucose-fructose oxidoreductase to metabolize sucrose, fructose and glucose as well as probably mannose, raffinose, and sorbitol. Genes for lactose, maltose, or cellobiose utilization were not determined. Insight into the genome showed that glucose can only be metabolized by the Entner-Doudoroff pathway because key enzymes of other pathways are missing, such as the 6-phosphofructokinase of the EMP pathway.

A total of 54 ORFs were predicted to encode for transport and secretory proteins, transcriptional regulators, and oxidoreductases. Comparative microarray analysis indicated that several of the genes were absent in another *Zymomonas* species, ZM1, but most of the 54 ORFs were actively transcribed in ZM4 in association with ethanol production. Because *Z. mobilis* is a facultative anaerobe and therefore grows in the presence of oxygen, different oxidative stress-related genes were found in the genome, such as a glutathione reductase, a glutathione synthase, a γ-glutamylcysteine synthetase, a catalase, and a superoxide dismutase, as well as several additional genes that respond to oxidative stress.

A further genome sequenced *Zymomonas* strain is a flocculating mutant of ZM4, strain ZM401, which is able to perform a self-immobilization within a fermenter without the consumption of supporting materials, thereby forming a high-density culture improving ethanol productivity. The strain was also suitable for high-gravity fermentation, increasing the ethanol production per titer. Genome comparison of ZM4 and ZM401 showed the presence of 28 single-nucleotide polymorphisms (SNPs) in strain ZM401; 48.3 % were localized in coding regions and 51.7 % (30 in total) in noncoding regions (Zhao et al. 2012). Further details of transcriptome analysis of strain ZM401 are provided in the literature (Jeon et al. 2012).

A further genome sequence of the *Zymomonas mobilis* strain ATCC 29191 shared an average nucleotide identity of 97 % with ZM4. The genome of ATCC 29191 was 95.1 kb smaller than that of the ZM4. A total of 51 genes were unique for strain ATCC 29191 and 115 genes for ZM4. Genes unique in ATCC 29191 included a helicase, a transporter, and tellurium resistance genes (Desiniotis et al. 2012).

Nonchromosomal Genetic Elements, Such as Plasmids, Prophages, Phages, Superintegrons, Genomic Islands

Plasmids in Sphingomonads

Plasmids play an important role in sphingomonads because many genes of different degradation pathways are located on plasmids. For example, genes encoding enzymes involved in degradation pathways of biphenyl, naphthalene, m-xylene, and p-cresol were detected on megaplasmids in *Sphingomonas aromaticivorans* F199^{T} (184 kb) or other sphingomonads, which were isolated from the same habitat (Romine et al. 1999). Basta et al. (2005) investigated 17 different xenobiotic-degrading sphingomonad strains; all contained 2–5 plasmids in sizes of 50–500 kb. On several of those plasmids, genes for different metabolic degradation pathways were located.

The high-molecular-weight polycyclic aromatic hydrocarbon degrading *Sphingomonas aromaticivorans* F199 ^{T} contains two plasmids pNL1 (180 kb) and pNL2 (480 kb) (Romine et al. 1999). The smaller aromatic catabolic plasmid pNL1 was characterized in detail by full-length sequence analysis (Romine et al. 1999). Plasmid pNL1 encoded 186 ORFs. Among those, 76 genes were associated with functions related to aromatic compounds: aromatic catabolism, transport, and cell membrane alteration. Genes associated with the degradation pathways of biphenyl, naphthalene, m-xylene, and p-cresol were found to be localized in 15 gene clusters on the plasmids. Several ORFs of pNL1, distributed among the catabolic genes, showed high homology to gene coding for bacterial efflux pump genes, including genes encoding for cytoplasmic and outer membrane proteins and membrane fusion proteins as compounds of a typical efflux pump. The plasmid contained intron-associated maturases in the replication region, which suggested that the plasmid (pNL1) can perform integration and excision actions between the plasmid and the chromosome or among different plasmids with the chromosome and/or other portions of the plasmid. The plasmid harbors gene clusters of all genes necessary for a conjugative plasmid transfer to other bacteria. This was proven by Romine et al. (1999) with the transfer of the plasmid from the host *Sphingomonas aromaticivorans* F199^{T} to another *Sphingomonas* strain.

Ochou et al. (2008) isolated and characterized two small cryptic indigenous plasmids, pYAN-1 (4.9 kb) and pYAN-2 (4.7 kb), from *Sphingobium yanoikuyae* that contained genes encoding proteins with predicted functions associated with mobilization (*mob* genes) and replication (rep genes). Based on the detailed characterization of the two plasmids, Ouhou and coauthors were able to construct a shuttle vector between *E. coli* and sphingomonads, which was proven by the use of *Novosphingobium capsulatum* as a model host using electroporation-transformation. The construction vector enabled the genetic manipulation of sphingomonads. In a similar manner, Tani et al. (2011) characterized a small crypic plasmid pSM103mini that originated from *Sphingopyxis macrogoltabidus*

strain 103. The plasmid is 4.3 kb in size with a G+C content of 57.5 %. Only four ORFs were identified, including genes encoding a resolvase and the replication protein RepA. A complex repeat sequence and the other two ORFs are associated with the maintenance of the plasmid in a cell. Based on the plasmid, the authors constructed a shuttle vector (pHSG398-fused plasmid, pHSG-SN103mini) between *Sphingopyxis* strains. However, the plasmid was not stable in a tested *Sphingomonas* species, *Sphingomonas terrae*.

Indigenous plasmids in a size range of 1.5 kb to >40 kb have been described for *Zymomonas* spp. Antibiotic-resistant or heavy-metal-resistance genes were also found to be localized on these plasmids (for reference, see Sprenger et al. 1993). Plasmids that originate from *Zymomonas mobilis* strains were used for the constructing of shuttle vectors that can be used for homologous and heterologous gene expression in *Z. mobilis* for metabolic engineering (Conway et al. 1987; Sprenger et al. 1993).

Phenotypic Analyses

Table 25.2

Sphingomonas Yabuuchi, Yano, Oyaizu, Hashimoto, Ezaki, Yamamoto 1990; Emend. Yabuuchi et al. 1999; Yabuuchi et al. 2002; Busse et al. 2003; Chen et al. 2012a

The genus *Sphingomonas* was proposed by Yabuuchi et al. (1990) based on the 16S rRNA gene sequence phylogeny and the presence of a unique sphingoglycolipid and ubiquinone Q-10 as the major respiratory quinone. The proposal of the genus was based on five species: the type species *Sphingomonas paucimobilis* (previously "*Pseudomonas paucimobilis*"; Holmes et al. 1977), *Sphingomonas parapaucimobilis*, *Sphingomonas yanoikuyae*, *Sphingomonas adhaesiva*, and *Sphingomonas capsulatum*. At the time of writing (June 2013), the species *Sphingomonas* contained 61 species.

Cells are Gram-negative, non-sporulating, and ovoid to rod-shaped, including straight, oval, short, thin, slightly curved, or pleomorphic short rods. Cell size is between 0.3–1.1 × 0.8–5.0 µm. Strains are nonmotile or motile; for some strains, motility was only obtained in the early exponential growth phase. Cells are motile by means of (single) polar, subpolar or peritious flagella. Reproduction occurs by binary fission. Budding formation can occur (*Sphingomonas jaspsi*, Asker et al. 2007a). The presence of polar fimbriae leads to rosette-like aggregation in some species. One species shows a dimorphic life cycle, including a prosthecate stage, non-motile cell and a flagellated, motile cell stages (*Sphingomonas leidyii*; Chen et al. 2012a). Cells of *Sphingomonas molluscorum* (Romanenko et al. 2007) show plait-like structures on the cell surface.

Colonies are white, cream, pale-yellow, yellow, deep-yellow, yellow-orange, pink-yellow, orange, dark-orange, reddish, or red-colored, circular, and (slightly or low) convex. Some are smooth, have an entire margin, and are glistening; some are also mucoid and slimy. The colony diameter is in the range of 0.1–2 mm; mucoid or slimy colonies are often larger, with a diameter of 6–8 mm [e.g. *Sphingomonas sanxanigenens* (Huang et al. 2009a) or *Sphingomonas endophytica* (Huang et al. 2012)]. Acetone-extracted cellular pigments were characterized by λmax at 458 and 476/478 nm with a slight inflexion at 425 nm (Busse et al. 2003); λmax at 452–454 and 480–482 nm with a shoulder at 431 nm (Xie and Yokota 2006; Ohta et al. 2004); λmax at 451 nm with two shoulders at 423 and 475 nm (Huo et al. 2011); or λmax at 477 and 450 nm (An et al. 2013). Carotenoids were identified as zeaxanthin (λmax at 451 and 476 nm) and nostoxanthin (λmax at 449 and 475 nm) (Asker et al. 2007a) and for *Sphingomonas astaxanthinifaciens* as dihydroastaxanthin and astaxanthin (Asker et al. 2007b).

Strains are strictly aerobic with oxygen as the thermal terminal electron acceptor and chemo-organotrophic. They are catalase positive with one exception (*Sphingomonas oryziterrae*, Chung et al. 2011), and oxidase-positive or − negative. Aesculin is most often hydrolyzed, but a prolonged incubation can be necessary to obtain a positive reaction.

All strains grow at 15–28 °C—most up to 4 °C, often also at 37 °C, some at 42 °C, or even at 55 °C (*Sphingomonas laterariae*, Kaur et al. 2012). The pH range for growth can be between pH 4 and 12. The pH optimum for growth is between pH 6.5 and 9 (for most species, pH 7–8). Most strains grow without additional NaCl, 0 % (w/v). Several strains are not able to grow in the presence of 1 % or 2 % (w/v) NaCl; some can grow up to a NaCl concentration of 5 %. For some exceptions, growth occurs still at 8 % NaCl (*Sphingomonas rubra*, Huo et al. 2011).

Ubiquinone Q-10 is the predominant respiratory quinone; minor amounts of Q-9 and Q-8 can also be detected. The major nonpolar cellular fatty acids are $C_{18:1}\omega 9t$ and $C_{18:1}\ \omega 7c$, followed by saturated C16:0 and/or C17:1. The major 2-hydroxy fatty acids are 2-OH C14:0 or 2-OH C15:0. No 3-hydroxy fatty acids were detected. Sphingoglycolipids are present, replacing lipopolysaccharide (LPS) in the cell wall. The SGL types obtained are glucuronosyl-(1→1)-ceramide sphingoglycolipid (SGL-1), and galacturonosyl-β(1 → 1)-ceramide sphingoglycolipid (in several species). The major polyamine is *sym*-homospermidine. Minor to trace amounts of spermidine, spermine, putrescine, cadaverine, 1,3-diaminopropane, and agmatine occur in some species. *sym*-Homospermidine as major polyamine is a characteristic feature that clearly differentiated the genus *Sphingomonas sensu stricto* from the genera *Sphingobium*, *Novosphingobium*, and *Sphingopyxis* (Busse et al. 1999; Takeuchi et al. 2001). The genera *Sphingosinicella* and *Sphingomicrobium*, however, shared this chemotaxonomic feature (Geueke et al. 2007; Kämpfer et al. 2012), which hampered the differentiation of the genus *Sphingomonas* from those genera.

Besides sphingoglycolipid, the polar lipid profiles contain phosphatidylglycerol as the predominant lipid, with moderate to large amounts of phosphatidyl ethanolamine,

Table 25.2

Overview of distinctive characteristics for genera of the family *Sphingomonadaceae*

	Sphingomonas	*Novosphingobium*	*Sphingobium*	*Sphingopyxis*	*Blastomonas*	*Sandaracinobacter*	*Sandarakinorhabdus*	*Sphingosinicella*	*Stakelama*	*Sphingomicrobium*	*Parasphingopyxis*	*Sphingorhabdus,*	*Zymomonas*
Habitats	Freshwater and marine, soil, plant-associated and anthropogenic habitats	Freshwater and marine, soil, plant-associated habitats	Freshwater and marine, soil, plant-associated habitats	Freshwater, marine, and soil habitats	Freshwater	Freshwater	Freshwater	Freshwater, soil, vermicompost, wastewater treatment plant	Marine system (sediments)	Marine system (coastal and surface water)	Marine system (tube-worm)	Freshwater and marine habitats	
Number of Species	63	23	34	16	2	1	1	4	2	4	1	4	1 (3 subsp.)
BChl *a*	Bchl *a*, pufM, pufL, (*Sphingomonas kaistensis*)	nd	nd	nd	Bchl *a*, pufM, pufL LH-I (867), LH-II (absent)	Bchl *a*, LH-I (867), LH-II (absent)	BChl *a*, LH-I (865) LH-II (800-837).	pufM detected in one species (*Sphinosinicella microcystinivorans*)	—	—	—	—	—
Ubiquinone	Q-10, minor Q-9, Q-8	Q-10, minor Q-9	Q-10, minor Q-9	Q-10, minor Q-8 and Q-9	Q-10, Q-9 is absent	Q-9 and Q-10	Q-10, minor Q-9	Q-10, minor Q-9, Q-8	Q-10	Q-10, minor Q-9	Q-10	Q-10, minor Q-9	
Polyamine	HSPD	SPD	SPD	SPD	SPD	—	Not analyzed	HSPD	SPD	HSPD (HSPD and SPD)[a]	Not detected	SPD (SPD and AGM)[b]	
Characteristic hydroxy fatty acids	2-OH 14:0 and/or 2-OH C15:0	2-OH C14:0	2-OH 14:0	2-OH 14:0 2-OH 15:0 and/or 2-OH C16:0	2-OH C14:0,	iso-2-OH C14:0	iso-2-OH C14:0 (2-OH C15:0; 2-OH C16:0)	2-OH C14:0	2-OH C14:0	2-OH C18:1 (2-OH C14:0, 2-OH C15:0, 2-OH C16:0 not detected)	2-OH C16:0 (2-OH C14:0 is absent)	2-OH C14:0	
G+C content	62–68	60–67	58.2–67	63–69.2 (53.3–57.3)[c]	64.8–65.2	68.5	64.3	59.4–65.0	61.4–66.5	63.4–65.2 (70.6)	60.1	52.6–57.8	47.5–49.5

BChl *a*, bacteriochlorophyll a, LH-I light harvesting complex I

[a]*Sphingomicrobium marinum* (Shahina et al. 2013b)

[b]*Sphingopyxis marina* DSM 22363^T (Kim et al. 2008)

[c]Two species *Sphingopyxis wooponensis* and *Sphingopyxis riguri*, which need to be reclassified to the genus *Sphingorhabdus* with respect to 16S rRNA gene sequence phylogeny

diphosphatidylglycerol, phosphatidyldimethylethanolamine, and phosphatidyl choline; different amounts of phosphatidylmonomethylethanolamine; and different amounts of unidentified polar lipids (Busse et al. 1999; Busse et al. 2003). Bacteriochlorophyll *a* was determined in one species, *Sphingomonas kaistensis* (Kim et al. 2007). However, a few species were investigated respectively. Detailed characteristics of the BChl *a* showed that three types are present in that species: BChl-a esterified with phytol (BChl a_P), esterified with farnesol (BChl *a*F), and esterified with geranylgeraniol (BChl *a*GG). In parallel genes for the photosynthetic type II reaction center, *pufL* and *pufM* genes were detected. The BChl *a* content was approximately 200 times lower as that determined for AAPs (Jiao et al. 2003; Kolber et al. 2001), with a ratio of 1:60–1:600 BChl *a* to carotenoid content (Kim et al. 2007).

Characteristic 16S rRNA gene signature nucleotides are at the following positions 52:359 (C:G), 134 (G), 593 (G), 987:1218 (G:C), and 990:1215 (U:G). The DNA G+C content ranges from 62 mol% to 68 mol%. The type species is *Sphingomonas paucimobilis* (formerly "*Pseudomonas paucimobilis*") (Table 25.3).

Habitats: Several environments of anthropogenic origin were found, including veterinary clinics, surfaces of air humidifiers, air samples from open buildings and the Russian space station Mir, whirled-up dust of cow farms, laboratory contaminants, wastewaters of a sequential batch reactor treatment and leather plants, wastewater treatment plants, HCH dump sites, hydrocarbon-contaminated soils, PCP-contaminated groundwater, 4,200-year-old ice, biological soil crust, the surface layer of desert soil, alpine soil, paddy soil, forest soil, topsoil of a cornfield and other agricultural soils (including soils of a ginseng field), glacier cryoconite collected from the Stubai Glacier, rhizosphere of a rice field, roots of *Malus* spp. (apple), *Oryza sativa*, or *Prunus persica* (peach), tissue of *Artemisia annua* L, ear of *Setaria viridis*, brown spots of yellow Spanish melon fruits, phyllosphere of *Cynara cardunculus* L. var. *sylvestris* or *Acacia caven*, specimen of the crab *Paralithodes camtschatica*, marine sponge, hymeniacidon flavia, millipede hind gut, marine bivalve (*Anadara broughtoni*), tidal flat sediment, water of freshwater lakes, natural mineral water, prealpine freshwater lakes, freshwater sediments, eutrophic fountains with algal blooms, and freshwater cyanobacterial mats of thermal springs.

Nitrogen Fixation

The capacity of nitrogen fixation was so far only detected for one *Sphingomonas* species, *Sphingomonas azotifigens*. The first evidence for nitrogen fixation was given for *Sphingomonas trueperi* NBRC 100456^T, which was formerly known as '*Pseudomonas azotocolligans*' (Anderson 1955). A later study, however, showed that the type strain was not able to fix nitrogen (Hill and Postgate 1969); this was supported by the study of Xie and Yokota (2006), in which neither acetylene reduction nor the *nifH* gene could be detected for the type strain of *S. trueperi*. A high capacity for acetylene reduction and amplification of the *nifH* gene was obtained for three strains (including the type strain) of *S. azotifigens*, which were all isolated from nonsterilized roots of *Oryza sativa*.

Sequence analysis of the partial *nifH* gene sequence showed the highest sequence similarities (98 %) to the respective sequences of uncultured diazotrophic bacteria detected in dead above-ground biomass of *Spartina alterniflora* (Lovell et al. 2001). Phylogenetic trees calculated based on partial *nifH* gene sequences showed that the *nifH* genes of the *S. azotifigens* strains cluster with *nifH* genes of one cluster of sequences originating either from *Alphaproteobacteria* or *Betaproteobacteria*,with highest sequence similarity to *nifH* genes of species from the genus *Bradyrhizobium*. This cluster was distinct from two monophyletic clusters of *nifH* genes of either nitrogen-fixing *Alphaproteobacteria* or *Gammaproteobacteria* (Xie and Yokota 2006).

The *nifH* gene sequences of *S. azotifigens* were distinct from those of many other *Alphaproteobacteria nifH* gene sequences. Based on this finding, Xie and Yokota (2006) concluded that the three strains of *S. azotifigens* may have obtained the *nifH* genes by lateral gene transfer. Further evidence for nitrogen fixing *Sphingomonas* spp. was given by Videira et al. (2009), who determined acetylene reduction and *nifH* gene sequences in 22 out of 42 isolates that were phylogenetically closely affiliated (based on 16S rRNA gene sequence) to the genus *Sphingomonas*. The strains were isolated from surface-sterilized plant tissue of roots and the aereal part of two rice varieties grown in two soils in Brazil. Four of the isolates showed *nifH* genes identical to the *nifH* gene found in the *S. azotifigens* isolates. The occurrence of nitrogen-fixing sphingomonads was suggested in several studies by the investigation of the rhizosphere of millet or sorghum (Hebbar et al. 1992), rice (Engelhard et al. 2000), and rice seeds (Mano et al. 2006). The ability of nitrogen fixation, however, was not proofed for the isolates. Engelhardt et al. (2000) suggested the abundance of nitrogen-fixing *Sphingomonas paucimobilis* bacteria in the rhizosphere of rice, but no nitrogenase activity was shown. Adhikart et al. (2001) amplified a partial *nifD* gene fragment form a single isolate of *Sphingomonas trueperi* but also did not show any nitrogenase activity.

Exopolysaccharides (Sphingans)

Some *Sphingomonas* species can produce water-soluble heteropolysaccharides by the fermentation of sugars that are excreted into the medium (exopolysaccharides, or EPSs). The characteristic feature of those EPSs is the ability to produce agar-like structures. The formation of EPSs was first described for *Pseudomonas* species (Kang et al. 1982); for *Sphingomonas* species, EPS formation was first reported by Pollock (1993), who described the release of so-called sphingans by the *Sphingomonas* species.

The two most studied sphingan-producing *Sphingomonas* species are *Sphingomonas paucimobilis* and *Sphingomonas elodea*, formerly described as "*Pseudomonas paucimobilis*" and

Table 25.3
Differentiating morphological and physiological characteristics of *Sphingomonas* species

	Sphingomonas abaci[a]	*Sphingomonas adhaesiva*[b]	*Sphingomonas aerolata*[a]	*Sphingomonas aestuarii*[c]	*Sphingomonas alpina*[d]	*Sphingomonas aquatilis*[a]	*Sphingomonas asaccharolytica*[a]	*Sphingomonas astaxanthinifaciens*[a,k]	*Sphingomonas aurantiaca*[a]	*Sphingomonas azotifigens*[e]	*Sphingomonas changbaiensis*[f]	*Sphingomonas cynarae*[g]	*Sphingomonas desiccabilis*[f]	*Sphingomonas dokdonensis*[a]	*Sphingomonas echinoides*[a]	*Sphingomonas endophytica*[h]	*Sphingomonas faeni*[a]	*Sphingomonas fennica*[i,j]	*Sphingomonas formosensis*[j]	*Sphingomonas ginsengisoli*[k]	*Sphingomonas ginsenosidimutans*[l]	*Sphingomonas glacialis*[m]	*Sphingomonas haloaromaticamans*[i]	*Sphingomonas hankookensis*[g]	*Sphingomonas histidinilytica*[n]	*Sphingomonas indica*[o]	*Sphingomonas insulae*[g]	*Sphingomonas japonica*[p]	*Sphingomonas jaspsi*[q,p]	*Sphingomonas jejuensis*[q]
Pigmentation	o	y	o	y	Y	y	ly	dr	o	yo	y	o	y	y	y	y	o	ly	ly	ro	y	yo	y	y	c	y	y	yo	dy	or
Motility	–	+	+	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	nd	–	–	–		–	+	–	+	+	+	–
Oxidase	–	+	+	+	+	+	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+		+	–	+	+	+	+	–
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	nd	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	v*	–	nd	–	–	–	–	(+)	–	–	–	–	+
ß-Galactosidase	nd	nd	+	+	+	+	+	–	(+)	+	–	+	+	–	+	nd	(+)	–	+	–	–	+	nd	–	nd	–	–	w	–	+
Aesculine	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+	–	+	–		+	+	+	+	+	–	–	+	+	w	+
Gelatine	nd	–	–	+	+	–	–	+	–	v	+	+	+	+	–	+	–	–	+	+	–	–	nd	–	–	–	–	s	(+)	+
Starch	nd	nd	–	–	–	nd	v	+	–	+	nd	nd	nd	+	nd	–	–	nd		nd	–	nd	nd	(+)	–	–	–	–	+	nd
Tween 80	nd	nd	nd	nd	nd	nd	+	+	nd	+	nd	nd	nd	+	–	nd	nd	nd	+	nd	nd	+	nd	nd	nd	+	ND	+	+	nd
DNase	nd	–	(+)	nd	nd	nd	+	ng	v	+	nd	nd	nd	nd	nd	nd	(+)	–	–	nd	+	nd	nd	nd	nd	nd	nd	nd	ng	nd
Urease	–	nd	–	–	–	–	–	v	–	nd	+	–	–	–	–	nd	–	+	–	+	+	–	nd	–	–	nd	–	–	+	nd
Indole activity	–	nd	–	–	–	–	–	–	–	–	–	–	–	–	–	nd	–	nd	nd	–	–	–	nd	–	–	–	nd	nd	–	+
Glucose fermentaion	–	nd	–	nd	–	–	–	–	–	nd	nd	+	nd	–	–	nd	–	nd	nd	–	–	–	nd	–	–	–	+	–	–	nd
Arginine dihydrolase	–	nd	–	nd	–	–	–	v	–	–	–	–	–	–	–	nd	–	nd	nd	+	+	–	nd	–	–	nd	–	–	+	nd
Assimilation of																														
Glucose	–	+	+	+	+	–	–	–	+	+	(+)	+	+	–	(+)	+	+	–	+	+	+	+	+	+	–	nd	+	–	+	+
L-Arabinose	–	–	+	–	+	+	(+)	–	+	+	–	–	+	–	+	+	+	–	+	–	–	+	+	+	+	nd	+	–	–	+
D-Mannose	–	+	+	+	+	–	(+)	–	+	nd	–	+	+	+	–	+	+	–	–	–	+	–	–	+	+	nd	+	–	+	–
N-Acethyl-D-glucosamine	–	+	+	–	–	–	+	–	+	nd	–	–	nd	–	+	nd	+	–	+	–	+	–	+	+	–	nd	+	–	–	–
Maltose	–	+	+	+	+	+	+	–	+	+	–	(+)	+	–	v	+	+	–	–	+	–	+	+	+	–	nd	+	–	+	–
Gluconate	(+)	–	+	–	–	–	–	–	+	–	+	(+)	–	–	v	nd	+	–	+	–	–	–	–	+	–	nd	–	–	–	–

Adipate	–	–	+	–	–	–	–	–	+	–	+	–	nd	+	–	nd	+	–	–	–	+	–	–	–	nd	nd	–	–	–	–
Malic acid	+	+	v	–	+	–	–	–	+	–	(+)	–	nd	–	+	nd	–	–	+	–	+	w	–	+	–	nd	+	+	–	–
Citrate	–	–	v	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	+	–	–	–	–	+	–	–	+	–	–	–
D-Mannitol	–	nd	–	nd	–	–	–	–	–	nd	–	–	–	–	–	–	–	nd	nd	–	–	–	–	–	–	nd	–	–	+	nd
Caprate	–	–	–	–	–	–	–	–	–	nd	–	–	nd	–	–	nd	–	nd	–	–	–	–	nd	–	–	nd	–	–	–	nd
Acetate	–	nd	–	nd	nd	–	–	–	–	+	nd	nd	nd	–	–	nd	–	+	nd	–	–	nd	+	nd	nd	nd	nd	–	–	nd
G+C content (mol%)	nd	nd	65.4	ND	64.1	63	64.8	63.3	64.7	66 – 68	65.8	66.1	ND	66.9	65.8	63.3	63.1	63.6	62.8	66.8	65.6	67.9	66.1	67.2	66.9	65.8	67	nd	63.3	69.4

	Sphingomonas jinjuensis[r]	*Sphingomonas kaistensis*[f,k]	*Sphingomonas koreensis*[a,l]	*Sphingomonas laterariae*[s]	*Sphingomonas leidyi*[t]	*Sphingomonas mali*[a]	*Sphingomonas melonis*[a]	*Sphingomonas molluscorum*[f,p]	*Sphingomonas mucosissima*[p]	*Sphingomonas oligophenolica*[f,m]	*Sphingomonas oryziterrae*[r]	*Sphingomonas panni*[g]	*Sphingomonas parapaucimobilis*[u]	*Sphingomonas paucimobilis*[u]	*Sphingomonas phyllosphaerae*[l]	*Sphingomonas pituitosa*[f]	*Sphingomonas polyaromaticivorans*[v]	*Sphingomonas pruni*[a]	*Sphingomonas pseudosanguinis*[v,w]	*Sphingomonas roseiflava*[u,x]	*Sphingomonas rubra*[y]	*Sphingomonas sanguinis*[u]	*Sphingomonas sanxanigenens*[z]	*Sphingomonas sediminicola*[k]	*Sphingomonas soli*[a]	*Sphingomonas starnbergensis*[aa]	*Sphingomonas trueperi*[f,u]	*Sphingomonas wittichii*[i,u]	*Sphingomonas xinjiangensis*[ab]	*Sphingomonas yabuuchiae*[w]	*Sphingomonas yunnanensis*[i]
Pigmentation	do	or	y	c	cl	ly	dy	y	o	py	dy	y	y	y	y	dy	y	ly	y	py	r	y	w	y	y	cw	y	(y)	y	dy	y
Motility	–	–	+	–	+	+	–	–	–	–	–	–	+	+	+	+	–	+		–	+	+	–	–	–	–	+	+	+	–	+
Oxidase	–	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	+	+	–	+	–	–	+	+	+	+	+	+	–	+
Catalase	+	+	+	+	+			+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Nitrate reduction	–	–	–	+	–	–	–	+	–	+	–	–	nd	–	–	–	–	–	–	+	nd	nd	+	nd	–	–	nd	nd	–	–	–
ß-Galactosidase	nd	–	+		–	+	+	+	–	nd	–	+	nd	+	+	+	–	+	–	+	–	nd		–	–	–	nd	nd	+	+	–
Aesculine	+	–	–	–	+	+	+	+	–	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+		+	+	+	+	+
Gelatine	–	–	–	–		–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	–
Starch	+	nd	+	–	–	–	–	–	+	nd	+	–	+	+	–	–	nd	+	nd	+	–	+	–	nd	nd	–	–	–	–	+	+
Tween 80	–	nd	nd	w	nd	+	–	+	ng	+	–	nd	+	+	nd	+	+	+	nd	+	–	+	nd	nd	nd	nd	–	–	–	+	nd
DNase	nd	nd	–	nd	nd	+	ng	nd	nd	nd	nd	nd	–	–	–	–	nd	ng	nd	–	–	–	nd	nd	nd	nd	(+)	–	nd	nd	–
Urease	+	v	v	+	–	–	–	–	–	–	–	–	nd	–	+	–	nd	–	nd	–	–	nd	–	nd	–	–	–	nd	–	–	+
Indole activity	nd	–	–	–	–	–	–	–	–	–	nd	–	nd	–	–	–	–	–	–	–	–	nd	–	–	–	nd	–	nd	–	nd	–
Glucose fermentaion	nd	–	–	nd	–	–	–	nd	nd	–	nd	–	–	–	–	nd	–	–	–	–	–	+	nd	–	–	nd	+	+	–	(+)	–
Arginine dihydrolase	nd	v	v	nd	–	–	–	–	–	–	nd	–	nd	–	+	–	–	–	–	–	–	nd	nd	+	–	–	–	nd	–	–	+

Table 25.3 (continued)

	Sphingomonas jinjuensis[r]	*Sphingomonas kaistensis*[f,k]	*Sphingomonas koreensis*[a,l]	*Sphingomonas lateriariae*[s]	*Sphingomonas leidyi*[t]	*Sphingomonas mali*[a]	*Sphingomonas melonis*[a]	*Sphingomonas molluscorum*[f,p]	*Sphingomonas mucosissima*[p]	*Sphingomonas oligophenolica*[f,m]	*Sphingomonas oryziterrae*[r]	*Sphingomonas panni*[g]	*Sphingomonas parapaucimobilis*[u]	*Sphingomonas paucimobilis*[u]	*Sphingomonas phyllosphaerae*[l]	*Sphingomonas pituitosa*[f]	*Sphingomonas polyaromaticivorans*[y]	*Sphingomonas pruni*[q]	*Sphingomonas pseudosanguinis*[y,w]	*Sphingomonas roseiflava*[u,x]	*Sphingomonas rubra*[y]	*Sphingomonas sanguinis*[u]	*Sphingomonas sanxanigenens*[z]	*Sphingomonas sediminicola*[k]	*Sphingomonas soli*[a]	*Sphingomonas starnbergensis*[aa]	*Sphingomonas trueperi*[f,u]	*Sphingomonas wittichii*[i,u]	*Sphingomonas xinjiangensis*[ab]	*Sphingomonas yabuuchiae*[w]	*Sphingomonas yunnanensis*[j]
Assimilation of																															
Glucose	–	+	(+)	+	+	+	+	+	+	v	+	+		+	+	+	+	+	+	+	w	nd	+	–	+	+	+	–	+	+	+
L-Arabinose	–	–	–	nd	–	(+)	+	+	+	+	–	+		+	+	+	+	+	+	+	nd	nd	+	+	+	+	+	–	+	+	–
D-Mannose	–	+	–	+	v	+	+	+	–	v	–	(+)	+	+	+	+	+	+	+	w	nd	+	+	–	–	+	+	+	+	+	+
N-Acethyl-D-glucosamine	–	+	+	+	+	+	+	+	nd	+	nd	(+)	+	+	+	+	+	+	–	+	–	–	nd	–	–	+	–	+	+	w	+
Maltose	–	+	+	nd	+	+	+	+	–	v	nd	(+)		+	+	+	+	+	+	+	w	nd	–	–	+	+	+	–	+	+	+
Gluconate	nd	(+)	–	nd	–	+	–	+	nd	+	nd	–		–	–	–	nd	+	–	+	–	–	nd	–	–	–	–	nd	–	+	–
Adipate	nd	+	–	nd	–	+	–	–	nd	–	nd	–		–	–	–	nd	–	nd	w	nd	nd	nd	–	–	–	–	–	–	w	–
Malic acid	nd	+	+	nd	+	–	+	–	nd	–	nd	+	+	+	+	+	–	(+)	+	+	nd	–	nd	–	–	w	+	+	+	+	+
Citrate	nd	–	–	–	–	–	–	–	–	–	nd	+	+	+	+	–	–	–	+	+	–	–	–	–	–	–	–	+	+	+	+
D-Mannitol	–	+	–	nd	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	nd	–	–	+	–	+	–	–	–
Caprate	–	–	–	nd	–	–	–	–	nd	–	–	–	nd	+	–	–	–	–	–	–	–	nd	nd	–	–	w	–	nd	–	–	–
Acetate	nd	–	–	nd	nd	–	–	–	+	nd	nd	–	nd	nd	–	nd	nd	–	–	nd	–	+	–	–	–	nd	nd	nd	nd	+	–
G+C content (mol%)	61	65.4 – 65.9	66	61.1	67	65.4 – 65.9	65.0	68.3	nd	64.1	63.3	nd		63.7	61	64.5	61.8	65.4		66 – 68	65.6		66.4	67.9	63.9	64.1	65.6	nd	63.3	66.1	66

– negative reaction, + positive, (+) or *w* weak positive, *y* variable reaction, *ng* no growth, *nd* not determined; *o* orange, *y* Yellow, *ly* light yellow, *dy* Dark-yellow, *dr* dark-red, *ro* red-orange, *py* pale yellow, *c* cream, *cl* colourless, *w* white, *cw* Cream white, *s* slow reaction, *ng* no growth

Data were taken from: [a]Asker et al. (2007a); [b]Lee et al. (2001); [c]Roh et al. (2009); [d]Margesin et al. (2012); [e] Xie and Yokota (2006); [f]Zhang et al. (2010b); [g]Talà et al. (2013); [h]Huang et al. (2012); [i]Wittich et al. (2007); [j]Lin et al. (2013); [k]An et al. (2013); [l]Choi et al. (2011); [m]Zhang et al. (2011); [n]Nigam et al. (2010); [o]Niharika et al. (2012); [p]Romanenko et al. (2007); [q]Park et al. (2011); [r]Chung et al. (2011); [s]Kaur et al. (2012); [t]Chen et al. (2012); [u]Yabuuchi et al. (2001); [v]Luo et al. (2012); [w]Kämpfer et al. (2007); [x]Yun et al. (2000); [y]Huo et al. (2011); [z]Huang et al. (2009a); [aa]Chen et al. (2013); [ab]An et al. (2011)

"*Pseudomonas elodea*". Several *Sphingomonas* strains were identified to produce sphingans of different structures. Phylogenetically, those *Sphingomonas* strains are closely related, forming a distinct cluster in 16S rRNA gene sequence-based trees (Fialho et al. 2008). *Sphingomonas* sp. ATCC 31555 produces welan (S-130), *Sphingomonas* strain ATCC 31961 rhamsan (S-194), S*phingomonas* sp. ATCC 53159 diutan (S-657), and *Sphingomonas* sp. ATCC 31554, ATCC 31853, and ATCC 21423 produce three different, yet-unnamed polysaccharides: S-88, S-198, and S-7, respectively (as reviewed by Fialho et al. 2008). All sphingans produced by those strains share the same backbone structure—a tetrasaccharide of (β1–3) or (β1–4) linked X–glucose–glucuronic acid–glucose–X subunits (where X can either be L-rhamnose or L-mannose) (Pollock 1993; Fialho et al. 2008). The most studied and commercially produced sphingan is gellan, which is composed of tetrasaccharides with repeating units of D-glucose, L-rhamnose, and D-glucuronic acid at a ratio of 2:1:1 with O-acetyl and L-glyceryl moieties on the D-glucosyl residue adjacent to the D-glucuronyl residues.

The heteropolysaccharide found in a culture of "*Pseudomonas elodea*" consists of a repeating linear tetrasaccharide of D-glucose, D-glucuronic acid, and L-rhamnose but without side chains (Jansson et al. 1983). The production of an unusual sphingan was recently described for *Sphingomonas cynarae* containing glucose, rhamnose, mannose, and galactose; glucuronic acid, commonly found in sphingans, was not detected (Talà et al. 2013). *Sphingomonas sanxanigenens* NX02^T produces the sphingan Ss (Huang et al. 2009a), which has the unique feature of precipitating by an increase of pH.

The biosynthesis of sphingans was most intensively studied for gellan. The respective biosynthetic pathway can be separated into three steps. In the first intracellular step, nucleotide sugar precursors are synthesized, followed by a second step in which the precursors are assembled. The formed tetrasaccharide units are thereby linked to the inner membrane. In a third step, the tetrasaccharides are translocated to the periplasmic space, polymerized, and exported through the outer membrane. More details with respect to the steps of the synthesis pathway can be found in Fialho et al. (2008). The genes responsible for the specific steps in the synthesis pathways have been identified. As shown by Fialho et al. (2008), a high homology was found between genes of synthesis gene clusters occurring in different *Sphingomonas* strains, *S. elodea* ATCC 31461 (gellan gene cluster; Harding et al. 2004), *Sphingomonas* sp. ATCC 31554 (S-88 sps cluster; Yamazaki et al. 1996), and from *Sphingomonas* sp. ATCC 53159 (diutan dps cluster; Coleman et al. 2008).

Blastomonas Sly and Cahill 1997

Gr. n. *blastos*, bud shoot; Gr. fem. n. *monas*, a unit, monad; N.L. fem. n. *Blastomonas*, a budding monad.

The genus *Blastomonas* was described initially by Sly and Cahill (1997) and Yurkov et al. (1997), and it was emended by Hiraishi et al. (2000). Currently (June 2013), two species, *Blastomonas natatoria* (Sly 1985; Sly and Cahill 1997) and *Blastomonas ursincola* (Yurkov et al. 1997; Hiraishi et al. 2000), are assigned to the genus.

Cells are Gram-negative, nonsporulating, and ovoid or rod-shaped. They are motile by means of a polar flagella. Reproduction occurs by budding or asymmetric cell division. Cells are single or paired; some form rosette-like aggregates. Stalks and prosthecae are not found. Colonies are yellow to orange-red colored, convex, smooth, and opaque. Carotenoids are present. Respective absorption spectra show characteristic λmax at 430, 458, and 485 nm (in vivo spectra). Members of the genus are strictly aerobic chemoorganotrophs and facultative photo-organotrophs. No growth occurs under anaerobic conditions in the light. Synthesis only light-harvesting complex I (LH-I)-type antenna with a λmax of 867 nm; LH-II is not present. Cells produce BChl *a*. Photosynthetic genes encoding proteins of RC/LH-1, *pufM*, and *pufL* genes are detectable by PCR amplification (sequence variation between the species of 4 % and 5 %) (Hiraishi et al. 2000)

Cells are catalase and oxidase positive. Nitrate is not reduced. No acid production occurs in Hugh-Leifson's OF medium. Optimal growth occurs at temperatures between 30 °C and 35 °C and pH 7.0–7.5. Growth in the presence of 3 % NaCl is variable. Strains can hydrolyze starch and tween 80. The hydrolysis of aesculin, casein, and gelatin is variable. Cells can utilize D-xylose, D-glucose, maltose, acetate, propionate, butyrate, succinate, fumarate, and glutamate. The utilization of L-arabinose, D-sorbitol, and malate is variable. Cells are negative for the utilization of D-fructose, D-mannose, cellobiose, lactose, methanol, propanol, formate, lactate, citrate, gluconate, phenylacetate, benzoate, p-cresol, dichlorophenol, dibenzofuran, dibenzo-p-dioxin, and naphthalene (Hiraishi et al. 2000). The type species is sensitive to tetracycline, polymyxin B, and chloramphenicol but resistant to penicillin, streptomycin, bacitracin, and kanamycin. Distinctive morphological and physiological characteristics are summarized in *Table 25.4*.

The predominant cellular fatty acid is C18:1 (d9). The major hydroxyl fatty acid is 2-OH C14:0. Further 2-hydroxyfatty acids can be detected in minor amounts, including 2-OH C15:0 and 2-OH C16:0; 3-hydroxy fatty acids are absent. The predominant polyamine is spermidine. Monosaccharide-type glycosphingolipids are present. Ubiquinone-10 is the major respiratory quinone; Q-9 was not detected. The genomic DNA G+C content is 64.8–65.2 mol%. The type species is *Blastomonas natatoria*. Habitates are freshwater environments.

Novosphingobium Takkeuchi, Hamana and Hiraishi 2001

No.vo.sphin.go´bi.um. L. adj. novus new; Gr. adj. sphingos of sphinx; Gr. n. bios life, living; N.L. fem. n. *Novosphingobium* new sphingosine-containing life.

The genus *Novosphingobium* was proposed by Takeuchi et al. (2001) based on six species (cluster III) of the genus *Sphingomonas sensu lato*, "*Sphingomonas rosa*", "*S. subarctica*", "*S. stygia*", "*S. subterranean*", "*S. aromaticivorans*" and "*S. capsulata*," which were reclassified as *Novosphingobium rosa*, *N. subarcticum*, *N. stygium*, *N. subterraneum*, *N. aromaticivorans*,

Table 25.4
Physiological characteristics differentiating the two *Blastomonas* species, *Blastomonas natatoria* and *Blastomonas ursinicola*

	Balstomonas natatoria (DSM 3183^T/IFO 15649^T)	*Blastomonas ursincola* DSM 9006^T
Growth with 3 % NaCl	+	–
Hydrolysis of		
Aesculin	+	–
Casein	–	+
Gelatin (on agar plate)	(+)	–
DNA	(+)	–
Assimilation of		
L-Arabinose	+	–
D-Xylose	++	+
D-Glucose	+	[+]
D-Sorbitol	–	[+]
Malate	–	[+]

Data are from Hiraishi et al. (2000). + positive, (+) weakly positive, (–) negative reaction. Symbols for carbon nutrition tests: ++ good growth, + moderate growth, [+] poor growth, – no or only very less growth. Both strains were positive for catalase, oxidase, starch hydrolysis; Tween 80 hydrolysis; utilization of maltose, ++; acetate, +; propionate, +; butyrate, +; pyruvate, ++; succinate, [+]; fumarate, [+]; and glutamate, ++ and showed negative reactions for: indole production, H_2S production, nitrate reduction, phenylalanine deaminase, urease, gelatin liquefaction in gelatin tube, acid from glucose in OF test, chitin hydrolysis, and utilization of D-fructose, D-mannose, cellobiose, lactose, methanol, propanol, formate, lactate, citrate, gluconate, phenylacetate, benzoate, p-cresol, dichlorophenol, dibenzofuran, dibenzo-p-dioxin, and naphthalene

and *N. capsulatum*. At the time of writing (July 2013), 23 *Novosphingobium* species were described with validated names.

Cells are Gram-negative, nonsporulating, and rod-shaped. They are motile or nonmotile; for some strains, motility was only obtained in the early exponential growth phase. Cells are motile by the means of a polar flagella. Cell size is in the range of 0.1–1.0 × 0.6–4.0 µm. Colonies are off-white, pale-yellow, whitish-brown, yellow, or yellow-orange; mostly small, convex, circular, opaque, smooth; and have a diameter of 1–2 mm. Colonies can also be shiny, nontransparent, contain an entire margin, or have mucous. Methanol:acetone-soluble pigments have a characteristic λmax at 453 and 480 nm. The major carotenoid is nostoxanthin (Glaeser et al. 2009).

All strains grow at a temperature range between 15 °C and 32 °C; some also grow at 4 °C and 37 °C, with one growing at 41 °C (*Novosphingobium indicum*, Yuan et al. 2009) The optimum temperature for growth is 25 °C and 30 °C. Growth occurs at a wide pH range, from pH 4.5 to 10. The optimum pH for growth is pH 6.0–7.0. Salinity-dependent growth differs among species. Some are salt-sensitive (freshwater species, Glaeser et al. 2009, 2013) and can only grow at very low NaCl (%w/v) concentrations of ≤ 0.25 (0.5) % (w/v) NaCl. Other strains require at least 1 % NaCl and grow up to 6 % NaCl (*Novosphingobium pentaromativorans*; Sohn et al. 2004). Other strains grow in a range of 0–4 % and even 5 % (w/v) NaCl. Strains are strictly aerobic and chemo-organotrophic. A broad range of sugars and amino acids can be assimilated by different species. Morphological and physiological differences among the type strains are depicted in *Table 25.5*.

The predominant respiratory quinone is ubiquinone Q-10; minor to trace amounts of Q-9 have also been detected. The predominant saturated fatty acid is C18:1; C16:0 is present as a minor component. The major 2-hydroxy fatty acid is 2-OH C14:0. Other 2-hydroxy fatty acids, 2-OH C12:0, 2-OH C13:0, 2-OH C15:0, and 2-OH C16:0 were also detected in minor to trace amounts. GSLs are present. Spermidine is the major polyamine component. Minor to trace amounts of putrescine, spermine, cadaverine, 1,3-diaminopropane, or agmatine can be present. *sym*-Homospermidine was not detected at all. Polar lipid profiles share sphingoglycolipids, phosphatidylgylcerin, and phosphatidylethanolamine; most often diphosphatidylglycerol, phosphatidylcholine, phosphatidyldimethylethanolamine, and phosphatidylmonomethyl ethanolamine are also present as well as phosphor-, glycol-, and aminolipids. Some species contained a yellow pigment in the polar lipid profile, positive stained with α-naphthol (Glaeser et al. 2009, 2013; Kämpfer et al. 2002a).

Characteristic 16S rRNA signature nucleotides are present at positions 52:359 (U:A), 134 (G), 593 (U), 987:1218 (A:U) and 990:1215 (U:G). Two species (*Novosphingobium acidiphilum* and *Novosphingobium nitrogenifigens*) contain a characteristic 21-bp signature gap in the 16S rRNA gene sequence between base positions 1256 and 1278 (*E. coli* numbering, Brosius et al. 1978) (Addison et al. 2007; Glaeser et al. 2009). This 21-bp gap is not recognized in any other species of the family *Sphingomonadaceae*, but it occurred frequently in other members of the class *Alphaproteobacteria* (Glaeser et al. 2009). The type species is *Novosphingobium capsulatum* (Yabuuchi et al. 1990). The DNA G+C content is in the range of 60–67 mol%.

Habitats include saturated Atlantic coastal plain terrestrial subsurface sediments, roots of rose trees, clinical specimens, stocked distilled water, surface water and sediments of

Table 25.5

Morphological and physiological characteristics differentiating *Novosphingobium* species

Characteristics	*Novosphingobium acidiphilum*[a]	*Novosphingobium aromaticivorans*[b,c]	*Novosphingobium barchaimii*[d]	*Novosphingobium capsulatum*[b,c]	*Novosphingobium fuchskuhlense*[e]	*Novosphingobium hassiacum*[c]	*Novosphingobium indicum*[f]	*Novosphingobium lentum*[g]	*Novosphingobium lindaniclasticum*[d]	*Novosphingobium mathurense*[d]	*Novosphingobium naphthalenivorans*[d]	*Novosphingobium nitrogenifigens*[h]	*Novosphingobium panipatens*[d]	*Novosphingobium pentaromaticivorans*[c]	*Novosphingobium resinovorum*[d]	*Novosphingobium rosa*[b,c]	*Novosphingobium sediminicola*[i]	*Novosphingobium soli*[d]	*Novosphingobium stygium*[b,c]	*Novosphingobium subarcticum*[b,c]	*Novosphingobium subterraneum*[b,c]	*Novosphingobium taihuense*[j]	*Novosphingobium tardaugens*[g]
Pigmentation	o-y	y	y	y	y	y	y	p-y	y	y	y	p-y	ly	y	y	y	y	y	y	y	y	y	w-b
Mobility	+		–	–	–	+	PF (–)	–	–	F (+)		–	–	–			–	+	–		–	–	–
Oxidase	+	+	+	–	+	+	–	+	+	–	+	+	–	–	+		+	+		+	+	–	–
Catalase	–		+	nd	–	+	+	+	+	–	+	+	–	+	+		+	–	+	+	+	+	+
Hydrolysis of:																							
Aesculin	+	+	+	+	–	–	–	–	+	+	(+)	–	–	+	+	(+)	+	–	+	+	+	+	–
Assimilation of:																							
L-Arabinose	(+)	–	–	(+)	–	–	–	–	+	+	+	(+)	(+)	–	+	+	+	+	–	+	(+)	+	–
Cellobiose	+	+	nd	+	+	+	–	–	nd	nd	nd	+	nd	–	nd	+	nd	nd	–	–	+	+	–
D-Fructose	(+)	–	+	–	–	–	–	–	+	+	–	+	(+)	+	nd	+	nd	(+)	–	–	–	+	–
D-Galactose	+	–	+	+	–	–	–	–	+	+	–	–	(+)	–	nd	–	nd	–	–	+	–	–	–
D-Glucose	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	–	+	+	+	–
D-Mannose	+	+	+	(+)	–	(+)/–	+	–	nd	nd	–	+	ND	–	–	+	+	–	–	–	+	+	–
Maltose	+	+	+	+	–	–	+	–	–	+	+	+	(+)	–	–	+	+	–	–	+	+	+	–
L-Rhamnose	+	+	+	+	(+)	–	–	–	–	+	–	–	–	+	–	+	+	–	–	+	+	+	–
Sucrose	+	+	+	+	–	+	–	–	nd	+	–	–	–	+	–	+	+	–	–	–	+	+	–
D-Xylose	–	+	nd	+	–	–	nd	–	nd	nd	nd	–	nd	–	nd	+	nd	nd	–	+	+	+	–

Table 25.5 (continued)

Characteristics	*Novosphingobium acidiphilum*[a]	*Novosphingobium aromaticivorans*[b, c]	*Novosphingobium barchaimii*[d]	*Novosphingobium capsulatum*[b, c]	*Novosphingobium fuchskuhlense*[e]	*Novosphingobium hassiacum*[c]	*Novosphingobium indicum*[f]	*Novosphingobium lentum*[g]	*Novosphingobium lindaniclasticum*[d]	*Novosphingobium mathurense*[d]	*Novosphingobium naphthalenivorans*[d]	*Novosphingobium nitrogenifigens*[h]	*Novosphingobium panipatens*[d]	*Novosphingobium pentaromaticivorans*[c]	*Novosphingobium resinovorum*[d]	*Novosphingobium rosa*[b, c]	*Novosphingobium sediminicola*[i]	*Novosphingobium soli*[d]	*Novosphingobium stygium*[b, c]	*Novosphingobium subarcticum*[b, c]	*Novosphingobium subterraneum*[b, c]	*Novosphingobium taihuense*[j]	*Novosphingobium tardaugens*[g]
DL-Lactate	–	–	nd	–	+	–	nd	–	nd	nd	nd	+	nd	–	–	–	–	–	–	+	–	nd	+
L-Malate	+	–	–	+	–	–	nd	–	+	–	–	+	–	–	+	+	–	+	–	+	–	nd	–
2-Oxoglutarate	+	–	nd	–	nd	–	nd	–	nd	nd	nd	–	nd	–	nd	–	nd	nd	–	–	–	nd	–
L-Histidine	–	–	nd	+	+	–	nd	–	nd	+	–	+	(+)	–	–	+	–	–	–	+	–	nd	–
L-Leucine	(+)	+	nd	+	–	–	nd	–	nd	–	–	–	+	–	nd	–	nd	+	–	+	–	nd	–
L-Phenylalanine	–	–	nd	–	–	–	–	–	nd	nd	nd	–	nd	+	nd	–	nd	–	–	–	–	–	–
L-Proline	+	+	–	–	–	+	+	–	nd	+	+	+	+	+	+	–	+	+	–	+	+	+	–

nd Not determined, *o-y* orange to yellow pigmented, *y* yellow, *p-y* pale-yellow, *w-b* whitish-brown, + positive reaction, (+) slightly positive, – negative reaction

[a]Glaeser et al. (2009); [b]Yabuuchi et al. (2002); [c]Kämpfer et al. (2002); [d]Saxena et al. (2013); [e]Glaeser et al. (2013); [f]Yuan et al. (2009); [g]Tiirola et al. (2005); [h]Addison et al. (2007); [i]Baek et al. (2011); [j]Liu et al. (2005)

freshwater lakes, sewage ponds, deep-sea water, fluidized-bed bioreactor treating PCP-contaminated groundwater, HCH-contaminated dumpsites, oil-, polychlorinated dibenzo-p-dioxins (PCDD)/F- and PAH-contaminated soils and sediments, activated sludge of a sewage treatment plant, and pulp and paper wastewater.

Nitrogen Fixation The ability to fix nitrogen was observed for one species in the genus *Novosphingobium*, *Novosphingobium nitrogenifigens* (Addison et al. 2007). The type strain Y88^T (originally isolated from pulp and paper wastewater), grown on nitrogen-limited mineral medium, showed a positive reaction for acetylene reduction and *nifH* was detected with degenerated primers (Addison et al. 2007). All genes required for nitrogen fixation were determined in the genome of the strain, *nifA*, *–D*, *–E*, *–H*, *–K*, *–N*, *–Q*, *–U*, *–W*, and *–X*, and *fixA* and *fixU* as well as genes encoding cofactors of the biosynthesis proteins (Strabala et al. 2012). Along with *S. azotifigens, N. nitrogenifigens* is one of the two species of the *Sphingomonadaceae* in which nitrogen fixation was proven.

Sphingobium Takkeuchi, Amana and Hiraishi 2001 Emend Li et al. 2013

Sphin.gobi.um. Gr. adj. Sphingos of sphinx; Gr. n. bios life, living; N.L. fem. n. Sphingobium sphingosine-containing life.

The genus *Sphingobium* was proposed based on three species of the genus *Sphingomonas sensu lato*, "*Sphingomonas yanoikuyae*", "*Sphingomonas herbicidovorans*", and "*Sphingomonas chlorophenolica*" representing the cluster IV of the genus *Sphingomonas sensu lato*, which were reclassified as *Sphingobium yanoikuyae*, *Sphingobium herbicidovorans*, and *Sphingobium chlorophenolica* (Yabuuchi et al. 2001). At the time of writing (July 2013), the genus contained 34 species (http://www.bacterio.net/).

Cells are Gram-negative, nonsporulating, and rod-shaped. Cell size is in the range of 0.1–1.5 × 0.7–3.5 μm. Cells can be nonmotile or motile by means of a polar, single lateral, or peritrichious flagella. Colonies are white, whitish-brown, pale, and creamy-yellow, yellow, circular, and smooth; some colonies are also convex, domed, or slimy. Two species, *Sphingobium indicum* and *Sphingobium chungbukense*, produce a water-soluble brown pigment (λmax = 532 nm; Pal et al. 2005). Some strains from rosette structures on complex medium and branched thread or hypha-like aggregates on selective media (*Sphingobium aromaticiconvertens*). Capsules can be formed under specific growth conditions; for example, cells of *Sphingobium aromaticiconvertens* from capsules after growth on dibenzofuran. Cells are strictly aerobic and have a chemo-organotrophic metabolism. Most grow at temperatures between 15 °C and 30 °C (optimal growth is mainly at 28–30 °C); most also grow at 37 °C, and some also at 40 °C. The pH range of growth is between pH 5 and 10, with an optimum at pH 7.0. Salinity-dependent growth occurs in a range of 0–5 % (w/v) NaCl; some only grow at low salinity, whereas others require at least 2 % (w/v) NaCl. Most species are positive for catalase activity and negative for nitrate reduction (Li et al. 2013).

Morphological and physiological features differentiating *Sphingobium* species are summarized in ❯ *Table 25.6*. The predominant respiratory quinone is ubiquinone Q-10, but minor to trace amounts of Q-9 can also be detected. The dominant fatty acid is C18:1; C16:0 occurs in minor amounts. The major 2-hydroxy fatty acid is 2-OH C14:0. In some species, 2-OH C15:0 and 2-OH C16:0 were also detected. The major polyamine is spermidine. Minor to trace amounts of putrescine, spermine, cadaverine, 1, 3-diaminopropane, and agmatine can be present. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipids, with only a few exceptions. Phosphatidyldimethylethanolamine, phosphatidylmonomethyl ethanolamine, and phosphatidylcholine are also found among species of the genus. Characteristic 16S rRNA signatures are present at positions 52:359 (U:A), 134 (G), 593 (U), 987: 1218 (A:U) and 990:1215 (U:G). The DNA G +C content is 58.2–67.0 mol% (values below 60 °C were only detected for *Sphingobium suberifaciens* and *Sphingobium rhizovicinum*). The type species is *Sphingobium yanoikuyae* (Takeuchi et al. 2001).

Habitates include oil-, chlorophenol-, and HCH-contaminated soils, γ-HCH-treated upland soils, HCH dumpsite sides, hydrocarbon-contaminated sawmill soil, chemically contaminated freshwater sediment, sewage-treatment wastewater, homemade kitchen-waste vermicompost, copper mine soil, activated sludge, fly ash dumping site of a thermal power plant, wastewater treatment system in a pesticide manufactory, rhizosphere soil of rice or *Fortunella hindsii* (Champ. ex Benth.) swingle, corked lettuce roots, spring water, river water or sediment, freshwater lake surface water, and sediments. Type strains of several *Sphingobium* species were isolated from HCH-contaminated sides and show HCH-degrading activities in different proportions regarding overall and HCH-isomere-specific degrading activities.

Sphingopyxis Takkeuchi, Hamana and Hiraishi 2001 Emend Baik et al. 2013

Sphin.go.pyx´is. Gr. adj. sphingos of sphinx; Gr. n. pyxis jewel box; N.L. fem. n. Sphingopyxis jewel box of sphingolipid-containing life.

The genus *Sphingopyxis* was proposed based on two species of the genus *Sphingomonas sensu lato*, "*Sphingomonas macrogoltabidus*" and "S*phingomonas terrae*," representing the cluster IV of the genus *Sphingomonas sensu lato*. The species were reclassified as *Sphingopyxis macrogoltabidus* and *Sphingopyxis terrae*. Three *Sphingopyxis* species, *Sphingopyxis marina*, *Sphingopyxis litoris* (both Kim et al. 2008), and *Sphingopyxis flavimaris* (Yoon and Oh 2005), were recently transferred to the new genus *Sphingorhabdus* by Jogler et al. (2013). At the time of writing (July 2013), the genus *Sphingopyxis* contained 16 species with validated names (http://www.bacterio.net/).

Cells are Gram-positive, nonsporulating, and ovoid to rod-shaped. Cell size is in the range of 0.2–0.9 × 0.5–3.0 μm. Ultrasmall cells (<0.1 μm^3) are characteristic for strains of *Sphingopyxis alaskensis*, which represents oligotrophic marine

Table 25.6

Morphological and physiological characteristics differentiating *Sphingobium* species

Characteristics	*Sphingobium abikonense*[a]	*Sphingobium amiense*[b, c]	*Sphingobium aromaticiconvertens*[d]	*Sphingobium baderi*[e]	*Sphingobium boeckii*[c]	*Sphingobium chinhatense*[f]	*Sphingobium chlorophenolicum*[b]	*Sphingobium chungbukense*[g]	*Sphingobium cloacae*[h]	*Sphingobium cupriresistens*[i]	*Sphingobium czechense*[j]	*Sphingobium faniae*[h]	*Sphingobium fontiphilum*[k]	*Sphingobium francense*[l]	*Sphingobium fuliginis*[m]	*Sphingobium herbicidovorans*[b]	*Sphingobium indicum*[i, l]	*Sphingobium japonicum*[l]	*Sphingobium jiangsuense*[m]	*Sphingobium lactosutens*[a]	*Sphingobium limneticum*[c]	*Sphingobium lucknowense*[n]	*Sphingobium olei*[o]	*Sphingobium qiguonii*[p, q]	*Sphingobium quisquiliarum*[r]	*Sphingobium rhizovicinum*[s]	*Sphingobium scionense*[c]	*Sphingobium suberifaciens*[c]	*Sphingobium ummariense*[h]	*Sphingobium vermicomposti*[c, h, t]	*Sphingobium vulgare*[Th, u]	*Sphingobium wenxiniae*[Th, t]	*Sphingobium xenophagum*[h, v]	*Sphingobium yanoikuyae*[Tb, h]
Pigmentation	y	cy	ly	w	cy	y	nd	y	cw	Y	y	py	y	Y	y		y	y	cw	y	y	y	y	gw	y	y	y	w	y	y	y	y	ly	gw
Motility	+	+	–	nd	+	–	–	+	–	–	+	–	–	–	–	+	–	–	–	–	+	+	nd	–	–	+	–	+	–	–	+	–	+	+
Assimilation of:																																		
D-Glucose	+	+	–	+	+	nd	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
L-Arabinose	+	–	–	nd	+	nd	–	+	–	+	–	+	–	+	+	+	+	+	+	+	+	+	nd	+	+	–	+	+	nd	–	+	+
D-Mannose	+	–	–	+	–	nd	–	+	–	–	nd	+	nd	–	nd	–	–	–	–	–	–	nd	–	+	nd	+	–	–	–	–	–	–	–	–
Malate	nd	–	–	nd	–	nd	–	–	–	nd	nd	+	–	nd	nd	+	nd	nd	–	nd	+	nd	+	–	nd	+	+	–	–	–	–	+	+	+
Maltose	+	+	–	+	–	nd	–	+	–	+	+	–	–	nd	+	+	nd	nd	+	nd	–	+	+	+	nd	+	+	–	+	–	–	–	+	+
Citrate	+	–	–	nd	–	–	–	–	–	–	–	–	–	–	nd	–	–	nd	–	+	–	–	–	+	–	+	+	–	–	–	–	nd	+	+
Aesculin hydrolysis	+	–	–	+	+	–	+	+	–	+	–	–	+	+	–	+	–	+	+	+	nd	–	+	–	+	+	+	nd	+	+	nd	–	–	+
Activity of:																																		
Oxidase	nd	+	+	+	+	+	+	+	+	–	+	+	+	+	+	nd	+	+	+	nd	+	+	nd	+	+	–	–	+	nd	+	+	–	–	–
Catalase	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	nd	+	–	–	+	+	–	+	+	–	+	+	+	+	+
Urease	+	–	nd	nd	–	nd	+	nd	+	–	–	+	–	nd	–	–	+	nd	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+
b-Galactosidase	nd	–	+	nd	+	nd	–	–	–	+	nd	+	–	nd	–	–	nd	nd	–	nd	+	–	–	–	–	+	+	+	+	+	–	–	–	+
Nitrate reduction	–	–	–	+	–	+	–	nd	–	+	–	–	–	nd	+	–	–	–	–	w	–	–	–	–	–	+	+	+	–	–	–	–	–	–

– negative, + positive, *nd* not determined, *y* yellow, *cy* cream yellow, *w* weight, *cw* cream weight, *gw* greyish weight, *ly* light yellow, *p-y* pale yellow

[a]Kumari et al. (2009); [b]Ushiba et al. (2003); [c]Chen et al. (2013); [d]Wittich et al. (2007); [e]Kaur et al. (2013); [f]Dadhwal et al. (2009); [g]Kim et al. (2000); [h]Wang et al. (2011); [i]Li et al. (2013); [j]Niharika et al. (2013); [k]Sheu et al. (2013); [l]Pal et al. (2005); [m]Prakash and Lal (2006); [n]Garg et al. (2012); [o]Young et al. (2007); [p]Zhang et al. (2012); [q]Yan et al. (2010); [r]Bala et al. (2010); [s]Young et al. (2008); [t]Wang et al. (2011); [u]Baek et al. (2010); [v]Stolz et al. (2000)

ultramicrobacteria. Cells may be motile or nonmotile; motility by means of a single polar or subpolar flagella. Intracellular accumulation of polyhydroxyalkanoates (mainly 3-hydroxybutyric acid, with minor amounts of 3-hydroxyvaleric acid) was obtained during growth in mineral medium containing 0.1 % glucose for *Sphingophyxis chilensis*, *S. macrogoltabidus*, and *S. alaskensis* (Godoy et al. 2003). The three species can be differentiated from *Sphingomonas adhaesiva*, which accumulated, under the same conditions, 60 % 3-hydroxybutyric acid and 3 % 3-hydroxyvaleric acid (Godoy et al. 2003).

Colonies are beige, yellowish, yellow or orange pigmented, circular, convex with entire margins, smooth, opaque, glistening, and/or nonglossy, with a diameter between 0.8 and 5 mm. Acetone-soluble pigments for bright-yellow pigmented cells of *Sphingopyxis witflariensis* are characterized by λmax at 452 and 478 nm (Kämpfer et al. 2002b). Strains are strictly aerobic and chemo-organotrophic. Strains of *Sphingopyxis alaskensis* were obligate oligotrophic after the first isolation, but they turned out to be facultative oligotrophic (Schut et al. 1993, 1995). Cells are catalase-variable (Baik et al. 2013); nitrate reduction is variable.

All strains grow at temperatures between 10 °C and 30 °C; some also at 35–37 °C and 42 °C (*Sphingopyxis ginsengisoli*, Lee et al. 2008; *Sphingopyxis soli*, Choi et al. 2010b) and even at 48 °C (*Sphingopyxis alaskensis*, Godoy et al. 2003). One species is psychrophilic and cold-adapted (*Sphingopyxis bauzanensis*) and grows at temperatures between 1 °C and 25 °C (Zhang et al. 2010). Growth occurs at a wide pH range (pH 4.5 to 11), with optimal growth between pH 6 and 8. Salinity requirements depend on the original habitat of strains. Most strains grow in the presence of 0–3 % (w/v) NaCl. Freshwater species (*Sphingopyxis rigui* and *Sphingopyxis wooponensis*) show optimal growth at 0 % and in the presence of up to 1 % (w/v) NaCl. For marine species (*Sphingopyxis alaskensis* or *Sphingopyxis baekryungensis*), growth occurs up to 5–10 % NaCl (w/v), with an optimum between 2 % and 3 % (w/v) NaCl. Morphological and physiological characteristics differentiating *Sphingopyxis* and *Sphingorhabdus* species are listed in *Table 25.7*.

The predominant respiratory quinone is ubiquinone Q-10; minor amounts of ubiquinone Q-9 and Q-8 can also be present. The major cellular fatty acids are C18:1, C16:0, and C16:1. The major 2-hydroxy fatty acids are 2-OH C14:0, 2-OH C15:0, and/or 2-OH C16:0. GSLs are present. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, sphingoglycolipid, and different unknown glycolipids and/or phospholipids. Some also contain phosphatidylcholine and minor amounts of phosphatidylmonomethylethanolamine. Spermidine is the major polyamine; some also contain minor to trace amounts of putrescine or spermine, as well as cadaverine or 1,3-diaminopropane. Characteristic 16S rRNA signatures are present at positions 52:359 (C:G), 134 (G), 593 (U), 987:1218 (G:C), and 990:1215 (U:G).

The type species is *Sphingopyxis macrogoltabida* (Takeuchi et al. 1993, 2001). The DNA G+C content is 63–69.2 mol%. Lower values of 53.3–57.3 mol% were obtained for *Sphingopyxis wooponensis* and *Sphingopyxis rigui* (Baik et al. 2013). Habitats include underground water, seawater, soil partially contaminated with hydrocarbons or HCH, ginseng field soil, river-sediment contaminated with chlorophenolic compounds, wetlands, freshwater, mineral water, wastewater treatment plant granules, or activated sludge.

Biodegradation of recalcitrant natural and anthropogenic xenobiotic and polyaromatic compounds by *Sphingomonas*, *Sphingobium*, *Sphingopyxis*, and *Novosphingobium* species A specific feature of sphingomonads of the genera *Sphingomonas*, *Sphingobium*, *Sphingopyxis*, and *Novosphingobium* is the ability to degrade recalcitrant compounds of natural or anthropogenic origin, including biphenyl, naphthalene, fluorene, and several substituted variants of those compounds; phenanthrene(s), pyrene, as well as diphenylether, furan, dibenzo-p-dioxin, and several chlorinated variants; carbazole, estradiol, polyethylene glycols, chlorinated phenols, nonylphenols, and different herbicides and pesticides (reviewed by Stolz 2009). The specific adaptation of sphingomonads to the degradation of those compounds is indicated by the high frequency in which sphingomonads are isolated from respective contaminated habitats. The replacement of LPS in the outer membrane by SGLs and the excretion of anionic EPS (sphingans) are important features, which contribute to the ability of sphingomonads to degrade aromatic compounds that are often strongly hydrophobic (Stolz 2009).

For example, the type strain of *Sphingobium yanoikuyae* B1^T degrades anthracene, toluene, naphthalene, phenanthrene, biphenyl, and m-xylene (Yabuuchi et al. 1990; Khan et al. 1996); *Novosphingobium aromaticivorans* F199^T degrades naphthalene, toluene, cresols, biphenyl, fluorene, and dibenzothiophene, whereas another *N. aromaticivorans* strain was capable of degrading 2-methylnaphthalene, 2,3-dimethylnaphthalene, acenaphthene, anthracene, fluoranthene, and phenanthrene (Shi et al. 2001). The type strains of *Novosphingobium subterraneum* and *Novosphingobium stygium* were capable of degrading biphenyl, toluene, dibenzothiophene, and fluorene (Balkwill et al. 1997; Takeuchi et al. 2001). Several other *Sphingomonas* sp. were found to degrade differently methylated naphthalenes (Bramucci et al. 2002; Sabaté et al. 1999, 2003), acenaphthene, or acenaphthylene (Pinyakong et al. 2004; Kouzuma et al. 2006) or several other bi- or polycyclic aromatic compounds such as phenanthrene (Dutta et al. 1998; Liu et al. 2004; Pinyakong et al. 2000), fluorene (Wattiau et al. 2001), chrysene (Willison 2004), tetralin (1,2,3,4-tetrahydronaphthalene; Hernáez et al. 1999), or carbazole (Habe et al. 2002; Kilbane et al. 2002; Shepherd and Lloyd-Jones 1998). Other sphingomonads were found to degrade compounds that are problematic because of their estrogenic activity, which can be quite frequently found in effluents of sewage plants; for example, 17ß-estradiole can be degraded by a *Novosphingobium tardaugens* strain (Fujii et al. 2003a). Type strains of *Sphingopyxis macrogoltabidus*, *Sphingopyxis terrae*, and *Sphingomonas sanguinis* were capable of degrading polyethylene glycols (PEG)

Table 25.7
Differentiating morphological and physiological characteristics for species of the genera *Sphingopyxis*, *Sphingorhabdus*, and *Parasphingopyxis*, which are phylogenetically closely related to each other

Characters	*Sphingopyxis alaskensis* [a]	*Sphingopyxis baekryungensis* [b]	*Sphingopyxis bauzanensis* [c]	*Sphingopyxis chilensis* [a]	*Sphingopyxis ginsengisoli* [d]	*Sphingopyxis granuli* [a]	*Sphingopyxis macrogoltabidab* [b]	*Sphingopyxis panacterrae* [e]	*Sphingopyxis panacterrulae* [f]	*Sphingopyxis rigui* [d]	*Sphingopyxis soli* [g]	*Sphingopyxis taejonensis* [b]	*Sphingopyxis terrae* [b]	*Sphingopyxis ummariensis* [h]	*Sphingopyxis wittfariensis* [b]	*Sphingopyxis wooponensis* [d]	*Sphingorhabdus planktonica* [i]	*Sphingorhabdus marina* [i]	*Sphingorhabdus litoris* [i]	*Sphingorhabdus flavimaris* [i]	*Parasphingopyxis lamellibrachiae* [j]
Pigmentation	y-b	o	o	y	y	w	y-w	y	p-y	ly	y	p-y	ly	y	y, tr	y	o-y,tr	y	y	y	o-y
Motility	+	+	+	+	+	+	+	nd	+	–	+	+	+	+	+	–	–	+	+	+	**+**
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	**+**
Catalase	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	–
Nitrate reduction	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**+**
Aesculin	+	+	+	+	+	nd	–	–	+	+	+	–	+	+	–			w	w	–	–
Arginine dihydrolase	– [7]	nd	–	– [4]	+[4]	nd	–	–	nd	+	–	– [4]	nd	nd	– [4]	+	–	+	+	nd	nd

Enzymes:																					
Urease	v	–	–	–[4]	–[4]	nd	–	–	–	–	+	–[4]	–	–	–	–	–	+	+	–	–
(a)b-Glucosidase	+/–[7]	–	nd	+	+	+	+	nd	+	–	–	w	nd	nd	–	–	+	w	w	nd	nd
Protease	nd	nd	nd	nd	nd	nd	–	nd	nd	–	nd	nd	nd	nd	–	–	–	+	–	–	nd
b-Galactosidase	–/+	–	–	–	–[4]	–	+	–	–	+	–	–[4]	–	–	–	+	+	–	+	nd	nd
Assimilation of:																					
Glucose	+	+	+	+	+[4]	–	+	+	+	–	+	+	+	+	–/+[4]	–	+	+	–	–	nd
L-Arabinose	–[7]	+	–	+[4]	–[4]	–	+	–	–	–	–	–	–	+	–	–	–	+	–	–	nd
N-Acetylglucosamine	–	nd	–	+/–[7]	–[4]	–	–	–	+	–	–	–	–	nd	–	–	–	–	–	nd	nd
Maltose	+	–	+	+	+[4]	–	+	–	+	–	+	–[4]	–	+	+	–	–	–	–	–	nd
Mannose	–	–/+[5]	–	+	–[4]	–	–	+	–	–	–	–	–	+	–	–		–	–	–	nd
Potassium gluconate	–[7]	nd	–	+	–[4]	nd	–	–	–	–	–	–	–	nd	–	–	–	–	–	nd	nd
Capric acid	–[7]	nd	–	–	–[4]	nd	+	+	–	–	–	w[4]	–	nd	+	–	–	–	–	nd	nd
Adipic acid	–	nd	nd	+/–[7]	w[6]	–	–	–	–	–	–	–	–	nd	–	–	–	+	–	nd	nd
Malic acid	–[7]	W	+	–	+[4]	–	–	+	+	–	–	+[4]	+	nd	–	–	–	+	+	–	nd
Trisodium citrate	–[7]	nd	–	–	–[4]	nd	–	–	–	nd	–	–	–	–	–	nd	–	+	+	–	–
DNA G+C content (mol%)	65	63	64.4	65.5	69.2	63.7	63.9	68.1	62.3	57.3	65.9	63	nd	68	nd	53.3	55.7	56.5	52.6	57.8	60.1

V variable reaction, + positive reaction, *w* weak reaction, – negative reaction, *nd* not determined, *y* yellow, *y-b* yellow to beige, *o* orange, *w* weight, *y-w* yellowish, *p-y* pale yellow, *o-y* yellow to orange, *tr* transparent
Data were taken from references added to the species name or as otherwise indicated by labeling the result respectively: [a]Kim et al. (2005); [b]Kämpfer et al. (1997, 2002); [c]Zhang et al. (2010); [d]Baik et al. (2013); [e]Lee et al. (2008); [f]Srinivasan et al. (2010); [g]Choi et al. (2010b); [h]Sharma et al. (2010); [i]Jogler et al. (2013); [j]Uchida et al. (2012)

(Takeuchi et al. 1993). An overview of several more compounds degraded by sphingomonads is given in ❯ *Table 25.8*, which was adapted from Stolz (2009).

One important feature of sphingomonads is the degradation of insecticides and herbicides. One of the most studied insecticides that can be degraded by several sphingomonads is y-hexachlorocyclohexane (HCH; lindane). The degradation of HCH (1, 2, 3, 4, 5, 6–hexachlorocyclohexane) is a common feature of several sphingomonads that were isolated from HCH-contaminated or HCH-dumping sites; among those eight *Sphingobium* species with validated names, *Sphingobium baderi* (Kaur et al. 2013), *Sphingobium chinhatense* (Dadhwal et al. 2009), *Sphingobium czechense* (Niharika et al. 2013a), *Sphingobium francense* (Pal et al. 2005), *Sphingobium japonicum* (Pal et al. 2005), *Sphingobium lactosutens* (Kumari et al. 2009), *Sphingobium lucknowense* (Garg et al. 2012), *Sphingobium quisquiliarum* (Bala et al. 2010), and *Sphingobium ummariense* (Singh and Lal 2009). Beside *Sphingobium* species strains phylogenetically placed into the genera *Sphingomonas*, *Novosphingobium* and *Sphingopyxis* are also HCH-degrading sphingomonads, including the species *Novosphingobium lindaniclasticum* (Saxena et al. 2013), *Novosphingobium barchaimii* (Niharika et al. 2013b), and *Sphingopyxis ummariensis* (Sharma et al. 2010).

Hexachlorocyclohexane is an organochlorine insecticide that has been applied since the 1940s. Two forms are available. First, technical-grade HCH which is a mixture of mainly four isomers, α-, β-, γ-, and δ-HCH. Only γ-HCH thereby shows the insecticidal activity; however, all isomers are persistent in the environment and toxic for insects, birds, and mammals. Gamma-HCH only makes up 6–10 % of the technical-grade HCH (Willett et al. 1998; Alvarez et al. 2012). The second form of HCH is lindane, which is almost pure γ-HCH. The degradation pathways of HCH isomers were mainly studied for three *Sphingobium* species, *Sphingobium japonicum* UT26^T, *Sphingobium indicum* B90A ^{T}, and *Sphingobium francense* Sp + (Böltner et al. 2005; Nagata et al. 2007). All currently compared sphingomonads shared the same genes involved in the degradation pathway (Lal et al. 2006; Nagata et al. 2007). The degradation pathway of y-HCH was primarily studied for *Sphingobium japonicum* UT26^T.

Gene-coding enzymes or other proteins involved in the degradation pathways are called lin-genes (*linA* to *linN*). Several of those genes were cloned and characterized in detail. *LinA* is a dehydrochlorinase (Imai et al. 1991) involved in the first steps of the upstream degradation pathways (Lal et al. 2006). Different variants of the genes are known (*linA*/*linA1*/*linA2*; Lal et al. 2006). The stereo selectivity of LinA is responsible for the selective declorination of HCH isomers, including α-, γ-, and δ-HCH but not β- or ε-HCH (Lal et al. 2006). Further enzymes involved in the next steps of the upstream pathway are a halidohydrolase (haloalkane dehalogenase) coded by *linB* (Nagata et al. 1993b) and a dehydrogenase coded by *linC* or *linX* variants (Nagata et al. 1994).

The initial steps of the degradation pathway of different HCH isomers can be more or less complex depending on the isomers partially including both enzymes LinA and LinB (Sharma et al. 2006; Nagata et al. 1993a; Trantirek et al. 2001; Wu et al. 2007). Genes involved in the downstream pathway of HCH degradation are *linD* coding a reductive dechlorinase (Miyauchi et al. 1998), *linE* coding a ring-cleavage oxygenase (Miyauchi et al. 1999), and *linF* coding a maleylacetate reductase. The genes *linG* and *linH* code the alpha and beta-subunits of an acyl-CoA transferase and *linJ* codes a thiolase (Nagata et al. 2007). Two *lin* genes were identified to be transcriptional regulators of the LysR-family (*linR;* Miyauchi et al. 2002) and the IclR-family (*linL*; Nagata et al. 2007). The gene cluster *lin*KLMN was found in a homologous arrangement in several sphingomonads strains; it codes a putative ABC transporter involved in the HCH degradation pathway (Endo et al. 2005; Nagata et al. 2007). The structure of the ABC transporter was predicted as followed: the membrane protein LinK was localized in the inner membrane linked to a cytoplasmic ATPase (LinL). *LinM* codes a periplasmatic protein and *linN* a lipoprotein located at the outer membrane associated with LinK (Nagata et al. 2007).

Sphingobium spp. have been the most investigated HCH-degrading bacteria so far. Genome sequence analysis (see above) of the three most studied strains showed that *lin* genes are either located on the chromosomes and/or on plasmids, which varied between the different strains. Several of the *lin* genes were associated with an insertion element (IS6100), which is a genetic mobile element that may explain the spread of *lin* genes among several different *Sphingobium* species by horizontal gene transfer (see the genome section within this chapter). It was furthermore discussed that the presence of glycosphingolipids in the outer membrane instead of lipopolysaccharides might facilitate the assimilation of hydrophobic HCH because SGLs provide a membrane surface of higher hydrophobicity (Lal et al. 2006).

Other sphingomonads were found to degrade herbicides as carbofuran (Feng et al. 1997a; Kim et al. 2004; Yan et al. 2007), isoproturon (Sørensen et al. 2001; Bending et al. 2003), or further insecticides as chlorpyrifos (Li et al. 2007). The type strain of *Sphingobium herbicidovorans* was capable of the complete degradation of both enantiomers of the herbicide 2-(4-chloro-2-methylphenoxy)propionic acid (mecoprop) in an enantio selective manner (Zipper et al. 1996). An intensively studied *Sphingomonas*-type strain degrading nitrodiphenyl ether herbicides is *Sphingomonas wittichii* RW1^T, which is able to degrade PCDD, dibenzofurans (DF), and diphenyl ethers. Those compounds are occur worldwide in water and soil pollutants; they are considered to be potential mutagens and endocrine disruptors. The aerobic PCDD and DF degradation pathway of strain RW1^T has been intensively studied (e.g. Wittich et al. 1992; Bünz and Schmidt 1997; Wittich 1998; Hong et al. 2002; Nam et al. 2005; Keum et al. 2008).

Polyphenol degradation was obtained for the type strain of *Sphingophyxis chilensis*, Godoy et al. 2003, which was isolated from a sediment of a chlorophenol-polluted river (Godoy et al. 1999). The type strain of *Sphingobium chlorophenolicum* (formerly *Sphingomonas chlorophenolica*) was isolated from

Table 25.8

Overview of xenobiotic and recalcitrant compounds degraded by sphingomonads strains

Strains	Degraded compounds	Reference
Sphingobium yanoikuyae B1	Anthracene, toluene, naphthalene, phenanthrene, biphenyl, m-xylene	Yabuuchi et al. (1990); Khan et al. (1996)
"*Sphingomonas paucimobilis*" EPA505	Fluoranthene, anthracene, phenanthrene, (substituted) naphthalene(s)	Mueller et al. (1997)
Novosphingobium aromaticivorans F199^T	Naphthalene, toluene, cresols, biphenyl, fluorene, dibenzothiophene	Balkwill et al. (1997); Takeuchi et al. (2001)
Novosphingobium aromaticivorans B0695	2-Methylnaphthalene, 2,3-dimethylnaphthalene, acenaphthene, anthracene, fluoranthene, phenanthrene	Shi et al. (2001)
Novosphingobium subterraneum	Biphenyl, (methyl-) toluene(s), naphthalene, dibenzothiophene, fluorene	Balkwill et al. (1997); Takeuchi et al. (2001)
Novosphingobium stygium	Biphenyl, toluene, dibenzothiophene, fluorene	Balkwill et al. (1997); Takeuchi et al. (2001)
"*Sphingomonas paucimobilis*" Q1	Naphthalene, biphenyl, xylene, toluene	Furukawa et al. (1983)
Sphingomonas sp. LB126	Fluorene	Wattiau et al. (2001)
Sphingomonas paucimobilis 2,322	Phenanthrene	Dutta et al. (1998)
Sphingomonas sp. strain ZL5	Phenanthrene	Liu et al. (2004)
Sphingomonas sp. P2	Phenanthrene	Pinyakong et al. (2000)
Sphingomonas sp. ASU1	2,6-Dimethylnaphthalene	Bramucci et al. (2002)
Sphingomonas sp. strain JS5	2-Methylphenanthrene	Sabaté et al. (1999)
Sphingomonas sp.	S3,6-Dimethylphenanthrene	Sabaté et al. (2003)
Sphingomonas sp. A4	Acenaphthene, acenaphthylene	Pinyakong et al. (2004); Kouzuma et al. (2006)
Sphingomonas sp. CHY-1	Chrysene	Willison (2004)
Sphingopyxis macrogoltabidus TFA	Tetralin (1,2,3,4-tetrahydronaphthalene)	Hernáez et al. (1999)
Sphingomonas sp. KA1	Carbazole	Habe et al. (2002)
Sphingomonas sp. GTIN11	Carbazole	Kilbane et al. (2002)
Sphingomonas sp. CB3	Carbazole	Shepherd and Lloyd-Jones (1998)
Sphingomonas sp. Lep 1	4-Methylquinoline	Pfaller et al. (1999)
"*Sphingomonas paucimobilis*" SYK-6	2,2′-Dihydroxy-3,3′- dimethoxy-5,5′-dicarboxy biphenyl	Masai et al. (2007)
Sphingomonas paucimobilis TMY1009	trans-4-Hydroxystilbene	Kamoda et al. (2003)
Sphingobium herbicidovorans MH^T	2-(2,4-Dichlorophenoxy) propionic acid (dichlorprop), 2,4-dichlorophenoxyacetic acid	Zipper et al. (1996)
Sphingomonas sp. HV3	2,4-Dichlorophenoxyacetic acid (2,4D), 2-methyl-4-chlorophenoxyacetic acid (MCPA)	Yrjälä et al. (1998)
Sphingomonas sp. TFD44	2,4-Dichlorophenoxyacetic acid (2,4D)	Fulthorpe et al. (1995)
Sphingobium chlorophenolica	Pentachlorophenol, 2,4,6-trichlorophenol	Nohynek et al. (1995)
Novosphingobium subarcticum KF1^T	2,3,4,6-Tetrachlorophenol, 2,4,6-trichlorophenol	Nohynek et al. (1996); Takeuchi et al. (2001)
Novosphingobium sp. K39	2,3,4,6-Tetrachlorophenol, 2,4,6-trichlorophenol	Männistö et al. (1999)
Sphingomonas wittichii RW1	(Chlorinated) Dibenzo-p-dioxin(s) and dibenzofuran(s)	Wittich et al. (1992); Yabuuchi et al. (2001)
Sphingomonas sp. HH69	(Acetoxy-, hydroxy) Dibenzofuran(s)	Harms et al. (1995)
Sphingomonas sp. SS3	(4-Chloro-, 4-fluoro-)Diphenylether	Schmidt et al. (1992b)
Sphingobium xenophaga BN6	(Substituted) naphthalene-2-sulfonate(s) (2-NS)	Stolz et al. (2000); Pal et al. (2006)
Sphingobium xenophaga QYY	Bromamine (1-amino-4-bromoanthraquinone-2-sulfonic acid)	Qu et al. (2006)

Table 25.8 (continued)

Strains	Degraded compounds	Reference
Sphingomonas subarctica SA1	Sulfanilic acid	Perei et al. (2001); Magony et al. (2007)
Sphingobium japonicum (*Sphingomonas paucimobilis*) UT26^T	γ-Hexachlorocyclohexane (lindane)	Pal et al. (2005); Nagata et al. (2007)
Sphingobium indicum (*Sphingomonas paucimobilis*) B90^T	γ-Hexachlorocyclohexane (lindane)	Kumari et al. (2002)
Sphingomonas sp. CF06	Carbofuran	Feng et al. (1997a, b)
Sphingomonas sp. SB5	Carbofuran	Kim et al. (2004)
Sphingomonas sp. SRS2	Isoproturon [3-(4-isopropylphenyl)-1, 1,-dimethylurea]	Sørensen et al. (2001); Bending et al. (2003)
Sphingomonas sp. TTNP3	Branched nonylphenols	Tanghe et al. (1999)
Sphingobium xenophaga Bayram	Branched nonylphenols	Gabriel et al. (2005)
Sphingomonas cloacae S-3	Nonylphenol	Fujii et al. (2001, 2003b)
Novosphingobium tardaugens ARI-1	β-Estradiol	Fujii et al. (2003a)
Sphingopyxis macrogoltabidus	Polyethylene glycol 4,000	Takeuchi et al. (1993)
Sphingopyxis terrae	Polyethylene glycol 6,000	Takeuchi et al. (1993)

Adapted from Stolz (2009)

soils contaminated with wood-preserving chlorophenols and was intensively studied because it is one of the most effective bacteria capable of a complete degradation pathway of pentachlorphenols (PCP) (Saber and Crawford 1985). The initial step of PCP mineralization is an oxidative dechlorination of PCP to tetrachlorop-benzoquinone (TeCB) by a 4-monooxygenase (PcpB). In the following step, the intermediate product TeCB is reduced by a tetrachlorobenzoquinone reductase (PcpD) to tetrachloro-p-hydroquinone (TeCH), which is further converted to 2,3,6-trichloro-p-hydroquinone (TCH) and 2,6-dichloro-p-hydroquinone (DiCH) by reductive dehalogenase (PcpC) and reductive dechlorination. A ring cleavage of DiCH by a 1,2-dioxygenase (PcpA) leads to the formation of 2-chloromaleylacetate (2-CMA), which is subsequently converted to succinyl-CoA and acetyl-CoA by a reductive dechlorination via a maleylacetate reductase (PcpE). Succinyl-CoA and acetyl-CoA can then be further mineralized through the Kreb's cycle (Pathway description: Orser and Lange 1994; Chanama and Chanama 2011).

Sandarakinobacter Yurkov et al (1997)

San.da.ra.ci.no.bac'ter. Gr. adj. sandaracinos, orange-colored; Gr. n. bacter, rod; M.L. masc. n. *sandaracinobacter*, orange-colored rod.

The genus contains only one species, *Sandaracinobacter sibiricus* (formerly "*Erythromonas sibricius*," formerly "*Erythrobacter sibiricum*"). The type strain was originally isolated as the first obligately aerobic Bchl *a*-containing freshwater bacterium from thin microbial mats that were formed near underwater hydrothermal vents in a river (Shiba and Simidu 1982).

Cells are Gram-negative, nonsporulating, and rod-shaped. Rods are thin and long and can form chains. Cells are motile by means of subpolar flagella (up to three). Cell size is in the range of 0.3–0.5 × 1.5–2.5 μm or longer. Cells divide by binary vision. Colonies are intensively yellow-orange pigmented. Carotenoid and Bchl *a* are produced. In vivo absorption spectra show absorption peaks with λmax at 424, 450, and 474 nm for carotenoids. The photosynthetic apparatus consists of a RC with a tightly bound tetraheme cytochrome c (cyt c) and a LH-I complex. The RC and LH-I are located in the cytoplasmatic membranes and showed absorption peaks with λmax at 750, 799, and 857 nm and λmax at 867 nm, respectively. The tetraheme cyt c is characterized by a molecular weight of 44.0 kDa and represents an immediate electron donor. Additionally, a soluble cyt c of 14.0 kDa and a membrane-bound cyt c of 30.0 kDa (cyt of bc_1 complex) are present (Yurkov et al. 1997). Storage compounds are polyphosphates, polysaccharide, and poly-β-hydroxybutyrate.

Cells have aerobic chemoorganotrophic and facultative photoheterotrophic metabolisms. They cannot grow anaerobically in the light. Cells are oxidase-positive and catalase-negative. A ribulose-bisphosphate carboxylase is not detectable. Fermentation or denitrification activities were not detected.

The optimal temperature for growth is 25–30 °C. Growth is strongly inhibited in the presence of 1.0 % (w/v) NaCl (salt-sensitive). The optimum pH is between 7.5 and 8.5. *Sandaracinobacter sibiricus* grows best on butyrate, sucrose, casein hydrolysate, and yeast extract; well on acetate and maltose; and shows weak growth on media containing glucose, fructose, pyruvate, propionate, or glycerol. Ribose, sorbitol, benzoate, fumarate, formate, succinate, citrate, malate, methanol, and ethanol are not utilized. Tween 60 is hydrolyzed but not gelatin or starch. Morphological and physiological characteristics differentiating *Sandaracinobacter sibiricus* and *Sandarakinorhabdus limnophila* are depicted in *Table 25.9*. *Sandaracinobacter sibiricus* is

Table 25.9

Differentiation of physiological characteristics of the genera *Sandaracinobacter* and *Sandarakinorhabdus*

Characteristics	*Sandaracinobacter sibiricus* RB16-17^T	*Sandarakinorhabdus limnophila* So47^T
Environment	Benthic	Planktonic
Capsule formation	+	–
Motility	+	–
Color	Yellow–orange	Orange–red
Cytochrome oxidase	–	+
λmax carotenoids (nm)	424, 450, 474	420, 460, 480
Carotenoid composition	nd	Nostoxanthin, 2 unidentified carotenoids (strain dependent: keto-nostoxanthin)
Catalase	–	+
Metabolism	Strictly aerobic (facultative photoheterotroph)	Strictly aerobic
Assimilation of:		
Maltose	+	–
Fructose	+	–
Acetate	+	+/–
Propionate	+	–
Pyruvate	+	–
Butyrate	–	–
Succinate	–	+/–
Lactate	–	–/+
Fumarate	–	–/+
Methanol	–	–/+

Data from Yurkov and Gorlenko (1990) for *Sandaracinobacter sibiricus* and Gich and Overmann (2006) for *Sandarakinorhabdus limnophila*. *nd* not determined. All strains were isolated from freshwater environments. No information on enzymic activities (other than those involved in glycolysis and the tricarboxylic acid cycle)

resistant to chloramphenicol, fusidic acid, streptomycin, amikacin, bacitracin, kanamycin, neomycin, and novobiocin. It is sensitive to penicillin, ampicillin, tetracycline, polymyxin B, erythromycin, nalidixic acid, lincomycin, mycostatin, aureomycin, and vancomycin. A high level of tellurite resistance occurs depending on the carbon source in the medium. Up to 1,200 pg mL^{-1} tellurite can be tolerated in a medium containing acetate or yeast extract as a sole organic source. Tellurite is reduced and transformed into metallic tellurium, which is accumulated inside the cells.

Ubiquinone Q-9 and Q-10 are the major respiratory quinones. Enaquinones are not present (Gogotov and Gorlenko 1995). The DNA base composition is 68.5 mol% G+C (determined by thermal denaturation). The habitat is freshwater algobacterial mats near hydrothermal sulfide-containing vents along a river bottom.

Sandarakinorhabdus Gich and Overmann 2006

San.da.ra´ki.no.rhab´dus. Gr. adj. *sandarakinos* of orange colour; Gr. fem. n. *rhabdos* rod; N.L. fem. n. *Sandarakinorhabdus* orange-coloured rod.

The genus *Sandarakinorhabdus* contains only one species, *Sandarakinorhabdus limnophila*—a phototrophic, Bchl *a* containing planktonic freshwater species. The type strain so42^T and two further strains of *Sandarakinorhabdus limnophila* were isolated from a mesotrophic freshwater lake, Lake Starnberger See in Bavaria, Germany (Gich and Overmann 2006).

Cells are Gram-negative, non-spore-forming, rod-shaped, and nonmotile. They grow as single cells. Capsules are not present. Cell division occurs by binary fission. Colonies are orange–red-pigmented with smooth surfaces; they form a mucilaginous texture on agar plates and have a diameter of 1–3 mm. Cells absorb at 430–490 nm (λmax at 420, 460, and 480 nm) and at 800–865 nm (λmax at 800, 837 and 865 nm), indicating the presence of carotenoids and BChl *a*P, respectively. Nostoxanthin was identified as the main carotenoid. Two unidentified carotenoids containing keto groups are also present. In some strains, keto-nostoxanthin was detected as an additional carotenoid.

Absorption maxima at 800 and 865 nm and a shoulder at 837 nm indicated the presence of two photosynthetic complexes—the photosynthetic, light-harvesting complexes LH-I (865 nm) and LH-II (800–837 nm, B800–830 type LH-II). Cells can contain polyphosphate granules and exhibit strong enzymatic activity for acid and alkaline phosphatases. They are cytochrome-oxidase negative and catalase positive. Strains are obligatory

aerobic and chemoorganoheterotrophic. Fermentation or anaerobic growth with nitrate or sulfate was not detected. Strains are facultative oligotrophs that grow in the presence of a number of short-chain organic acids and amino acids as electron donors and carbon sources. Cells grow well in media containing 0.1 % peptone or yeast extract.

The predominant respiratory lipoquinone is ubiquinone Q-10; minor amount of ubiquinone Q-9 was also detected. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, glycolipids, and two sphingoglycolipids (SGL-1 and SGL-2). Polyamines were not analyzed. The predominant cellular fatty acids are C16:1 ω7c and C18:1 ω7c, followed by C16:0. The most abundant 2-hydroxy fatty acid is iso-2-OH C14:0; minor to trace amounts of 2-OH C15:0 and 2-OH C16:0 and trace amount of a 3-hydroxy fatty acid iso-3-OH C16:0 were also detected. The G+C content of the genomic DNA is 64.3 mol% (determined by high-performance liquid chromatography).

Sphingocinicella Maruyama, Park, Ozawa, Tanaka, Sumino, Hamana, Hiraishi and Kato 2006, Emend Geueke et al. 2007; Yasir et al. 2010

Sphin.go.si´ni.cel´la. N.L. n. *sphingosinum* sphingosine; L. fem. n. cella a store-room and in biology a cell; N.L. fem. n. *Sphingosinicella* sphingosine-containing cell.

The genus *Sphingosinicella* was proposed by Maruyama et al. (2006) and comprised four species at the time of writing (July 2013): *Sphingosinicella microcystinivorans* (Maruyama et al. 2006), *Sphingosinicella xenopeptidilytica* (Geueke et al. 2007), *Sphingosinicella soli* (Yoon et al. 2008), and *Sphingosinicella vermicomposti* (Yasir et al. 2010).

Cells are Gram-negative, non-spore-forming, and rod-shaped. Cell size is the range of 0.3–0.8 × 0.6–4.0 μm. Cells are motile by means of polar flagella. Colonies are yellow or pale-yellow, circular, convex, smooth, sticky, or glistening with a diameter between 0.5 and 1.0 mm (either investigated on tryptone soy agar [TSA] or half-strength R2A). Acetone/methanol-soluble pigments are characterized by λmax 448–449 and 476 nm (*Sphingosinicella xenopeptidilytica*, Geueke et al. 2007). Strains are strictly aerobic and chemoorganotrophic. All strains are oxidase positive. They are variable with respect to catalase activity, nitrate reduction, and aesculin hydrolysis.

Growth conditions shared by all members of the genus are growth at temperatures between 10 °C and 37 °C with an optimal growth temperature around 30 °C; no growth is obtained at 44 °C. Growth occurs at pH values between 6.0 and 9.5, but not above pH 11. The optimal pH for growth was between 7.5 and 8.0. All strains grow with 0–1 % (w/v) NaCl, but not in the presence of >2.5 % (w/v) NaCl. Specific metabolic features are the ability to degradation microcystin (*Sphingsinicella microcystinivorans*, Maruyama et al. 2006) or beta-peptides, H-βhVal-βhAla-βhLeu-OH, or H-βhAla-βhLeu-OH, which is shared by strains of the species *Sphingosinicella microcystinivorans* and *Sphingosinicella xenopeptidilytica* (Geueke et al. 2007). The ability of beta-peptide degradation is associated with beta-peptidyl aminopeptidase activity, which is variable among species (Yasir et al. 2010). Species-differentiating physiological characters are summarized in *Table 25.10*.

The predominant respiratory quinone is ubiquinone Q-10; minor amounts of Q-8 and Q-9 can be present. The major polyamine is *sym*-homospermidine as determined for the genus *Sphingomonas*. The major fatty acids are C18:1 ω7c and C16:1 ω7c (by gas chromatographical analysis using the Sherlock MIDI software detected as summed feature C16:1 ω7c and/or iso-C15:0 2-OH), and minor amounts of C16:0. The predominating 2-hydroxy fatty acid is 2-OH C14:0, but trace amounts of 2-0H C12:0, 2-0H C15:0, 2-0H C16:0, and 2-0H C16:1 can also occur. 3-Hydroxy fatty acids are absent. Glycosphingolipids are produced. All strains share diphosphatidylglycerol (partially in low amounts), phosphatidylethanolamine, phosphatidylmonomethylethanolamine, and sphingoglycolipid in their polar lipid patterns; differences can be found in the content of unidentified phospholipids and the presence of glycolipids.

The characteristic 16S rRNA gene sequence signature nucleotides are the same as for the genus *Sphingomonas*: 52:359 (C:G), 134 (G), 593 (G), 987:1218 (G:C), and 990:1215 (U:G). The signature nucleotides in the 16S rRNA gene sequence described previously (Geueke et al. 2007) are variable from species to species within the genus *Sphingosinicella* (Yasir et al. 2010). The DNA G+C content ranges between 59.4 mol% and 65.0 mol% (including G+C values for all species). The type species is *Sphingosinicella microcystinivorans* (Maruyama et al. 2006).

Members of the genus *Sphingosinicell*a were isolated from the water of a toxic *Microcystis* blooming lake (Maruyama et al. 2006), an aeration tank of a wastewater treatment plant (Geueke et al. 2007), alkaline soil (Yoon et al. 2008), and vermicompost (Yasir et al. 2010).

Microcystin Degradation The ability to degrade microcystin is shared by several strains of the species ***Sphingosinicella microcystinivorans***. Microcystin is a hepatotoxic substance produced by several cyanobacteria of the genera *Microcystis*, *Anabaena*, *Nostoc*, and *Oscillatoria* (=Planktothrix); it can cause serious diseases in humans and animals (Jochimsen et al. 1998; Kuiper-Goldman et al. 1999).

The species was proposed based on three freshwater strains all capable of microcystin degradation. The type strain Y2^T was isolated from the eutrophic freshwater lake (Lake Suwa, Japan) during a bloom of toxic *Microcystis* (Park et al. 2001); the other two strains (MDB2 and MDB3) were isolated from the Tenryu River in Japan (Maruyama et al. 2006). Detailed characterization of the type strain showed that it is able to use microcystin as a sole carbon source and degrades several types of microcystin: microcystin-RR, –YR, –LR, and its isomer 6(Z)-Adda (Park et al. 2001). All strains contain the gene *mlrA* encoding a microcystinase; the hydrolytic enzyme opens the cyclic peptide of microcystin. MlrA is therefore necessary for the initial steps in microcystin degradation (Saito et al. 2003).

Microcystin degradation and *mlrA* genes were not detected in the other *Sphingosinicella* species (Geueke et al. 2007;

Table 25.10
Physiological characteristics differentiating *Sphingosinicella* species

Characteristics	*Sphingo sinicella microcystinivorans*	*Sphingo sinicella xenopeptidilytica*	*Sphingo sinicella soli*	*Sphingo sinicella vermicomposti*
Nitrate reduction	+	+	–	–
Growth at 2.0 % NaCl	+	+	+	–
Catalase	+	+	+	–
Oxidase	+	+	+	w
Aesculine	–	–	–	+
Detection (by PCR) of:				
mlrA, mlrB	+	–	–	–
puf	+	–	–	–
Assimilation of:				
Tween 80	+	+	–	–
Tween 40	+	+	+	–
ß-Hydroxybutyric acid	+	+	+	–
DL-Lactic acid	+	+	–	–
D-Alanine	–	+	–	+
L-Alaninamide	–	–	–	+
L-Ornithine	–	–	+	–
L-Proline	+	+	–	–
D-Serine	+	+	–	–
Trisodium citrate	–	+	–	–
Enzyme activity (API ZYM)				
Valine arylamidase	–	–	+	+
Cystine arylamidase	–	–	–	+
Trypsin	–	–	–	+
Acid phosphatase	–	+	–	+
N-acetyl-**b**glucosaminidase	+	+	+	–
Susceptibility to antibiotics				
Ampicillin	–	+	–	+
Streptomycin	+	–	+	–
DNA G+C content (mol%)	63.6–63.7[a]	65.0[b]	65.1[c]	59.4

[a]Maruyama et al. (2006); [b]Geueke et al. (2007), [c]Yoon et al. (2008)
Data from Yasir et al. (2010) unless indicated otherwise. + positive, – negative, *w* weak positive, *PCR* polymerase chain reaction

Yoon et al. 2008; Yasir et al. 2010). However, *mlrA* charring sphingomonads were also detected in other genera of the *Sphingomonadaceae*, such as the strains most closely related (>99 % 16S rRNA gene sequence similarity) to the type strain of *Sphingopyxis alaskensis* (Ho et al. 2012) or other *Sphingopyxis* and also *Novosphingobium* type strains (Ho et al. 2007).

***Stakelama* Chen, Zheng, Wang, Yan, Hao, Du and Jiao 2010**
Sta.ke.la´ma. N.L. fem. n. *Stakelama* arbitrary name derived from State Key Laboratory of Marine Environment Science.

The genus *Stakelama* comprises two species, *Stakelama pacifica* (type species; Chen et al. 2010) and *Stakelama sediminis* (Thawng et al. 2013). The type strain of *Stakelama pacifica* JLT832^T was isolated from surface water of the Pacific Ocean and the type strain of *Stakelama sediminis* CJ70^T from a tidal flat sediment in Korea.

Cells are Gram-negative, non-spore-forming, and rod-shaped. They are weakly motile by means of a flagella or non-motile. Colonies are gold–yellow, circular, small (about 1 mm in diameter), smooth or glossy, and opaque. Acetone-soluble pigments are characterized by λmax at 455 and 485 nm (*Stakelema sediminis*). All strains have a strictly aerobic metabolism. Cells are positive for catalase and variable for oxidase activity. Aesculin is hydrolyzed if tested (only for *Stakelama sedimins*).

Strains are negative for the hydrolysis of gelatin and starch and negative for indole production. Growth generally occurs between 5 °C and 20 °C up to 37 °C (optimal growth between 28 °C and 30 °C); at a pH ranges between pH 6 and 9 or pH 5.0 and 10.0 (optimal pH range from pH 6 to 8); and in the presence of 0–5 % (w/v) NaCl (with different optima between 0–1 % and 2 %). Further species-differentiating physiological properties are given in Table 25.11.

The respiratory quinone is ubiquinone Q-10; other ubiquinones were not detected. The predominant polyamine is spermidine; minor amounts of cadaverine and putrescine and trace amounts of spermine and 1,3-diaminopropane are partially present. *sym*-Homospermidine is absent. The major polar lipids are sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and one phospholipid (data from Thawng et al. 2013). The polar lipid profile can either contain only SGL (*Stakelama pacifica*) or SGL1 and 2 (*Stakelama sediminis*). The species can be further differentiated by the presence of specific aminophospholipids. The predominant fatty acids are C18:1 ω7c, 2-OH C14:0, and C16:0. Trace amounts of 2-OH C15:0 are also detected (Thawng et al. 2013). No further 2-hydroxy and no 3-OH fatty acids are present. The DNA G+C content ranges between 61.4 mol% and 66.5 mol%. The type species is *Stakelama pacifica*.

Sphingomicrobium Kämpfer, Arun, Young, Busse, Kassmannhuber, Rosselló-Móra, Geueke, Rekha and Chen 2012

Sphin.go.mi.crob´i.um. N.L. n. *sphingosinum* (from Gr. gen. n. *sphingos* of sphinx, and suff. *-ine*) sphingosine; N.L. pref. *sphingo-* pertaining to sphingosine; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Sphingomicrobium* a sphingosine-containing microbe.

The genus *Sphingomicrobium* contained four species at the time of writing: *Sphingomicrobium lutaoense* (Kämpfer et al. 2012), *Sphingomicrobium astaxanthinifaciens* (Shahina et al. 2013a), *Sphingomicrobium marinum*, and *Sphingomicrobium flavum* (Shahina et al. 2013b).

Cells are Gram-negative, non-spore-forming, and rod-shaped; they are either nonmotile or motile and flagellated. Cell size is in the range of 0.3–1.1 × 1.2–3.1 μm. Colonies are round, with a diameter of approximately 0.5 mm, and light to bright yellowish or orange-reddish colored. The orange-reddish color is specific for the type strain of *Sphingomicrobium astaxanthinifaciens*, which is able to produce high amounts of astaxanthin (40 μg g^{-1} dry weight; Shahina et al. 2013a).

Cells are oxidase and catalase positive. An aerobic metabolism is observed. They grow on MA 2,216 but not on TSA; some species could weakly grow on R2A or nutrient agar (NA). Growth occurs between 20 °C and 40 °C (optimum at 30–37 °C); *Sphingomicrobium flavum* can also grow at 10 °C and *Sphingomicrobium marinum* even at 45 °C (Shahina et al. 2013b). The pH range of growth is pH 6.5–10 (only *Sphingomicrobium flavum* can grow up to pH 9); the growth is optimum between pH 6.5 and 8.5. Salinity-dependent growth is species-dependent between 1–2 % and 1–7 % (w/v) NaCl; all species grow at 1–2 % NaCl, with optimum growth at 1 %. Species-differentiating morphological and physiological characteristics are given in Table 25.12.

The major respiratory quinone is ubiquinone Q-10; minor amounts of Q-9 can also be present. The predominant polyamines are *sym*-homospermidine and (for *Sphingomicrobium marinum*) *sym*-homospermidine and spermidine (Shahina et al. 2013a, b). Minor amounts of spermidine, putrescine, caderverine, and trace amounts of spermine can also be detected. *sym*-Homospermidien, as the main polyamine, is shared with the genera *Sphingomonas* and *Sphingosinicella* but clearly distinguishes the genus from other genera of the *Sphingomonadaceae* (Busse et al. 1999; Takeuchi et al. 2001; Geueke et al. 2007; Kämpfer et al. 2012). The predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, sphingoglycolipid, and three to eight unidentified glycolipids (GL 1–8). Some species also contain aminolipids (*Sphingomicrobium flavum* and *Sphingomicrobium marinum*; Shahina et al. 2013b) or lipids that are not stained with the staining reagents for amino-, phosphate- or glucosyl-groups).

Phosphatidylmonomethylethanolamine and phosphatidyldimethylethanolamine were not detected. The absence of phosphatidylmonomethylethanolamine and phosphatidyldimethylethanolamine was only reported for a few species of the genus *Sphingomonas*, including *Sphingomonas haloaromaticamans* (Wittich et al. 2007) and *Sphingomonas glacialis* (Zhang et al. 2011); it therefore distinguished species of the genus *Sphingomicrobium* from other genera. The predominant fatty acids of *Sphingomicrobium* species are C18:1 ω7c and 2-OH C18:1, which was not obtained by any other *Sphingomonadaceae*. The 2-hydroxy fatty acids 2-OH C14:0, 2-OH C15:0, and 2-OH C16:0 were not detected, in contrast to all other genera of the *Sphingomonadaceae*. No 3-hydroxy fatty acids were detected, as obtained from all other genera of the *Sphingomonadaceae*. DNA G+C content is 63.4–65.2 mol% (only higher for *Sphingomicrobium astaxanthinifaciens*, 70.6 %; Shahina et al. 2013a). The type species is *Sphingomicrobium lutaoense* (Kämpfer et al. 2012).

Habitats include coastal hot springs (*Sphingomicrobium lutaoense*) or seawater of 10 cm depth (*Sphingomicrobium astaxanthinifaciens*, S. *marinum*, and *S. flavum*) in Taiwan.

The type strain of *Sphingomicrobium lutaoense* (Kämpfer et al. 2012) was not able to hydrolyze the b-peptide H-bhVal-bhAla-bhLeu-OH, which clearly distinguished it from *Sphingosinicella xenopeptidilytica* (Geueke et al. 2007).

Sphingorhabdus Jogler et al. 2013

Sphin.go.rhab´dus. N.L. n. *sphingosinum* (from Gr. gen. n. *sphingos* of sphinx and suff. -ine) sphingosine; N.L. pref. *sphingo-* pertaining to sphingosine; Gr. fem. n. *rhabdos* rod; N.L. fem. n. *Sphingorhabdus* a sphingosine-containing rod.

The genus *Sphingorhabdus* is represented by four species (as of July 2013): *Sphingorhabdus planktonica* (type species) and three species that were formerly described as members of the genus *Sphingopyxis*, "*Sphingopyxis marina*", "*Sphingopyxis*

Table 25.11

Differentiation of physiological characteristics for species of the genus *Stakelama*

Characteristics	*Stakelama pacifica*	*Stakelama sediminis*
Motility	+	–
Optimum growth pH	pH 7.0	pH 6.0
Nitrate reduction	–	+
Oxidase	+	+
Valine arylamidase	+	–
Cystine arylamidase	+	–
N-Acetyl-β-glucosaminidase	–	+
β-Galactosidase (PNPG)	+	–
Acid production from:		
D-Galactose	+	–
D-Mannose	+	–
L-Rhamnose	+	–
Methyl α-D-glucopyranoside	+	–
D-Lactose	+	–
Melibiose	+	–
Sucrose	+	–
Melezitose	+	–
Raffinose	+	–
D-Lyxose	+	–
Assimilation of:		
Dextrin	–	+
Glycogen	+	–
Tween 80	–	+
α-D-Lactose	+	–
Melibiose	+	–
L-Rhamnose	+	–
Sucrose	+	–
D-Gluconic acid	+	–
D-Glucuronic acid	–	+
DL-Lactic acid	–	+
Succinic acid	+	–
L-Alanine	–	+
L-Alanyl glycine	–	+
Hydroxy-L-proline	+	–
L-Proline	–	+
DNA G+C content (mol%)	66.0	61.4

Both type strains were positive for β-glucuronidase; negative for acid production of L-xylose, methyl β-D-xylopyranoside, D-fructose, L-sorbose, methyl α-D-mannopyranoside, potassium gluconate and positive for acid production from starch. They were positive for the utilization of D-fructose, D-mannose, methyl β-D-glucoside, turanose, succinic acid monomethyl ester, bromosuccinic acid, L-alaninamide, L-glutamic acid, glycyl L-glutamic acid, and L-serine; and negative for α-ketobutyric acid. Data were from Thawng et al. (2013), except for the G+C content of *Stakelama pacifica*, which was taken from Chen et al. (2010)

litoris" (both Kim et al. 2008), and "*Sphingopyxis flavimaris*" (Yoon and Oh 2005), which were reclassified as *Sphingorhabdus marina*, *Sphingorhabdus litoris*, and *Sphingorhabdus flavimaris* by Jogler et al. (2013). The type strain of the species *Sphingorhabdus planktonica* G1A_585^T was isolated from an alpine oligotrophic freshwater lake (Walchensee, near Kochel in Bavaria, Germany; Jogler et al. 2013). Type strains of the other species were isolated from water samples of marine systems.

Cells are Gram-negative, non-spore-forming, and rod-shaped. Cell size is in the range of 0.7–1.0 × 0.4–2.5 μm. Cells are motile or nonmotile. Colonies are circular and convex with

Table 25.12

Differential phenotypic characteristics for species of the genus *Sphingomicrobium*

Characteristics	*Sphingo microbium lutaoense*	*Sphingo microbium astaxanthinifaciens*	*Sphingo microbium marinum*	*Sphingo microbium flavum*
Colony pigmentation	Light-yellow	Reddish-orange	Light-yellow	Bright-yellow
Growth in NaCl (% w/v) (optimum)	1–7 (3)	1–4 (3)	1–3 (1)	1–2 (1)
Reduction of nitrate to N_2	w	–	–	–
Acetoin production	+	w	w	w
Hydrolysis of:				
Starch	+	–	+	+
L-tyrosine	+	–	+	+
Tween-20	w	–	w	+
Egg yolk	w	w	w	+
Aesculin	–	+	+	–
Assimilation of:				
L-Arabinose	–	+	+	+
D-Mannose	–	+	+	+
Potassium gluconate	+	+	+	–
Adipic acid	+	+	+	–
Malate	+	+	+	–
Trisodium citrate	–	–	+	–
Acid production from:				
Esculin ferric citrate	w	+	+	+
Potassium-5-ketogluconate	w	+	w	+
Enzyme activities				
Lipase	+	–	–	–
Valine arylamidase	w	+	+	+
Cystine arylamidase	–	+	+	+
Trypsin	–	+	+	+
Acid phosphatase	–	+	+	–
α-galactosidase	w	–	–	–
β-galactosidase	w	–	–	–
α-glucosidase	w	–	–	–
β-glucosidase	w	–	–	–
N-acetyl-β-glucosaminidase	–	+	–	–
DNA G+C (mol%)	67.7 (63.4)	70.6	64.2	65.2

+ positive reaction, – negative reaction, *w* weak reaction. All strains are positive for catalase, oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, and naphthol-AS-BI-phosphohydrolase activities; as well as assimilation of D-glucose, D-mannitol, acetyl-glucosamine, D-maltose, and phenylacetic acid. All strains are negative for: D-glucose fermentation; reduction of nitrate to nitrite; DNase, α-chymotrypsin, β-glucuronidase, α-fucosidase, α-mannosidase, o-nitrophenyl-β-D-galactopyranoside, p-nitrophenyl-β-D463 galactopyranoside, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophane deaminase activities; citrate utilization; indole and H_2S production; assimilation of capric acid; oxidation/fermentation of mannitol, inositol, sorbitol, rhamnose, saccharose, melibiose, amygdalin and arabinose; hydrolysis of gelatin, chitin, xylan, CMC, casein and Tween-80; growth on TSA and R2A. Data were taken from Kämpfer et al. (2012) and Shahina et al. (2013a, b)

a diameter of 0.7–1.0 mm; they are yellow to yellowish (marine species) or orange and transparent (*Sphingorhabdus planktonica*). The carotenoid content is characterized by λmax at 427 (shoulder), 453 and 481 nm (peaks; *Sphingorhabdus planktonica*; Jogler et al. 2013). Cells are oxidase and catalase positive. All strains show an aerobic metabolism. Only a broad spectrum of substrates can be utilized.

Growth conditions differ depending on the origin of the type strains. The type strain of the freshwater species (*Sphingorhabdus planktonica*, Jogler et al. 2013) only grows

between 18 °C and 24 °C, but not at 11 °C and 27 °C, with NaCl concentration between 0 % and 2 % (w/v) NaCl. Marine species grow either between 15 °C and 34 °C (opt. 24–31 °C) or between 4 °C and 30 °C, but not at 37 °C. The marine species require sea salt in the medium, at least 2–3 % (w/v), with an optimum content between 2–3 % and 3–5 %; they can partially grow in the presence of up to 7 % sea salt content. Further differentiating morphological and physiological characteristics are given in Table 25.7 for the comparison between the type strains of the genera *Sphingopyxis* and *Parasphingopyxis*.

The major respiratory quinone is ubiquinone Q-10; trace amounts of Q-9 were also detected. The main polyamine is spermidine and (for one species) spermidine and agmatine (*Sphingopyxis marina* DSM 22363^T; Kim et al. 2008). All species share minor to trace amounts of putrescine and spermine, and some species contain also trace amounts of agmatine and cadaverine or 1,3-diaminopropane. Major nonpolar fatty acids are summed feature 8 (C18:1 ω7c and/or C18:1ω6c) and summed feature 3 (C16:1ω7c). The main 2-hydroxy fatty acid is 2-OH C14:0. Polar lipids shared by all species are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and sphingoglycolipids; some species also contain phosphatidylcholine, phosphatidyl monomethylethanolamine, phosphatidyldimethylethanol amine, and glycolipids. The characteristic 16S rRNA gene sequence signature nucleotides are at position 990:1215 (U:A) (Jogler et al. 2013). The G+C content is between 52.6 mol% and 57.8 mol%, including values for type strains of currently proposed *Sphingorhabdus* species. The type species is *Sphingorhabdus planktonica* (Jogler et al. 2013)

The four *Sphingorhabdus* species form a separate phylogenetic branch among members of the *Sphingomonadaceae* (based on the 16S rRNA gene sequence phylogeny) and can be distinguished form the genus *Sphingopyxis* by lower G+C values: 52.6–57.8 mol% for the genus *Sphingorhabdus* compared to 63–69.2 mol% for species remaining in the genus *Sphingopyxis*.

Habitates include surface water of freshwater lakes and marine habitats.

Parasphingopyxis Uchida et al. 2012

Pa.ra.sphin.go.py´xis. Gr. prep. *para* beside, alongside of, near, like; N.L. fem. n. *Sphingopyxis* a bacterial generic name; N.L. fem. n. *Parasphingopyxis* beside *Sphingopyxis*.

The genus *Parasphingopyxis* is currently represented by only one species, *Parasphingopyxis lamellibrachiae*. The type strain of *Parasphingopyxis lamellibrachiae*, strain JAMH 0132^T, was isolated from the trophosome of a tubeworm (*Lamellibrachia satsuma*) of the Kagoshima Bay, Japan. The closest related type strain is *Sphingopyxis baekryungensis* SW-150^T (95.1 % 16S rRNA gene sequence similarity). Sequence similarities to all other type strains of the genera *Blastomonas, Sphingomonas*, *Sphingosinicella*, and *Novosphingobium* are below 95 %.

Cells are Gram-negative, non-spore-forming, and rod-shaped. Cell size is 0.5–0.6 × 1.0–2.0 μm. Cells are motile. Colonies are orange–yellow. Optimal growth occurs at 28–30 °C, pH 6.5–7.5, and a salt concentration of 2 % (w/v) NaCl. Growth generally occurs at 5–36 °C, pH 6.0–9.5, and a salt concentration of 0.5–5 % (w/v) NaCl. No growth occurs in a medium containing less than 0.5 % (w/v) NaCl. Cells are oxidase positive and catalase negative. Aesculin is not hydrolyzed, but nitrate is reduced to nitrite. Differentiating morphological and physiological characteristics are given in Table 25.7 in comparison to type strains of the genera *Sphingopyxis* and *Sphingorhabdus*.

The predominant respiratory quinone is ubiquinone Q-10. Polyamines, including putrescine, spermidine, spermine, *sym*-homospermidine, cadaverine, and agmatine, were not detected. This clearly distinguishes this species/genus from all other members of the *Sphingomonadaceae*. The major fatty acids are C18:1 ω7c, C16:0 and 11-methyl C18:1 ω7c. 2-OH C14:0 is absent. The main 2-hydroxy fatty acid is 2-OH C16:0. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, and sphingoglycolipid. The type species is *Parasphingopyxis lamellibrachiae* (Uchida et al. 2012). The DNA G+C content of the respective type strain JAMH 0132^T is 60.1 mol%.

Zymomonas Kluyvera and Van Niel, 1936 Emend. Coton et al 2006

Gr. n. *zumê*, leaven, ferment; Gr. fem. n. *monas*, a unit, monad; N.L. fem. n. *Zymomonas*, intended to mean fermenting monad.

The genus *Zymomonas* contains only one species, which is represented by three subspecies: *Zymomonas mobilis* subsp. *mobilis* (Lindner 1928b; De Ley and Swings 1976), *Zymomonas mobilis* subsp. *pomaceae* (Millis 1951; De Ley and Swings 1976), and *Zymomonas mobilis* subsp. *francensis* (Coton et al. 2006). The latter subspecies was described in 2006 after a polyphasic analysis of *Zymomonas mobilis* strains isolated from French framboise ciders compared to several strains of the other subspecies, including the type strains.

Cells are Gram-negative, non-spore-forming, and rod-shaped. Rods are straight with rounded or ovoid ends, occasionally ellipsoidal, and single. Cell size measures 2–6 × 1.0–1.4 μm. Rosette-like cell aggregations, cell chains, and curved or U-shaped cells can occur. Usually, cells are nonmotile; some are motile by the means of one to four polar flagella. Motility can occur spontaneously. Membranes contain pentacyclic triterpenoids of the hopane series (Sahm et al. 1993). Intracellular lipids, glycogen, or poly-β-hydroxybutyrate were not obtained. Deep colonies are lenticular, regular, and contain entire edges; they are butyrous, white or cream colored, and have a diameter of 1–2 mm. Anaerobic surface colonies are spreading, contain entire edges, are convex or umbonate, and have a diameter of 1–4 mm (Swings and De Ley 1977). When incubated aerobically, colonies reach a maximum diameter of 1.5 mm or appear as microcolonies (Swings et al. 1997).

Cells are oxidase negative and catalase positive. They are negative for gelatinase activity but postitve for superoxide dismutase activity; negative for nitrate reduction and indole production; and negative for the hydrolysis of gelatin, Tween 60, and Tween 80. *Zymomonas* are facultative anaerobic; some strains are obligatory anaerobic and have a fermentative metabolism. They can grow chemoorganotrophic on glucose and fructose and ferment glucose or fructose to ethanol and CO_2,

as well as some organic acids such as lactic acid. Some strains can also utilize sucrose, but other carbon sources are not used. Gluconate can be degraded but does not serve as sole carbon or energy source (Strohdeicher et al. 1988). Sorbitol and gluconolactone are formed when grown on sucrose or mixtures of glucose and fructose by a so-far unique enzyme, glucose-fructose oxidoreductase.

Optimal growth occurs at 25–30 °C, reduced growth at 38 °C (74 % of the strains can grow), and weak growth at 40 °C (De Ley and Swings 1976; Swings and De Ley 1977). Slow growth occurs at 15 °C and no growth occurs at 4 °C (Millis 1951; Dadds et al. 1973). Growth at 36 °C can be used for phenotypic differentiation of the *Z. mobilis* subspecies, *Z. mobilis* subsp. *mobilis* (positive), *Z. mobilis* subsp. *pomaceae* (negative), and *Z. mobilis* subsp. *francensis* (negative or weak positive) (Swings et al. 1977; Coton et al. 2006). Further phenotypic features for subspecies differentiation is growth in the presence of 0.5 % (w/v) NaCl and 0.2 % bile salts: *Z. mobilis* subsp. *mobilis* (positive), *Z. mobilis* subsp. *pomaceae* and *Z. mobilis* subsp. *francensis* (negative) (Coton et al. 2006).

Zymomonas species are acid tolerant and grow in a pH range of pH 3.5–7.5. Most of *Zymomonas* strains (90 %) are able to grow between pH 3.8 and 7.5. At pH 3.5, at least 43 % of *Zymomonas* strains showed growth (Swings and De Ley 1977). In liquid standard medium, *Zymomonas* cannot grow at pH 3.0. A mixture of amino acids is required for optimal growth conditions; however, a specific single amino acid seemed not to be essential. *Zymomonas* requires biotin and pantothenate for growth.

The tightness of the subspecies is reflected by high DDH similarities (>76 % between *Z. mobilis* subsp. *mobilis* and *Z. mobilis* subsp. *pomaceae*) and high phenotypic similarity (Ssm ≥88 %) between those subspecies (De Ley and Swings 1976). *Zymomonas* subspecies have a very similar G+C content of 47.5–49.5 mol%. In contrast to other *Sphingomonadaceae* (mainly the genus *Sphingomonas*) 2-OH C14:0 is absent in *Zymomonas* species (Yabuuchi et al. 1990; Takeuchi et al. 1993; Balkwill et al. 1997).

Several genotypic features can be used to differentiate *Zymomonas mobilis* subspecies, including partial *gyrB* and *hsp60* and 16-23S rRNA ISR sequences, as well as genomic fingerprinting methods as RAPD-PCR with primer 1 and rep-PCR analysis with BOX and ERIC-PCRs as described in detail by Coton et al. (2006). The type species is *Zymomonas mobilis* (Lindner 1928a; previously "*Thermobacterium mobile*" Lindner 1928b). The genomic DNA G+C content is 47.5–49.5 mol%, which is much lower compared to all other members of the *Sphingomonadaceae*.

The natural habitats of *Zymomonas* include sugar-rich plant saps where *Zymomonas* ferments sugar to ethanol. Natural niches of the genus are acid palm wines, ciders, and beers, which fits well to the acidic tolerance obtained for *Zymomonas*. *Zymomonas* are used as fermenting agents in Agave sap, palm sap, and sugarcane juice; it occurs as a spoiler in beers, ciders, and perries; in honeybees and in opening honey.

Glucose and fructose metabolism *Zymomonas* ferment glucose anaerobically by the Entner-Doudoroff pathway (for references, see Conway 1992), followed by a pyruvate decarboxylation and reduction of acetaldehyde. Dissimilation of glucose under aerobic conditions leads to the formation of ethanol, acetaldehyde, and acetate. *Zymomonas* grow well in liquid media containing either D-glucose or D-fructose at 30 °C. The final pH of the medium reaches 4.8–5.2 by growth at 30 °C; a stronger acidification can be obtained by growth at higher temperatures. Half of the strains grow in the presence of up to 40 % glucose (Swings and De Ley 1977). Sucrose can also be fermented and used for growth by many strains (for references, see Preziosi et al. 1991; Sprenger 1996). However, the property to grow on sucrose can be lost if *Zymomonas* were subcultured on D-glucose (Shimwell 1950). Sucrose fermentation is also inducible (Dadds et al. 1973).

With respect to the industrial applications of *Zymomonas* species, both the glucose and sucrose metabolism of the genus *Zymomonas* were intensively studied but will not be discussed in detail here.

Ethanol Tolerance *Zymomonas* are characterized by a high tolerance to ethanol; strains can grows in the presence of 5 % ethanol, and even at higher concentrations (Swings and De Ley 1977). In batch cultures, up to 13 % (w/v) ethanol can be produced by *Zymomonas* (Rogers et al. 1982). Humanoids, pentacyclic triterpeniods, in the membranes of *Zymomonas* are thought to have a protective function against the produced ethanol. Up to 3 % of the membrane's dry weight or 40–50 % of total lipid content are humanoids (Sahm et al. 1993).

Sphingomonas, *Novosphingobium*, *Sphingopyxis*, and *Sphingobium* species

Isolation, Enrichment, and Maintenance Procedures

Members of the *Sphingomonadaceae* grow on a wide range of complex standard media, most often better in diluted variants. *Sphingomonadaceae* originate from a variety of habitats, including different soil habitats that are contaminated with (poly) aromatic compounds, freshwater and marine ecosystems, habitats associated with plants or eukaryotic hosts, or man-made environments. Based on this broad range of habitats, specifically adapted media are somewhat necessary to enrich and isolate sphingomonads.

Most members of the genera *Sphingomonas*, *Novosphingobium*, *Sphingobium*, and *Sphingopyxis* can be grown on R2A (Difco or Oxoide; containing 0.05 % (w/v) yeast extract, 0.05 % peptone, 0.05 % Casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K_2HPO_4, 0.005 % $MgSO_4$, and 1.5 % agar; pH, 7.2), nutrient agar (NA, Difco), TSA (Difco), 0.3 % PYE agar (containing 0.3 % peptone from casein and 0.3 % yeast extract, pH 7.2), or

peptone yeast extract glucose agar (PYGV; containing 0.025 % w/v of yeast extract and glucose; 5 mL L^{-1} of a vitamin solution, and 1.5 % agar; Staley 1968), Luria–Bertani agar (LB; containing per L: 10 g tryptone, 5 g yeast extract, 5 g NaCl, 1 g D-glucose, and 15 g agar), or HD medium (0.05 % casein peptone, 0.01 % glucose, 0.025 % yeast extract, in w/v). Some strains can be grown on full-strength medium, whereas others need to be grown on 2-fold, 5-fold, or 10-fold diluted variants of the listed complex media.

Species isolated from freshwater habitats are often very salt-sensitive and can only be grown on low-salt media, such as R2A or PYE, or in diluted variants of those. Another medium recommended for routine growth of freshwater *Novosphingobium* species is K7 medium, which contains per liter 1 g peptone, 1 g yeast extract, and 1 g glucose (Glaeser et al. 2009). For pH adjustment, it is recommended to use KCl instead of NaCl to avoid the input of sodium ions, which can inhibit growth.

Species from marine habitats grow well on marine agar 2216 or the respective marine broth (all from Difco); again, 2-, 5-, or 10-fold diluted variants may be necessary. Alternatively, a modified rich organic (RO) medium can be used (Yurkov et al. 1999). The RO medium contains per liter distilled water 1 g peptone, 1 g yeast extract, 1 g NaCl, 1 g sodium acetate, 0.3 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.05 g $CaCl_2 \cdot 7H_2O$, 0.3 g NH_4Cl, 0.3 g K_2HPO_4, 20 µg vitamin B12, and 1.0 mL element solution, and is adjusted to pH 8.0 (Chen et al. 2010). Most of the strains can grow at a pH around pH 7.0. Some strains that originate from acidic environments need a lower pH for growth. Incubation conditions that cover growth of most of the strains are 25–28 °C in the dark.

Many species originally isolated from soil or freshwater habitats as well as several other habitats, including human-impacted anthropogenic habitats, can be isolated by common serial dilution plating on R2A, NA, LB, TSA, or diluted variants of those media, and 1 % PTYG medium. Most often, incubation temperatures for primary isolation were in the range of 25–30 °C. Several strains that were isolated on diluted standard media could later be grown on undiluted media. Some strains were isolated on media supplemented with antibiotics. *Sphingomonas glacialis* was isolated on R2A supplemented with 100 µg mL^{-1} cycloheximide (Zhang et al. 2011). *Sphingomonas histidinilytica*, *Sphingopyxis ummariensis*, *Novosphingobium lindaniclasticum*, and *Sphingobium quisquiliarum* were isolated on LB agar amended with nystatin (30 µg mL^{-1}) and streptomycin (200 µg mL^{-1}) (Nigam et al. 2010; Sharma et al. 2010; Bala et al. 2010; Saxena et al. 2013). *Sphingopyxis witflariensis* was isolated on PTYG agar (Balkwill and Ghiorse 1985) containing 16 mg L^{-1} aztreonam (Kämpfer et al. 2002b). *Novosphingobium mathurense* and *Novosphingobium panipatense* were isolated on a minimal salt medium (MSM; Mohn et al. 2006; Vanbroekhoven et al. 2004) that was supplemented with 200 µg mL^{-1} streptomycin (Gupta et al. 2009). *Sphingomonas endophytica* was isolated on sodium propionate-asparagine agar (pH 7.2; Qin et al. 2009) contained nalidixic acid (25 mg L^{-1}), nystatin (50 mg L^{-1}), and cycloheximide (50 mg L^{-1}) (Huang et al. 2012).

Many freshwater species (*Sphingomonas starnbergensis*, *Sphingomonas leidyii*, *Novosphingobium acidiphilum*, *Sphingobium boeckii*, *Sphingobium limneticum*, and *Sphingorhabdus planktonica*) can be enriched using a basic synthetic freshwater medium (Bartscht et al. 1999). The basic medium is composed of 10 mM NH_4Cl, 10 mM KH_2PO_4, 100 mM KNO_3, 200 mM $MgSO_4 \cdot 7H_2O$, 100 mM $CaCl_2 \cdot 2H_2O$, 250 mM $CaCO_3$, 1 ml L^{-1} of SL 10 trace elements (Tschech and Pfennig 1984), and 10 ml L^{-1} vitamin solution (Balch et al. 1979) buffered with 10 mM HEPES (Bartscht et al. 1999) and supplemented with 20 amino acids, glucose, pyruvate, citrate, 2-oxoglutarate, and succinate (200 µM each), Tween 80 (0.001 % v/v), formate, acetate, and propionate (200 µM each) (Jaspers et al. 2001), For the enrichment of *Sphingomonas starnbergensis* (Chen et al. 2012b) and *Sphingorhabdus planktonica* (Jogler et al. 2013) signal molecules (cAMP, N-butyryl homoserine lactone, N-oxohexanoyl-di-homoserine lactone and ATP) were added at a 10 µM final concentration to stimulate growth (Bruns et al. 2002). For the isolation and purification of freshwater species on agar plates, it is recommended to used prewashed agar (12 g L^{-1}; washed three times with distilled water). The recommended agar media for isolation and cultivation are the basic synthetic freshwater medium, 1:10 diluted HD medium (0.05 % casein peptone, 0.01 % glucose, 0.025 % yeast extract, w/v), or YPG agar (0.075 % (w/v) yeast extract, 0.15 % glucose, and 0.075 %, 1.2 % pre-washed agar) supplemented with 5 % (v/v) 0.22-µm filter-sterilized or autoclaved habitat water. Several freshwater species were enriched using a dilution-to-extinction cultivation (Button 1993) in 96-well plates (Glaeser et al. 2009, 2013; Baek et al. 2010, 2011). *Novosphingobium sediminicola* and *Sphingobium vulgare* were enriched by dilution-to-extinction cultivation in unsupplemented lake water and were then purified on 10-fold diluted R2A (Baek et al. 2010, 2011). For dilution-to-extinction cultivation of freshwater, *Novosphingobium* species synthetic freshwater medium as described above or autoclaved lake water supplemented with 2.5 % (v/v) autoclaved YPG concentrate (3 % yeast extract, 6 % peptone, and 3 % glucose, adjusted to pH 6.5) can be used (Glaeser et al. 2009, 2013).

Marine *Sphingomonas* and *Sphingopyxis* species can be isolated on marine agar 2,216 (Difco, USA; e.g. *Sphingopyxis baekryungensis*; Yoon et al. 2005) or the half-strength variant with 1 % (w/v) additionally applied NaCl (*Sphingomonas jejuensis*, Park et al. 2011), or it can be pre-enriched in 0.22-µm filtered-autoclaved seawater (FAS) or synthetic seawater medium containing less than approximately 1 mg carbon per liter (*Sphingopyxis alaskensis*; Vancanneyt et al. 2001). *Sphingomonas molluscorum* was isolated on seawater medium containing (per liter) 5 g peptone, 2.5 g yeast extract, 1 g glucose, 0.2 g K_2HPO_4, 0.05 g $MgSO_4$, and 15 g agar in 750 ml seawater/250 ml distilled water (Romanenko et al. 2007).

Sphingomonas mucosissima and *Sphingomonas desiccabilis* originate from biological soil crusts and were isolated on BG11-PGY medium containing 10 % BG-11 mineral medium, 0.25 % peptone, 0.25 % yeast extract, 0.25 % glucose, and 15 %

agar (Reddy and Garcia-Pichel 2007). The BG-11 base is composed of $NaNO_3$ (1.5 g L^{-1}) $K_2HPO_4 \cdot 3H_2O$ (40 mg L^{-1}), $MgSO_4 \cdot 7H_2O$ (75 mg L^{-1}), $CaCl_2 \cdot 2H_2O$ (36 mg L^{-1}), citric acid (6 mg L^{-1}), ferric ammonium citrate (6 mg L^{-1}), EDTA (disodium magnesium) (1 mg L^{-1}), Na_2CO_3 (20 mg L^{-1}), and 1 mL L^{-1} trace metal solution, pH 7.4 (Rippka et al. 1979).

Sphingobium species inhabiting contaminated soils or dump sites can be isolated on a mineral salt medium supplemented with the respective contaminants. For example, S*phingobium abikonense*, *Sphingobium lactosutens*, and *Sphingobium ummariense* were isolated on MSM medium containing $(NH_4)_2HPO_4$ (10.5 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.2 g L^{-1}), $FeSO_4 \cdot 7H2O$ (0.01 g L^{-1}), K_2HPO_4 (0.1 g L^{-1}), and $Ca(NO_3)_2$ (0.01 g L^{-1}) supplemented with 5 µg mL^{-1} HCH (Kumari et al. 2009; Singh and Lal 2009). *Sphingopyxis chilensis* was enriched from contaminated river sediment by the incubation of 20 % river water in chloride-free minimal saline medium supplemented with 2,4,6-dichlorophenol (TCP) (Godoy et al. 1999, 2003); *Novosphingobium naphthalenivorans* was enriched in dibenzofuran-containing liquid medium and isolated on dibenzofuran-coated agar plates (Futamata et al. 2004; Kubota et al. 2005) and *Novosphingobium pentaromativorans* in MM2 broth containing (per liter of aged sea water, pH 7.2; ZoBell 1946) 18 mM $(NH_4)_2SO_4$, 1 µM $FeSO_4 \cdot 7H_2O$, and 100 µL 1 M KH_2PO_4/Na_2HPO_4 buffer solution, supplemented with 1,000 p. p.m. of a final mixture of PAHs consisting of equal quantities of naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene (Sohn et al. 2004).

Strains can be stored lyophilized for years or can be preserved at − 80 °C after suspension in standard liquid medium supplemented with 7 % DSMO or 15–20 % glycerol after shock freezing in liquid nitrogen.

Blastomonas

The type strain of *Blastomonas natatoria* was isolated from a swimming pool using a Millipore *Pseudomonas* Count Water Tester (Sly and Hargreaves 1984). *Blastomonas* can be grown on diluted media, such as Staley's peptone yeast glucose agar, on which *Blastomonas* form pink colonies or on nutrient or R2A, on which *Blastomonas natatoria* form yellow colonies (Sly 1985). Recommended media for the cultivation of *Blastomonas* are DSM medium 364 (PYEA, containing per L 10 g peptone, 10 g yeast extract, 5 g NaCl, and 15 g agar; adjusted to pH 7.2), 535 (trypticase soy broth; 30 g L^{-1}), 1,015 (YPGA; yeast extract, peptone, glucose each 7 g L^{-1}). The recommended incubation temperature is 28 °C (http://www.straininfo.net/strains/36294/browser).

Cultures of *Blastomonas* can be preserved for long-term storage at − 80 °C in cryo-cultures containing liquid cultures grown in sucrose peptone broth, mixed with 10 % glycerol, and shock frozen in liquid nitrogen. Media for *Blastomonas ursincola* require a supplementation with 20 µg mL^{-1} vitamin B12 (Yurkov et al. 1997).

Sandaracinobacter

Sandaracinobacter sibiricus was isolated from a thin microbial mat, which was formed around underwater hydrothermal vents on a river bottom (Yurkov and Gorlenko 1990). *Sandaracinobacter sibiricus* was isolated by direct inoculation of homogenized diluted mat samples on agar plates of rich organic (RO) medium (Yurkov et al. 1994) containing yeast extract (1 g L^{-1}), Bacto peptone (1 g L^{-1}), sodium acetate (1 g L^{-1}), KCl (0.3 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), $CaCl \cdot 2H_20$ (0.05 g L^{-1}), NH_4CL (0.3 g L^{-1}), and K_2HPO_4 (0.3 g L^{-1}) supplemented with 20 mg L^{-1} vitamin B12 and 1.0 mL L^{-1} trace elements. Incubation was at 30 °C and pH 7.6–7.8.

Sandaracinobacter sibiricus in liquid cultures taken from late logarithmic growth phase and agar surface cultures remain viable after storage at 4 °C for at least 2 months. For long-term storage, cultures need to be frozen in liquid nitrogen and stored at − 70 °C after 30 % glycerol is added to a culture of mid-logarithmic growth phase. The species can also be preserved lyophilized.

Sandarakinorhabdus

The genus *Sandarakinorhabdus* is only represented by one freshwater species, *Sandarakinorhabdus limnophila*. The species was isolated as described for several other freshwater *Sphingomonadaceae* in synthetic freshwater medium buffered with 10 mM HEPES and supplemented with formate, acetate, propionate, succinate, citrate, α-ketoglutarate, and pyruvate (200 µM each; all sodium salts); an amino acid mixture containing all 20 amino acids (200 µM each); 0.001 % Tween 80, and 40 µM glucose (Jaspers et al. 2001). Dilution-to-extinction cultivation (Bartscht et al. 1999) or application of the MicroDrop cultivation technique may be required (Bruns et al. 2003) to enrich *Sandarakinorhabdus* species. Purification can be done on plates containing synthetic freshwater medium and a 10-vitamin mixture (Bartscht et al. 1999) and 1 % (w/v) peptone. For routine cultivation, half-strength R2A (=DSM medium 830; http://www.dsmz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html) and incubation at 15 °C are recommended. Strains can be at maintained at − 80 °C resuspendend in growth medium containing 50 % glycerol or 7 % DMSO for at least 7 months.

Sphingorhabdus

The genus *Sphingorhabdus* contains species that originate either from freshwater or from marine habitats. The freshwater species, *Sphingorhabdus planktonica* (represented by one strain) (Jogler et al. 2013), can be pre-enriched as described for other freshwater sphingomonads in basic synthetic freshwater medium buffered with 10x HEPES and supplemented with formate, acetate,

propionate, succinate, citrate, α-ketoglutarate, and pyruvate (200 µM each; all sodium salts); an amino acid mixture containing all 20 amino acids (200 µM each); 0.001 % Tween 80, and 40 µM glucose (Jaspers et al. 2001). Additional supplementation with 10 µM of the inducers cAMP, N-butyryl homoserine lactone, and N-oxohexanoyl-DL-homoserine lactone (Bruns et al. 2002) may be necessary. Subsequent isolation can be done on agar (1 %, w/v) containing artificial freshwater, 1:10 diluted HD medium (0.05 % casein peptone, 0.01 % glucose, 0.025 % yeast extract; w/v), and 10 mM HEPES buffer (pH 7). Incubation temperature is 20 °C. For cultivation, it is recommended to used freshwater HD medium adjusted to pH 8.3 (=DSM medium 1312; http://www.dsmz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html) at 20 °C. It is required to keep the agar surface moist; an incubation time of 10–15 days is needed. The species cannot grow on R2A, NA, or CASO agar (Jogler et al. 2012).

The marine *Sphingorhabdus* species (*Sphingorhabdus marina*, *Sphingorhabdus litoris*, and *Sphingorhabdus flavimaris*) were isolated on marine agar 2216 (MA; Difco) incubated at 30 °C. The same medium and growth conditions are recommended to maintain those strains. For long-term preservation, MA cultures suspended with 20 % (w/v) glycerol can be stored at − 80 °C.

Sphingosinicella

Most of the *Sphingosinicella species* were isolated on NA or diluted NA (Yoon et al. 2008; Yasir et al. 2010; Maruyama et al. 2006). Pre-enrichments may be necessary; *Sphingosinicella vermicomposti* was pre-enriched in nutrient broth of 0.08 g L^{-1} (Difco) supplemented with 20 % (w/v) 0.2-µm filter-sterilized aqueous extract of vermicompost (Yasir et al. 2010). *Sphingosinicella xenopeptidilytica* can be pre-enriched in mineral salt medium supplemented with β-peptides as carbon source (Schreiber et al. 2002; Geueke et al. 2005, 2007). Beside nutrient agar (NA), *Sphingosinicella* species can grow on several complex media including R2A or the half-strength variant and TSA. Pre-enrichments and subcultivations can be done at 30 °C.

Stakelama

Species of the genus *Stakelama* were isolated from different environments and therefore different isolation conditions are required. The marine species *Stakelama pacifica* can be isolated on seawater medium (SW) containing, per liter of 0.22 µm-filtered seawater, 1 g peptone, 1 g yeast extract, 20 µg vitamin B12, and 1 ml of a trace element solution (ES) and cultured on modified rich organic (RO) medium (Yurkov et al. 1999) containing, per liter of distilled water, 1 g peptone, 1 g yeast extract, 20 g NaCl, 1 g sodium acetate, 0.3 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.05 g $CaCl_2 \cdot 7H_2O$, 0.3 g NH_4Cl, 0.3 g K_2HPO_4, 20 µg vitamin B12, and 1.0 ml ES, adjusted to pH 8.0 at 28 °C (Chen et al. 2010). The same conditions are recommended for cultivation of this species.

The second species, *Stakelama sediminis*, can be isolated and routinely grown on R2A at 30 °C (Thawng et al. 2013). Cultivation on LB and nutrient agar is also possible, but not on TSA. The species can be maintained at − 80 °C as a suspension in R2A broth supplemented with 20 % (v/v) glycerol.

Sphingomicrobium

Sphingomicrobium species were isolated from coastal or marine surface waters using marine agar 2,216 (MA; Difco) at temperatures between 30 °C and 45 °C (Kämpfer et al. 2012; Shahina et al. 2013a, b). The same media and growth conditions are also recommended for subsequent cultivation. Furthermore, it is recommended to grow the species on modified MA medium (=DSM medium 514a; http://www.dsmz.de/catalogues/-catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html) at 28 °C; the addition of a vitamin solution can thereby accelerate growth. Strains can be preserved lyophilized or by the addition of 20 % glycerol to fresh cultures, shock freezing in liquid nitrogen, and storage at − 80 °C.

Parasphingopyxis

The type strain of *Parasphingopyxis lamellibrachiae* (single strain of this genus) was isolated from the *Trophosome* tissue of a marine annelid tubeworm (*Lamellibrachia satsuma*). The tissue was homogenized in normal-strength artificial seawater (ASW) containing NaCl (27.5 g L^{-1}), KCl (0.7 g L^{-1}), $MgCl_2 \cdot 6H_2O$ (5.4 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (6.8 g L^{-1}), $CaCl_2 \cdot 2H_2O$ (1.4 g L^{-1}), and $NaHCO_3$ (0.2 g L^{-1}) (Miyazaki et al. 2008) and spread on marine agar 2,216 (MA; Difco) diluted with 10-fold 1× ASW and supplemented with 1.2 % (w/v) agar and incubated at 20 °C. For cultivation of *Parasphingopyxis lamellibrachiae*, marine agar 2,216 or the respective broth and incubation at 20 °C are recommended.

Zymomonas

The growth medium recommended for *Zymomonas* species is the *Zymomonas* DSM medium 10 (http://www.dsmz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html) containing in Bacto peptone (10.0 g L^{-1}), yeast extract (10.0 g L^{-1}), glucose (20.0 g L^{-1}), and, if required, agar (15 g L^{-1}). A medium for *Zymomonas* enrichment and detection in breweries is the *Zymomonas*

detection medium described by Dadds (1971), containing malt extract (3 g L^{-1}), yeast extract (3 g L^{-1}), D-glucose (20 g L^{-1}), peptone (5 g L^{-1}), and actidione (0.02 g L^{-1}) in distilled water. The pH should be adjusted to 4.0. Ethanol should be added in a final concentration of 3 % (v/v). An enrichment at 30 °C is recommended. The enrichment of *Zymomonas* is indicated by gas production after 2–6 days of incubation.

Further media recommended to grow *Zymomonas mobilis* in liquid culture contains glucose (20 g L^{-1}) and yeast extract (5 g L^{-1}). Incubation at 30 °C is recommended. *Zymomonas mobilis* strains can grow on zymomonads-pimaricine (ZP) agar (Coton and Coton 2003) under anaerobic conditions at 30 °C (incubation time of ~5 days). The medium contains glucose (20 g L^{-1}), peptone (5 g L^{-1}), yeast extract (3 g L^{-1}), malt extract (3 g L^{-1}), and agar (15 g L^{-1}). The pH should be adjusted to 4.8 using 10 % citric acid. If the medium is used to enrich and isolate *Zymomonas mobilis*,the growth of yeast and lactic acid bacteria can be inhibited by the addition of 100 mg L^{-1} pimaricine and 30 mg L^{-1} penicillin (ZPP medium; Coton and Coton 2003).

Zymomonas strains grow easily in liquid media containing either D-glucose or D-fructose. The nitrogen source for growth can be supplied as peptone, yeast extract, nutrient broth, beer, palm juice, or apple juice or a mixture of 20 amino acids. Groups of amino acids, individual amino acids, NH_4Cl, or $(NH_4)SO_4$ can also serve as nitrogen sources, but they are not verified for different *Zymomonas* strains. Growth factors required by the *Zymomonas* strains are biotin and pantothenate. Only some strains need additional growth factors, such as vitamin B2, lipoic acid, riboflavin, and folic acid for growth (Van Pee et al. 1974). *Zymomonas* can be maintained as living cells in standard medium at room temperature if they are transferred every 2–3 weeks. Lyophilized cells can be maintained over years.

Ecology

Sphingomonads, especially *Sphingomonas*, *Novosphingobium*, *Sphingopyxis*, and *Sphingobium* species were isolated from a broad range of habitats. Some of the most abundant habitats are explored here in more detail.

Habitats Contaminated with Recalcitrant (Poly)Aromatic Contaminants

Several sphingomonads were isolated in high proportions from a broad range of soils or other habitats contaminated with different recalcitrant (poly)aromatic compounds of anthropogenic origin. Potential reasons for the adaptation of sphingomonads to those habitats and their metabolic versatility for the degradation of a broad range of contaminants were already discussed in this chapter.

Sphingomonadaceae in Aquatic Systems

Freshwater Habitats

Sphingomonads represent typical members of freshwater bacterioplankton communities (Glöckner et al. 2000; Zwart et al. 2002; Newton et al. 2011; Gich et al. 2005). Several species of the genera *Sphingomonas*, *Novosphingobium*, and *Sphingopyxis* were isolated from freshwater systems, such as *Sphingomonas aquatilis* and *Sphingomonas koreensis* (Lee et al. 2001), *Sphingomonas jaspsi* (Asker et al. 2007a), *Sphingomonas astaxanthinifaciens* (Asker et al. 2007b), *Sphingomonas starnbergensis* (Chen et al. 2012b), *Sphingopyxis taejonensis* (Lee et al. 2001), *Sphingopyxis rigui* and *Sphingopyxis wooponensis* (Baik et al. 2013), *Novosphingobium acidiphilum* (Glaeser et al. 2009), *Novosphingobium fuchskuhlense* (Glaeser et al. 2013), *Sphingobium boeckii* and *Sphingobium limneticum* (Chen et al. 2013), and *Sphingobium xenophagum* (Stolz et al. 2000). All or several species of the genera *Blastomonas*, *Sandaracinobacter*, *Sandarakinorhabdus*, and *Sphingosinicella* were also isolated from freshwater systems. *Sandaracinobacter sibiricus* was isolated from a freshwater algobacterial mat near a hydrothermal vent (Yurkov et al. 1997). *Sandarakinorhabdus limnophila* and *Sphingorhabdus planktonica* were isolated from two freshwater lakes located in Germany (Gich and Overmann 2006; Jogler et al. 2012). The type species *Sphingosinicella microcystinivorans* was isolated from another freshwater lake during a toxic *Microcystis* bloom (Maruyama et al. 2006).

A broad range study of environmental 16S rRNA gene sequences ubiquitously occurring in freshwater habitats was performed by Newton et al. (2011), covering all freshwater 16S rRNA gene sequences of the primary databases. The study provided an excellent overview of *Sphingomonadaceae* phylotypes (linked to proposed species if available), which were found to be unique in freshwater systems. Two lineages of freshwater *Alphaproteobacteria* were phylogenetically placed into the *Sphingomonadaceae*. The lineage alfIII represented environmental sequences phylogenetically placed into the genus *Sphingomonas sensu stricto*. The linage alfIV, which was much more abundant with respect to the freshwater sequences, showed an overlaps with the genus *Novosphingobium*. Two tribes NovoA1 and NovoA2 (both of the lineage alfIV-A) represent the two freshwater species *Novosphingobium acidiphilum* (Glaeser et al. 2009) and *Novosphingobium fuchskuhlense* (Glaeser et al. 2013), respectively. The fourth-abundant sphingomonads freshwater tribe was Phyx (alfIV-B), which phylogenetically shows an overlap with the genus *Sphingopyxis*. Another tribe was assigned, including further *Sphingomonadaceae*-related phylotypes.

Several studies of surface water bacterioplankton showed the abundance of *Sphingomonadaceae* in freshwater systems in more detail. The abundance and persistence of the *Novosphingobium* species *Novosphingobium acidiphilum* (Glaeser et al. 2009) in the small humic-acid rich Lake Grosse Fuchskuhle was shown in several environmental studies by molecular cultivation-independent and cultivation-dependent studies

(Glöckner et al. 2000; Burkert et al. 2003; Allgaier and Grossart 2006; Hutalle-Schmelzer et al. 2010; Glaeser et al. 2009, 2010). The ecological niche of this species seemed to be linked to the utilization of small organics substances formed by the degradation (photolysis by sunlight exposure) of humic substances, which are highly abundant in the lake. The adaptation to reactive oxygen species, especially the short-lived singlet oxygen, seemed to play an important role in the adaptation of the species to this ecosystem (Glaeser et al. 2010).

A high diversity of freshwater sphingomonads was found by Jogler et al. (2011), who investigated the population structure of *Sphingomonadaceae* in two freshwater lakes in Germany, Lake Walchensee and Lake Starnberger See. The authors determined several different phylotypes based on 16S rRNA gene sequence analysis; among those, several subpopulation were found represented by different 16S -23S rRNA gene internal transcribed spacer (ITS)-types. Cultivation of representative strains of different ITS types of the predominant phylotype (G1A, later on described as *Sphingorhabdus planktonica*; Jogler et al. 2013) showed high physiological diversity regarding substrate utilization; however, this could not be linked to niche separation of the specific subpopulations (ecotypes).

A second predominant genotype obtained in the study of Jogler et al. (2011), genotype GA7, represents the species *Sandarakinorhabdus limnophila* (Gich and Overmann 2006). Several mechanisms for niche separation of closely related *Sphingomonadaceae* were discussed, including differences in the ability to degrade refractory high-molecular weight compounds, resistance to ultraviolet light, planktonic or sessile lifestyles, aerobic oxygenic phototrophic, or differences in the susceptibility of bacteriophage attach. Freshwater sphingomonads were also found associated with higher freshwater organisms, such as the green algae *Desmodesmus armatus* and the diatom *Stephanodiscus minutulus* (Eigemann et al. 2013). The detected phylotypes were phylogenetically most closely related to the genera *Sphingomonas* and *Sphingopyxis*. An ecological molecular cultivation-independent study of aerobic anoxygenic phototrophs in the surface waters of freshwater lakes targeting the *pufM* gene gave evidence for freshwater-associated *Sphingomonadaceae* of the genera *Blastomonas* and *Sphingomonas* containing *pufM*, indicating that phototrophy may be a common metabolic feature of freshwater *Sphingomonas* in different lakes (Salka et al. 2011).

Marine Habitats

Marine species were mainly found in the genus *Sphingopyxis* (Godoy et al. 2003; Yoon et al. 2005), the dissected genus *Sphingorhabdus* (Yoon and Oh 2005; Kim et al. 2008), and the genus *Sphingomicrobium*, *Sphingomicrobium lutaoense* (Kämpfer et al. 2012), and *Stakelama pacifica* (Chen et al. 2010). The species *Sphingopyxis alaskensis* (Godoy et al. 2003), formerly described as "*Sphingomonas alaskensis*" (Vancanneyt et al. 2001), is thereby one of the most well-investigated marine sphingomonads, representing a model organism for an oligotrophic marine lifestyle. Seven strains representing this species were isolated from seawater of the Resurrection Bay, a deep fjord of the Gulf of Alaska (Vancanneyt et al. 2001). The strains were oligotrophic ultramicrobacteria (<0.1 m^3), which were adapted to the nutrient-limited marine habitats (oligotrophs).

A genome-sequencing approach was performed to understand the lifestyle of this marine species in detail. In particular, carbon and nitrogen utilization were investigated with respect to the ability to adapt to heterotrophic growth under nutrient-depleted (oligotrophic) conditions (Williams et al. 2009). It was found that *Sphingopyxis alaskensis* has the physiological capacity to exploit an increase of an ambient nutrient availability and can thereby achieve high-population densities in its marine habitat. Several further metabolic features and adaptation mechanisms of marine bacteria to their environment were also investigated using *Sphingopyxis alaskensis*, as a model taxa, such as the adaptation to cold marine environments or the response to solar irradiation studied by quantitative proteomics (Ting et al. 2010; Matallana-Surget et al. 2009), as well as the adaptation to UV exposure investigated in detail by analysis of the effects of growth temperature and starvation state of the bacterium (Matallana-Surget et al. 2010)

Other marine sphingomonads were found in association with higher marine organisms. For example, *Sphingomonas jejuensis* was isolated from the marine sponge *Hymeniacidon flavia* (Park et al. 2011), *Sphingomonas molluscorum* from the marine bivalve *Anadara broughtoni* (Romanenko et al. 2007), and *Sphingomonas japonica* from the marine crustacean *Paralithodes camtschatica* (Romanenko et al. 2009). Sphingomonads were also found to be the dominating fraction of epiphytic bacterial biofilm communities that inhabit the surfaces of the green macroalgae *Ulvacean marine*, which is found worldwide in coastal tidal and subtidal ecosystems (Burke et al. 2011). The detected sphingomonad phylotypes were phylogenetically closely related to the genus *Sphingopyxis*. In that comparative study, however, *Sphingomonadaceae* were not detected in the surrounding seawater.

A marine *Novosphingobium* isolate was found to be able to grow on the aromatic part of fuels as a carbon and energy source. A detailed investigation of the substrate utilization of the strain showed that it could grow in a surprisingly wide range of aromatic compounds, including mono-, bi-, tri-, and tetracyclic aromatic hydrocarbons and heterocyclic compounds (Notomista et al. 2011).

Phyllosphere and Rhizosphere/Root-Associated Sphingomonads

Several species of the genus *Sphingomonas* were isolated from habitats associated with plants, including the phyllosphere and rhizosphere as well as root compartments. Some *Sphingobium* species were also isolated from the rhizosphere. *Sphingomonas cynarae* was isolated from the phyllosphere of *Cynara*

cardunculus, a wild cardoon, which is a native Mediterranean plant that is considered to be a wild ancestor of the artichoke and cultivated cardoon (Talà et al. 2013). *Sphingomonas phyllosphaerae* was isolated from the phyllosphere of *Acacia caven* (Rivas et al. 2004) and *Sphingomonas roseiflava* from the ear of *Setaria viridis* (*Gramineae*) (Yun et al. 2000). *Sphingomonas asaccharolytica* was isolated from roots of a *Malus* spp. (apple) (Takeuchi et al. 1995), strains of *Sphingomonas azotifigens* from roots of *Oryza sativa* (Xie and Yokota 2006), and *Sphingomonas jinjuensis* and *Sphingomonas oryziterrae* were both isolated from the rhizosphere of rice growing in a conventionally-managed rice field (*S. jinjuensis*) and a no-tillage rice field (Chen et al. 2011). *Sphingomonas pruni* was isolated from roots of *Prunus persica* (peach) (Takeuchi et al. 1995). The *Sphingobium* species isolated from the rhizosphere include *Sphingobium indicum*, which was isolated from the rhizosphere of rice in India (Pal et al. 2005), and *Sphingobium rhizovicinum*, which was isolated from the rhizosphere soil of *Fortunella hindsii* (Champ. ex Benth.) Swingle (Young et al. 2008).

Several studies investigating bacteria inhabiting the phyllosphere of plants reported the abundance of *Sphingomonas* species in this habitat (Kim et al. 1998; Idris et al. 2004; Enya et al. 2007; Fürnkranz et al. 2008). A metaproteogenomic approach of natural microbial communities growing in the phyllosphere of *Arabidopsis thaliana* and agriculturally grown clover and soybean plants indicated that *Sphingomonas* species occur in high abundance as natural inhabitants in the phyllosphere of several plant species beside methylotrophic bacteria (Delmotte et al. 2009). In a subsequent study, the antagonistic potential of *Sphingomonas* strains isolated from the respective phyllosphere samples were tested with the plant-pathogen model *Arabidopsis thaliana* and *Pseudomonas syringae* pv. *tomato* DC3000. Several of the investigated *Sphingomonas* isolates prevent disease symptom formation and kept the cell numbers of the pathogens lower compared to axenic plants. Similar effects were obtained for several plant-derived *Sphingomonas* species, including *Sphingomonas melonis* and *Sphingomonas phyllosphaerae*, which were tested in parallel. Based on those results, the authors concluded that a competitive phyllosphere colonization and suppression of pathogen growth in the phyllosphere may be a common trait of phyllosphere colonizing *Sphingomonas*.

Plant protection by *Sphingomonas* was also shown against *Xanthomonas campestris* pv. *campestris* LMG 568, another well-established plant pathogen (Buell 2002). Concurrence for photo-assimilates leaked by the plant leaves, such as glucose, fructose, and sucrose, was determined to be one of the important antagonistic features for protecting *Sphingomonas* against *P. syringae* pv. tomato DC3000, which metabolized the same substrates. This is supported by the fact that membrane proteins assigned to *Sphingomonas* spp. and involved in transport processes of various carbohydrates were abundant proteins in the metaproteomic approach (Delmotte et al. 2009).

Sphingomonas also occurs as endophytes in plant root tissue. One endophytic *Sphingomonas* species was already described—*Sphingomonas endophytica* (Huang et al. 2012), which was isolated from surface-sterilized tissue of *Artemisia annua* L. An endophytic strain that showed plant-growth promotion is *Sphingomonas* strain YM22. This strain was isolated from surface-sterilized roots of a copper-resistant plant of the species *Commelina communis*. The strain showed a high tolerance to Cu (0.5 mM), Pb (5 mM), and Zn (10 mM); it produced indol acetic acid (IAA) and high amounts of siderophores, and shows 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Sun et al. 2010). Plant growth promotion of this strain was shown in a pot inoculation experiment with maize (*Zea mays*) plants, indicated by an increase of the plant biomass (including root and above-ground tissues) compared to noninoculated controls (Sheng et al. 2012). Furthermore, the strain was able to colonize the rhizosphere of maize and increase the proportion of water-extractable copper in the rhizosphere soil after the application of $Cu_2(OH)_2CO_3$.

Another endophytic plant growth promoting *Sphingomonas* strains (S12) was isolated from surface-sterilized rice roots (Adhikari et al. 2001). The strain was phylogenetically closely related to *Sphingomonas trueperi*. Pot experiments showed an increase in plant height and dry weight of inoculated rice seedlings compared to the nontreated seedlings. Furthermore, a reduced disease incidence was obtained for inoculated plants. Strain S12 was also capable of fixing nitrogen, which was proved by the detection of *nifD* and the ability to reduce acetylene. Further endophytic *Sphingomonas* were isolated from surface-sterilized chili pepper seeds (*C. annuum* L. cv. Bukang) (Kang et al. 2007). *Sphingomonas* were also detected in the mycorrhizosphere involved in mineral weathering in a forest soil (Uroz et al. 2007).

Sphingomonads in Activated Sludge

Several sphingomonads were isolated from activated sludge, including *Sphingomonas hankookensis* (Yoon et al. 2009), *Sphingopyxis terrae* (Takeuchi et al. 1993), *Sphingopyxis witflariensis* (Kämpfer et al. 2002b), and *Novosphingobium tardaugens* (Fujii et al. 2003a), *Sphingobium faniae* is a pyrethroid-degrading bacterium isolated from activated sludge treating wastewater from pyrethroid manufacture (Guo et al. 2010), *Sphingobium wenxiniae* a synthetic pyrethroid (SP)-degrader isolated from activated sludge of an SP-manufacturing wastewater treatment facility (Wang et al. 2011), or *Sphingobium jiangsuense* isolated from a wastewater treatment system in a pesticide manufacture (Zhang et al. 2012).

Fluorescence in situ hybridization (FISH) studies of activated sludge samples of different wastewater treatment plant targeted *Sphingomonadaceae* by the use of a specific fluorescent probes (Neef et al. 1999). Up to 50 % of the detected sphingomonads cells were located in flogs or the activated sludge. The ability to produces slime and/or capsules and the release of EPS in general, as well as the hydrophobicity of the outer cell layer (due to the presence of SGLs), suggested that sphingomonads contribute to the formation of aggregates in activated sludge.

Zymomonas

Zymomonas mobilis was isolated from different alcoholic beverages. *Zymomonas* spp. were either considered to be the active agent in natural fermentation processes as in agave or palm sap or the active agent in bioreactors for ethanol and acetaldehyde production. However, *Zymomonas* spp. are also considered to be spoilage bacteria in ciders, beers, and perries (Swings and De Ley 1977; Buchholz et al. 1987; Coton and Coton 2003).

Pathogenicity and Clinical Relevance *[Including Antibiotic Sensitivity]*

Only a few *Sphingomonas* species are known to be clinically relevant—mainly ***Sphingomonas paucimobilis*** (formerly "*Pseudomonas paucimobilis*") and (in lower abundance) *Sphingomonas parapaucimobilis*. Those species are known as primary opportunistic bacteria that are proposed to be persistent nosocomial infecting agents (Ryan and Adley 2010). *Sphingomonas paucimobilis* was isolated from several different clinical specimens, such as wounds, blood, cerebrospinal or peritoneal fluids, urine, the vagina, and the cervix (Holmes et al. 1977; Morrison and Shulman 1986; Reina et al. 1991; Lin et al. 2010; Mutlu et al. 2011; Lanoix et al. 2012; Souto et al. 2012). Clinical isolates identified as *Sphingomonas parapaucimobilis* were also isolated from clinical specimens, including sputum, urine, and the vagina (Yabuuchi et al. 1990).

Reported clinical syndromes associated with *Sphingomonas paucimobilis* are primary bacteremia and septicemia, bloodstream infections, intravascular catheter infections, peritoneal dialysis-associated peritonitis, urinary tract infections, biliary tract infection, cutaneous infection, ventilator-associated pneumonia, peritonitis, meningitis, myositis, osteomyelitis, septic arthritis, endophthalmitis, cervical adenitis, bromohidrosis, and diarrheal disease (Toh et al. 2011; Ryan and Adley 2010; Maragakis et al. 2009; Lin et al. 2010). Reported infections caused by *Sphingomonas paucimobilis* were either hospital acquired or related to nosocomial outbreaks. Infections were often caused by contaminated solutions of distilled water, hemodialysis fluid, or sterile drug solutions (Ryan and Adley 2010). With respect to neonatal infections in intensive care units, Lemaitre et al. (1996) isolated *Sphingomonas paucimobilis* from the tracheal secretions of mechanically ventilated babies (84 individuals) at a neonatal intensive care unit over a 2-year period. The ventilator equipment was thereby identified as source of maintenance and spread of *Sphingomonas paucimobilis*.

No literature reports were found where *Sphingomonas paucimobilis* infections were linked to the death of patients (Ryan and Adley 2010; Lin et al. 2010). *Sphingomonas paucimobilis* is reported to have a low clinical virulence, but infection can lead to a septic shock. Both, healthy and immune-compromised individuals, can be infected (Lin et al. 2010). Community-acquired infections, diabetes mellitus, and alcoholism were identified as significant risk factors for primary bacteremia caused by *Sphingomonas paucimobilis* (Toh et al. 2011).

Clinically relevant *Sphingomonas* strains were reported to be susceptible to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, and aminoglycosides, but only partially susceptible to other antimicrobial agents, such as fluoroquinolones (Fass and Barnishan 1980; Hsueh et al. 1998; Reina et al. 1991). Ammendolia et al. (2004) investigated in detail the interaction of *Sphingomonas paucimobilis* with epithelial cells. The contact of *Sphingomonas paucimobilis* with the epithel cells altered the cell surface, caused numerous microvilli extensions, and membrane ruffles occurred. Confocal laser scanning microscopy showed that actin microfilaments were involved in the attachment and entry of the bacteria into the HeLa cells.

A further *Sphingomonas* isolate was reported to be associated with a clinical infection of a woman with sickle cell disease—***Sphingomonas mucosissima*** (Angelakis et al. 2009). Minimum inhibitory concentration (MIC) values were determined for several antibiotics: cefotaxime (1 mg L^{-1}), amoxicillin–clavulanic acid (1 mg L^{-1}), vancomycin (2–3 mg L^{-1}), imipenem (0.064 mg L^{-1}), ceftazidime (4–5 mg L^{-1}), amikacin (1 mg L^{-1}), ciprofloxacin (3 mg L^{-1}), and trimethoprim-sulfamethoxazole (0.047 mg L^{-1}). The patient was successfully treated with intravenous administration of ceftriaxone for 5 days. The intravenous catheter of the patient was thought to be the source of the infection.

Plant Pathogens

Few *Sphingomonas* were identified to be plant pathogens. ***Sphingomonas melonis*** was described as the causative agent of the brown-spot disease of yellow Spanish melons (*Cucumis melo* var. *inodorus* Naud.) cultivated in greenhouses in Almeria, Spain (Buonaurio et al. 2002). After infection of yellow Spanish melon fruits and leaves with cells of the type strain of *S. melonis*, desease symptoms were observed on the fruits. ***Sphingobium suberifaciens*** (formerly "*Rhizomonas suberifaciens*" or "*Sphingomonas suberifaciens*") was isolated and described as the causative agent of corky root rot of lettuce (van Bruggen et al. 1989, 1990). Initial symptoms are yellow bands occurring on young roots, which later form dark greenish-brown and corked areas that cover most of the tap root and main lateral roots. The infected roots became brittle and break off easily (van Bruggen et al. 1988). Furthermore, ***Novosphingobium rosa*** (formerly *Sphingomonas rosa*, "*Agrobacterium rhizogenes*") was isolated from hairy roots of *Rose* sp. and described as a plant pathogen (Sawada et al. 1993).

Zymomonas

For the genus *Zymomonas*, humans, animals, or plants pathogens are not currently known. However, antagonistic activity of *Zymomonas* spp. is indicated. Lindner (1929, 1931) recommended to use *Zymomonas* spp. as a supplement for

human nutrition, such as in form of yoghurt additives. For some *Zymomonas* strains, antagonistic activity against bacteria and fungi was determined in vitro, and the therapeutic application of *Zymomonas* for chronic enteric and gynecological infections was reported (Swings and De Ley 1977; Falcao de Morais et al. 1993). In mice, *Zymomonas mobilis* showed 60 % protection to an experimental infection with *Schistosoma mansoni* after oral application of *Zymomonas* cultures (Santos Jde et al. 2004).

Antibiotic Sensitivity

Sensitivities to antibiotics were studied for a broad range of *Sphingomonadaceae*. A total of 33 type strains were tested by Yabuuchi et al. (2001), including type strains of the genera *Sphingomonas* (12), *Sphingobium* (6), *Novosphingobium* (6), *Sphingopyxis* (2), and *Blastomonas* (2) species. In all, 38 antibiotics were tested. The type strains resisted to up to 58 % of the tested antibiotics. A total of 91–100 % was susceptible to tetracylines, amikacin, gentamicin, panipenem, and imipenem. Differences in susceptibilities to amoxicillin (67 %) and amoxicillin/clavulanic acid (91 %) suggest the presence of penicillinase in these species. Genera-dependent differences were obtained for antibiotics of the chinolone/fluorochinolone group. All type strains of the genus *Novosphingobium* were at least somewhat resistant to norfloxacin, ciprofloxacin, ofloxacin, and levofloxacin.

Vaz-Moreira et al. (2011) studied the antibiotic-resistance pattern of 86 *Sphingomonadaceae* isolated from drinking water systems, such as drinking water treatment plants, tap water, cup filters of dental chairs, demineralization filters, including antibiotics of fife classes, several beta-lactams and aminoglycosides, a fluoroquinolone, a polymyxin, and a sulfonamide. As identified by 16S rRNA gene and *atpD* sequence analysis, 27 *Sphingomonas*, 28 *Sphingobium*, 12 *Novosphingobium*, 7 *Sphingopyxis*, and 12 *Blastomonas* strains were investigated in the study. The antibiotic resistance level was especially high for colistin (90 %) and beta-lactam antibiotics. The highest prevalence values for antibiotic resistance were obtained for strains phylogenetically assigned to the genera *Sphingomonas* and *Sphingobium* with respect to beta-lactams, ciprofloxacin, and cotrimoxazole. Resistance patterns were mainly linked to species rather than to strains or isolation size. *Sphingomonadaceae* in water systems may be a source of clinically relevant antibiotic resistance. For example, a high prevalence of polymyxin (polymyxin B) and beta-lactams resistance was determined for clinical isolates of *Sphingomonas paucimobilis* (Sader and Jones 2005) (*Table 25.13*).

Applications

Sphingomonadaceae, especially of the genera *Sphingomonas* and *Sphingobium*,are biotechnologically interesting for two main reasons. First, sphingomonads have the potential to be applied for bioremediation of contaminated aquifers or soils because several sphingomonads can degrade a variety of xenobiotic compounds. Second, sphingomonads produce agar-like EPS, sphingans, which are of interest for food, pharmaceutical, and industrial applications.

Bioremediation by *Sphingomonas*

A variety of sphingomonads were found to be candidates for bioremediation of wastewater or contaminated dumping sites. For example, *Sphingomonas wittichii* RW1^T was used for bioremediation of PCDD in contaminated incinerator fly ash (Nam et al. 2005). In that study, a reduction of 75.5 % was obtained for the toxic of PCDDs after 15 days of treatment. Because sphingomonads that will be used for bioremediation need to cope with several environmental stress factors, further study is needed to overcome related problems. For example, the effects of changes in the water potential and respective adaptive responses of *Sphingomonas wittichii* RW1^T was investigated in detail by Johnson et al. (2011) using transcriptome and membrane fatty acid analysis. Bioengineering can be applied to increase the efficiency of the degradation pathways.

Sphingomonas strain sp. FG03 isolated from phenol-contaminated soils was positively tested for phenol degradation. The strain showed a high efficiency of phenol degradation with a removal rate of 95 % within 3 h in the presence of 800 mg L^{-1} phenol. Both free suspended and immobilized cells showed the respective activity. However, immobilized cells showed a better performance and were thereby indicated to be applicable for treating phenol-contaminated wastewater (Liu et al. 2009). The alkylphenol-degrading *Sphingomonas* sp. strain TTNP3 was proven for bioaugmentation in a membrane bioreactor of nonylphenol (Kolvenbach and Corvini 2012). The ability of *Sphingomonas* sp. TTNP3 to degrade a variety of alkylphenols, with potential reverse effects on living organisms by actin with the endocrine system, were summarized by Kolvenbach and Corvini (2012).

Production and Application of Exopolysaccharide (Sphingans)

Extracellular polysaccharides (EPS) produced by *Sphingomonas* species (sphingans) are gelling agents that are of interest for food, pharmaceutical, and industrial applications, including environmental bioremediation processes. A broad range of patents were published in recent years with respect to gellans. Fialho et al. (2008) reported of more than 800 patents worldwide.

Gellan gum produced by *Sphingomonas elodea* is one of the most important sphingans; it is commercially procured by C.P. Kelco in Japan and the USA (Prajapati et al. 2013). The strain used for production was isolated from the surface of a plant of the genus *Elodea* as an outcome of an extensive screening approach performed by Kelco (Kang and Veeder 1981). Gellan is produced in a fermentative process at a laboratory scale. The production of gellan is a growth-dependent process with a final

yield of approximately 10 g gellan L^{-1}. By the release of gellan into the growth medium, the viscosity of the medium increases, which makes it difficult to separate the produced gellan from the cells. Gellan can be precipitated from diluted cell cultures by the use of isolpropyl alcohol. Thereafter, gellan is suspended in water, further purified by dialysis, and finally lyophilized. Then, it can be suspended in water to produce gels.

The physical properties of gellan are dependent on the EPS concentration, the temperature, the water content, and the concentration of monovalent and divalent cations in the medium. Gels are formed with various cations. An important role in the rheology of the gellan gel matrix is therefore played by the acyl substituents. Deacylation of the native gellan gum can be used to modify the characteristics of gellan to produce either soft, elastic, and thermoreversible or hard-to-rough gels (Rinaudo and Milas 2000). Gellases can be used to modify native gellans; gellan lyasis (described as shinganases) are produced by several gram-positive and gram-negative bacteria as extracellular eliminase-type endoenzymes that cleave ß-D-glucosyl (1- > 4) ß-D-glucuronysyl linkages of the EPS and can thereby degrade EPS, used in biotechnolocially gellan production to modifiy the viscosity of EPS (Giavasis et al. 2000; White et al. 1996).

Gellan is applied as a gelling, stabilizing, and suspending agent in the food and medical industries in the United States and Europe. The advantage of gellan is its thermal and acid stability, the ability to adjust gel elasticity and rigidity, its high transparency, and good flavor. The stage of acyl content—no, low, or high acyl content—differentiates the commercially available gellan forms. The main products are Gelrite®, Kelcogel® F, or Kelcogel® LT100. Gelrite is an alternative gelling agent to agar, which is often applied in plant tissue cultures or used for the cultivation of thermophilic bacteria (Lin and Casida 1984). It also can be used by the process of biodegradation as a gel agent to encapsulate bacteria for bioaugmentation (Moslemy et al. 2003, 2004). Kelcogels is applied as a gelling, stabilizing, and suspending agent in the food industry (E number E418) or in the personal care industry as a supplement for lotions, creams, and toothpastes (Sutherland 2001). In the biomedical sector, gellan is applied as a pharmaceutical excipient to several different drugs, with several more possible applications also being tested (for more details, see Fialho et al. 2008).

The sphingan Ss, produced by *Sphingomonas sanxanigenens* $NX02^T$ (Huang et al. 2009a), can be precipitated by acidification (pH 3.0). This allows for much more cost-effective production compared to gellan, which cannot be precipitated as easily (Huang et al. 2009b). The sphingan produced by *Sphingomonas sanxanigenens* was used in China to recover petroleum by water flooding; thereby, it acts as a mud and thickening agent (Huang et al. 2009b).

Bioethanol Producing Capacity of *Zymomonas mobilis*

Zymomonas mobilis was originally used in tropical areas to produce spirituous beverages from plant sap (Swings and De Ley 1977). The unique metabolic capacity of *Zymomonas mobilis* to efficiently produce ethanol from simple sugars compounds made this species interesting for a biotechnological application to produce ethanol. *Zymomonas mobilis* has thereby several advantages compared to yeast: *Z. mobilis* cells have higher specific rates of glucose uptake and ethanol production; the yield of produced ethanol is higher compared to a low yield of bacterial biomass, anaerobic production is possible, and *Zymomonas* spp. are able to grow in the presence of relatively high amounts of ethanol. Some authors have studied extensively the kinetics of ethanol fermentation in both batch and continuous cultures at high glucose concentrations (Lee et al. 1979; Rogers et al. 1982). Attempts to broaden the narrow substrate and product spectrum of *Z. mobilis* have yielded strains that can utilize carbon sources such as mannitol (Buchholz et al. 1988) after chemical mutagenesis. Introduction of heterologous genes from various microorganisms has led to strains that can utilize D-xylose (Feldmann et al. 1992; Zhang et al. 1995), D-mannose (Weisser et al. 1996), L-arabinose (Deanda et al. 1996), β-glucosides, or lactose (Sprenger 1993).

Because *Zymomonas mobilis* is genetically assessable, severally metabolically engineered strains of *Zymomonas mobilis* were constructed for efficient bioethanol production. In the mid-1980s several heterologous genes were cloned to extend the substrate spectrum of *Zymomonas mobilis* (for review, see Rogers et al. 2007). With the elucidation of the enzymatic background of the Entner–Doudoroff pathway of *Zymomonas mobilis* in the 1980s, genes of complete pathways were introduced into *Zymomonas* cells to enable the utilization of further sugar compounds xylose and arabinose for ethanol production via the Entner-Doudoroff pathway. For example, Zhang and co-workers (1995) introduced genes encoding the xylose isomerase, xylulokinase, transaldolase, and transketolase to produce a functional metabolic pathway of xylose utilization. The introduction of this metabolic pentose pathway enabled *Zymomonas* strains to convert xylose to central intermediates of the Enter-Doudoroff pathway and to ferment it thereby to ethanol.

Another example is the introduction of five *E. coli* genes encoding L-arabinose isomerase, L-ribulokinase, L-ribulose-5-phosphate-4-epimerase, transaldolase, and transketolase generating an L-arabinose fermenting strain (Deanda et al. 1996). Glucose, xylose, and arabinose co-fermenting strains were later constructed by Mohagheghi et al. (1998). Several other strains were produced by genetic engineering. For example, Yanase et al. (2012) developed a genetically engineered *Zymomonas mobilis* strain that was able to produce bioethanol from complex cellolytic material (wood hydrolysates rich in glucose, mannose, and xylose as major sugar components) by the introduction of genes encoding enzymes from the catabolic mannose and xylose metabolism of *E. coli* into *Zymomonas mobilis*. Several recombinant plasmids harboring *E. coli*-derived genes (*xylA*, *xylB*, *tal*, and *tktA*) are necessary to ferment several further sugar substrates, including mannose, xylose, and glucose.

With the publication of the complete genome sequence of *Z. mobilis* ZM4 in 2005 (Seo et al. 2005), the ability for genetic

Table 25.13

Antibiotic susceptibility tests reported for several species of the genera *Sphingomonas*, *Sphingopyxis*, *Novosphingobium*, and *Sphingobiur*

Species	Amoxicillin	Ampicillin	Piperacillin	Ampicillin/ Clavulanic acid	Oxacillin	Penicillin G	Moxalactam	Streptomycin	Kanamycin	Gentamycin	Amikacin	Neomycin	Dibekacin	Doxycycline	Tetracycline	Oxytetracycline	Minocycline	Novobiocin	Rifampicin	Rifamycin	Ciprofloxacin	Levo floxacin	Nalidixic acid
Sphingomonas adhaesiva	S(25)	S(10)	IM(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				IM(5)	S(5)	
Sphingomonas alpina		R(100)				R(100)		R(100)	S(100)						S(100)				R(100)				
Sphingomonas asaccharolytica	S(25)	S(10)	S(100)	S(20/10)		S(10)	IM(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				R(5)	R(5)	
Sphingomonas cynarae										S(10)											S(5)		
Sphingomonas desiccabilis										R(10)													
Sphingomonas dokdonensis		R(10)				R(20U)		R(50)	S(30)	S(30)		S(30)			S(30)			R(5)					
Sphingomonas echinoides	S(25)	S(10)	S(100)	S(20/10)		S(10)	S(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingomonas glacialis		R(30)				R(100)		R(30)	S(30)						S(30)				S(30)				
Sphingomonas hankookensis		R(10)				R(20U)		R(50)	S(30)	S(30)		S(30)			S(30)			S(30)					
Sphingomonas histidinilytica	S(10)	S(30)				S(10)			S(30)	S(10)	S(30)	R(30)				S(30)		S(30)	S(5)		S(5)		S(30)
Sphingomonas indica		R(10)				R(10)		R(10)	R(30)	R(10)	S(30)				S(30)	S(30)			S(5)		S(5)		S(30)
Sphingomonas jinjuensis		S(10)				R(10)		S(10)	S(30)	S(10)					R(10)				R(10)				
Sphingomonas koreensis	R(25)	R(10)	R(100)	R(20/10)		R(10)	R(30)			R(10)	R(30)		R(30)	S(10)	S(30)		S(30)				R(5)	S(5)	
Sphingomonas laterariae		S(10)				R(10)			S(30)	S(10)	S(30)				S(30)	S(30)			S(5)		S(5)		R(10)
Sphingomonas leidyi		R(10)			R(5)	R(10)					S(30)				S(30)								
Sphingomonas mali	S(25)	S(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				R(5)	S(5)	
Sphingomonas melonis	R(25)	R(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingomonas molluscorum						R(10)		R(10)		S(10)				S(30)	S(30)				S(30)		S(5)		
Sphingomonas mucosissima						R(10)		R(10)		S(10)				S(30)	S(30)			S(30)	S(30)		S(5)		
Sphingomonas natatoria	S(25)	S(10)	S(100)	S(20/10)		S(10)	R(30)			S(10)	S(30)			S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingomonas oryziterrae		S(10)				R(10)		S(10)	R(30)	R(10)					R(10)				R(10)				
Sphingomonas parapaucimobilis	R(25)	R(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingomonas paucimobilis	S(25)	S(10)	R(100)	S(20/10)		S(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingomonas pituitosa	R(25)	R(10)	R(100)	S(20/10)		S(10)	S(30)			S(10)	S(30)			S(10)	S(30)		R(30)				IM(5)	S(5)	
Sphingomonas pruni	R(25)	R(10)	R(100)	R(20/10)		R(10)	IM(30)			S(10)	S(30)		S(30)	S(10)	S(30)		R(30)				IM(5)	S(5)	
Sphingomonas roseiflava	S(25)	S(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingomonas rubra	S(10)	R(10)				R(10)		R(10)	S830)	S(10)		S(30)			S(30)			S(30)	S(5)				
Sphingomonas sanguinis	R(25)	R(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingomonas starnbergensis	-					S(10)			S(30)	S(10)	S(30)	S(30)			S(30)								
Sphingomonas trueperi	S(25)	S(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				IM(5)	S(5)	
Sphingomonas wittichii	S(25)	S(10)	S(100)	S(20/10)		S(10)	R(30)			S(10)	S(30)		S(30)	S(10)	IM(30)		S(30)				R(5)	R(5)	
Tested type strains	17	23	13	13	1	26	13	9	10	24	18	5	11	15	25	3	13	5	9		18	13	3
R Total number	6	12	10	2	1	19	10	7	2	3	1	1	1	0	2	0	2	1	2		3	1	1
% of total tested type strains	35.3	52.2	76.9	15.4	100	73.1	76.9	77.8	20	12.5	5.6	20	9.1	0	8	0	15.4	20	22.2		16.7	7.7	33.3
Sphingopyxis alaskensis	IM(25)	R(10)	R(100)	IM(20/10)		R(10)	R(30)			R(10)	R(30)		R(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingopyxis bauzanensis		S(50)				S(100)		R(100)	R(100)*						S(100)				S(100)				
Sphingopyxis macrogoltabida		R(10)	R(100)	IM(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				R(5)	S(5)	
Sphingopyxis rigui		S(10)				R(10IU)		R(10)	S(30)	R(10)	S(30)				R(30)								R(30)
Sphingopyxis taejonensis	IM(25)	R(10)	R(100)	R(20/10)		R(10)	R(30)			S(10)	IM(30)		IM(30)	S(10)	S(30)		S(30)				IM(5)	S(5)	
Sphingopyxis terrae	S(25)	S(10)	S(100)	S(20/10)		S(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingopyxis ummariensis		R(10)				S(10)			S(30)	S(10)		S(30)			S(30)	S(30)		S(30)	S(5)				
Sphingopyxis witflariensis		S(10)				R(10IU)		R(10)	S(30)	S(10)	S(300IU)				R(30)								
Sphingopyxis wooponensis		S(10)				S		R(10)	S(30)	S(10)	S(30)				R(30)								R(30)
Tested type strains	4	9	4	4		9	4	4	5	8	6	2	4	4	9	1	4	1	2		4	4	2
R Total number	1	4	3	1		5	4	4	1	2	1	0	1	0	3	0	0	0	0		1	0	2
% of total tested type strains	25	44	75	25		56	100	100	20	25	17	0	25	0	33	0	0	0	0		25	0	100
Novosphingobium aromaticivorans	S(25)	S(10)	S(100)	S(20/10)		S(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				R(5)	IM(5)	
Novosphingobium barchaimii		S(10)				R(10)		S(10)	S(30)	R(10)	R(30)				S(30)	S			S(5)		S(5)		S(30)
Novosphingobium capsulatum	S(25)	S(10)	IM(100)	S(20/10)		S(10)	IM(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				R(5)	S(5)	
Novosphingobium hassiacum		S(10)				R(10IU)		R(10)	S(30)	S(10)					S(30)								
Novosphingobium indicum		S(10)	S(100)		R(1)	S(10)		R(10)	S(30)	S(10)		S(10)			S(30)				S(5)		S(5)		
Novosphingobium naphthalenivorans		S(10)	S(100)		R(1)	S(10)		R(10)	S(30)	S(10)		S(10)							S(5)				

Norfloxacin	Ofloxacin	Sparfloxacin	Tosufloxacin	Cefa.dor	Cefmetazol	Chephalothin	Cefazolin	Lincomycin	Erythromycin	Oleandomycin	Clarithromycin	Roxithromycin	Polymyxin B	vancomycin	cefotaxime	Cefoperazon	Ceftazidim	Aztreonam	Carumonam	Sulfamethoxazol + riTrimethoprim	Cotrimoxazol	Imipenem	Metropenem	Panipenem	Flomoxef	Chloramphenicol	trimetroprim
R(10)	IM(5)	S(5)	IM(5)	S(30)	IM(30)		R(30)		R(15)		S(15)	R(15)	R(300)		S(30)	S(75)	S(30)	R(30)	R(30)	R		S(10)	S(10)	S(10)	R(30)		R(5)
																										R(100)	R(100)
R(10)	R(5)	IM(5)	IM(5)	S(30)	S(30)		R(30)		S(15)		IM(15)	IM(15)	R(300)		S(30)	R(75)	S(30)	S(30)	S(30)	S		S(10)	IM(10)	S(10)	R(30)		R(5)
													100U													R(30)	
						R(30)		R(15)		S(15)		R (100U)														S(100)	
R(10)	S(5)	R(5)	R(5)	S(30)	S(30)		S(30)		R(15)		S(15)	R(15)	R(300)		S(30)	S(75)	S(30)	R(30)	R(30)	IM		S(10)	S(10)	S(10)	S(30)		R(5)
																										S(30)	
						R(30)		R(15)		S(15)			W(100)													S(100)	
													S(300)	S(30)												S(30)	
													S(30)	S(30)												S(30)	
									S(15)	S(15)				S(30)												S(30)	
R(10)	R(5)	IM(5)	R(5)	R(30)	R(30)		R(30)		R(15)		R(15)	R(15)	R(300)		R(30)	R(75)	R(30)	R(30)	R(30)	R		R(10)	R(10)	R(10)	R(30)		R(5)
													S(300)	S(30)												S(30)	
								R(15)						S(30)	R(30)			R(30)								R(30)	
R(10)	S(5)	S(5)	IM(5)	S(30)	R(30)		R(30)		R(15)		S(15)	R(15)	R(300)		S(30)	R(75)	R(30)	R(30)	R(30)	S		S(10)	S(10)	S(10)	R(30)		R(5)
IM(10)	S(5)	S(5)	S(5)	R(30)	R(30)		R(30)		IM(15)		S(15)	IM(15)	R(300)		R(30)	R(75)	R(30)	R(30)	R(30)	S		S(10)	S(10)	R(10)	R(30)		R(5)
						R(30)			R(2)				R(300)	S(30)				R(30)								S(30)	R(5)
						R(30)			R(2)				R(300)	S(30)				R(30)								S(30)	R(5)
S(10)	S(5)	S(5)	R(5)	S(30)	IM(30)		S(30)		S(15)		S(15)	S(15)	IM(300)		S(30)	S(75)	S(30)	IM(30)	R(30)	S		S(10)	S(10)	S(10)	IM(30)		R(5)
									S(15)	S(15)				R(30)												S(30)	
R(10)	S(5)	S(5)	S(5)	R(30)	R(30)		R(30)		S(15)		S(15)	S(15)	IM(300)		R(30)	R(75)	R(30)	R(30)	R(30)	S		S(10)	S(10)	S(10)	R(30)		R(5)
IM(10)	S(5)	S(5)	S(5)	S(30)	R(30)		R(30)		S(15)		S(15)	S(15)	IM(300)		S(30)	S(75)	S(30)	IM(30)	S(30)	S		S(10)	S(10)	S(10)	S(30)		R(5)
R(10)	S(5)	S(5)	S(5)	IM(30)	S(30)		R(30)		S(15)		IM(15)	IM(15)	R(300)		S(30)	R(75)	R(30)	S(30)	S(30)	IM		R(10)	R(10)	S(10)	R(30)		S(5)
R(10)	R(5)	S(5)	S(5)	R(30)	R(30)		R(30)		R(15)		R(15)	R(15)	R(300)		IM(30)	R(75)	S(30)	R(30)	S(30)	R		S(10)	S(10)	S(10)	IM(30)		R(5)
S(10)	S(5)	S(5)	S(5)	S(30)	S(30)		S(30)		S(15)		S(15)	S(15)	S(300)		S(30)	R(75)	S(30)	R(30)	IM(30)	S		S(10)	S(10)	S(10)	S(30)		S(5)
									S(15)						S(10)											S(30)	
S(10)	S(5)	S(5)	S(5)	R(30)	R(30)		R(30)		S(15)		S(15)	S(15)	R(300)		R(30)	R(75)	R(30)	R(30)	R(30)	S		IM(10)	S(10)	S(10)	R(30)		R(5)
								R(15)	S(15)					S(30)	S(30)			R(30)								S(30)	
R(10)	IM(5)	S(5)	S(5)	S(30)	R(30)		R(30)		IM(15)		S(15)	R(15)	R(300)		IM(30)	R(75)	IM(30)	R(30)	R(30)	R		S(10)	S(10)	IM(10)	R(30)		R(5)
R(10)	R(5)	S(5)	R(5)	S(30)	S(30)		S(30)		R(15)		R(15)	R(15)	IM(300)		S(30)	IM(75)	S(30)	R(30)	S(30)	R		S(10)	S(10)	S(10)	S(30)		R(5)
13	13	13	13	13	13	4	13	4	19	4	13	13	20	9	16	13	13	17	13	13		13	13	13	13	13	15
8	3	1	4	5	8	4	9	4	7	0	3	6	11	1	5	9	6	14	8	4		2	2	2	7	1	13
61.5	23.1	7.7	30.8	38.5	61.5	100	69.2	100	36.8	0	23.1	46.2	55	11.1	31.3	69.2	46.2	82.4	61.5	30.8		15.4	15.4	15.4	53.8	7.7	86.7
S(10)	S(5)	S(5)	S(5)	R(30)	R(30)		R(30)		IM(15)		S(15)	R(15)	S(300)		R(30)	IM(75)	R(30)	R(30)	S(30)	R		S(10)	R(10)	S(10)	R(30)		R(5)
																										S(100)	S(100)
S(10)	S(5)	S(5)	S(5)	R(30)	R(30)		R(30)		S(15)		S(15)	IM(15)	S(300)		R(30)	R(75)	S(30)	R(30)	R(30)	S		S(10)	R(10)	S(10)	R(30)		S(5)
									S(15)			R(300IU		S(30)												S(30)	
R(10)	IM(5)	S(5)	IM(5)	R(30)	R(30)		R(30)		R(15)		R(15)	R(15)	S(300)		R(30)	R(75)	R(30)	R(30)	R(30)	R		S(10)	R(10)	IM(10)	R(30)		R(5)
IM(10)	S(5)	S(5)	IM(5)	IM(30)	R(30)		S(30)		IM(15)		R(15)	R(15)	R(300)		S(30)	S(75)	S(30)	R(30)	R(30)	R		S(10)	IM(10)	S(10)	R(30)		R(5)
									S(15)					S(30)												S(30)	
												S(300IU)														R(30)	
									S(15)			R(300IU)														S(30)	
4	4	4	4	4	4		4		7		4	4	7	2	4	4	4	4	4	4		4	4	4	4	5	5
1	0	0	0	3	4		3		1		2	3	3	0	3	2	2	4	3	3		0	3	0	4	1	3
25	0	0	0	75	100		75		14		50	75	43	0	75	50	50	100	75	75		0	75	0	100	20	60
R(10)	R(5)	R(5)	R(5)	IM(30)	R(30)		R(30)		IM(15)		S(15)	IM(15)	S(300)		S(30)	S(75)	S(30)	IM(30)	S(30)	IM		S(10)	S(10)	S(10)	S(30)		R(5)
													S	S(30)												S(30)	
R(10)	R(5)	IM(5)	R(5)	S(30)	S(30)		S(30)		IM(15)		S(15)	R(15)	IM(300)		S(30)	IM(75)	S(30)	R(30)	R(30)	S		S(10)	S(10)	S(10)	S(30)		R(5)
												S(300IU)														S(30)	
R(10)	S(5)						S(30)	R(2)	S(15)				R(30U)	S(30)		S(30)					S(25)						
								R(2)	S(15)							S(30)					S(25)						

Table 25.13 (continued)

Species	Amoxicillin	Ampicillin	Piperacillin	Ampicillin/ Clavulanic acid	Oxacillin	Penicillin G	Moxalactam	Streptomycin	Kanamycin	Gentamycin	Amikacin	Neomycin	Dibekacin	Doxycycline	Tetracycline	Oxytetracycline	Minocycline	Novobiocin	Rifampicin	Rifamycin	Ciprofloxacin	Levo floxacin	Nalidixic acid
Novosphingobium pentaromativorans		S(10)	S(100)		R(1)	S(10)		R(10)	S(30)	S(10)		S(10)							S(5)				
Novosphingobium rosa	R(25)	R(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				R(5)	R(5)	
Novosphingobium stygium	S(25)	S(10)	S(100)	S(20/10)		S(10)	S(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				R(5)	R(5)	
Novosphingobium subarcticum	S(25)	S(10)	S(100)	S(20/10)	R(1)	S(10)	R(30)	R(10)	S(30)	S(10)	S(30)	S(10)	S(30)	S(10)	S(30)		S(30)		S(5)		R(5)	R(5)	
Novosphingobium subterraneum	S(25)	S(10)	S(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				R(5)	R(5)	
Tested type strains	6	11	9	6	4	1	6	6	6	1	7	4	6	6	9	1	6		4		8	6	1
R Total number	1	1	1	0	4	4	4	5	0	1	1	0	0	0	0	0	0		0		6	4	0
% of total tested type strains	17	9	11	0	100	36	67	83	0	9	14	0	0	0	0	0	0		0		75	67	0
Sphingobium abikonense		R(10)				R(10)			S(30)	S(30)	S(30)	S(30)			S(30)	S(30)			S(5)		S(5)		
Sphingobium baderi		R(10)				S(10)		R(10)	S(30)	S(10)	S(30)				S(30)	S(30)			S(5)		S(5)		R(30)
Sphingobium boeckii					R(5)				S(30)		S(30)			S(30)	S(30)								
Sphingobium chinhatense		R(10)							S(30)						S(30)								
Sphingobium chlorophenolicum	S(25)	S(10)	S(100)	S(20/10)		S(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingobium chungbukense	S(25)	R(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingobium cloacae	R(25)	R(10)	R(100)	IM(20/10)		R(10)	S(30)			S(10)	S(30)		R(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingobium cupriresistens	S(10)	R(10)			R(1)	R(10)		S(10)	S(30)			S(30)			S(30)			S(5)	S(5)				S(30)
Sphingobium czechense		R(10)				R(10)		R(10)	S(30)	S(10)	S(30)				S(30)	S(30)			S(5)		S(5)		S(30)
Sphingobium faniae		R(50)				S(30)		R(50)		S(15)					S(10)								
Sphingobium fontiphilum		R(10)				R(10)		R(10)	S(30)	S(10)					S			S(30)	S(5)				S(30)
Sphingobium francense		R(100)							S(50)						S(15)								
Sphingobium fuliginis	R(10)	R(10)				R(10)		R(10)	S(30)	S(10)		S(30)			S(30)	S(30)		S(30)		S(5)			S(30)
Sphingobium herbicidovorans	R(25)	R(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingobium indicum		R(100)							S(50)						S(15)								
Sphingobium japonicum		R(100)							S(50)						S(15)								R
Sphingobium lactosutens		R(10)				R(10)			S(30)	S(10)	S(30)	S(30)			S(30)	S(30)			S(5)		S(5)		
Sphingobium limneticum					R(5)				S(30)		S(30)	S(30)		S(30)	S(30)								
Sphingobium lucknowense		R(10)							S(30)	S(10)	S(30)				S(30)	S(30)			S(5)		S(5)		
Sphingobium quisquiliarum		R(10)							S(30)	S(10)	S(30)				S(30)	S(30)			S(5)		S(5)		
Sphingobium rhizovicinum					R	R				S					S				R			R	
Sphingobium suberifaciens	S(25)	S(10)	S(100)	S(20/10)		S(10)	S(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Sphingobium ummariense	R(10)	R(10)				R(10)		R(10)	S(30)	S(30)		S(30)			S(30)	S(30)		S(30)		S(30)			S(30)
Sphingobium vermicomposti	S(25)									S(10)					S(30)						S(5)		
Sphingobium wenxiniae		R(100)				S(30)		R(100)	S(30)	S(15)					S(10)								
Sphingobium xenophagum	R(25)	R(10)	R(100)	S(20/10)		R(10)	R(30)			S(10)	S(30)		S(30)	S(10)	IM(30)		S(30)				S(5)	S(5)	
Sphingobium yanoikuyae	S(25)	S(10)	R(100)	S(20/10)		S(10)	R(30)			S(10)	S(30)		S(30)	S(10)	S(30)		S(30)				S(5)	S(5)	
Tested type strains	11	23	7	7	4	18	7	8	17	20	15	6	7	9	27	8	7	4	9	2	14	7	7
R Total number	5	20	5	0	4	12	5	7	0	0	0	0	1	0	0	0	0	0	1	0	0	1	2
% of total tested type strains	45	87	71	0	100	67	71	88	0	0	0	0	14	0	0	0	0	0	11	0	0	14	29

Numbers in parenthesis represent concentrations of tested antibiotics in mg L^{-1} or (where indicated) in reactivity units (U), *S* susceptible, *IM* intermediate, *R* resistant

Norfloxacin	Ofloxacin	Sparfloxacin	Tosufloxacin	Cefaclor	Cefmetazol	Chephalothin	Cefazolin	Lincomycin	Erythromycin	Oleandomycin	Clarithromycin	Roxithromycin	Polymyxin B	vancomycin	cefotaxime	Cefoperazon	Ceftazidim	Aztreonam	Carumonam	Sulfamethoxazol + riTrimethoprim	Cotrimoxazol	Imipenem	Metropenem	Panipenem	Flomoxef	Chloramphenicol	trimetroprim
								R(2)	S(15)							S(30)					S(25)						
R(10)	R(5)	R(5)	R(5)	S(30)	R(30)		R(30)		IM(15)		S(15)	IM(15)	R(300)		S(30)	R(75)	S(30)	S(30)	S(30)	R		S(10)	R(10)	R(10)	R(30)		R(5)
R(10)	R(5)	S(5)	R(5)	S(30)	S(30)		S(30)		S(15)		S(15)	S(15)	R(30)		S(30)	S(75)	S(30)	S(30)	S(30)	R		S(10)	S(10)	S(10)	S(30)		R(5)
R(10)	R(5)	R(5)	R(5)	R(30)	R(30)		R(30)	R(2)	S(15)		S(15)	R(15)	S(30)		S(30)	S(30)	IM(30)	S(30)	IM(30)	R	S(25)	S(10)	IM(10)	IM(10)	IM(30)		R(5)
R(10)	R(5)	IM(5)	R(5)	IM(30)	R(30)		R(30)		IM(15)		S(15)	S(15)	R(30)		S(30)	S(75)	S(30)	S(30)	S(30)	R		S(10)	S(10)	S(10)	S(30)		R(5)
7	7	6	6	6	6		7	4	9		6	6	9	2	6	9	6	6	6	6	4	6	6	6	6	2	6
7	6	3	6	1	4		4	4	0		0	2	4	0	0	1	0	1	1	4	0	0	1	1	1	0	6
100	86	50	100	17	67		57	100	0		0	33	44	0	0	11	0	17	17	67	0	0	17	17	17	0	100
													S(30)	S(30)												S(30)	
														S(30)												S(30)	
S(10)	S(5)					R(30)		R(15)	S(15)					S(30)				S(30)				S(10)				S(30)	
									S(15)																		
S(10)	S(5)	S(5)	S(5)	S(30)	S(30)		R(30)		R(15)		R(15)	R(15)	S(30)		S(30)	S(75)	S(30)	R(30)	R(30)	S		S(10)	S(10)	S(10)	S(30)		R(5)
S(10)	S(5)	S(5)	S(5)	S(30)	S(30)		R(30)		S(15)		S(15)	S(15)	S(30)		S(30)	R(75)	S(30)	R(30)	R(30)	R		S(10)	S(10)	S(10)	S(30)		R(5)
R(10)	S(5)	S(5)	S(5)	S(30)	S(30)		R(30)		IM(15)		R(15)	R(15)	S(30)		S(30)	R(75)	R(30)	S(30)	S(30)	S		R(10)	R(10)	S(10)	R(30)		S(5)
						R(20)		R(2)	S(15)					S(30)	S(30)											S(30)	R(5)
													S(30)	S(30)												S(30)	
									S(10)																	S(30)	
																				S						S(30)	
									S(10)																	S(100)	
									R(15)					R(30)													
S(10)	S(5)	S(5)	S(5)	R(30)	S(30)		R(30)		S(15)		S(15)	S(15)	S(30)		S(30)	R(75)	S(30)	IM(30)	R(30)	R		S(10)	S(10)	S(10)	S(30)		R(5)
									S(10)																	S(25)	
									R(10)																	S(25)	
													S(30)	S(30)												S(30)	
						R(30)	R(30)	R(15)	S(15)					S(30)				R(30)				S(10)				S(30)	
													S(300)	S(30)												S(30)	
													S(300)	S(30)												S(30)	
R									R					R							S						
R(10)	S(5)	S(5)	S(5)	S(30)	S(30)		S(30)		S(15)		S(15)	IM(15)	S(30)		S(30)	S(75)	S(30)	R(30)	S(30)	S		S(10)	S(10)	S(10)	S(30)		R(5)
									R(10)					S(30)												S(30)	
						S(30)											S(30)			R			S(10)				
									S(10)																	S(30)	
S(10)	S(5)	S(5)	IM(5)	S(30)	S(30)		R(30)		IM(15)		S(15)	IM(15)	S(30)		S(30)	R(75)	S(30)	IM(30)	IM(30)	R		S(10)	S(10)	S(10)	S(30)		R(5)
IM(10)	S(5)	S(5)	IM(5)	S(30)	R(30)		R(30)		IM(15)		S(15)	R(15)	S(30)		S(30)	S(75)	S(30)	R(30)	R(30)	S		S(10)	S(10)	S(10)	R(30)		R(5)
9	8	7	7	7	7	4	8	3	19		7	7	12	12	8	7	8	9	7	9	1	9	8	7	7	16	8
3	0	0	0	1	1	3	7	3	5		2	3	0	2	0	4	1	5	4	4	0	1	1		2	0	7
33	0	0	0	14	14	75	88	100	26		29	43	0	17	0	57	13	56	57	44	0	11	13	0	29	0	88

modification further increased. Besides the genetically engineered *Zymomonas mobilis*, bacteria genes of *Zymomonas* are also used for the genetic engineering of other bacteria. Codon-optimized genes encoding the pyruvate decarboxylase and alcohol dehydrogenase of *Z. mobilis* were introduced into *Lactococcus lactis* to enable a high-yield ethanol formation by this model species (Solem et al. 2013).

Beside ethanol production, *Zymomonas mobilis* can also be used for the production of other substances, such as L-alanine (Uhlenbusch et al. 1991), carotenoids (Misawa et al. 1991), and sorbitol (Liu and Blaschek 2010). A recombinant strain containing a plasmid for the overexpression of glucose–fructose oxidoreductase (GFOR) significantly increased the yield of sorbitol and gluconic acid from glucose and fructose. By the application of divalent metals as Zn^{2+} to this process, enzymes of the Entner–Doudoroff pathway were inhibited and the yield of ethanol as byproduct could be decreased, increasing in parallel the yield of sorbitol.

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26 The Family *Xanthobacteraceae*

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Abstract
The family *Xanthobacteraceae*, established in 2005 on the basis of 16S rRNA phylogeny, is affiliated with the *Alphaproteobacteria*. Currently (May 2012) it encompasses 7 genera (*Xanthobacter* [type genus], *Ancylobacter*, *Azorhizobium*, *Labrys*, *Pseudolabrys*, *Pseudoxanthobacter*, and *Starkeya*) and 28 species. All members grow as aerobic chemoheterotrophs, but facultative chemolithoautotrophy with hydrogen and/or reduced sulfur compounds is found in many species. Nitrogen fixation is widespread; the genus *Azorhizobium* entirely consists of N_2-fixing symbionts that live in association with leguminous plants. Some species can grow on unusual substrates such as alkenes, halogenated aliphatic and aromatic compounds, terpenes, thiophenes, or polyaromatic compounds. Representatives of the family can be found worldwide in freshwater lakes and streams, soils, wetlands, and in polluted sites.

Taxonomy, Historical and Current

Family *Xanthobacteraceae* Lee, Liu, Anzai, Kim, Aono, and Oyaizu 2005, 1916[VP]

Xan.tho.bac.te.ra'ce.ae. N.L. masc. n. *Xanthobacter*, type genus of the family; suff. *–aceae*, ending to denote a family; N.L. fem. pl. n. *Xanthobacteraceae*, the *Xanthobacter* family.

Gram-negative, rod-shaped chemoorganotrophic or facultatively chemolithoautotrophic bacteria, motile or nonmotile. Do not form spores. Some species fix N_2. The major isoprenoid quinone is Q-10. The family *Xanthobacteraceae* was circumscribed on the basis of phylogenetic analysis of 16S rRNA sequences. The family is phenotypically, metabolically, and ecologically diverse.

Type genus: *Xanthobacter*.

The mol% G+C of the DNA varies between 61 and 69.

The family *Xanthobacteraceae* was created in 2005, based on 16S rRNA sequence comparisons (Lee et al. 2005). At the time of writing (May 2012), the family contained 7 genera with 28 species (Tables 26.1–26.7): *Xanthobacter* [type genus] (8 species, the names of 2 of which were thus far effectively but not validly published), *Ancylobacter* (7 species, including one whose name was effectively but not yet validly published), *Azorhizobium* (2 species), *Labrys* (7 species), *Pseudolabrys* (1 species), *Pseudoxanthobacter* (1 species), and *Starkeya* (2 species).

The genus *Xanthobacter* was established by Wiegel et al. (1978) based on numerical taxonomy comparisons of organisms assigned at the time to the genus *Corynebacterium*. The species *Corynebacterium autotrophicum* (Baumgarten et al. 1974) was renamed *Xanthobacter autotrophicus*, and proposed as the type species of the new genus. A comparative study showed that the

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Table 26.1
The genera classified within the family *Xanthobacteraceae*, as of May 2012

Genus	Number of species	Type species	General properties
Xanthobacter [type genus]	8[a]	*Xanthobacter autotrophicus*	Rod-shaped, sometimes twisted or pleomorphic cells. Refractile (polyphosphate) and lipid (poly-β-hydroxybutyrate) bodies are evenly distributed in the cells. Aerobic, with a strictly respiratory type of metabolism. Colonies are opaque and generally slimy, yellow due to the presence of zeaxanthin dirhamnoside. Nearly all strains can grow chemolithoautotrophically in mineral media under an atmosphere of H_2, O_2, and CO_2, as well as chemoorganoheterotrophically on methanol, ethanol, n-propanol, n-butanol, and various organic acids as carbon sources. The carbohydrate utilization spectrum is limited. Some strains can use substituted thiophenes as sole carbon, energy, and sulfur sources. N_2 is fixed under a decreased O_2 pressure
Ancylobacter	7[a]	*Ancylobacter aquaticus*	Curved rods. Rings are occasionally formed prior to cell separation. Cells are encapsulated. Some strains produce gas vesicles. Generally nonmotile or motile by means of a single polar flagellum. Obligately aerobic with a strictly respiratory type of metabolism. Colonies are white to cream colored. Chemoorganotrophic, using a variety of sugars or salts of organic acids as carbon sources. Chemolithotrophic growth has been reported on molecular hydrogen. Some strains are facultatively methylotrophic, using methanol and formate
Azorhizobium	2	*Azorhizobium caulinodans*	Motile, short, rod-shaped cells, showing peritrichous flagella on solid medium and one or more lateral flagella in liquid medium. Obligately aerobic. N_2 is fixed under microaerobic conditions. Among sugars, only glucose is oxidized; the favorite carbon substrates are organic acids such as lactate or succinate. Grow also on malonate and on proline. Starch is not hydrolyzed. Denitrification is not observed. Nodulate the stems and roots of leguminous plants of the genus *Sesbania*
Labrys	7	*Labrys monachus*	Motile or nonmotile cells; may or may not possess triangular radial symmetry. They may have two to three short prosthecae, can be aerobes or facultative anaerobes. Cells divide by budding. Most strains are obligately aerobic, chemoorganotrophic. Use carbohydrates and some organic acids as sole carbon and energy sources. May be facultative methylotrophs
Pseudolabrys	1	*Pseudolabrys taiwanensis*	Non-motile, short, rod-shaped cells that multiply by division and not by budding. Methanol, methylamine, formaldehyde, and formamide are not used as sole carbon sources
Pseudoxanthobacter	1	*Pseudoxanthobacter soli*	Motile, short, rod-shaped cells that accumulate poly-β-hydroxybutyrate as polar inclusion bodies
Starkeya	2	*Starkeya novella*	Nonmotile, short, rod-shaped cells. Strictly aerobic facultative chemolithoautotrophs that grow on thiosulfate and tetrathionate but not on elemental sulfur or thiocyanate. Neutrophilic and mesophilic. Growth is also observed with formate. Some strains may degrade methylated sulfides

[a]Including species whose names have been effectively but not yet validly published

nitrogen-fixing facultative chemolithotrophic isolate known as *Mycobacterium flavum* strain 301 (Federov and Kalininskaya 1961) can be classified within the new genus as *Xanthobacter flavus* (Malik and Claus 1979). Later 16S rRNA sequence comparison confirmed the phylogenetic relationship. Based on 16S rRNA phylogeny, the species *Blastobacter viscosus* 7d (Loginova and Trotsenko 1980) and *Blastobacter aminooxidans* 14a (Doronina et al. 1984) were moved from the genus *Blastobacter* (*Bradyrhizobiaceae*) to the genus *Xanthobacter* as *X. viscosus* and *X. aminoxidans* (Doronina and Trotsenko 2003).

The genus name *Ancylobacter* was proposed by Raj (1983) as a substitute for the genus name *Microcyclus* (Ørskov 1928). The name *Microcyclus* was earlier used for a fungus in 1904, and therefore the name was considered illegitimate, even though it appeared in the Approved Lists of Bacterial Names of 1980. The strain described by Ørskov as the type of *Microcyclus aquaticus* has been lost. Subsequently, new similar strains were isolated by Ørskov, and one of these strains (ATCC 25396) was proposed as the neotype strain of *M. aquaticus*, now *A. aquaticus* comb. nov. (Ørskov 1957; Larkin and Borrall 1979; Staley et al. 2005).

Table 26.2

Comparison of selected characteristics of the members of the genus *Xanthobacter*

Character	*X. autotrophicus* [a, b]	*X. agilis* [b, c]	*X. aminoxidans* [d]	*X. flavus* [b, e]	*X. polyaromaticivorans* [f]	*X. tagetidis* [g]	*X. viscosus* [d]	*X. xylophilus* [a,h]
Basonym	*Corynebacterium autotrophicum*		*Blastobacter aminooxidans*	Formerly assigned to *Mycobacterium flavum* (a name not found in the approved lists of names of 1980)	(A name effectively but not yet validly published)		*Blastobacter viscosus*	(A name effectively but not yet validly published)
Type strain	ATCC 35674	ATCC 43847	ATCC BAA-299	DSM 338	NR	DSM 11105	ATCC BAA-298	VKM B-2535
Cell size (μm)	0.4 × 1.0	0.7 × 1.1–3.6	0.8–1.0 × 1.5–3.5	0.5–0.7 × 1.0–2.5	NR	0.5 × 1.0	0.5–0.9 × 1.0–3.2	0.4 × 0.7
Motility	–	+	–	–	–	+	–	–
Growth at 15 °C	w	+	+	w	–	+	+	+
Growth at 37 °C	w	–	–	w	+	+	–	–
Growth at 45 °C	d, w	–	–	–	–	–	–	–
pH range for growth	5.0–9.0	NR	6.5–8.5	NR	NR	6.8–8.7	6.5–8.5	4.8–6.8
Growth with H_2 as energy source	+	+	+	+	+	+	+	–
Growth with thiosulfate as energy source	+	+	NR	+	NR	+	NR	–
N_2 fixation	+	+	+	+	+	+	+	–
Growth on formate, acetate, propionate, butyrate, pyruvate, succinate, fumarate	+	+	NR	+	+	+	Formate, butyrate, pyruvate: +	NR
α-ketoglutarate	+	+	NR	+	NR	+	+	NR
Methylamines	+	+	+	+	NR	NR	–	NR
Citrate	+	–	–	d	NR	+	–	+
Lactate	+	–	+	+	NR	NR	NR	NR
Malonate	d	–	NR	+	NR	NR	NR	NR
Lactose, sorbose, raffinose, rhamnose	+	–	NR	–	–	–	NR	–
Fructose	+	–	NR	d	–	+	+	–
Glucose	+	–	+	d	–	+	+	–
Mannose	d	–	NR	d	–	+	NR	–
Sucrose	d	–	NR	–	–	+	NR	–

Table 26.2 (continued)

Character	*X. autotrophicus* [a, b]	*X. agilis* [b, c]	*X. aminoxidans* [d]	*X. flavus* [b, e]	*X. polyaromaticivorans* [f]	*X. tagetidis* [g]	*X. viscosus* [d]	*X. xylophilus* [a,h]
Nitrate reduced to nitrite	+	–	+	+	+	W	+	NR
Urease	d	NR	–	–	–	NR	–	NR
G+C content of DNA (mol%)	65–70	68–69	69.1	68–69	65	68–69	66.3	63.6
Sample source and site	Soil, mud, water	Lake water, Switzerland	Sewage purification system of a paper mill, Russia	Turf podzol soil, USSR	Crude oil tank sludge	Roots of *Tagetes* plants	Activated sludge of a paper mill, Russia	Acidic water with decaying spruce wood

Data taken from: [a]Baumgarten et al. (1974); Wiegel et al. (1978)
[b]Wiegel (2005); Wiegel (2006)
[c]Jenni and Aragno (1987)
[d]Doronina and Trotsenko (2003)
[e]Malik and Claus (1979)
[f]Hirano et al. (2004)
[g]Padden et al. (1997)
[h]Zaichikova et al. (2010a)
NR not reported, *w* weak, *d* 11–89 % of the strains are positive
Additional data on growth substrates are given by Wiegel (2005, 2006) and are given in the original species descriptions

Table 26.3

Comparison of selected characteristics of the members of the genus *Ancylobacter*

Character	*A. aquaticus* [a]	*A. abiegnus* [b]	*A. dichloromethanicus* [c]	*A. oerskovii* [d]	*A. polymorphus* [e]	*A. rudongensis* [f]	*A. vacuolatus* [e, g]
Basonym	*Micrococcus aquaticus*	(A name effectively but not yet validly published)					Previously described as *'Renobacter vacuolatum'*
Type strain	ATCC 25396	VKM B-2563	DSM 21507	DSM 18746	DSM 2457	JCM 11671	DSM 1277
Cell size (μm)	0.3–1.0 × 1.0–3.0	0.65–0.9 × 1.35–1.50	0.4–0.5 × 0.7–0.9	0.5–0.6 × 0.9–1.7	0.8–1.0	0.6–0.8	0.8–1.0
Rod morphology	Curved	Cocci	Curved	Pleomorphic	Curved	Curved	Curved
Maximum growth temperature on slant	34	25	37	40	42	40	37
Growth at 3 % NaCl	–	–	–	+	+	+	–
Nitrate reduction	+	NR	–	–	+	–	+
Urease	+	NR	+	+	+	+	+
Autotrophic growth with H_2	+	–	+	–	+	–	+
Utilization of L-fucose	–	–	+	+	–	–	+
Arabinose	–	–	+	+	+	+	+
D-mannose	–	–	NR	+	–	–	–
L-rhamnose	–	–	–	+	–	–	–
D-malate	+	+	–	+	+	W	–
Malonate	+	NR	NR	+	–	+	+
Gluconate	+	+	NR	+	–	–	+
Citrate	+	+	–	–	–	+	–
Oxalate	+	+	NR	+	+	+	+
Dichloromethane	–	NR	+	NR	–	–	–
Gelatin hydrolysis	–	NR	–	–	+	+	+
G+C content of DNA (mol%)	66–69	66.8	64.5	68.0	65.5	68.2	65.5
Sample source and site	Woodlake waters, freshwater ponds	Acidic water with decaying spruce wood	Contaminated soil, chemical plant, Russia	Soil	River mud	Roots of *Spartina anglica*, China	Soil, Russia

Data taken from: [a]Ørskov (1928); Raj (1983)
[b]Zaichikova et al. (2010b)
[c]Firsova et al. (2009)
[d]Lang et al. (2008)
[e]Xin et al. (2006)
[f]Xin et al. (2004)
[g]Nikitin (1971)
w weakly positive, *NR* not reported
Additional data on growth substrates are given by Staley et al. (2005) and are given in the original species descriptions

Table 26.4
Comparison of selected characteristics of the members of the genus *Azorhizobium*

Character	*A. caulinodans*[a]	*A. doebereinerae*[b]
Type strain	LMG 6465	LMG 9993
Cell size (μm)	0.5–0.6 × 1.5–2.5	0.6–0.9 × 1.5
Motility	Peritrichous flagella on solid medium; one lateral flagellum in liquid medium	2–4 lateral flagella
Host	*Sesbania rostrata* (stem)	*Sesbania virgata* (root) can form ineffective root nodules in *S. rostrata*, *Macroptilium atropurpureum* and pseudonodules on *S. rostrata* stems
N_2 fixation	+	+
Growth on glucose	+	–
Sucrose	–	–
Mannitol	–	–
DL-lactate	+	+
L-leucine	+	–
G+C content of DNA (mol%)	66–68	NR
Sample source and site	Root and stem nodules of *Sesbania rostrata*	Root nodules of *Sesbania virgata*, Brazil

Data taken from: [a]Dreyfus et al. (1988)
[b]de Souza Moreira et al. (2006)
NR not reported
Additional data on growth substrates are given by Kuykendall (2005) and are given in the original species descriptions

Ancylobacter vacuolatus (Xin et al. 2006) was earlier described as *Renobacter vacuolatum* (Nikitin 1971).

Taxonomic rearrangement of obligately or facultatively chemolithoautotrophic organisms that oxidize reduced sulfur compounds, earlier classified as members of the genus *Thiobacillus*, led to the removal of *T. novellus* (Starkey 1934) (a member of the *Alphaproteobacteria*) from the genus *Thiobacillus* (*Betaproteobacteria*) and its reclassification as *Starkeya novella* gen. nov., comb. nov. (Kelly et al. 2000).

Phylogenetic Structure of the Family and Its Genera

Figure 26.1 shows a Neighbor Joining tree of the type strains of the 28 species of the family *Xanthobacteraceae*. The family is associated with the *Alphaproteobacteria*, close relatives being the families *Hyphomicrobiaceae*, *Bradyrhizobiaceae*, *Beijerinckiaceae*, *Methylocystaceae*, and *Acetobacteraceae*.

The family *Xanthobacteraceae*, originally with five genera (*Xanthobacter*, *Azorhizobium*, *Ancylobacter*, *Labrys*, and *Starkeya*) was proposed by Lee et al. (2005) based on 16S rRNA comparisons of the members of the *Alphaproteobacteria*. It is interesting to note that in an earlier phylogenetic analysis that did not include *Xanthobacter* spp., *Labrys monachus* did not cluster with *Starkeya novella* (*Thiobacillus novellus*) and with *Ancylobacter aquaticus* (Fritz et al. 2004). The close phylogenetic relationship between *Xanthobacter* and *Azorhizobium* was first reported in 1996 (Rainey and Wiegel 1996; Wiegel 2005). Already then, it was noted that members of *Xanthobacter* and *Azorhizobium* are intermixed in the tree, as seen also in *Fig. 26.1*. A Maximum Likelihood (RAxML) tree constructed (not shown) placed the two species of *Azorhizobium* on a single branch surrounded by members of the genus *Xanthobacter*. Both the Neighbor Joining and the Maximum Likelihood trees place the two *Starkeya* species between the *Ancylobacter* branches.

Genome Analysis

At the time of writing (May 2012), three genome sequences of members of the *Xanthobacteraceae* had been published (*Table 26.8*): the type strain of *Azorhizobium caulinodans* (Lee et al. 2008), the type strain of *Starkeya novella*, and *Xanthobacter autotrophicus* Py2, a strain that can grow on alkenes (van Ginkel and de Bont 1986) and has many interesting physiological and biochemical features as described below. The chromosomes are 4.77–5.37 Mbp in length and contain 4,483–4,847 predicted genes. *X. autotrophicus* Py2 in addition contains a 316-kb plasmid encoding 308 predicted proteins. Of the 4,717 predicted proteins encoded by the 5.37 Mbp genome of *A. caulinodans*, 3.7 % are unique for this organism. Most nodulation functions as well as a putative type-IV secretion system are found in a distinct "symbiosis region" (Lee et al. 2008).

Phages

Wilke and Schlegel (1979) described three phages infecting *Xanthobacter autotrophicus* strain GZ29. Two lytic phages CA1 and CA2 have heads of 61–68 nm diameter and tails of 98–100 and 166–175 nm length, respectively. A third phage designated CA3, with a head diameter of 37–43 nm, a 43–50 nm tail, and a small DNA molecule of 3.3 kDa, did not form plaques and was detected only by its transducing activity and by electron microscopy.

Forty-three plaque-forming phages against the stem-nodulating *Azorhizobium caulinodans* were isolated from rhizosphere soil of different leguminous plant species. They all had a head and short (14–18 nm) non-contractible and non-flexible tails and were assigned to the *Podoviridae* (Sharma et al. 2008).

Table 26.5

Comparison of selected characteristics of the members of the genus *Labrys*

Character	*L. monachus*[a]	*L. methylaminiphilus*[b]	*L. miyagiensis*[c]	*L. neptuniae*[d]	*L. okinawensis*[c]	*L. portucalensis*[e]	*L. wisconsinensis*[f]
Type strain	ATCC 43932	ATCC BAA-1080	NBRC 101365	LMG 23578	DSM 18385	LMG 23412	DSM 19619
Cell size (μm)	1.1–1.5	0.7–1.0 × 1.0–1.2	NR	0.7–0.9 × 1.2–1.5	NR	0.5–1.0 × 0.8–1.0	1.0–1.5 × 2.0-3.0
Motility	+	–	+	–	+	–	+
Temperature range (°C)	5–40	10–35	15–32	15–35	15–32	16–37	10–40
NaCl growth range (%)	0–0.7	NR	0–0.3	NR	0–0.3	NR	0–1.2
Capsule formation	–	+	+	+	+	+	+
Anaerobic growth	–	–	–	–	–	–	+
Oxidase	+	+	NR	–	+	+	+
Catalase	w	+	w	w	w	+	w
Growth on D-ribose	+	NR	NR	NR	NR	NR	+
D-xylose	+	NR	+	NR	+	NR	+
Sucrose	+	+	+	–	+	NR	+
Arabinose	+	NR	+	+	+	+	+
Cellobiose	+	NR	+	NR	+	NR	+
Fructose	+	+	+	+	+	NR	+
Lactose	–	+	NR	–	NR	+	–
G+C content of DNA (mol%)	65–68	65.7	61.0–61.4	62.7	62.3	62.9	NR
Sample source and site	Silt, Lake Mustjarv, Estonia	Sediment, Lake Washington, WA, USA	Grassland soil, Japan	Freshwater pond, Taiwan	Root nodule of *Entada phaseoloides*, Japan	Polluted sediment, Portugal	Water of Lake Michigan, WI, USA

Data taken from: [a]Vasilyeva and Semenov (1984)
[b]Miller et al. (2005)
[c]Islam et al. (2007)
[d]Chou et al. (2007)
[e]Carvalho et al. (2008)
[f]Albert et al. (2010)
w weakly positive, *NR* not reported
Additional data on growth substrates are given by Vasilyeva (2005) and are given in the original species descriptions

Table 26.6
Comparison of selected characteristics of the members of the monospecific genera *Pseudolabrys* and *Pseudoxanthobacter*

Character	*Pseudolabrys taiwanensis* [a]	*Pseudoxanthobacter soli* [b]
Type strain	CCUG 51779	DSM 19599
Cell size (μm)	NR	0.2 × 2.0
Motility	–	+
Mode of reproduction	Division	Budding
N_2 fixation	NR	+
Temperature range (°C)	15–37	10–37 (opt. 37)
pH range	4.2–8.5	5.5–10.0 (opt. 7.0)
Growth on methanol or methylamine	–	–
Formaldehyde, formamide	–	
D-ribose	NR	+
Fumarate	+	+
L-malate	w	+
DL-lactate	+	NR
3-hydroxybenzoate	+	–
Predominant quinones	Q-10	Q-10, Q-9, and Q-8
G+C content of DNA (mol%)	67	68.4
Sample source and site	Soil, Taiwan	Soil, Taiwan

Data taken from: [a]Kämpfer et al. (2006)
[b]Arun et al. (2008)
w weakly positive, *NR* not reported
Additional data on growth substrates are given in the original species descriptions

Table 26.7
Comparison of selected characteristics of the members of the genus *Starkeya*

Character	*S. novella* [a]	*S. koreensis* [b]
Basonym	*Thiobacillus novellus*	
Type strain	ATCC 8093	KCTC 12212
Cell size (μm)	0.4–0.8 × 0.8–2.0	0.4–0.8 × 0.8–2.0
Cell morphology	Short rods, coccoidal, or ellipsoidal	Highly curved rods, rings
Motility	–	–
Temperature optimum (°C)	25–30	28–30
pH optimum	7.0–8.0	6.5–8.0
Growth with 3 % NaCl	w	–
Growth on thiosulfate or tetrathionate	+	+
Formate	+	+
Methanol	+	+
Methylamine	–	–
Sucrose	–	–
Maltose	–	–
Citrate	–	+
Malate	–	–
Succinate	–	–
G+C content of DNA (mol%)	67.9	69
Sample source and site	Soil	Rice straw, S. Korea

Data taken from: [a]Starkey (1934), Kelly et al. (2000)
[b]Im et al. (2006)
w weakly positive, *NR* not reported
Additional data on growth substrates are given by Kelly and Wood (2005) and are given in the original species descriptions

Phenotypic Analyses

The Properties of the Genera and Species of *Xanthobacteraceae*

Phenotypically the members of the family *Xanthobacteraceae* are quite diverse. With the other members of the *Alphaproteobacteria*, they share a Gram-negative type of cell wall, presence of ubiquinone Q-10 as the major respiratory quinone (with Q-9, Q-8, and Q-11 sometimes found in minor amounts), and other chemotaxonomic traits such as the types of fatty acids present. Predominant polyamines are putrescine and *sym*-homospermidine, as characterized in *Xanthobacter autotrophicus* 7c and CB2, *Azorhizobium caulinodans*, and *Labrys wisconsinensis* (Hamana et al. 1990; Wiegel 2006; Albert et al. 2010). They are all aerobes, although *Labrys wisconsinensis* was described as a facultative anaerobe as growth was obtained anaerobically on plate count broth supplemented with 0.075 % agar (Albert et al. 2010). Chemolithoautotrophic growth is widespread within the family, with hydrogen and/or reduced sulfur compounds serving as electron donor and energy source. Another property found in many of the genera is the ability to fix N_2.

Genus *Xanthobacter* Wiegel, Wilke, Baumgarten, Opitz, and Schlegel 1978, 580[AL]

Xan.tho.bac'ter Gr. adj. *xanthos*, yellow; N.L. masc. n. *bacter*, rod, staff; N.L. masc. n. *Xanthobacter*, yellow rod.

Rod-shaped, sometimes twisted cells, 0.4–1.0 × 0.8–6.0 μm, nonmotile or motile by peritrichous flagella. Gram-negative type of cell wall. Pleomorphic cells are sometimes produced on media containing succinate and other tricarboxylic acid cycle intermediates, whereas coccoid cells as well as cells up to 10 μm long are produced on media containing an alcohol as the sole

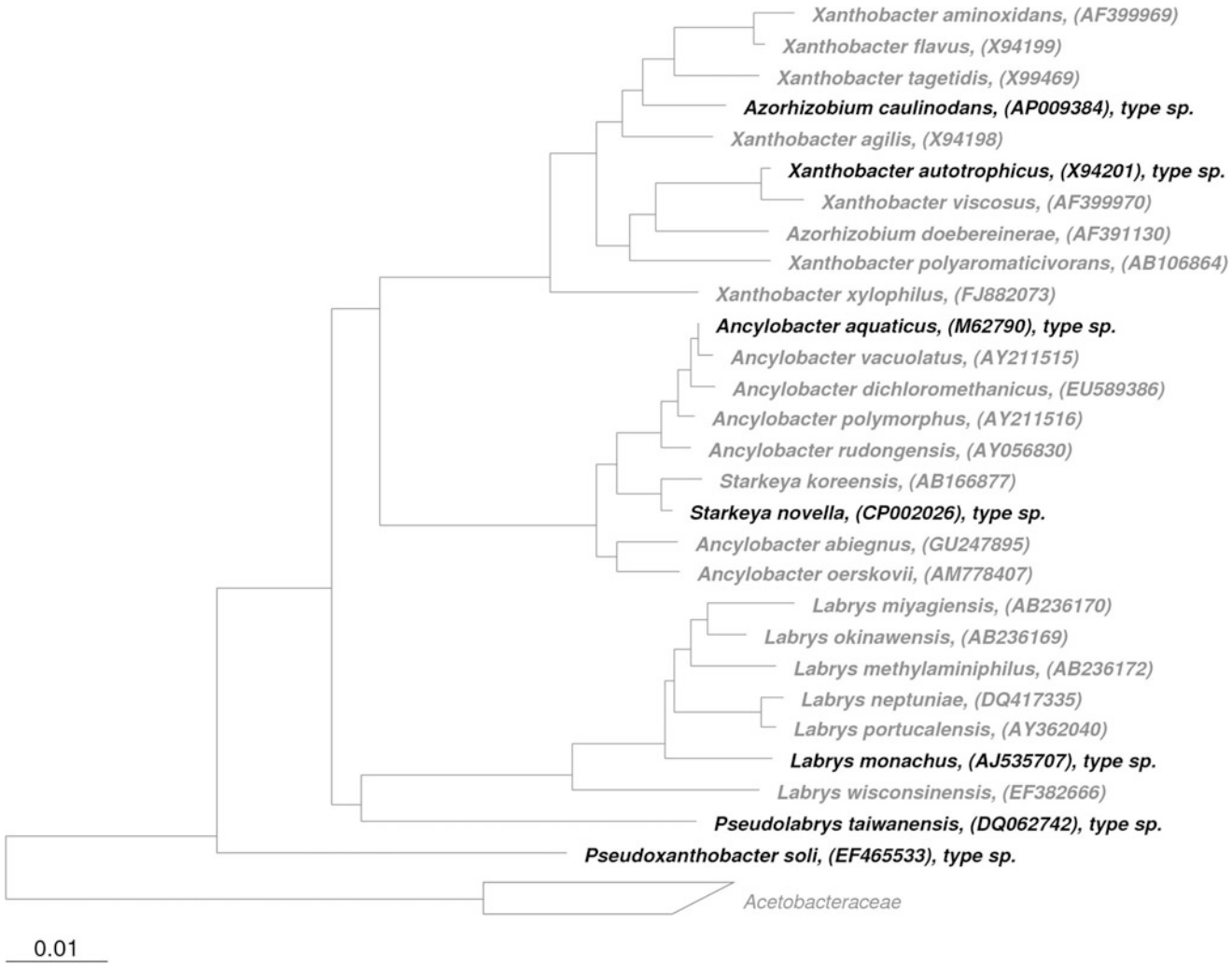

Fig. 26.1

Phylogenetic reconstruction of the family *Xanthobacteraceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence dataset and alignment were used according to the All-Species Living Tree Project (*LTP*) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. *Scale bar* indicates estimated sequence divergence

carbon source. Refractile (polyphosphate) and lipid (poly-β-hydroxybutyrate) bodies are evenly distributed in the cells. Due to polyphosphate granules, the Gram reaction frequently appears falsely to be positive or variable. Aerobic, with a strictly respiratory type of metabolism. Neutrophilic and mesophilic. Colonies are opaque and generally slimy, yellow due to the presence of zeaxanthin dirhamnoside. Most strains can grow chemolithoautotrophically in mineral media under an atmosphere of H_2, O_2, and CO_2, as well as chemoorganoheterotrophically on methanol, ethanol, n-propanol, n-butanol, and various organic acids as carbon sources. The carbohydrate utilization spectrum is limited. Some strains can use substituted thiophenes as sole carbon, energy, and sulfur sources. When degrading aliphatic epoxides, tested strains contain coenzyme M, which otherwise is a typical coenzyme of the obligate anaerobic methanogenic archaea. N_2 is fixed under a decreased O_2 pressure.

The mol% G+C of the DNA is 65–70.

Type species: *Xanthobacter autotrophicus*.

The genus *Xanthobacter* currently contains eight species: *X. autotrophicus*, *X. agilis*, *X. aminoxidans*, *X. flavus*, *X. polyaromativorans* (a name effectively but not yet validly published), *X. tagetidis*, *X. viscosus*, and *X. xylophilus* (a name effectively but not yet validly published). The main features of the members of the genus *Xanthobacter* are summarized in *Table 26.2*.

Table 26.8
Properties of the sequenced genomes of members of the *Xanthobacteraceae* (as of May 2012)

Property	*Xanthobacter* sp. Py2[a]	*Azorhizobium caulinodans* ORS571^{T} [b]	*Starkeya novella* DSM 506^{T}
Accession number	NC_009720 (chromosome) NC_009717 (plasmid)	AP009384	NC_014217 CP002026
Genome length (bp)	5,308,934 (chromosome)	5,369,772	4,765,023
G+C content	67.5 (chromosome)	67	67.9
Extrachromosomal elements	Plasmid pXAUT01 (316,164 bp; G+C content 65.3)	0	NR
% Coding bases	NR	89	NR
Number of predicted genes	4,847 (chromosome) 308 (plasmid)	~4,779	4,483
Predicted protein-coding genes	NR	4,717	4,431
% of proteins with putative function	NR	3,588	NR
Number of 16S rRNA genes	2	3	1

[a]For the description of the isolate, see van Ginkel and de Bont (1986)
[b]Data taken from Lee et al. (2008)
NR not reported

Additional comments:

- Irregular twisted cells (*X. tagetidis*) and branched cells (*X. autotrophicus*, *X. flavus*) are commonly found during growth on tricarboxylic acid cycle intermediates. Branching cells do not show septa at the branching points (Wiegel 2005).
- Most members of the genus multiply by symmetric division, but *X. viscosus* and *X. aminoxidans* reproduce by budding (Doronina and Trotsenko 2003). *X. polyaromaticivorans*, a species that degrades polycyclic and heterocyclic aromatic compounds, is atypical as it shows no autotrophic growth (Hirano et al. 2004).
- *X. flavus* was originally described as nonmotile. However, reexamination of the type strain showed peritrichous flagella in exponential cultures grown on methanol, ethanol, *n*-propanol, isopropanol, butanol, or gluconate, but not in media containing citrate, fumarate, malate, succinate, glutamate, glutamine, yeast extract, or in cells growing autotrophically on $H_2 + CO_2$ (Reding et al. 1992).
- Cellular fatty acids are high in $C_{18:1}$ and include $C_{18:0}$, 3-OH $C_{16:0}$, and (in *X. agilis*) $C_{19:0}$ cyclo ω7*c* (Urakami et al. 1995).
- *X. polyaromaticivorans* is slightly pinkish-orange, probably due to a zeaxanthin with terminal substitutions different from zeaxanthin dirhamnoside (Wiegel 2006).
- *X. xylophilus*, isolated from acidic low-salinity dystrophic water with decaying spruce wood, is moderately acidophilic, and uses organic acids (citrate, oxalate, succinate, gluconate), as well as xylose and xylan (Zaichikova et al. 2010a).
- *X. tagetidis* is associated with the roots of marigold (*Tagetes*) plants, which accumulate high concentrations of thiophenes. It grows on substituted thiophenes, and also is a facultative autotroph that can grow on H_2, thiosulfate, or sulfide as electron donors. It fixes CO_2 using ribulose bisphosphate carboxylase/oxygenase (Padden et al. 1997).
- All strains of *X. autotrophicus* and *X. flavus* produce an α-polyglutamine capsule located between the cell wall and the slime (Wiegel 2005).
- Two methanol degrading isolates 25P and 32P, classified as strains of *X. autotrophicus*, were described by Doronina et al. (1996).

Genus *Ancylobacter* Raj 1983, 397^{VP}

An.cy.lo.bac'ter. Gr. adj. *ankulos*, crooked, curved; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Ancylobacter*, a curved rod.

Curved rods, 0.3–1.0 × 1.0–3.0 μm. Rings (0.9–3.0 μm outer diameter) are occasionally formed prior to cell separation. Cells are encapsulated. Some strains produce gas vesicles. Gram-negative. Generally nonmotile or motile by means of a single polar flagellum. Obligately aerobic with a strictly respiratory type of metabolism. Colonies are white to cream colored. Oxidase and catalase positive. Chemoorganotrophic, using a variety of sugars or salts of organic acids as carbon sources. Chemolithotrophic growth has been reported on molecular hydrogen. Some strains are facultatively methylotrophic, using methanol and formate.

The mol% G+C of the DNA is 66–69.

Type species: *Ancylobacter aquaticus*.

The genus *Ancylobacter* currently contains seven species: *A. aquaticus*, *A. abiegnus* (a name effectively but not yet validly published), *A. dichloromethanicus*, *A. oerskovii*, *A. polymorphus*, *A. rudongensis*, and *A. vacuolatus*. The main features of the members of the genus *Ancylobacter* are summarized in *Table 26.3*.

Additional comments:

- Overviews of the biology of *Ancylobacter* were published by Raj (1989) and by Staley et al. (2005).

- Staley et al. (2005) consider the type strain of *A. aquaticus* to be avacuolate, despite a claim to the contrary (Raj 1977).
- Autotrophic growth of several strains was reported with hydrogen (Namsaraev and Nozhevnikova 1978; Malik and Schlegel 1981) or with thiosulfate as energy source (Stubner et al. 1998). CO_2 fixation is mediated by ribulose bisphosphate carboxylase/oxygenase (Loginova et al. 1978; Firsova et al. 2009).
- *A. dichloromethanicus*, isolated from contaminated soil, uses dichloromethane, methanol, formate, formaldehyde, and a range of larger carbon sources (Firsova et al. 2009).
- The major fatty acid of *A. aquaticus* is $C_{18:1}$; further present $C_{16:0}$, $C_{18:0}$ $C_{19:0}$ cyclo. The major respiratory quinone is Q-10, with minor amounts of Q-9 and Q-11 (Urakami and Komagata 1986).

Genus *Azorhizobium* Dreyfus, Garcia, and Gillis 1988, 97[VP]

A.zo.rhi.zo'bi.um. N.L. n. *azotum* [from Fr. n. *azote* (from Gr. prep. *a*, not; Gr. n. *zôê*, life; N.Gr. n. *azôê*, not sustaining life)], nitrogen; N.L. pref. *azo-*, pertaining to nitrogen; N.L. neut. n. *Rhizobium*, a bacterial generic name; N.L. neut. n. *Azorhizobium*, a nitrogen (using) *Rhizobium*.

Motile, short, rod-shaped Gram-negative cells, showing peritrichous flagella on solid medium and one or more lateral flagella in liquid medium. Obligately aerobic. N_2 is fixed under microaerobic conditions when nicotinic acid is provided. Oxidase- and catalase-positive; urease-negative. Among sugars, only glucose is oxidized; the favorite carbon substrates are organic acids such as lactate or succinate. Grow also on malonate and on proline. Starch is not hydrolyzed. Denitrification is not observed. Nodulate the stems and roots of leguminous plants of the genus *Sesbania*.

The mol% G+C of the DNA of the type species is 66.

Type species: *Azorhizobium caulinodans*.

The genus *Azorhizobium* currently contains two species: *A. caulinodans* and *A. doebereinerae*. The main features of the members of the genus *Azorhizobium* are summarized in *Table 26.4*.

Additional comments:

- Overviews of the properties of nitrogen-fixing stem nodules of *Sesbania* and the biology of *Azorhizobium* were published by Dreyfus and Dommergues (1981), Dreyfus et al. (1984), Goormachtig et al. (1998), and Kuykendall (2005).
- *A. doebereinerae* was isolated from root nodules of Brazilian woody species *Sesbania virgata*. Its colonies on YMA agar are similar to those of *A. caulinodans*: scant extracellular polysaccharide, fast to intermediate growth rate, and causing alkalinization of the medium. Neither *A. caulinodans* nor *A. doebereinerae* use mannitol or sucrose, compounds used by most *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Bradyrhizobium* spp. However, they can use DL-lactate. *A. doebereinerae* differs from *A. caulinodans* as it does not use either L-leucine or D-glucose (de Souza Moreira et al. 2006).

Genus *Labrys* Vasilyeva and Semenov 1985, 375[VP] (Effective Publication: Vasilyeva and Semenov 1984, p. 92 (Russian edition)); Emended Islam, Kawasaki, Nakagawa, Hattori and Seki, 2007, 556; Emended Albert, Waas, Langer, Pavlons, Feldner, Rosselló-Mora and Busse, 2010, 1757)

La'brys. N.L. masc. n. *Labrys* (from Gr. n. *labrus*), double-headed ax, an organism resembling a double-headed ax by the shape of the cell.

Motile or nonmotile cells; can be rod-shaped, and may or may not possess triangular radial symmetry. They may have two to three short prosthecae and can be aerobes or facultative anaerobes. Cells divide by budding. Buds are produced directly from the mother cell at the tip of the triangle that lacks prosthecae. In this stage, the cell resembles a double-headed ax. Most strains are aerobic, chemoorganotrophic. Use carbohydrates and some organic acids as sole carbon and energy sources. Oxidase and catalase positive. May be facultative methylotrophs. The primary polar lipids are diphosphatidylglycerol, phosphatidylmonomethylethanolamine, and phosphatidylcholine. The predominant fatty acids are $C_{19:0}$ cyclo ω8*c*, $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ω7*c*. The major ubiquinone is Q-10, and the major polyamine is *sym*-homospermidine.

The mol% G+C of the DNA is 61–68.

Type species: *Labrys monachus*.

The genus *Labrys* currently contains seven species: *L. monachus*, *L. methylaminiphilus*, *L. miyagiensis*, *L. neptuniae*, *L. okinawensis*, *L. portucalensis*, and *L. wisconsinensis*. The main features of the members of the genus *Labrys* are summarized in *Table 26.5*.

Additional comments:

- *L. neptuniae* was isolated from root nodules of the pan-tropical aquatic legume *Neptunia oleracea* (Chou et al. 2007).
- *L. portucalensis* is a fluorobenzene-degrading bacterium obtained from industrially contaminated sediment in northern Portugal. Fluorobenzene is used as a solvent in the pharmaceutical industry, as an insecticide, and as a reagent for plastic and resin polymers production (Carvalho et al. 2005, 2008).
- *L. okinawensis* and *L. miyagiensis* are budding bacteria isolated from rhizosphere habitats in Japan (Islam et al. 2007).
- *Labrys methylaminiphilus* was isolated from freshwater Lake Washington sediment following enrichment on

methylamine. It also uses a variety of mono- and disaccharides, organic acids, aromatic compounds, and alcohols. Methanol is not used. It also grows on polymers such as agarose and humic acid (Miller et al. 2005).
– Some species accumulate considerable amounts of poly-β-hydroxybutyrate (Vasilyeva 2005).

Genus *Pseudolabrys* Kämpfer, Young, Arun, Shen, Jäckel, Rosselló-Mora, Lai, and Rekha 2006, 2470[VP]

Pseu.do.la'brys. Gr. adj. *pseudês* false; N.L. masc. n. *Labrys* a bacterial genus name; N.L. masc. n. *Pseudolabrys* the false *Labrys*.

Nonmotile, short, rod-shaped Gram-negative cells that multiply by division and not by budding. Methanol, methylamine, formaldehyde, and formamide are not used as sole carbon sources. The major fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$, and $C_{19:0}$ cyclo ω8*c*.

The mol% G+C of the DNA of the type species and only species described is 67.

Type species: *Pseudolabrys taiwanensis*. The main features of the single member of the genus *Pseudolabrys* are summarized in Table 26.6.

Genus *Pseudoxanthobacter* Arun, Schumann, Chu, Tan, Chen, Lai, Kämpfer, Shen, Rekha, Hung, Chou, and Young 2008, 1573[VP]

Pseu.do.xan.tho.bac'ter. Gr. adj. *pseudês* false; N.L. masc. n. *Xanthobacter* a bacterial genus name; N.L. masc. n. *Pseudoxanthobacter* the false *Xanthobacter*.

Motile, short, rod-shaped Gram-negative or Gram-variable cells that accumulate poly-β-hydroxybutyrate as polar inclusion bodies. The major quinone is Q-10. The characteristic diamino acid of the peptidoglycan is *meso*-diaminopimelic acid. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine, phosphatyidyldimethylethanolamine, and an unknown aminolipid. The predominant fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo.

The mol% G+C of the DNA of the type species and only species described is 68.

Type species: *Pseudoxanthobacter soli*. The main features of the single member of the genus *Pseudoxanthobacter* are summarized in Table 26.6.

Additional comments:

– *P. soli* is a nitrogen-fixing species isolated from soil. Its major respiratory quinone is Q-10, with minor amounts of Q-9 and Q-8. The organism can fix N_2 and multiplies by budding (Arun et al. 2008).

Genus *Starkeya* Kelly, McDonald, and Wood 2000, 1800[VP]

Star.key'a. N.L. fem. n. *Starkeya*, named after Robert L. Starkey, who made important contributions to the study of soil microbiology and sulfur biochemistry.

Nonmotile, short, rod-shaped Gram-negative cells. Colonies grow on thiosulfate agar. Biotin is required. Strictly aerobic and facultative chemolithoautotrophic. Oxidize and grow on thiosulfate and tetrathionate but not on elemental sulfur or thiocyanate. Neutrophilic and mesophilic. Contain ubiquinone Q-10. Major cellular fatty acids are $C_{18:1}$ and $C_{19:0}$ cyclo. Growth is also observed with formate. Some strains may degrade methylated sulfides. Isolated from soils and presumably widely distributed.

The mol% G+C of the DNA is 67–69.

Type species: *Starkeya novella*.

The genus *Starkeya* currently contains two species: *S. novella* and *S. koreensis*. The main features of the members of the genus *Starkeya* are summarized in Table 26.7.

Additional comments:

– For optimal autotrophic development, biotin is required. When grown on formate, high levels of ribulose bisphosphate carboxylase/oxygenase are expressed (Kelly and Wood 2005).
– *S. novella* uses a variety of sugar alcohols, amino acids, carboxylic acids, and fatty acids for heterotrophic growth; reduced sulfur compounds serving as electron donors include thiosulfate, tetrathionate, dimethylsulfide, and dimethylsulfoxide (Kelly et al. 2000).
– A *S. novella* isolate from sewage was shown to oxidize methanethiol, dimethylsulfide, and dimethyldisulfide (Cha et al. 1999).

Isolation, Enrichment, and Maintenance Procedures

Strategies for the enrichment and isolation of *Xanthobacter* species can be based on the ability to fix N_2 under chemolithoautotrophic growth conditions under a gas mixture of H_2 (10 %), N_2 (70–75 %), CO_2 (10 %), and air (5–10 %), and on the specific yellow color and the characteristic "fried-egg" appearance of colonies caused by slime production on media containing 1 % succinate and nutrient broth, and the appearance of branched cells in such colonies (Wiegel 2005, 2006). Some isolates were obtained by chance, such as *X. autotrophicus* strain 7c, which was isolated from an enrichment for propane-oxidizing bacteria, using black mud of a pond in Germany as inoculum; the *Xanthobacter* obtained did not use propane. *X. tagetidis* can be reproducibly enriched from *Tagetes* (marigold) roots using thiophene-2-carboxylate or thiophene-2-acetate as substrates (Padden et al. 1997).

For enrichment and isolation of gas-vacuolated strains of *Ancylobacter*, 100 ml of a freshwater source can be added to a sterile aluminum foil-covered beaker containing 10 mg Bacto peptone (Difco). After 2 weeks incubation, at room temperature the culture is plated onto a hydrolysate medium containing glucose (Van Ert and Staley 1971; Staley et al. 2005). For the isolation of motile variants of gas-vacuolate strains of *Ancylobacter aquaticus*, selection on soft agar plates can be used: Growth radiating from the center of the colony consists of motile cells (Lara and Konopka 1987).

Ancylobacter oerskovii and two strains of *Ancylobacter polymorphus* were isolated from soil after enrichment with oxalate. All known species of the genus appear to use oxalate as the sole source of carbon (Lang et al. 2008), a finding that can probably be used as the basis for selective isolation procedures. Enrichment on dichloromethane as carbon and energy source, amended with a low concentration of yeast autolysate, was successfully applied for the isolation of *A. dichloromethanicus* (Firsova et al. 2009).

Azorhizobium grows well on nitrogen-free agar media, distinguishing the genus from other legume-nodulating bacteria (*Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*) (Dreyfus et al. 1988). Lactate and succinate are preferred substrates for growth (Kuykendall 2005). Selective inhibitors are also useful in studies of *Azorhizobium* and related plant-associated nitrogen-fixing bacteria: *Azorhizobium* is resistant to carbencillin; nalidixic acid inhibits *Azorhizobium* but allows growth of *Rhizobium* (Robertson et al. 1995).

Low nutrient concentrations may favor growth of *Labrys* spp. in enrichment cultures. This, *L. miyagiensis*, isolated from grassland soil in Japan, grows in 1/100 diluted nutrient broth but is inhibited by full-strength nutrient broth medium (Islam et al. 2007). *L. methylaminiphilus*, a facultatively methylotrophic bacterium obtained from sediment of Lake Washington, WA, USA, was enriched using mineral medium with 0.01 % methylamine (Miller et al. 2005). The type strain of *L. monachus* was isolated from silt samples from Lake Mustjarv (Estonia) using horse manure extract, obtained by heating 1 % (w/v) dry manure in distilled water. The sediment is left to settle and a liquid medium is prepared from the supernatant (Vasilyeva 2005). Some other *Labrys* species were obtained on nonselective media. *L. neptuniae* was isolated from root nodules of the tropical aquatic legume *Neptunia oleracea* on yeast extract —mannitol agar (Chou et al. 2007). Finally, *L. wisconsinensis* was isolated from Lake Michigan water as a colony that grew on Difco Plate Count Agar—a nonselective general medium for heterotrophic bacteria (Albert et al. 2010).

The type strains (and thus far only isolates) of the soil bacteria *Pseudoxanthobacter soli* and *Pseudolabrys taiwanensis* were both isolated as colonies on nutrient agar, without any prior enrichment or selection procedure (Arun et al. 2008; Kämpfer et al. 2006).

No selective isolation procedures for *Starkeya* spp. have yet been tested. Vitamins may be important in determining the outcome of enrichment and isolation experiments: For optimal autotrophic growth, *S. novella* requires biotin, and for optimal heterotrophic growth yeast extract, biotin, or other vitamins such as pantothenate may be required, depending on the substrate (Kelly et al. 2000). *S. koreensis* was isolated from rice straw using R2A agar, a nonselective general low-nutrient plating medium (Im et al. 2006).

Maintenance

Cultures can be maintained if they are refrigerated at 4 °C for a number of weeks. For long-term preservation, lyophilization is recommended.

Physiological and Biochemical Features

Physiologically and biochemically there are a number of noteworthy features within in the family *Xanthobacteraceae*: the ability of some strains of *Xanthobacter*, *Ancylobacter*, and *Labrys* to degrade chlorinated and brominated alkanes, alkenes, and aromatic compounds; the degradation of thiophenes by *X. tagetidis*; the ability of *Xanthobacter* spp. to grow on polyaromatic compounds; and the growth of some *X. autotrophicus* strains to grow on ethylene, propylene, and other alkenes.

Xanthobacter autotrophicus GJ10 was isolated on 1,2-dichloroethane as the sole carbon and energy source. It possesses a hydrolytic haloalkane dehydrogenase with a broad substrate specificity that degrades dichloroethane to 2-chloroethanol. This intermediate is further metabolized via chloroacetaldehyde to chloroacetic acid, which is dechlorinated by a second dehalogenase to glycolate. A similar strain GJ11 was isolated from sediment of the River Rhine (Janssen et al. 1984, 1985). *Xanthobacter* sp. strain TM1, isolated from a wastewater treatment plant receiving domestic and pharmaceutical effluent in Portugal, degrades dichloromethane, chloroacetic acid, dichloroethane, 2-chloroethanol, 2-fluorobenzoate, 3-fluorobenzoate, 4-fluorobenzoate, 2-chlorobenzoate, 4-chlorobenzoate, and methanol (Emanuelsson et al. 2009). *Xanthobacter* sp. strain ENV481, isolated from a microcosm model with aquifer soil and groundwater from a landfill, New Jersey, can degrade bis (2-chloroethyl) ether, a compound used as a solvent for fats and greases, a cleaning fluid for textiles, a constituent of paints and varnishes, and an insecticide (McClay et al. 2007). *Xanthobacter flavus* strain 14p1, isolated from river sludge in Germany, degrades 1,4-dichlorobenzene but no other aromatic or chloroaromatic compounds. The degradation pathway starts with dioxygenation, followed by ring opening via *ortho* cleavage of dichlorocatechol to 2,5-dichloro-*cis*,*cis*-muconic acid (Spiess et al. 1995; Spiess and Görisch 1996; Sommer and Gorisch 1997). *X. autotrophicus* strain GJ10 grows on 1,2-dichloroethane,

bromochloromethane, dibromomethane, and 1-bromo-2-chloroethane. A novel pathway of degradation of dihalomethanes to formaldehyde was proposed. Cells growing on 1,2-dichloroethane converted 2-fluoroethanol and 1-chloro-2-fluoroethane to 2-fluoroacetate (Torz et al. 2007). *Labrys portucalensis* can grow on fluorobenzene (Carvalho et al. 2008). Co-metabolism of chlorinated compounds may also occur: *X. autotrophicus* strains GJ10 and Py2 degrade trichloroethylene during growth on propene (Reij et al. 1995; Inguva and Schreve 1999). When grown on propylene, *Xanthobacter* strain Py2 can degrade trichloroethylene, 1-chloroethylene (vinyl chloride), 1,3-dichloropropylene, 2,3-dichloropropylene, and other related compounds. Addition of propylene oxide, propionaldehyde, and glucose enhanced the rate of degradation of chlorinated alkenes (Ensign et al. 1992).

Several isolates of the genus *Ancylobacter* degrade chlorinated aliphatic compounds. *A. dichloromethanicus* uses dichloromethane (Firsova et al. 2009). *A. aquaticus* strain GJ10 (Janssen et al. 1984, 1985) and strains AD25 and AD27, isolated from slurries of brackish water sediment and activated sludge, respectively (van den Wijngaard et al. 1992), degrade 1,2-dichloroethane. Strains AD25 and AD27 also use 2-chloroethylvinylether as sole carbon and energy source. Such chlorinated ethers are synthesized for the production of anesthetics, sedatives, and cellulose ethers (van den Wijngaard et al. 1993). Other chlorinated compounds broken down by *Ancylobacter* strains include 2-chloroethanol, chloroacetate, and 2-chloropropionate (Staley et al. 2005; van den Wijngaard et al. 1992). The organochlorine fungicide pentachloronitrobenzene (PCNB) could be degraded by *Labrys portucalensis* strain pcnb-21, isolated from a PCNB-polluted soil in China (Li et al. 2011). *Labrys* sp. strain Wy1, isolated from soil in a rubber estate in Malaysia, can use the herbicide 2,2-dichloropropionate (2,2-DCP) as sole source of carbon (Wong and Huyop 2011).

Xanthobacter tagetidis was isolated from the roots of marigold (*Tagetes*) plants. *Tagetes* species accumulate thiophenes in the roots at concentrations up to 1 % of the root mass. *X. tagetidis* grows on thiophenes such as thiophene-2-carboxylate, thiophene-3-carboxylate, on analogs of these compounds (pyrrole-2-carboxylate, furan-2-carboxylate), and on the condensed thiophene dibenzothiophene (Padden et al. 1997).

Xanthobacter polyaromaticivorans 127 W grows on a range of polycyclic and heterocyclic aromatic compounds under extremely low oxygen concentrations. Polycyclic aromatic hydrocarbons used include anthracene, fluorene, naphthalene, phenanthrene, dibenzothiophene, dibenzofuran, and biphenyl. It also degrades dibenzothiophene (Hirano et al. 2004). Another *Xanthobacter* strain, isolated from forest soil in the UK by enrichment with cyclohexane vapor as the carbon source, metabolizes cyclohexane via cyclohexanol, cyclohexanone, and 1-oxa-2-oxocycloheptane (ε-caprolactone) to adipic acid (Trower et al. 1985).

Biochemically one of the most interesting processes performed by some *Xanthobacter* isolates is the degradation of alkenes. Alkene-utilizing *Xanthobacter* strains were obtained from enrichment cultures with propene and 1-butene. A monooxygenase was found to be involved, forming 1,2-epoxyalkanes as intermediate (van Ginkel and de Bont 1986). Alkene degradation and the metabolism of the epoxide intermediates was studied in-depth in *X. autotrophicus* strain Py2, which grows on ethylene, propylene, and butylene (Small and Ensign 1997). The involvement of coenzyme M (2-mercaptoethanesulfonate) in the process came as a big surprise. Coenzyme M has been known for many decades as a central component of the biochemical pathway of methanogenesis in archaea, but it was never before found in any other organisms. *Xanthobacter* strain Py2 possesses a linear megaplasmid that encodes enzymes of aliphatic alkene and epoxide metabolism and coenzyme M biosynthesis. Epoxidation of propylene to epoxypropane is followed by a sequence of three reactions resulting in epoxide ring opening and carboxylation to form acetoacetate. Coenzyme M plays a central role in epoxide carboxylation by serving as the nucleophile for epoxy ring opening and as the carrier of the C_3 unit that is finally carboxylated to acetoacetate, releasing the coenzyme (Sluis and Ensign 1997; Krum and Ensign 2001; Krishnakumar et al. 2008; Pandey et al. 2011). Shotgun proteomics revealed proteins specific to growth on propylene, including the enzymes necessary for the biosynthesis of coenzyme M (Broberg and Clark 2010).

Another substrate metabolized via an epoxide intermediate is the terpene limonene, used by *Xanthobacter* sp. C20. This strain was isolated from the River Rhine using cyclohexane as the sole carbon and energy source. It possesses a novel bioconversion pathway in which limonene is converted to limonene-8,9-epoxide in a reaction that involves cytochrome P-450 (van der Werf et al. 2000).

Ecology

Members of the genus *Xanthobacter* may be ubiquitous in wet soil and sediments. They can be found in freshwater (*X. agilis*), wet soil containing decaying organic material (*X. autotrophus*, *X. flavus*), compost of root balls of *Tagetes* (*X. tagetidis*) (Padden et al. 1997), and they are associated with plant roots including wetland rice (Oyaizu-Masuchi and Komagata 1988; Reding et al. 1991; Wiegel 2005, 2006). One *X. flavus* originated from marine sediment (Lidstrom-O'Connor et al. 1983; Meijer et al. 1990). *X. xylophilus* is moderately acidophilic (opt. 5.5, range 4.8–6.8) and was found in acidic low-salinity dystrophic water with decaying spruce wood (Zaichikova et al. 2010a). *Xanthobacter* species may play an important role in the degradation of toxic organic compounds in polluted environments. *X. viscosus* and *X. aminoxidans* were found in activated sludge of a water treatment plant processing paper mill pulp (Loginova and Trotsenko 1980; Doronina et al. 1984; Doronina and Trotsenko 2003). *X. autotrophicus* was suggested to be involved in the biodegradation of toluene in a freshwater stream in Delaware (Tay et al. 1999).

Ancylobacter aquaticus is found in freshwater habitats, including ponds, creeks, and lakes (Van Ert and Staley 1971; Konopka et al. 1976) and in rice paddies and soil environments (Stubner et al. 1998). *A. abiegnus* was isolated from dystrophic

Table 26.9
Sensitivity of species of the family *Xanthobacteraceae* to selected antibiotics

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ampicillin	–	NR	–	+	–	–	+	–	–	+	+	–	+	NR	NR	+
Chloramphenicol	–	+	–	+	–	–	NR	–	–	NR	NR	–	NR	–	NR	NR
Erythromycin	–	NR	–	NR	NR	NR	NR	–	–	–	–	NR	–	–	–	NR
Novobiocin	+	NR	NR	NR	–	–	–	NR	NR	NR	NR	+	NR	NR	NR	NR
Penicillin	+	NR	–	–	NR	NR	NR	NR	–	+	+	–	+	NR	–	NR
Tetracycline	NR	NR	NR	–	NR	NR	NR	NR	–	+	+	+	+	-	+	+
Kanamycin	NR	NR	NR	NR	+	NR	NR	NR	–	+	+	+	+	–	NR	+
Streptomycin	NR	NR	NR	NR	+	+	+	+	+	NR	NR	+	NR	NR	NR	–

Species: *1 Xanthobacter autotrophicus*, *2 X. agilis*, *3 X. flavus*, *4 X.tagetidis*, *5 X. xylophilus*, *6 Ancylobacter abiegnus*, *7 A. dichloromethanicus*, *8 A. oerskovii*, *9 Labrys methylaminiphilus*, *L monachus*, *10 L. myagiensis*, *11 L. neptuniae*, *12 L. okinawensis*, *13 L. portucalensis*, *14 Pseudoxanthobacter soli*, *15 Starkeya koreensis*
+ sensitive, – resistant, *NR* not reported
Data were derived from the species descriptions (see *Table 26.2–26.7*) and from Wiegel (2005, 2006)

waters with decaying spruce wood (Zaichikova et al. 2010b). *Ancylobacter* species may also be involved in the degradation of toxic compounds. *A. dichloromethanicus*, a facultatively methylotrophic bacterium that can grow on dichloromethane, was isolated from contaminated soil in Russia (Firsova et al. 2009). *Ancylobacter* sp. strain XJ-412-1 degrades the herbicide metsulfuron-methyl. It was isolated from an agricultural soil in China, which had been exposed to sulfonylurea herbicides for many years (Lu et al. 2011).

Azorhizobium is associated with stem- or root nodules of *Sesbania* and some other leguminous plants (Dreyfus and Dommergues 1981; Dreyfus et al. 1984, 1988; Kuykendall 2005; de Souza Moreira et al. 2006). A comparative study of rhizosphere and non-rhizosphere soils in four vegetation zones of Senegal showed *Azorhizobium* to be more abundant on leaves and stems than *Rhizobium* in three out of the four vegetation zones. Approximately 90 % of the stem nodules and 39–48 % of the root nodules on *S. rostrata* were formed by *Azorhizobium* (Robertson et al. 1995).

Little is known about the distribution of *Labrys* species in nature. They have been recovered from unpolluted freshwater environments (Vasilyeva 2005; Albert et al. 2010) as well as from polluted sites (e.g., the fluorobenzene-degrading *L. portucalensis* (Carvalho et al. 2008). *L. neptuniae* was isolated from root nodules of the aquatic pan-tropical legume *Neptunia oleracea*, but it probably is not the dominant bacterium there: Over 95 % of the colonies of *Neptunia*-associated bacteria in Taiwan that developed on yeast extract—mannitol agar belonged to *Allorhizobium undicola* (Chou et al. 2007).

Hardly anything is known about the distribution and the ecological role of the genera *Pseudolabrys* and *Pseudoxanthobacter*. It is also known little about the ecological niches where *Starkeya* species may be important. The type strains of *S. novella* and *S. koreensis* were isolated from soil and from rice straw, respectively (Starkey 1934; Im et al. 2006), not from environments rich in sulfide.

Pathogenicity, Clinical Relevance

No pathogenic bacteria are known within the *Xanthobacteraceae*. The plant-associated species of the genus *Azorhizobium* that colonize the stems of *Sesbania* and some other leguminous plants live in symbiosis with their host, and they were never shown to cause harm to their plant hosts (Dreyfus et al. 1984; Kuykendall 2005).

Table 26.9 summarizes data on the sensitivity of members of the *Xanthobacteraceae* to different antibiotics. Such data are not available for all members of the family, and the information present does not show any clear patterns, possibly except for the fact that all species tested were sensitive to erythromycin. Wiegel (2006) commented that the available data do not suggest antibiotic typing as a valid method for identification of *Xanthobacter*. This may be the case for other genera of the family as well. The resistance of *Azorhizobium* to carbencillin was mentioned above and allows the selective isolation with the exclusion of *Rhizobium* (Robertson et al. 1995).

Application

As shown above, many representatives of the genera *Xanthobacter*, *Ancylobacter*, and *Labrys* degrade toxic compounds: chlorinated alkanes, alkenes, polyaromatic compounds, thiophenes, etc., and the bacteria may be involved in the biodegradation of such compounds in polluted environments. Many such stains were isolated from sites polluted with such chemicals. However, no applications based on the use of such bacteria in bioremediation operations are known.

The polysaccharide slime produced by *Xanthobacter* species may have interesting biotechnological applications. Wiegel (2006) mentioned the possible use of these polysaccharides as drag-reducing substances for minimizing friction in turbulent flows in pipelines and water turbines or as viscosifiers in oil fields.

An intriguing application of gas-vacuolated *Anyclobacter aquaticus* was proposed: Mosquitocidal toxin genes from *Bacillus sphaericus* and *Bacillus thuringiensis* var. *israelensis* were introduced into *Ancylobacter* by electroporation. The transformed cells exhibited significant toxicity toward mosquito larvae. Such transgenic *Ancylobacter* could be released in water bodies infested with mosquito larvae, and due to the buoyancy of the cells conferred by the gas vesicles the toxin will accumulate at the water surface (Yap et al. 1994). No information could be found whether this interesting idea has ever been developed into field applications.

Starkeya novella, due to its potential to oxidize sulfide and methylated sulfur compounds, and its adaptability to a broad pH range (5–10), could be applied in biofilters for the removal of bad-smelling sulfur compounds, for example, from piggery wastewater (Chung et al. 1997).

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Betaproteobacteria

27 The Family *Alcaligenaceae*

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Abstract
Alcaligenaceae, a family in the order *Burkholderiales* of the β-*Proteobacteria*, comprises the genera *Achromobacter*, *Advenella*, *Alcaligenes*, *Bordetella*, *Brackiella*, *Candidimonas*, *Castellaniella*, *Derxia*, *Kerstersia*, *Oligella*, *Paenalcaligenes*, *Paralcaligenes*, *Parapusillimonas*, *Pelistega*, *Pigmentiphaga*, *Pusillimonas*, and *Taylorella*. The cultures are Gram-negative asporogenous rods, which have been recovered from a variety of habitats, including animals and human diseases, e.g., *Bordetella pertussis* from whooping cough, and the terrestrial and aquatic environments. The major isoprenoid quinone is Q-8. The dominant fatty acids include $C_{16:0}$ and $C_{17:0}$ cyclo. The G+C content of the DNA is 36–73 mol%. 16S rRNA gene sequencing evidence has been used to delineate members of the family.

Taxonomy, Historical and Current

Short Description of the Family

Alcaligenaceae (Al.ca.li.ge.na'ce.ae. M.L. masc. N. *Alcaligenes* type genus of the family; M.L. masc. Pl. N. *Alcaligenaceae* the *Alcaligenes* family).

The family *Alcaligenaceae* was proposed by DeLey et al. (1986) to accommodate *Alcaligenes* and *Bordetella*, with the former as the type genus, which were regarded as closely related by a numerical phenotypic study. 16S rRNA sequencing with support from chemotaxonomic and phenotypic differences pointed to two distinct lineages within *Alcaligenes*, the first comprising *Alc. alcaligenes* and *Alc. faecalis* and the second the *Alc. defragrans* lineage, which was proposed as a new genus, *Castellaniella* (Kämpfer et al. 2006). Separately, a polyphasic study of nine *Alc. faecalis*-like organisms from human clinical material led to the recognition of a new genus, *Kerstersia* (Coenye et al. 2003b). Then, *Advenella* was named after a polyphasic study of 14 isolates obtained from human and veterinary samples (Coenye et al. 2005). Other additions included *Oligella*, which was proposed as a new genus to include taxa previously recognized as *Moraxella urethralis* (= *Oligella urethralis*) and CDC Group IVe (=*Oligella ureolytica*) (Rossau et al. 1987). *Parapusillimonas granuli* was recovered from environmental samples, i.e., granules in a wastewater-treatment bioreactor associated with alcohol fermentation (Kim et al. 2010). *Taylorella* was proposed as a new genus to accommodate organisms previously classified as *Haemophilus equigenitalis*, which were originally equated with *Haemophilus* because of the

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G+C ratio of the DNA (Taylor et al. 1978; Sugimoto et al. 1983). Moreover, the pattern of respiratory quinones and cellular fatty acids was different to *Haemophilus* (Sugimoto et al. 1983). Most recently, *Candidimonas* was proposed to accommodate two strains from sewage sludge compost that were equated with the family but could not be placed in any of the current genera (Vaz-Moreira et al. 2011).

Phylogenetic Structure of the Family and Its Genera

The phylogenetic tree revealed that the *Alcaligenaceae* belongs in the β-*Proteobacteria*, forming a distinct clade within the order *Burkholderiales* (Fig. 27.1) with component taxa revealing >95 % 16S rRNA sequence homology (Coenye et al. 2005). Comparative 16S rRNA sequencing pointed to two lineages within *Alcaligenes*, with *Alc. defragrans* comprising a second line, which was subsequently elevated to a new genus, as *Castellaniella* (Kämpfer et al. 2006).

Molecular Studies

Many studies have addressed 16S rRNA sequence homologies of organisms now regarded as members of the *Alcaligenaceae*. Homologies of <99 % have often been used to justify new species designations. Thus, sequence similarities between strain Se-1111R^T (=*Bor. petrii*) and other *Bordetella* spp. were in the range of 97.9–98.6 %, which supported the delineation of a new species, i.e., *Bor. petrii* (Von Wintzingeroda et al. 2001). Yet, *Bor. holmesii* F5101 was 99.5 % similar to *Bor. pertussis* (Weyant et al. 1995).

Five isolates were subjected to sequencing of the 16S rRNA gene and determined to show 93.5–95.3 % homology with *Alcaligenaceae* representatives leading to the proposal for a new genus, *Tetrathiobacter* (Ghosh et al. 2005), which were later reclassified to *Advenella* (Gibello et al. 2009). Sequencing of the 16S rRNA gene revealed that five isolates, LMG 22250^T, R-16599, R-20008, R-18191, and R-20007, which were named as *Advenella*, i.e., *A. incenata*, formed a single, stable phylogenetic lineage. RAPD and protein profiling data indicated multiple albeit as yet unnamed species which shared >97 % 16S rRNA gene sequence homology (Coenye et al. 2005). A 98.5 % sequence homology with *Adv. kashmirensis* was instrumental in leading to the delineation of DPN7^T as a new species, *Adv. mimigardefordensis* (Wübbeler et al. 2006).

Sequence data highlighted two lineages within *Alcaligenes*, with the second proposed as a new genus, *Castellaniella* (Kämpfer et al. 2006). Moreover, Ho-11^T shared a sequence homology of 97.8 % with *Cas. denitrificans* and was elevated into a new species as *Cas. caeni* (Liu et al. 2008). *Cas. daejeonensis* was closest in terms of homology to other castellaniellas; specifically there was 98.6 % 16S rRNA sequence homology with *Cas. denitrificans* (Lee et al. 2010a). DCT36^T shared 98.4 % homology with *Cas. caeni* and was elevated to a new species as *Cas. ginsengisoli* (Kim et al. 2009).

Sequence homology revealed that GT was most closely related to *Alc. faecalis* at 98.7 % homology leading to the delineation of a new subspecies, i.e., *Alc. faecalis* subsp. *parafaecalis* (Schroll et al. 2001). Furthermore, *Alc. faecalis* subsp. *faecalis* ATCC 8750^T shared 97 % and 96 % sequence similarity with *Alc. faecalis* sub. *phenolicus* and *Alc. faecalis* subsp. *parafaecalis* type strains, respectively (Rehfuss and Urban 2005). SC-089^T and SC-092^T were equated with *Alcaligenaceae*, but not with any of the existing genera leading to the description of *Candidimonas* (Vaz-Moreira et al. 2011). Subsequently, B59^T was reported to have 97.7 % and 97.6 % 16S rRNA gene sequence homology with *Can. nitroreducens* and *Can. humi*, respectively, leading to the recognition of a new species, i.e., *Can. bauzanensis* (Zhang et al. 2012). Separately, 16S rRNA gene sequencing of *Brackiella oedipodis* showed 92–93 % homology with other members of the family (Willems et al. 2002). Moreover, HL-12^T belonged in *Derxia* and displayed 98.8 % and 99.1 % sequence homology with two isolates of *D. gummosa*, leading to the description of a new species, *D. lacustris* (Chen et al. 2013). Meanwhile, comparative examination of 16S rRNA gene sequences of an organism revealed <95 % similarity with other groups in the *Alcaligenaceae*, the discrimination of which was supported by chemotaxonomy and led to the description of *Paenalcaligenes* (Kämpfer et al. 2010). Similarly, sequence analyses of a representative strain of *Pel. europaea* revealed that *T. equigenitalis* was the nearest neighbor (similarity = ~94.8 %) (Vandamme et al. 1998). Sequence data for *T. asinigenitalis* revealed high intraspecific homology between three isolates (>99.8 %) and a value of 97.6 % homology with *T. equigenitalis* (Jang et al. 2001).

16S rRNA gene sequencing of *K. gyiorum* LMG 5906^T with LMG 5890 revealed 99.3 % similarity (Vandamme et al. 2012). Use of the *gyrB* gene revealed gene sequence homologies of 98.9–99.2 % between *K. gyiorum* LMG 5906^T with LMG 5890, the latter strain of which was elevated into a new species as *K. similis* (Vandamme et al. 2012).

16S rRNA gene sequencing data served to link a single isolate, GR24-5^T, with the family *Alcaligenaceae*, revealing the highest similarity with *Parapusillimonas granuli* (homology = 97.1 %) leading to the proposition for a new taxon, i.e., *Paralcaligenes ureilyticus* (Kim et al. 2011). *Parapusillimonas granuli* was described after a polyphasic study including the results of 16S rRNA gene sequencing pointed to a relationship with *Pus. noertemannii* (similarity = 97.30 %). The original culture, B201^T, of *Pus. harenae* was most closely related to *Pus. ginsengisoli* with a 98.6 % 16S rRNA sequence homology (Park et al. 2011). Sequencing of the 16S rRNA gene revealed that *Pusillimonas* was related to the *Alcaligenaceae* albeit at 94–96 % homology (Stolz et al. 2005).

Pig. kullae demonstrated a 95–96 % 16S rRNA sequence homology with *Achromobacter*, *Alcaligenes*, and *Bordetella* pointing to membership of *Alcaligenaceae* (Blümel et al. 2001). K110^T demonstrated a 99.4 % sequence homology with *Pig. kullae* and was named as a new species, *Pig. daeguensis* (Yoon et al. 2007). *Pig. litoralis* was most closely related to other

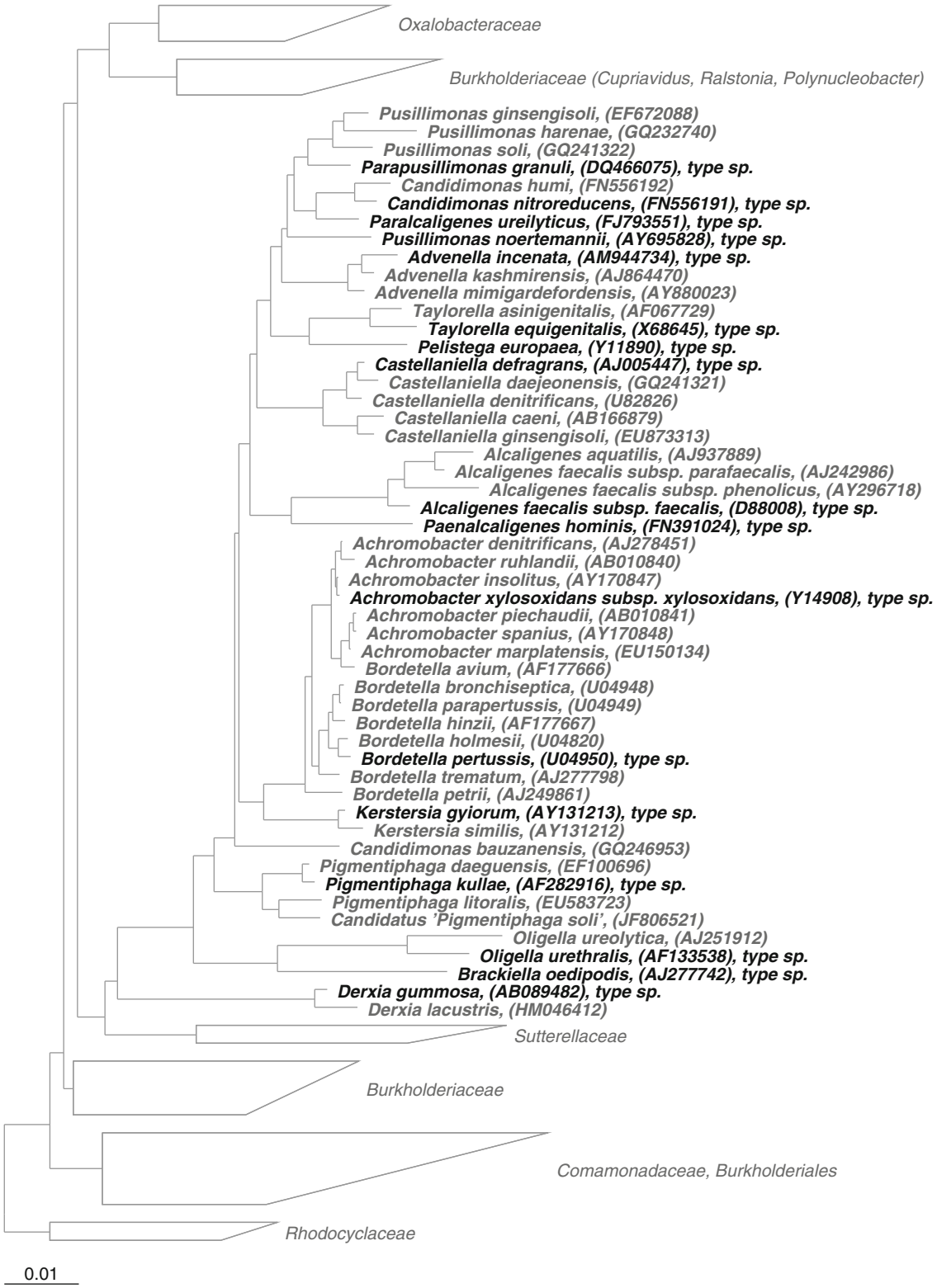

Fig. 27.1

Phylogenetic reconstruction of the family *Alcaligenaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. *Scale bar* indicates estimated sequence divergence

pigmentiphaga (Chen et al. 2009). The highest similarity of BS12^T was with *Pig. litoralis* (98.3 %) and led to the delineation of a new species, i.e., *Pig. soli* (Lee et al. 2011).

DNA-DNA Hybridization Studies

Numerous hybridization studies have involved representatives of the *Alcaligenaceae*. Thus, DNA:DNA confirmed the separation of *Ach. insolitus* from *Ach. spanius* and other achromobacters (Coenye et al. 2003a). *Adv. mimigardefordensis* shared 41 % homology with *Adv. kashmirensis* and much lower values with other representatives of the family (Wübbeler et al. 2006). Hybridization data pointed to the association of *Alc. denitrificans* with *Achromobacter*, leading to its reclassification as *Ach. denitrificans* comb. nov. (Coenye et al. 2003b). A DNA reassociation of 56 % was recorded between *Alc. faecalis* subsp. *parafaecalis* G^T and the type strain of *Alc. faecalis* (Schroll et al. 2001). Similarly, data for *Alc. faecalis* subsp. *phenolicus* J^T revealed homology values of 59.5 % and 60.2 % with *Alc. faecalis* subsp. *faecalis* ATCC 8750^T and *Alc. faecalis* subsp. *parafaecalis* G^T, respectively, thus indicating the distinctiveness of J^T from the other subspecies (Rehfuss and Urban 2005).

DNA:DNA hybridization suggested a close relationship between *Bordetella bronchiseptica*, *Bor. parapertussis*, and *Bor. pertussis* (DeLey et al. 1986) [Kloos et al. (1981) considered these taxa to be inseparable by DNA:DNA hybridization] but pointed to the uniqueness of other genera, notably *Parapusillimonas* which shared a homologies of <62 % with other taxa in the family (Kim et al. 2010). *Bor. petrii* shared 22.5–35.1 % DNA:DNA homology with other bordetellas (Von Wintzingeroda et al. 2001), which was well below the threshold of 70 %, which is taken as evidence of including within a species (Wayne et al. 1987). Similarly, *Bor. hinzii* demonstrated low binding homologies with other bordetellas and alcaligenes (Vandamme et al. 1995). *Bor. trematum* was distinct from other bordetellas; the highest DNA:DNA homology was with *Bor. bronchiseptica* at 30 % (Vandamme et al. 1996).

D. lacustris was related at 48.6 % and 51.5 % DNA homology with *D. gummosa* pointing to separate species status (Chen et al. 2013). The DNA:DNA relatedness of *Paralcaligenes ureilyticus* GR24-5^T was 23 % with *Parapusillimonas granuli* (Kim et al. 2011). *T. equigenitalis* hybridized with *T. asinigenitalis* to 23 % and 24 % indicated separate species status (Jang et al. 2001). Incidentally, *T. equigenitalis* displayed negligible DNA homologies with *Haemophilus* in which the organism was originally classified (Sugimoto et al. 1983). DNA:DNA relatedness between *Pus. harenae* and *Pus. ginsengisoli* and *Pus. soli* was 30.2 ± 5.4 % and 4.9 ±1.8 %, respectively, pointing to the distinction between the three species (Park et al. 2011).

DNA:DNA homology values of 23.7 2 % and 21.5 3.5 % between BZ59^T and *Can. nitroreducens* SC-089^T and *Can. humi* SC-092^T, respectively, were well below the threshold to justify inclusion with the same species (Wayne et al., 1987). The outcome was the recognition of a new species, i.e., *Can. bauzanensis* (Zhang et al. 2012).

Ribotyping

Ribotyping has been effective in epidemiological studies by distinguishing between different species and strains. Using 113 cultures from 11 host species worldwide, *Bor. bronchiseptica* was characterized by ribotyping with restriction enzyme *PvuII*, resulting in the recognition of 16 ribotypes containing 5–7 restriction fragments of 1.8–5.6 kb in size. Most [88 %] isolates from pigs were equated with ribotype 3 whereas those from dogs [74.1 %] belonged in ribotype 4, although there was not any correlation with geographical origin (Register et al. 1997). Furthermore, ribotyping with restriction enzyme *PvuII* was effective in allowing diagnostic laboratories to discriminate avian [=turkey]-respiratory-disease-causing *Bor. avium* from *Bor. hinzii* (Register et al. 2003).

Genome Sequencing

The genome sequences have been published for a range of *Alcaligenaceae* representatives, including *Ach. piechaudii* (Trimble et al. 2012), *Ach. xylosoxidans* (Jakobsen et al. 2010; Strnad et al. 2011), *Alc. faecalis* subsp. *faecalis* (Phung et al. 2012), *Bor. avium*, *Bor. bronchiseptica*, *Bor. parapertussis* (Sebaihia et al. 2006), *Bor. pertussis* (Zhang et al. 2011), *Pusillimonas* (Cao et al. 2011), *T. asinigenitalis*, and *T. equigenitalis* (Hebert et al. 2011; Hauser et al. 2012). The draft sequences of *Ach. piechaudii* HLE and *Alc. faecalis* subsp. *faecalis* NCIMB 8687 indicated genomes of 6.89 Mb (Trimble et al. 2012) and 3.9 Mb in 186 contigs, respectively (Phung et al. 2012). *Bor. avium* strain 197 N was reported to possess the smallest genome [3,732,255 bp] and gene complement [3417 predicted coding sequences] of any of the *Bordetella* sequenced to date (Sebaihia et al. 2006). >1,000 genes encoding surface and secreted proteins including genes for the synthesis of a type I secretion system, hemagglutinins, a polysaccharide capsule, two large genes for secreted proteins, and unique genes for both lipopolysaccharide and fimbria biogenesis in *Bor. avium* were absent from *Bor. bronchiseptica* (Sebaihia et al. 2006). The *T. asinigenitalis* genome comprises a single circular chromosome of 1,638,559 bp and 1,534 coding sequences (Hebert et al. 2012). The *T. equigenitalis* genome encodes many proteins associated with pathogenicity, including hemagglutinin-related proteins and a type IV secretion system (Hebert et al. 2012).

Phages

Bacteriophages have been recognized in *Achromobacter* (Jones and Pretorius 1981), *Alc. eutrophus* (Walthermauruschat and Mayer 1978; Faelen et al. 1993), and *Bordetella* (Paloheimo et al. 1987; Dai et al. 2010; Yuan et al. 2013). For example, a non-plaque forming prophage with 42 kb double-stranded DNA was recovered from *Alc. eutrophus* (Faelen et al. 1993).

Phenotypic Analyses

A comparison of *Alcaligenaceae* genera is given in Table 27.1.

Achromobacter Yabuuchi and Yano 1981

A. chro. mo. bac' ter Gr. adj. *Achromus* colorless; M. L. n. *bacter* the masc. equivalent of Gr. neut. N. *bactrum* a rod or staff; M. L. masc. N. *Achromobacter* colorless rodlet.

Cultures comprise straight obligately aerobic asporogenous chemoorganotrophic, motile by means of peritrichous flagella Gram-negative rods of 0.8–1.2 × 2.5–3.0 μm in size. Catalase and oxidase are produced, but not arginine dihydrolase, lysine or ornithine decarboxylase, or phenylalanine deaminase. DNA, gelatin, and urea are not degraded. Carbohydrates are usually not utilized. The dominant polyamines are putrescine and 2-hydroxyputrescine. The respiratory quinone is Q-8. The major fatty acids are $C_{12:0}$ 2OH, $C_{14:0}$ 3OH, $C_{16:0}$, $C_{16:1}$, and $C_{17:0}$ cyclo. The G+C ratio of the DNA is 65–70 mol%. The type species is *Achromobacter xylosoxidans* (Yabuuchi and Yano 1981). The distinguishing traits of *Achromobacter* species are given in Table 27.2.

Ach. xylosoxidans was divided into two subspecies, namely, *denitrificans* and *xylosoxidans*. The former was reclassified to *Ach. denitrificans* initially from *Alc. denitrificans* (Rüger and Tan 1983) and subsequently from *Ach. xylosoxidans* subsp. *denitrificans* (Yabuuchi et al. 1998).

Ach. xylosoxidans subsp. *xylosoxidans* comprises strictly aerobic, asporogenous motile by peritrichous flagella straight Gram-negative rods of 0.8–1.2 × 2.5–3.0 μm in size. Catalase and oxidase are produced, but not arginine dihydrolase or lysine decarboxylase. Nitrates are reduced. Simmons citrate is utilized. Neither [rabbit] blood DNA, gelatin, nor urea is degraded. Acid is produced slowly and weakly from D-arabinose, galactose, glucose, and mannose. The respiratory quinone is Q-8. The major fatty acids are $C_{12:0}$ 2OH, $C_{14:0}$ 2OH, $C_{14:0}$ 3OH, $C_{16:0}$, $C_{16:1}$, and C_{17} cyclo. The G+C ratio of the DNA is 65–70 mol%. The type strain is ATCC 27061^T (Yabuuchi and Yano 1981).

Ach. denitrificans comprises Gram-negative rods that are motile by peritrichous flagella. Acylamidase, catalase, and oxidase are produced, but not arginine dihydrolase, H_2S, lysine or ornithine decarboxylase, or phenylalanine deaminase. Growth occurs at 25–41 °C, in 0–5 % (w/v) NaCl and on MacConkey agar. Nitrates are reduced to gas. Neither aesculin, blood, DNA, gelatin, starch, Tween 80, nor urea is degraded. Simmons citrate but not malonate is utilized. Acid is produced from ethanol and glycerol, but not from adonitol, D-arabinose, L-arabinose, cellobiose, dulcitol, fructose, galactose, glucose, inositol, inulin, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose, or xylose. Adipic acid, DL-malic acid, phenylacetate, and sodium citrate are assimilated, but not *N*-acetyl-D-glucosamine, L-arabinose, *n*-capric acid, glucose, maltose, D-mannitol, D-mannose, or potassium gluconate. The dominant respiratory quinone is Q-8. The type strain is 15173^T, which has a G+C ratio of the DNA of 68.5 mol% (Yabuuchi et al. 1998).

Ach. insolitus produces white to light brown flat or slightly convex colonies with smooth edges that comprise Gram-negative, small (1–2 μm in length), and coccoid cells that occur singly, in pairs, or in short chains. Motility may occur. Growth occurs at 28–37 °C and in 0–4.5 % (w/v) NaCl. Catalase, esterase (C4), leucine arylamidase, and oxidase are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, chymotrypsin, cysteine or valine arylamidase, ester lipase (C8), α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, H_2S, indole, lipase (C14), lysine or ornithine decarboxylase, α-mannosidase, phosphoamidase, or trypsin. Neither aesculin, blood, DNA, gelatin, starch, nor urea is degraded. Nitrates are reduced. Growth occurs on acetamide. Acetate, aconitate, adipate, azelate, butyrate, citraconate, citrate, L-cysteine, fumarate, gluconate, glutarate, DL-glycerate, L-histidine, DL-3-hydroxybutyrate, isobutyrate, isovalerate, itaconate, DL-lactate, L-leucine, D- and L-malate, mesaconate, L-norleucine, pimelate, L-proline, pyruvate, sebacate, L-serine, suberate, succinate, *meso*-tartrate, L-threonine, tryptamine, L-tryptophan, *n*-valerate, and L-valine are assimilated, but not *N*-acetylglucosamine, adonitol, amygdalin, D- or L-arabinose, arbutin, cellobiose, diaminobutane, dulcitol, D-fructose, galactose, D-glucose, glycerol, inositol, inulin, lactose, maltose, mannitol, D-mannose, D-melezitose, melibiose, raffinose, L-rhamnose, ribose, salicin, sucrose, D-tartrate, trehalose, or D-xylose. The major cellular fatty acids are $C_{12:0}$, $C_{12:0}$ 2-OH, $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:0}$, $C_{18:1}$ *ω7c*, and summed features 2 and 3. The G+C content of the DNA is 64.9–65.5 mol%. The type strain is LMG 6003^T = API 201-3-84^T = CCUG 47057^T, which has a G+C ratio of the DNA of 64.9 mol% (Coenye et al. 2003a).

Ach. marplatensis comprises asaccharolytic, motile Gram-negative rods of 0.5–1.0 μm in length. Growth occurs at 15–40 °C, but not at 4 °C or 42 °C, and on MacConkey agar, but not in 6.5 % (w/v) NaCl. Acid and alkaline phosphatases, catalase, leucine arylamidase, oxidase, and phosphoamidase are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cysteine or valine arylamidase, esterase (C4), esterase (C8), esterase lipase, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, H_2S, indole, lipase (C14), lysine or ornithine decarboxylase, α-mannosidase, trypsin, or tryptophan deaminase. Nitrates and nitrites are reduced. The Voges-Proskauer reaction is negative. Tyrosine but not aesculin, casein, DNA, elastin, gelatin, lecithin, starch, Tween 80, or urea is degraded. Citrate, gluconate (weakly), malate, and phenylacetate are assimilated. Growth does not occur with *N*-acetylglucosamine, amygdalin, arabinose, arginine, caprate, glucose, inositol, lactose, lactulose, maltotriose, maltose, mannitol, mannose, melibiose, *p*-nitrophenyl β-D-galactopyranoside, raffinose, rhamnose, sorbitol, or sucrose. The predominant fatty acids are $C_{16:0}$, $C_{16:1}$ *ω7c*, and $C_{17:0}$ cyclo. The type strain is CCM 7608^T = CCET 7342^T = CCUG 56371^T (Gomila et al. 2011).

Table 27.1
Comparison of the phenotypic traits of *Alcaligenaceae* genera

Character	*Achromobacter*	*Advenella*	*Alcaligenes*	*Bordetella*	*Brackiella*	*Candidimonas*
Micromorphology	Gram-negative straight rods	Gram-negative small rods	Gram-negative rods and coccobacilli	Gram-negative coccobacilli	Gram-negative coccobacilli	Gram-negative short rods
Growth media	TSA	NA, TSA	LB, TSA	Complex, often with blood	Columbia agar	PCA
Growth temperature (°C)	28–37	30–37	23–35	(25)–37–(42)	30–37	10–40
Tolerance to NaCl [% w/v]	0–4	0–3				0–3
Metabolism	Obligately aerobic; chemoorganotrophic	Aerobic [acid is not always produced]	Obligately aerobic; chemoorganotrophic [produces alkali from organic acids and amides]	Strictly aerobic or facultatively anaerobic	Aerobic to microaerophilic	Aerobic or anaerobic; chemoorganotrophic
Motility	+ [peritrichous flagella]	variable	+ [peritrichous flagella]	+ or – [peritrichous flagella]	–	+ or –
Production of catalase and oxidase	+	+	+	+	+	
Production of arginine dihydrolase and lysine and ornithine decarboxylase	–	–	–		–	–
Nitrate reduction	+	+ or –	–	–	+	+
Degradation of DNA and gelatin	–	–	–	–	–	–
Polyamines	Putrescine, 2-hydroxyputrescine		Putrescine, 2-hydroxyputrescine			
Polar lipids						Phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine, diphosphatidylglycerol
Respiratory quinone	Q-8		Q-8	Q-8		Q-8
Dominant fatty acids	$C_{12:0}$ 2OH, $C_{14:0}$ 3OH, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$ cyclo	$C_{12:0}$, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1\omega7c}$, summed features 2 and 3	$C_{12:0}$ 2OH, $C_{14:0}$ 3OH, $C_{16:0}$, $C_{17:0}$ cyclo	$C_{16:0}$ and $C_{16:1}$ $\omega7c$ or $C_{17:0}$ cyclo	$C_{16:0}$, $C_{18:1}$ $\omega7c$, $C_{19:0\ cyclo}$ $\omega8c$	$C_{16:0}$, $C_{17:0}$ cyclo, summed feature 3
G + C ratio of the DNA	65–70 mol%	53.6–57.7 mol%	56–60 mol%	60–69 mol%		61.6–65 mol%

Species	*Ach. xylosoxidans* *Ach. denitrificans* *Ach. insolitus* *Ach. marplatensis* *Ach. piechaudii* *Ach. ruhlandii* *Ach. spanius*	*Adv. incenata* *Adv. kashmirensis* *Adv. mimigardefordensis*	*Alc. faecalis* subsp. *faecalis* *Alc. faecalis* subsp. *parafaecalis* *Alc. faecalis* subsp. *phenolicus* *Alc. aquatilis*	*Bor. pertussis* *Bor. avium* *Bor. bronchiseptica* *Bor. hinzii* *Bor. holmesii* *Bor. parapertussis* *Bor. petrii* *Bor. trematum*	*Bra. oedipodis*	*Can. bauzanensis* *Can. nitroreducens* *Can. humi*

Characteristic	*Castellaniella*	*Derxia*	*Kerstersia*	*Oligella*	*Paenalcaligenes*
Micromorphology	Gram-negative rods	Gram-negative pleomorphic rods	Gram-negative coccoid cells	Gram-negative small rods	Gram-negative rods
Growth media	LB, NA, R2A, TSA	R2A	NA, TSA	NA supplemented with blood, serum or yeast autolysate	NA
Growth temperature (°C)	(10)–30–(42)	15–40	28–42	42	30–42
Tolerance to NaCl [% w/v]	0–5	0–2(4)			
Metabolism	Facultatively anaerobic	Strictly respiratory; autotrophic—fixes molecular nitrogen [*D. lacustris* fixes nitrogen microaerophilically]		Does not produce acid in the oxidative-fermentative medium	Respiratory
Motility	+	+ [polar flagella]	May occur	Mostly nonmotile; some cultures of *O. ureolytica* motile with peritrichous flagella	+
Production of catalase and oxidase	+	+ [*D. gummosa* does not produce catalase]	+ [oxidase is not produced]	+	+
Production of arginine dihydrolase and lysine and ornithine decarboxylase	−			−	
Nitrate reduction	+	−	−	+	
Degradation of DNA and gelatin	− [gelatin—maybe weakly degraded]	−		−	
Polyamines	Putrescine				
Dominant polar lipids	Phosphatidylglycerol, diphosphatidylglycerol	Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol			Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine
Respiratory quinone	Q-8	Q-8			Q-8
Dominant fatty acids	$C_{16:0}$, $C_{16:1}\omega7c$, $C_{17:0}$ cyclo, $C_{18:1}$ $\omega7c$, and/or $C_{14:0}$ 3-OH	$C_{12:0}$ 3-OH, $C_{14:0}$ 2-OH, $C_{14:0}$ 3-OH, $C_{16:0}$, $C_{18:1}$ $\omega7c$, summed feature 3	$C_{14:0}$, $C_{14:0}$ 2-OH, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:0}$, $C_{18:1}$ $\omega7c$, $C_{19:0}$ cyclo $\omega8c$, summed features 2 and 3	$C_{16:0}$, $C_{18:1}$, $C_{19:cyc}$	$C_{16:0}$, $C_{17:0}$ cyclo, summed feature 3

Table 27.1 (continued)

Characteristic	*Castellaniella*	*Derxia*	*Kerstersia*	*Oligella*	*Paenalcaligenes*
G + C ratio of the DNA	63–66 mol%	69.2–72.6 mol%	61.5–62.9 mol%	46–47 mol%	57 mol%
Species	*Cas. defragrans* *Cas. caeni* *Cas. denitrificans* *Cas. daejeonensis* *Cas. ginsengisoli*	*D. gummosa* *D. lacustris*	*K. gyiorum*	*O. urethralis* *O. ureolytica*	*Pae. hominis*

Characteristic	*Paralcaligenes*	*Parapusillimonas*	*Pelistega*	*Pigmentiphaga*
Micromorphology	Gram-negative rods	Gram-negative rods	Gram-negative variably shaped cells	Gram-negative rods
Growth media	R2A	R2A	Blood agar, TSA	LB, NA, TSA in 50 % seawater
Growth temperature (°C)	10–30	25–37	37–42	(4)–30–42–(46)
Tolerance to NaCl [% w/v]	0–2			0–5
Metabolism	Strictly aerobic	Facultatively anaerobic	Microaerophilic; glucose is oxidized to alkali	
Motility	+ [single polar flagella]	+ [with 3 flagella]	−	+ [with single subterminal flagella] or −
Production of catalase and oxidase	+	+	+	+
Production of arginine dihydrolase and lysine and ornithine decarboxylase	−	Arginine dihydrolase is produced	− [arginine dihydrolase may be produced by some cultures]	− [arginine dihydrolase may be produced]
Nitrate reduction	−	−	−	−
Degradation of DNA and gelatin	−		− [gelatin is attacked by most cultures]	−
Polyamines	Cadaverine, putrescine, 2-hydroxyputrescine	Putrescine		
Dominant polar lipids	Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine			Phosphatidylethanolamine

Respiratory quinone	Q-8	Q-8		Q-8
Dominant fatty acids	$C_{16:0}$, $C_{17:0}$ cyclo, summed feature 1	$C_{16:0}$, $C_{17:0}$ cyclo, summed features 3 and 5	$C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ $\omega7c$, $C_{16:1}$ $\omega5c$, $C_{16:0}$ (3-OH), summed features 3 and 7	$C_{16:0}$
G + C ratio of the DNA	55.1 mol%	67.9 ± 0.1 mol%	~42–43 mol%	65.5–68.8 mol%
Species	*Paralcaligenes ureilyticus*	*Parapusillimonas granuli*	*Pel. europaea*	*Pig. kullae* *Pig. daeguensis* *Pig. litoralis* *Pig. soli*

Characteristic	*Pusillimonas*	*Taylorella*
Micromorphology	Small Gram-negative rods	Gram-negative coccobacilli
Growth media	NA, R2A	Complex media, e.g., chocolate agar, ECA
Growth temperature (°C)	(15)–30–40–(45)	30–42
Tolerance to NaCl [% w/v]	0–6	
Metabolism	Oxidative	Microaerophilic, chemoorganotrophic
Motility	+ [polar or lateral flagella]	–
Production of catalase and oxidase	+	+
Production of arginine dihydrolase and lysine and ornithine decarboxylase	–	–
Nitrate reduction	+ or –	–
Degradation of DNA and gelatin	–	–
Polyamines	Putrescine, spermidine, and 2-hydroxyputrescine	
Dominant polar lipids	Two unknown aminolipids, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine	
Respiratory quinone	Q-8	
Dominant fatty acids	$C_{16:0}$, $C_{17:0}$ cyclo, summed feature 2	$C_{16:0}$, $C_{18:0}$, $C_{19:0}$ 10-methyl, summed features 3, 4, and 7
G + C ratio of the DNA	53.1–61.8 mol%	36.5–37.8 mol%
Species	*Pus. noertemannii* *Pus. ginsengisoli* *Pus. harenae* *Pus. soli*	*T. equigenitalis* *T. asinigenitalis*

Table 27.2
Differentiating phenotypic properties of *Advenella*

Characteristics	*Adv. incenata*	*Adv. kashmirensis*	*Adv. mimigardefordensis*
Nitrate reduction	–	+	–
Esterase (C4)[a]	+	+	–
Assimilation of			
Glycerol[b]	+	–	+
2-Ketogluconate[b]	+	–	–

[a]Data from API ZYM
[b]Data from API 20NE and API ID 32GN

Ach. piechaudii produces small (1 mm in diameter) unpigmented, smooth colonies with entire edges on tryptone soya agar (TSA) after 48 h incubation and comprises obligately aerobic, straight asporogenous, nonencapsulated, motile with 2–8 peritrichous flagella Gram-negative rods of 0.5–1.0 x 1.0–1.5 µm in size that occur singly. Growth occurs at room temperature to 37 °C [optimally at 28–30 °C] but not at 5 °C or 42 °C and in 0–7 % (w/v) NaCl. Catalase, H_2S, and oxidase are produced, but not arginine dihydrolase, β-D-galactosidase, indole, lysine or ornithine decarboxylase, phenylalanine deaminase, or phosphatase. Tributyrin and tyrosine are degraded, but not aesculin, casein, DNA, gelatin, starch, Tween 20 or 80, or urea. Nitrates are reduced to nitrite. Utilizes citrate, but not malonate. Assimilates D-gluconate, isovalerate, itaconate, mesaconate, and *n*-valerate, but not carbohydrates. Produces acid from ethanol, but not adonitol, L-arabinose, D-cellobiose, dulcitol, D-fructose, D-glucose, glycerol, *m*-inositol, lactose, maltose, mannitol, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose, or D-xylose. The dominant polyamines are putrescine and 2-hydroxyputrescine. The fatty acid profile comprises $C_{12:0}$ 2OH, $C_{14:0}$, $C_{14:0}$ 3OH, $C_{16:0}$, $C_{16:0}$ 2OH, $C_{17:0}$ cyclo, $C_{18:0}$, $C_{18:1}$, and $C_{19:0}$ cyclo. The type strain is CIP 60.75^T = ATCC 43552^T = IAM 12591^T = LMG 1873^T, which has a G+C ratio of the DNA of 64–65 mol% (Kiredjian et al. 1986; Yabuuchi et al. 1998).

Ach. ruhlandii comprises asporogenous, nonfermentative motile with peritrichous flagella Gram-negative rods that grow on MacConkey agar, and at 41 °C, and in the presence of 5 % (w/v) NaCl. Growth occurs autotrophically with hydrogen. Catalase and oxidase are produced, but not arginine dihydrolase, lysine or ornithine decarboxylase, or phenylalanine deaminase. Nitrate is reduced to nitrite. Aesculin, DNA, gelatin, starch, Tween 80, and urea are not degraded. Adipic acid, capric acid, citrate, gluconate, glucose, malic acid, and phenylacetate are assimilated. Alkali is produced in Simmons citrate. Acid is produced from D- and L-arabinose, glucose, D-ribose, and D-xylose, but not from adonitol, cellobiose, dulcitol, fructose, galactose, glycerol, inositol, inulin, lactose, maltose, mannitol, melezitose, melibiose, raffinose, salicin, sorbitol, sucrose, or trehalose. The type strain is ATCC 15749^T = DSM 653^T = IAM 12600^T, which has a G+C ratio of the DNA of 68.1 mol% (DeLey et al. 1986; Yabuuchi et al. 1998).

Ach. spanius produces white to light brown flat or slightly convex colonies with smooth edges that comprise small (1–2 µm long) Gram-negative coccoid cells that occur singly, in pairs, and as short chains. Motility may occur. Catalase, esterase (C4), leucine arylamidase, and oxidase are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, chymotrypsin, cysteine or valine arylamidase, ester lipase (C8), α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, H_2S, indole, lipase (C14), lysine or ornithine decarboxylase, α-mannosidase, phosphoamidase, or trypsin. Nitrates are reduced to nitrites. Neither aesculin, blood, DNA, gelatin, starch, nor urea is degraded. Growth occurs at 28–37 °C and in 0–4 % (w/v) NaCl, but does not occur on acetamide. β-alanine, DL-3-aminobutyrate, diaminobutane, 2-ketoglutarate, tryptamine, and L-tyrosine are assimilated, but not acetate, *N*-acetylglucosamine, aconitate, adipate, adonitol, aesculin, amygdalin, D- or L-arabinose, arbutin, benzoate, butyrate, caprate, *n*-caproate, cellobiose, citrate, L-citrulline, dulcitol, erythritol, D-fructose, D- or L-fucose, fumarate, galactose, gluconate, D-glucose, glycerol, glycine, heptanoate, L-histidine, *p*-hydroxybenzoate, inositol, inulin, isobutyrate, L-isoleucine, DL-kynurenine, lactose, L-leucine, L-lysine, maltose, mannitol, D-mannose, D-melezitose, melibiose, mesaconate, L-methionine, L-ornithine, pelargonate, L-proline, propionate, pyruvate, raffinose, L-rhamnose, ribose, salicin, L-serine, sorbitol, succinate, sucrose, L-threonine, trehalose, or D-xylose. The major fatty acids are $C_{12:0}$ 2-OH, $C_{14:0}$, $C_{16:0}$, $C_{16:0}$ 2-OH, $C_{17:0}$ cyclo, $C_{18:0}$, $C_{18:1}$ $\omega7c$, and summed features 2 and 3. The G+C content of the DNA is 64.9 mol%. The type strain is LMG 5911^T = API 198-2-84^T = CCUG 47062^T, which has a G+C ratio of the DNA of 64.9 mol% (Coenye et al. 2003a).

Advenella Coenye et al. 2005

Ad.ven.el'la. L. n. *advena a* stranger, a foreigner; L. dim. ending –*ella*; N. L. fem. n. *Advenella* the little stranger—the source of the cultures is unknown.

Cultures comprise Gram-negative small (1–2 µm in length) rods or coccoid-shaped cells that occur singly, in pairs, or short chains. Motility may occur. Colonies on nutrient agar are light brown and flat or slightly convex with smooth edges. Cultures do not always produce acid in the oxidative-fermentative test medium, but produce catalase, leucine arylamidase, and oxidase, but not amylase, arginine dihydrolase, chymotrypsin, C_{14}-lipase, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, *N*-acetyl-β-glucosidase, β-glucuronidase, H_2S, indole, lysine or ornithine decarboxylase, α-mannosidase, trypsin, or valine arylamidase. Neither aesculin, blood, DNA, gelatin nor Tween 80 is degraded. Citrate is utilized. Growth occurs at 30 °C and 37 °C in 0–3 % (w/v) NaCl. The dominant fatty acids are $C_{12:0}$, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1\omega7c}$, and summed features 2 and 3. The G+C ratio of the DNA is 53.6–57.7 mol%. The type species is *Advenella incenata*

Table 27.3
Differentiating phenotypic properties of *Achromobacter*

Characteristics	*Ach. xylosoxidans*	*Ach. denitrificans*	*Ach. insolitus*	*Ach. spanius*	*Ach. marplatensis*	*Ach. piechaudii*	*Ach. ruhlandii*
Growth at 22 °C			–	–	+	+	+
Growth at 42 °C	+	Weak	+	–	–	Weak	+
Nitrite reduction	+	+	–	–	+	–	–
Utilization as source of carbon							
D-glucose	+	–	–	–	–	–	+
Utilization of acetamide	+	–	+	–	+	–	–

(Coenye et al. 2005). The distinguishing traits of *Advenella* species are given in *Table 27.3*.

Adv. incenata cultures match the genus description and also produce alkaline phosphatase and utilize L-malate, but do not reduce nitrates. Adipate, D-arabinose, D- and L-fucose, galactose, gluconate, glycerol, 2-ketogluconate, rhamnose, ribose, and D- and L-xylose are assimilated, but not D-adonitol, D- or L-arabitol, dulcitol, erythritol, D-fructose, gentiobiose, glycogen, inositol, inulin, D-lyxose, melezitose, melibiose, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, raffinose, sorbitol, L-sorbose, starch, sucrose, tagatose, trehalose, turanose, or xylitol. The G+C ratio of the DNA is 54.0–54.5 mol%. The type strain is LMG 22250^T = CCUG 45225^T (Coenye et al. 2005; Gibello et al. 2009).

Twelve cultures were classified into a newly described genus, *Tetrathiobacter*, as *Tet. kashmirensis* (Ghosh et al. 2005) but were subsequently reclassified to *Advenella* as *Adv. kashmirensis* (Gibello et al. 2009). Characteristics include the ability to reduce nitrate to nitrite and to utilize ammonium salts and urea but not glutamate, aspartate, or nitrate as nitrogen sources. Neither yeast extract nor vitamins are required for growth. Growth occurs in 1 M NaCl. Esterase (C4) and acid [weakly] and alkaline phosphatase are produced, but not cystine arylamidase or esterase lipase (C8). Acetate, adipate, D- and L-arabinose, citrate, L-cysteine, D-fructose, D- and L-fucose, D-galactose, gluconate, D-glucose, L-glutamate, D-lactose, L-lysine, malate, rhamnose, ribose, succinate, and D- and L-xylose are assimilated chemoorganotrophically, but not D-adonitol, D- or L-arabitol, benzoate, dulcitol, erythritol, gentiobiose, glycerol, glycogen, L-histidine, inositol, inulin, L-isoleucine, 2-ketogluconate, L-leucine, D-lyxose, *myo*-DL-lactate, D-mannitol, melezitose, melibiose, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, oxalate, D-raffinose, L-serine, sorbitol, L-sorbose, starch, sucrose, tagatose, L-threonine, trehalose, turanose, L-tyrosine, or xylitol. There is strain-dependent utilization of L-arginine, L-aspartic acid, D-maltose, D-mannose, and L-tryptophan and as the sole source of carbon. The major fatty acids are $C_{16:0}$, $C_{18:1}$ *ω7c*, and summed feature 3. The type strain is LMG 22695^T = MTCC 7002^T, which has a G+C content of the DNA of 55.1 mol% (Ghosh et al. 2005).

Tet. mimigardefordensis (Wübbeler et al. 2006) was reclassified as *Adv. mimigardefordensis* (Gibello et al. 2009). Colonies are white to lime yellow becoming yellower with age, circular and flatter than convex, and comprising oxidative, motile Gram-negative coccoid rods of 1.5–2 μm in length. Growth occurs at 15–40 °C and is best at 30–37 °C. Alkaline [weakly] and acid phosphatase, catalase, esterase lipase (C8), and oxidase are produced, but not cystine arylamidase or esterase (C4). Adipate, D-arabinose, D and L-fucose, D-galactose, gluconate, glycerol, DL-lactate, melezitose, ribose, and D- and L-xylose are assimilated, but not D-adonitol, D- or L-arabitol, dulcitol, erythritol, D-fructose, gentiobiose, glycogen, inositol, inulin, 2-ketogluconate, D-lyxose, melibiose, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, raffinose, D-rhamnose, sorbitol, L-sorbose, starch, sucrose, tagatose, trehalose, turanose, or xylitol. The major fatty acids are $C_{16:0}$, $C_{18:1}$ *ω7c*, and $C_{16:1}$ *ω7c* and/or iso-$C_{15:0}$ 2-OH. The type strain is DSM 17166^T = LMG 22922^T, which has a G+C ratio of the DNA of 55.1 mol% (Wübbeler et al. 2006; Gibello et al. 2009).

Alcaligenes Castellani and Chalmers 1919

Al.ca.li'ge.nes Arabic *al* the; Arabic n. *gally* the ash of saltwort; French n. *alkali* alkali; Gr. v. *gennaio* to produce; M. L. masc. N. *Alcaligenes* alkali-producing [bacteria] (Castellani and Chalmers 1919).

Cultures on nutrient agar are nonpigmented and comprise obligately aerobic, chemoorganotrophic [alkali is produced] motile with 1–9, occasionally up to 12, peritrichous flagella Gram-negative rods/coccobacilli of 0.5–1.2 × 1.0–3.0 μm in size that occur mostly singly. Poly-β-hydroxybutyrate may be accumulated intracellularly. Catalase and oxidase are produced, but not indole. Aesculin, cellulose, DNA, and gelatin are not degraded. Carbohydrates are not usually utilized. Chemoorganotrophic. Alkali is produced from organic salts and amides. The dominant respiratory quinone is Q-8. The predominant polyamines are putrescine and

Table 27.4
Differentiating phenotypic properties of *Alcaligenes*

Characteristics	*Alc. faecalis* subsp. *faecalis*	*Alc. faecalis* subsp. *parafaecalis*	*Alc. faecalis* subsp. *phenolicus*	*Alc. aquatilis*
Growth at 42 °C	+	–	+	–
Nitrite reduction	+	–	+	+
Assimilation of				
Glycogen[a]	–	weak	–	+
L-Histidine[a]	+	–	+	+
L-Proline[a]	+	+	+	–
L-Serine[a]	+	–	–	+
L-Tryptophan[a]	+	–	+	–

V variable
[a]Data from API 20NE and API ID 32GN

2-hydroxyputrescine. The major fatty acids are $C_{12:0}$ 2OH, $C_{14:0}$ 3OH, $C_{16:0}$, and $C_{17:0}$ cyclo. The G+C ratio of the DNA is 56–60 mol%. The type species is *Alcaligenes faecalis* with *Alc. faecalis* subsp. *faecalis* as the type subspecies (Kersters and DeLey 1984). The distinguishing traits of *Alcaligenes* species are given in Table 27.4.

Alc. faecalis is subdivided into subspecies, namely, *faecalis*, *parafaecalis*, and *phenolicus*.

Alc. faecalis subsp. *faecalis* produces nonpigmented to grayish-white colonies with irregular edges that comprise asporogenous Gram-negative peritrichously flagellated rods. Catalase and oxidase are produced. Neither gelatin, starch, Tween 80, nor urea is degraded. Nitrites but not nitrates are reduced. 2-hydroxyputrescine is present in the polyamine pattern. Acid is not produced from D-glucose or D-xylose. Acetate is used as a source of carbon, but not *N*-acetylglucosamine, adipate, adonitol, amygdalin, L-arabinose, cellobiose, D-fructose, D-fucose, D-galactose, D-gluconate, D-glucose, inulin, itaconate, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, L-rhamnose, D-ribose, pimelate, salicin, suberate, sucrose, *m*-tartrate, trehalose, or D-xylose. The major fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:0}$ $\omega 7c$, and summed features 2 and 3. The G+C ratio of the DNA is 55.9–59.4 mol%. The type strain is ATCC 8750^T = DSM 30030^T = NCIMB 8156^T = CCM 1052^T = CIP $60\text{-}80^T$ (Schroll et al. 2001; Van Trappen et al. 2005).

Cultures of *Alc. faecalis* subsp. *parafaecalis* develop circular, entire, low-convex, smooth colonies with irregular edges and a diameter of ≤2 mm after incubation at 37 °C for one day and comprise alkali-producing asporogenous, motile Gram-negative rods of 0.75–1.0 μm × 1.5–3.0 μm in size. L-alanine aminopeptidase, acid and alkaline phosphatase, catalase, esterase (C4), lipase (C8) [weakly], lipase (C14) [weakly], leucine and valine arylamidase, naphthol-AS-BI-phosphohydrolase, and oxidase are produced, but not *N*-acetyl-β-glucosaminidase, chymotrypsin, cystine arylamidase, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, H_2S, indole, lysine or ornithine decarboxylase, α-mannosidase, trypsin, or tryptophan deaminase. Growth occurs on MacConkey agar, and not at 42 °C. The Voges-Proskauer reaction is negative. Nitrates are not reduced. Citrate is utilized. Gelatin is degraded, but not casein, DNA, Tween 80, or urea. Acetate, butyrate, γ-butyrolactone, citrate, ethanol, heptanoate, γ-hydroxybutyric acid, L-lactate, propionate, and valerate are assimilated. The major respiratory isoprenoid quinone is Q-8. The main component in the polyamine pattern is putrescine. The major fatty acids are $C_{16:0}$, $C_{16:1}$, and $C_{17:0}$ cyclo (Schroll et al. 2001; Van Trappen et al. 2005). The G+C content of the DNA is 56 mol%. The type strain is DSM 13975^T = CIP 106866^T.

Alc. faecalis subsp. *phenolicus* produces white, translucent, irregular colonies with spreading edges on TSA plates and comprises asporogenous, alkali-producing motile [by peritrichous flagella] Gram-negative coccobacilli of 0.75–1.5 × 0.6–0.8 μm in size. Growth occurs at 23 °C, 30 °C, 37 °C, and 42 °C [optimally at 30 °C]. Grows on M9 mineral medium in 0.5 % (v/v) phenol as a sole source of carbon. Catalase is produced. Nitrites [anaerobically] but not nitrates are reduced. Acetate, D-alanine, L-alanine, L-asparagine, L-aspartic acid, butyrate, *cis*-aconitic acid, citrate, ethanol, L-glutamic acid, L-histidine, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, α-ketovaleric acid, L-lactate, D,L-lactic acid, L-leucine, malonic acid, L-ornithine, phenol, L-phenylalanine, L-proline, propionate, pyruvate, succinic acid, and L-tryptophan are used as sole sources of carbon, but not *N*-acetyl-D-glucosamine, *N*-acetyl-D galactosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, citric acid, α-cyclodextrin, dextrin, *i*-erythritol, formic acid, D-fructose, L-fucose, D-galactose, gentiobiose, glycogen, α-D-glucose, α-hydroxybutyric acid, *m*-inositol, itaconic acid, α-ketobutyric acid, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-raffinose, L-rhamnose, D-serine, L-serine, D-sorbitol, sucrose, L-threonine, thymidine, D-trehalose, turanose, or xylitol. Acid is not produced from arabinose, cellobiose, galactose, glucose, lactose, maltose, D-mannitol, raffinose, sorbitol, sucrose, or D-xylose. The major fatty acids are $C_{12:0}$, $C_{12:0}$ 2-OH, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:0}$, $C_{18:1}$ $\omega 7c$, and summed features 2 and 3. The type strain is DSM 16503^T = NRRL B-41076^T, which has a G+C ratio of the DNA

Table 27.5
Differentiating phenotypic properties of *Bordetella*

Characteristics	*Bor. pertussis*	*Bor. avium*	*Bor. bronchiseptica*	*Bor. hinzii*	*Bor. holmesii*	*Bor. parapertussis*	*Bor. petrii*	*Bor. trematum*
Motility	–	+	–	+	–	–	–	+
Growth on MacConkey agar	–	+	+	+	–	+	+	+
Oxidase	+	+	+	+	–	–	+	–
Hemolysis	+	–	+		–	+	–	
Urea degradation	+	–	+	–	–	–	–	–
Assimilation of								
Adipate[a]	–	+	v	+	–	–	+	+
Caprate[a]	–	–	v	+	–	–	–	–
D-Gluconate[a]	–	–	–	–	–	–	+	–
L-Malate[a]	–	+	v	+	–	–	+	+
Phenylacetate[a]	–	+	+	+	–	–	–	+

V variable
[a]Data from API 20NE and API ID 32GN

of 54.8 mol% (Rehfuss and Urban 2005; Van Trappen et al. 2005; Rehfuss and Urban 2005).

Alc. aquatilis produces non- and yellow-pigmented, circular, low-convex, and smooth colonies with spreading, irregular edges of 2–5 mm in diameter after incubation on TSA at 28 °C for 48 h. Cultures comprise motile by peritrichous flagella Gram-negative rods of 0.7–1.1 × 1.0–2.5 μm in size. Growth occurs at 4–35 °C [optimally at 18–24 °C]. Catalase [mostly] and oxidase are produced, but not arginine dihydrolase, β-galactosidase, H_2S, indole or lysine, or ornithine decarboxylase. Nitrates are not reduced, but some strains reduce nitrite. Aesculin, gelatin, starch, and urea are not degraded. Alkali is produced from asparagine, citrate, and glutamine. Sodium acetate and sodium citrate are mostly utilized. In most cases, acids are not produced from carbohydrates. Growth does not occur on *N*-acetylglucosamine, adipate, L-alanine, L-arabinose, L-fucose, gluconate, glucose, 3-hydroxybutyrate, 4-hydroxybenzoate, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, malonate, maltose, mannitol, mannose, D-melibiose, L-proline, propionate, rhamnose, D-ribose, salicin, D-sorbitol, and D-sucrose. Growth is observed on acetate, caprate (mostly), citrate, glycogen, histidine, 3-hydroxybenzoate, DL-lactate, malate, phenylacetate, L-serine, suberate, and valerate. The dominant fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}$ *ω7c*, and summed features 2 and 3. The type strain is LMG 22996^T = CCUG 50924^T, which has a G+C ratio of the DNA of 56 mol% (Van Trappen et al. 2005).

Bordetella Moreno-Lòpez 1952

Bor. de. tel' la. M. L. dim. Ending *–ella*; M. L. fem. N. *Bordetella* after Jules Bordet, who first isolated the pertussis organism jointly with O. Gengou (Moreno-López 1952).

The emended description of *Bordetella* is that cultures comprise nonmotile or motile by peritrichous flagella Gram-negative, catalase-positive, coccobacilli of 0.2–0.5 × 0.5–2.0 μm in size that occur singly, in pairs, and occasionally in chains and may exhibit bipolar staining. Cultures grow strictly aerobically, facultatively anaerobically, and nonfermentatively. One species, *Bor. petrii*, is able to grow anaerobically under conditions favoring respiratory nitrate and selenate reduction. Citrate is assimilated aerobically; one species assimilates D-gluconate. The major respiratory isoprenoid quinone is Q-8. The type species of the genus is *Bordetella pertussis*. The G+C content of the DNA is 60–69 mol% (Von Wintzingeroda et al. 2001). The distinguishing traits of *Bordetella* species are given in Table 27.5.

Bor. pertussis produces minute colonies on potato glycerol blood agar and comprises nonmotile encapsulated coccobacilli of 0.2–0.5 × 0.5–1.0 μm in size. Growth does not occur at 25 °C or 42 °C or on MacConkey agar. Chymotrypsin, ester lipase (C8), naphthol-AS-BI-phosphohydrolase, oxidase, serine arylamidase, and possibly catalase are produced, but not alkaline phosphatase, cystine or valine arylamidase, glutamic decarboxylase, or trypsin. Nitrates are not reduced. Neither gelatin nor urea is degraded. DL-alanine, DL-aspartic acid, L-glutamic acid, glycine, L-proline, and L-serine are utilized, but not citrate. The major fatty acids are $C_{16:0}$ and $C_{16:1}$ *ω7c*. The G+C ratio of the DNA is 66–70 mol%. The type strain is ATCC 9797^T.

Bor. avium produces unpigmented colonies that comprise strictly aerobic, chemoorganotrophic capsulated rods of 0.4–0.5 × 1–2 μm in size that occur singly or in pairs and are motile by peritrichous flagella. Either of two distinct colony types may occur, namely, a small [<1 mm in diameter] glistening colony with entire edges after incubation for 24 h or larger, circular, smooth convex colonies with entire edges. Growth occurs

optimally at 37 °C and on MacConkey agar, nutrient agar (NA), and salmonella-shigella agar. Nitrates are not reduced. Acid and alkaline [weakly] phosphatase, catalase, esterase (C4), esterase lipase (C8) [weak], oxidase, and phosphoamidase are produced, but not *N*-acetyl-β-D-glucosaminidase, α-L-fucosidase, α- or β-D-galactosidase, α- or β-D-glucosidase, β-D-glucuronidase, indole, lipase (C14), α-D-mannosidase, phenylalanine deaminase, or trypsin. Neither aesculin, gelatin, nor urea is degraded. Growth occurs on L-aspartate, citrate, fumarate, L-glutamate, glutarate, α-ketoglutarate, L-malate, L-proline, pyruvate, and succinate, but not *N*-acetyl glucosamine, adonitol, aesculin, amygdalin, D- or L-arabinose, arbutin, L-arginine, benzoate, butyrate, D-cellobiose, dulcitol, D-fructose, D-galactose, D-gentiobiose, D-gluconate, D-glucose, glycine, glycogen, inulin, L-histidine, *m*-inositol, itaconate, lactose L-leucine, L-lysine, D-malate, malonate, D-maltose, D-mannitol, D-mannose, D-melezitose, D-melibiose, L-methionine, L-ornithine, L-phenylalanine, raffinose, L-rhamnose, D-ribose, salicin, L-serine, sorbitol, sucrose, L-threonine, trehalose, D- or L-tryptophan, L-valine, or D- or L-xylose. The G+C content of the DNA is 61.6–62.6 mol %. The type strain is ATCC 35086^T, which has a G+C ratio of the DNA of 61.6 mol% (Kersters et al. 1984).

Bor. bronchiseptica comprises motile Gram-negative coccobacilli, which grow on blood and MacConkey agar and at 25 °C and 42 °C. Catalase, oxidase, and serine arylamidase are produced, but not chymotrypsin, cystine, proline or valine arylamidase, lipase (C14), phosphatase, or trypsin. Nitrates are reduced to nitrite. Urea is degraded, but not gelatin. Tetrazolium is reduced. DL-alanine, DL-aspartic acid, citrate, L-glutamic acid, glycine L-proline, and L-serine are utilized. The major fatty acids are $C_{16:0}$, $C_{16:1}$ *ω7c*, and $C_{17:0}$ cyclo. The G+C content of the DNA is 67–79 mol%. The type strain is ATCC 19395^T (Vandamme et al. 1996).

Bor. hinzii may produce two distinct colony types including round, raised, glistening, grayish colonies of ~2 mm in diameter after incubation for 48 h at 37 °C in 5 % CO_2. The second type is characterized by the presence of flat, dry, crinkled colonies that are ≤5 mm in diameter. Cultures comprise Gram-negative, asporogenous rods that are motile by peritrichous flagella. Oxidase and catalase are produced, but not arginine dihydrolase, β-galactosidase, or indole. Nitrates are not reduced. Neither aesculin nor gelatin is degraded. Urease activity is strain dependent. Alkali is produced from acetamide, adipate, malonamide, malonate, valerate, and propionamide. Production of alkali from glycine is strain dependent. Acid is not produced from glucose. Adipate, citrate, malate, and phenylacetate are assimilated, but not *N*-acetyl-glucosamine, L-arabinose, D-gluconate, D-glucose, maltose, D-mannitol, or D-mannose. Assimilation of caprate is strain dependent. Q-8 and trace amounts of Q-9 are the respiratory quinones. The major fatty acid components are $C_{16:0}$, $C_{17:0}$ cyclo, and summed feature 3. The DNA base composition is 65–67 mol%. The type strain is LMG 13501^T, which has a G+C content of the DNA of 66 mol% (Vandamme et al. 1995).

Bor. holmesii, formerly known as CDC nonoxidizer group 2, produces brown, soluble, pigmented [diffusing around the colonies on heart infusion tyrosine agar], round, punctate, convex, semi-opaque colonies on rabbit blood agar after incubation at 35 °C for 3 days and comprises asaccharolytic, nonmotile, small, Gram-negative coccoid cells/rods; longer rods are observed sometimes. Growth occurs sometimes slowly on MacConkey agar. Catalase is produced weakly or not at all; oxidase is not produced. Urea is not degraded, nor are nitrates reduced. Carbohydrates are not utilized. The major fatty acids are $C_{16:0}$ and $C_{17:0}$ cyclo, with smaller quantities of $C_{18:0}$. The G+C ratio of the DNA is 61.5–62.3 mol%. The type strain is CDC F5101 = ATCC51541^T, which has a G+C ratio of the DNA of 61.9 mol% (Weyant et al. 1995).

Bor. parapertussis comprises strictly aerobic, nonmotile, Gram-negative rods, which produce brown diffusible pigment on peptone agar. Growth occurs on blood and MacConkey agars. Catalase is produced, but not oxidase or phosphatase. Nitrates are not reduced. Urea is degraded, but not gelatin. DL-alanine, DL-aspartic acid, citrate, L-glutamic acid, glycine, L-proline, and L-serine are utilized. The major fatty acids are $C_{16:0}$ and $C_{17:0}$ cyclo. The G+C ratio of the DNA is 66–70 mol%. The type strain is ATCC 15311^T, which has a G+C ratio of the DNA of 68.6 mol%.

Bor. petrii produces white/cream colonies comprising Gram-negative, asporogenous, nonmotile, circular to rod-shaped cells of 0.4–0.7 × 1.0–2.8 μm in size that have fimbria of different diameters. Catalase and oxidase is produced, but not indole or urease. Neither aesculin, blood, nor gelatin is degraded. Tetrazolium is reduced. Nitrates are not reduced. Growth occurs aerobically, microaerophilically, and anaerobically, the latter of which is nonfermentative, possibly attributed to denitrification or to the reduction of selenate to elemental sulfur. Growth occurs at 30 [aerobically] and 37 °C [aerobically and microaerophilically] and on MacConkey agar. The Voges-Proskauer reaction is negative. Adipate, citrate, D-gluconate, and L-malate are assimilated, but not *N*-acetylglucosamine, adonitol, amygdalin, L-arabinose, arbutin, caprate, cellobiose, erythritol, gentiobiose, D-glucose, glycerol, glycogen, inositol, inulin, lactose, maltose, mannose, mannitol, melezitose, melibiose, methyl β-D-xyloside, methyl α-D-glucoside, raffinose, rhamnose, ribose, saccharose, salicin, sorbitol, starch, trehalose xylitol, and L- and D-xylose. The major respiratory isoprenoid quinone is Q-8. The type strain is DSM 12804^T = CCUG 43448 T, which has a G+C ratio of the DNA of 63.8 mol% (Von Wintzingeroda et al. 2001).

Bor. trematum produce convex, circular, and grayish cream-white colonies with entire edges on blood agar, which comprise motile by peritrichous flagella, asporogenous, encapsulated Gram-negative rods of 0.5–0.6 × 1–1.8 μm in size; the longest rods are ≤2.4 μm long in 16–24 h cultures. Alkaline phosphatase, ester lipase (C8), naphthol-AS-BI-phosphohydrolase, and serine and valine arylamidase are produced, but not cystine or proline arylamidase, lysine decarboxylase, oxidase, or trypsin. Growth occurs microaerophilically but not anaerobically, and on MacConkey agar, and at 25 °C and 42 °C. Urea is not degraded. Adipate, aesculin, L-malate, and phenylacetate are assimilated, but not D-gluconate, caprate, D-glucose, or D-xylose. The major fatty acids are $C_{16:O}$, $C_{17:O}$ cyclo, and summed feature 3. The G+C ratio of the DNA is 64–65 mol%. The type strain is LMG 13506^T, which has a G+C ratio of the DNA of 65 mol% (Vandamme et al. 1996).

Brackiella Willems et al. 2002

Bra'cki.ella L. n. *Brackiella* after Manfred Brack, a German pathologist.

Cultures of *Bra. oedipodis* comprise aerobic to microaerophilic nonmotile Gram-negative asporogenous slightly ovoid coccobacilli of 0.9 × 1.1 μm; actively growing cells before dividing were up to 1.8 μm in length. Cells are surrounded by densely studded fimbriae of 280 nm in length. Growth occurs at 30–37 °C, but not at 4 °C, 40 °C, or 42 °C, on 5 % (v/v) sheep blood agar but without evidence of hemolysis. Two colony types are observed after 24 h, namely, small (up to 0.5 mm in diameter) and large (up to 1 mm in diameter), but after 4 h all colonies are of 1–2 mm in diameter. Catalase and oxidase is produced. Nitrates are reduced to nitrites. Urea is attacked. Acid is produced from maltose and D-mannose. Acetate is not utilized. One major outer membrane protein (OMP) of 45 kDa and three smaller and minor OMPs have been identified. The lipopolysaccharide profile comprises a ladder of repeating units with two strong bands at 8.4 kDa and other bands at 10 and 12 kDa and minor bands at 21–26 kDa. The major fatty acids are $C_{19:0\ cyclo}$ ω8*c*, $C_{18:1}$ ω7*c*, and $C_{16:0}$.

The type species is *Brackiella oedipodis*, which in addition to the genus characteristics produces L-alanine aminopeptidase and ornithine decarboxylase, degrades starch, and is positive for the methyl red test, but does not produce arginine dihydrolase, β-D-galactosidase, β-D-glucuronidase, indole, H_2S, phenylalanine deaminase, pyrase, or lysine decarboxylase; decompose aesculin, DNA, gelatin, or Tween 80; produce acid from adonitol, L-arabinose, cellobiose, dulcitol, erythritol, glycerol, *myo*-inositol, lactose, mannitol, melibiose, methyl-α-D-glucoside, mucate, raffinose, L-rhamnose, saccharose, salicin, D-sorbitol, trehalose, or D-xylose; utilize Simmons citrate or the Voges-Proskauer reaction; and grow on MacConkey agar, on KCN, or in 6.5 % (w/v) NaCl. The type strain is LMG 19451^T = DSM = 13743^T = NCIMB 13739^T.

Candidimonas Vaz-Moreira et al. 2011

Can.di.di.mo'nas. L. adj. *Candidus -a –um* white; L. fem. N. *monas* a unit, monad; N.L. fem. No. *Candidimonas* a unit (rod) that produces white colonies.

Cultures contain asporogenous, motile or nonmotile, Gram-negative short-curved rods. Catalase and oxidase are produced. Mesophilic or psychrophilic. Chemoorganotrophic. Respiratory metabolism. Nitrates are reduced. Amino acids and organic acids are utilized as the sole sources of carbon. Ubiquinone Q-8 is the respiratory quinone. The polar lipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine, and diphosphatidylglycerol. The major fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and summed feature 3. The G+C content of the DNA is 64–65 mol%. The type species is *Candidimonas nitroreducens* (Vaz-Moreira et al. 2011; Zhang et al. 2012). The distinguishing traits of *Candidimonas* species are given in Table 27.6.

Table 27.6
Differentiating phenotypic properties of *Candidimonas*

Characteristics	*Can. nitroreducens*	*Can. bauzanensis*	*Can. humi*
Anaerobic growth	+ [slow and weak]	+	–
Motility	–	+	–
Nitrate reduction to nitrate	+	+	–

Can. bauzanensis produces smooth, round, convex, cream-white colonies of 1–1.5 mm in diameter after incubation on R2A for 5 days. Colonies comprise Gram-negative rods of 0.7–0.9 × 1.2–1.9 μm in length. Growth occurs facultatively, anaerobically, and at 1–37 °C but not at 42 °C, at pH 6–8, and in 0–3 % (w/v) NaCl. Nitrates are reduced to nitrogen gas. Esterase (C4), esterase lipase (C8) [weakly], acid phosphatase [weakly], naphthol-AS-BI-phosphohydrolase, and leucine arylamidase are produced, but not *N*-acetyl-β-glucosaminidase, alkaline phosphatase, arginine dihydrolase, α-chymotrypsin, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, H_2S, indole, lysine or ornithine decarboxylase, α-mannosidase, trypsin, or tryptophan deaminase. The Voges-Proskauer reaction is negative. Urea, but not aesculin, gelatin, skimmed milk, or starch, is degraded. Citrate is not utilized. Adipic acid, L-arabinose, D-glucose, malic acid, potassium gluconate, and trisodium citrate (weakly) are assimilated, but not *N*-acetylglucosamine, L-alanine, L-arabinose, capric acid, citrate, L-fucose, D-glucose, glycogen, L-histidine, inositol, maltose, D-mannitol, D-mannose, or phenyl acetic acid. Neither amygdalin, L-arabinose, D-glucose, inositol, D-mannitol, melibiose, L-rhamnose, D-sorbitol, nor sucrose is fermented. The polyamines are dominated by putrescine and spermidine. The isoprenoid quinone is Q-8. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine, and diphosphatidylglycerol. The predominant fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and summed feature 3. The type strain is BZ59^T = DSM 22805^T = LMG 26046^T = CGMCC 1.10190^T, which has a G+C ratio of the DNA of 61.6 mol% (Zhang et al. 2012).

Can. nitroreducens produces circular, convex, white colonies of ~1 mm in diameter after incubation on plate count agar for 48 h. Colonies comprise Gram-negative coccobacilli of 0.53 ± 0.11 μm in length. Growth occurs anaerobically [slowly] and at 15–40 °C [optimally at 30 °C], at pH 5–8 [optimally at pH 7], and in up to 3 % (w/v) NaCl [optimally in 1 % w/v NaCl]. Nitrates are reduced to nitrites. Esterase (C4), esterase lipase (C8), acid and alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, and leucine and valine arylamidase are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, H_2S, indole, lipase (C14), lysine or ornithine decarboxylase,

α-mannosidase, trypsin, or tryptophan deaminase. The Voges-Proskauer reaction is negative. Neither aesculin, gelatin, nor urea is degraded. Simmons citrate is not utilized. Glucose is oxidized, but not amygdalin, L-arabinose, inositol, D-mannitol, melibiose, L-rhamnose, D-sorbitol, or sucrose. Adipate, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxybutyric acid, itaconic acid, lactic acid, malate (weakly), phenylacetate, potassium gluconate (weakly), propionic acid, sodium acetate, and valeric acid are assimilated, but not *N*-acetylglucosamine, L-alanine, L-arabinose, capric acid, citrate, L-fucose, D-glucose, glycogen, L-histidine, inositol, maltose, D-mannitol, D-mannose, melibiose, potassium 2-ketogluconate, potassium 5-ketogluconate, L-proline, L-rhamnose, D-ribose, salicin, L-serine, sodium malonate, D-sorbitol, suberic acid, sucrose, or trisodium citrate. The predominant fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}$ *ω7c*, and summed feature 2. The type strain is SC-089^T = CCUG 55806^T = LMG 24812^T, which has a G+C ratio of the DNA of 64 mol% (Vaz-Moreira et al. 2011).

Can. humi produces circular, convex, white colonies of ~1 mm in diameter after incubation on plate count agar (PCA) for 48 h. Colonies comprise Gram-negative coccobacilli of 0.5 ± 0.09 μm in size. Growth occurs at 15–40 °C [optimally at ~30 °C], at pH 5–8 [optimally at pH 7], and in up to 3 % (w/v) NaCl [optimally in 1 % w/v NaCl]. Anaerobic growth does not occur. Nitrates are not reduced. Esterase (C4), esterase lipase (C8), acid and alkaline phosphatase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, and leucine and valine arylamidase are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, cystine arylamidase, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, H_2S, indole, lipase (C14), lysine or ornithine decarboxylase, α-mannosidase, trypsin, or tryptophan deaminase. Simmons citrate is not utilized. The Voges-Proskauer reaction is negative. Neither aesculin, gelatin, nor urea is degraded. Adipate, citrate 3-hydroxybenzoic acid, 3-hydroxybutyric acid, itaconic acid, lactic acid, phenylacetate, potassium 2-ketogluconate, propionic acid, sodium acetate, trisodium citrate, and valeric acid are assimilated, but not *N*-acetylglucosamine, L-alanine, L-arabinose, capric acid, L-fucose, D-glucose, glycogen, L-histidine, 4-hydroxy benzoic acid, inositol, malate, maltose, D-mannitol, D-mannose, melibiose, potassium gluconate, potassium 5-ketogluconate, L-proline L-rhamnose, D-ribose, salicin, L-serine, sodium malonate, D-sorbitol, suberic acid, or sucrose. The major fatty acids are $C_{16:0}$, $C_{18:1}$ *ω7c*, and summed features 2 and 3. The type strain is SC-092^T = CCUG 55807^T = LMG 24813^T, which has a G+C ratio of the DNA of 65 mol% (Vaz-Moreira et al. 2011).

Castellaniella Kämpfer et al. 2006

Cas.tel.la'ni.el.la. N. L. fem. dim. n. *Castellaniella* named after Sir Aldo Castellani, an Anglo-Italian bacteriologist who first described *Alcaligenes*.

On NA with incubation at 25–30 °C for 24 h, cultures comprise Gram-negative, facultatively anaerobic and denitrifying, motile rods of 1.3–2.0 × 0.2–0.8 μm. Polar lipids comprise phosphatidylglycerol and diphosphatidylglycerol with minor quantities of two unknown aminolipids and an unknown polar lipid. The polyamines consist mostly of putrescine with moderate amounts of 2-hydroxyputrescine. The main component of the quinone system is ubiquinone Q-8. The major fatty acids are $C_{16:0}$, $C_{16:1}$*ω7c*, $C_{17:0}$ cyclo, $C_{18:1}$ *ω7c*, and/or $C_{14:0}$ 3-OH (Kämpfer et al. 2006). The type species is *Cas. defragrans*. The distinguishing traits of *Castellaniella* species are given in Table 27.7.

Cas. defragrans (basonym = *Alcaligenes defragrans*) matches the genus description. The type strain is CCUG 39790^T = DSM 12141^T = CIP 105602^T which has a G+C ratio of the DNA of 66.9 mol% (Kämpfer et al. 2006).

Cas. caeni produces brownish 1–2 mm diameter smooth, transparent, circular, convex colonies on R2A agar after 2 days, which contain facultatively anaerobic, Gram-negative, nonmotile rods of 0.3–0.5 × 1.2–2.0 μm in size. Oxidase is produced but not arginine dihydrolase, catalase, β-galactosidase, H_2S, indole, lipase, lysine and ornithine decarboxylase, or tryptophan deaminase. The Voges-Proskauer reaction is positive. Growth occurs on MacConkey agar, NA, and TSA, at 10–37 °C [optimally at 30 °C] and at pH 5.0–8.5 [optimally at pH 6.5–7.0]. NaCl is not required for growth, but cultures can grow in 5 % (w/v) NaCl. Nitrates are reduced to nitrogen anaerobically. Aesculin is degraded, but not carboxymethylcellulose, casein, chitin, DNA, gelatin, starch, urea, or xylan. Acetate, adipate, D-adonitol, L-alanine, D-arabinose, L-arginine, L-asparagine, L-aspartate, caprate, cellobiose, L-cysteine, formate, fumarate, L-fucose, D-galactose, D-glucose, L-glutamate, L-glutamine, glutarate, glycine, glycerol, L-histidine, DL-3-hydroxybutyrate, L-isoleucine, lactate, L-lysine, malate, methanol, phenylacetate, L-proline, propionate, L-serine, suberate, succinate, L-threonine, L-valine, and valerate are utilized, but not *N*-acetylglucosamine, amygdalin, L-arabinose, benzoate, citrate, dextran, dulcitol, ethanol, fructose, gluconate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, inositol, inulin, itaconate, 2-ketogluconate, 5-ketogluconate, D-lactose, L-leucine, D-lyxose, maleic acid, malonate, maltose, D-mannitol, mannose, melibiose, L-methionine, oxalate, L-phenylalanine, pyruvate, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, sucrose, tartrate, trehalose, L-tryptophan, L-tyrosine, xylitol, L-xylose, or D-xylose. Acid is not produced from amygdalin, L-arabinose, glucose, inositol, D-mannitol, melibiose, L-rhamnose, D-sorbitol, or sucrose. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylglycerol. The major fatty acids are summed features 4 and 7. The type strain is KCTC 12197^T = LMG 23411^T, which has a G+C content of the DNA of 63.5 mol% (Liu et al. 2008).

Apart from the genus characteristics, colonies of *Cas. denitrificans* are beige and circular with an entire edge. Growth occurs at 30–42 °C but not at 4 °C. A wide range of compounds are utilized as sole sources of carbon and include acetate, *cis*-aconitate, *trans*-aconitate, L- and β-alanine, 4-aminobutyrate, L -aspartate, citrate, fumarate, D-gluconate (weak), glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, L-leucine, L-malate, mesaconate, L-ornithine, 2-oxoglutarate, L-proline, propionate,

Table 27.7
Differentiating phenotypic properties of *Castellaniella*

Characteristics	*Cas. defragrans*	*Cas. caeni*	*Cas. denitrificans*	*Cas. daejeonensis*	*Cas. ginsengisoli*
Motility	+	–	+	+	+
Alkaline phosphatase[a]	–	–	+	–	+
Assimilation of					
N-acetyl-D-glucosamine[b]	–	–	–	–	+
Adipate[b]	–	+	–	+	+
Citrate[b]	+	–	+	–	+
L-Fucose[b]	–	+	–	–	–
Gluconate[b]	+	–	+	+	+
D-Glucose[b]	–	+	–	+	+
L-Histidine[b]	–	+	–	+	–
3-Hydroxybutyrate[b]	+	–	+	+	+
Itaconate[b]	–	–	+	–	–
D-Mannose[b]	–	–	–	+	+
L-Serine[b]	+	+	–	+	+
Suberate[b]	–	+	–	–	–

[a]Data from API ZYM
[b]Data from API 20NE and API ID 32GN

pyruvate, and L-serine, but not *N*-acetylgalactosamine, *N*-acetylglucosamine, adipate, adonitol, L-arabinose, L-arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, L-histidine, 3-hydroxybenzoate, *i*-inositol, D-maltose, D-mannitol, D-mannose, α-D-melibiose, phenylacetate, L-phenylalanine, putrescine, L-rhamnose, D-ribose, salicin, L-serine, D-sorbitol, D-sucrose, D-trehalose, L-tryptophan, or D-xylose. Acid is not produced from adonitol, L-arabinose, D-arabitol, cellobiose, dulcitol, erythritol, glucose, inositol, lactose, maltose, D-mannitol, D-mannose, methyl D-glucoside, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, or D-xylose. The type strain is DSM 11046^T = CCUG 39541^T (Kämpfer et al. 2006).

Cas. daejeonensis produces 0.5–2.0 mm diameter circular, smooth, convex, shiny, transparent, yellowish colonies on R2A agar after incubation at 30 °C. Cultures comprise facultatively anaerobic, Gram-negative, asporogenous, motile rods, 0.3–0.5 × 1.4–1.7 μm in size. Catalase and oxidase are produced, but not acid or alkaline phosphatase, cysteine or valine arylamidase, α-fucosidase, β-galactosidase, α-mannosidase, or naphthol-AS-BI-phosphohydrolase. Growth occurs on Luria-Bertani (LB) agar, R2A agar, NA, and TSA at 30–42 °C [optimally at 30 °C] but not at 20 °C or 45 °C and at pH 5.0–9.0 [optimally at 6.5–7.0]. Nitrate is reduced to nitrogen gas. Gelatin and casein (weakly) are degraded, but not so chitin, DNA, hydroxyethyl cellulose, starch, or xylan. Acetate, adipate, L-alanine, caprate, gluconate, D-glucose, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, inositol, 2-ketogluconate, L-proline, malonate, D-mannitol, D-mannose, phenylacetate, L-rhamnose, D-ribose, L-serine, D-sorbitol, and valerate are assimilated, but not *N*-acetyl-D-glucosamine, citrate, itaconate, L-fucose, or suberate. Acid is produced from D-arabitol, erythritol, gluconate, mannose, and lactose. The major fatty acids are $C_{16:0}$ and summed features 4 and 7. The principle respiratory quinone is Q-8. Putrescine is the dominant polyamine. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylglycerol. The type strain is KCTC 22454^T = JCM 16240^T, which has a G+C ratio of the DNA of 66.2 mol% (Lee et al. 2010a).

Cas. ginsengisoli produces yellow, smooth, circular, convex colonies on R2A after 3 days and comprises Gram-negative, motile rods of 0.7–0.8 × 1.6–2.5 μm in size. Growth occurs on LB agar, NA, R2A agar, and TSA at 25–42 °C [optimally at 30 °C in LB broth] and at pH 5.0–9.0 [optimally at pH 6.5–7.0 in LB broth]. Acid and alkaline phosphatase, catalase, esterase (C4), esterase lipase (C8), β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and oxidase are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, lipase (C14), α- and β-galactosidase, α- and β-glucosidase, β-glucuronidase, α-mannosidase, or trypsin. Nitrates are reduced to nitrogen gas. Neither gelatin nor urea is degraded. Acetate, *N*-acetyl-D-glucosamine, adipate, L-alanine, L-arabinose, caprate, citrate, gluconate, D-glucose, 3- and 4-hydroxybenzoate, DL-3-hydroxybutyrate, lactate, L-malate, maltose, D-mannose, D-mannitol, phenylacetate, L-proline, propionate, L-serine and *n*-valerate are utilized, but not L-fucose, glycogen, L-histidine, inositol, itaconate, 2- or 5-ketogluconate, malonate, melibiose, L-rhamnose, D-ribose, salicin, D-sorbitol, sucrose, or suberate. The polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylglycerol. The major fatty acids are $C_{16:0}$, $C_{18:1}\omega7c$, and summed feature 4. The type strain is KCTC 22398^T = JCM 15515^T, which has a G+C ratio of the DNA of 63.7 mol% (Kim et al. 2009).

Table 27.8
Differentiating phenotypic properties of *Derxia*

Characteristics	*D. gummosa*	*D. lacustris*
Catalase	–	+
Esterase lipase (C8)[a]	+	–
N-acetyl-β-glucosaminidase[a]	+	–

[a]Data from API ZYM

Derxia Jensen et al. 1960

Derx' i. a. M.L. fem. N. *Derxia* after H.G. Derx, a Dutch microbiologist.

Cultures are initially slimy and semitransparent becoming larger, opaque and wrinkled, and dark brown in color. These comprise aerobic (strictly respiratory), Gram-negative, motile [polar flagella], pleomorphic rods of 1.0–1.2 × 3.0–6.0 μm in size that occur singly or in short chains and fix molecular nitrogen. Growth occurs at 15–40 °C but not at 50 °C [optimally at 25–35 °C], at pH 5.5 ~9.0 but not at pH 4.4. Catalase is not produced. The G+C ratio of the DNA is 69.2–72.6 mol%. The type species is *Derxia gummosa* (Jensen et al. 1960). The distinguishing traits of *Derxia* species are given in Table 27.8.

D. gummosa produces yellow irregular colonies with curled edges on R2A agar and comprises obligately aerobic, encapsulated, motile by single polar flagella Gram-negative rods, which contain intracellular poly-β-hydroxybutyrate granules. Growth occurs at 10–45 °C [optimally at 20–30 °C] and in 0–2 % (w/v) NaCl [optimally in 1 % w/v NaCl]. *N*-acetyl-β-glucosaminidase and esterase lipase (C8) are produced, but not catalase. Molecular nitrogen is fixed aerobically and in decreased oxygen levels. Growth occurs as a facultative hydrogen autotroph. Adipate, arabinose, and mannitol are assimilated. The major respiratory quinone is Q-8. The dominant fatty acids are $C_{12:0}$ 3-OH, $C_{14:0}$ 2-OH, $C_{14:0}$ 3-OH, $C_{16:0}$, $C_{18:1}$ *ω7c*, and summed feature 3. The G+C ratio of the DNA is 69.2–72.6 mol%. The type strain is ATCC 15994^T, which has a G+C ratio of the DNA of 69.2 mol% (Jensen et al. 1960; De Smedt et al. 1980; Xie and Yokota 2004; Chen et al. 2013).

D. lacustris produces round, convex, smooth, light-yellow [becoming brownish yellow and brownish red with further incubation], glistening, translucent colonies with entire edges of 0.4–1.8 mm in diameter on R2A agar after incubation at 25 °C for 48 h. These comprise asporogenous, aerobic, motile encapsulated with a thick capsule Gram-negative rods of 1.0–1.2 × 2.0–4.0 μm in size, which contain intracellular poly-β-hydroxybutyrate granules. Molecular nitrogen is fixed microaerophilically. Growth occurs at 15–40 °C [optimally at 25–35 °C], at pH 6.0–7.0 [optimally at pH 6.0], and in 0–4 % (w/v) NaCl [optimally in 0–1 % w/v NaCl]. Alkaline and acid phosphatase, catalase, esterase (C4), β-glucosidase, leucine and valine arylamidase, naphthol-AS-BI-phosphohydrolase, and oxidase are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, esterase lipase (C8), α-fucosidase, α- or β-galactosidase, α-glucosidase, β-glucuronidase, indole, lipase, α-mannosidase, or trypsin. Nitrates are not reduced. Tween 60 is degraded, but not aesculin; casein; DNA; gelatin; Tween 20, 40, or 80; or urea. Gluconate, glucose, and malate are assimilated, but not *N*-acetylglucosamine, adipate, arabinose, caprate, citrate, maltose, mannitol, mannose, or phenylacetate. Acid is not produced from glucose. The major respiratory quinone is Q-8. The polar lipid profile comprises phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, two unknown aminophospholipids, and 7 unknown phospholipids. The major fatty acids are $C_{12:0}$ 3-OH, $C_{14:0}$ 2-OH, $C_{14:0}$ 3-OH, $C_{16:0}$, $C_{18:1}$ *ω7c*, and summed feature 3. The type strain is BCRC 80208^T = KCTC 23311^T, which has a G+C ratio of the DNA of 72.0 mol% (Chen et al. 2013).

Kerstersia Coenye et al. 2003b

Kers.ters'ia. N.L. fem. n. *Kerstersia* after K. Kersters, an eminent Belgian microbiologist.

Cultures on NA are white to light brown, flat or slightly convex with smooth edges, and comprise small (1–2 μm) coccoid cells that occur singly, in pairs, and as short chains. Motility may occur. Catalase is produced, but not β-galactosidase, oxidase, or urease. Growth occurs at 28 °C and 42 °C. The fatty acid profile includes $C_{14:0}$, $C_{14:0}$ 2-OH, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:0}$, $C_{18:1}$ *ω7c*, $C_{19:0}$ cyclo *ω8c*, and summed features 2 and 3. The G+C content of the DNA is 61.5–62.9 mol%. The type species is *Kerstersia gyiorum*. The distinguishing characteristics of *Kerstersia* species are included in Table 27.9.

K. gyiorum matches the genus description, and in addition caprylate is assimilated, but not aconitate, adipate, L-cysteine, glutarate, isobutyrate, L-isoleucine, isovalerate, 2-ketoglutarate, L-leucine, L-methionine, pimelate, L-threonine, or L-valine. The G+C content of the DNA is 62.7–62.9 mol%. The type strain = LMG 5906^T = CCUG 47000^T and has a G+C content of 62.9 mol% (Coenye et al. 2003b).

K. similis produces white to light brown flat or slightly convex colonies with a swarming appearing on NA that comprise nonmotile, Gram-negative coccobacilli of ~0.3–0.5 × 0.85–1.3 μm in size that occur singly or in pairs. Growth occurs at 28–42 °C and in 0–4.5 % (w/v) NaCl. Catalase is produced, but not arginine dihydrolase, β-galactosidase, H_2S, lysine or ornithine decarboxylase, or oxidase. Neither aesculin, DNA, gelatin, starch, or urea is degraded. Nitrates are not reduced. Acetate, α-D-alanine, α-L-alanine, L-aspartate, butyrate, caprate, *n*-caproate, citrate, fumarate, L-glutamate, heptanoate, *p*-hydroxybenzoate, DL-3-hydroxybutyrate, 2-ketoglutarate [weakly], DL-lactate, D-malate, L-malate, L-norleucine, L-ornithine, pelargonate, phenylacetate, L-phenylalanine, L-proline, propionate, pyruvate, succinate, L-tryptophan, L-tyrosine, and *n*-valerate are assimilated, but not adonitol, aesculin, amygdalin, D-arabinose, L-arabinose, arbutin, cellobiose, dulcitol,

Table 27.9
The distinguishing phenotypic traits of *Kerstersia* species

Characteristic	*K. gyiorum*	*K. similis*
Assimilation of 2-ketoglutarate	–	+ [weak]
Oxidation of D-galacturonic acid	+	–
Oxidation of D-glucuronic acid	+	–
Oxidation of D-serine	–	+

erythritol, D-fucose, L-fucose, galactose, D-glucose, inositol, inulin, 2-ketogluconate, 3-ketogluconate, lactose, malate, malonate, maltose, mannitol, D-mannose, melezitose, melibiose, oxalate, raffinose, L-rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, or D-xylose. The fatty acid profile includes $C_{14:0}$, $C_{14:0}$ 2-OH, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:0}$, $C_{18:1}$ $\omega 7c$, $C_{19:0}$ cyclo $\omega 8c$, and summed features 2 and 3. The type strain = LMG 5890^T = CCUG 46999^T and has a G+C content of 61.5 mol% (Vandamme et al. 2012).

Oligella Rossau et al. 1987

O.lig.el'la. Gr. Adj. *Oligos*, little; scanty; M.L. dim. Ending *–ella*; M.L. fem. N. *Oligella*, referring to a small bacterium with limited nutritional properties.

Cultures comprise slowly developing, aerobic, noncapsulated, asporogenous, small, Gram-negative oxidase and usually catalase-positive rods of $\leq$1 μm in length, which are often in pairs. Cells are mostly nonmotile, but some (*O. ureolytica*) are motile by peritrichous flagella. Carbohydrates are not fermented or oxidized. H_2S and indole is not produced, nor is gelatin attacked. The major fatty acids are $C_{16:0}$ and $C_{18:1}$. The G+C content of the DNA is 47 ± 1 mol%. The type species is *O. urethralis*.

Cultures of *O. urethralis* comprise Gram-negative, aerobic, nonmotile coccobacilli, which grow at 42 °C but do not produce acid in the oxidative-fermentative medium and do not attack urea, reduce nitrates, or grow on *p*-hydroxybenzoate. Neither acetamide, aesculin, starch, or Tween 80 is attacked, nor is arginine hydrolase or arginine or ornithine decarboxylase produced. The type strain is ATCC 17960^T, and the G+C content of the DNA is 46.1–47.5 mol%.

Cultures of *O. ureolytica* contain motile cells that reduce nitrate to nitrite, utilize citrate, and produce urease and grow on *p*-hydroxybenzoate but not at 42 °C. $C_{19:cyc}$ is present in moderate quantities. The type strain is ATCC 43534^T = CCUG 1465^T = LMG 6519^T, and the G+C content of the DNA is 46–47 mol%.

Paenalcaligenes Kämpfer et al. 2010

Pa. en. al. ca. li' ge. nes. L. adv. *paene* nearly, almost; N. L. masc. n. *Alcaligenes* a bacterial genus name; N. L. masc. n. *Paenalcaligenes* almost *Alcaligenes*.

After 24 h incubation on NA, cultures comprise Gram-negative, motile rods of 1.3–2.0 × 0.2–0.8 mm in size, which have a respiratory metabolism and produce oxidase. The quinone system is ubiquinone Q-8 with smaller amounts of Q-7. Polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and an unidentified phospholipid. The major fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and summed feature 3. The type species is *Paenalcaligenes hominis*.

Pae. hominis has the genus characteristics, and additionally on NA, colonies are beige and circular and have an entire edge. Growth occurs at 30–42 °C but not at 4 °C. L-alanine-*p*-nitroanilide is hydrolyzed, but not *p*-nitrophenyl (pNP) β-D-galactopyranoside, pNP β-D glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP β-D-xylopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, L-aniline pNA, γ-L-glutamate pNA, and L-proline pNA. Acetate, *cis*- and *trans*-aconitate, pyruvate, fumarate, DL-3- hydroxybutyrate, and DL-lactate are used as sole sources of carbon, but not *N*-acetylgalactosamine, *N*-acetylglucosamine, adipate, adonitol, L-alanine, β-alanine, 4-aminobutyrate, L-arabinose, L-arbutin, L-aspartate, cellobiose, citrate, D-fructose, D-galactose, D-glucose, D-gluconate, glutarate, L-histidine, 3-hydroxybenzoate, L-malate, L-leucine, maltose, D-mannose, α-melibiose, L-ornithine, L-phenylalanine, L-proline, propionate, L-rhamnose, D-ribose, L-serine, sucrose, salicin, trehalose, D-xylose, myo-inositol, maltitol, D-mannitol, phenylacetate, D-sorbitol, or L-tryptophan. Acid is not produced from adonitol, L-arabinose, D-arabitol, cellobiose, dulcitol, erythritol, glucose, inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, methyl D-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, or D-xylose (Kämpfer et al. 2010). The type strain is CCUG $53761A^T$ = CCM 7698^T, which has a G+C content of the DNA of 57 mol%.

Paralcaligenes Kim et al. 2011

Par.al.ca.li'ge.nes. Gr. Prep. *para* beside; M.L. masc. n. *Alcaligenes* a bacterial genus name; M.L. masc. n. *Paralcaligenes* beside *Alcaligenes*.

Cultures comprise Gram-negative motile (with a single polar flagellum) strictly aerobic catalase- and oxidase-positive rods. The isoprenoid quinone is Q-8. The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine, and an unknown aminophospholipid. The major polyamines are cadaverine, putrescine, and 2-hydroxyputrescine. The dominant fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and summed feature 1. The type species is *Paralcaligenes ureilyticus*.

After incubation on R2A agar plates at 28 °C for 2 days, colonies of *Paralcaligenes ureilyticus* are white, circular, and convex and comprise rods of ~1.2–1.8 × 0.4–0.5 μm. Acid and alkaline phosphatase, catalase esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and urease are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cystine or valine arylamidase,

esterase lipase (C8), α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, indole, lipase (C14), α-mannosidase, oxidase, or trypsin. Nitrates are not reduced. Growth occurs at 10–30 °C (optimally at 28 °C) and at pH 4.0–8.0 (optimally at pH 6.0–7.0) and in 0–2 % (w/v) NaCl (optimally at 0–1 %). Aesculin, casein, carboxymethylcellulose, DNA, gelatin, tyrosine, and starch are not degraded. Glucose is not fermented. 3-hydroxybutyric acid, itaconic acid, lactic acid, potassium 2-ketogluconate, L-proline, propionic acid, sodium acetate, and valeric acid are assimilated, but not *N*-acetylglucosamine, adipic acid, L-alanine, L-arabinose, capric acid, L-fucose, D-glucose, glycogen, L-histidine, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, inositol, malic acid, D-maltose, D-mannitol, D-mannose, D-melibiose, phenylacetic acid, potassium gluconate, potassium 5-ketogluconate, L-rhamnose, D-ribose, D-saccharose, salicin, L-serine, sodium malonate, D-sorbitol, suberic acid, or trisodium citrate. The type strain is KACC 13888^T = DSM 24591^T, which has a G+C ratio of the DNA of 55.1 mol% (Kim et al. 2011).

Parapusillimonas Kim et al. 2010

Pa.ra.pu.sil.li.mo' nas. Gr. prep. *Par* a like; N.L. fem. n. *Pusillimonas* name of bacterial genus; N.L. fem. n. *Parapusillimonas* a bacterium like *Pusillimonas*.

Cultures comprise Gram-negative rods, which produce catalase and oxidase. Ubiquinone Q-8 is present. The major cellular fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and summed features 3 and 5. The major polyamine is putrescine. The polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. The G+C content of the DNA is 67.9 ± 0.1 mol%. The type species is *Parapusillimonas granuli*.

Parapusillimonas granuli matches the genus description, and when grown on R2A agar at 30 °C for 5 days, colonies are beige and circular with a diameter of 2 mm and comprise facultatively anaerobic rods of 0.9 × 1.5 μm in size, which are motile by means of three flagella per cell and possess fimbriae. Arginine dihydrolase, esterase (C4 and C8), leucine and valine arylamidase, and urease are produced, but not H_2S, indole, *N*-acetyl-ß-glucosaminidase acid or alkaline phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α- or ß-galactosidase, ß-glucuronidase, α- or ß-glucosidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, or trypsin. Nitrates are not reduced. Growth occurs at 25–37 °C [optimally at 30 °C] and at pH 6.5–7.5 [optimally at pH 7]. Utilizes adipate, L-alanine, citrate, gluconate, D-glucose, glycogen, L-histidine, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxybutyric acid, inositol, lactic acid, malate, phenylacetate, potassium 2-ketogluconate, L-proline, propionic acid, L-serine, sodium acetate, D-sorbitol, and valeric acid, but not L-arabinose, caprate, L-fucose, maltose, D-mannitol, melibiose, potassium 5-ketogluconate, L-rhamnose, D-ribose, salicin, sodium malonate, or sucrose. The type strain is KCTC 12668^T = LMG 24012^T, which has a G+C ratio of the DNA of 67.9 ± 0.1 mol% (Kim et al. 2010).

Pelistega Vandamme et al. 1998

Pe.li'ste.ga. Gr. n. *peleia* pigeon; Gr. fem. n. *stege* house, stay, residence; M.L. fem. n. *Pelistega* refers to the bacteria living in pigeons.

Cultures comprise Gram-negative, asporogenous, encapsulated, microaerophilic, nonmotile cells with variable morphologies. The major fatty acid components are $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ *ω7c*, $C_{16:1}$ *ω5c*, $C_{16:0}$ (3-OH), and summed features 3 and 7. The G+C ratio of the DNA is ~42–43 mol%. Type species is *Pelistega europaea*.

Pel. europaea produces gray-white to yellowish, convex, circular, smooth colonies with entire edges on blood agar and comprises microaerophilic, nonmotile, Gram-negative, asporogenous, capsulated cells with variable morphologies of 0.2–0.4 × 1–2 μm in size. Special growth factors are not required. Growth does not occur on MacConkey agar. Growth occurs at 37 °C and 42 °C, but not at 24 °C. Alkaline and acid phosphatase, catalase, esterase C4, ester lipase C8 and alanine, arginine, L-aspartic acid, glycine and leucine arylamidase, and oxidase are produced universally, whereas arginine dihydrolase, lipase, naphthol-AS-BI-phosphohydrolase, proline, pyroglutamic acid and tyrosine arylamidase, and urease are strain dependent, and *N*-acetyl-β-glucosaminidase, chondroitin sulfatase, chymotrypsin, cystine, leucyl glycine, glutamyl glutamic acid, histidine, phenylalanine, serine and valine arylamidase, α-fucosidase, α- and β-galactosidase, α- and β-glucosidase, β-glucuronidase, hyaluronidase, indole, lipase C14, lysine or ornithine decarboxylase, α-maltosidase, α-mannosidase, phenylalanine deaminase, and trypsin not at all. Nitrates are not reduced. The Voges-Proskauer reaction and methyl red test is negative. Aesculin and gelatin (mostly) are degraded, but not DNA or lecithin. Glucose is oxidized to alkali. Simmons citrate is utilized by some strains, but not malonate. L-malate is assimilated, but not *N*-acetyl-D-glucosamine, adipate, L-arabinose, caprate, D-gluconate, D-glucose, maltose, D-mannose, D-mannitol, or phenylacetate. Acid is not produced from *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D- and L-arabitol, cellobiose, dextrin, dulcitol, D-fructose, D-galactose, galacturonate, D-glucose, inositol, lactose, lactulose, maltose, D-mannitol, D-mannose, *myo*-inositol, palatinose, rhamnose, salicin, D-sorbitol, L-sorbose, sucrose, trehalose, or D-xylose. The major fatty acid components are $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ *ω7c*, $C_{16:1}$ *ω5c*, $C_{16:0}$ (3-OH), and summed features 3 and 7. Four genomovars are recognized. The type strain is LMG 10982 T, which has a G+C content of the DNA of 43 mol% (Vandamme et al. 1998).

Pigmentiphaga Blümel et al. 2001

Pig.men'ti.pha.ga. L. n. *pigmentum* dye; Gr. n. *phagos* eater; N.L. fem. adj. *Pigmentiphaga* eating dyes.

The genus and type species description were combined by Blümel et al. (2001) and amended by Yoon et al. (2007) to comprise cells that are motile or nonmotile. The common major

Table 27.10
Differentiating phenotypic properties of *Pigmentiphaga*

Characteristics	*Pig. kullae*	*Pig. daeguensis*	*Pig. litoralis*	*Pig. soli*
Motility	+	–	–	+
Facultatively anaerobic	–	–	+	
Growth at 4 °C	–	–	+	–
Growth at 40 °C	+	+	–	+
Alkaline phosphatase[a]	–	–	+	+
α-Chymotrypsin[a]	+	+	–	–
Cystine arylamidase[a]	–	–	+	–
Esterase (C4)[a]	+	+	–	+
α-Glucosidase[a]	+	–	+	–
β-Glucosidase[a]	–	–	+	–

[a]Data from API ZYM

polar lipid is phosphatidylethanolamine. The G+C content of the DNA is 67.4–68.8 mol%. The type species is *Pigmentiphaga kullae*. The distinguishing traits of *Pigmentiphaga* species is given in Table 27.10.

Pig. kullae produces opaque, circular, and convex colonies, which comprise catalase- and oxidase-positive, Gram-negative, motile, asporogenous rods of 1.3–4 × 0.7–1.2 μm in size. Growth occurs at 30–42 °C, but not at 4 °C. L-alanine-*p*-nitroanilide is hydrolyzed, but not *p*-nitrophenyl β-D-galactopyranoside, *p*-nitrophenyl β-D-glucuronide, *p*-nitrophenyl α-D-glucopyranoside, *p*-nitrophenyl β-D-glucopyranoside, *p*-nitrophenyl β-D-xylopyranoside, bis-*p*-nitrophenyl phosphate, bis-*p*-nitrophenyl-phenylphosphonate, bis-*p*-nitrophenyl phosphorylcholine, L-aniline *p*-nitroanilide-L-glutamate-*p*-nitroanilide, or L-proline *p*-nitroanilide. Acetate, *cis*-aconitate, *trans*-aconitate, adipate, L-aspartate, azelate, citrate, fumarate, glutarate, 3-hydroxybenzoate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, itaconate, DL-lactate, mesaconate, 2-oxoglutarate, propionate, pyruvate, and suberate are used as sole sources of carbon, but not *N*-acetylgalactosamine, *N*-acetylglucosamine, adonitol, L-alanine 4-aminobutyrate, L-arabinose, L-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, L-histidine, *i*-inositol, L-leucine, maltitol, D-maltose, D-mannitol, D-mannose, α-D-melibiose, L-ornithine, phenylacetate, L-phenylalanine, putrescine, L-rhamnose, D-ribose, D-sorbitol, D-sucrose, salicin, L-serine, D-trehalose, L-tryptophan, or D-xylose. Acids are not produced from adonitol, L-arabinose, D-arabitol, cellobiose, dulcitol, erythritol, glucose, inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, methyl D-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, or D-xylose. The major isoprenoid quinone is ubiquinone Q-8. The major polar lipid is phosphatidylethanolamine, with lesser amounts of phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are $C_{10:0}$ 3-OH, $C_{14:0}$ 2-OH, $C_{16:0}$, $C_{16:0}$ 2-OH, $C_{17:0}$ cyclo, $C_{19:0}$ cyclo *ω8c*, and summed features 3 and 7. The type strain is DSM 13608^T = NCIMB 13708^T, which has G+C content of the DNA of 68.5 ± 0.3 mol% (Blümel et al. 2001).

Pig. daeguensis produces yellow-white circular to slightly irregular 1.0–2.0 mm diameter colonies on TSA after incubation at 37 °C for 3 days and contains Gram-negative, nonmotile, asporogenous rods of 0.3–0.6 × 0.7–2.5 μm in size. Growth occurs at 15–46 °C [optimally at 37 °C] and not at all at 10 °C or 47 °C, at pH 5.0–9.0 [optimally at pH 7.0–8.0] and not at pH 4.5 or 9.5, and in 0–5 % (w/v) NaCl [optimally in 0.5 % (w/v) NaCl]. Anaerobic growth does not occur on TSA or on TSA supplemented with nitrate. Arginine dihydrolase (weakly), catalase, α-chymotrypsin, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase (weakly), and oxidase are produced, but not *N*-acetyl-β-glucosaminidase, acid or alkaline phosphatase, cystine or valine arylamidase, α-fucosidase, α- or β-galactosidase, β-glucuronidase, α- or β-glucosidase, lipase (C14), lysine or ornithine decarboxylase, α-mannosidase, trypsin, or tryptophan deaminase. Nitrates are not reduced. Hypoxanthine is degraded, but not aesculin; casein; gelatin starch; Tween 20, 40, 60, and 80; tyrosine; urea; or xanthine. Adipate, adonitol, citrate, and malate are utilized, but not *N*-acetylglucosamine, aesculin, amygdalin, L-arabinose, D-arabinose, D- and L-arabitol, arbutin, caprate, cellobiose, dulcitol, erythritol, fructose, D- and L-fucose, gentiobiose, galactose, gluconate, glucose, glycogen, glycerol, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, maltose, mannitol, mannose, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, phenylacetate, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, starch, sucrose, D-tagatose, trehalose, D-turanose, xylitol, D-xylose, or L-xylose. The predominant ubiquinone is Q-8. The major polar lipids are phosphatidylglycerol, phosphatidylethanolamine, and two unidentified aminolipids. The major fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and cyclo $C_{19:0}$ *ω8c*. The type strain is KCTC 12838^T = JCM 14330^T, which has a G+C ratio of the DNA of 67.4 mol% (Yoon et al. 2007).

Pig. litoralis produces yellow-pigmented, convex, non-translucent, glistening, circular colonies with slightly irregular edges of 1–2 mm in diameter after incubation on TSA at 28 °C for 3 days. Cultures comprise nonmotile, asporogenous, facultatively anaerobic, Gram-negative, straight to slightly curved rods of ~1.0–2.5 × 0.5–0.8 μm in size. Acid and alkaline phosphatase, arginine dihydrolase, catalase, cystine arylamidase, esterase lipase (C8), α- and β-glucosidase, lysine and ornithine decarboxylase, naphthol-AS-BI-phosphohydrolase, and oxidase are produced, but not *N*-acetyl-β-glucosaminidase, α-chymotrypsin, esterase (C4), α-fucosidase, α- or β-galactosidase, β-glucuronidase, H_2S, indole, leucine arylamidase, lipase (C14), α-mannosidase, trypsin, or valine arylamidase. Growth occurs at 4–35 °C [optimally at 25–30 °C] at pH 5.0–10.0 [optimally at pH 7.0] and in 0–5 % (w/v) NaCl [optimally at 0.5–1 %]. Aesculin is degraded, not but casein; gelatin; hypoxanthine; starch; L-tyrosine; Tween 20, 40, 60, and 80; urea; or

xanthine. The Voges-Proskauer reaction is positive. Nitrate is not reduced. Citrate is utilized. Arabinose, glucose, inositol, malate, maltose, mannitol, mannose, rhamnose, sorbitol, and sucrose are utilized, but not *N*-acetylglucosamine, adipic acid, amygdalin, capric acid, gluconate, melibiose, or potassium phenylacetic acid. Acid is produced from D-fructose, D-glucose, glycerol, D-lactose, maltose, D-mannitol, D-ribose, sucrose, trehalose, and D-xylose. The predominant respiratory quinone is ubiquinone Q-8. The polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and an unidentified phospholipid. The dominant fatty acids are $C_{16:0}$, $C_{16:1}$, cyclo $C_{17:0}$, and $C_{18:1}\omega7c$. The type strain is JSM 061001^T = CCTCC AA207034^T = KCTC 22165^T, which has a G+C ratio of the DNA of 65.5 mol% (Chen et al. 2009).

Pig. soli produces pale yellow circular, convex, smooth colonies on LB after 3 days, which comprise short aerobic, motile [single subterminal flagella] Gram-negative rods of 0.7–1.0 × 0.5–0.7 μm in size. Growth occurs strongly on TSA, LB, and NA and weakly on R2A agar, at 15–42 °C but not at 4 °C [optimally at 30 °C], at pH 5–8, and up to 3 % (w/v) NaCl. Acid and alkaline phosphatase, catalase, esterase (C4), esterase (C8), leucine and valine arylamidase, naphthol-AS-BI-phosphohydrolase, and oxidase are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cysteine arylamidase, α-fucosidase, α- and β-galactosidase, α- and β-glucosidase, β-glucuronidase, indole, lipase (C14), protease (gelatin), α-mannosidase, trypsin, and urease. Nitrates are not reduced. Growth occurs on acetate, adipate, caprate, citrate, D-glucose, glycogen, D,L-3-hydroxybutyrate, 4-hydroxybenzoate, L-malate, and suberate, but not *N*-acetyl-D-glucosamine, L-alanine, L-arabinose, gluconate, L-fucose, L-histidine, 3-hydroxybenzoate, itaconate, 2-ketogluconate, 5-ketogluconate, D,L-lactate, D-maltose, D-melibiose, malonate, D-mannitol, D-mannose, myo-inositol, propionate, L-rhamnose, D-ribose, salicin, L-serine, D-sorbitol, D-sucrose, phenylacetate, L-proline, or *n*-valerate. Acid is produced in D-adonitol, aesculin, amygdalin, erythritol, ferric citrate, glycerol, 2-ketogluconate, 5-ketogluconate, D-lyxose, α-methyl-D-glucopyranoside, β-methyl-D-xylose, salicin, and L-xylose, but not from *N*-acetyl-glucosamine, amidon, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, D-cellobiose, dulcitol, D-fructose, D-fucose, L-fucose, D-galactose, gentiobiose, gluconate, D-glucose, glycogen, inositol, inulin, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, α-methyl-D-mannopyranoside, D-melezitose, D-raffinose, L-rhamnose, D-ribose, D-sucrose, D-sorbitol, L-sorbose, D-tagatose, D-trehalose, D-turanose, xylitol, or D-xylose. The major quinone is Q-8. The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The predominant cellular fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and summed features 3 and 8. The type strain = KCTC 23577^T = JCM 17666^T = KEMB 9004-082^T, which has a G+C ratio of the DNA of 67.3 mol% (Lee et al. 2011). Since its description in the Journal of Microbiology, the name has not been validated in the International Journal of Systematic and Evolutionary Microbiology.

Pusillimonas Stolz et al. 2005

Pu.sil.li.mo' nas. L. adj. *pusillus* very small/min; Gr. fem n. *monas* unit/monad; N.L. fem. n. *Pusillimonas* very small monad/unicell, referring to the small size of cells and colonies of the type species.

Cultures comprise small Gram-negative, oxidase-positive oxidative rods. The major isoprenoid quinone is ubiquinone Q-8. Putrescine, spermidine, and 2-hydroxyputrescine are the major polyamines. Major polar lipids are two unknown aminolipids, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine (Stolz et al. 2005). The major fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and summed feature 2 (Park et al. 2011). The G+C content of the DNA is in the range of 53.1–61.8 mol% (Park et al. 2011). The type species is *Pusillimonas noertemannii* (Stolz et al. 2005). The distinguishing traits of *Pusillimonas* species are given in Table 27.11.

Pus. noertemannii develops brownish circular colonies with entire edges on NA and comprises Gram-negative polarly flagellated rods of 1–1.5 × 0.5–0.8 μm in size, which grow at 30–42 °C but not 4 °C. L-alanine-*p*-nitroanilide is hydrolyzed, but not L-aniline-*p*-nitroanilide, γ-L-glutamate-*p*-nitroanilide, *p*-nitrophenyl β-D-galactopyranoside, *p*-nitrophenyl β-D-glucuronide, *p*-nitrophenyl α-D-glucopyranoside, *p*-nitrophenyl β-D-glucopyranoside, bis-*p*-nitrophenyl phosphate, bis-*p*-nitrophenyl-phenylphosphonate, bis-*p*-nitrophenyl-phosphoryl choline, *p*-nitrophenyl β-D-xylopyranoside, or L-proline–*p*-nitroanilide. Acetate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, DL-lactate, 2-oxoglutarate propionate, and pyruvate are used as the sole sources of carbon, but not *N*-acetylgalactosamine, *N*-acetylglucosamine, *cis*-aconitate, *trans*-aconitate, adipate, adonitol, L-alanine, 4-aminobutyrate, L-arabinose, L-arbutin, L-aspartate, D-cellobiose, citrate, D-fructose, fumarate, D-galactose, gluconate, D-glucose, glutarate, L-histidine, 3-hydroxybenzoate, *i*-inositol, itaconate, L-leucine, L-malate, maltitol, D-maltose, D-mannitol, D-mannose, α-D-melibiose, L-ornithine, phenylacetate, L-phenylalanine, putrescine, L-rhamnose, D-ribose, salicin, L-serine, D-sorbitol, D-sucrose, D-trehalose, L-tryptophan, or D-xylose. Acid is not produced from adonitol, L-arabinose, D-arabitol, cellobiose, dulcitol, erythritol, glucose, inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, methyl D-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, or D-xylose. The dominant fatty acids comprise $C_{16:0}$, $C_{17:0}$, and $C_{19:0}$ cyclo $\omega8c$. The type strain = DSM 10065^T = NCIMB 14020^T, which has a G+C content of the DNA of 61.8 mol% (Stolz et al. 2005).

Pus. ginsengisoli produces pale yellow, circular colonies with entire edges after growth on NA at 30 °C for 5 days and comprises Gram-negative, aerobic, motile rods of 0.5–0.8 × 0.3–0.6 μm. Grows at 15–40 °C (optimally at 30 °C), but not at 4 °C or 42 °C, and at pH 5.5–9.0 (optimally at pH 7.0). Acid and alkaline phosphatase, catalase, α-chymotrypsin, cystine and leucine and valine arylamidase, esterase (C4), esterase (C8), naphthol-AS-BI-phosphohydrolase, oxidase, and trypsin are produced, but not *N*-acetyl-β-glucosaminidase, arginine

Table 27.11
Differentiating phenotypic properties of *Pusillimonas*

Characteristics	*Pus. noertemannii*	*Pus. ginsengisoli*	*Pus. harenae*	*Pus. soli*
Nitrate reduction	–	+		
N-acetyl-β-glucosaminidase[a]	+	–	–	
Acid phosphatase[a]	(+)	–	+	+
Alkaline phosphatase[a]	–	+	+	+
α-Chymotrypsin[a]	–	+	+	–
Cystine arylamidase[a]	–	+	+	–
Leucine arylamidase[a]	–	+	+	+
Valine arylamidase[a]	–	+	+	–
α-Galactosidase[a]	+	–	–	
α-Glucosidase[a]	+	–	–	
Naphthol-AS-BI-phosphohydrolase[a]	–	+	+	+
Trypsin[a]	–	+	Weak	–
Urease[a]	+	–	+	
Assimilation of				
Adipate[b]	–	+	+	+
L-Alanine[b]	–	+		+
Caprate[b]	+	–	–	–
Citrate[b]	–	+	–	+
D-Glucose[b]	+	–	–	
3-Hydroxybutyrate[b]	+	–		+
4-Hydroxybutyrate[b]	+	–		+
m-Inositol[b]	–	–		+
Malonate[b]	–	+/–		+
Phenylacetate[b]	–	+		+
Propionate[b]	–	+		+
D-Ribose[b]	–	+/–		+
Suberate[b]	+	–		+
n-Valerate[b]	–	Weak		+

[a]Data from API ZYM
[b]Data from API 20NE; API ID 32GN

dihydrolase, α-fucosidase, α- or β-galactosidase gelatinase, α- or β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, or urease. Acetate, adipate, L-alanine, citrate, 3-hydroxybutyrate, itaconate, DL-lactate, L-malate, phenylacetate, L-proline, propionate, L-serine, and *n*-valerate are assimilated, but not *N*-acetyl-D-glucosamine, L-arabinose, caprate, L-fucose, gluconate, D-glucose, glycogen, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 2-ketogluconate, 5-ketogluconate, maltose, D-mannitol, D-mannose, melibiose, *myo*-inositol, L-rhamnose, salicin, D-sorbitol, suberate, or sucrose. The predominant quinone is Q-8. The predominant polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The major fatty acids are $C_{19:0}$ cyclo *ω8c*, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and summed features 2 and 8. The type strain is KCTC 22046^T = JCM 14767^T, which has a G+C ratio of the DNA of 57.3 mol% (Srinivasan et al. 2010).

Pus. harenae develops ivory, round, convex colonies with entire edges on R2A agar and comprises strictly aerobic, Gram-negative, motile [with two lateral flagella] rods of 0.5–0.7 × 0.6–0.9 μm in size. Growth occurs at 15–45 °C [optimally at 30 °C], at pH 5.0–9.0 [optimally at pH 6.0–7.5], and in 0–6 % (w/v) NaCl [optimally in 0–3 % w/v NaCl]. Acid phosphatase, alkaline phosphatase (weak), catalase, α-chymotrypsin (weak), esterase (C4), esterase lipase (C8), cysteine, leucine and valine arylamidases, naphthol-AS-BI-phosphohydrolase, oxidase, and trypsin (weak) are produced, but not *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, α-mannosidase, indole, or lipase (C14). Nitrates are not reduced. Urea is degraded, but not aesculin or gelatin. Adipic acid and malic acid are assimilated, but not *N*-acetylglucosamine,

L-arabinose, capric acid, D-glucose, maltose, D-mannitol, D-mannose, phenyl acetic acid, potassium gluconate, or trisodium citrate. Acid is not produced from glucose. The major cellular polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, an unidentified phospholipid, and three aminolipids. The major acids are $C_{16:0}$, $C_{17:0}$ cyclo, $C_{19:0}$ cyclo *ω8c*, and summed feature 2. The type strain is KACC 14927^T = JCM 16917^T, which has a G+C ratio of the DNA of 53.1 mol% (Park et al. 2011).

Pus. soli comprises asporogenous Gram-negative catalase- and oxidase-positive motile rods of 0.7–1.0 × 0.3–0.5 μm in size. Growth occurs optimally at 30 °C and at pH 6.5–7.0 [range = pH 5.0–9.0]. Nitrates are reduced to gaseous nitrogen. Neither casein, cellulose, chitin, DNA, starch, nor xylan is attacked. Acetate, DL-3-hydroxybutyrate and DL-lactate are utilized, but not *N*-acetyl-D-glucosamine, L-arabinose, L-fucose, gluconate, D-glucose, glycogen, 2-ketogluconate, 5-ketogluconate, maltose, D-mannitol, D-mannose, melibiose, salicin, or sucrose. Esterase (C4) and esterase lipase (C8) are produced, but not *N*-acetyl-β-glucosaminidase, α-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, lipase (C14), or α-mannosidase. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and two unknown aminolipids. The major fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo, and $C_{19:0}$ cyclo *ω8c*. The type strain = KCTC 22455^T = JCM 16386^T, which has a G+C ratio of the DNA of 59.4 mol% (Lee et al. 2010b).

Taylorella Sugimoto et al. 1983

Tay.lor. el'la M. L. gen. n. *Taylorella*, named after C.E.D. Taylor who first studied the organism.

Cultures comprise microaerophilic [growth in 5–10 % CO_2], chemoorganotrophic, asporogenous, nonmotile, Gram-negative coccobacilli to rods that do not grow on conventional media, and poorly if at all on blood agar. Good growth occurs on chocolate agar but is not stimulated with X- and V-factors. Growth occurs at 30–42 °C [optimally at 35–37 °C]. Catalase, oxidase, phosphatase, and phosphoamidase are produced, but not arginine dihydrolase, H_2S, indole, or lysine or ornithine decarboxylase. Nitrates are not reduced. Neither DNA, gelatin, lipids, nor urea is degraded. Acid is not produced from carbohydrates. The type strain is *T. equigenitalis*. The distinguishing traits of *Taylorella* species is given in Table 27.12.

T. equigenitalis matches the genus description. In addition, cultures produce circular, convex, smooth, gray-white colonies with entire edges of 1.0–1.5 mm diameter on Eugon agar supplemented with heated blood after incubation for 3–4 days. Leucine and lysine arylamidase are produced, but not *N*-acetyl-glucosaminidase, chymotrypsin, esterase (C4 and C8), α- or β-fucosidase, α- or β-galactosidase, α- or β-glucosidase, β-glucuronidase, α-mannosidase, phenylalanine deaminase, or trypsin. The G+C ratio of the DNA is 36.5–37.0 mol% (Sugimoto et al. 1983). The type strain is NCTC 11184^T (Sugimoto et al. 1983).

Table 27.12
Differentiating phenotypic properties of *Taylorella*

Characteristics	*T. equigenitalis*	*T. asinigenitalis*
Valine arylamidase[a]	+ [weak]	–
Naphthol-AS-BI-phosphohydrolase[a]	+ [weak]	–

[a]Data from API ZYM

T. asinigenitalis produces tan colonies of ~1 mm in diameter that are entire, convex, opaque, and sometimes viscid in consistency after growth on ECA for 48 h. Cultures comprise Gram-negative, nonmotile coccobacilli that are catalase- and oxidase-positive and asaccharolytic and do not reduce nitrate. Alkaline phosphatase and leucine arylamidase are produced, but not indole and urease; aesculin is not degraded, nor is the methyl red test or Voges-Proskauer reaction positive. Growth does not occur on MacConkey agar or ECA medium incubated in air or anaerobically at 37 °C. Growth occurs microaerophilically and in air with 5–7 % CO_2 at 37 °C, but not at 24 °C or 42 °C. Growth is not stimulated by hemin or NAD. The major fatty acids are $C_{16:0}$, $C_{18:0}$, $C_{19:0}$ 10-methyl, and summed features 3, 4, and 7. The type strain is ATCC 700933^T = LMG 19572^T, and the G+C ratio of the DNA is 37.8 mol% (Jang et al. 2001).

Isolation, Enrichment, and Maintenance Procedures

Comparatively simple to complex media may be needed to isolate and culture the component taxa of *Alcaligenaceae*. Many representatives grow well on TSA with incubation at 25–37 °C for 1–2 days. Examples include *Ach. insolitus*, *Ach. spanius* and *Alc. denitrificans*, *Advenella*, *Kerstersia*, *Bor. avium*, *Bor. hinzii*, *Bor. trematum*, and *Pig. daeguensis* (Kersters et al. 1984; Vandamme et al. 1995; 1996; 2012; Coenye et al. 2003a, b; 2005; Van Trappen et al. 2005; Yoon et al. 2007). *Pel. europaea* needed microaerophilic conditions with TSA incubated at 36–37 °C (Vandamme et al. 1998). *Pig. litoralis* produced colonies on TSA prepared in 50 % seawater with incubation at 28 °C for 14 days (Chen et al. 2009). NA supported the growth of *Castellaniella* with incubation at 30 °C (Kämpfer et al. 2006). *Candidimonas* was cultured on PCA or R2A agar with incubation at 25–30 °C for 48 h (Vaz-Moreira et al. 2011; Zhang et al. 2012). *Pig. kullae* produced colonies on LB agar within 3 days at 37 °C (Blümel et al. 2001) whereas *Alc. faecalis* subsp. *parafaecalis* needed 48 h incubation (Schroll et al. 2001), and *Pus. soli* involved incubation at 30 °C for 2 weeks (Lee et al. 2010b). *Alc. faecalis* subsp. *phenolicus* and *Parapusillimonas granuli* were cultured on R2A agar with incubation at 30 °C (Rehfuss and Urban 2005; Kim et al. 2010). Similarly, *D. lacustris* was isolated on R2A agar after incubation at 25 °C for

3 days (Chen et al. 2013), whereas *Paralcaligenes ureilyticus* involved incubation at 28 °C for 5 days (Kim et al. 2011). Subculturing was achieved on nutrient agar but rarely on TSA and not at all on MacConkey agar (Kim et al. 2011). *Pig. soli* and *Pus. ginsengisoli* grew initially and were isolated on tenfold dilute R2A with incubation at 30 °C (Srinivasan et al. 2010; Lee et al. 2011). *Bra. oedipodis* was found using Columbia agar with cultivation aerobically at 37 °C for 24 h (Willems et al. 2002). *Cas. caeni* and *Cas. daejeonensis* were recovered on R2A agar with incubation at 30 °C for 2 weeks (Liu et al. 2008; Lee et al. 2010a). Also, *Cas. ginsengisoli* was recovered on R2A agar and was routinely cultured on LB agar with incubation at 30 °C (Kim et al. 2009). *Bor. bronchiseptica* may be isolated on Levine eosin-methylene blue agar (Smith and Baskerville 1979) whereas *Bor. petrii* was recovered anaerobically on RAMM medium of Shelton and Tiedje (1984) with incubation at 30 °C for 7 days (Von Wintzingeroda et al. 2001). *Pus. harenae* was recovered using marine 2216E agar with incubation aerobically at 25 °C for 5 days and grown routinely on R2A agar at 30 °C for 3 days (Park et al. 2011).

D. gummosa was recoverable on a nitrogen-free mineral medium supplemented with mannitol (Jensen et al. 1960). *Ach. marplatensis* was isolated in a mineral salt basal medium supplemented initially with 5 mg L^{-1} increasing to 100 mg $L^{-1}$of pentachlorophenol as the sole source of carbon (Gomila et al. 2011). *Adv. kashmirensis* was recovered from soil supplemented with 5 % sodium thiosulfate and 5 % elemental sulfur powder with incubation at 30 °C for 2 weeks with intermittent moistening with sterile water (Ghosh et al. 2005). The enriched samples were added to a modified basal and mineral salts solution (MSTY) with 20 mM sodium thiosulfate and 0.05 % yeast extract with incubation at 30 °C on a rotary shaker until phenol red indicator turned from red to yellow. Serial dilutions were plated on MSTY agar within incubation at 30 °C. The cultures were maintained in LB broth (Ghosh et al. 2005). *Adv. mimigardefordensis* was recovered by enrichment culture of mature compost involving a mineral salts medium supplemented with 0.5 % (w/v) 3, 3′-dithiodipropionic acid as the sole source of carbon with incubation at 30 °C for ∼1 week. Afterwards, serial dilutions were spread over mineral salts medium agar plates incorporating 0.5 % (w/v) 3, 3′-dithiodipropionic acid with incubation at 30 °C for 7 days (Wübbeler et al. 2006).

Richer media were required for some bordetellas, namely, *Bor. avium*, which grew well on Columbia agar supplemented with 7 % (v/v) defibrinated ox blood and on veal infusion agar (Kersters et al. 1984; Vandamme et al. 1995; 1996). *Bor. holmesii* was cultured on heart infusion agar supplemented with 5 % (v/v) rabbit blood in a candle jar at 35 °C (Weyant et al. 1995). *Bor. pertussis* and *Bor. parapertussis* were recoverable from human specimens on brilliant green medium modified with 16–20 % blood with incubation at 35–37 °C for up to 7 days (Kendrick and Eldering 1969). *Oligella* grew slowly on nutrient agar especially supplemented with blood, serum, or yeast autolysate (Rossau et al. 1987). *Pae. hominis* was discovered using blood agar with incubation at 37 °C with subculturing on nutrient agar at 30 °C for 24 h (Kämpfer et al. 2010). *Pus. noertemannii* grew strongly on chocolate-blood agar with incubation at 37 °C for 3–4 days, less well on sheep blood agar, and weakly on LB and NA (Stolz et al. 2005). *T. asinigenitalis* was cultured in 5–7 % CO_2 on Eugon agar supplemented with 10 % (v/v) laked horse blood and containing clindamycin and trimethoprim (Jang et al. 2001). In comparison, *T. equigenitalis* grew on Eugon agar with 5 % (v/v) Fildes digested sheep blood (Sugimoto et al. 1983).

Ecology

Habitat

Alcaligenaceae representatives occupy a diverse range of ecological niches from water and soil to animals and display a range of roles including the degradation of macromolecules, nitrogen fixation, and pathogenesis. *Ach. marplatensis* came from soil contaminated with pentachlorophenol in Argentina (Gomila et al. 2011), whereas *Adv. kashmirensis* was recovered from orchard soil and characteristically oxidized reduced sulfur compound, namely, tetrathionate and thiosulfate (Ghosh et al. 2005). *Adv. mimigardefordensis* was associated with compost and was capable of utilizing 3,3′-dithiodipropionic acid (Wübbeler et al. 2006). *Alcaligenes* spp. have been found in soil, water, hospitals, and clinical material (Coenye et al. 2003b). In particular, *Alc. aquatilis* was cultured from estuarine sediment and a salt marsh in Germany and the USA, respectively (Van Trappen et al. 2005). A solvent-tolerant strain capable of converting acetone-butanol residues into poly-β-hydroxybutyrate was recovered from water in a eutrophic garden pond, and identified as a new center of variation in *Alc. faecalis*, and named as *Alc. faecalis* subsp. *parafaecalis* (Schroll et al. 2001). In comparison, *Alc. faecalis* subsp. *phenolicus* is a phenol-degrading denitrifier, which was first identified in a graywater bioprocessor in the Johnson Space Center (Rehfuss and Urban 2005). *Bor. petrii* was recovered from an anaerobic dechlorinating bioreactor enriched from river sediment and was capable of aerobically reducing selenate to elemental selenium (Von Wintzingeroda et al. 2001). *Can nitroreducens*, *Can. humi*, and *Can. bauzanensis* were present in sewage sludge compost and soil, respectively (Vaz-Moreira et al. 2011; Zhang et al. 2012). *Cas. defragrans* was cultured from soil and demonstrated the ability to utilize alkenoic monoterpenes (Foss et al. 1998) and degrade phenol and taurine (Baek et al. 2003; Ruff et al. 2003). The denitrifier *Cas. caeni* was found in sludge in a leachate treatment plant in South Korea (Liu et al. 2008) whereas *Cas. daejeonensis* and *Cas. ginsengisoli* were isolated from oil-contaminated soil and ginseng field surface soil also in South Korea (Kim et al. 2009; Lee et al. 2010b). *D. gummosa* and *D. lacustris* were isolated initially from soil and lake water, respectively, and demonstrated nitrogen-fixing traits (Jensen et al. 1960; Chen et al. 2005). *Par. granuli* was obtained from environmental samples, namely, granules within a wastewater-treatment bioreactor in an alcohol fermentation factory in South Korea (Kim et al. 2010). *Pig. daeguensis* originated from wastewater from a dye works in South Korea (Yoon et al. 2007).

Moreover, *Pig. kullae* decolorized azo dyes, i.e., 1-(4′-carboxyphenylazo)-4-naphthol, as the sole source of carbon and energy (Blümel et al. 2001). *Pig. litoralis* was recovered from tidal flat sediment in China (Chen et al. 2009), and *Pig. soli* was found in South Korean soil (Lee et al. 2011). *Pus. harenae* was initially present in beach sand from the Taean coast of South Korea (Park et al. 2011). In comparison, *Pus. noertemannii* was isolated from river water and degraded 5-aminosalicylate (Stolz et al. 2005). Another member of the same genus, *Pus. soli* was recovered from farm soil (Lee et al. 2010b) whereas *Pus. ginsengisoli* was cultured from soil in a ginseng field located in South Korea (Srinivasan et al. 2010). *Paralcaligenes ureilyticus* was isolated from soil cultivated with Korean ginseng (Kim et al. 2011).

Bordetella spp. have been often isolated from epithelial cilia of the respiratory tract and from blood, being pathogenic for warm-blooded animals including humans. Thus, *Bor. holmesii* was obtained from blood cultures of young human adults (Weyant et al. 1995). *Bra. oedipodis* was recovered from the enlarged heart of a cotton-topped tamarind (*Saguinus oedipus*), which was devoid of disease signs and had died suddenly after a tooth extraction (Willems et al. 2002). *Kerstersia* was originally recovered from human clinical material (Coenye et al. 2003b) whereas *O. urethralis* was cultured from human urine, being considered as commensals of the genitourethral tract (Rossau et al. 1987). *T. asinigenitalis* was described from the genital tract of male donkeys in the USA (Jang et al. 2001).

Pathogenicity, Clinical Relevance

Many *Alcaligenaceae* representatives have been recovered from animals, with some taxa associated with disease as opportunists and primary pathogens. *Ach. insolitus* and *Ach. spanius* have been recovered from human clinical samples, with the former from wounds and urine and the latter from blood (Coenye et al. 2003a). *Bor. hinzii* has been recovered from humans and the respiratory tracts of poultry, but not necessarily linked with pathogenicity (Vandamme et al. 1995). Also, *T. asinigenitalis* was isolated from the genital tract of male donkeys but there was not any evidence of disease (Jang et al. 2001). *Ach. xylosoxidans* may be pathogenic of compromised patients (Yabuuchi and Yano 1981), including the cause of respiratory tract infections in cystic fibrosis patients (Lambiase et al. 2011) and prosthetic valve endocarditis in catheterized dialysis patients (Ahmed et al. 2009), and may be attributed with specific infections, such as osteomyelitis (Ozer et al. 2012) and ocular infections (Reddy et al. 2009). *Alc. faecalis* has been recovered from chickens and linked with respiratory disease (Berkhoff et al. 1984). Of the more serious pathogens, *Bor. pertussis*, *Bor. parapertussis*, *Bor. bronchiseptica*, *Bor. avium*, and *Bor. holmesii* are the causal agents of whooping cough, a milder form of disease, a severe respiratory disease mostly in dogs [= kennel cough] and pigs, coryza (= rhinotracheitis) in turkey poults and other birds, and septicaemia in humans, respectively (LeLey et al. 1986; Weyant et al. 1995; Willems et al. 2002). *Bor. avium* has been recovered initially from the respiratory tracts of turkeys and other birds, being associated with coryza/rhinotracheitis in turkey poults (Kersters et al. 1984). *Pel. europaea* was associated with respiratory disease in pigeons (Vandamme et al. 1998). The first cultures of *Bor. trematum* was obtained from wound and ear infections (Vandamme et al. 1996) whereas *Bra. oedipodis* was cultured from an enlarged heart [= endocarditis] of a cotton-topped tamarind, which died suddenly after a tooth extraction (Willems et al. 2002). *Kerstersia* was recovered from human clinical material, namely, leg/ankle wounds, sputum, feces, and neck abscess (Coenye et al. 2003b; Vandamme et al. 2012), whereas *Pen. hominis* was sourced from human blood of an 85-year-old male (Kämpfer et al. 2010). Finally, *T. equigenitalis* was associated with contagious equine, which is a serious venereal disease of horses (Platt et al. 1977; Sugimoto et al. 1983).

Application

Waste Treatment and Removal

Alc. faecalis, which were recovered from green water, nitrified heterotrophically ammonia into nitrite via formation of hydroxyl amine. This was oxidized to nitrous oxide using oxygen or nitrite as electron acceptor (Velusamy and Krishnani 2013). *Bordetella* Sulf-8 metabolized thiosulfate or sulfide into sulfate and degraded H_2S in a biotrickling filter system. In batch experiments, sulfur was removed at a rate of 88 mg S g^{-1} cells h^{-1} (Nisola et al. 2010). *Pigmentiphaga*-related DNA sequences were associated with naphthalene and phenanthrene degradation in contaminated soil (Jones et al. 2011). *T. kashmirensis*, which was recovered from soil, tolerated 64 mM selenite and detoxified it by aerobic reduction to insoluble elemental red selenium (Hunter and Manter 2008). Tannin-degrading *T. kashmirensis* was considered to have a role in the composting of olive mill waste (Federici et al. 2011).

Bioremediation

A thermophilic isolate of *Alc. faecalis*, which was recovered from crude oil-contaminated soil in India, produced a new class of glycolipid biosurfactant in medium supplemented with 2 % (v/v) diesel as the carbon and energy source. The biosurfactant demonstrated high emulsifying activity against hydrocarbons and was considered to have potential value in the bioremediation of hydrocarbon spillages (Bharali et al. 2011). *Achromobacter* was identified as part of a microbial consortium of six isolates involved in the bioremediation of hydrocarbon-rich industrial wastewater effluents, with possible value to petroleum refineries and the petrochemical industry (Gargouri et al. 2011). Also, *Achromobacter* in the presence of mineral salts solution was associated with 92 % bioremediation of polycyclic aromatic hydrocarbon-contaminated soil within 20 days; there was 99 % remediation after 8 weeks (Cutright and Lee 1994).

Family representatives have been attributed with a range of other bioremediation activities, including involvement in the aerobic degradation of glyphosate-contaminated soils by *Achromobacter* (Ermakova et al. 2010), the decoloration of dark brown-colored distillery effluent [due to the presence of high concentrations of melanoidin, which is an amino carbonyl polymer, phenolics, heavy metals, and sulfate] by *Ach. xylosoxidans* (Chaturvedi et al. 2006), the biotransformation of the herbicide with 100 $\mu g\ L^{-1}$ of terbuthylazine in a groundwater microcosm by *Adv. incenata* (Caracciola et al. 2010), the degradation of naphthalene and toluene in liquid culture by *Bor. petrii* (Bianchi et al. 2005), the mineralization of 1.2,4-trichlorobenzene in soil by *Bor. petrii* (Wang et al. 2007), and the in situ removal of nitrate in groundwater by *Castellaniella* (Spain et al. 2007).

Formation of Added-Value Products

Adv. mimigardefordensis SHX22 was genetically modified to produce poly(3-mercaptopropionic acid) (PMP) homopolymer. SHX22 accumulated PMP to ~25 % (w/w) of cell dry weight when grown in a mineral salts medium containing glycerol as the carbon source in addition to 3,3′-dithiodipropionic acid as a precursor (Xia et al. 2012).

Enzymes

Alcaligenaceae representatives are associated with the production of a diverse range of enzymes. For example, *Achromobacter* has been described as producer of collagenolytic enzymes, i.e., zinc-containing metalloenzymes, which have importance in biotechnology and medicine (Demina and Lysenko 1996).

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28 The Family *Burkholderiaceae*

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Abstract
The family *Burkholderiaceae* belongs to the order *Burkholderiales* within the class *Betaproteobacteria*. It contains the genera *Burkholderia* (the type genus), *Chitinimonas*, *Cupriavidus*, *Lautropia*, *Limnobacter*, *Pandoraea*, *Paucimonas*, *Polynucleobacter*, *Ralstonia*, *Thermothrix*, and *Wautersia*. The family is characterized by the presence of ecologically extremely diverse organisms and contains truly environmental saprophytic organisms, phytopathogens, opportunistic pathogens, as well as primary pathogens for humans and animals.

Taxonomy: Historical and Current

Short Description of the Family

Burk.hol.de.ri.a'ce.ae. M.L. fem. n. *Burkholderia* type genus of the family, *-aceae* ending to denote family; M.L. pl. n. *Burkholderiaceae*, the *Burkholderia* family.

The description is an emended version of the description given in *Bergey's Manual*, 2nd edition (Garrity et al. 2005). Family is phenotypically, metabolically, and ecologically extremely diverse and includes both strict aerobic and facultatively anaerobic chemoorganotrophs, as well as obligate and facultative chemolithotrophs. Based on phylogenetic analysis of 16S rRNA gene sequences, the family *Burkholderiaceae* contains the genera *Burkholderia* (the type genus), *Cupriavidus*, *Lautropia*, *Limnobacter*, *Pandoraea*, *Paucimonas*, *Polynucleobacter*, *Ralstonia*, and *Thermothrix*. In addition to these genera, the family also contains the more recently described genera *Chitinimonas* and *Wautersia*. Together with the families *Alcaligenaceae*, *Comamonadaceae*, and *Oxalobacteraceae*, the family *Burkholderiaceae* belongs to the order *Burkholderiales* within the class *Betaproteobacteria*.

Taxonomic History and Phylogenetic Structure of the Family and Its Genera

Historically, the taxonomy of the family *Burkholderiaceae* has its basis in studies on the genus *Pseudomonas* (reviewed by Kersters et al. 1996). Due to its broad and vague phenotypic definition, a lot of incompletely characterized, polarly flagellated, rod-shaped, aerobic, Gram-negative bacteria were placed in the genus *Pseudomonas*, but following results obtained by rRNA-DNA hybridizations, the genus was divided into five so-called rRNA groups by Palleroni et al. (1973). Later De Vos et al. (1985) demonstrated that these five groups were only distantly

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related to each other and that the genuine genus *Pseudomonas* had to be restricted to the group containing the type species *Pseudomonas aeruginosa* (rRNA group I or the *Pseudomonas fluorescens* rRNA branch). The seven species ([*Pseudomonas*] *solanacearum*, [*Pseudomonas*] *pickettii*, [*Pseudomonas*] *cepacia*, [*Pseudomonas*] *gladioli*, [*Pseudomonas*] *mallei*, [*Pseudomonas*] *pseudomallei*, and [*Pseudomonas*] *caryophylli* belonging to rRNA group II (the so-called solanacearum rRNA branch)) were transferred to the new genus *Burkholderia* in 1992 by Yabuuchi et al. [*Burkholderia*] *pickettii* and [*Burkholderia*] *solanacearum* were subsequently transferred to the novel genus *Ralstonia* (Yabuuchi et al. 1995). The genus *Burkholderia* belongs to rRNA superfamily III *sensu* De Ley (1992) or subgroup beta-3 of the *Betaproteobacteria sensu* Woese (1987). In the last decades, the genus *Burkholderia* has been expanded considerably, now containing well over 60 species (see http://www.bacterio.cict.fr/b/burkholderia.html for an up-to-date overview). The closest neighbor of the genus *Burkholderia* is the genus *Pandoraea*, described by Coenye et al. in 2000. Besides the previously described species [*Burkholderia*] *norimbergensis*, the genus initially contained four other species (*Pandoraea apista*, *Pandoraea pnomenusa, Pandoraea pulmonicola*, and *Pandoraea sputorum*) and was more recently expanded with the species *Pandoraea thiooxydans* (Anandham et al. 2010) and *Pandoraea oxalativorans*, *Pandoraea faecigallinarum*, and *Pandoraea vervacti* (Sahin et al. 2011). Besides these named species, the genus *Pandoraea* also contains four unnamed genomospecies (Coenye et al. 2000; Daneshvar et al. 2001).

A second major phylogenetic cluster within the family contains the genera *Cupriavidus, Ralstonia*, and *Wautersia*. As already mentioned above, the genus *Ralstonia* was created to accommodate two misclassified *Pseudomonas* species (*Ralstonia pickettii* and *Ralstonia solanacearum*) as well as a misclassified *Alcaligenes* species (*Ralstonia eutropha*) (Yabuuchi et al. 1995). Over the next few years, a number of other *Ralstonia* species were described (either novel species or previously misclassified taxa), including *Ralstonia basilensis* (Steinle et al. 1998; Goris et al. 2001), *Ralstonia campinensis* (Goris et al. 2001), *Ralstonia gilardii* (Coenye et al. 1999), *Ralstonia insidiosa* (Coenye et al. 2003), *Ralstonia mannitolilytica* (De Baere et al. 2001), *Ralstonia metallidurans* (Goris et al. 2001), *Ralstonia oxalatica* (Sahin et al. 2000), *Ralstonia paucula* (Vandamme et al. 1999), *Ralstonia respiraculi* (Coenye et al. 2003b), *Ralstonia syzygii* (formerly known as [*Pseudomonas syzygii*]) (Vaneechoutte et al. 2004), and *Ralstonia taiwanensis* (Chen et al. 2001). In 2004 Vaneechoutte et al. reported that two sublineages (>4 % dissimilarity in the 16S rRNA gene sequence) could be distinguished within the genus *Ralstonia* and proposed to reclassify the species belonging to the so-called *Ralstonia eutropha* lineage (*R. basilensis*, *R. campinensis*, *R. eutropha*, *R. gilardii*, *R. metallidurans*, *R. oxalatica*, *R. paucula*, *R. respiraculi*, and *R. taiwanensis*) in the novel genus *Wautersia*, with *Wautersia eutropha* as the type species. However, in 2004, Vandamme and Coenye demonstrated that *W. eutropha* (the type species of the genus *Wautersia*) is a later heterotypic synonym of *Cupriavidus necator* (the type species of the genus *Cupriavidus*, described by Makkar and Casida in 1987). According to Rule 37a of the Bacteriological Code, this meant that the name *Wautersia* had to be replaced by *Cupriavidus*, and all *Wautersia* species were renamed as *Cupriavidus* species. Only a single species remains in the genus *Wautersia, Wautersia numazuensis* (described in 2005 by Kageyama et al.), but its close phylogenetic relationship with *Cupriavidus* species strongly suggests it should be reclassified in this genus. Since its creation, three novel species were added to the genus *Cupriavidus*, namely, *Cupriavidus pinatubonensis* and *Cupriavidus laharis* (Sato et al. 2006) and *Cupriavidus pampae* (Cuadrado et al. 2010). Estrada-de Los Santos et al. (2012) recently proposed the name *Cupriavidus alkaliphilus* for plant-associated isolates. At the time of writing, this name has not been validated yet.

Besides the *Burkholderia/Pandoraea* group and the *Cupriavidus/Ralstonia/Wautersia* group, several other genera belong to the family *Burkholderiaceae*, namely, the genera *Chitinimonas*, *Lautropia*, *Limnobacter*, *Paucimonas*, *Polynucleobacter*, and *Thermothrix*.

The genus *Chitinimonas* (Chang et al. 2004), containing the species *Chitinimonas taiwanensis* (Chang et al. 2004) and *Chitinimonas koreensis* (Kim et al. 2006), forms a separate branch in the 16S rRNA-based phylogenetic tree and is only distantly related to other members of this family. Actually, this genus appears to be more closely related to members of the *Neisseriaceae* and its exact phylogenetic affiliation probably will need to be revised.

Limnobacter and *Lautropia* species (*Limnobacter thiooxidans, Limnobacter litoralis*, and *Lautropia mirabilis*) (Gerner-Smidt et al. 1994; Spring et al. 2001; Lu et al. 2011) occupy a position between both major phylogenetic groups but are more closely associated with the *Burkholderia/Pandoraea* group.

The genus *Paucimonas* (containing a single species, *Paucimonas lemoignei*) (Jendrossek 2001) appears to occupy a position somewhat between the *Burkholderiaceae* and the *Oxalobacteraceae*.

The genus *Polynucleobacter* contains five species (*Polynucleobacter acidophilus, Polynucleobacter cosmopolitanus, Polynucleobacter difficilis, Polynucleobacter necessarius* [with subspecies *necessarius* and *asymbioticus*], and *Polynucleobacter rarus*) (Heckmann and Schmidt 1987; Hahn et al. 2009, 2010, 2011a, b, 2012). Although it occupies a distinct position within the tree, its closest relatives are members of the *Cupriavidus/Ralstonia/Wautersia* group.

Finally, the genus *Thermothrix*, containing the species *Thermothrix azorensis* and *Thermothrix thiopara* (Odintsova et al. 1996; Caldwell et al. 1976), appears to be unrelated to any other member of this family and its deep-branching position suggests it should be reclassified.

Molecular Analyses

DNA-DNA Hybridization Studies

The vast majority of descriptions of species of the genera belonging to the family *Burkholderiaceae* include results of

DNA-DNA hybridization experiments. Overall species can easily be distinguished from each other as DNA-DNA binding values between representatives of different species are below 30 %. However, within the *Burkholderia cepacia* complex, representatives of different species generally have DNA-DNA hybridization values between 30 % and 60 %, while values obtained from strains belonging to the same species are generally higher than 70 %. DNA-DNA binding values obtained with other *Burkholderia* species are generally below 30 % (Coenye et al. 2001). The same appears to be true to some extent for *Pandoraea* species (with values up to 45 % between strains from different species) (Coenye et al. 2000) and certain *Ralstonia* species, e.g., hybridization values up to 58 % between *Ralstonia pickettii* and *Ralstonia mannitolilytica* (De Baere et al. 2001).

Phylogenetic Analysis Based on Housekeeping Genes

Ribosomal RNA genes evolve slowly, and due the associated limited taxonomic resolution, analysis of the 16S rRNA gene often does not allow to reliably distinguish between closely related species (Fox et al. 1992; Palys et al. 2000). This problem is most obvious in the genera *Burkholderia* (especially in the *Burkholderia cepacia* complex) and in the genus *Pandoraea* (*Fig. 28.1*). The sequencing of several "housekeeping" genes (i.e., protein-coding genes with an essential function) (e.g., *gyrB*, *rpoB*, *infB*, and *recA*) has been proposed to complement the phylogenetic information obtained from sequencing the 16S rRNA gene (Eisen 1995; Yamamoto and Harayama 1995, 1998; Venkateswaran et al. 1998; Hedegaard et al. 1999; Maréchal et al. 2000). The housekeeping genes most often used in phylogenetic analyses of taxa of the family *Burkholderiaceae* are *recA* (encoding recombinase A) and *gyrB* (encoding gyrase B).

Phylogenetic analyses based on the *recA* gene have played an important role in taxonomic studies of the genus *Burkholderia* and in the development of diagnostic PCR assays (see below), especially for the *Burkholderia cepacia* complex (Mahenthiralingam et al. 2000; Payne et al. 2005). In phylogenetic trees based on (nearly) complete *recA* sequences, most *Burkholderia cepacia* complex species will form discrete clusters that can easily be discerned from other species, unlike in trees based on 16S rRNA gene sequences. A notable exception is *Burkholderia cenocepacia*, in which four different *recA* lineages (designated IIIA through IIID) can be found (Vandamme et al. 2003; Vanlaere et al. 2009). Other protein-coding housekeeping genes that have been used for deducing relationships between *Burkholderia* species include *hisA* (encoding a 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase involved in histidine biosynthesis) (Papaleo et al. 2010) and *parB* and *repA*, two genes involved in the partitioning and replication, respectively, of the multireplicon *Burkholderia cepacia* complex genomes (Drevinek et al. 2008).

Sequence analysis of the gene encoding the gyrase B protein (*gyrB*) was shown to be very useful to deduce phylogenetic relationships between *Pandoraea* species. Sequence analysis of *gyrB* confirmed the separate status of *Pandoraea* genomospecies 1, 3, and 4 and suggested a close relationship between *Pandoraea* genomospecies 2 and *Pandoraea sputorum* (Coenye and LiPuma 2002; Sahin et al. 2011).

Multilocus Sequence Analysis

Initially developed as a typing tool, the sequence analysis of multiple (protein-coding) housekeeping genes (multilocus sequence analysis, MLSA) has emerged as a powerful taxonomic tool (Gevers et al. 2005; Coenye et al. 2005). Within the genus *Burkholderia*, MLSA schemes have been developed for the *Burkholderia pseudomallei/Burkholderia mallei/Burkholderia thailandensis* group (Godoy et al. 2003) and the *Burkholderia cepacia* complex (Baldwin et al. 2005). In order to keep up with the increasing taxonomic complexity of the *Burkholderia cepacia* complex and to be able to include other *Burkholderia* species, an "expanded" MLSA scheme was developed and published in 2009 (Spilker et al. 2009). The use of degenerate primers allowed expansion of the *Burkholderia cepacia* complex MLSA scheme to include other clinically relevant (e.g., *Burkholderia gladioli*) or important environmental (e.g., *Burkholderia glumae*) species. In the *Burkholderia cepacia* complex MLSA scheme, the following genes are used: *atpD* (encoding ATP synthase β chain), *gltB* (large subunit of glutamate synthase), *gyrB* (gyrase B), *recA* (recombinase A), *lepA* (GTP-binding protein), *phaC* (acetoacetyl-CoA reductase), and *trpB* (subunit B of tryptophan synthase). In the *Burkholderia pseudomallei* MLSA scheme, the following genes are used: *aceA* (encoding acetyl coenzyme A reductase), *gltB* (large subunit of glutamate synthase), *gmhD* (ADP glycerol-mannoheptose epimerase), *lepA* (GTP-binding elongation factor), *lipA* (lipoic acid synthetase), *narK* (nitrite extrusion protein), and *ndh* (NADH dehydrogenase).

MALDI-TOF MS

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of intact cells has recently emerged as a powerful tool for the characterization and identification of microorganisms. Most studies so far have focussed on the *Burkholderia cepacia* complex. In their pioneering study, Vanlaere et al. (2008) demonstrated that MALDI-TOF MS is a powerful approach for the rapid identification of *Burkholderia cepacia* complex bacteria. Seventy-five strains belonging to the nine at that time established *Burkholderia cepacia* complex species as well as some species commonly misidentified as belonging to the *Burkholderia cepacia* complex were included. All *Burkholderia cepacia* complex strains clustered together and could be separated from non-*Burkholderia cepacia* complex strains. Within the *Burkholderia cepacia* complex, most strains

Fig. 28.1

Phylogenetic reconstruction of the family *Burkholderiaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al., 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40% maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

grouped in species-specific clusters, except for *Burkholderia anthina* and *Burkholderia pyrrocinia* strains which constituted a single cluster. Subsequent studies in the context of cystic fibrosis microbiology confirmed that MALDI-TOF MS is a useful addition to the toolbox to identify *Burkholderia cepacia* complex species and related taxa (including *Ralstonia* and *Cupriavidus* species) (Degand et al. 2008; Minan et al. 2009; Fernández-Olmos et al. 2012; Desai et al. 2012). Two recent studies also demonstrated that this technique is appropriate for the confirmation of *Burkholderia pseudomallei* (Inglis et al. 2012; Lau et al. 2012).

PCR-Based Assays for Identification of Species Belonging to the Family *Burkholderiaceae*

Several candidate PCR assays have been developed for the identification of the *Burkholderia cepacia* complex species, but most of these assays were developed before the recognition that several species constitute the *Burkholderia cepacia* complex, and most relied on published DNA sequence data derived from analyses of culture collection strains that, in retrospect, are poorly representative of the total diversity within this group. However, in 1999, LiPuma et al. and Bauernfeind et al. (1999) presented rRNA-gene-based PCR assays which allowed the identification of *Burkholderia multivorans* and *Burkholderia vietnamiensis* and *Burkholderia cepacia, Burkholderia cenocepacia*, and *Burkholderia stabilis* as a group (but the latter three species could not be differentiated from each other). They also developed a PCR assay that, in retrospect, showed a positive reaction for the majority of *Burkholderia, Ralstonia, Cupriavidus, and Pandoraea* isolates investigated (unpublished data). Around the same time, Whitby et al. (2000) described an rRNA-gene-based PCR assay for the identification of *Burkholderia gladioli*. Vermis et al. (2004) developed a 16S rRNA-based assay for the identification of *Burkholderia dolosa*. 16S rRNA-gene-based PCR assays with excellent sensitivity and specificity were also developed for the genus *Pandoraea* as a whole and a number of individual *Pandoraea* species (*Pandoraea sputorum, Pandoraea norimbergensis, Pandoraea pnomenusa, and Pandoraea apista/Pandoraea pulmonicola*) (Coenye et al. 2001a). Similar assays were developed for *Ralstonia pickettii* and *Ralstonia mannitolilytica* (Coenye et al. 2002) and *Ralstonia insidiosa* (Coenye et al. 2003a).

Due to the inherent limitations imposed by the use of the 16S rRNA gene, the possibility to use alternative targets for PCR-based identification tools has been explored. The *recA* gene was identified as such a target that turned out to be extremely useful for the differentiation of *Burkholderia* species, including members of the *Burkholderia cepacia* complex. Analysis of *recA* sequences from a large number of isolates enabled the design of a *Burkholderia* genus-specific *recA* PCR as well as the design of a *Burkholderia cepacia* complex-specific *recA* PCR (Mahenthiralingam et al. 2000; Payne et al. 2005). In addition, PCR assays were developed for a number of individual *Burkholderia cepacia* complex species, including *Burkholderia cepacia, Burkholderia multivorans, Burkholderia cenocepacia, Burkholderia stabilis* and *Burkholderia vietnamiensis* (Mahenthiralingam et al. 2000), *Burkholderia ambifaria* (Coenye et al. 2001), and *Burkholderia dolosa* (Vermis et al. 2004). A thorough evaluation by Vermis et al. (2002) (508 *Burkholderia cepacia* complex isolates representing nine species) revealed that the *recA*-based assays for the identification of *Burkholderia multivorans, Burkholderia cenocepacia*, and *Burkholderia ambifaria were most efficient but that the assay directed against Burkholderia cepacia* lacked sensitivity and cross-reacted with all *Burkholderia pyrrocinia* isolates examined. In an attempt to develop a single PCR assay to distinguish all *Burkholderia cenocepacia* subgroups from other *Burkholderia cepacia* complex species, Drevinek et al. (2008) explored the sequence diversity in the *parB* and *repA* genes. The *Burkholderia cenocepacia repA* sequences were distinct from other species, which enabled the design of a species-specific multiplex PCR that allowed to identify the former species with 100 % sensitivity and 93 % specificity.

Ribotyping and Riboprinting

In ribotyping, chromosomal restriction fragment length polymorphisms (RFLP) are detected by probing restriction-digested genomic DNA with rRNA (Stull et al. 1988; Bingen et al. 1994) and RFLPs result mainly from nucleotide sequence variation in regions flanking rRNA genes. A modification of ribotyping, referred to as PCR ribotyping, employs PCR to amplify the 16S–23S intergenic spacer region of the bacterial rRNA operon to detect sequence length polymorphisms therein (Kostman et al. 1995). Both ribotyping and PCR ribotyping were used in early investigations of *Burkholderia cepacia* complex epidemiology (LiPuma et al. 1988, 1990, 1994; Dasen et al. 1994). Moissenet et al. (2001) evaluated PCR ribotyping as a tool for species and strain differentiation within the genera *Ralstonia* and *Cupriavidus* but concluded that the resolution was insufficient. The generation of RiboPrint patterns for *Burkholderiaceae* using the RiboPrinter microbial characterization system has been evaluated in a limited number of studies. Brisse et al. (2000) showed that this method was potentially useful for species-level identification within the *Burkholderia cepacia* complex. The method was also evaluated for typing of *Burkholderia pseudomallei* (Inglis et al. 2002) and *Burkholderia mallei* (Grif et al. 2003). Riboprinting has occasionally been used for other taxa within this family, including *Cupriavidus pauculus* (Clermont et al. 2001).

REP-PCR

The genomes of most bacteria contain specific conserved repetitive sequences. Versalovic et al. (1991) showed that these repeats can be used to amplify the intervening DNA sequences. Three main sets of repeats have been used for typing: (i) repetitive extragenic palindromic (REP) elements

Table 28.1
Selection of strains of the family *Burkholderiaceae* for which a complete genome sequence is available. Data obtained from the Genome Atlas Database (http://www.cbs.dtu.dk/services/GenomeAtlas/)

Species and strain designation	No. of replicons	Total size (bp)	No. of genes	No. of 16S rRNA genes	No. of tRNA genes	%GC
Burkholderia ambifaria AMMD	4	7,528,567	6,617	6	69	66.8
Burkholderia ambifaria MC40-6	4	7,642,536	6,697	6	68	66.4
Burkholderia cenocepacia AU 1054	3	7,279,116	6,477	6	67	66.9
Burkholderia cenocepacia HI2424	4	7,702,840	6,919	6	67	66.8
Burkholderia cenocepacia J2315	4	8,055,782	6,485	6	73	66.9
Burkholderia cenocepacia MC0-3	3	7,971,389	7,008	6	67	66.4
Burkholderia glumae BGR1	6	7,284,683	5,773	5	66	67.9
Burkholderia mallei ATCC 23344	2	5,835,527	5,025	4	56	68.5
Burkholderia mallei NCTC 10229	2	5,742,303	5,510	4	56	68.5
Burkholderia mallei NCTC 10247	2	5,848,380	5,852	4	55	68.5
Burkholderia mallei SAVP1	2	5,232,401	5,189	5	55	68.4
Burkholderia multivorans ATCC 17616	4	7,008,622	6,259	5	65	66.7
Burkholderia phymatum STM815	4	8,676,562	7,496	6	62	62.3
Burkholderia phytofirmans PsJN	3	8,214,658	7,241	6	63	62.3
Burkholderia pseudomallei 1106a	2	7,089,249	7,183	4	59	68.3
Burkholderia pseudomallei 1710b	2	7,308,054	6,347	4	60	68.0
Burkholderia pseudomallei 668	2	7,040,403	7,230	4	59	68.3
Burkholderia pseudomallei K96243	2	7,247,547	5,855	4	60	68.1
Burkholderia thailandensis E264	2	6,723,972	5,634	4	58	67.6
Burkholderia vietnamiensis G4	8	8,391,070	7,617	6	67	65.7
Burkholderia xenovorans LB400	3	9,731,138	8,702	6	64	62.6
Cupriavidus metallidurans CH34	4	6,913,352	6,319	4	62	63.5
Cupriavidus necator H16	3	7,416,678	6,626	5	61	66.3
Cupriavidus taiwanensis	3	6,476,522	1,611	5	63	67.0
Polynucleobacter necessarius subsp. *asymbioticus* QLW-P1DMWA-1	1	2,159,490	2,077	1	38	44.8
Polynucleobacter necessarius subsp. *necessarius* STIR1	1	1,560,469	1,508	1	37	45.6
Ralstonia pickettii 12D	5	5,685,358	5,361	3	54	63.3
Ralstonia pickettii 12 J	3	5,325,729	4,952	3	55	63.6
Ralstonia solanacearum GMI1000	2	5,810,922	5,120	4	57	67.0

(35–40 bp), (ii) enterobacterial repetitive intergenic consensus (ERIC) sequences (124–127 bp), and (iii) BOX elements (154 bp) (Olive and Bean 1999). Depending on which sequences are targeted, these assays are referred to as REP-, ERIC-, or BOX-PCR, respectively. Collectively, these methods are referred to as rep-PCR. Targets most commonly used for the typing of *Burkholderia cepacia* complex organisms are the ERIC (Liu et al. 1995; Cimolai and Trombley 1996; Seo and Tsuchiya 2005) and BOX elements (Chen et al. 2001; LiPuma et al. 2002; Biddick et al. 2003; Coenye et al. 2004; Seo and Tsuchiya 2005). This approach has also been used to investigate *Burkholderia pseudomallei* (Currie et al. 2007) (BOX), *Ralstonia solanacearum* (Smith et al. 1995; Norman et al. 2009; Stevens and van Elsas 2010; Xue et al. 2011) (ERIC, REP, and BOX), *Ralstonia pickettii* and *Ralstonia insidiosa* (Ryan et al. 2011) (BOX), and *Pandoraea apista* (Atkinson et al. 2006) (ERIC, REP, and BOX). Finally, BOX-PCR was also used to compare root-nodule isolates with reference isolates of *Burkholderia phymatum*, *Burkholderia tuberum*, *Burkholderia mimosarum*, *Burkholderia nodosa*, *Burkholderia sabiae*, *Burkholderia caribensis*, and *Cupriavidus taiwanensis* (Liu et al. 2011).

Genome Comparison

Genome sequences are currently available for many species of this family, but clinically relevant species like *B. cenocepacia*, *B. mallei*, and *B. pseudomallei*,

phytopathogens like *R. solanacearum*, and strains from species with potential for specific applications like *C. necator* are overrepresented. A selection of genomes of representatives of the *Burkholderiaceae* and their key properties are shown in *Table 28.1*. More information can be found in reviews by Mahenthiralingam and Drevinek (2007) and Vandamme and Dawyndt (2011).

Many members of the *Burkholderiaceae* (most notably species from the genera *Burkholderia*, *Cupriavidus*, and *Ralstonia*) have large and unusual multireplicon genomes that lie at the basis of the remarkable phenotypic diversity demonstrated by these bacteria (Mahenthiralingam and Drevinek 2007). Early research carried out by Cheng and Lessie (1994) showed that *B. multivorans* ATCC 17616 contained three large circular replicons of 3.4, 2.5, and 0.9 Mb. Each of these replicons contained rRNA genes, so they could be considered as real chromosomes. Rodley et al. (1995) showed that the type strain of *B. cepacia* and strains belonging to *B. glumae* and *B. glathei* also contained multiple replicons, as did strains belonging to *R. pickettii*, *R. solanacearum*, and *R. eutropha*. Further research by Lessie et al. (1996) showed that representatives of all members of the *B. cepacia* complex contain two or three chromosomes, with the total genome size ranging from 4.7 to 8.1 Mb. Research from others also demonstrated the multiple replicon structure in *B. gladioli* (Wigley and Burton 2000). Interestingly, large genomes have been described as disproportionately enriched in regulation and secondary metabolism genes and depleted in protein duplication, DNA replication, cell division, and nucleotide metabolism genes in comparison to small-sized genomes (Konstantinidis and Tiedje 2004). This may explain why species with large genomes dominate in environments where resources are scarce but diverse, such as in soils (Konstantinidis and Tiedje 2004). Recent work demonstrated that the smallest chromosome in the *B. cepacia* complex (chromosome 3) is not essential, but should be considered as a large plasmid that encodes virulence, secondary metabolism, and other accessory functions in *B. cepacia* complex bacteria (Agnoli et al. 2012). Based on a bioinformatics approach, Juhas et al. (2012) recently claimed that the core genome of the order *Burkholderiales* consists of 649 genes. All but two of these identified genes were located on the largest chromosome (chromosome 1) of *B. cenocepacia*, including the known essential genes *infB*, *gyrB*, *ubiB*, and *valS*, as well as the so far uncharacterized genes BCAL1882, BCAL2769, BCAL3142, and BCAL3369.

There is a growing interest in using information derived from whole-genome sequencing projects for taxonomic purposes (Coenye and Vandamme 2003b; Coenye et al. 2005; Gevers et al. 2005). Several of the approaches were evaluated by Vanlaere et al. (2009; on 13 genomes) and by Vandamme and Dawyndt (2011; on 44 genomes) to determine their ability to contribute to our taxonomic understanding of the *Burkholderiaceae* (with a strong focus on the genus *Burkholderia*). Parameters that have been included in these studies are average nucleotide identity (ANI), average amino acid identity (AAI), and core gene identity (CGI) (Konstantinidis and Tiedje 2005a, b, 2007; Goris et al. 2007). ANI and CGI analysis of 44 genomes (43 *Burkholderia* genomes + *C. metallidurans* CH34) resulted in highly correlated similarity matrices for both methods ($r^2 = 0.95$) (Vandamme and Dawyndt 2011). Trees based on these similarity matrices showed a much better resolved picture of the phylogenetic relationships of more distantly related species. For example, the *B. cepacia* complex and the *B. mallei*/*B. pseudomallei* group were extremely well resolved with high bootstrap values and both formed distinct lines of descent (Vandamme and Dawyndt 2011).

Table 28.2
Phenotypic characteristics that allow the differentiation of *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Burkholderia thailandensis* (Based on data compiled by LiPuma et al. (2011))

	B. mallei	*B. pseudomallei*	*B. thailandensis*
Growth at 42 °C	–	+	+
Gas from nitrate	–	+	+
Acid from maltose	–	+	+
Acid from mannitol	–	+	+
Acid from arabinose	ND	–	+
Motile	No	Yes	Yes

+, >90 % of isolates are positive; –, <10 % of isolates are positive; *ND*, not determined

Phenotypic Analyses

Burkholderia Yabuuchi et al. 1993 Emend. Gillis et al. 1995

Burk.hold.er'i.a. M.L. dim. ending *-ia;* M.L. fem. n. *Burkholderia*, named after W. H. Burkholder, the American bacteriologist who first discovered the etiological agent of sour skin of onions.

The following genus description was presented in 1995 by Gillis et al. and was based on the 11 species described at that time. *Burkholderia* cells are Gram-negative, nonfermentative, straight rods that have a single polar flagellum or a tuft of polar flagella. A single species, *B. mallei*, is atrichous and nonmotile. Catalase is produced, and oxidase activity varies between species. The cellular fatty acids are characterized by the presence of 3-hydroxy C16:0. The type strains of several species are characterized by the presence of two types of ornithine lipids. Most species grow at 40 °C. All species can grow with the following substrates as sole carbon sources: glucose, glycerol, inositol, galactose, sorbitol, and mannitol. Some species are pathogenic for humans, animals, or plants. They are isolated from plant material, soil, or clinical samples and can be recognized on the basis of 16S rRNA characteristics. Most strains accumulate polyhydroxybutyrate as carbon reserve material and are capable of *ortho* cleavage of protocatechuate. The G + C content is 59.0–69.5 mol%. The type species is *Burkholderia cepacia*.

Table 28.3
Phenotypic characteristics that allow the differentiation of members of the *Burkholderia cepacia* complex and *Burkholderia gladioli* (Based on data compiled by LiPuma et al. (2011))

	Bam	Ban	Bar	Bcen	Bce	Bco	Bdi	Bdo	Blaa	Blae	Bme	Bmu	Bpy	Bse	Bst	Bubo	Bvi	Bgl
Oxidase	+	+	+	+	+	v	+	+	v	+	+	+	v	+	+	+	+	v
Growth on MacConkey	+	+	+	v	v	+	+	+	+	+	+	+	+	+	+	+	v	+
Acid from																		
Maltose	+	+	+	v	v	v	+	+	+	+	+	+	+	+	+	+	+	–
Lactose	+	+	v	+	+	+	+	+	+	+	+	+	+	+	v	+	+	–
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	v	+	v	+
Sucrose	+	v	v	+	v	+	v	–	v	+	v	–	v	v	–	+	+	–
Adonitol	+	v	+	v	v	+	v	+	v	+	+	+	+	+	+	–	–	+
Nitrate reduction	v	v	v	v	–	v	+	+	v	–	–	+	v	–	–	v	v	v
Lys decarboxylase	+	v	v	+	+	+	+	–	+	+	+	v	+	v	+	–	+	–
Orn decarboxylase	–	–	+	v	v	–	–	–	v	–	–	–	v	v	+	–	–	–
Esculin hydrolase	v	–	–	v	v	v	–	–	v	–	+	–	–	v	–	–	–	v
Gelatinase	+	–	+	v	v	+	v	–	v	v	+	–	v	+	+	+	–	v

+, >90 % of isolates are positive; –, <10 % of isolates are positive; v, between 10 % and 90 % of strains are positive; *ND*, not determined
Bam B. ambifaria, Ban B. anthina, Bar B. arboris, Bcen B. cenocepacia, Bce B. cepacia, Bco B. contaminans, Bdi B. diffusa, Bdo B. dolosa, Blaa B. lata, Blae B. latens, Bme B. metallica, Bmu B. multivorans, Bpy B. pyrrocinia, Bse B. seminalis, Bst, B. stabilis, Bub B. ubonensis, Bvi B. vietnamiensis, Bgl B. gladioli

Phenotypic characteristics distinguishing the closely related species *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Burkholderia thailandensis* are shown in Table 28.2. Phenotypic characteristics distinguishing the members of the *Burkholderia cepacia* complex and *Burkholderia gladioli* from each other are shown in Table 28.3.

Chitinimonas Chang et al. 2004 Emend. Kim et al. 2006

Chi.ti.ni.mo'nas N.L. n. *chitinum* chitin; Gr. n. *monas* unit, monad; N.L. fem. n. *Chitinimonas*, a chitin-utilizing monad.

Cells are Gram-negative rods and motile by means of single polar flagella. Poly-β-hydroxybutyrate granules are stored as reserve material. Endospores are not formed. Grows well by using chitin as the exclusive carbon, nitrogen and energy source both under aerobic and anaerobic conditions. Growth occurs at 4–39 °C, pH 4 to 10, and 0–1 % NaCl salinity. Catalase and oxidase activity is present. Nitrate is reduced, no indole production or glucose fermentation. Esculin, gelatin, and urea may or may not be hydrolyzed. The major fatty acid components are 16:0, 18:1 ω7*c*, and summed feature 3 (16:1 ω7*c* or 15 iso 2-OH or both). The type species is *Chitinimonas taiwanensis*.

Cupriavidus Makkar and Casida 1987 Emend. Vandamme and Coenye 2004

Cup.ri.a.vi'dus. L. n. *cuprum* copper; L. adj. *avidus* eager for, loving; M.L. neut. N. *Cupriavidus* lover of copper.

Cells are Gram-negative, peritrichously flagellated rods, and chemoheterotrophic or chemolithotrophic. The metabolism is oxidative. Several amino acids are used as sole carbon and nitrogen sources. Catalase and oxidase activity is produced. Resistance to various metals is widespread. The respiratory quinone Q8 has been reported in [*Ralstonia*] *eutropha* (Yabuuchi et al. 1995). The DNA G + C content is between 63 and 69 mol%. Species occur in soil and human-clinical specimens, particularly in samples from debilitated patients. The type species is *Cupriavidus necator*. Characteristics to differentiate *Cupriavidus* and *Ralstonia* species are shown in Table 28.4.

Lautropia Gerner-Smidt et al. 1995

Lau.tro'pia, in honor of the late Hans Lautrop, Danish bacteriologist.

Gram-negative cocci occur in at least three forms: small encapsulated cocci (1–2 μm in diameter), often occurring in clusters of 10 to more than 100 cells; small unencapsulated cocci (1–2 μm in diameter), motile by the action of a tuft of three to nine flagella; and large (>5 μm in diameter) spheroplast-like cells. They are facultative aerobes but grow best under aerobic conditions with no requirement for CO_2. They are mesophilic, growing at temperatures between 30 °C and 44 °C; nonpigmented; grow on most enriched media, especially on chocolate and Levinthal, TGY, and Tween 80 agar; and grow slower with no hemolysis on horse-blood agar. Three colony morphologies can be observed, flat, dry, and circular colonies predominating in young cultures, becoming larger, wrinkled, crisp, and crateriform on prolonged incubation and smooth,

Table 28.4

Phenotypic characteristics that allow the differentiation of *Cupriavidus* and *Ralstonia* species. Based on data reported by Gillis et al. (1995), Goris et al. (2001), Coenye et al. (2003a), Coenye et al. (2003b), Vaneechoutte et al. (2004), Sato et al. (2006), Cuadrado et al. (2010), and LiPuma et al. (2011)

	Rp	Rm	Ri	Rsyz	Rsol	Cr	Cg	Cp	Ccam	Cbas	Cmet	Cnec	Cpina	Clah	Cpam	Ctai	Coxa	Calk
Nitrate reduction	+	–	+	v	+	v	–	–	+	v	v	v	+	+	–	–	+	–
Urease activity	+	+	v	ND	–	–	–	+	+	–	–	v	+	+	–	–	–	–
Assimilation of																		
Citrate	+	+	+	v	+	–	–	+	–	+	+	+	+	+	+	+	+	–
N-acetylglucosamine	+	+	–	ND	–	–	–	–	–	–	–	+	–	–	–	–	v	–
Phenylacetate	+	–	+	–	–	–	v	+	+	+	+	+	+	–	–	+	–	+
Acid from																		
L-Arabinose	+	+	ND	–	–	ND	–	–	–	–	–	+	–	–	–	–	–	ND
Glucose	+	+	–	–	+	–	–	–	–	–	–	+	–	–	+	–	–	ND
Growth at 41 °C	v	+	ND	–	–	ND	+	v	+	–	–	+	–	+	–	+	+	+
Motility	+	+	ND	–	+	ND	+	+	–	–	+	–	–	–	+	+	+	–

+, >90 % of isolates are positive; –, <10 % of isolates are positive; v, between 10 % and 90 % of strains are positive; *ND*, not determined

Rp Ralstonia pickettii, Rm Ralstonia mannitolilytica, Ri Ralstonia insidiosa, Rsyz Ralstonia syzygii, Rsol Ralstonia solanacearum, Cr Cupriavidus respiraculi, Cg Cupriavidus gilardii, Cp Cupriavidus pauculus, Ccam Cupriavidus campinensis, Cbas Cupriavidus basilensis, Cmet Cupriavidus metallidurans, Cnec Cupriavidus necator, Cpina Cupriavidus pinatubonensis, Clah Cupriavidus laharis, Cpam Cupriavidus pampae, Ctai Cupriavidus taiwanensis, Coxa Cupriavidus oxalaticus, Calk Cupriavidus alkaliphilus

glistering, raised, round, mucoid colonies. The colony diameter varies between pinpoint size and more than 5 mm; all colony types may occur in all sizes, largest in older cultures; and colonies are usually adherent to the substrate. Growth in broth is granular with a coarse sediment and granules adherent to the side of the tube. Biochemically, strains are oxidase, catalase, and urease positive; reduce nitrate and nitrites; produce polysaccharide on sucrose agar; ferment glucose, fructose, maltose, sucrose, and mannitol; and do not ferment lactose, trehalose, raffinose, inulin, salicin, adonitol, dulcitol, sorbitol, inositol, xylose, rhamnose or arabinose, or hydrolyse starch. Most strains hydrolyse esculin. They may produce β-xylosidase, but not β-galactosidase or β-glucuronidase, do not decarboxylate lysine or ornithine, and do not produce arginine decarboxylase/dihydrolase or phenylalanine deaminase. They are VP, gelatinase, and H_2S negative, do not hydrolyze hippurate or Tween 80, and are sensitive to penicillin G, ampicillin, piperacillin, cefuroxime, gentamicin, and erythromycin. Mean mol% (G + C) is 65 (range 64.6–65.4). The habitat is the human oral cavity. The type species is *Lautropia mirabilis*.

Limnobacter Spring et al. 2001 Emend. Lu et al. 2011

Lim.no.bac'ter. Gr. n. *limnos* lake; M.L. n. *bacter* rod; M.L. masc. n. *Limnobacter* lake rod, referring to the isolation of the type species from lake sediment.

Cells are Gram-negative, slightly curved rods that are motile by single polar flagella. Polyhydroxybutyrate is stored as a reserve material. Endospores are not formed. *Limnobacter* are strictly aerobic and oxidase and catalase positive. Growth occurs between 4 °C and 44 °C. Carboxylic acids and amino acids are used as energy and carbon sources, but no carbohydrates or polyols are used. They are not able to grow autotrophically. Thiosulfate is oxidized to sulfate in the presence of an organic carbon source. Major fatty acids are 18:1ω7*c*, 16:1ω7*c*, 16:0, and 10:0 3-OH. The major ubiquinone is Q-8. The G + C content is between 55 % and 59 %. The type species is *Limnobacter thiooxidans*.

Pandoraea Coenye et al. 2000

Pan.do.rae'a. N.L. fem. n. *Pandoraea*, referring to Pandora's box in Greek mythology, which was the origin of diseases of mankind and thus to the surprisingly diverse members of this genus.

Pandoraea cells are Gram-negative, nonsporulating straight rods of 0.5–0.7 by 1.5–4.0 µm. They occur single and are motile by means of a single polar flagellum. Catalase activity is present. Growth is observed at 30 °C and 37 °C. Nitrite is not reduced. There are no denitrification, no β-galactosidase or DNAse activity, no liquefaction of gelatin, no esculin hydrolysis, no indole production, and no hydrolysis of Tween 80. Additional characteristics are listed in the Results section above. The following fatty acid components are present: 12:0, 12:0 2OH, 16:0, 17:0 cyclo, 16:0 2OH, 16:0 3OH, 19:0 cyclo ω8c, 18:1 2OH, summed feature 3, summed feature 4, and summed feature 7. The G + C content is between 61.2 and 64.3 mol%. Strains of this

Table 28.5
Phenotypic characteristics that allow the differentiation of *Pandoraea* species (Based on data reported by Coenye et al. (2000), Anandham et al. (2010), and Sahin et al. (2011))

	Pnor	Ppul	Ppno	Papi	Pspu	Pthio	Poxa	Pver	Pfae
Growth at 42 °C	−	+	+	+	v	+	−	+	+
Enzymatic activity									
Urease	v	−	+	+	v	+	+	+	−
C4-esterase	v	−	v	v	v	+	+	+	+
C8-esterase	+	−	+	+	+	−	+	−	+
Assimilation of									
Caprate	v	+	+	v	v	−	−	+	+
D-Gluconate	+	+	+	+	+	ND	−	+	+

+, all isolates are positive; −, none of isolates are positive; v, result is isolate dependent; *ND*, not determined
Pnor Pandoraea norimbergensis, *Ppul Pandoraea pulmonicola*, *Ppno Pandoraea pnomenusa*, *Papi Pandoraea apista*, *Pspu Pandoraea sputorum*, *Pthio Pandoraea thiooxydans*, *Poxa Pandoraea oxalativorans*, *Pver Pandoraea vervacti*, *Pfae Pandoraea faecigallinarum*

genus are isolated from human clinical samples (mostly cystic fibrosis patients) and the environment and do not cause soft rot on onions. The clinical data that are presently available indicate that at least some of these organisms may cause chronic infection in and have been transmitted among cystic fibrosis patients. The type species is *Pandoraea apista*. Phenotypic characteristics allowing the differentiation of the different *Pandoraea* species are shown in Table 28.5.

Paucimonas Jendrossek 2001

Pau.ci.mo'nas. L., adj. *paucus* little, few; Gr. fem. *monas* unit, cell; *Paucimonas* bacterium with restricted (few) catabolic capacities.

Paucimonas cells are Gram-negative with chemoorganotrophic and strictly respiratory metabolism and are catalase and oxidase positive. Preferred carbon sources are organic acids. Most sugars (e.g., glucose, fructose, xylose), sugar acids (e.g., gluconate), polyalcohols (e.g., sorbitol, glycerol), alcohols (e.g., ethanol, butanediol, phenol), amino acids, polypeptides (e.g., nutrient broth, gelatin, Luria-Bertani broth), or polysaccharides (e.g., starch, cellulose) do not support growth. PHB and related homopolyesters and/or copolymers of hydroxyalkanoic acids can be accumulated intracellularly. Chemolithoautotrophic or phototrophic growth has not been observed. Type species of the genus is *Paucimonas lemoignei*.

Polynucleobacter Heckmann and Schmidt 1987 Emend. Hahn et al. 2009

Po.ly.nuc'le.o.bac.ter. Gr. adj. *polys* numerous; L. masc. n. *nucleus* nut, kernel; N.L. masc. n. *bacter* the equivalent of the Gr. neut. n. *bactron* a rod; N.L. masc. *Polynucleobacter* rod with many nucleoids.

It harbors endosymbiotic strains of several *Euplotes* species and free-living strains dwelling in the water column of freshwater lakes, ponds, and streams and is essential for their host species and nonmotile. Not all strains affiliated with the genus possess multiple nucleoid-like structures as indicated by the genus name. The type species is *Polynucleobacter necessarius*.

Ralstonia Yabuuchi et al. 1996

Ral.sto'n.ia. M.L. dim. *-ia* ending; M.L. fem. n. *Ralstonia* named after Ericka Ralston, the American bacteriologist who first described *Pseudomonas pickettii*.

Ralstonia cells are Gram-negative rods; have no formation of endospores; are nonmotile or motile by means of a single polar or peritrichous flagella; are aerobic, with a respiratory metabolism with oxygen as terminal electron acceptor; and are able to grow on ordinary peptone media. The colony color is beige for most species. They are oxidase and catalase positive and lysine and ornithine decarboxylase negative. The major ubiquinone is Q-8. Cellular lipids contain two kinds of phosphatidylethanolamine. Major fatty acids are C16:0, a mixture of C18:0 ω9t and C18:1 ω7c, and C14:0 3OH. The G + C content is between 64 and 68 mol%. The type species is *Ralstonia pickettii*. Characteristics to differentiate *Ralstonia* and *Cupriavidus* species are shown in Table 28.4.

Thermothrix Caldwell et al. 1981

Ther.mo'thrix. Gr. adj. *thermos*, hot; Gr. fem. n. *thrix*, hair; N.L. fem. n. *Thermothrix*, hot hair.

Thermothrix cells are Gram-negative rods, mobile with a single polar flagellum, and thermophiles with growth temperatures ranging from 63 °C to 86 °C. They are aerobic and are

facultative or obligate chemolithotrophs. Under unfavorable conditions (lack of oxygen, pH around 8.5, or temperature close to maximum growth temperature), cells will form filaments and are isolated from geothermal sources where the pH is close to neutral. The type species is *Thermothrix thiopara*.

Wautersia Vaneechoutte et al. 2004

Wau.ter′si.a. L. fem. n. *Wautersia* named in honor of the Belgian microbiologist Georges Wauters.

Wautersia cells are Gram-negative rods that are motile by means of peritrichous flagella. They are aerobic, form smooth colonies that reach 1–2 mm within 48 h at 30°C on blood agar, and are positive for catalase and oxidase. Glucose is neither acidified nor assimilated. They are susceptible to colistin. Cellular fatty acids are of the saturated and monounsaturated straight-chain types, mainly C16:1ω9*c*, C16:0, C18:1ω11*c*, and C14:0. The type species of the genus is *Wautersia eutropha*. As mentioned above, only *Wautersia numazuensis* still remains in this genus, with all other species (including the type species *Wautersia eutropha*) being reclassified as *Cupriavidus* species. It is clear that *Wautersia numazuensis* should also be reclassified as a *Cupriavidus* species and that the use of the name *Wautersia* should be discontinued.

Isolation, Enrichment, and Maintenance Procedures

Most members of the family *Burkholderiaceae* grow on a wide range of media, including (but not limited to) nutrient agar, tryptic soy agar, Mueller-Hinton agar, Columbia agar, and LB agar, with or without blood. However, the use of selective media can be required to recover relevant species (*Burkholderia pseudomallei*, *Burkholderia cepacia* complex) from clinical samples. Note that attempts to grow *Polynucleobacter necessarius* subsp. *necessarius* outside its eukaryotic host have so far been unsuccessful (Hahn et al. 2009). Similarly, several obligate endosymbionts of *Psychotria* plants (and other *Rubiaceae*) have been described, but since attempts to obtain these in pure culture were unsuccessful, they have been described as "Candidatus Burkholderia kirkii" (Van Oevelen et al. 2002), "Candidatus Burkholderia calva" and "Candidatus Burkholderia nigropunctata" (Van Oevelen et al. 2004), "Candidatus Burkholderia andongensis" and "Candidatus Burkholderia petitii" (Lemaire et al. 2011), "Candidatus Burkholderia harborii" and "Candidatus Burkholderia schumanniana" (Verstraete et al. 2011), and, finally, "Candidatus Burkholderia hispidae," "Candidatus Burkholderia rigidae," and "Candidatus Burkholderia schumannianae" (Lemaire et al. 2012).

Several different media have been developed for the selective isolation of *Burkholderia cepacia complex* isolates from sputum of cystic fibrosis, e.g., *Pseudomonas cepacia* (PC) medium (containing 300U polymyxin B per ml and 100 µg ticarcilline per ml) (Gilligan et al. 1985); oxidation-fermentation agar supplemented with lactose, 300U polymyxin B per ml, and 0.2U of bacitracin per ml (OFPBL agar) (Welch et al. 1987); and *B. cepacia* selective agar (BCSA) (containing 1 % lactose and 1 % sucrose in an enriched base of casein and yeast extract with 600U of polymyxin B per ml, 10 µg of gentamicin per ml, and 2.5 µg vancomycin per ml) (Henry et al. 1997). BCSA was reported superior compared to OFPBL and PCA for the rapidity and quality of recovery of *B. cepacia* complex organisms from cystic fibrosis respiratory specimens and was more inhibitory towards other organisms (Henry et al. 1999). A comparison of three commercially available "*Burkholderia cepacia*" media (MAST Diagnostics, Bootle, Merseyside, United Kingdom; LAB M Ltd., Bury, United Kingdom; and Oxoid Ltd., Basingstoke, United Kingdom), through the analysis of 142 clinical and environmental isolates, showed that BCSA and Mast *B. cepacia* medium supported the growth of *Burkholderia cepacia* complex isolates most efficiently (Vermis et al. 2003b). BCSA and MAST were also compared in terms of sensitivities and specificities for the isolation of *Burkholderia cepacia* complex species from sputum specimens from cystic fibrosis patients; in that study, BCSA was found to be as sensitive as MAST agar but more selective (Wright et al. 2001). It should be noted that several *Pandoraea*, *Ralstonia*, and *Cupriavidus* isolates will also grow on BCSA. The selectivity of the abovementioned media for the isolation of environmental *Burkholderia cepacia* complex isolates may be much lower (Carson et al. 1988), and therefore the use of other media, like PCAT medium (containing azelaic acid and tryptamine) (Burbage and Sasser 1982), may be recommended, although not all *Burkholderia cepacia* complex species grow on the latter medium (Vermis et al. 2003a). An enrichment medium, based on the ability of *Burkholderia cepacia* complex, isolates to use L-threonine and L-arabinose as carbon sources, and their insensitivity to polymyxin B and 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridan (C-390), was developed by Vermis et al. (2003b) and successfully used to demonstrate the presence of *Burkholderia cepacia* complex in water and soil samples (Vermis et al. 2003b; Vanlaere et al. 2005). However, other bacteria were also recovered (including isolates belonging to the genera *Pandoraea, Chryseobacterium, Comamonas, Ralstonia, Herbaspirillum*, and *Pseudomonas*) following this enrichment procedure. Ashdown medium (containing crystal violet and gentamicin as selective agents) is typically used for the isolation of *Burkholderia pseudomallei* (LiPuma et al. 2011). If enrichment is required, an enrichment broth consisting of Ashdown medium supplemented with 50 mg of colistin was found to be superior to standard enrichment broth such as tryptic soy broth and increases recovery of *Burkholderia pseudomallei* (LiPuma et al. 2011).

For short-term preservation (several weeks up to months), stab cultures in semisolid medium or cultures on slants (with storage at 4 °C) are appropriate. For long-term storage, media containing glycerol (20 % v/v) and storage at −80 °C or in liquid nitrogen are recommended.

Ecology

For reviews of the ecology of *Burkholderia* species, see Coenye and Vandamme (2003) and Compant et al. (2008). Members of the genus *Burkholderia* occupy diverse ecological niches and can be found in the soil, in water, and in (close) association with plants, animals, and fungi. Several *Burkholderia* species (including *Burkholderia vietnamiensis*) are capable of nitrogen fixation and several *Burkholderia* species are capable of forming root nodules on several plants. Others, including *Burkholderia gladioli* and *Burkholderia glumae*, are notorious plant pathogens (Gonzalez et al. 2007), as is *Ralstonia solanacearum* (Genin and Boucher 2004; Mansfield et al. 2012). Much less is known about the ecology of other genera of this family.

Pathogenicity: Clinical Relevance

Burkholderia pseudomallei and *Burkholderia mallei*

The family *Burkholderiaceae* contains two highly pathogenic organisms, *Burkholderia pseudomallei* and *Burkholderia mallei* (LiPuma et al. 2011). *Burkholderia pseudomallei* causes melioidosis, an infection characterized by a wide range of clinical manifestations, ranging from asymptomatic colonization to fulminant sepsis. The most common presentations of melioidosis include pneumonia, soft-tissue infection, abscesses of liver and spleen, and septicemia (Dance 1991; LiPuma et al. 2011). *Burkholderia pseudomallei* is a saprophytic organism, broadly distributed in soil and water in Southeast Asia and northern Australia. The majority of infected people acquire the organism through percutaneous inoculation on exposure to contaminated soil or water, although the possibility of inhalation or ingestion as modes of infection requires further investigation (Dance et al. 2000; Haase et al. 1995; Currie et al. 2001). Sporadic cases have resulted from person-to-person or animal-to-person spread (Dance 2000). Exposure in endemic areas is quite frequent due to the organism's ubiquity, and latent infections are common. Thus, it is difficult to accurately determine what sort of environmental exposure poses the greatest risk of melioidosis. Sporadic cases of human melioidosis occur in regions outside the endemic area, such China, Korea, the Philippines, Indonesia, India, and West Africa. Most cases in Europe and North America are thought to be imported by immigrants or international travelers (Dorman et al. 1998; Dance et al. 1999). Several reports describe the occurrence of *B. pseudomallei* in European cystic fibrosis patients. In these cases, the organisms were most likely acquired during travel, although this could not always be confirmed (Visca et al. 2001; Schulin and Steinmetz 2001; O'Carroll et al. 2003; Engelthaler et al. 2011).

Burkholderia mallei is primarily a pathogen in horses, in which it causes glanders, a disease characterized by fever, inflammation of the nasal mucosa, necrosis, and obstruction of the oropharynx. In humans, infection can be limited to subcutaneous tissues or can disseminate to cause sepsis (LiPuma et al. 2011). If inhaled, *Burkholderia mallei* can cause pneumonia with necrosis of the tracheobronchial tree (Srinivasan et al. 2001). *Burkholderia mallei* can be spread via contact with infected animals or through exposure in research laboratories (Srinivasan et al. 2012; CDC 2000). Glanders has been virtually eliminated in the Western world due to stringent infection control measures, including the immediate slaughter of affected animals. However, research interest in this species due to recent concerns about biological warfare may result in an increasing risk of occupational exposure.

Respiratory Tract Infections in Cystic Fibrosis Patients

Members of the *Burkholderia cepacia* complex and phenotypically similar species (including *B. gladioli* and *Pandoraea, Cupriavidus*, and *Ralstonia* species) are notorious for causing respiratory tract infections in cystic fibrosis patients. The reader is referred to several detailed reviews on this topic for more information (LiPuma 2005, 2010, 2011; Coenye and LiPuma 2003).

Application

Burkholderia cepacia Complex Bacteria as Biocontrol, Bioremediation, and Plant-Growth-Promoting Agents

The biological control of plant diseases, insects, and nematodes by microorganisms (both bacteria and fungi) has been proposed as an alternative or a supplement to chemical pesticides, and the use of introduced biological control could have enormous ecological and economic benefits. The two traditional approaches used for biological control of soilborne plant pathogens in the field have been (1) crop rotations, to allow time for resident antagonists to "sanitize" the soil or for propagules of specialized pathogens to die, and (2) the addition of organic amendments to soil, to stimulate resident antagonists. However, the greatest progress towards biological control of soilborne plant pathogens has been made with antagonists introduced with the planting material, i.e., biological control with plant-associated microorganisms. It has often been shown that *Burkholderia cepacia* complex strains can be used to control seedling and root diseases in vitro and in field tests and could replace chemical alternatives like captan, thiram, PCNB, benlate, and thiabendazole (LiPuma and Mahenthiralingam 1999). Field tests have shown that *Burkholderia cepacia* complex can colonize the rhizosphere of several crops, including corn, maize, rice, pea, sunflower, and radish and thereby significantly can increase the crop yield, even in the absence of pathogens (see, e.g., McLoughlin et al. 1992; Parke et al. 1991; Bowers and Parke 1993; Hebbar et al. 1998; see Balandreau and Mavingui (2007) for a review on this topic).

In addition, the exceptional metabolic versatility of this organism can be used for bioremediation purposes. Constituents of crude oils (including polycyclic aromatic compounds), herbicides (including 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid, the principal component of "Agent Orange") TCE, and ether derivatives used as gasoline additives can be degraded by several *Burkholderia cepacia* complex isolates (Kilbane et al. 1982; Folsom et al. 1990; Krumme et al. 1993; Bhat et al. 1994). Well-characterized biodegradative strains belonging to the *Burkholderia cepacia* complex include G4 (Nelson et al. 1987; Folsom et al. 1990; Shields et al. 1991; Leahy et al. 1996; McClay et al. 1996; Massol-Deya et al. 1997) and CRE-7 (Mueller et al. 1996). Potentially useful strains have also been identified in other *Burkholderia* species, including the species *Burkholderia xenovorans* (strain LB400, degradation of biphenyl and polychlorinated biphenyls) (Haddock et al. 1993; Seeger et al. 1995; Billingsley et al. 1997; Master and Mohn 1998; Bopp 1985; Goris et al. 2004) and *Burkholderia kururiensis* (trichloroethylene) (Zhang et al. 2000). For more information, readers are referred to Denef (2007) for a review on this topic. Another extensively studied strain with demonstrated biodegradative capacities is *Cupriavidus necator* JMP134 (for a recent review, see Perez-Pantoja et al. 2008).

However, many strains used or under development for those purposes are taxonomically poorly characterized and their potential hazard to people with cystic fibrosis is unclear (Govan and Vandamme 1998; Holmes et al. 1998; Vidaver et al. 1999; Govan et al. 2000). Until more is known about the organisms currently used or under development for agricultural applications and the potential risks for cystic fibrosis patients, widespread use of these organisms has been forbidden by several national regulatory agencies, including the United States Environmental Protection Agency (EPA).

Heavy-Metal Resistance in the Genus *Cupriavidus*

Many members of the genus *Cupriavidus*, including *Cupriavidus metallidurans*, *Cupriavidus campinensis*, and *Cupriavidus basilensis*, are known for their metal resistance (Goris et al. 2001). The high resistance of certain *Cupriavidus* strains to cadmium, copper, zinc, cobalt, lead, and mercury has attracted considerable attention and opened the possibilities of developing bacterial biosensors for contamination and the development of novel approaches for the bioremediation of contaminated water and soils. For more information, the reader is directed to the reviews of Mergeay et al. (2003), Diels et al. (2009), and von Rozycki and Nies (2009) and the references therein.

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29 The Family *Comamonadaceae*

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Abstract
The family *Comamonadaceae* is a large and diverse bacterial family belonging to the order *Burkholderiales*. It is regularly enlarged with new genera and species and currently comprises over 100 species in the following 29 genera: *Acidovorax*, *Albidiferax*, *Alicycliphilus*, *Brachymonas*, *Caenimonas*, *Comamonas*, *Curvibacter*, *Delftia*, *Diaphorobacter*, *Extensimonas*, *Giesbergeria*, *Hydrogenophaga*, *Hylemonella*, *Lampropedia*, *Limnohabitans*, *Macromonas*, *Malikia*, *Ottowia*, *Polaromonas*, *Pseudacidovorax*, *Pseudorhodoferax*, *Ramlibacter*, *Rhodoferax*, *Simplicispira*, *Tepidicella*, *Variovorax*, *Verminephrobacter*, *Xenophilus*, and *Xylophilus*. These genera form a phylogenetic cluster with a relative level of 16S rRNA gene sequence similarity of 93–97 %, yet harbor a remarkable phenotypic diversity that includes aerobic organotrophs, anaerobic denitrifiers and Fe^{3+}-reducing bacteria, hydrogen oxidizers, photoautotrophic and photoheterotrophic bacteria, and fermentative bacteria. Most are environmental bacteria from water and soil habitats; however, some *Comamonadaceae* are also pathogens, and others have been described from earthworms, rumen fluid, and human clinical samples. Most are mesophiles, although a few have been found in Antarctic habitats and some in hot springs.

Taxonomy, Historical and Current

Short Description of the Family

Co.ma.mo.na.da'ce.ae. N.L. fem. n. *Comamonas*, type genus of the family; suff. *-aceae*, suffix to denote a family; N.L. fem. pl. n. *Comamonadaceae*, the *Comamonas* family.

The family *Comamonadaceae* belongs to the *Burkholderiales* order in the *Betaproteobacteria* class of the phylum Proteobacteria. It was proposed in 1991 as a formal taxon for the so-called [*Pseudomonas*] *acidovorans* rRNA complex (Willems et al. 1991a), one of the five distinct clusters that had been recognized in the

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genus *Pseudomonas* on the basis of rRNA characteristics (Palleroni et al. 1973; De Vos and De Ley 1983). The *Pseudomonas acidovorans* rRNA group comprised at least 10 *Pseudomonas* species and also *Alcaligenes paradoxus*, *Xanthomonas ampelina*, *Comamonas terrigena*, and several *Aquaspirillum* species (De Vos and De Ley 1983; De Vos et al. 1985; Willems et al. 1987, 1991c). At the time of its description, the family comprised five genera. With the exception of *Comamonas* which is a revived genus (De Vos et al. 1985), most genera of the new family had been recently created for species that were originally placed in other genera based on phenotypic similarities: *Acidovorax* and *Hydrogenophaga* (both for several former *Pseudomonas* species), *Xylophilus* (formerly *Xanthomonas ampelina*), and *Variovorax* (formerly *Alcaligenes paradoxus*). The family also comprised several *Aquaspirillum* and phytopathogenic *Pseudomonas* species that had been phylogenetically misnamed at genus level (Willems et al. 1991a). These have since been transferred to the other genera *Simplicispira* (Grabovich et al. 2006), *Giesbergeria* (Grabovich et al. 2006), *Comamonas* (Wauters et al. 2003), *Curvibacter* (Ding and Yokota 2004), and *Acidovorax* (Willems et al. 1992). In addition many new genera and species have been described over the years so that the *Comamonadaceae* currently comprise 29 genera and 104 species.

The single most salient common feature of the members of this family is their membership of a phylogenetic cluster in the 16S rRNA gene phylogeny of the Betaproteobacteria. They occur in various natural or man-made habitats, pristine as well as polluted, ranging from soil, freshwater, groundwater, activated sludge, and industrial processing water (*Albidiferax*, *Alicycliphilus*, *Caenimonas*, *Curvibacter*, *Comamonas*, *Delftia*, *Diaphorobacter*, *Extensimonas*, *Giesbergeria*, *Simplicispira*, *Hydrogenophaga*, *Hylemonella*, *Lampropedia*, *Limnohabitans*, *Macromonas*, *Malikia*, *Ottowia*, *Polaromonas*, *Pseudacidovorax*, *Pseudorhodoferax*, *Rhodoferax*, *Variovorax*, *Xenophilus*). Some are plant associated (*Simplicispira psychrophila*) and several are plant pathogens (*Acidovorax*, *Xylophilus*). Others are found in clinical samples (*Acidovorax*, *Comamonas*, *Delftia acidovorans*), inside associated with chironomid egg masses (insects in freshwater habitats) (*Brachymonas*), in nephridia of earthworms (*Verminephrobacter*), or in the gut of termites (*Comamonas odontotermitis*). Other members have been found in sulfur or sulfide springs (*Comamonas thiooxidans*, *Giesbergeria kuznetsovii*), in hot springs (*Tepidicella*), in polar or other extremely cold habitats (*Polaromonas*, *Rhodoferax antarcticus*) including the Antarctic Ocean (*Polaromonas vacuolata*), and in desert soil (*Ramlibacter*).

From this wide range of habitats, it comes as no surprise that morphological and physiological features vary considerably between genera and species. The family includes oligotrophs living in freshwater as well as very versatile heterotrophs that can metabolize complex organic pollutants, psychrophiles, as well as mild thermophiles. Most are free-living environmental saprophytes, but others are plant or animal associated including plant pathogens. Most have an aerobic heterotrophic metabolism, yet some are able to denitrify and use nitrates (some *Comamonas*, *Hydrogenophaga*, *Simplicispira*, and *Acidovorax* species, *Albidiferax*, *Alicycliphilus*, *Brachymonas denitrificans*, *Diaphorobacter nitroreducens*, *Ottowia thiooxydans*, and *Variovorax ginsengisoli*) or Fe(III) (*Albidiferax*, *Comamonas guangdongensis*) as terminal electron acceptors and some are able to oxidize thiosulfate (some *Comamonas* species, *Ottowia*) and grow autotrophically on hydrogen (*Hydrogenophaga* and some environmental *Acidovorax* species, *Variovorax paradoxus*) or carbon monoxide (some *Hydrogenophaga pseudoflava* strains), photoauto- or photoheterotrophically (*Rhodoferax*).

There are some common chemotaxonomic features such as the presence of the straight chain fatty acids C16:0, C16:1ω7c, and C18:1ω17c and the hydroxy fatty acids C10:0 3-OH and/or C8:0 3-OH and Q-8 as the main respiratory quinone; however, these features are also present in many other Betaproteobacteria and are therefore not diagnostic.

Phylogenetic Structure of the Family and Its Genera

In the 16S rRNA gene phylogeny, the *Comamonadaceae* family forms a single large cluster in the phylum Betaproteobacteriaceae. Inside this large cluster, the species of each of the majority of the different genera group together per genus. However, *Acidovorax*, *Comamonas*, and *Hydrogenophaga* species each form large clusters that also comprise the species of one or two genera as separate subclusters or lineages (*Fig. 29.1*) as discussed below, and the taxonomy of these groups may therefore require further changes in the future.

The *Acidovorax* species make up two clusters, one comprising the environmental species *Acidovorax defluvii*, *Acidovorax radicis*, *Acidovorax facilis* (type species), *Acidovorax temperans*, *Acidovorax delafieldii*, *Acidovorax soli*, and *Acidovorax caeni* and the other uniting the plant-pathogenic species *Acidovorax avenae*, *Acidovorax cattleyae*, *Acidovorax oryzae*, *Acidovorax citrulli*, *Acidovorax anthurii*, and *Acidovorax valerianellae*. Both these clusters are each other's nearest neighbors; however, several phylogenetic algorithms place *Verminephrobacter eiseniae* and *Xylophilus ampelinus* with the environmental and the plant-pathogenic *Acidovorax* cluster, respectively (*Fig. 29.1*). *Xylophilus ampelinus* is a pathogen of grapevines and is yellow pigmented as are the plant-pathogenic *Acidovorax* species. In contrast to these plant pathogens, it is a very slow-growing organism that does not grow at temperatures above 30 °C (Willems et al. 1987, 1992). The placement of *Verminephrobacter* close to the environmental *Acidovorax* species was also reported by Pinel et al. (2008) who proposed this genus for symbiotic bacteria from the nephridia of lumbricid earthworms. In addition to 16S rRNA gene sequences, they also used partial *recA*, *gap*, and *rpoC* gene sequences to study the phylogenetic relationships. Their findings suggest that nephridial isolates, environmental and phytopathogenic *Acidovorax* species may form three sister clusters and indicate that with more data, further taxonomic rearrangements of the current genus *Acidovorax* may follow in the future (Pinel et al. 2008).

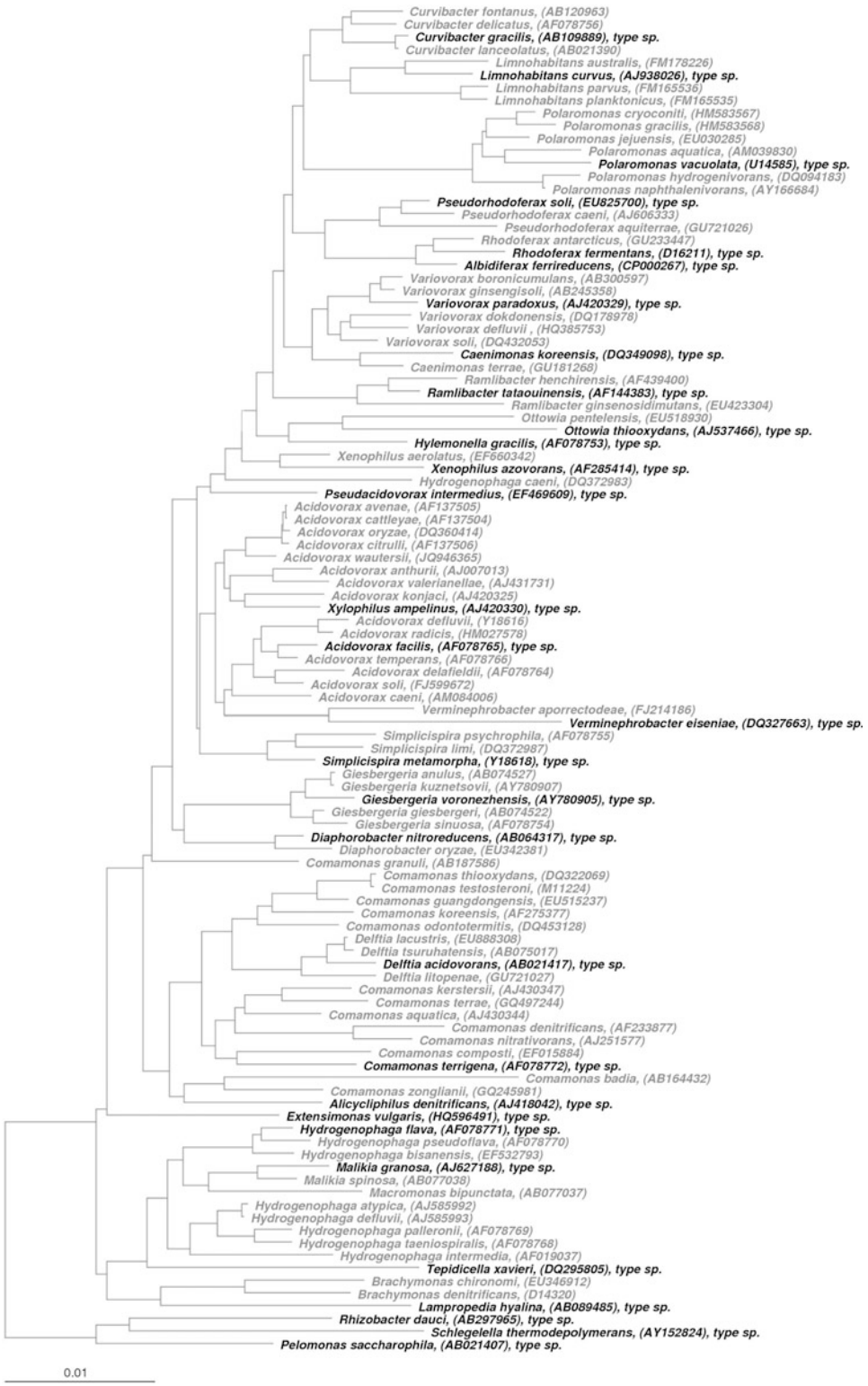

Fig. 29.1

Phylogenetic reconstruction of the family *Comamonadaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes–Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

The genus *Comamonas*, originally proposed by Davis and Park in 1962, was not included in the Approved Lists of Bacterial Names (Skerman et al. 1980). The genus was revived by De Vos et al. (1985) who studied the relationships of a series of *Comamonas*, *Vibrio*, and *Lophomonas* (also not on the Approved Lists) sp. strains with uncertain taxonomic affiliation. Initially only one species, *Comamonas terrigena*, was proposed (revived) for strains from a hay infusion, soil, and blood (De Vos et al. 1985). Tamaoka et al. (1987) then transferred *Pseudomonas acidovorans* and *Pseudomonas testosteroni* to *Comamonas* as separate species. Later three subgroups were described in *Comamonas terrigena*, one of them comprising the type strain of *Aquaspirillum aquaticum* (Willems et al. 1991c). These subgroups were since recognized as separate species with the proposal of *Comamonas aquatica* and *Comamonas kerstersii* (Wauters et al. 2003). *Comamonas acidovorans* was later placed in a separate genus, *Delftia* (Wen et al. 1999). At present the genus *Comamonas* comprises 14 species and two further species are proposed (Zhang et al. 2013a; Sun et al. 2013). The 16 *Comamonas* species make up three closely related clusters with *Comamonas granuli* at the periphery: the first cluster comprising *Comamonas terrigena* (type species), *Comamonas aquatica*, *Comamonas kerstersii*, *Comamonas denitrificans*, *Comamonas nitrativorans*, *Comamonas terrae*, and *Comamonas composti*; the second one *Comamonas badia* and *Comamonas zongliani;* and the third one *Comamonas odontotermitis*, *Comamonas koreensis*, *Comamonas thiooxydans*, *Comamonas testosteroni*, and *Comamonas guangdongensis* (Fig. 29.1). The second cluster has *Alicycliphilus denitrificans* as its nearest neighbor. The genus *Delftia*, which currently comprises four species, forms a separate cluster that is the nearest neighbor of this third *Comamonas* cluster (Fig. 29.1). The position of some species such as *Comamonas granuli*, *Comamonas badia*, and *Alicycliphilus denitrificans* varies with different phylogenetic algorithms and additional information (e.g., of other genes) may therefore in future lead to further taxonomic rearrangements.

The *Hydrogenophaga* species (four former *Pseudomonas* species and five more recently created species) form two related subclusters in the phylogenetic tree: one subcluster comprising *Hydrogenophaga flava* (type species), *Hydrogenophaga pseudoflava*, and *Hydrogenophaga bisanensis* and the other *Hydrogenophaga atypica, Hydrogenophaga defluvii, Hydrogenophaga palleronii*, *Hydrogenophaga taeniospiralis*, and *Hydrogenophaga intermedia*. These subclusters are separated by two *Malikia* species and *Macromonas bipunctata* (Fig. 29.1). The position of *Hydrogenophaga caeni* varies with the algorithm used: neighbor-joining analysis placed it outside the *Hydrogenophaga* main cluster whereas maximum likelihood placed it within this cluster. *Hydrogenophaga* species are found in aquatic or soil habitats and activated sludge, as are *Malikia* and *Macromonas* species (Spring et al. 2005; Dubinina et al. 2005). The phylogenetic position of these latter two genera also varies with the phylogenetic algorithm used, however: the *Malikia* cluster and the *Macromonas bipunctata* lineage group inside or at the periphery of the *Hydrogenophaga* cluster. Also here, additional new information may lead to taxonomic rearrangements in the future.

The genus *Macromonas* was created in 1924 (Utermöhl and Koppe 1924) and included on the Approved Lists of Bacterial Names (Skerman et al. 1980). However, strains of the type species of *Macromonas*, *Macromonas mobilis*, are not available, and its characteristics and phylogenetic position can thus not be verified. The position of the only other species, *Macromonas bipunctata*, which is morphologically and ecologically similar to the type species, has been established by 16S rRNA gene sequence analysis as close to the genus *Hydrogenophaga* and relatives (Dubinina et al. 2005) and is confirmed here in Fig. 29.1. Until strains of the type species are isolated for comparison, it therefore seems sensible to consider this genus as a member of the family *Comamonadaceae* for now (Dubinina et al. 2005).

The genus *Aquaspirillum* was proposed by Hylemon et al. (1973) for aerobic freshwater spirilla with a G+C range of 49–65 mol% in a large study which revised the taxonomy of the genus *Spirillum*. Several of the species were later shown to belong to the *Comamonadaceae* (Willems et al. 1991a) and were in recent years placed in separate genera that occupy separate positions in the family phylogeny. *Aquaspirillum delicatum* was accommodated in the genus *Curvibacter* as *Curvibacter delicatus* (Ding and Yokota 2004). *Hylemonella gracilis* was created to accommodate *Aquaspirillum gracile* (Spring et al. 2004). *Giesbergeria* was created in 2006 to accommodate two *Aquaspirillum* species that had previously been proposed but not validly published (Grabovich et al. 2006). In addition the authors transferred three valid but generically misnamed *Aquaspirillum* species (*Aquaspirillum anulus, Aquaspirillum giesbergeri*, and *Aquaspirillum sinuosum*) to the genus. The genus thus comprised five species: the type species *Giesbergeria voronezhensis*, *Giesbergeria kuznetsovii*, *Giesbergeria anulus*, *Giesbergeria giesbergeri*, and *Giesbergeria sinuosa* (Grabovich et al. 2006). In the same study, a second genus *Simplicispira* was created to host two further generically misnamed *Aquaspirillum* species as *Simplicispira metamorpha*, the type species, and *Simplicispira psychrophila* (Grabovich et al. 2006). Since then a third species, *Simplicispira limi*, has been described (Lu et al. 2007).

Albidiferax ferrireducens was originally proposed as a new species in the genus *Rhodoferax* because of its phylogenetic grouping with members of this genus (Finneran et al. 2003). However, the inability to perform anoxygenic photosynthesis and lack of photosynthetic pigments later led to its transfer to a new genus *Albidoferax* (Ramana and Sasikala 2009), corrected to *Albidiferax* on validation of the name. *Albidiferax* ferrireducens does form a single and tight cluster with the two *Rhodoferax* species in the 16S rRNA gene phylogeny (Fig. 29.1). Sequence similarity between the three species is 97.25–98.5 % (Ramana and Sasikala 2009; Madigan et al. 2000).

The above paragraphs illustrate that the family *Comamonadaceae* comprises many species that were previously assigned to other genera (or species). Some come from old genera that were created using mostly phenotypic

Table 29.1
Overview of name changes

Current name	Former names	References
Acidovorax citrulli	*Pseudomonas pseudoalcaligenes* subsp. *citrulli*	Schaad et al. (2008)
Acidovorax avenae	*Pseudomonas avenae, Pseudomonas rubrilineans, Acidovorax avenae subsp. avenae*	Willems et al. (1992)
Acidovorax cattleyae	*Pseudomonas cattleyae, Acidovorax avenae subsp. cattleyae*	Schaad et al. (2008)
Acidovorax delafieldii	*Pseudomonas delafieldii*	Willems et al. (1990)
Acidovorax facilis	*Pseudomonas facilis*	Willems et al. (1990)
Acidovorax konjaci	*Pseudomonas pseudoalcaligenes subsp. konjaci*	Willems et al. (1992)
Albidiferax ferrireducens	*Rhodoferax ferrireducens*	Ramana and Sasikala (2009)
Comamonas aquatica	*Aquaspirillum aquaticum, Comamonas terrigena* group 2	Wauters et al. (2003)
Comamonas kerstersii	*Comamonas terrigena* group 3	Wauters et al. (2003)
Comamonas terrigena	*Comamonas terrigena* group 1	Willems et al. (1991c)
Comamonas testosteroni	*Pseudomonas testosteroni*	Tamaoka et al. (1987)
Curvibacter delicatus	*Aquaspirillum delicatum, "Spirillum delicatum"*	Ding and Yokota (2004)
Curvibacter lanceolatus	*Pseudomonas lanceolata*	Ding and Yokota (2004)
Delftia acidovorans	*Pseudomonas acidovorans, Comamonas acidovorans*	Wen et al. (1999)
Giesbergeria anulus	*Aquaspirillum anulus, "Spirillum anulus"*	Grabovich et al. (2006)
Giesbergeria giesbergeri	*Aquaspirillum giesbergeri, "Spirillum giesbergeri"*	Grabovich et al. (2006)
Giesbergeria sinuosa	*Aquaspirillum sinuosum, "Spirillum sinuosum"*	Grabovich et al. (2006)
Hydrogenophaga flava	*Pseudomonas flava, "Hydrogenomonas flava"*	Willems et al. (1989)
Hydrogenophaga palleroni	*Pseudomonas palleroni*	Willems et al. (1989)
Hydrogenophaga pseudoflava	*Pseudomonas pseudoflava, Pseudomonas carboxydoflava*	Willems et al. (1989)
Hydrogenophaga taeniospiralis	*Pseudomonas taeniospiralis*	Willems et al. (1989)
Hylemonella gracilis	*Aquaspirillum gracile, "Spirillum gracile"*	Spring et al. (2004)
Malikia spinosa	*Pseudomonas spinosa*	Spring et al. (2005)
Simplicispira metamorpha	*Aquaspirillum metamorphum, "Spirillum metamorphum"*	Grabovich et al. (2006)
Simplicispira psychrophila	*Aquaspirillum psychrophilum, "Spirillum psychrophilum"*	Grabovich et al. (2006)
Variovorax paradoxus	*Alcaligenes paradoxus*	Willems et al. (1991a)
Xylophilus ampelinus	*Xanthomonas ampelina*	Willems et al. (1987)

characterization. The introduction of the 16S rRNA sequence as an essential indication for the phylogenetic placement has led to many taxonomic transfers and the creation of new genera. An overview of name changes is given in *Table 29.1*. Furthermore, the correct placement of the old genus *Lampropedia* (Schroeter 1886) in the *Comamonadaceae* was deduced from studies of the rRNA genes (Lee et al. 2004). It was originally mainly recognized as a separate genus because of its conspicuous tablet-shaped arrangement of cells. It is found in water rich with organic material and in rumen fluid. Only one species has been described, *Lampropedia hyalina*, with several strains available in collections. Most of these strains in cultivation have lost the capacity to form tablets, and Lee et al. (2004) suggest this feature should therefore not be decisive for species description.

Brachymonas forms one of the deeper branching lineages of the family *Comamonadaceae* (Wen et al. 1999 and *Fig. 29.1*). In addition to the two valid species, *Brachymonas denitrificans* (Hiraishi et al. 1995) and *Brachymonas chironomi* (Halpern et al. 2009), an isolate enriched from an oil refinery wastewater sludge using cyclohexane as carbon source was described tentatively as a new species "*Brachymonas petroleovorans*," however, without a species description or type strain assignment (Rouvière and Chen 2003).

Comments on the Membership of the Family

Xylophilus is not listed as a member of the family in the List of Prokaryotic names with Standing in Nomenclature (www.bacterio.cict.fr). However, phylogenetically, it does belong to the *Comamonadaceae* cluster (*Fig. 29.1*), and it was assigned to the family in the original description (Willems et al. 1991a).

In the original description of the family, in a separate section at the end of the paper, the nearest neighbors of the *Comamonadaceae* were discussed (Willems et al. 1991a). These include the facultatively photolithotrophic organism

Rubrivivax gelatinosus, the hydrogen oxidizers *Pseudomonas saccharophila* (now *Pelomonas saccharophila*) and *Alcaligenes latus* (currently *Azohydromonas lata*), and the sheathed manganese-oxidizing bacteria belonging to the genera *Leptothrix* and *Sphaerotilus*. In the past these were reported to be closely related or to belong to the acidovorans rRNA complex (De Vos and De Ley 1983; Willems et al. 1991b). At the time of the description of the family, they were explicitly excluded because they appeared less related than the other family members according to DNA–rRNA hybridizations and rRNA oligonucleotide catalogues and because their inclusion would result in a very widely defined family (Willems et al. 1991a).

Meanwhile the family *Comamonadaceae* has become more diverse by the inclusion of many new genera; however, the phylogeny of 16S rRNA gene sequences which is now available places *Rubrivivax* and relatives in a separate cluster near the *Comamonadaceae* (Fig. 29.1). Several additional genera have been described that group in this part of the phylogenetic tree. Some, such as *Kinneretia asaccharophila* gen. nov., sp. nov., isolated from a freshwater lake, was described as a member of the *Rubrivivax* branch of the family *Comamonadaceae* (Gomila et al. 2010). *Caldimonas* was described as "related to the *Rubrivivax* subgroup and the family *Comamonadaceae*" (Takeda et al. 2002). Indeed, *Caldimonas*, *Kinneretia*, *Pelomonas*, *Roseateles*, and *Schlegelella* are listed as members of the family in the List of Prokaryotic names with Standing in Nomenclature (www.bacterio.cict.fr). However, phylogenetically, they are not part of the *Comamonadaceae* cluster (Fig. 29.1) and constitute neighboring clusters that include the genera *Kinneretia* (1 species), *Pelomonas* (3 sp.), *Mitsuaria* (1 sp.), *Roseateles* (3 sp.), *Paucibacter* (1 sp.), *Inhella* (2 sp.), *Leptothrix* (5 sp.), *Sphaerptilus* (3 sp.), *Vitreoscilla* (3 sp.), *Rubrivivax* (2 sp.), *Ideonella* (2 sp.), *Aquincola* (1 sp.), *Azohydromonas* (2 sp.), *Aquabacterium* (5 sp.), *Caldimonas* (2 sp.), Schlegelella (2 sp.), and *Tepidimonas* (4 sp.)

Comment on the genus *Caenibacterium*: The genus *Caenibacterium* was created for an organism isolated from a thermophilic aerobic digester of municipal sludge for which the name *Caenibacterium thermophilum* was proposed (Manaia et al. 2003). The species has since been shown to be a later synonym of *Schlegelella thermodepolymerans* (Lütke-Eversloh et al. 2004). *Caenibacterium* and *Schlegelella* are listed as members of the family in the List of Prokaryotic names with Standing in Nomenclature (www.bacterio.cict.fr). However, as pointed out above, *Schlegelella* does not belong to the phylogenetic cluster of the *Comamonadaceae* family.

Molecular Analyses

DNA–DNA Hybridization Studies

In nearly all multispecies genera of the family, DNA–DNA hybridizations with existing species have been performed to justify proposals of new species. In most cases the values obtained between species are low (less than 45 %). Recent examples include the genus *Acidovorax* where DNA–DNA hybridization values between *Acidovorax soli* strain BL21^T and other *Acidovorax* strains between 12 % and 28 % (Choi et al. 2010) and the genus *Acidovorax* where hybridizations between *Acidovorax radices* N35^T and eight other *Acidovorax* species were between 25 % and 56 %. With the closely related. *Simplicispira Metamorpha* DSM 1837^T, 39 % was Obtained (Li et al. 2011).

Occasionally higher values are reported between species, and in those cases the polyphasic approach provides additional information to help decide on species status. For example, in the genus *Delftia*, hybridization values between the type strains of *Delftia acidovorans* and *Delftia tsuruhatensis* were just below 69 %. Nevertheless, they were regarded as separate species because of phenotypic and chemotaxonomic differences (Shigematsu et al. 2003). *Delftia lacustris* 332^T showed 36–66 % DNA–DNA hybridization with the type strains of both other *Delftia* species (Jørgensen et al. 2009), and more recently, *Delftia litopenaei* wsw-7^T showed 40–50 % DNA–DNA relatedness with the type strains of the other species (Chen et al. 2012).

Other Sequence Analyses

Whereas in the 16S rRNA phylogeny the family constitutes a radiation of mostly equally related genera, studying more variable genes might shed some light on closer relationships that may exist between some genera but that are not strongly reflected in the phylogeny of conserved 16S rRNA genes. However, genes other than the 16S rRNA genes have rarely been used to study the members of the family *Comamonadaceae*. This may change as more complete genomes can be mined, but at present only a few studies have used other genes to study diversity of species.

Many members of the *Comamonadaceae* are capable of accumulating polyhydroxyalkanoates such as PHB in the cell. Chen et al. (2012) compared the polyhydroxyalkanoate synthase gene (phaC, partial sequence of 606 bp) in several of these organisms and found the phylogeny largely congruent with that of 16S rRNA genes with separate clusters for the genera *Acidovorax*, *Alicycliphilus*, *Variovorax*, *Delftia*, *Polaromonas*, and *Comamonas*.

For *Verminephrobacter* strains, the *recA*, *gap*, and *rpoC* sequences were determined and compared with those of environmental and plant-associated members of the genus *Acidovorax*. The phylogenies revealed that *Verminephrobacter* strains form a coherent cluster among strains and clusters of the *Acidovorax* species with uncertain branching orders, possibly indicating the heterogeneity of the current genus *Acidovorax* (Pinel et al. 2008).

Genome Comparisons

Over the last 5 years, 33 complete or draft genome sequences have been reported for representatives of 10 genera of the family (Table 29.2). These genomes mostly comprise 1 chromosome (although a *Variovorax paradoxus* strain has two chromosomes, Table 29.2), no plasmids or sometimes 1–8 plasmids; reported sizes range from 3.62 Mb for *Hylemonella gracilis* ATCC 19624^T to 7.01 Mb for *Acidovorax* sp. CF316 (Table 29.2). In most cases, a strain of some particular interest was sequenced; however, nine type strains are also included (Table 29.2). For many of these sequences, detailed analyses are still underway. Several have, however, already been explored in more detail, and the most striking observations published. Those that have been reported on carry one to three rRNA operons (see below).

Xie et al. (2011) reported the genome of *Acidovorax avenae* subsp. *avenae* RS-1, isolated from rice shoots and capable of causing bacterial strip of rice. This strain contains three rRNA operons; pathogenicity-related genes involved in hypersensitivity response, type III secretion, synthesis of fimbriae, and adhesion proteins are present. The genome comprises 301 ORFs that are not present in other *Acidovorax* genomes and that are clustered apparently forming several genomic islands in this strain (Xie et al. 2011). An isolate from soil and capable of degrading polychlorinated biphenyl, *Acidovorax* sp. KKS102, was reported to have a circular chromosome with three rRNA operons (Ohtsubo et al. 2012), whereas *Acidovorax* sp. NO1, from gold mine soil, had a single rRNA operon (Huang et al. 2012). This latter strain is a facultative anaerobic nitrate reducer that can oxidize arsenite aerobically as well as anaerobically. Its genome contains the *aio* operon for arsenite oxidation, three *ars* operons for arsenite resistance operons, and a denitrification pathway that lacks nitrite reductase (*nir*) (Huang et al. 2012). *Acidovorax* strain TPSY, an organism obtained from groundwater and capable of facultatively anaerobic mixotrophic nitrate-dependent Fe(II) oxidation, has been referred to as "*Acidovorax ebreus*," although no species description was published (Byrne-Bailey et al. 2010). Its genome was reported to be a single circular chromosome, carrying the expected metabolic genes and also genes for resistance against lead, arsenite, and mercury; genes involved in twitching motility, but no known pathogenicity; and genes such as type I and type III secretion systems (Byrne-Bailey et al. 2010).

The genomes of two strains of *Alicycliphilus denitrificans*, K601^T and BC, have been sequenced. Both have been isolated from different wastewater treatment plants and are able to degrade cyclic hydrocarbons using either oxygen or nitrate as terminal electron acceptors. Strain BC can also use chlorate as terminal electron acceptor. Both strains carry a circular chromosome; K601^T also hosts one plasmid (75 k), and BC hosts two plasmids (79 kbp and 120 kbp). Genes involved in benzene degradation are located on the chromosome in both strains, while in strain BC, the chlorate reductase and chlorite dismutase genes are located on the megaplasmid that is absent from strain K601 (Oosterkamp et al. 2011).

Comamonas testosteroni ATCC 11996^T grows aerobically on steroids and aromatic compounds. Its genome comprises a circular chromosome that carries one rRNA operon and degradation genes for these compounds; it has an incomplete pentose phosphate pathway that may account for the inability to metabolize most sugars (Gong et al. 2012). A second strain of this species, strain CNB-2 from activated sludge, carries – in addition to its circular chromosome – a large plasmid that contains the genes required for 4-chlorobenzene degradation (Ma et al. 2009). The genome encodes for enzymes that degrade short chain fatty acids and aromatic and cyclic hydrocarbons. Like the type strain, it carries an incomplete pentose phosphate pathway. Many genes are involved in transport functions (22 %). Four different terminal oxidase complexes were detected, functioning at different oxygen concentrations. Furthermore, nitrate can serve as an alternative electron acceptor at low oxygen concentrations. These genes are thought to contribute to the competitiveness of this organism in the soil and wastewater environment (Ma et al. 2009).

The genome of *Hydrogenophaga* sp. strain PBC, an organism from textile wastewater and capable of degrading 4-aminobenzene sulfonate, was reported to carry three rRNA operons and the necessary genes for the degradation of 4-aminobenzene sulfonate as well as other aromatic compounds (Gan et al. 2012).

Polaromonas naphthalenivorans CJ2^T was recovered from coal-tar waste contaminated sediments. Its genome consists of a circular chromosome (4.4 Mb, 63 mol% G+C) and eight plasmids with a lower mol% G+C and sizes ranging from 353 bp to 6.4 kbp (Yagi et al. 2009). The chromosome carries two rRNA operons, genes for several central pathways (Embden–Meyerhof–Parnas glucolytic pathway, Entner–Doudoroff pathway, citric acid cycle, Calvin cycle for CO_2 fixation using type II RuBisCO) and several pathways for metabolizing aromatic compounds. The plasmids encode 845 genes, and their function differs with three of the plasmids not carrying genes for metabolism or energy production; one plasmid harboring many unknown genes and one plasmid carrying mostly genes involved in conjugation and degradation. The genome carries 309 mobile genetic elements that indicate a mosaic nature for the genome. A comparison with other genomes revealed that about 60 % of the genes of this organism were homologous to genes in other Betaproteobacteria (max. E-value 10^{-5}, min. identity 30 %) (Yagi et al. 2009).

De Luca et al. (2011) report on the genome of *Ramlibacter tataouinensis* TTB310^T, an organism from a semiarid region with an unusual cell cycle that involves immobile cyst-like cells that can multiply and vegetative cells that are highly motile. Its genome is a circular chromosome with a high mol% G+C of 70; it carries one rRNA operon and a number of genes reflecting possible adaptations to desert life such as genes for carotenoid biosynthesis, exopolysaccharide synthesis and hydrolysis, trehalose synthesis, type IV pili involved in gliding, blue and red light sensors, and a complex network of two component systems that may be involved in the control of the cell cycle in relation to fast environmental changes (De Luca et al. 2011).

Table 29.2

Overview of genome sequences available for members of the *Comamonadaceae*

Organism	Genome structure	Size (Mb)	GC%	Genes	Proteins	GenBank	References
Acidovorax avenae subsp. *avenae* ATCC 19860^T	1 Ch.	5.48	68.8	4851	4737	CP002521.1	JGI Project ID: 4085254
Acidovorax avenae subsp. *avenae* RS-1	156 Contigs	5.52	68.7	–		AFPT00000000	Xie et al. (2011)
Acidovorax citrulli AAC00-1	1 Ch.	5.35	68.5	4858	4709	CP000512.1	JGI Project ID: 4001427
Acidovorax citrulli ZJU1106	2303 Contigs	4.63	67.8	–		AMRP00000000	Institute of Biotechnology, Zhejiang University, China
Acidovorax delafieldii 2AN	638 Contigs	4.84	65	4942	4891	ACQT00000000	JGI Project ID: 4085645
"Acidovorax ebreus" TPSY	1 Ch.	3.8	66.8	3609	3479	CP001392.1	Byrne-Bailey et al. (2010)
Acidovorax radicis N35^T	30 Contigs	5.53	–	5099	5044	AFBG00000000	Helmholtz Zentrum Muenchen, Department Microbe-Plant Interactions
Acidovorax radicis N35v	33 Contigs	5.53	64.8	–		AFBH00000000	Helmholtz Zentrum Muenchen, Department Microbe-Plant Interactions
Acidovorax sp. KKS102	1 Ch.	5.2	64.9	4803	4742	CP003872.1	Ohtsubo et al. (2012)
Acidovorax sp. JS42	1 Ch., 2 pl.	4.59	66.1	4383	4155	CP000539.1, CP000540, CP000541	JGI Project ID: 4001426
Acidovorax sp. CF316	315 Contigs	7.09	67.8	6782	6724	AKJX00000000	Brown et al. (2012)
Acidovorax sp. NO-1	161 Contigs	5.01	–	4729	4679	AGTS00000000	Huang et al. (2012)
Albidiferax ferrireducens T118^T	1 Ch., 1 pl.	4.97	59.6	4554	4417	CP000267.1, CP000268.1	US DOE Joint Genome Institute
Alicycliphilus denitrificans K601^T	1 Ch., 1 pl.	5.07	67.9	4899	4696	CP002657.1, CP002658.1	Oosterkamp et al. (2011)
Alicycliphilus denitrificans BC	1 Ch., 2 pl.	4.84	68	4709	4542	CP002449.1, CP002450.1, CP002451.1	Oosterkamp et al. (2011)
Comamonas testosteroni CNB-1	1 Ch., 1 pl.	5.46	61.4	4979	4891	CP001220.1, EF079106.1	Ma et al. (2009)
Comamonas testosteroni ATCC 11996^T	63 Contigs	5.41	61.5	5056	4984	AHIL00000000	Gong et al. (2012)
Comamonas testosteroni KF-1	1 Contig	6.03	61.8	5600	5492	AAUJ02000001	JGI Project ID: 4002763
Comamonas testosteroni NBRC 100989	153 Contigs	5.59	61.9	–		BAEC01000001-BAEC01000153	Biotechnology National Institute of Technology and Evaluation, NITE, Tokyo
Comamonas testosteroni S44	112 Contigs	5.53	61.4	5218	5154	ADVQ00000000	State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Hubei
Delftia acidovorans SPH-1	1 Ch.	6.77	66.5	6153	6040	CP000884.1	JGI Project ID: 4002762

Table 29.2 (continued)

Organism	Genome structure	Size (Mb)	GC%	Genes	Proteins	GenBank	References
Delftia sp. Cs1-4	1 Ch.	6.69	66.7	6029	5861	CP002735.1	JGI Project ID: 4086526
Hydrogenophaga sp. PBC	148 Contigs	5.14	68.4	5009	4964	AJWL00000000	Gan et al. (2012)
Hylemonella gracilis ATCC 19624^T	115 Contigs	3.62	–	3388	3342	AEGR00000000	California Institute of Technology Millard and Muriel Jacobs Genetics and Genomics Laboratory
Polaromonas naphthalenivorans CJ2^T	1 Ch., 8 pl.	5.37	61.7	5063	4929	CP000529.1, CP000530.1, CP000531.1, CP000532.1, CP000533.1, CP000534.1, CP000535.1, CP000536.1, CP000537.1	Yagi et al. (2009)
Polaromonas sp. CF318	159 Contigs	5.01	–	4805	4752	AKIV00000000	Brown et al. (2012)
Polaromonas sp. JS666	1 Ch., 2 pl.	5.9	62	5626	5453	CP000316.1, CP000317.1, CP000318.1	JGI Project ID: 3634502
Ramlibacter tataouinensis TTB310^T	1 Ch.	4.07	70	3926	3880	CP000245.1	De Luca et al. (2011)
Variovorax paradoxus S110	2 Ch.	6.75	67.5	6453	6279	CP001635.1, CP001636.1	JGI Project ID: 4083788
Variovorax paradoxus EPS	1 Ch.	6.55	66.5	6087	5952	CP002417.1	JGI Project ID: 4083792
Variovorax sp. CF313	173 Contigs	6.03	67	5650	5597	AKIW00000000	Brown et al. (2012)
Verminephrobacter aporrectodeae subsp. *tuberculatae* At4^T	1082 Contigs	4.68	–	4867	4823	AFAL00000000	Kjeldsen et al. (2012)
Verminephrobacter eiseniae EF01-2^T	1 Ch., 1 pl.	5.6	65.3	5109	4947	CP000542.1, CP000543.1	JGI Project ID: 4001428

Data obtained from the NCBI genome pages http://www.ncbi.nlm.nih.gov/genome

The complete genomes of *Verminephrobacter aporrectodeae* and *Verminephrobacter eiseniae*, two species that live as extracellular endosymbionts in earthworms, have been compared with those of two closely related free-living strains, *Acidovorax avenae* subsp. *citrulli* strain AAC00-1 and *Acidovorax* sp. JS42. The comparison revealed that genome size was not reduced and showed no A-T bias in the symbionts. Positive selection was detected in genes involved in DNA metabolism, tRNA modification, and TonB-dependent iron uptake, indicating that these may be involved in functions important in symbiosis. The comparison revealed Average Nucleotide Identity (ANI) values of 82.2 ± 6.4 % between both *Verminephrobacter* strains and approximately 76 ± 8 % of these species with *Acidovorax* strains (Kjeldsen et al. 2012).

In a recent study comparing the genes encoding oxygenases involved in aromatics biodegradation in 80 Burkholderiales genomes, 16 *Comamonadaceae* were included (Pérez-Pantoja et al. 2012). The authors report in detail on the presence and organization of various central and peripheral degradative pathways. Within the family *Comamonadaceae*, *Comamonas*, *Variovorax*, *Delftia*, and *Polaromonas* strains have a quite distinguishable catabolic pattern involving the protocatechuate *meta*-cleavage pathway, whereas other genera of this family, such as *Acidovorax* and *Alicycliphilus*, have a restricted aromatic catabolic potential (Pérez-Pantoja et al. 2012).

Phenotypic Analyses

A comparison of some general features of the members of the *Comamonadaceae* is given in *Table 29.3*.

Acidovorax Willems et al. (1990), 394^{VP}

A.ci.do'vo.rax, N.L. neut. n. *acidum* (from L. adj. *acidus*, sour) an acid; L. adj. *vorax*, voracious; N.L. masc. n. *Acidovorax*, acid-devouring (bacteria).

Table 29.3

Morphological and chemotaxonomic characteristics of the genera of *Comamonadaceae*. Data taken from the original descriptions and references as given in the main text

Genus	Source	# Species	Morphology	Motility	Metabolism	Temperature preference	Major fatty acids	Major hydroxy fatty acids	G+C content
Acidovorax	Water, soil, plants, clinical samples	15	Straight to slightly curved rods	+, polar flagellum	Aerobic chemoorganotrophic (some strains fac. hydrogen oxidizers; some strains capable of nitrate respiration)	Mesophilic	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/C16:1 ω7c)	C8:0 3-OH, C10:0 3-OH	62–70
Albidiferax	Coastal aquifer sediments	1	Rods	+, single polar flagellum	Facultatively anaerobic chemoorganotrophic; respiration using Fe(III)-nitrilotriacetic acid, Mn(IV) oxide, fumarate, nitrate and oxygen	Mesophilic, psychrotolerant	ND	ND	59.5
Alicycliphilus	Municipal sewage plant	1	Short rods	+	Oxidative using oxygen, nitrate or nitrite at TAE	Mesophilic	C16:1 ω7c, C16:0, C18:1 ω7c	C10:0 3-OH	66
Brachymonas	Activated sludge, chironomid egg mass	2	Cocci or short rods	−	Aerobic chemoorganotrophic, fac. Anaerobic using nitrate	Mesophilic, psychrotolerant	C16:1 ω7c, C16:0, C18:1 ω7c	C10:0 3-OH	60–65
Caenimonas	Activated sludge, paddy soil	2	Rods or curved rods	−	Aerobic chemoorganotrophic	Mesophilic	SF 3 (C16:1ω7c and/or iso-C15:0 2-OH), C16:0	C10:0 3-OH	62.7–68.7
Comamonas	Soil, water, clinical	16	Rods	− or +, polar flagella	Aerobic, non-fermentative, chemoorganotrophic	Mesophilic	C16:0, C18:1 ω7c, SF3 (C16:1 ω7c/C15:0 iso 2-OH)	C10:0 3-OH	61–70
Curvibacter	Well water or distilled water	4	Slightly curved rods	− or 1 to 6 polar flagella	Aerobic chemoorganotrophic	Mesophilic	C16:0, SF3 (iso-C15:0 2-OH/C16:1 ω7c), C18:1 ω7c	C8:0 3-OH, C10:0 3-OH (species dependent)	62–67
Delftia	Aquatic habitats, soil, clinical samples	4	Straight to slightly curved rods	+, polar or bipolar tufts of 1 to 5 flagella	Aerobic, non-fermentative, and chemoorganotrophic	Mesophilic, some psychrotolerant	C16:0, C17:0 cyclo, C18:1w7c, SF3 (C16:1ω7c and/or C16:1ω6c)	C10:0 3-OH	63–66
Diaphorobacter	Activated sludge, sediment from paddy field	2	Rods	+, polar flagellum	Aerobic, one species able to denitrify	Mesophilic	C16:1ω7c, C16:0, C18:1 ω7c	C10:0 3-OH	63–65
Extensimonas	Industrial wastewater	1	Short rods, elongating at higher temperature	+, polar flagellum	Aerobic, chemoorganotrophic	Moderately thermophilic	SF3 (C16:1 ω7c/C15:0 iso 2-OH), C17:0 cyclo	C10:0 3-OH	68.8

Giesbergeria	Pond water, sulfide spring, wastewater	5	Spirilla	+, bipolar tufts of flagella	Aerobic, chemoorganotrophic	Mesophilic	C16:1, C16:0	(C10:0 3-OH)	56.5–60
Hydrogenophaga	Water, soil	9	Rods	+, one or two polar to subpolar flagella	Aerobic, chemoorganotrophic	Mesophilic	C16:0, C18:1 ω7c, SF3 (C16:1 ω7c/C15:0 iso 2-OH)	C8:0 3-OH, C10:0 3-OH (species dependent)	64–69
Hylemonella	Pond water	1	Spirilla	+, bipolar tufts of flagella	Aerobic, chemoorganotrophic	Mesophilic	C16:0, C16:1 ω7c, C18:1 ω7c	C10-3OH, C12:0 2-OH (species dependent)	65
Lampropedia	Water rich in organic material, rumen fluid	1	Coccoid cells in tablet formation	−, Twitching may occur	Aerobic chemoorganotrophic	Mesophilic	C14:0, C16:0, C18:1 ω7c	C10:0 3-OH, C12:0 3-OH	63–67
Limnohabitans	Freshwater	4	Straight or curved rods	−	Aerobic chemoorganotrophic, facultative anaerobic	Mesophilic, some species psychrotolerant	C16:0, C16:1 ω7c/ω6c	C8:0 3-OH	55–60
Macromonas	Freshwater environments with low oxygen and hydrogen sulfide concentrations	2	Large irregular bean-shaped or cylindrical cells with inclusions	+, polar tuft of flagella	Aerobic chemoorganotrophic	Mesophilic	C16:1 ω7c, C17:1 ω6c, C18:1 ω7c, C16:0 (M. bipunctata)	-	67.6 (M. bipunctata)
Malikia	Activated sludge, river water	2	Straight to slightly curved rods	+, one or two polar flagella	Aerobic chemoorganotrophic	Mesophilic, psychrotolerant	C16:0, C16:1 ω7c, C18:1 ω7c	C8:0 3-OH	66–67
Ottowia	Activated sludge	2	Rods	−	Aerobic, chemoorganotrophic, mixotrophic with thiosulfate	Mesophilic, psychrotolerant	C16:0, C16:1 ω7c, C18:1 ω7c	C10-3OH, C12:0 2-OH (species dependent)	59–69
Polaromonas	Antarctic ocean, glacier cryoconite, sediment, soil, water	7	Coccoid to rod shaped	+ or −	Aerobic chemoorganotrophic	Psychrotrophic or mesophilic	C16:0, SF3 (C16:1 ω7c and/or iso-C15:0 2-OH), C18:1 ω7c	C8:0 3-OH, C10:0 3-OH	52–64
Pseudacidovorax	Soil	1	Short rods	+, polar flagellum	Facultative aerobic chemoorganotrophic	Mesophilic	C16:0, SF3 (C16:1 ω7c and/or iso-C15:0 2-OH), C18:1 ω7c	C8:0 3-OH, C10:0 3-OH, C16:1 2-OH, C18:1 2-OH	70.1
Pseudorhodoferax	Soil, activated sludge, groundwater	3	Short rods	+, polar Flagellum	Aerobic, Chemoorganotrophic	Mesophilic	C16:0, C18:1 ω7c, summed feature 3 (C16:1 ω7c and/or C15:0 iso 2-OH)	C10:0 3-OH	67.6–70.1

Table 29.3 (continued)

Genus	Source	# Species	Morphology	Motility	Metabolism	Temperature preference	Major fatty acids	Major hydroxy fatty acids	G+C content
Ramlibacter	Soil	2	Straight to curved rods and coccoid cells	− or +, peritrichous flagella	Aerobic, chemoorganotrophic	Mesophilic	C16:0, C17:0 cyclo, SF4 (C16:1 ω7c and/or C15:0 iso 2-OH), SF7 (C18:1 ω7c and/or ω9t and/or ω12t)	C10:0 3-OH, C12:0 3-OH	66.6–69.6
Rhodoferax	Ditch water, activated sludge, aquatic microbial mat	2	Curved to spiral-shaped rods	+, one or more polar flagella	Fac. anaerobic Photoheterotrophic or photoautotrophic, aerobic heterotrophs or anaerobic fermenters	Mesophilic, psychrotolerant (one species)	C16:0, C16:1	C8:0 3-OH	60–61.5
Simplicispira	Freshwater, wastewater, Antarctic mosses	3	Slightly curved rods to spirilla	+, bipolar tufts of flagella or a single polar flagellum	Aerobic, organotrophic, some facultative anaerobic with denitrification	Mesophilic	C16:1, C16:0, C18:1	C10:0 3-OH	63–65
Tepidicella	Hot spring runoff	1	Short rods	+, polar flagellum	Aerobic organotrophic	Moderately thermophilic	C16:0, C18:1 ω7c, C17:0 cyclo	C8:0 3-OH	64.9–65.5
Variovorax	Soil, sewage	6	Straight to slightly curved rods to oval-shaped cells	+, one to multiple Peritrichous flagella	Aerobic chemoorganotrophic (some *V. paradoxus* are fac. hydrogen oxidizers; *V. ginsengisoli* is capable of nitrate respiration)	Mesophilic	C16:0, C17:0 cyclo, SF4 (C16:1 ω7c and/or iso-C15:0 2-OH), SF7 (C18:1 ω7c and/or ω9t and/or ω12t)	C10:0 3-OH	65–71
Verminephrobacter	Nephridia of earthworm	2	Rods	− or +, polar flagellum	Aerobic chemoorganotrophic, prefers low oxygen tension	Mesophilic	C16:0, C16:1 ω7c, C18:1 ω7c, C17:0 cyclo	C10:0 3-OH	67
Xenophilus	Soil, air	2	Straight to curved rods	+, polar flagellum	Aerobic organotrophic	Mesophilic	C16:0, SF (C16:1 ω7c and/or iso-C15:0 2-OH), C18:1 ω7c, C17:0 cyclo	C8:0 3-OH, C10:0 3-OH, C16:1 2-OH, C16:0 2-OH, C18:1 2-OH	69–70.4
Xylophilus	Vitis vinifera, diseased	1	Straight to curved rods	+, a polar flagellum	Aerobic organotrophic	Mesophilic	C16:0, SF (C16:1 ω7c and/or iso-C15:0 2-OH), C18:1 ω7c, C17:0 cyclo	C8:0 3-OH	68–69

The genus *Acidovorax* contains 15 species that either originate from the environment (soil, water) (*Acidovorax caeni, Acidovorax defluvii, Acidovorax delafieldii, Acidovorax facilis, Acidovorax soli, Acidovorax temperans*) or are phytopathogenic (*Acidovorax anthurii, Acidovorax avenae, Acidovorax cattleyae, Acidovorax citrulli, Acidovorax konjaci, Acidovorax oryzae, Acidovorax valerianellae*) or plant-associated species (*Acidovorax radicis*). *Acidovorax temperans* and *Acidovorax wautersii* were also isolated from clinical samples. They can be regarded as versatile saprophytes, aerobic chemoorganotrophs, although *Acidovorax caeni* and some strains of *Acidovorax delafieldii* (not the type strain) and *Acidovorax temperans* (including the type strain) are able to grow anaerobically using nitrate as terminal electron acceptor (Willems et al. 1990; Heylen et al. 2008). *Acidovorax facilis* strains and some strains of *Acidovorax delafieldii* (though not the type strain) are capable of autotrophic growth using hydrogen as an energy source (Willems et al. 1990).

Additional features and differentiating characteristics of the species are shown in ❯ *Table 29.4*.

Albidiferax corrig. Ramana and Sasikala (2009), 2647[VL]

Al.bi.di.fe'rax, L. adj. *albidus*, whitish; L. adj. *ferax* fertile; N.L. masc. n. *Albidiferax*, whitish and fertile.

Albidiferax ferrireducens (originally described as *Rhodoferax ferrireducens*) was isolated from coastal aquifer sediments by anaerobic enrichment culturing in a mineral medium with acetate as electron donor and Fe(III) nitrilotriacetic acid (NTA) as electron acceptor (Finneran et al. 2003). Cells are rods, 3–5 µm by 1 µm, and are motile by means of a single polar flagellum. In addition to acetate, alternative electron donors with the same electron acceptor include lactate, propionate, pyruvate, and malate (all 20 mM) and succinate (10 mM) and benzoate (1 mM). However, formate, butyrate, ethanol, methanol, or glycerol (all 20 mM); caproate, isobutyrate, valerate, butanol, or propanol (all 10 mM); or hydrogen (160 kPa) could not be used. Electron acceptors that can be used with lactate or acetate as electron donor included Fe(III)–NTA and Mn(IV) oxide (both 10 mM), fumarate (20 mM), and atmospheric oxygen. Not utilized were poorly crystalline Fe(III) oxide (100 mM); Fe(III) citrate (50 mM); anthraquinone-2, 6-disulfonate (AQDS) or chromium(VI) (both 5 mM); cobalt-EDTA (0.05 mM); uranium(VI) (2.5 mM); elemental sulfur (1 g l^{-1}); nitrite, selenate, or selenite (all 10 mM); or sulfate, sulfite, or thiosulfate (all 20 mM); and 1 % oxygen. Nitrate (20 mM) can serve as an electron acceptor with lactate, but not with acetate (Finneran et al. 2003).

Cells produce polyhydroxyalkanoate inclusions when grown in carbon-rich medium. No growth on yeast extract (0.1 %) and no autotrophic growth by converting H_2 and CO_2 to acetate. No phototrophic growth and no fermentative growth (Finneran et al. 2003).

Temperature and pH range for growth are 4–30 °C and 6.7–7.1. Optimal growth occurs at a temperature of 25 °C and pH 7.0. Anaerobic growth with Fe(III) reduction is possible at 4 °C, indicating *Albidiferax ferrireducens* is psychrotolerant (Finneran et al. 2003).

Genomic G+C content is 59.5 mol% (Finneran et al. 2003). No fatty acid data have been reported.

Alicycliphilus Mechichi et al. (2003), 149[VP]

A.li.cyc.li'phi.lus. Gr. adj. *aliphos* fat; L. n. *cyclus*, circle or ring; N.L. pref. *alicycli* referring to circular fat-like organic compounds; N.L. adj. *philus* (from Gr. adj. *philos*), friend; N.L. masc. adj. *Alicycliphilus* alicyclic compound-liking, referring to the substrates used for the isolation of this organism.

Alicycliphilus was first isolated from a municipal sewage plant by anaerobic enrichment with cyclohexanol and nitrate. Only one species has been described. Cells are short rods (0.6 by 1–2 µm), motile, and catalase and oxidase positive. Oxidative metabolism using a wide range of organic substrates and oxygen, nitrate or nitrite but not sulfate, sulfite or fumarate as terminal electron acceptors. Best growth (aerobically or anaerobically) at 28–30 °C and at pH 7.2–7.4 (Mechichi et al. 2003).

Alicycliphilus denitrificans K601^T is able to use the following substrates under anoxic conditions: cyclohexanol, cyclohexanone, 1,3-cyclohexanedione, 2-cyclohexenone, 1,3-cyclohexanediol (cis and trans), monocarboxylic acids (C2–C7), adipate, pimelate, 5-oxocaproate, citrate, 2-oxoglutarate, succinate, L-malate, propionate, crotonate, L-lactate, pyruvate, and fumarate. It is unable to use the following substrates: aniline, phenol, benzoate, 2-aminobenzoate, 2-hydroxybenzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, resorcinol, hydroxyquinol, *m*-cresol, *o*-cresol, *p*-cresol, vanillate, naphthoate, indole, 1,2-cyclohexanediol, 1,2-cyclohexanedione, 2-hydroxycyclohexanone, 1,4-cyclohexanedione, cyclohexane, formate, D-glucose, D-fructose, D-xylose, and aliphatic alcohols (C1–C8). Under aerobic conditions, the following compounds are used: propionate, L-malate, aniline, fumarate, indole, vanillic acid, acetate, 4-hydroxybenzoate, *m*-cresol, *o*-cresol, *p*-cresol, crotonate, D-glucose, L-lactate, and pyruvate. The following compounds are not used aerobically: 4-aminobenzoate, benzoate, resorcinol, 2-naphthoate, biphenyl 2-carboxylate, 2-aminobenzoate, 3-hydroxybenzoate, gentisate, protocatechuate, hydroxyquinol, 3-fluorobenzoate, and 3-chlorobenzoate (Mechichi et al. 2003). *Alicycliphilus denitrificans* strain BC is able to degrade benzene, toluene, phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, and acetate using chlorate or oxygen as an electron acceptor but not using nitrate. It can use acetate, lactate, pyruvate, succinate, propionate, butyrate, malate, citrate, fumarate, glycerol, and yeast extract with nitrate as terminal electron acceptor. In contrast to the type strain, it cannot use cyclohexanol (Weelink et al. 2008). A further strain of *Alicycliphilus denitrificans*, KN Bun08, was recently described; it is capable of aerobic as well as anaerobic growth using nitrate with the following substrates: acetone, acetate, DL-lactate, pyruvate, succinate, propionate, butyrate, malate, citrate, fumarate, 3-hydroxybutyrate, ethanol, xylose, glucose, and fructose,

Table 29.4

Comparison of selected characters of the species of *Acidovorax*. Data taken from the descriptions in the references listed in the table. Fatty acid data are consensus data from these references

Characteristic	*Acidovorax anthurii* CFBP 3232^T	*Acidovorax avenae* LMG 2117^T	*Acidovorax caeni* LMG 24103^T	*Acidovorax cattleyae* LMG 2364^T	*Acidovorax citrulli* LMG 5376^T	*Acidovorax defluvii* BSB411^T	*Acidovorax delafieldii* ATCC 17505^T	*Acidovorax facilis* ATCC 11228^T	*Acidovorax konjaci* LMG 5691^T	*Acidovorax oryzae* FC – 143^T	*Acidovorax radicis* N35^T	*Acidovorax soli* BL21^T	*Acidovorax temperans* CCUG 11779^T	*Acidovorax valerianellae* CFBP 4730^T	*Acidovorax wautersii* LMG 26971^T
References	Gardan et al. (2000)	Willems et al. (1992), Schaad et al. (2008)	Heylen et al. (2008)	Willems et al. (1992)	Willems et al. (1992)	Schulze et al. (1999)	Willems et al. (1990)	Willems et al. (1990)	Willems et al. (1992)	Willems et al. (1990)	Li et al. (2011)	Choi et al. (2012)	Willems et al. (1990)	Gardan et al. (2003), Schaad et al. (2008)	Vaneechoutte et al. (2013)
Source	*Anthurium* affected by bacterial leaf spot	Diseased grasses	Activated sludge	Diseased *Phalaenopsis* and *Cattleyae*	Diseased Cucurbitaceae	Activated sludge	Soil, water, clinical samples	Soil	Diseased *Amorphophallus rivieri* cv. Konjac	Diseases rice plants	Wheat roots	Landfill soil	Clinical samples, activated sludge	Diseased *Valerianella locusta*	Clinical and environmental samples
Motility	Motile with a polar flagellum	Motile with a polar flagellum	Motile	Motile with a polar flagellum	Motile with a polar flagellum	Nonmotile	Motile with a polar flagellum	Motile with a polar flagellum	Motile with a polar flagellum	Motile with a polar flagellum	Motile with a polar flagellum	Motile with a polar flagellum	Motile with a polar flagellum	Motile with a polar flagellum	Motile with a polar flagellum
Cell shape	Rods	Straight to slightly curved rods	Rods	Straight to slightly curved rods	Straight to slightly curved rods	Rods to ovoid or round cells	Straight to slightly curved rods	Straight to slightly curved rods	Straight to slightly curved rods	Straight to slightly curved rods	Straight to slightly curved rods	Straight to slightly curved rods	Straight to slightly curved rods	Straight rods	Rods
Cell size	0.2–0.7 × 1–5 μm	0.2–0.8 × 1–5 μm	0.9 × 1.8 μm	0.2–0.8 × 1–5 μm	0.2 –0.8 × 1–5 μm	0.4–1.2 × 0.8–2.5 μm	0.2–0.7 × 1–5 μm	0.2–0.7 × 1–5 μm	0.2–0.8 × 1–5 μm	0.2–0.7 × 1–5 μm	0.2–0.7 × 1–5 μm	0.2–0.7 × 1–5 μm	0.2 –0.7 × 1–5 μm		0.2–0.7 × 1.0–5.0 μm
Colony morphology	Circular, raised, entire margin, creamy-white, brown diffusible pigment	Convex, smooth to slightly granular, beige to faint yellow	Round, smooth, yellow-brown	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Convex, smooth to slightly granular, beige to faint yellow	Circular, white to cream with a clear margin, slightly raised, slow growing	Smooth, convex, nonpigmented
Temperature range for growth			15–37								10–35	10–42			20–37
Growth at 4 °C			–								–	–		–	
Growth at 37 °C			+			–	+	–			–	+	+		+
Growth at 42 °C							+	–				+	–	–	–

Optimal growth						28					30	30			
pH range			5.5 − 10.5								5–9	6 –9			
Growth at pH 5			−									−			
Growth at pH 10			+								−	−			
Optimal pH											7	7			
Growth without NaCl											+	+			
Growth with 1 % NaCl			+								+	+			
Growth with 2 % NaCl			+			−					+	−			
Growth with 3 % NaCl			−				−	−			−	−	−		
Oxidase	+	+	+	+	+	+	+	+	+		+	−	+	+	+
Catalase	+		+			+	+	+			−	−	+	+	+
Urease	+		−									+w			‘+
Major fatty acids (>10 %)	C15:0, C16:0, C16:1 ω7c, C18:1 ω7c	C16:0, C16:1 ω7c, C18:1 ω7c	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/ C16:1 ω7c)	C16:0, C16:1 ω7c, C18:1 ω7c	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/C16:1 ω7c)	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/C16:1 ω7c)	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/ C16:1 ω7c)	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/ C16:1 ω7c)	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/ C16:1 ω7c)	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/ C16:1 ω7c)	C16:0, C16:1 ω7c/t	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/ C16:1 ω7c)	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/ C16:1 ω7c)	C16:0, C16:1 ω7c, C18:1 ω7c	C16:0, C18:1 ω7c, SF3 (iso-C15:0 2-OH/ C16:1 ω7c)
Important fatty acids (5–10 %)	C17:0				C15:0		C14:0	C12:0, C14:0, C10: 3-OH			C18:1 ω7c/t		C12:0		
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH	C10:0 3-OH	C8:0 3-OH, C10:0 3-OH	C10:0 3-OH	C10:0 3-OH	C8:0 3-OH, C10:0 3-OH	C8:0 3-OH, C10:0 3-OH	C8:0 3-OH, C10:0 3-OH	C10:0 3-OH	C10:0 3-OH		C10:0 3-OH	C8:0 3-OH, C10:0 3-OH	C10:0 3-OH	C10:0 3-OH
DNA G+C content (mol%)	63.5	69.8	65.7	68.6	68.4	62	65.6	64.7	68.4		64.9	60.9	62.4	64.5	67.1
Alkaline phosphatase, esterase C4			w									+			−
Esterase lipase C8			+									+			
Lipase C14, valine arylamidase, cysteine arylamidase, α-chymotrypsin, acid phosphatase			−									−			
Leucine arylamidase			+									+			
Trypsin			+									−			−
Naphthol-AS-BI-phospho hydrolase			+									+			

Table 29.4 (continued)

Characteristic	*Acidovorax anthurii* CFBP 3232^T	*Acidovorax avenae* LMG 2117^T	*Acidovorax caeni* LMG 24103^T	*Acidovorax cattleyae* LMG 2364^T	*Acidovorax citrulli* LMG 5376^T	*Acidovorax defluvii* BSB411^T	*Acidovorax delafieldii* ATCC 17505^T	*Acidovorax facilis* ATCC 11228^T	*Acidovorax konjaci* LMG 5691^T	*Acidovorax oryzae* FC – 143^T	*Acidovorax radicis* N35^T	*Acidovorax soli* BL21^T	*Acidovorax temperans* CCUG 11779^T	*Acidovorax valerianellae* CFBP 4730^T	*Acidovorax wautersii* LMG 26971^T
α-Galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase			–									–			
β-Galactosidase			–			–	–	–			–	–	–		
α-Glucosidase, β-glucosidase			–			–						–			
Reduction nitrate to nitrite		+	+	+(–[a])	–(+[a])	+[a]	+	+	+	+		+	+	–	–
Reduction nitrate to nitrogen		–[a]	+	–[a]	–[a]	+[a]	–	–	–[a]			–	+	–	–
Indole production = tryptophanase	–		+				–	–			–	–	–		
Fermentation glucose			–				–	–			–	–	–	–	
Arginine dihydrolase		–	–				–	–			–	–	–	–	–
Urease	+	+[a]	–	+[a]	+[a]	–[a]	+[a]	+[a]	+[a]			+w	–[a]	+	+
Pyrrolidonyl aminopeptidase		+		–	+	+	+	+	+				+		+
Hydrolysis aesculin	–		–			–	–	–			–	–	–	–	–
Hydrolysis gelatin		w	–	–	+(–[a])		–	+	–	w		–	–	–	–
Hydrolysis of DNA							–	–			–	–	–		
Hydrolysis of casein (skimmed milk)	–										–	–			
Hydrolysis of starch		–					–	–			–	–	–		
Alkaline reaction in litmus milk		+								+				–	
Alkalinization of citrate in Simmons agar		–		+	–	–	–	–	–				–		+
Acid from lactose[a]		–		–	–	–	–	–	–				–		+
Acid from rhamnose[a]		–		–	–	–	–	–	–				–		+
Acid from arabinose	+	+[a]		+[a]	–[a]	–[a]	+[a]	–[a]	–[a]				–[a]		+
Use of															
D-glucose	–	+	–	+	+	–	+	+	–	+		+	+	+	

N-acetyl glucosamine	–		–									–			
Potassium gluconate			–			–	+	+				–	–	+	
Capric acid		–	–	–	–		–	–	–		–	–	–		
Adipic acid		+	–	+	+	w	+	–	+	+		+	+	+(– Schaad)	
Malic acid			+				–	–	+	+	+	+	–	+	
Sodium tri-citrate		+	–	+	d	–		–	+	–		–	d	–	
Phenylacetate		–	–	–	–	–	–	–	–		–	–	–		
α-Cyclodextrin, dextrin			–								–	–			
Glycogen			+				–	–			–	–	-		
Tween 40			+								+	+			
Tween 80			+				+	–			+	+	+		
N-Acetyl-D-galactosamine			–								–	–			
N-Acetyl-D-glucosamine	–	–	–	–	–	–	–	–	–		–	–	–		
Adonitol		–	–	–	–	–	–	–	–		–	–	–		
L-Arabinose		+	–	+	+	–	+	+	–		+	–	–		
D-Arabitol		–	–	+	–		+	+	+	+	+	–	+	+	
D-Cellobiose		–	–	–	–	–	–	–	–		–	–	–		
i-Erythritol			–								–	–			
D-Fructose		+	–	+	+	–	+	+	+	+	+	–	+	+	
L-Fucose		+	–	–	–		–	–	–		–	–	–		
D-Galactose		+	–	+	+	–	+	+	–		+	–	–		
Gentiobiose			–				–	–			–	–	–		
α-D-Glucose	–	+	–	+	+	–	+	+	–	+	+	+	+		
m-Inositol	–	–	–	–	–		–	–	–		–	–	–		
α-D-Lactose			–				–	–			–	–	–		
Lactulose			–								–	–			
Maltose		+	–	–	–	–	–	–	–	–	–	–	–	+	
D-Mannitol	–	+	–	+	–	–	+	+	+	+	+	–	+	+	
D-Mannose		+	–	–	–	–	+	+	–		+	–	–		
D-Melibiose		–	–	–	–	–	–	–	–	–	–	–	–		
β-Methyl-D-glucoside		–	–	–	–		–	–	–		–	–	–		
D-Psicose			–								+	–			
D-Raffinose		–	–	–	–		–	–	–	–	–	–	–		
L-Rhamnose		–	–	–	–		–	–	–		–	–	–		
D-Sorbitol		+	–	+	–	–	+	+	–	+	+	–	+	–	
Sucrose	–	–	–	–	–	–	–	–	–		–	–	–		
D-Trehalose	–	–	–	–	–	–	–	–	–		–	–	–		

Table 29.4 (continued)

Characteristic	*Acidovorax anthurii* CFBP 3232^T	*Acidovorax avenae* LMG 2117^T	*Acidovorax caeni* LMG 24103^T	*Acidovorax cattleyae* LMG 2364^T	*Acidovorax citrulli* LMG 5376^T	*Acidovorax delftii* BSB411^T	*Acidovorax delafieldii* ATCC 17505^T	*Acidovorax facilis* ATCC 11228^T	*Acidovorax konjaci* LMG 5691^T	*Acidovorax oryzae* FC − 143^T	*Acidovorax radicis* N35^T	*Acidovorax soli* BL21^T	*Acidovorax temperans* CCUG 11779^T	*Acidovorax valerianellae* CFBP 4730^T	*Acidovorax wautersii* LMG 26971^T
Turanose		−	−	−	−	−	−	−	−		−	−	−	+	
Xylitol		−	−	−	−	−	−	−	−		−	−	−		
Methyl pyruvate			+								+	+			
Mono − methyl succinate			+	+							+	+			
Acetic acid	+	+	+	+	−	−	−	−	−		+	−	+		
cis-Aconitic acid			+								−	+	−		
Citric acid			−			−	−	−			−	−	−		
Formic acid	+		+								+	+			
D-Galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid			−								−	−	−	+	
D-Gluconic acid			−			−	+	+	+	+	+	−	−	+	
α-Hydroxybutyric acid			+								+	−			
β-Hydroxybutyric acid			+			+	+	+	+	+	+	+	+	+	
γ-Hydroxybutyric acid			+								−	−			
p-Hydroxyphenyl acetic acid			−								+	−			
Itaconic acid		−	−	−	−	−	−	−	−		−	−	−		
α-Ketobutyric acid			+								+	−			
α-Ketoglutaric acid		+	+	+	+	+	+	−	+	−	+	+	d	+	
α-Ketovaleric acid			+								−	−			
D,L-Lactic acid			+			−	+	+	+	+	+	+	+	+	
Malonic acid		+	−	−	−		−	−	−		−	−	−		
Propionic acid		+	+	+	−	−	−	+	+		+	−	+		
Quinic acid			−								+	−			
D-Saccharic acid			−								−	−			

Sebacic acid, succinic acid		+	+	+	+		+	+	+	+	+	+	+	+	
Bromosuccinic acid			+								+	−			
Succinamic acid			−								+	+			
Glucuronamide			−								−	+			
L-Alaninamide			−								−	−			
D-Alanine		+	+	+	w		−	−	+		+	−	−		
L-Alanine		+	+	+	+		+	+	+		+	+	+	+	
L-Alanylglycine			+								w	−			
L-Asparagine			−								+	+			
L-Aspartic acid		+	+	+	+	−	+	+	+		+	+	+	+	
L-Glutamic acid		+	+	+	+		+	+	+	+	+	+	+	+	
Glycyl-L-aspartic acid			−								−	−			
Glycyl-L-glutamic acid			−								+	−			
L-Histidine		+	−	+	−	−	+	+	+		+	−	+	+	
Hydroxy-L-proline			−								+	−			
L-Leucine		+	+	+	+	−	+	+	+	+	+	+	+	+	
L-Ornithine			−			−	+	−			−	−	−		
L-Phenylalanine			−			−					+	−		+	
L-Proline		+	+	+	+	−	+	+	+	+	+	+	+	+	
L-Pyroglutamic acid			+								−	−			
D-Serine			−								−	−			
L-Serine		+	+	+	+	−	+	+	+		−	+	−		
L-Threonine		+	+	+	−		+	+	+	+	+	+	+		
D,L-Carnitine			−								−	−			
γ-Aminobutyric acid	+	+	−	+	+	−	+	+	+		−	−	+	+	
Urocanic acid			−								+	−			
Inosine, uridine, thymidine, putrescine			−								−	−			
Phenylethylamine			−				+	+			−	−	+		
2-Aminoethanol	+		−				−	−			−	−	−	−	
2,3-Butanediol			−								−	−			
Glycerol	+		+				+	+			+	−	+	+	
D,L-α-Glycerol phosphate, glucose-1-phosphate			−								−	+			
Glucose-6-phosphate			−								−	−			
D-Xylose	−	+		−	−	−	−	−	−	+			−	−	
D-Ribose	−	+		+	+	−	+	+	+	+			−	−	

Table 29.4 (continued)

Characteristic	*Acidovorax anthurii* CFBP 3232^T	*Acidovorax avenae* LMG 2117^T	*Acidovorax caeni* LMG 24103^T	*Acidovorax cattleyae* LMG 2364^T	*Acidovorax citrulli* LMG 5376^T	*Acidovorax defluvii* BSB411^T	*Acidovorax delafieldii* ATCC 17505^T	*Acidovorax facilis* ATCC 11228^T	*Acidovorax konjaci* LMG 5691^T	*Acidovorax oryzae* FC – 143^T	*Acidovorax radicis* N35^T	*Acidovorax soli* BL21^T	*Acidovorax temperans* CCUG 11779^T	*Acidovorax valerianellae* CFBP 4730^T	*Acidovorax wautersii* LMG 26971^T
Citraconate		+		+	+		+	–	+	+			d	+	
β-Alanine		+				–	+	+					+		
Isobutyrate		+		+	+		–	–	+				+		
Isovalerate		+		+	–		+	+	+				+		
Pimelate		+		+	+		+	–	+				+	+	
L-Tryptophan	–	+		+	–	–	–	+	+				–	+	
Ethanol		–								+				+	
D-Fucose		–		+	+		+	–	–	+	+	–	–		
Lipase		+								+				–	
PHB accumulation	+					+								+	

[a]Data from Vaneechoutte et al. (2013)

but not formate; in aerobic conditions, it can also use the aromatic compounds benzoate, phenol, aniline, resorcinol, and cresol and, more slowly, also alicyclic compounds including cyclohexanol, cyclohexanediols, and cyclohexanediones (Dullius et al. 2011).

The major fatty acids reported for the type strain are C16:1ω7c, C16:0, and C18:1 ω7c. Present in smaller amounts were C10:0 3-OH and C12:0, C15:0 and cyclo C17:0. Genomic G+C content is 66 mol% (Mechichi et al. 2003).

Brachymonas Hiraishi et al. (1995), 879[VL]

Bra.chy.mo'nas. Gr. adj. *brachus*, short; L. fem. n. *monas*, a monad, unit; N.L. fem. n. *Brachymonas*, a small short unit.

Brachymonas was created for small coccobacilli isolated from activated sludge that are aerobic chemoorganotrophs with the capacity to also grow anaerobically with the respiration of nitrate to nitrogen (Hiraishi et al. 1995). Originally one species, *Brachymonas denitrificans*, was proposed; however, a second species was proposed later, *Brachymonas chironomi*, isolated from chironomid egg masses. This latter species is unable to grow anaerobically with nitrate, although it does reduce nitrate to nitrite (Halpern et al. 2009).

Brachymonas species are nonmotile coccobacilli or rods, oxidase and catalase positive, urease negative, and negative for lysine and ornithine decarboxylase. Both reduce nitrate to nitrogen in the case of *Brachymonas denitrificans* and to nitrite in the case of *Brachymonas chironomi*. Neither species produces acid from carbohydrates (Hiraishi et al. 1995; Halpern et al. 2009).

Additional characters and differentiating features of both species are shown in ➲ *Table 29.5*.

Caenimonas Ryu et al. (2008), 1067[VP]

Cae'ni.mo.nas. L. n. *caenum* mud, sludge; L. fem. n. *monas*, a unit, monad; N.L. fem. n. *Caenimonas*, monad isolated from sludge.

Caenimonas koreensis has been described for strain KCTC 12616^T isolated from activated sludge (Ryu et al. 2008). Recently a second species, *Caenimonas terrae*, was proposed for a paddy soil isolate (Kim et al. 2012). These authors also proposed an emendation of the genus *Caenimonas* which is incorporated in the following description of the genus.

The organism is strictly aerobic. Cells are rods to curved rods. *Caenimonas koreensis* cells are nonmotile; motility is not reported for *Caenimonas terrae* (Kim et al. 2012). Colonies on R2A are circular and white. Chemoorganotrophic metabolism. *Caenimonas koreensis* is unable to use hydrogen as an energy source; for *Caenimonas terrae*, hydrogen oxidation was not reported (Kim et al. 2012). Catalase positive or negative and oxidase positive. Both species are positive for hydrolysis of gelatin, and *Caenimonas koreensis* also hydrolyzes Tween 20 (Ryu et al. 2008). Both species oxidize thiosulfate to sulfate but are negative for glucose fermentation, indole production, arginine dihydrolase, and hydrolysis of aesculin, casein, Tween 80, starch, xanthine, hypoxanthine, CM-cellulose, and chitin (Ryu et al. 2008; Kim et al. 2012).

Both are positive for the assimilation of saccharose, 3-hydroxybutyrate, and proline, but negative for assimilation of D-mannose, adipate, phenylacetic acid, rhamnose, inositol, itaconate, sodium malonate, lactate, potassium-5-ketogluconate, salicin, and fucose (Kim et al. 2012).

Both species produce in API test systems alkaline phosphatase, leucine arylamidase, esterase (C4), esterase lipase (C8), and naphthol-AS-BI-phosphohydrolase. Neither species produces lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase (Ryu et al. 2008; Kim et al. 2012).

The major respiratory quinone is ubiquinone Q-8. Major fatty acids are summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH) and C16:0; hydroxy fatty acid is C10:0 3-OH.

The G+C content of genomic DNA is 62.7–68.7 mol% (Ryu et al. 2008; Kim et al. 2012).

Additional characters and differentiating features of *Caenimonas* species are shown in ➲ *Table 29.6*.

In addition, *Caenimonas koreensis* hydrolyzes urea and is negative for H_2S or acetoin production, lysine decarboxylase, ornithine decarboxylase, and assimilation of capric acid and weakly produces tryptophan deaminidase. It produces acid from raffinose, myo-inositol, D-lactose, L-arabinose, and D-fructose, however, not from D-glucose, sorbitol, sucrose, rhamnose, amygdalin, melibiose, D-galactose, D-mannose, D-mannitol, arbutin, or salicin (Ryu et al. 2008).

Caenimonas koreensis utilizes D-fructose, malonate, and maleate as sole carbon sources, but not glycerol, D-mannitol, or D- or L-tryptophan (Ryu et al. 2008). Sole carbon source utilizations are not reported for *Caenimonas terrae* (Kim et al. 2012).

Comamonas (ex Davis and Park **1962**) De Vos et al. (1985), 450[VP]

Co'ma.mo.nas. L. n. *coma*, hair (of the head), hair; L. fem. n. *monas*, a unit, monad; N.L. fem. n. *Comamonas*, cell with a polar tuft of flagella.

The genus *Comamonas* comprises 16 species originating from water, soil, activated sludge, and clinical samples. All are oxidase and catalase positive. Aerobic chemoorganotrophic metabolism.

Comamonas denitrificans is a facultative anaerobic organism capable of denitrification of nitrate to nitrogen. Five strains of this species possess a nitrite reductase containing hemes c and d (=NirS) (Gumaelius et al. 2001). *Comamonas nitrativorans* is also a facultative anaerobe capable of reducing nitrate to nitrogen (Etchebehere et al. 2001). *Comamonas guangdongensis* is able to use Fe(III) as an alternative terminal electron acceptor while using citrate, glycerol, glucose, or sucrose as the electron donor. It can transform 2,4-D to 4-chlorophenol, and under anaerobic conditions, Fe(III) reduction and 2,4-D degradation occur simultaneously (Wu et al. 2009).

Table 29.5
Characteristics of the species of *Brachymonas*

Characteristic	*Brachymonas denitrificans* JCM 9216^T	*Brachymonas chironomi* LMG 24400^T
References	Hiraishi et al. (1995)	Halpern et al. (2009)
Source	Activated sludge	Chironomid egg mass in a waste stabilization pond
Cell size	0.6–1 × 1.2–2 μm	0.75–1.2 × 1–3 μm
Morphology	Smooth, convex, opaque, with entire margin, cream, beige, or pale yellow	Beige to brown colored colonies are circular, smooth, convex, and opaque with entire margins
Metabolism	Aerobic or anaerobic chemoorganotrophic with nitrate	Aerobic chemoorganotrophic
Anaerobic growth with	Nitrate	–
Temperature range for growth (°C)	10–40	18–37
Optimal growth temperature (°C)	30–35	30
pH range	5–9	5–9
Optimal pH	7–7.5	6–8
Growth with 0–2 % NaCl	+	+
Growth with 3 % NaCl	+	–
Major fatty acids (>10 %)	C16:1ω7c, C16:0, C18:1ω7c	C16:1ω7c, C16:0, C18:1ω7c
Important hydroxy fatty acids (> = 1 %)	C16:0 3-OH	–
Quinones	Q-8, RQ-8	Q-8 (Q-7, Q-9)
Polar lipids[ab]	PEA, PG, PS, DPG, UPL1, UPL2, UAPL	PEA, PG, PS
DNA G+C content (mol%)	64.9	60
Utilization of		
Acetate, lactate	+	
Benzoate, butyrate, glutarate, glycolate, propionate	w	
Caproate, caprylate	–	
Citrate	–/+[b]	–
Formate	–	
Fumarate, pyruvate, succinate	+	
Gluconate	–/+[b]	–
Malate	w/+[b]	–
Malonate, pelargonate, tartrate	–	
Adipate	+	–
Adonitol, i-arabinose, cellobiose, D-fructose, glycerol, lactose	–	
Arabinose, maltose	+	–
Ethanol	+	
D-Glucose, mannitol	–/+[b]	–
D-Mannose	–/+[b]	
Methanol, sorbitol, sucrose, D-xylose	–	
Propylene glycol, D-ribose	w	
L-Alanine	+	
Aminobutyrate, L-arginine	–	
L-Asparagine, L-glutamate, L-leucine, L-phenylalanine, L-proline	+	
L-Glycine, L-histidine, L-lysine, i-ornithine, L-tryptophan	–	
N-Acetyl-glucosamine	+	–

Table 29.5 (continued)

Characteristic	*Brachymonas denitrificans* JCM 9216^T	*Brachymonas chironomi* LMG 24400^T
Indole production	–	–
Voges–Proskauer	–	
Phenylalanine deaminase	–	
Tryptophan deaminase	+	–
Hydrolysis of starch, alginate, chitin, casein, tributyrin, Tween 80	–	
Hydrolysis of gelatin	–	–
Acetoin production	–	+

[a]PEA phosphatidylethanolamine, PG phosphatidylglycerol, PS phosphatidylserine, UPL unknown phospholipid, UAPL unknown aminophospholipid
[b]Result according to Halpern et al. (2009)

Table 29.6
Characteristics of *Caenimonas* species

Characteristic	*Caenimonas koreensis* KCTC 12616^T	*Caenimonas terrae* KACC 13365^T
References	Ryu et al. (2008), Kim et al. (2012)	Kim et al. (2012)
Source	Activated sludge	Paddy soil
Shape	Rods	Curved rods
Cell size	0.4–0.6 × 0.8–2.0 μm	0.6–0.7 × 1.4–2.3 μm
Temperature range for growth (°C)	10–35	10–40
Optimal growth temperature	30	28–30
pH range for growth	6.0–9.0	5.0–8.0
Optimal pH	7.0–8.0	7.0
Major fatty acids (>10 %)	SF 3 (C16:1ω7c and/or iso-C15:0 2-OH), C16:0	C16:0, SF 3 (C16:1 ω7c and/or iso-C15:0 2-OH), C17:0 cyclo
Polar lipids[a]	PEA, PG, DPG, UAL	PEA, PG, DPG
Polyamines	2-Hydroxyputrescine, putrescine	2-Hydroxyputrescine, putrescine, spermidine
DNA G+C content (mol%)	62.7	68.7
Catalase	+	–
Nitrate reduction	+[a] or –[b]	–
Valine arylamidase	w[a] or –[b]	–
Acid phosphatase	w[a] or +[b]	–
Urease	+[a] or –[b]	–
Hydrolysis of tyrosine	–[a] or +[b]	–
Assimilation of		
N – Acetylglucosamine	–[a] or +[b]	+
Maltose, glucose, L-arabinose, ribose, sorbitol	–	+
Melibiose	+	–
Mannitol	–[a] or +[b]	–
Trisodium citrate, potassium-2-ketogluconate, malate, sodium acetate	–	+
Potassium gluconate	–[a] or +[b]	+
Glycogen	–	+
Propionate	+	–
Histidine	+	–

[a]Data from Ryu et al. (2008)
[b]Data from Kim et al. (2012)

In liquid culture, *Comamonas badia* grows in a flocculated state with cells embedded in a polysaccharide matrix comprising glucosamine, glucose, mannose, galactose, and rhamnose (Tago and Yokota 2004).

Comamonas kerstersii is able to grow at temperatures up to 44 °C (Wauters et al. 2003).

Comamonas thiooxidans is an aerobic heterotroph and is also able to grow mixotrophically with the oxidation of thiosulfate in mineral salts–thiosulfate medium supplemented with arabinose, citrate, succinate, pyruvate, acetate, glucose, or galactose (Pandey et al. 2009).

Comamonas aquatica, *Comamonas kerstersii*, *Comamonas koreensis*, *Comamonas odontotermitis*, *Comamonas terrae*, *Comamonas terrigena*, and *Comamonas testosteroni* are negative for lysine and ornithine decarboxylase. *Comamonas* species generally use very few sugars and prefer to grow on organic or amino acids, although *Comamonas koreensis* is reported to utilize glucose, tagatose, and mannitol and oxidize several other sugars in Biolog GN tests (Chang et al. 2002) and *Comamonas thiooxidans* utilizes glucose, galactose, and lactose (Narayan et al. 2010).

Additional characters and differentiating features of *Comamonas* species are shown in ❯ *Table 29.7*.

Curvibacter Ding and Yokota (2004), 2228[VP]

Cur.vi.bac'ter. L. adj. *curvus*, curved or crooked; N.L. masc. n. *bacter*, rod; N.L. masc. n. *curvibacter*, curved rod.

The genus *Curvibacter* comprises four species that occur in freshwater wells. All are aerobic chemoorganotrophs. *Curvibacter fontanus* is reported to grow better in microaerophilic conditions (Ding and Yokota 2010). Published descriptions for the well-water isolates are relatively limited and are summarized in ❯ *Table 29.8*.

Delftia Wen et al. (1999), 573[VP]

Delf.'tia. N.L. fem. n. *Delftia* referring to the city of Delft, the site of isolation of the type species, and in recognition of the pioneering role of Delft research groups in the development of bacteriology.

Strains of the genus *Delftia*, which currently comprises four species, are particularly versatile organotrophs, and the utilization of many different carbon sources has been reported in a number of studies (Palleroni 1984; Tamaoka et al. 1987; Willems et al. 1991c; Wen et al. 1999; Jørgensen et al. 2009; Shigematsu et al. 2003; Chen et al. 2012), with corroborating results and also with some contrasting results, probably as a consequence of differences in procedures. In the most recent new species description, Chen et al. (2012) have ensured comparability by including the four type strains under identical conditions in all tests. Therefore, these data have been included here. All four type strains are able to accumulate poly-β-hydroxybutyrate and are positive for oxidase, catalase, DNase, alkaline phosphatase, C8 esterase lipase, leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities; hydrolysis of Tweens 20, 40, and 60; nitrate reduction; and assimilation of glucose, mannitol, gluconate, adipate, malate, and phenylacetate. All four type strains are negative for C14 lipase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, and urease activities; hydrolysis of aesculin, starch, urea, gelatin, chitin, CM-cellulose, corn oil, and Tween 80; indole production; and glucose fermentation. All strains are sensitive to sulfamethoxazole plus trimethoprim, chloramphenicol, tetracycline, novobiocin, and nalidixic acid. Additional differentiating features of the species are shown in ❯ *Table 29.9*.

The genus description dates from the time when the type species was the only *Delftia* species (Wen et al. 1999). It describes the positive hydrolysis of Tween 80 (lipase) and absence of growth on glucose as genus characteristics. However, the youngest species, *Delftia litopeniae* is negative for the former and positive for the latter (Chen et al. 2012). The genus description contains a number of features that have not been reported for most other species of the genus: hydrolysis of acetamide (positive for *Delftia acidovorans* and *Delftia tsuruhatensis*), absence of denitrification (negative except for *Delftia litopeniae* for which no data are available), absence of autotrophic growth with H_2 (negative for *Delftia acidovorans*), negative levan formation from sucrose (negative for *Delftia acidovorans*), meta-cleavage of protocatechuate (positive for *Delftia acidovorans* and *Delftia tsuruhatensis*), the presence of Q-8 as main quinone (only reported for *Delftia acidovorans* and *Delftia litopeniae*), the presence of Q-7 and Q-9 as minor quinones, the absence of menaquinone, and the presence of putrescine and 2-hydroxyputrescine as main polyamines (only reported for *Delftia acidovorans*). The genus description also describes the motility as driven by polar or bipolar tufts of 1–5 flagella. Again this feature has not been reported for other species, although all are motile.

Diaphorobacter Khan and Hiraishi (2003), 936[VL]

Di.a.pho.ro.bac'ter. Gr. adj. *diaphoros*, different, advantageous, profitable; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Diaphorobacter*, distinguished and profitable rod, referring to usefulness in nitrogen removal.

Two *Diaphorobacter* species have been described, *Diaphorobacter nitroreducens* and *Diaphorobacter oryzae*, from activated sludge and paddy field sediments, respectively (Khan and Hiraishi 2002; Pham et al. 2009). Both species are aerobic rods and motile with one polar flagellum. Strains of *Diaphorobacter* are positive for oxidase, catalase, nitrate reduction, arginine dihydrolase, protease, and urease and utilization of acetate, propionate, 3-hydroxybutyrate, potassium gluconate, L-alanine, DL-lactate, valerate, L-proline, and malate. They do not produce acid from glucose and are negative for β-galactosidase and hydrolysis of starch, casein, and DNA and do not utilize citrate, malonate, and most sugars, although the type strain of *Diaphorobacter oryzae* is reported to use D-glucose (Pham et al. 2009). *Diaphorobacter nitroreducens* is able to degrade

Table 29.7

Characteristics of the species of *Comamonas*

Part 1								
Characteristic	*Comamonas aquatica* LMG 2370^T	*Comamonas badia* IAM 14839^T	*Comamonas composti* LMG 24008^T	*Comamonas denitrificans* ATCC 700936^T	*Comamonas granuli* KCTC 12199^T	*Comamonas guangdongensis* KACC 16241^T	*Comamonas jiangduensis* KACC 16697^T	*Comamonas kerstersii* LMG 3475^T
References	Wauters et al. (2003), Willems et al. (1991c), Chou et al. (2007)	Tago and Yokota (2004), Chou et al. (2007)	Young et al. (2008)	Gumaelius et al. (2001), Chou et al. (2007)	Kim et al. (2008)	Zhang et al. (2013a)	Sun et al. 2013	Wauters et al. (2003), Willems et al. (1991c), Chou et al. (2007)
Source	Environmental and clinical samples	Activated sludge	Food waste compost	Activated sludge	Granules from a wastewater treatment bioreactor	Subterranean forest sediment	Soil from rice field	Mostly clinical samples
Shape	Rods	Rods	Rods	Straight to slightly curves rods	Rods	Short rods	Short rods	Rods
Motility	Polar or bipolar tufts of flagella	One polar flagellum	Motile	Bipolar tufts of flagella	Motile	Peritrichous flagella	Peritrichous flagella	Polar or bipolar tufts of flagella
Cell size	0.3–0.5 × 1–4 μm	0.8–0.9 × 1–2.5 μm	0.5 × 1.0–2.0 μm	1–2 × 2–6 μm	0.5–0.7 × 1.2–4.0 μm	0.3–0,4 × 1.2–1.5 mm	0.3–0.4 × 1.2–1.5 mm	0.3–0.5 × 1–4 μm
Pigmentation	Nonpigmented	Pink colonies	Nonpigmented	Yellow-white colonies		Pale yellow	Pale yellow	
Metabolism	Aerobic, non-fermentative, chemoorganotrophic	Aerobic, chemoorganotrophic	Aerobic, facultative anaerobic on NA, capable of thiosulfate oxidation	Fac. anaerobic with denitrification to nitrogen	Aerobic, non-fermentative, chemoorganotrophic	Facultative anaerobic	Aerobic, heterotrophic	Aerobic, non-fermentative, chemoorganotrophic
Anaerobic growth		–		Using nitrate	-	Using Fe(III)		
Temperature (°C) at which growth is reported	28–30	20–30	20–35	20–37	20–42	30–32	4–42	30–42
Temperatures without growth (°C)	42	37		4	4		45	
pH range					6.0–8.5		6.5–9.5	
Optimal pH			6–8			6.5–7.0		
% NaCl that permits growth			0–4.5	0.02	0–1 %	0–1	0–3	
Nitrate reduction	+		+	+	+	–	–	+

Table 29.7 (continued)

Part 1								
Characteristic	*Comamonas aquatica* LMG 2370^T	*Comamonas badia* IAM 14839^T	*Comamonas composti* LMG 24008^T	*Comamonas denitrificans* ATCC 700936^T	*Comamonas granuli* KCTC 12199^T	*Comamonas guangdongensis* KACC 16241^T	*Comamonas jiangduensis* KACC 16697^T	*Comamonas kerstersii* LMG 3475^T
Major fatty acids (>10 %)	C16:1ω7c, C16:0, C18:1ω9c	C16:1ω7c, C16:0, C18:1ω7c	C16:0, C18:1ω7c, SF3 (C16:1ω7c/ C15:0 iso 2-OH)	C16:0, C18:1ω7c, SF3 (C16:1ω7c/ C15:0 iso 2-OH)	C16:0, C18:1ω7c, SF3 (C16:1ω7c/C15:0 iso 2-OH)	C16:0, SF3 (C16:1ω7c/ C16:1ω6c), SF8 (C18:1ω7c, C18:1ω6c), C17:0 cyclo	C16:0, SF3 (C16:1ω7c/ C16:1ω6c), SF8 (C18:1ω7c, C18:1ω6c)	C16:1ω7c, C16:0, C18:1ω9c
Important fatty acids (5–10 %)			C10:0 3-OH				C17:0 cyclo, C14:0	
Important hydroxy fatty acids (> = 1 %)	C10: 3-OH	C10:0 3-OH, C16:0 2-OH	C10:0 3-OH	C10: 3-OH	C10:0 3-OH	C10:0 3-OH, C16:0 2-OH, C16:1 2-OH	C10:0 3-OH	C10: 3-OH
DNA G+C content (mol%)	64	66.3	62.8	60.8	68.4	64.8	65.3	61
Quinones		Q8			Q8	Q8	Q8	
Reduction nitrate to nitrite	+		+	+	+	−	−	+
Reduction nitrate to nitrogen	−	−		+	−	−	−	−
Alkaline phosphatase	−	−	+	+		−	−	−
Esterase C4			+			−	+	
Esterase lipase C8	+	+	+	−		+	−	+
Lipase C14	−	−	+	−		−	−	−
Leucine arylamidase			+			+	+	
Valine arylamidase	−	+	+	+		−	−	+
Cysteine arylamidase	+	−	−	−		−	−	−
Acid phosphatase	+	−	+	+		−	−	+
Naphthol-AS-BI-phosphohydrolase			+			+	+	
Arginine dihydrolase	−		−		+	−	−	−
Urease	−		−		+	−	−	−

Hydrolysis aesculin			–		–	–	–	
Hydrolysis gelatin	–	–	–		–	–	–	–
PHB/PHA accumulation				+				
Use of								
Gluconate	+	+	+	–	–	–	–	–
Capric acid	–	–	–	–	–	–	–	–
Adipic acid	+	+	+	–	–	–	+	+
Malic acid	+	+	+	–	+	–	+	+
Trisodium citrate	–	+	–	–	–	–	–	–
Phenylacetate	–	+	–	–	–	–	–	–
Testosterone	–							–
β-Alanine								
L-Tryptophan				–				
L-Histidine				–				
Glycolate				+				
Suberate	+				–			+
L-Arginine				+				
2-Aminobenzoate, caprylate, pelargonate								
Benzoate								
L-Malonate				+				
L-Lysine				+				
Oxidation of (in Biolog GN2)								
Glycogen			+			–	–	
Tween 40	–	+	+	–		+	+	+
Tween 80	+	–	+	–		+	+	+
N-Acetyl-D-galactosamine	–	+	–	–		–	–	–
Acetate	+	–	+	–		+	+	+
cis-Aconitate	–	–	+	+		+	–	–
Citric acid			+			–	–	
Formic acid			+			+	+	
D-Galactonic acid lactone			–			–	–	

Table 29.7 (continued)

Part 1								
Characteristic	*Comamonas aquatica* LMG 2370^T	*Comamonas badia* IAM 14839^T	*Comamonas composti* LMG 24008^T	*Comamonas denitrificans* ATCC 700936^T	*Comamonas granuli* KCTC 12199^T	*Comamonas guangdongensis* KACC 16241^T	*Comamonas jiangduensis* KACC 16697^T	*Comamonas kerstersii* LMG 3475^T
D-Gluconic acid			–			–	–	
D-Glucosaminic acid			–			–	–	
3-Hydroxybutyrate			+			+	+	
4-Hydroxybutyrate	+	+	+	+		+	–	+
p-Hydroxyphenyl acetic acid			–			+	–	
Itaconate	+	–	+	–		–	+	+
D,L-Lactic acid, sebacic acid			+			+	+	
Propionate	+	+	+	–		+	+	–
Quinic acid			–			–	–	
D-Saccharic acid			–			–	–	
D-Alanine			–			+	–	
L-Alanine			–			+	–	
L-Asparagine			+			–	–	
L-Aspartic acid			–			–	–	
L-Glutamic acid			+			–	–	
Glycyl-L-aspartate	–	+	–	+		–	–	–
Glycyl-L-glutamate	–	+	–	–		–	–	–
Hydroxy-L-proline	–	–	+	–		–	–	–
L-Leucine			+			+	+	
L-Ornithine	–	+	–	–		–	–	–
L-Phenylalanine	–	–	–	–		+	–	+
L-Proline			–			+	+	
L-Pyroglutamic acid			+			+	–	
D-Serine	–	+	–	–		–	–	–
L-Serine	–	+	–	–		–	–	–
L-Threonine	–	–	–	+		+	+	+

Urocanic acid			+			+	–	
Glycerol			–			–	+	
D,L-α-glycerol phosphate			–			–	–	
Susceptibility to								
Ampicillin	S	R	S	S			R	R
Gentamicin	S	S	R	S			R	S
Penicillin G	S	S	S	S			R	S
Rifampicin	S	R	S	S			R	S
Streptomycin	S	R	R	S			R	S

Part 2								
Characteristic	*Comamonas koreensis* KCTC 12005^T	*Comamonas nitrativorans* DSM 13191^T	*Comamonas odontotermitis* LMG 23579^T	*Comamonas terrae* KCTC 22606^T	*Comamonas terrigena* LMG 1253^T	*Comamonas testosteroni* LMG 1786^T	*Comamonas thiooxidans* DSM 17888^T	*Comamonas zonglianii* DSM 22523^T
References	Chang et al. (2002), Chou et al. (2007)	Etchebehere et al. (2001), Chou et al. (2007)	Chou et al. (2007)	Chitpirom et al. (2012)	De Vos et al. (1985), Willems et al. (1991c), Chou et al. (2007)	Tamaoka et al. (1987), Willems et al. (1991c), Chou et al. (2007)	Narayan et al. (2010), Pandey et al. (2009)	Yu et al. (2011)
Source	Wetland sediment	Denitrifying reactor from a landfill leachate treatment system	Gut of termite *Odontotermes formosanus*	Arsenic-contaminated soil	Hay infusion	Soil	Sulfur spring sediment	Phenol-contaminated soil
Shape	Straight to slightly curved rods	Curved rods	Rods	Rods	Straight to slightly curved rods	Straight to slightly curved rods	Rods	Short rods
Motility	Nonmotile	Bipolar tufts of polar flagella	Motile	Polar flagellum	Polar or bipolar tufts of flagella	Motile	Motile	Nonmotile
Cell size	0,4–0.6 × 1–1.5 μm	0.8–2.5 μm	0.5–1 × 2–2.5 μm	Not reported	0.3–0.5 × 1–4 μm	0.5–0.7 × 1.1–4.4 μm	0.75–0.9 – 1–1.2 μm	0.6–0.8 × 0.9–1.5 μm
Pigmentation	Nonpigmented	Cream		Nonpigmented	Nonpigmented		Cream	Pale yellow
Metabolism	Non-ferm entative, aerobic, facultative anaerobic on nutrient agar	Aerobic, chemoorg anotrophic, facultatively anaerobic using nitrate	Aerobic, chemoorg anotrophic	Aerobic, chemoorg anotrophic	Aerobic, non-fermentative, chemoorg anotrophic	Aerobic, heterotrophic, capable of thiosulfate oxidation	Aerobic, heterotrophic, capable of mixotrophic growth using thiosulfate oxidation	Aerobic, chemoorg anotrophic
Anaerobic growth with	+	Nitrate	–		–			

Table 29.7 (continued)

Part 2								
Characteristic	*Comamonas koreensis* KCTC 12005^T	*Comamonas nitrativorans* DSM 13191^T	*Comamonas odontotermitis* LMG 23579^T	*Comamonas terrae* KCTC 22606^T	*Comamonas terrigena* LMG 1253^T	*Comamonas testosteroni* LMG 1786^T	*Comamonas thiooxidans* DSM 17888^T	*Comamonas zonglianii* DSM 22523^T
Temperature (°C) at which growth is reported	30–37	30	20–37	30–40	28–37	28–37	12–40	15–43
Temperatures without growth	4. 42			4	42	42		
pH range				5–11			6.0–8.5	6–0
Optimal pH	7	7	7 (6–9)					7–8
Reported growth with NaCl	0.03		0–2 %	0.045	0.5–1.5 %	0.5–1.5 %		0–1 %
Growth factors required					Methionine, nicotinamide	−		
Nitrate reduction	+	+	+	−	+	+	+	−
Major fatty acids (>10 %)	C16:1ω7c, C16:0, C17:0 cyclo	C16:0, C18:1ω7c, SF3 (C16:1ω7c/C15:0 iso 2-OH)	C16:0, C18:1ω7c, SF3 (C16:1ω7c/ C15:0 iso 2-OH)	C16:0, C17:0 cyclo, SF3 (C16:1ω7c/ C16:1ω6c), SF8 (C18:1ω7c, C18:1ω6c)	C16:1, C16:0, C18:1	C16:1, C16:0, C18:1	C16:0, C18:1ω7c, C16:1ω7c	C16:0, C17:0 cyclo, C18:1ω7c, SF3 (C16:1ω7c/C15:0 iso 2-OH)
Important fatty acids (5–10 %)	C15:0, C18:1		C17:0 cyclo					
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH, C16:0 2-OH	C10: 3-OH	C10:0 3-OH, C16:0 2-OH	C10:0 3-OH, C16:0 2-OH, C16:1 2-OH	C10:0 3-OH, C16:0 2-OH	C10:0 3-OH, C16:0 2-OH	C10:0 3-OH, C16:0 2-OH, C16:1 2-OH	C10:0 3-OH, C16:0 2-OH, C16:1 2-OH
DNA G+C content (mol%)	66	Not reported	61.6	69.6	64	62.5	61.4	65.2
Quinones	Q8	Q-8		Q8	Q8	Q8		
Reduction nitrate to nitrite	+	+	+	−	+	+		−
Reduction nitrate to nitrogen	−	+	−	−	−	−		−
Alkaline phosphatase	+	−	+		−	−		+
Esterase C4	−		+		+	+		+

Esterase lipase C8	+	+	+		+	+		+
Lipase C14	+	−	−		−	−		−
Leucine arylamidase	+		+		+			+
Valine arylamidase	+	+	+		+	+		+
Cysteine arylamidase	−	−	−		−	+		−
Acid phosphatase	+	+	+		+	+		+
Naphthol-AS-BI-phospho-hydrolase	+		+		+	+		+
Arginine dihydrolase	−		−	−	−	−		+
Urease	−		−	+	−	−	+	+
Hydrolysis aesculin			−	+	−	−		−
Hydrolysis gelatin	−		−	−	−	−	−	+
PHB/PHA accumulation			−		+	+		
Use of								
Gluconate	+	−	+	+	+	+	+	+
Capric acid	−	−	−	−	−	+		+
Adipic acid	+	+	−	+	+	+		+
Malic acid	+	+	+	+	−	+		−
Trisodium citrate	+	−	+	−	−	+		+
Phenylacetate	−	+	+	−	−	−		−
Testosterone					−	+		
β-Alanine					+	−		
L-Tryptophan					−	+	−	
L-Histidine	−				−	+		
Glycolate					−	+		
Suberate					+	+		
L-Arginine	−				−	−	+	
2-Aminobenzoate, caprylate, pelargonate					−	+		

Table 29.7 (continued)

Part 2								
Characteristic	*Comamonas koreensis* KCTC 12005^T	*Comamonas nitrativorans* DSM 13191^T	*Comamonas odontotermitis* LMG 23579^T	*Comamonas terrae* KCTC 22606^T	*Comamonas terrigena* LMG 1253^T	*Comamonas testosteroni* LMG 1786^T	*Comamonas thiooxidans* DSM 17888^T	*Comamonas zonglianii* DSM 22523^T
Benzoate		+			−	+		
L-Malonate		−			−	−		
L-Lysine					−	−	−	
Oxidation of (Biolog GN2)								
Glycogen	−		+					
Tween 40	+	+	+		−	+		+
Tween 80	+	+	+		−	+		+
N-Acetyl-D-galactosamine	−	+	−		−	−		
Acetate	+	+	+		−	+	+	+
cis-Aconitate	−	+	−		+	−		
Citric acid	−		+				+	+
Formic acid	−		+					
D-Galactonic acid lactone	+		−					+
D-Gluconic acid	+		+				+	+
D-Glucosaminic acid	−		−					+
3-Hydroxybutyrate	−		+					+
4-Hydroxybutyrate	+	−	+		+	−		+
p-Hydroxyphenyl acetic acid	−		−					+
Itaconate	− (+)[a]	−	−		−	−		−
D,L-Lactic acid, sebacic acid	−		+					+
Propionate	+ (−)[a]	+	+		+	+	−	+
Quinic acid	+		−					+
D-Saccharic acid	+		−					
D-Alanine	−		+					
L-Alanine	−		+				−	+
L-Asparagine	−		+				−	+
L-Aspartic acid	−		+				+	+

L-Glutamic acid	–		+				+	+
Glycyl-L-aspartate	–	–	+		–	+	–	-
Glycyl-L-glutamate	+	–	+		–	+	–	–
Hydroxy-L-proline	+	–	+		–	–		–
L-Leucine	–		+				+	+
L-Ornithine	–	–	+		–	–		
L-Phenylalanine	+	+	+		–	–		+
L-Proline	–		+				–	+
L-Pyroglutamic acid	–		+				–	+
D-Serine	+	–	–		–	–		
L-Serine	+	–	–		–	–	+	
L-Threonine	+	+	+		–	+	–	
Urocanic acid	–		–					+
Glycerol	–		–	–				+
D,L-α-Glycerol phosphate	–		–					+
Susceptibility to								
Ampicillin	R	S	R	R	S	R		R
Gentamicin	S	S	S	S	S	R		R
Penicillin G	R	S	R	R	S	R		R
Rifampicin	S	S	R		S	S		R
Streptomycin	S	S	S	S	S	R		R

Table 29.8
Characteristics of the type strains of *Curvibacter* species

Characteristic	*Curvibacter gracilis* IAM 15033^T	*Curvibacter delicatus* IAM 14955^T	*Curvibacter lanceolatus* IAM 14947^T	*Curvibacter fontanus* IAM 15072^T
References	Ding and Yokota (2004)	Ding and Yokota (2004), Hylemon et al. (1973)	Ding and Yokota (2004), Leifson (1962b)	Ding and Yokota (2004)
Source	Well water	Distilled water	Distilled water	Well water
Shape	Slightly curved rods	Slightly curved rods	Slightly curved rods	Spirilla to curved rods
Motility	Nonmotile	1–6 polar flagella	1 polar flagellum	ND
Cell size	0.5–1.4 × 1.1–2.8 mm	0.3 × 0.7 mm	0.6 × 1.8 mm	0.4–0.5 × 1.1–2.4 mm
Colonies	Yellow-brown	Yellow-brown	Yellow-brown	
Temperature range	9–40	9–40	9–40	
Optimal temperature for growth (°C)	25–30	30–32	20–30	25–30
pH range	5.0–8.0	5.5–8.5	Neutrophilic	
Optimal pH for growth	6–7			7
Major cellular fatty acids (>10 %)	C16:0, SF3 (iso-C15:0 2-OH/C16:1 ω7c), C18:1 ω7c	C16:0, SF3 (iso-C15:0 2-OH/C16:1 ω7c), C18:1 ω7c	C16:0, SF3 (iso-C15:0 2-OH/C16:1 ω7c), C18:1 ω7c	C16:0, SF3 (iso-C15:0 2-OH/C16:1 ω7c), C15:0
Important fatty acids (5–10 %)		C19:0		C18:1 ω7c, C17:0 CYCLO
Hydroxy fatty acids	C8:0 3-OH	C8:0 3-OH	C8:0 3-OH	C10:0 3-OH
Quinones	Q-8			Q-8
DNA G+C content (mol%) 66 62 66	66.2	62.2	66	66.6
Catalase	+	+	+	
Oxidase	+	+	+	
Phosphatase	+	+	+	
Growth with 3 % NaCl	–	–	–	
API 20 NE test:				
L-Arginine	+	–	–	–
Urea	+	–	+	+
Aesculin	–	+	–	+
Gelatin	–	+	–	+
p-Nitrophenyl β-D-galactopyranoside	–	w	–	
Glucose	w	w	–	+
D-Mannose	–	–	w	–
Mannitol	–	w	–	+
Maltose	–	w	–	+
Gluconate	+	–	–	+

poly-3-hydroxybutyrate and poly(3-hydroxybutyrate-co-hydroxyvalerate) under aerobic and anaerobic denitrifying conditions; it also breaks down 1-chloro-4-nitrobenzene (Khan and Hiraishi 2002). *Diaphorobacter oryzae* is reported to reduce nitrate, but it is not clear whether true anaerobic denitrification was tested (Pham et al. 2009). Major respiratory quinone is Q-8 (Khan and Hiraishi 2002; Pham et al. 2009).

Additional characters and differentiating features of the type strains of the two *Diaphorobacter* species are given in *Table 29.10*.

Extensimonas Zhang et al. (2013b). 2065^{VP}

Ex.ten.si.mo.'nas. L. part. *extensus*, extended; L. fem. n. *monas*, a unit, monad; N.L. fem. n. *Extensimonas*, extended unit.

Extensimonas with a single species, *Extensimonas vulgaris*, was created for a bacterium from industrial wastewater that grows at 15–50 °C (Zhang et al. 2013b).

Cells are short rods (0.8–0.9 × 1.3–1.9 μm) that elongate when grown at high temperatures, motile with a polar flagellum. Cells may contain polyphosphate granules. *Extensimonas vulgaris*

Table 29.9

Comparison of selected characters of the species of *Delftia*

Characteristic	*Delftia acidovorans* ATCC 15668^T	*Delftia lacustris* 32^T	*Delftia litopenaei* wsw-7^T	*Delftia tsuruhatensis* T7^T
Source	Soil, sediment, activated sludge, crude oil, oil brine, water, and various clinical samples[a]	Freshwater[b]	Freshwater shrimp culture pond	Activated sludge[c]
Growth pH				
Range	5.0–8.0	5.0–9.0	5.0–9.0	5.0–9.0
Optimum	6.0–7.0	6.0–7.0	6.0–7.0	7.0
NaCl concentration for growth (%)				
Range	0–4.0	0–5.0	0–3.0	0–4.0
Optimum	0–2.0	0–2.0	0–1.0	0–2.0
Optimal growth temperature (°C)	20–30	25–35	25–35	20–25 (35[c])
Temperature growth range (°C)	No growth at 4, nor at 41[a]	3–37[b]	4–40	10–40[c]
Nitrate reduction to nitrite	+	+	+	+
Hydrolysis of				
Peptidoglycan	+	+	−	+
Casein	−	−	−	+
Enzyme activities:				
C4 esterase	+	+	−	+
Valine arylamidase	−	+	−	+
α-Glucosidase	−	+	−	−
β-Glucosidase	−	+	−	−
Arginine dihydrolase	−	−	−	− (+[c])
Gelatinase	−	−	−	−
Assimilation of				
Glucose	−	−	+	−
Arabinose	+	+	−	+
Mannose	+	+	−	−
N-Acetylglucosamine	+	+	−	+
Maltose	−	−	−	+
Caprate	−	−	−	+
Citrate	+	+	−	+
Susceptibility to[d]				
Kanamycin	S	R	S	S
Penicillin G	S	R	S	R
Gentamicin	S	R	S	R
Streptomycin	S	R	S	R
Ampicillin	R	R	S	R
Rifampicin	S	R	R	R
Major fatty acids (>10 %)	C16:0, C18:1 ω7c, C17:0 cyclo, SF3 (C16:1 ω7c and/or C16:1 ω6c)	C16:0, C18:1ω7c, C17:0 cyclo, SF3 (C16:1 ω7c and/or C16:1 ω6c)	C16:0, C18:1 ω7c, SF3 (C16:1 ω7c and/or C16:1 ω6c)	C16:0, C18:1 ω7c, C17:0 cyclo, SF3 (C16:1 ω7c and/or C16:1 ω6c)

Table 29.9 (continued)

Characteristic	*Delftia acidovorans* ATCC 15668^T	*Delftia lacustris* 32^T	*Delftia litopenaei* wsw-7^T	*Delftia tsuruhatensis* T7^T
Important hydroxy fatty acids	C10:0 3-OH[e]	C10:0 3-OH	C10:0 3-OH	C10:0 3-OH
Important polar lipids[f]	PEA, PG, DPG, UAL, UPL1, UPL2 and UPL3	PEA, PG, DPG, UAL, UPL1 and UPL2	PEA, PG, DPG, UAL, UPL1, UPL2, UPL3, UPL4	PEA, PG, DPG, UAL, UPL1 and UPL2
DNA G+C content (mol%)	67[a]	65.3[b]	67.6	66.2[c]

Data taken from Chen et al. (2012) unless indicated otherwise
[a]Data taken from Wen et al. (1999)
[b]Data taken from Jørgensen et al. (2009)
[c]Data taken from Shigematsu et al. (2003)
[d]S Sensitive, R resistant
[e]Presence of 8:0 3-OH is reported by Wen et al. (1999) in the genus description, at a time when *D. acidovorans* was the only species in the genus. It was indeed reported in *Delftia acidovorans* cells grown on Columbia Blood Agar (Willems et al. 1989) but was not detected when this and other *Delftia* species were grown on R2A (Chen et al. 2012)
[f]Phosphatidylethanolamine (PEA), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), unidentified aminolipid (UAL), unidentified phospholipids (UPL1, UPL2, UPL3, UPL4)

Table 29.10
Characteristics of the two *Diaphorobacter* species

Characteristic	*Diaphorobacter nitroreducens* LMG 24467^T	*Diaphorobacter oryzae* JCM 11421^T
References	Khan and Hiraishi (2002)	Pham et al. (2009)
Source	Activated sludge from sewage treatment plant	Paddy field sediment
Cell size	0.7–0.9 × 1.0–1.8 mm	0.5–0.8 × 1.3–1.8 mm
Pigmentation	Crème to beige	
Metabolism	Aerobic, facultatively anaerobic with denitrification	Aerobic, heterotrophic
Anaerobic growth with	Nitrate	Not reported
Temperature range	20–40	7–35
Optimal temperature	28–35	
pH range	5–9	
Optimal pH	7–8	
Major fatty acids (>10 %)	C16:1ω7c, C16:0, C18:1ω7c	C16:0, C17:0 cyclo, SF4 (C16:1w7c/iso-C15:0 2-OH), SF7 (C18:1ω7c/ω9t/ω12t)
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH	C10:0 3-OH
DNA G+C content (mol%)	65.0	62.9
Aerobic assimilation of		
Adipate, suberate	+	−
2-Ketogluconate, caprate, histidine	−	+

grows at NaCl concentrations of 0–2 %, temperatures of 15–50 °C, and a pH range of 5.5–8.5. Optimal growth conditions were 0.5 % NaCl, 48 °C, and pH 7.0–7.5. Strictly aerobic; sodium thiosulfate, sodium sulfite, sodium sulfate, sodium nitrite, sodium nitrate, and $FeCl_3$ are not used as accessory electron acceptors. Heterotrophic metabolism. No growth factors required. The following substrates are used as sole carbon sources: alanine, glutamate, asparagine, succinate, citrate, malonate, fumarate, and salicylate; not used are formate, acetate, and DL-lactate. Sugars or alcohols are not used. The following complex substrates support growth: yeast extract, peptone, and casamino acids. Catalase, oxidase, and urease are positive. Using API ZYM test, the following enzymes were present: alkaline phosphatase, esterase C4, lipase C8, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphohydrolase, and naphthol-AS-BI-phosphohydrolase. Nitrate is reduced to nitrite; nitrite is not reduced. Hydrolysis of starch, casein, and gelatin is negative.

The major quinone is Q-8. Major fatty acids are C16:0; SF3 (C16:1ω7c/iso-C15:0 2-OH), C17:0 cyclo, and C18:1ω7c; C10:0 3-OH is the main hydroxy fatty acid, although several 2-hydroxy fatty acids are also present at more than 1 %: C16:0 2-OH, C16:1 2-OH, and C18:1 2-OH. Polar lipids include phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unknown aminoglycolipid, an unknown glycolipid, and two unknown phospholipids. The G+C content of the genomic DNA is 68.8 mol% (Zhang et al. 2013b).

Giesbergeria Grabovich et al. (2006), 571[VP]

Gies.ber.ger'i.a. N.L. fem. n. *Giesbergeria* named after the researcher G. Giesberger, who made a great contribution to the study of the physiology of heterotrophic spirilla.

Cells of *Giesbergeria* are spiral shaped and motile by means of bipolar tufts of flagella. They can accumulate poly-3-hydroxybutyrate and some also accumulate polyphosphate and elemental sulfur. Cells are aerobic, catalase and oxidase positive, neutrophilic and mesophilic, and unable to grow with 3 % NaCl or to hydrolyze casein or starch. Aerobic chemoorganotrophs that do not reduce nitrate to nitrate. Not able to use nitrates, sulfates, thiosulfate or fumarate as electron acceptors. No indole production. Unable to use sugars such as glucose, fructose, maltose, arabinose, xylose, or galactose.

Giesbergeria kuznetsovii and *Giesbergeria voronezhensis* can use ammonium salts, casein hydrolysate, yeast extract, peptone, aspartate, glutamate, and cysteine as nitrogen sources. For the other species, this information was not reported (Grabovich et al. 2006; Hylemon et al. 1973).

The major quinone is Q-8 (Grabovich et al. 2006).

Additional characteristics of the *Giesbergeria* species are shown in ❯ *Table 29.11*.

Hydrogenophaga Willems et al. (1989), 329[VP]

Hy.dro.ge.no'pha.go. N.L. n. hydrogenum (from Gr. n. hydoor, water; Gr. n. gennao, to create), hydrogen, that which produces water; Gr. v. phagein, to eat; N.L. fem. n. Hydrogenophaga, eater of hydrogen.

Hydrogenophaga strains are all rod shaped with cell sizes of 0.3–0.6 by 0.6–5.5 µm. They are motile by means of one or two polar to subpolar flagella, although flagellation was not reported for all species. Chemoorganotrophic, aerobic metabolism; some species are able to grow anaerobically using nitrate as terminal electron acceptor (❯ *Table 29.12*); some species are able to grow autotrophically using hydrogen, and some can oxidize thiosulfate (❯ *Table 29.12*). Some *Hydrogenophaga pseudoflava* strains, but not the type strain, are capable of N2 fixation (Jenni et al. 1989). The strains previously classified as "*Pseudomonas carboxydoflava*" are capable using CO for mixotrophic growth on organic substrates or for autotrophic growth with CO_2. For CO-autotrophic growth, the presence of molybdopterin cytosine dinucleotide is required as a cofactor (Meyer and Schlegel 1983; Volk et al. 1994). All have Q-8 as major quinone. *Hydrogenophaga* strains can use various carbon sources; all species can use lactate and 3-hydroxybutyrate (although this data is unavailable for *H. caeni*); all species can use gluconate except for *Hydrogenophaga caeni*. Additional differentiating features for the different species are shown in ❯ *Table 29.12*.

Hylemonella Spring et al. (2004), 104[VP]

Hy.le.mo.nel'la. N.L. fem. dim. n. *Hylemonella*, named after Philip B. Hylemon, who made important contributions to the taxonomy of freshwater spirilla.

Hylemonella cells are slender spirilla (diameter 0.2–0.3 µm, wavelength 2.8–3.5 µm, helix diameter 0.5–2.1 µm, length 3.5–14 µm) and motile with bipolar tufts of flagella. No internal storage of polyhydroxyalkanoates. Colonies are pinpoint sized and unpigmented. No growth at temperatures below 15 °C or above 42 °C. Metabolism is aerobic and chemoorganotrophic. No anaerobic growth with nitrate. No chemolithoautotrophic growth with hydrogen. No oxidation of thiosulfate to sulfate. Growth factors are required. Oxidase, urease, and catalase positive. Negative for hydrolysis of hippurate, casein, and starch. Uses only carbohydrates as carbon sources. Strains of *Hylemonella gracilis* (formerly *Aquaspirillum gracile*), the only species, were found not to utilize any of the sole carbon and nitrogen sources that were tested by Hylemon et al. (1973) and therefore are thought to have complex nutritional requirements. Acid is produced from D-glucose, D-galactose, and L-arabinose. *Hylemonella gracilis* can reduce nitrate to nitrite. Major respiratory quinone is Q-8. Major fatty acids are C16:1, C16:0, C18:0, C10:1, and C17:0, and hydroxyl fatty acids are C10:0 3-OH and C12:0 3-OH. Polyamines are 2-hydroxyputrescine and putrescine, and G+C content of DNA is 65 mol% (Spring et al. 2004; Hylemon et al. 1973; Sakane and Yokota 1994; Hamana et al. 1994).

Lampropedia Schroeter (1886), 151[AL]

Lam.pro.pe.di.a. Gr. adj. *lampros*, bright; Gr. neut. n. *pedion*, a plain; N.L. fem. n. *Lampropedia*, a shining flat sheet (of cells).

Lampropedia cells grow as square sheets of 16, 32, or 64 coccoid cells (1–2 µm), arising through the synchronous division of the cells in two planes and surrounded by an envelope layer. Occasionally cells occur in pairs or tetrads. The ability to grow with conspicuous tablet morphology is often lost in laboratory cultures. Requires biotin and thiamine for growth. Cells are unpigmented and do not carry flagella but can display twitching motility. Tolerant to 1.5 % NaCl; pH range 6–9; temperature range 10–40 °C. Poly-3-hydroxybutyrate granules are accumulated in the cells and some strains accumulate polyphosphate under cyclic aerobic–anaerobic conditions. Strictly aerobic chemoorganotrophic metabolism. No nitrate reduction. Oxidase and catalase are positive. The only species, *Lampropedia hyalina*, uses only intermediates of the tricarboxylic acid cycle as energy sources and ammonium salts and some

Table 29.11
Characteristics of the five *Giesbergeria* species

	Giesbergeria anulus	*Giesbergeria giesbergeri*	*Giesbergeria kuznetsovii*	*Giesbergeria sinuosa*	*Giesbergeria voronezhensis*
Reference	Grabovich et al. (2006), Hylemon et al. (1973)	Grabovich et al. (2006), Hylemon et al. (1973)	Grabovich et al. (2006)	Grabovich et al. (2006), Hylemon et al. (1973)	Grabovich et al. (2006)
Type strain	CIP 107877^T	DSM 9157^T	DSM 12827^T = D-412	DSM 11556^T	DSM 12825^T = D-419
Source	Pond water	Pond water	Sulfide spring	Pond water	Wastewater
Cell diameter (mm)	0.8–1.4	0.7–1.4	1.2–1.5	0.6–0.9	1.3–2.1
Helix wavelength (mm)	5.0–13.0	4.5–8.4	4.8–8.0	8.6–10.5	7.9–14.7
Helix diameter (mm)	1.7–4.5	1.2–5.0	1.9–4.0	1.4–3.5	2.9–6.8
Helix length (mm)	5.0–13.0	4.0–40.0	8.0–60.0	5.0–42.0	9.2–26.6
Elemental sulfur accumulated in the presence of sulfide			+		+
Major fatty acids (>10 %)	C16:1, C16:0	C16:1, C15:0 anteiso, C16:0, C18:1	C16:1, C16:0	C16:1, C16:0, C17:1, C18:1	C16:1, C16:0
Important fatty acids (5–10 %)	C18:1	C18:0	C18:1	C15:0 anteiso	C12:0, C14:0
Important hydroxy fatty acids (> = 1 %)					C10:0 3-OH
Temperature range (°C)	3–36	8–36	7–36	9–37	7–36
Optimal temperature (°C)			28		30
pH range			6.0–8.5		6.0–9.0
G+C content (mol%)	58–59	57–58	56.5	57–59	57.8–60
Urease	–	+	+	+	+
Assimilation of					
Acetate, lactate, fumarate, succinate	+	v	+	+	+
Aconitate, asparagine, glycine, histidine, isocitrate, leucine, methionine, proline, tryptophan, tyrosine	–	–	+	–	v
Alanine, arginine, cysteine, glutamine, glycerol, propanol, lysine, ornithine, phenylalanine, serine, valine, butanol, ethanol	–	–	+	–	–
Aspartate, 2-oxaloglutarate	–	–	+	–	+
Caproate, propionate	–	–	+	–	
Citrate	–	–	–	–	v
Glutamate	+	–	+	–	
3-Hydroxybutyrate	–	–	–	–	
Malate, pyruvate	v	v	+	+	+
Oxaloacetate	–	–	+	+	+
H_2S production from cysteine	+	v	+	+	+

Table 29.12

Differentiating features of the nine *Hydrogenophaga* species

	Hydrogenophaga atypica DSM 15342^T	*Hydrogenophaga bisanensis* CCUG 54518^T	*Hydrogenophaga caeni* DSM 17962^T	*Hydrogenophaga defluvii* DSM 15341^T	*Hydrogenophaga flava* DSM 619^T	*Hydrogenophaga intermedia* DSM 5680^T	*Hydrogenophaga palleronii* LMG 2366^T	*Hydrogenophaga pseudoflava* LMG 5945^T	*Hydrogenophaga taeniospiralis* LMG 7170^T
References	Kämpfer et al. (2005)	Yoon et al. (2008)	Chung et al. (2007)	Kämpfer et al. (2005)	Jenni et al. (1989), Lalucat et al. (1982), Palleroni (1984), Willems et al. (1989)	Contzen et al. (2000)	Jenni et al. (1989), Lalucat et al. (1982), Palleroni (1984), Willems et al. (1989)	Jenni et al. (1989), Lalucat et al. (1982), Palleroni (1984), Willems et al. (1989)	Jenni et al. (1989), Lalucat et al. (1982), Palleroni (1984), Willems et al. (1989)
Source	Activated sludge	Wastewater of a textile dye works	Activated sludge	Activated sludge	Mud from ditch	Wastewater	Water	Water river	Soil
Optimum temperature (°C)	25	30–37	30	25	30	30	30	35–38	33 or 37
Growth at 41 °C	–	+	–	–	–	w	–	+	
Yellow pigmentation	+	+	–	+	+	+	+	+	+
Denitrification	–	+	+	–	–	–	–	+	+
Nitrate reduction	+	+	+	+	+	+	–	+	+
Nitrite reduction		–	–		–		–	+	+
Autotrophic growth with H2	–	–	–	+	+	–	+	+	+
Oxidation of thiosulfate to sulfate	–	+	+	–	–	+	+	–	–
Hydrolysis of									
Aesculin		–	–		+			+	–
Gelatin		–	–		–		–	–	+
Tween 80		–	+		–		+	+	+
Utilization/ assimilation of									
L-Arabinose, sucrose, D-galactose, D-mannose, cellobiose	–	–	–	–	+	–	+	+	–
D-Fructose	–	+	–	–	+	–	+	+	–
Sorbitol	–	–	+	–	+	–	+	+	–
Mannitol	–	–	–	–	+	+	–	+	+
Maltose	–	–	+	–	+	–	–	+	–
D-Xylose	–	–		–	–	–	–	+	+
L-Histidine	–		+	+	+	–	–	+	–
2-Ketoglutarate	+			–	v	+	v	v	+

Table 29.12 (continued)

	Hydrogenophaga atypica DSM 15342^T	*Hydrogenophaga bisanensis* CCUG 54518^T	*Hydrogenophaga caeni* DSM 17962^T	*Hydrogenophaga defluvii* DSM 15341^T	*Hydrogenophaga flava* DSM 619^T	*Hydrogenophaga intermedia* DSM 5680^T	*Hydrogenophaga palleronii* LMG 2366^T	*Hydrogenophaga pseudoflava* LMG 5945^T	*Hydrogenophaga taeniospiralis* LMG 7170^T
Major fatty acids (>10 %)	C15:1w6c, C17:0 cyclo, SF3 (C16:1ω7c and/or iso-C15:0 2-OH)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), C18:1ω7c	C16:0, SF3 SF3 (C16:1ω7c and/or iso-C15:0 2-OH)	C15:0, C15:1w6c, C17:0 cyclo, SF3 (C16:1ω7c and/or iso-C15:0 2-OH)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), SF6 (C18:1ω7c, C18:1ω9t and/or C18:1ω12t)	C15:0, C16:0, C17:0, C17:0 cyclo	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), SF6 (C18:1ω7c, C18:1ω9t and/or C18:1ω12t)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), SF6 (C18:1ω7c, C18:1ω9t and/or C18:1ω12t)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), SF6 (C18:1ω7c, C18:1ω9t and/or C18:1ω12t)
Important fatty acids (5–10 %)	C15:0, C17:0, SF6 (C18:1ω7c, C18:1ω9t and/or C18:1ω12t)		C15:0	C16:0, C17:0, SF6 (C18:1ω7c, C18:1ω9t and/or C18:1ω12t)		SF3 (C16:1ω7c and/or iso-C15:0 2-OH), SF6 (C18:1ω7c, C18:1ω9t and/or C18:1ω12t)	C17:0 cyclo		
Important hydroxy fatty acids (> = 1 %)	C8:0 3-OH		C8:0 3-OH, C10:0 3-OH	C8:0 3-OH, C9:0 3-OH	C8:0 3-OH, C10:0 3-OH	C8:0 3-OH, C9:0 3-OH	C8:0 3-OH	C10:0 3-OH	
G+C content (mol%)	64	64.8		65	66.7	68.6	67.3	66.4	64.8

Fatty acid data taken from Yoon et al. (2008), Kämpfer et al. (2005). Cells were grown on YPG agar (Contzen et al. 2000) at 28 °C for 48 h. For *H. caeni* data are for cells grown on R2A (no data on YPG were available) (Chung et al. 2007)

w, weak response; v, varies in different reports; ND, no data

amino acids as sole nitrogen source (Lee et al. 2004; Murray 2005). It is able to grow on Green Top Agar medium, LB medium, and R2A medium and is positive for assimilation of acetate, DL-3-hydroxybutyrate, and propionate (Lee et al. 2004).

DNA G+C content is 63–67 mol%, and major fatty acids are C14:0, C16:0, and C18:1ω7c, and important hydroxyl fatty acids are C10:0 3-OH and C12:0 3-OH (Lee et al. 2004).

Limnohabitans Hahn et al. (2010a), 1361[VP]

Lim.no.ha'bi.tans. Gr. n. *limne*, lake; L. part. adj. *habitans*, inhabiting; N.L. part. adj. used as a masc. n. *Limnohabitans*, lake dweller, referring to the type of ecosystem from which the type species was first isolated.

Limnohabitans are nonmotile, straight, or curved rods that are found in the water column in freshwater habitats where they can be very abundant. No gas vesicles have been reported. Aerobic, facultatively anaerobic on nutrient broth, soytone, and yeast extract (NSY) medium (Hahn et al. 2004). Chemoorganotrophic; no data available on nitrate reduction, denitrification, or accumulation of storage materials. Nonpigmented and oxidase and catalase positive or negative (species dependent). Not halotolerant. Metabolic capabilities of some members of the genus may be more diverse: two *Limnohabitans* strains not belonging to the current species (Kasalicky 2012) were recently shown to possess a photosynthesis gene cluster, RuBisCO, CO dehydrogenase, ammonia monooxygenase, and sulfur-oxidizing genes (Zeng et al. 2012).

Major quinone is Q-8. Major fatty acids are C16:0 and C16:1ω7c/ω6c; also present though in lower amounts are C12:0 and C18:1ω7c/ω6c; main hydroxy fatty acid is C8:0 3-OH. DNA G+C content is 55–50 mol% (Hahn et al. 2010a, b; Kasalicky et al. 2010).

Differentiating features of the four *Limnohabitans* species are given in ❯ *Table 29.13*. It should be noted that to overcome inefficient growth on media with a single carbon source, the utilization of substrates was tested in one-tenth strength NSY medium which contains nutrient broth, soy peptone, and yeast extract (Kasalicky et al. 2010; Hahn et al. 2004).

Macromonas Utermöhl and Koppe in Koppe (1924), 632[AL]

Ma.cro.mo'nas. Gr. adj. *makros*, large; Gr. fem. n. *monas*, a unit, monad; N.L. fem. n. *Macromonas*, a large monad.

Macromonas cells are large, irregular pear- or bean shaped, cylindrical, or slightly bent. They are characterized by large inclusions of calcium oxalate. In addition, sulfur granules may be present. Motile by means of a polar tuft of flagella. Two species have been described: *Macromonas mobilis*, the type species, and *Macromonas bipunctata*. No pure cultures are available of the type species, *Macromonas mobilis*, whereas axenic strains are available of the second species, *Macromonas bipunctata*.

Both species have similar cells that differ notably in size with the type species being the larger: cells of *Macromonas mobilis* are 6–14 × 10–30 µm and cells of *Macromonas bipunctata* are 2.2–4 × 3.3–6.5 µm. Cells carrying more inclusions move more slowly.

In *Macromonas bipunctata*, in addition to sulfur and calcium oxalate granules, also calcium polyphosphate and poly-3-hydroxybutyrate have been reported, the type and amount of inclusions depending on the growth conditions. *Macromonas bipunctata* was isolated from the sediment of an aeration tank in a sewage treatment plant. It is an aerobic chemoorganotroph that prefers moderate concentrations of growth substrates (5–10 mM). Carbon metabolism comprises the tricarboxylic acid cycle and glyoxylate cycle. Many organic acids are good substrates. They include acetate, benzoate, fumarate, malate, oxaloacetate, and succinate but not citrate, isocitrate, aconitate, malonate, and glycolate. Sugars, most alcohols, and amino acids are not utilized. Ammonium, peptone, casein hydrolysate, and glutamate, but not nitrate and nitrite, can be used as sole nitrogen sources. *Macromonas bipunctata* can oxidize sulfide to sulfur and thiosulfate to tetrathionate by means of hydrogen peroxide; however, these processes do not provide useful energy. Vitamins are required for growth and the addition of sulfides, thiosulfate, or catalase improves growth and survival of cultures. Optimal temperature is 28 °C and optimal pH range is 7.5–8.2. No anaerobic growth with nitrate, sulfate, thiosulfate, or fumarate as terminal electron acceptors (Dubinina et al. 2005).

Malikia Spring et al. (2005), 627[VP]

Ma.li'ki.a. N.L. fem. n. *Malikia*, named after Kuhrsheed A. Malik, for his contributions to our knowledge of the cultivation and taxonomy of hydrogen-oxidizing and polyhydroxyalkanoate-accumulating proteobacteria.

Malikia was proposed as a new genus for a strain isolated from activated sludge which was named the type species, *Malikia granosa*. In the same study, the *Pseudomonas spinosa* was shown to represent a second species in the same genus and was thus renamed as *Malikia spinosa* (Spring et al. 2005).

Straight to slightly curved rods and motile by means of one to three polar flagella. In nutrient-rich media, filamentous cells of up to 50 µm length may occur. Under nitrogen-limited conditions, cells grown on acetate (*Malikia granosa*) or maltose (*Malikia spinosa*) accumulate granules of polyhydroxyalkanoates (PHAs). Under carbon-limited conditions, PHA granules are degraded and polyphosphate granules accumulate. Nonpigmented. Strictly aerobic chemoorganotrophic. Unable to grow anaerobically or phototrophically. Oligotrophic. A variety of substrates (tested at concentrations of 0.1 %) can be used by *Malikia granosa*; *Malikia spinosa* is more restricted in its food sources. Both species can utilize D-glucose, but not D-fructose, D-mannitol, formate, or glycerol. Oligonitrophilic: able to

Table 29.13
Differentiating features of the four *Limnohabitans* species

Character	*Limnohabitans australis* DSM 21646^T	*Limnohabitans curvus* DSM 21645^T	*Limnohabitans parvus* DSM 21592^T	*Limnohabitans planctonicus* DSM 21594^T
References	Hahn et al. (2010b)	Hahn et al. (2010a, b)	Hahn et al. (2010b), Kasalicky et al. (2010)	Hahn et al. (2010b), Kasalicky et al. (2010)
Source	Freshwater pond	Freshwater lake	Freshwater reservoir	Freshwater reservoir
Shape	Curved rods	Curved rods	Short rods	Rods
Size (mm)	0.4–0.5 × 1.0–1.7	0.4–0.5 × 1.0–1.75	0.3 × 0.6	0.3 × 0.9
Temperature range (°C)	12–36	4–34	4–34	4–34
Maximum NaCl concentration (%)	0.2	0.5	0.5	0.5
Catalase	–	+	+	+
Major fatty acids (>10 %)	C16:1ω7c/ω6c	C16:0 and C16:1ω7c/ω6c	C16:0 and C16:1ω7c/ω6c	C16:0 and C16:1ω7c/ω6c
Important fatty acids (5–10 %)	C12:0, C16:0		C18:1ω7c/ω6c	C18:1ω7c/ω6c
Important hydroxy fatty acids (> = 1 %)	C8:0 3-OH	C8:0 3-OH	C8:0 3-OH, C12:0 3-OH	C10:0 3-OH, C12:0 3-OH
DNA G+C content (mol%)	55.8	55.5	59.9	59.9
Utilization of				
Ethanol	–	w	–	w
Glycerol, L-glutamine	–	–	w	+
Glyoxylate	w	–	–	w
Glycolate	–	–	–	w
Acetate	+	+	–	+
Propionate, D-ribose, D-galactose	–	w	–	–
Pyruvate, succinate	+	+	w	+
DL-Malate, citrate	–	+	+	+
Malonate	w	–	–	–
Oxaloacetate, L-histidine, L-phenylalanine, L-serine	–	–	–	+
Fumarate	w	+	w	+
L-Glutamate, L-proline	–	–	+	+
L-Tryptophan	–	–	+	w
D-Glucose	w	+	+	+
D-Mannose	–	+	w	+
Sucrose	–	–	–	–
D-Gluconate	+	+	–	–

grow with 0.1 % yeast extract as sole bound nitrogen source. Unable to grow autotrophically using hydrogen or thiosulfate. Oxidase and catalase positive. Nitrate is reduced to nitrite. Growth factors are required. Both species can utilize adipate, gluconate, L-malate, D-mannitol, D-glucose, and sucrose; neither can use citrate, glycolate, glyoxylate, malonate, oxaloacetate, 2-oxoglutarate, succinate, adonitol, glycerol, arabinose, D-galactose, D-fructose, lactose, D-glutamate, or L-histidine (Spring et al. 2005). Additional substrate utilization patterns and differentiating features of both species are shown in *Table 29.14*.

Major respiratory quinone is Q-8, and important fatty acids are C16:0, C16:1w7c, and C18:1ω7c, and main hydroxyl fatty acid is C8:0 3-OH. The DNA G+C content is 66–67 mol% (Spring et al. 2005).

Ottowia Spring et al. (2004), 103^{VP}

Ot.to'wi.a. N.L. fem. n. *Ottowia*, named after Johannes C. G. Ottow, who made several interesting contributions to our knowledge of denitrification in soil and activated sludge.

Table 29.14
Differentiating features of *Malikia granosa* and *Malikia spinosa*

Character	*Malikia granosa* DSM 15619^T	*Malikia spinosa* DSM 15801^T
Source	Activated sludge	River water
Cell size	1.3 × 3.5–6 μm	1.1 × 3–6 μm
Optimal growth temperature (°C)	35	32
Optimal pH	6.5–7.0	7.5–8
Maximum growth temperature (°C)	40	36
Maximum NaCl concentration (%)	0.5	1
Utilization of		
Acetate, benzoate, DL-lactate, propionate, pyruvate, D-mannose, L-proline	+	–
Fumarate, D-maltose	–	+
%G+C (mol%)	67	66

Data from Spring et al. (2005)

Table 29.15
Differentiating features of both *Ottowia* species

	Ottowia thiooxydans DSM 14619^T	*Ottowia pentelensis* DSM 21699^T
References	Spring et al. (2004)	Felföldi et al. (2011)
Source	Activated sludge, municipal water treatment plant	Activated sludge for coke plant effluent
Shape	Straight to slightly curved rods	Short rods
Size	0.8 × 2–6 mm	0.5–0.7 × 1.0–1.7 mm
Pigmentation	Pale yellow	Beige
Denitrification	+	–
Anaerobic growth with nitrate	+	–
Reduction of nitrate to nitrite	+	–
Reduction of nitrate to nitrous oxide	+	
Major fatty acids (>10 %)	C16:0, C16:1ω7c, C18:1ω7c	C16:0, C16:1ω7c, C18:1ω7c, cyclo C17:0
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH, C12:0 2-OH	C10:0 3-OH
Temperature range (°C)	2–29	4–37
Optimal temperature (°C)	25–27	20–28
pH range	5.6–9.8	5–12
G+C content (mol%)	59.3	68.5
Utilization of		
Fumarate	+	–
Gluconate	–	+
DL-Lactate	+	–
Adipic acid	–	+
Citrate	–	+

Ottowia cells are nonmotile straight to slightly curved rods that accumulate poly-3-hydroxybutyrate internally. They are aerobic chemoorganotrophs and can convert thiosulfate to sulfate to grow mixotrophically but not autotrophically. No autotrophic growth with hydrogen. *Ottowia thiooxydans*, but not *Ottowia pentelensis*, is able to denitrify reducing nitrate and nitrite to nitrous oxide. Growth factors are required for *Ottowia thiooxydans*, but this information is unavailable for *O. pentelensis*. Oxidase and catalase are positive. Substrate utilization is restricted to some organic acids including malic acid and phenyl acetic acid but not malonate; most sugars and sugar alcohols are not utilized. Major respiratory quinone is Q-8 (Spring et al. 2004; Felföldi et al. 2011). Additional and differentiating features of the two species are shown in *Table 29.15*.

Polaromonas Irgens et al. (1996), 825^{VP}

Po.lar.o.mo'nas. N.L. adj. *polaris* (from L. n. *polus*, the end of an axis, a pole; and L. suff. *-aris*, suffix denoting pertaining to), referring to the geographic poles; L. fem. n. *monas*, a monad, unit; N.L. fem. n. *Polaromonas*, polar bacterium.

Polaromonas was created by Irgens et al. (1996) for gas-vacuolated bacteria from Antarctic marine water samples, with initially just one species, *Polaromonas vacuolata*. Since 2004, a further six species have been described, most but not all capable of growing at 1 °C.

Polaromonas comprises coccoid to rod-shaped cells and nonmotile or motile cells. Many, though not all, species are psychrotrophic and can grow at 1 °C. Aerobic chemoorganotrophic metabolism. Anaerobic growth was not detected in tow of the species and was not tested in other species. Two of the species are able to grow autotrophically with hydrogen (Sizova and Panikov 2007), and one species is able to grow with naphthene as sole carbon and energy source (Jeon et al. 2004). Oxidase and catalase are positive. All species are able to utilize Tween 40 and Tween 80, except for the type species *Polaromonas vacuolata*, for which this information is not documented.

Additional features and differentiating characters of the species are shown in *Table 29.16*.

Major quinone is Q-8; major fatty acids are C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), and C18:1ω7c, and important fatty acids are C8:0 3-OH and C10:0 3-OH, although one of both may be absent from some species. The G+C content of genomic DNA is 52–64 mol%.

Table 29.16
Characteristics of the *Polaromonas* species

Character	*Polaromonas glacialis* DSM 24062^T	*Polaromonas cryoconiti* DSM 24278^T	*Polaromonas naphthalenivorans* DSM 15660^T	*Polaromonas aquatica* CCUG 39402^T	*Polaromonas jejuensis* KACC 12508^T	*Polaromonas hydrogenivorans* KACC 12646^T	*Polaromonas vacuolata* ATCC 51984^T
References	Margesin et al. (2012)	Margesin et al. (2012)	Jeon et al. (2004), Margesin et al. (2012)	Kämpfer et al. (2006), Margesin et al. (2012)	Weon et al. (2008), Margesin et al. (2012)	Sizova and Panikov (2007), Margesin et al. (2012)	Irgens et al. (1996), Kämpfer et al. (2006)
Source	Glacier cryoconite	Glacier cryoconite	Coal-tar contaminated sediment	Tap water	Soil	Forest soil Alaska	Antarctic ocean
Shape	Coccoid rods	Small oval-shaped rods	Cocci	Rods	Rods	Cocci	Cigar-shaped rods
Size	0.6–0.8 × 0.8–1.0 μm	0.7–0.9 × 1.1–1.4 μm	1–4 μm diameter	1–2 μm length	0.6 × 1–3 μm	0.8–2.8 μm diameter	0.8 × 2–3 μm
Motility	–	–	–	+	–	–	Polar flagellum
Gas vesicles			–	–		–	+
Pigmentation colonies	Creamy white	Creamy white	Beige	Beige	Pale yellow	White or beige	White
Cell inclusions			PHA granules, poly-P, polyglucose				
Metabolism	Aerobic chemoorganotrophic	Aerobic chemoorganotrophic	Aerobic chemoorganotrophic, facultatively autotrophic with hydrogen	Aerobic chemoorganotrophic	Aerobic chemoorganotrophic	Aerobic chemoorganotrophic, facultatively autotrophic with hydrogen	Aerobic chemoorganotrophic
Autotrophic growth with hydrogen			+			+	
Anaerobic growth			–		–		
Urease			–	–	–	–	+
Growth factors required							Amino acids
Slime production on R2A agar	+	–	+	–	–	+	
Growth at 1 °C	+	+	+	–	–	+	+
Growth at 30 °C	–	–	–	+	+	–	–

Optimal growth temperature (°C)		1–10	20	25–30	28–30	15–20	4
Maximum growth temperature (°C)		25	25			25	15
Growth pH 8	–	–	+	+	+	w	
Growth with 1 % NaCl	+	–	w	w	w	w	+
NaCl range for growth					0–1 %		0–6 %
Nitrate reduction	+	+	+	–	–	+	
Alkaline phosphatase	w	+	–	+	+	+	
Lipase (C14)	w	+	–	w	+	w	
α-Galactosidase	–	–	w	–	–	–	
β-Galactosidase	–	–		–	+	–	
Valine arylamidase	w	w	–	w	+	+	
Amylase	w	–	–	w	w	–	
Assimilation of							
D-Glucose	–	–	+	–	–	+	+
Sucrose	–	–	+	–	–	+	–
Potassium gluconate	–	w	–	–	+	–	
Adipic acid	–	w	–	+	–	–	
Malic acid	–	w	–	+	–	–	+
Trisodium citrate	–	–	–	–	+	–	+
Phenylacetic acid	–	–	–	–	+	–	
Lactic acid			–	+	+	+	+
L-Alanine			–	–	+		+
D-Sorbitol	–	–			–	+	+
Propionic acid			–	–	+		+
3-Hydroxybutyrate			–	–	+	+	+
Utilization of naphthene as sole carbon and energy source	–	–	+	–	–	–	

Table 29.16 (continued)

Character	*Polaromonas glacialis* DSM 24062^T	*Polaromonas cryoconiti* DSM 24278^T	*Polaromonas naphthalenivorans* DSM 15660^T	*Polaromonas aquatica* CCUG 39402^T	*Polaromonas jejuensis* KACC 12508^T	*Polaromonas hydrogenivorans* KACC 12646^T	*Polaromonas vacuolata* ATCC 51984^T
Major fatty acids (>10 %)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), C18:1w7c	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), C18:1w7c, C17:0 cyclo	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), C17:0 cyclo	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH)
Important fatty acids (5–10 %)					C18:1w7c	C18:1w7c	C18:1w7c
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH	C10:0 3-OH, C12:0 3-OH	C10:0 3-OH	C8:0 3-OH	C8:0 3-OH	C10:0 3-OH	C8:0 3-OH
%G+C (mol%)	61.3	60.7	61.5		63.7	62.5	52
Polar lipids[a]	PG, DPG, PEA, UAL, 3 UPL, 5 UPoL	PG, DPG, PEA, 3 UAL, UPoL	PEAS, PG, DPG	PEA, DPG, PG, PS, 6 UPoL, 3 UPL, 3 UAL			
Polyamines	Putrescine, 2-hydroxyputrescine	Putrescine, 2-hydroxyputrescine		Putrescine, 2-hydroxyputrescine			

[a]Phosphatidylethanolamine (PEA), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), unidentified aminolipid (UAL), unidentified phospholipid (UPL), unidentified polar lipids (UPoL)

Pseudacidovorax Kämpfer et al. (2008), 493[VP]

Pseu.da.ci.do.vo'rax. Gr. adj. *pseudes*, false; N.L. masc. n. *Acidovorax*, a bacterial genus name; N.L. masc. n. *Pseudacidovorax*, the false *Acidovorax*.

Cells are short rods and motile by means of a polar flagellum. Aerobic or facultatively anaerobic chemoorganotrophic metabolism. The type and only species, *Pseudacidovorax intermedius*, is able to grow at 15–36 °C, not at 10 or 45 °C, on several complex media such as nutrient agar, tryptic soy agar, and R2A where it forms beige-pigmented colonies. Oxidase and catalase are positive. Unable to denitrify, reduce nitrate, or grow autotrophically with hydrogen. Capable of fixing nitrogen; the *nif* H gene was most similar to that of *Burkholderia vietnamiensis* and *Pelomonas saccharophila* and thus grouped with other nitrogen-fixing Betaproteobacteria.

Pseudacidovorax intermedius is able to utilize several organic acids and few sugars including acetate, propionate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-alanine, L-aspartate, L-proline, *cis*- or *trans*-aconitate (weak), 4-aminobutyrate (weak), citrate (weak), L-arabinose (weak), D-glucose (weak), and D-fructose (weak). It is unable to use *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *p*-arbutin, D-cellobiose, D-gluconate, maltose, D-mannose, melibiose, L-rhamnose, D-ribose, sucrose, salicin, trehalose, D-xylose, adonitol, *i*-inositol, maltitol, D-mannitol, sorbitol, putrescine, adipate, itaconate, mesaconate, 2-ketoglutarate, suberate, β-alanine, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate, and phenylacetate.

Major fatty acids (>10 %) are C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), and C18:1ω7c; another important fatty acid (5–10 %) is C17:0 cyclo; main hydroxy fatty acids (> = 1 %) are C8:0 3-OH, C10:0 3-OH, C16:1 2-OH, and C18:1 2-OH. DNA G+C content is 70.1 mol% (Kämpfer et al. 2008).

Pseudorhodoferax Bruland et al. (2009), 2706[VP]

Pseu.do.rho.do.fer'ax. Gr. adj. *pseudes*, false; N.L. masc. n. *Rhodoferax*, a bacterial genus name; N.L. masc. n. *Pseudorhodoferax*, the false *Rhodoferax*.

At present the three species in this genus each consist of only a type strain. Colonies are circular and convex; those of *Pseudorhodoferax soli* TBEA3[T] are white, those of *Pseudorhodoferax caeni* SB1[T] are white to light yellow, and those of *Pseudorhodoferax aquiterrae* NAFc-7[T] are light yellow. All three strains are aerobic chemoorganotrophs that grow on complex media within 1–2 days. All species are able to accumulate polyhydroxybutyrate (Bruland et al. 2009; Chen et al. 2013).

Pseudorhodoferax strains are oxidase and catalase positive (Bruland et al. 2009; Chen et al. 2013). The data of Bruland et al. (2009) and Chen et al. (2013) are generally in good agreement. Positive for alkaline phosphatase, C4 esterase, C8 esterase lipase, C14 lipase, leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities and for hydrolysis of Tweens 20, 40, and 60 and assimilation of arabinose, mannitol, gluconate, D-fructose, propionate, and malate (Chen et al. 2013). All strains are negative for DNase, arginine dihydrolase, cysteine arylamidase, trypsin, alpha-chymotrypsin, alpha-galactosidase, beta-galactosidase, alpha-glucosidase, beta-glucosidase, *N*-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase activities, hydrolysis of aesculin, starch, gelatin, CM-cellulose, corn oil and indole production, glucose fermentation, assimilation of glucose, mannose, N-acetyl-glucosamine, and caprate (Chen et al. 2013). All strains are sensitive to chloramphenicol, kanamycin, tetracycline, novobiocin, and nalidixic acid and resistant to rifampicin, penicillin G, and ampicillin (Chen et al. 2013). In addition, *Pseudorhodoferax caeni* and *Pseudorhodoferax soli* strains were positive for growth with acetate, taurine, and succinate; both were negative for growth with maltose, D-mannose, inositol, sorbitol, rhamnose, sucrose, 3,3′-dithiodipropionic acid, L-cysteine, L-homocysteine, 3-mercaptopropionic acid, mercaptosuccinic acid, and thiolactic acid (Bruland et al., 2009). Both species are unable to ferment D-fructose and D-glucose in Hugh–Leifson oxidative/fermentative tests and are negative for protease (Bruland et al. 2009). Additional differentiating features of the three species are shown in ❯ *Table 29.17*.

Ramlibacter Heulin et al. (2003), 593[VP]

Ram.li.bac'ter. N.L. n. *ramlis* (from Arabic *raml*), sand; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Ramlibacter*, rod isolated from sandy soil.

Cells are rod shaped and may form coccoid cyst-like cells that are resistant to desiccation and vacuum. Coccoid cells are able to divide. Both types of cells may contain polyhydroxyalkanoate granules. Cells are motile with polar flagella or unflagellated, however, are motile on solid media by gliding. Optimal pH is 7–7.5 and optimal temperature for growth 30 °C; weak growth at 37 °C. *Ramlibacter ginsenosidimutans* grows at 25–37 °C, but not at 18 or 42 °C. Growth factors are required by *Ramlibacter tataouinensis* and *Ramlibacter henchirensis*.

Aerobic chemoorganotrophic metabolism. Catalase, oxidase, and β-glucosidase positive. Arginine dihydrolase and urease negative. Unable to denitrify and no reduction of nitrate to nitrogen. The following substrates are not utilized: arabinose, mannose, mannitol, *N*-acetylglucosamine, gluconate, caprate, adipate, citrate, and phenylacetate (Heulin et al. 2003; Wang et al. 2012).

G+C content of genomic DNA is 66.6–69.7 mol% (Heulin et al. 2003). Major fatty acids are C16:0, C17:0 cyclo, summed feature 4 (C16:1 ω7c and/or C15:0 iso 2-OH), and summed feature 7 (C18:1 ω7c/ω7c/ω7c). The hydroxyl fatty acids C10:0 3-OH and/or C12:0 3-OH are present in some of the species (Wang et al. 2012).

Additional characteristics of *Ramlibacter* species are shown in ❯ *Table 29.18*.

Table 29.17

Comparison of selected characters of the species of *Pseudorhodoferax*

Characteristic	*Pseudorhodoferax aquiterrae* NAFc-7Ta	*Pseudorhodoferax caeni* SB1^{Tb}	*Pseudorhodoferax soli* TBEA3^{Tb}
Source	Groundwater	Activated sludge	Soil
Cell size (μm)	0.6–0.9 × 1.3–1.7	0.9–1.0 × 1.5–3.3	0.8–0.9 × 1.4–2.5
Colony morphology	Light yellow, convex, round, smooth	White-light yellow, circular	White, circular, convex
Optimal NaCl range (%)	0–1	1	0–1
Utilization of			
3,3′-Thiodipropionic acid		−	+
3-Sulfinopropionate		−	+
Arabinose	+	−	+
Adipic acid	+	−	+
Citrate	−	+	−
Maltose	+	−[a]	−[a]
Phenyl acetate	−	+[a]	+[a]
Nitrate reduction	+	−	−
Fermentative degradation D-Fructose		−	−
Enzyme activities			
Beta-glucuronidase	−	−[a]	+[a]
Urease	+	−(+[a])	−(+[a])
Protease		−	−
Beta-galactosidase		−	−
Hydrolysis of Tween 80	+	−[a]	+[a]
Susceptibility to			
Sulfamethoxazole + trimethoprim	S	R	S
Streptomycin	S	R	S
Gentamicin	S	R	S
Quinones	Q-8	Q-8	Q-8
Major fatty acids (>10 %)	C16:0, SF3[c]	C16:0, C18:1ω7c, SF3[d]	C16:0, C17:0 cyclo, SF3[d]
Important fatty acids (5–10 %)	C17:0 cyclo	C17:0 cyclo, SF2[d], C10:0 3-OH	C18:1ω7c, SF2[d], C10:3-OH
Important hydroxy fatty acids	C10:0 3-OH	C10:0 3-OH	C10:0 3-OH
Polar lipids[ae]	PEA, PG, DPG, PS, UPL	PEA, PG, DPG, PS, UPL	PEA, PG, DPG, PS, UPL
DNA G+C content (mol%)	67.6	70.1	69.1

[a]Data from Chen et al. (2013)

[b]Data from Bruland et al. (2009)

[c]SF, summed feature: several fatty acids that cannot be separated by the Microbial Identification System. In Chen et al. (2013), summed feature 2 consisted of C16:1 iso I and/or C14:0 3-OH, and summed feature 3 consisted of C16:1 ω7c and/or C16:1 ω6c

[d]In Bruland et al. (2009), summed feature 2 consisted of C12:0 aldehyde, C16:1 iso I, and/or C14:0 3-OH. Summed feature 3 consisted of C16:1 ω7c and/or C15:0 iso 2-OH

[e]Phosphatidylethanolamine (PEA), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), unidentified phospholipid (UPL)

Rhodoferax Hiraishi et al. (1992), 191^{VL}

Rho.do.fer'ax. Gr. n. *rhodon*, the rose; L. adj. *ferax*, fertile; N.L. masc. n. *Rhodoferax*, red and fertile. *Rhodoferax* was created to accommodate photoheterotrophic bacteria from ditch water and activated sludge that were previously referred to as the "*Rhodocyclus gelatinosus*-like" group, with initially just one species, *Rhodoferax fermentans* (Hiraishi et al. 1991). A second species was later described from an aquatic microbial mat sample from Antarctica (Madigan et al. 2000).

Rhodoferax cells are curved to spiral-shaped cells that are motile with one or more flagella. Both species are facultatively anaerobic photoheterotrophs using a variety of substrates including glucose, fructose, pyruvate, lactate, acetate, succinate, fumarate, malate, citrate, and aspartate. *Rhodoferax antarcticus* is also able to grow slowly photoautotrophically using hydrogen; this property is not documented for *Rhodoferax fermentans*. Both species can also grow aerobically heterotrophically in the dark, and *Rhodoferax fermentans* is able to grow anaerobically in the dark by fermenting pyruvate, glucose, or fructose.

Table 29.18
Differentiating features of the *Ramlibacter* species

Characteristic	*Ramlibacter tataouinensis* DSM 14655^T	*Ramlibacter henchirensis* DSM 14656^T	*Ramlibacter ginsenosidimutans* DSM 23480^T
References	Heulin et al. (2003)	Heulin et al. (2003)	Wang et al. (2012)
Source	Desert soil	Desert soil	Agricultural soil
Shape	Rods and coccoid cysts	Rods and coccoid cysts	Straight to curved rods
Size	Rods 0.2 × 3 mm; cyst 0.8–0.9 mm diameter	Rods 0.2 × 3 mm; cyst 0.8–0.9 mm diameter	0.3–0.4 × 1–2 mm
Motility	Unflagellated, motile on solid media	Unflagellated, motile on solid media	Peritrichous flagella
Pigmentation	Yellow-orange	Yellow-orange	Yellowish
PHA granules	+	+	
Cysts formed	+	+	–
Hydrolysis of gelatin	–	+	
Reduction of nitrate to nitrite	+	+	–
β-Galactosidase	–	+	+
Esterase lipase (C8)	+	+	-
Utilization of			
Glucose	–	+	+
Maltose	–	–/w	w
Malate	–	–	w
Acetate	+	+	-
Pyruvate	+	+	
3-Hydroxybutyrate	+	+	–
4-Hydroxybutyrate	+	+	
DL-Lactate	+	+	–
Propionate	+	+	–
Tween 40	–	+	
Tween 80	–	+	
α-Ketovalerate	–	+	
α-Ketobutyrate	–	+	
Major fatty acids (>10 %)	C16:0, SF4 (C16:1ω7c and/or iso-C15:0 2-OH), SF7 (C18:1w7c/w9t/w12t)	C16:0, SF4 (C16:1ω7c and/or iso-C15:0 2-OH), SF7 (C18:1w7c/w9t/w12t), C17:0 cyclo	C16:0, SF4 (C16:1ω7c and/or iso-C15:0 2-OH), C17:0 cyclo
Important fatty acids (5–10 %)	C12:0, C18:0, C17:0 cyclo		
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH		C12:0 3-OH
DNA G+C content (mol%)	69.6	66.6	68.1

Bacteriochlorophyll a and spheroidene-type carotenoids are present as phototrophic pigments although no obvious intracytoplasmic photosynthetic membranes have been observed.

Rhodoferax antarcticus is a psychrotrophic species with optimal growth 12–18 °C, whereas *Rhodoferax fermentans* is a mesophilic species that grows best at 25 °C and 30 °C. Both species do not grow at 37 °C and both require growth factors. Both species are capable of nitrogen fixation and can use ammonium and glutamate as sole nitrogen sources. Both are freshwater organisms that can grow without added NaCl; with 1 % NaCl, *Rhodoferax fermentans* is unable to grow and *Rhodoferax antarcticus* grows weakly. Additional and differentiating features of both species are shown in *Table 29.19*.

The G+C content of DNA is 60–61.5 mol% (Hiraishi et al. 1991; Madigan et al. 2000). Major fatty acids and hydroxy

Table 29.19
Differentiating features for both *Rhodoferax* species

Characteristic	*Rhodoferax fermentans* JCM 7819^T	*Rhodoferax antarcticus* ATCC 700587^T
References	Hiraishi et al. (1991), Madigan et al. (2000)	Madigan et al. (2000)
Source	Activated sludge	Aquatic microbial mat, Antarctica
Shape	Curved rods	Curved to spiral-shaped rods
Size	0.6–0.9 × 1.5–3 μm	0.7 × 2–3 μm
Motility	Polar flagellum	One or more polar flagella
Pigments	Bacteriochlorophyll a, spheroidene carotenoids	Bacteriochlorophyll a, spheroidene carotenoids
Metabolism	Facultatively anaerobic photoorganotrophic, in the dark anaerobic fermentative growth or aerobic organotrophic growth	Facultatively anaerobic photoorganotrophic and photoautotrophic, aerobic organotrophic growth in the dark
Use of reduced sulfur compounds for photoautotrophic growth	–	
Use of hydrogen for photoautotrophic growth		+
Temperature range (°C)		0–25
Optimal growth temperature (°C)	25–30	12–18
pH range	5–9	
Optimal pH	6.5–7.0	
Growth factors required	Thiamine, biotin	Biotin
Phototrophic growth with		
Xylose, arabinose, galactose, mannose, gluconate, asparagine	+	
Mannitol, butyrate, glutamate, α-ketoglutarate, benzoate, propanol, butanol	+	–
Sucrose, valerate	–	+
Fermentative growth with		
Glucose, fructose	+	–
Pyruvate	+	
Major fatty acids	C16:0, C16:1	
Main hydroxy fatty acid	C8:0 3-OH	
Quinones	Q-8, RQ-8	
G+C content (mol%)	60.1	61.5

fatty acids have only been reported for *Rhodoferax fermentans* and include C16:0, C16:1, C18:1, and C8:0 3-OH (Hiraishi et al. 1991; Hiraishi and Imhoff 2005). Major quinones have also only been reported for *Rhodoferax fermentans* and consist of ubiquinone Q-8 and rhodoquinone RQ-8 (Hiraishi et al. 1991).

Simplicispira Grabovich et al. (2006), 575^{VP}

Sim.pli.ci.spi'ra. L. adj. *simplex–icis*, simple; L. fem. n. *spira*, a spiral; N.L. fem. n. *Simplicispira*, a simple spiral. *Simplicispira* cells are polymorphic rods or weakly curved rods to spiral-shaped cells. They are motile by means of bipolar tufts of flagella or a single flagellum. Cells are catalase and oxidase positive. Aerobic chemoorganotrophic metabolism. Two species are also facultative anaerobic with reduction of nitrate to nitrogen for *Simplicispira psychrophila* (Terasaki 1979) and to nitrite for *Simplicispira limi* (Lu et al. 2007). Neutrophilic and mesophilic. The major quinone is Q-8. All species are catalase and oxidase positive. Sugars are generally not utilized, except for maltose which is utilized by *Simplicispira limi*. The following substrates are not utilized by any of the species: glycerol, leucine, ornithine, phenylalanine, and serine; casein and starch are not hydrolyzed and indole is not produced (Lu et al. 2007; Hylemon et al. 1973; Terasaki 1979). Differentiating substrate utilization patterns as well as other distinctive features for the three species are shown in *Table 29.20*.

Table 29.20

Comparison of selected characters of the species of *Simplicispira*

Characteristic	*Simplicispira limi* DSM 17964^T	*Simplicispira metamorpha* DSM 1837^T	*Simplicispira psychrophila* LMG 5408^T
References	Lu et al. (2007)	Grabovich et al. (2006), Hylemon et al. (1973), Sakane and Yokota (1994)	Grabovich et al. (2006), Terasaki (1979), Sakane and Yokota (1994)
Source	Activated sludge	Infusion of freshwater mussels	Antarctic mosses
Motility	One polar flagellum	Bipolar tufts of flagella	Bipolar tufts of flagella
Cell shape	Rods	Short spirilla	Short, curved, spiral-shaped cells or rods
Cell diameter (μm)	0.7–0.8	0.7–1.3	0.7–0.9
Helix wavelength (μm)		7.5–12	5.5–6.5
Helix diameter (μm)		2.2–3.5	1.0–1.4
Helix length (μm)	1.5–1.7	3.5–11	1.5–14
Anaerobic growth with nitrate	+	−	+
Reduction of nitrate to nitrite	+	−	−
Reduction of nitrate to nitrogen	−	−	+
Major fatty acids (>10 %)	SF3 (C16:1ω7c/iso-C15:0 2-OH), C16:0, C18:1ω7c	C16:1, C16:0, C18:1	C16:1, C16:0, C18:1
Important fatty acids (5–10 %)	C18:1ω7c 11-methyl		
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH	C10:0 3-OH	C10:0 3-OH, C8:0 3-OH
Temperature range (°C)	10–40	3–38	2–26
Optimal temperature (°C)	30	30–32	20
pH range	6–9		5.5–9
G+C content (mol%)	63.3	63	64.6
Quinones	Q-8	Q-8	Q-8
Urease	+	−	+
Formation of poly-3-hydroxybutyrate		+	−
Assimilation of			
Acetate	−	+	−
Alanine	−	+	
Aspartate	−	+	
Butyrate		+	−
Citrate	+	−	−
Fumarate		+	−
Glutamate	−	+	
3-Hydroxybutyrate	+	−	
Lactate	−	+	−
Malonate	−	+	−
Maltose	+	−	−
Proline	+	−	
Propionate	−	v	−
Pyruvate		+	−
Succinate	+	+	−
Hydrolysis of gelatin	−	+	+
H_2S from cysteine	−	+	w

Tepidicella França et al. (2006), 56[VP]

Te.pi.di.cel'la. L. adj. *tepidus*, warm; L. fem. n. *cella*, chamber, cell; N.L. fem. n. *Tepidicella*, a cell living in a warm environment.

Only one species, *Tepicella xavieri*, has been described for two isolates from a hot spring runoff (França et al. 2006). Cells are rod-shaped, 0.5–1 × 1–2 μm, and motile with one polar flagellum. Growth is possible in the temperature range of 25–55 °C, with an optimum of 45 °C; pH range is 6.5–10.5 with an optimum of 8.5–9.0. These organisms can thus be regarded as slightly thermophilic and moderately alkaliphilic. Grows in the presence of 0–2 % NaCl.

Aerobic chemoorganotrophic metabolism. Non-fermentative. No anaerobic growth with nitrate, nitrite, Fe(III)-nitrilotriacetic acid, fumarate, sulfate, or thiosulfate. L-cysteine, thiosulfate, and tetrathionate are oxidized to sulfate without an increase in cell biomass. Negative for reduction of nitrate to nitrite.

Oxidase, catalase, DNAse, acid phosphatase, urease, alkaline phosphatase, esterase (C4), lipase (C8), leucine arylamidase, valine arylamidase, and naphthol-AS-BI-phosphohydrolase are positive; amylase and xylanase negative; aesculin, hippurate, and Tween 20 are hydrolyzed. Negative for cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Utilizes mainly organic and amino acids including succinate, acetate, lactate, pyruvate, citrate, malate, 2-ketoglutarate, aspartate, L-phenylalanine, L-lysine, L-leucine, L-serine, L-histidine, L-tryptophan, L-tyrosine, L-ornithine, L-proline, L-alanine, L-asparagine, L-glutamate, and L-glutamine. Sugars and polyols are not utilized, nor benzoate, glycine, L-valine, L-methionine, or L-threonine.

Major fatty acids are C16:0, C18:1ω7c, and C17:0 cyclo; also important (5–10 %) were C16:1ω7c and C19:0 cyclo ω8c; main hydroxy fatty acid was C8:0 3-OH.

Polar lipids consist of phosphatidylethanolamine and phosphatidylglycerol and main respiratory quinone is Q-8. G+C content of genomic DNA was 64.9–65.5 mol% (França et al. 2006).

Variovorax Willems et al. (1991), 447[VP]

Va.ri.'vo.rax. L. adj. varius, *various*; L. masc. adj. *vorax*, voracious; N.L. masc. n. *Variovorax*, [bacteria] devouring a variety [of substrates].

Strains of *Variovorax* are versatile chemoorganotrophs capable of using a wide range of organic compounds for growth. All are aerobic and *Variovorax ginsengisoli* was also reported to be capable of anaerobic respiration of nitrate (Im et al. 2010). Some strains of *Variovorax paradoxus* (Biotype I) are able to grow lithoautotrophically using hydrogen (Willems and Gillis 2005). When grown in TSB containing 100 mM boric acid (2 days at 25 °C), *Variovorax boronicumulans* BAM-48^T accumulated boric acid to a larger extent than related species did, although *Variovorax paradoxus* NBRC 15149^T and *Variovorax dokdonensis* KCTC 12544^T also showed some accumulation (Miwa et al. 2008).

The following description contains those characteristics of the genus that were reported consistently as present or absent in the individual species descriptions (Willems and Gillis 2005; Yoon et al. 2006; Kim et al. 2006; Miwa et al. 2008; Im et al. 2010; Jin et al. 2012), unless otherwise indicated. All *Variovorax* strains can assimilate L-arabinose (negative for *Variovorax dokdonensis* according to Yoon et al. 2006), histidine, 4-hydroxybenzoate, 3-hydroxybutyrate, 2-ketogluconate, and suberate. None of the strains assimilates *N*-acetylglucosamine, caprate (except *Variovorax boronicumulans* according to Miwa et al. 2008), maltose, rhamnose, inositol, sucrose, glycogen, L-serine, melibiose, or salicin. All strains are positive for catalase, oxidase, esterase lipase (C8), and leucine arylamidase but negative for nitrite reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, *N*-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, and trypsin. Additional and differentiating features of the *Variovorax* species are shown in ❯ *Table 29.21*.

Verminephrobacter Pinel et al. (2012), 2551[VL]

Ver.mi.ne.phro'bac.ter. L. n. *vermis*, a worm; Gr. n. *nephros*, a kidney; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Verminephrobacter*, earthworm-kidney (associated) bacteria.

Cells are short, straight rods and are nonmotile (*Verminephrobacter aporrectodeae*) or possess a polar flagellum (*Verminephrobacter eiseniae*, although in cultivation motility is rarely observed). Cells contain PHB granules. Aerobic chemoorganotrophic metabolism with a preference for low oxygen tension. Fumarate and nitrate are not used as alternative electron acceptors. *Verminephrobacter aporrectodeae* is able to ferment glucose and pyruvate; this feature was not documented for *Verminephrobacter eiseniae*. Both species are oxidase and catalase positive; urease negative. Nitrate is not reduced. Temperature range for growth is 10–30 °C. Moderate growth on complex media containing pyruvate and amino acids. Ammonium salts, urea, and nitrate can be used as nitrogen sources in defined media. Biotin is required for growth on defined mineral medium in the absence of amino acids. Both species utilize a variety of substrates including D-fructose, D-fucose, L-fucose, D-galactose, D-glucose, D-mannose, L-rhamnose, D-xylose, acetate, fumarate, lactate, malate, propionate, pyruvate, succinate, glycerol, L-alanine, L-aspartate, L-asparagine, L-glutamate, and L-glutamine. Neither species utilizes benzoate, citrate, glutarate, maleate, L-histidine, L-isoleucine, L-serine, L-threonine, L-lysine, L-methionine, and L-valine (Pinel et al. 2008; Lund et al. 2012).

Additional features of both species are shown in ❯ *Table 29.22*.

Within *Verminephrobacter aporrectodeae*, two subspecies have been proposed – though not validly published. They differ in the species of *Aporrectodea* they were isolated from (*Aporrectodea tuberculata* and *Aporrectodea caliginosa*) and also show small differences in preferred growth conditions (Lund et al. 2012).

Table 29.21

Comparison of selected characters[a] of the species of *Variovorax*. Data taken from Willems and Gillis (2005), Yoon et al. (2006), Kim et al. (2006), Miwa et al. (2008), Im et al. (2010), and Jin et al. (2012) were generally in good agreement unless otherwise indicated

Characteristic	*Variovorax boronicumulans* BAM-48^T	*Variovorax defluvii* 2C1-b^T and 2C1-21	*Variovorax dokdonensis* DS-43^T	*Variovorax ginsengisoli* Gsoil 3165^T	*Variovorax paradoxus* LMG 1797^T	*Variovorax soli* GH9-3^T
Source	Soil	Sewage	Soil	Agricultural soil	Soil	Greenhouse soil
Motility	Motile	Motile	Motile, peritrichous flagella	Motile	Motile, 1–3 flagella laterally inserted	Motile, 1–3 flagella
Cell shape	Rods	Oval or rod shaped	Oval or rod shaped	Rods	Straight to slightly curved rods	Short rods
Cell size	0.5–0.7 μm × 1.0–2.0 μm	0.4–0.5 μm × 0.8–2.0 μm	0.3–0.5 μm × 0.7–2.8 μm	0.3–0.5 μm × 1.5–3.0 μm	0.5–0.6 μm × 1.2–3.0 μm	0.5–0.7 μm × 1.0–1.5 μm
Morphology	Circular, entire margin, yellow	Circular, slightly convex, smooth, glistening, and yellow	Circular, slightly convex, smooth, glistening, pale yellow	Undulating, convex, embedded, yellowish	Glistening, yellow	Irregular, light yellow
Metabolism	Aerobic, chemoorganotrophic	Aerobic, chemoorganotrophic, non-fermentative	Aerobic chemoorganotrophic	Aerobic chemoorganotrophic, facultatively anaerobic using nitrate as TEA	Aerobic, chemoorganotrophic, facultative lithoautotrophic growth with hydrogen (some strains)	Aerobic, chemoorganotrophic
Anaerobic growth with	–	–	–	Nitrate	–	–
Temperature range for growth (°C)	4–37	8–37	10–40	4–30		10–35
Optimal growth (°C)	30	28–32	30			30
pH range	5–9	6–9	5–9.5	5.0–8.5		5–9
Optimal pH	7	7	7–8			7
Growth without NaCl	+		+	+		+
Growth with 1 % NaCl	+	+	+	+		+
Growth with 2 % NaCl	–	+	+	–		+
Growth with 3 % NaCl		–	+ (to 5 %)			+

Table 29.21 (continued)

Characteristic	*Variovorax boronicumulans* BAM-48^T	*Variovorax defluvii* 2C1-b^T and 2C1-21	*Variovorax dokdonensis* DS-43^T	*Variovorax ginsengisoli* Gsoil 3165^T	*Variovorax paradoxus* LMG 1797^T	*Variovorax soli* GH9-3^T
Nitrate reduction	−	+	−	+	+	+
Urease	−	−	+	−	− (+[a])	− (+[b])
Aesculin hydrolysis	−	+	−	−	−	−
Assimilation of						
Acetate	w	+	+	+	− (+[a])	+ (−[b])
Adipate	−	+	+	−	+	+
L-Alanine	w	w	+	+	−	+ (−[b])
L-Fucose	−	−	−	−	+	−
Glucose	+ (−[c])	−	+	−	+	− (+[b])
Gluconate	−	+	+	−	+	+
3-Hydroxybenzoate	+	+	+	− (+[d])	+	+
Itaconate	−	+	−	−	−	−
5-Ketogluconate	+	−	−	−	+	+ (−[b])
DL-Lactate	w	+	+	+	−	+
Malate	+	+	+ (−[e])	−	+	+
Malonate	−	+	−	+	−	−
Citrate	+	−	−	−	+	−
D-Mannitol	+	−	−	+	+	+
D-Mannose	−	−	−	+	+	−
Phenylacetate	−	−	−	+	+	+
L-Proline	+	−	+	+	+	+ (−[b])
Propionate	+	+	+	+	−	+
D-Ribose	+	−	−	−	+	−
D-Sorbitol	+ (−[c])	−	−	−	+	− (+[b])
Valerate	+	+	+	+	−	+
Enzyme activities						
Acid phosphatase	w	−	+	+	−	+
Alkaline phosphatase	+	−	+	w	+	+
Esterase (C4)	−	+	w	+	+	+
α-Glucosidase	−	−	+	+	+	−

Naphthol-AS-BI-phosphohydrolase	–	–	– (+[e])	–	–	+
Valine arylamidase	–	–	–	w	+	–
Susceptibility to						
Polymyxin B			+		+	
Streptomycin		–	+	+		
Chloramphenicol		–	+	+	+	
Gentamicin		–	+	–		
Tetracycline		–	+	+	+	
Kanamycin		–	+	+	+	
Neomycin		+	+	+	+	
Penicillin G		+	–	+		
Ampicillin		+	–	–	–	
Novobiocin		–	–	–	–	
Lincomycin		+	–	–		
Oleandomycin		–	–	+		
Erythromycin		–		+		
Main ubiquinone	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8
Major fatty acids (>10 %)	C16:0, C17:0 cyclo, SF4 (C16:1 ω7c and/or iso-C15:0 2-OH), SF7 (C18:1 ω7c and/or ω9t and/or ω12t)	C16:0, C17:0 cyclo, SF4 (C16:1ω7c and/or iso-C15:0 2-OH), SF7 (C18:1ω7c and/or ω9t and/or ω12t)	C16:0, C17:0 cyclo, SF4 (C16:1 ω7c and/or iso-C15:0 2-OH), SF7 (C18:1 ω7c and/or ω9t and/or ω12t)	C16:0, C17:0 cyclo, SF4 (C16:1 ω7c and/or iso-C15:0 2-OH)	C16:0, C17:0 cyclo, SF4 (C16:1 ω7c and/or iso-C15:0 2-OH), SF7 (C18:1 ω7c and/or ω9t and/or ω12t)	C16:0, C17:0 cyclo, SF4 (C16:1 ω7c and/or iso-C15:0 2-OH)
Important fatty acids (5–10 %)			C12:0	SF7 (C18:1 ω7c and/or ω9t and/or ω12t)		C10:0 3-OH, SF7 (C18:1 ω7c and/or ω9t and/or ω12t)
Important hydroxy fatty acids	C10:0 3-OH, C14:0 2-OH	C10:0 3-OH, C14:0 2-OH	C10:0 3-OH, C12:0 2-OH	C10:0 3-OH	C10:0 3-OH, C14:0 2-OH	C10:0 3-OH
DNA G+C content (mol%)	71.2	65.2–65.5	66.0	66.0	67.0	67.1

[a]Data from Willems and Gillis (2005)
[b]Data from Kim et al. (2006)
[c]Data from Miwa et al. (2008)
[d]Data from Im et al. (2010)
[e]Data from Yoon et al. (2006)

Table 29.22
Additional features of both *Verminephrobacter* species

Characteristic	*Verminephrobacter eiseniae* ATCC BAA-1489^T = DSM 19249^T	*Verminephrobacter aporrectodeae* LMG 25313^T
References	Pinel et al. (2008)	Lund et al. (2012)
Source	Nephridia of the earthworm species *Eisenia foetida*	Nephridia of the earthworm genus *Aporrectodea*
Shape	Short rods	Rods
Size	0.4–0.8 × 1.3–1.7 mm	0.8 × 2 mm
Motility	Polar flagellum	Nonmotile
Inclusions	PHB granules	PHB granules
Anaerobic growth with DMSO	–	
Anaerobic growth with nitrite, Fe(III)		–
Temperature range for growth (°C)	10–33	10–30
Optimal temperature for growth (°C)	25–28	20
pH range	6–8.2	6.0–8.5
Optimal pH	7.5–8.2	7
Range NaCl concentration	0–120 mM	0–171 mM
Optimal NaCl concentration	50–90 mM	0–100 mM
Hydrolysis of starch	–	
Hydrolysis of gelatin	–	
Growth factors needed	Biotin	
DNA G+C content (mol%)	67	
Major fatty acids (>10 %)	C16:0, C16:1ω7c, C18:1w7c, C17:0 cyclo	
Important fatty acids (5–10 %)	C14:0	
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH	
Fermentation of pyruvate, glucose		+
Utilization of		
L-Arabinose	+	–
D-Ribose	+	–
Fumarate	–	+
2-Oxoglutarate	+	–

Fatty acids have only been reported for *Verminephrobacter eiseniae*: major fatty acids (>10 %) are C16:0, C16:1ω7c, C18:1ω7c, and C17:0 cyclo, and also present in important amounts is C14:0; the hydroxyl fatty acid present is C10:0 3-OH (Pinel et al. 2008). The G+C content of the genomic DNA was only reported for *Verminephrobacter eiseniae* and is 67 mol% (Pinel et al. 2008).

Xenophilus Blümel et al. (2001), 1835^{VP}

Xe.no'phi.lus. Gr. adj. *xenos*, foreign; Gr. masc. n. *philos*, friend; N.L. masc. n. *Xenophilus*, friend of foreign compounds, referring to the isolation of the type species by enrichment on azo dyes.

Cells are straight to slightly curved, motile rods. For *Xenophilus aerolatus*, a polar flagellum was reported. Yellow pigmented. Aerobic, chemoorganotrophic metabolism. Oxidase and oxidase positive. Nitrate reduction negative. The following substrates are utilized by *Xenophilus azovorans* and *Xenophilus aerolatus*: itaconate, suberate, acetate, lactic acid, propionate, 3-hydroxybutyrate, 3-hydroxybenzoate, 4-hydroxybenzoate, dextrin, L-histidine, and L-proline; the following substrates are not utilized: sucrose, L-rhamnose, D-ribose, inositol, casein, methanol, tartaric acid, citrate, glycine, and L-serine. The main respiratory quinone was Q-8 (Blümel et al. 2001; Kim et al. 2010).

Additional carbon sources and other features of both species are given in *Table 29.23*.

Table 29.23
Additional and discriminating features for both *Xenophilus* species

Characteristic	*Xenophilus aerolatus* KACC 12602^T	*Xenophilus azovorans* DSM 13620^T
References	Kim et al. (2010)	Blümel et al. (2001), Kim et al. (2010)
Source	Air	Soil
Shape	Rods	Straight to curved rods
Size	0.6–0.8 × 1.0–1.4 mm	0.5–1 × 1–3 mm
Motility	One polar flagellum	+
Pigmentation	Yellow	Pale yellow
No growth at (°C)		1, 37, 42
Temperature range for growth (°C)	10–35	
Optimal temperature for growth (°C)	25–30	30
pH range	5.0–9.0	
Optimal pH	6.0–8.0	
Range NaCl concentration	0–2 %	
Optimal NaCl concentration	0–1 %	
Hydrolysis of hypoxanthine, tyrosine, Tween 80, xanthine	+	
Hydrolysis of casein, chitin, carboxymethyl cellulose, DNA, pectin, starch	–	
Urease	+	–
Utilization of		
Formic acid	(+)	+
D-Sorbitol, L-alanine, starch	+	–
D-Glucose, α-D-lactose, mannitol	–	+
L-Malic acid, ascorbic acid, succinic acid	–	+
DNA G+C content (mol%)	69	70,4
Major fatty acids (>10 %)	C16:0, SF3 (C16:1ω7c and/or iso-C15:0 2-OH), C18:1w7c, C17:0 cyclo	C16:0, SF4 (C16:1ω7c and/or iso-C15:0 2-OH), SF7 (C18:1w7c/w9t/w12t), C17:0 cyclo
Important hydroxy fatty acids (> = 1 %)	C10:0 3-OH, C16:0 2-OH, C16:1 2-OH, C18:1 2-OH	C8:0 3-OH, C10:0 3-OH, C16:1 2-OH, C16:0 2-OH, C18:1 2-OH
Main polyamines		2-hydroxyputrescine, putrescine, spermidine
Polar lipids[a]	DPG, PEA, PG, 2 UAL	PEA, PG, DPG, UPL
Plasmids		100 kb, 350 kb

[a]Phosphatidylethanolamine (PEA), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), unidentified aminolipid (UAL), unidentified phospholipid (UPL)

Xylophilus Willems et al. (1987), 428^{VP}

Xy.lo.phi'lus. Gr. n. *xylon*, wood; N.L. adj. *philus* (from Gr. adj. *philos*), friendly, loving; N.L. masc. adj. *Xylophilus*, friend of wood.

Cells are straight to slightly curved rods, 0.4–0.8 × 0.6–3.3 μm. Filamentous cells of more than 30 μm long may occur in older cultures. Motile with a polar flagellum. Colonies are yellow pigmented, circular, and slightly raised with an entire margin.

Strictly aerobic, chemoorganotrophic metabolism. Growth is generally very slow and poor, even at the optimal growth temperature of 24 °C. Temperature range for growth is 6–30 °C. Maximum NaCl concentration is 1 %. Requires L-glutamate (0.1 %) for growth in defined media.

Oxidase negative and catalase and urease positive. No reduction of nitrate. The following substrates are used for growth: acetate (0.2 % but not 0.5 %), citrate, DL-malate, succinate, DL-tartrate, mannitol, sucrose, and fumarate (Willems et al. 1987; Kim et al. 2010); the following substrates are not used: formate (weakly used according to Kim et al. [2012]), propionate, malonate, maleate, oxalate, benzoate, and calcium gluconate. Lipolysis of Tween 80, but no hydrolysis of gelatin, aesculin, starch, casein, arbutin, and sodium hippurate. Potato soft rot test negative. H_2S is formed from cysteine and weakly from thiosulfate. Negative for production of indole and ammonia, Voges–Proskauer test, arginine dihydrolase, lysine and ornithine decarboxylase, lecithinase, and acid production from glucose (Willems et al. 1987).

Major fatty acids include summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), C16:0, C18:1ω7c, and C17:0 cyclo. Hydroxy fatty acid C8:0 3-OH is present in significant amounts (Kim et al. 2010). The G+C content of the DNA ranges from 68 to 69 mol% (Willems et al. 1987).

Isolation, Enrichment and Maintenance Procedures

The genera and species of the family are metabolically very diverse and originate from a wide range of habitats. No single isolation or enrichment procedure is available to obtain all or most members of the family. The genera will therefore be discussed separately below.

Acidovorax comprises some environmental species and also several plant-associated/plant-pathogenic species. No single specific isolation protocol or selective medium is available. The environmental species can be encountered in soil, water, and occasionally in clinical samples. They will grow on nutrient agar. *Acidovorax defluvii* was isolated on R2A by whole-cell hybridization with a DNA probe targeting the beta1 subgroup of the Proteobacteria. It was grown on nutrient agar (Schulze et al. 1999). *Acidovorax soli* BL21^T was isolated from soil by dilution plating on Luria–Bertani agar and incubation at 30 °C for 3 days (Choi et al. 2010).

The plant-associated and plant-pathogenic species of *Acidovorax* can be isolated from plant material and will also grow on complex media such as nutrient agar. For example, *Acidovorax radicis* strain N35^T was isolated from the roots of wheat plants grown in agricultural soil, Neumarkt, Germany. Washed roots were surface sterilized, crushed, and plated on nutrient broth agar (no. 4, Fluka) (Li et al. 2011). For this strain two different colony types were observed: one showing the rough colonies on NB agar plates and forming flocks in liquid NB medium and the other forming smooth colonies and not flocculating in liquid medium. About 5 % of the rough-type colonies switch to the smooth type on NB agar plates. Reversal to the rough colony type was not observed. Cells of the smooth colony type had lost their flagellum (Li et al. 2011). Another example, *Acidovorax valerianellae* can be isolated from plants of *Valerianella locusta* affected by bacterial leaf spot. The bacterium is cultivated on YBGA (0.7 % yeast extract, 0.7 % Bacto-peptone, 0.7 % glucose, and 1.5 % agar; pH 7.3) at 25 °C (Gardan et al. 2003).

Albidiferax ferrireducens (originally named *Rhodoferax ferrireducens*) was isolated from sediments collected at 18 ft depth in Oyster Bay, VA, USA, by enrichment culturing. The enrichment medium (Lovley et al. 1993) contained 10 mM lactate as the electron donor and 100 mmol l^{-1} poorly crystalline Fe(III) oxide as the sole electron acceptor. Anaerobic tubes were flushed with N_2/CO_2 (80:20, v/v) to eliminate dissolved oxygen. The final pH was 6–7. The enrichment cultures were incubated at 20 °C in the dark and were transferred (10 % inoculum) at least five times. Isolation of pure cultures was performed using a similar medium solidified with agar that contained Fe(III) chelated with nitrilotriacetic acid rather than poorly crystalline Fe(III) oxide. Colonies were glossy white, smooth, round, and convex (Finneran et al. 2003).

Alicycliphilus strains have been isolated by enrichment using several specific carbon sources. *Alicycliphilus denitrificans* strain K601^T was isolated from a wastewater treatment plant in Konstanz, Germany, under denitrifying conditions and using the alicyclic compound cyclohexanol as sole carbon source (Dangel et al. 1988). The enrichment medium (also used for isolation and routine cultivation) consisted of l^{-1} distilled water: 1.08 g KH_2PO_4, 5.6 g K_2HPO_4, 0.54 g NH_4Cl, 0.15 g $CaCl_2{\cdot}2H_2O$, 0.2 g $MgSO_4{\cdot}7H_2O$, 1.27 g $NaNO_3$, 1 ml trace element solution SL-10 (Widdel et al. 1983), 1 ml selenite/tungstate solution (Tschech and Pfennig 1984), 1 ml vitamin solution VL-7 (Pfennig 1978), and 1 mM cyclohexanol. The final pH was 7.2–7.4. Oxygen was removed by applying several cycles of vacuum and flushing with oxygen-free nitrogen gas at room temperature. For aerobic growth, the same medium composition was used without $NaNO_3$. Cultures were routinely grown at 30 °C, and aerobically grown cultures were shaken at 120 r.p.m. (Mechichi et al. 2003). A further strain, BC, showing 99.7 % 16S rRNA gene sequence similarity with the type strain, was obtained by dilution culturing from enrichment cultures using benzene as a carbon source and chlorate as an electron acceptor (Weelink et al. 2008). A third strain, KN Bun08, was assigned to *Alicycliphilus denitrificans* based on its high rRNA gene sequence similarity with the type strain (99 %). This strain was isolated from sediment from a small tarn close to the University of Konstanz, Konstanz, Germany, through an enrichment culture using butanone as carbon source and nitrate as electron acceptor. The small whitish colonies of the acetone-degrading, nitrate-reducing strain KN Bun08 were purified by dilution culturing on agar with acetone plus nitrate (Dullius et al. 2011).

As can be expected from their different habitats, there is not a single isolation procedure for *Brachymonas* species. *Brachymonas denitrificans* was isolated from various types of wastewater sludge, the type strain from soybean curd waste sludge, and other strains from sewage sludge or activated sludge (Hiraishi et al. 1995). Strains do not require growth factors and grow well on nutrient agar or a mineral medium with a suitable carbon and energy source. Best growth with organic acids or amino acids rather than sugars (Hiraishi et al. 1995). *Brachymonas chironomi* was isolated from egg mass of chironomids, freshwater insects (Diptera, Chironomidae), from a waste stabilization pond in northern Israel. Washed egg mass was homogenized and dilutions were plated onto *m-Aeromonas* medium (Himedia) which promotes the growth of members of the genus *Aeromonas*. After incubation at 30 °C for 48 h, isolations were made (Halpern et al. 2009).

Caenimonas koreensis KCTC 12616^T was isolated from a serial dilution of a sample from activated sludge from a lab-scale sequencing batch reactor performing enhanced biological phosphorous removal with sodium acetate as a sole carbon source. Dilution samples were plated on R2A agar (Difco) and incubated at 20 °C for 7 days for isolation. Routine cultivation can be done on R2A agar at 30 °C (Ryu et al. 2008). Recently

a second species was proposed, *Caenimonas terrae*, from soil by serial dilution on R2A medium and incubation at 28 °C for 5 days. The organism can be maintained on glycerol stocks in liquid nitrogen or can be freeze-dried (Kim et al. 2012).

Comamonas strains have been isolated from various environmental sources (natural water or soil samples [De Vos et al. 1985; Narayan et al. 2010], soils with contamination [Chitpirom et al. 2012; Yu et al. 2011]), compost (Young et al. 2008), and wastewater treatment plants (Gumaelius et al. 2001; Tago and Yokota 2004), including bioreactors (Etchebehere et al. 2001; Kim et al. 2008); various clinical samples (De Vos et al. 1985; Willems et al. 1991c); and termite gut samples (Chou et al. 2007). Without a common selective medium, standard procedures of isolation using complex media can be used. Because these organisms are quite versatile, enrichment steps to select for particular metabolic traits can be used.

Curvibacter species have all been isolated from water. However, no single isolation medium or procedure is available. *Curvibacter delicatus* (originally *Spirillum delicatum*) and *Curvibacter lanceolatus* (originally *Pseudomonas lanceolata*) were isolated from distilled water samples using a salt-free agar containing ($g.l^{-1}$) 3 g Casitone, 1 g yeast extract, 1 g K_2HPO_4, and 15 g agar (pH 7.1). After drying overnight at 37 °C, 0.1 ml of sample diluted with sterile distilled water was plated and incubated for 2–5 days at 20 °C (Leifson 1962c) from distilled water. The procedure is not selective as many different gram-negative, polarly flagellated non-fermentative straight rods were obtained (Leifson 1962b). *Curvibacter gracilis* strain 7-1^T was isolated from well water and maintained on B104 medium containing 10 g polypepton, 2 g yeast extract, and 1 g $MgSO_4.7H_2O$, pH 7,0 (Ding and Yokota 2004). *Curvibacter fontanus* strains were isolated from well water and grew poorly in aerobic conditions but better under microaerophilic conditions (Ding and Yokota 2010). The PYMB medium used (polypeptone 1 g, yeast extract 0.2 g, $MgSO_4{\cdot}7H_2O$ 1 g, brain heart infusions 2 g, agar 15 g, water 1 l, pH 7) was enriched with 10 % sterile supernatant of a late logarithmic phase culture of *Micrococcus luteus* (Ding and Yokota 2010).

Also for *Delftia*, not a single isolation strategy is available. *Delftia acidovorans* (originally *Pseudomonas acidovorans* and *Comamonas acidovorans*) has been isolated from soil, mud, wood chips, and water using mineral medium with various aromatic compounds, hydrocarbons, and higher dicarboxylic acids (e.g., phenol, *m*-cresol, acetamide, muconic acid, *p*-hydroxybenzoate, indole, nicotinate, tryptophan, maleate, mesaconate, kerosene, crude oil) as sole carbon source (Gray and Thornton 1928; den Dooren de Jong 1926; Robert-Gero et al. 1969; Stanier et al. 1966). *Delftia lacustris* strain 332^T was isolated, after enrichment for peptidoglycan degraders, from filtered water (0.8 mm pore size) collected from the mesotrophic Lake Sjælsø, northern Zealand, Denmark. Microcosms were supplemented with peptidoglycan (2 mg l^{-1}) from *Bacillus* sp. strain A6 (Frette et al. 2004) purified according to the method of Pelz et al. (1998) and incubated for 7 days at 20 °C. Bacteria were isolated from the microcosms on 1/10 LB agar (Eisenstadt et al. 1994) and transferred to 1/10 LB agar supplemented with peptidoglycan (or with heat-denatured cell-wall material) from *Bacillus* sp. strain A6; strain 332^T formed distinct but opaque clearing zones. Routine cultivation is possible in LB broth or TSB in shake flasks incubated overnight at 22 °C or on PG-enriched media [tryptic soy agar or dilute tryptic soy broth amended with 2 mg peptidoglycan l^{-1}] (Jørgensen et al. 2009). *Delftia tsuruhatensis* strain T7^T was isolated from activated sludge from a domestic wastewater treatment plant in Japan by enrichment with terephthalate as sole carbon source (Shigematsu et al. 2003). *Delftia lacustris* strain 332^T was isolated from the mesotrophic freshwater Lake Sjælsø, northern Zealand, Denmark, by selection for peptidoglycan degradation (Jørgensen et al. 2009). *Delftia litopenaei* wsw-7^T was isolated from a freshwater sample from a shrimp culture pond in Pingtung, Southern Taiwan, by plating on R2A medium (Chen et al. 2012).

Diaphorobacter nitroreducens was isolated from activated sludge with pellets and flakes of poly(3-hydoxybutyrate-*co*-3-hydroxyvalerate) (PHBV) as the sole added substrate under anaerobic denitrifying conditions. Strains can be maintained on medium PBY (0.5 % Bacto-peptone (Difco), 0.3 % beef extract (Difco), and 0.1 % yeast extract (Difco)) at 28–30 °C with aeration through shaking. For denitrification experiments, a supplement of 0.2 % KNO_3 can be added (Khan and Hiraishi 2002). *Diaphorobacter oryzae* strains were obtained from sediment samples from rice fields through aerobic thiosulfate-oxidizing enrichment culturing in basal freshwater medium (Taylor et al. 1981) plus 1 mM thiosulfate at 20 °C, although the strains themselves do not oxidize thiosulfate (Pham et al. 2009).

Extensimonas was isolated from filtered wastewater by dilution plating on CM agar (containing, per liter distilled water, 0.5 g NaCl, 0.5 g yeast extract, 0.5 g beef extract, 1 g peptone, and 1.0 g glucose, pH 7.0–7.2) and incubating at 28 °C. After 3 days, colorless colonies were purified and maintained on the same medium. Longer-term storage is possible on the same medium plus 25 % glycerol at −80 °C (Zhang et al. 2013b).

Giesbergeria comprises five species from pond water, sulfur spring, and wastewater. *Giesbergeria anulus* (originally *Spirillum anulus*) and *Giesbergeria sinuosa* (originally *Spirillum* sinuosum) were isolated by supplementing a 100 ml water sample from a stagnant small pond with 1 g peptone or yeast autolysate and incubating at room temperature for 3–7 days. Further enrichment could be achieved by dilution of the medium (Williams and Rittenberg 1957). They can be maintained on peptone-succinate-salts (PSS) medium (Hylemon et al. 1973) or modified PSS medium (Grabovich et al. 2006). Strains can be preserved in liquid nitrogen as a dense suspension in PSS broth with 15 % glycerol (Hylemon et al. 1973).

Chemolithotrophic hydrogen-oxidizing strains of *Hydrogenophaga* can be isolated from soil or water by enrichment in a mineral medium (Palleroni and Doudoroff 1972) with incubation in an atmosphere of H_2, O_2, and CO_2. Heterotrophic strains can be isolated from soil or water by selection with various carbon sources or can be isolated using dilution plating on general media: for example, *Hydrogenophaga bisanensis* was

isolated from wastewater of a textile dye factory by means of dilution plating on nutrient agar at 30 °C (Yoon et al. 2008). *Hydrogenophaga caeni* was isolated from activated sludge involved in phosphorous removal, by dilution plating on R2A and incubation at 20 °C; subculturing was done at 30 °C (Chung et al. 2007).

Hylemonella gracilis was originally isolated (as *Spirillum gracile*) from pond water using a medium composed of (per l) 5 g peptone, 0.5 g yeast extract, 20 mg Tween 80, 0.1 g K_2HPO_4, and 10 g agar (pH 7.2). The authors used the small cell size as a selective feature by placing sterile cellulose ester filter disks (average pore size 0.45 µm) on the plates, adding 0.05 ml of pond water in the center, and incubating at room temperature for 1.5–5 h before removing the filters. Further incubation of at least 3 days resulted in spreading, semitransparent areas of subsurface growth of the spirilla (Canale-Parola et al. 1966).

Lampropedia strains can be found in water rich with organic matter, wastewater, and rumen fluid. They will grow on rich media, however, may be hard to isolate because the source of samples for isolation usually contains large numbers of other bacteria. The tablet-like sheets can be picked out using a microscope. In liquid culture, they form a hydrophobic and rumpled pellicle on the surface of cultures left aerobically at room temperature (Murray 2005). On solid media, a strain is still able to be transparent; strains that had lost this ability produced round colonies that may be slimy and glossy or matt and fry (Lee et al. 2004). They can be maintained by regular subculturing, although this may select for mutants. Lyophilization is possible with a fair degree of survival (Murray 2005).

Limnohabitans represents a group of freshwater bacteria, referred to as the R-BT065 freshwater cluster (Simek et al. 2001) that can be quite abundant in many habitats (Kasalicky et al. 2010; Newton et al. 2011). Isolation of strains in culture and subsequent species description was possible thanks to the filter and dilution–acclimatization method which eliminates fast-growing competing bacteria by filtration and allows for the adjustment of bacteria to higher nutrient concentrations by using gradually more concentrated NSY (nutrient broth, soytone, yeast extract) medium (up to 3 g l^{-1}) in consecutive subcultures (Hahn et al. 2004). Purity can be verified by plating on NSY agar.

Macromonas bipunctata cultures can be obtained using a semisolid medium comprising, per l distilled water, 1 g sodium acetate, 0.1 g calcium chloride, 0.1 g casein hydrolysate, 0.1 g yeast extract, 1 g agar with vitamins, trace elements, and 200 mg of freshly precipitated iron(II) sulfide added after sterilization (Dubinina et al. 2005). Inoculations in 10 ml portions should be incubated at 28 °C for 2–3 days when growth appears as a white surface film that can be streaked onto the same medium solidified with 10 g of agar to purify strains (Dubinina et al. 2005). Lyophilization is unsuccessful; however, cultures can be maintained on semisolid agar medium (0.15 % agar) with 1 g l^{-1} thiosulfate instead of FeS (Dubinina et al. 2005).

Malikia granosa was isolated from activated sludge from a wastewater treatment plant by dilution plating on solid medium of Rouf and Stokes (1964) which was designed to select for bacteria of the *Sphaerotilus/Leptothrix* group. The strain was selected by whole-cell hybridization (Spring et al. 2004) with an oligonucleotide probe LDI23a for the same group (Wagner et al. 1994). A second species, *Malikia spinosa*, is the former *Pseudomonas spinosa* which originates from river water and was isolated by enrichment of water samples with low amounts (0.01–0.1 %) of nutrients such as acetate, ammonium salts, and yeast extract and purification on salt-free nutrient agar (Leifson 1962a). No data are published on preservation. However, strains are available from culture collections as lyophilized cultures.

The same approach as used for the isolation of *Malikia granosa* also led to the isolation of a second organism that was described as *Ottowia thiooxydans* (Spring et al. 2004). More recently, *Ottowia pentelensis* was isolated from a laboratory-scale activated sludge for coke plant effluent using plate dilution on TC medium containing potassium thiocyanate (500 mg l^{-1}) and later maintained on TSA (Felföldi et al. 2010).

Polaromonas species have been isolated from cold habitats including water from the Southern Ocean, glacier cryoconites, and forest soil from Alaska but also from less cold environments such as a coal-tar contaminated aquifer, tap water from a paper mill, and soil. No specific isolation procedure targets *Polaromonas* in general, and several different procedures have been used to obtain the described species. Gosink and Staley (1995) recovered *Polaromonas vacuolata* from Antarctic sea ice and water samples by plating on seawater-*Cytophaga* medium (oligotrophic medium containing beef extract, yeast extract, tryptone, and succinate in half-strength artificial seawater (Irgens et al. 1989)). Plates were incubated at 4–6 °C for 3–8 weeks, and gas-vacuolate colonies were selected visually for their white appearance. *Polaromonas jejuensis* was isolated from soil samples by plating serial dilutions on R2A plates and incubating at 28 °C for 5 days (Weon et al. 2008).

Other species were obtained by selecting for a particular metabolic property. *Polaromonas hydrogenivorans*, which is capable of autotrophic growth with hydrogen, was selectively enriched from forest soil by using this property. An enrichment in mineral medium with H_2 and CO_2 as energy and carbon source, respectively, was incubated at 0.1–1 °C (Sizova and Panikov 2007).

Polaromonas naphthalenivorans was selected from samples of surface freshwater sediment obtained from groundwater flowing through naphthalene-rich coal-tar waste contamination. Serially diluted samples were plated onto minimal salts base agar medium (Stanier et al. 1966) and incubated in the presence of naphthalene vapor as the sole carbon source at 10 °C for 1 month. *Polaromonas naphthalenivorans* formed large (0.5 cm) mucoid colonies (Jeon et al. 2004).

Pseudacidovorax was isolated from soil by serial dilution and plating on nutrient agar. It can be maintained on tryptic soy agar and grows well at 28 °C (Kämpfer et al. 2008).

Pseudorhodoferax soli strain TBEA3^T was isolated from a soil enrichment using the thioether 3,3′-thiodipropionic acid as a sole source of carbon and energy (Bruland et al. 2009). *Pseudorhodoferax caeni* strain SB1^T was isolated using aniline-containing enrichment cultures from activated sludge after conjugal transfer of the aniline-degradative plasmid pNB2 (Bathe 2004). Both strains grow on complex media within 1–2

days. They can be grown on mineral salts medium (MSM; Schlegel et al. 1961) supplemented with a suitable carbon source, 0.5 % (w/v) 3,3′-thiodipropionic acid for *Pseudorhodoferax soli* TBEA3^T, or 1 % (w/v) sodium gluconate for both *Pseudorhodoferax soli* TBEA3^T and *Pseudorhodoferax caeni* SB1^T. To enhance growth on MSM, the medium can be supplemented with yeast extract (0.1 % w/v) or with a vitamin solution containing (l^{-1}) 20 mg biotin, 20 mg folic acid, 60 mg lipoic acid, 50 mg thiamine, 50 mg riboflavin, 50 mg nicotinic acid, 100 mg pyridoxal hydrochloride, 50 mg pantothenic acid, 50 mg vitamin B12, and 50 mg *p*-aminobenzoic acid (Mohn 1995). *Pseudorhodoferax aquiterrae* NAFc-7^T isolated groundwater from Tainan countryside, Southern Taiwan, by incubation on R2A agar (BD Difco) at 25 °C for 3 days (Chen et al. 2013).

Ramlibacter species were also isolated from soil. Heulin et al. (2003) isolated *Ramlibacter tataouinensis* and *Ramlibacter henchirensis* from desert soil while studying alterations to meteorite fragments. Isolations were performed by diluting a crushed sample in tenfold-diluted tryptic soy broth (TSB/10; 3 g l^{-1}) supplemented with 100 mM $CaCO_3$ (TTB medium) to mimic natural selective pressure (carbonated sandy soil). After 2 days incubation at 30 °C, the resulting culture was filtered using a 0.45 µm filter to select the smallest bacterial forms. The filtrate was diluted tenfold in tubes of TSB/10 and incubated for 5 days at 30 °C. Tubes in which associations of rod-shaped and spherical bacteria were microscopically observed were plated on TTA agar medium (TSB, 3 g l^{-1}; granulated agar, 15 g l^{-1}; CaCO3 100 mM) and stored at 30 °C for 1 month. *Ramlibacter* appeared as small yellow-orange colonies (0.1–0.5 mm in diameter), embedded in a medium, and all purified colonies contained the two cell types (rods and coccoid cells) (Heulin et al. 2003). An unvalidated species, "*Ramlibacter ginsenosidimutans*," that phylogenetically groups with other *Ramlibacter* species was isolated from soil of a ginseng field in China using serial dilution on modified xylan-nutrient agar (per liter: 0.02 g tryptone, 0.02 g yeast extract, 0.02 g malt extract, 0.02 g beef extract, 0.02 g casamino acid, 0.02 g soytone, 1.0 g xylan, 0.1 g sodium pyruvate, 0.3 g K2HPO4, 0.05 g MgSO4, 0.05 g CaCl2, and 15 g agar, pH 7.0) and incubation at 30 °C for 1 month. Routine cultivation was on R2A agar (Difco) or 1/10 TSA (trypticase soy agar; Difco) at 25 °C. Preservation was done in a glycerol solution [20 % (w/v)] at −70 °C (Wang et al. 2012).

Freshwater phototrophic *Rhodoferax* have been isolated from different sources, relatively rich in organic matter, including ditch water, activated sludge, and microbial mats from Antarctic ponds. Isolation by enrichment for purple nonsulfur bacteria can be made more selective for *Rhodoferax fermentans* by the addition of 0.5 mM EDTA (Hiraishi and Imhoff 2005). Suitable anaerobic incubation is under illumination at 1,000–2,000 lx at 28 °C for *Rhodoferax fermentans* and at 12–18 °C for *Rhodoferax antarcticus*. MYCA medium (Hiraishi et al. 1991) containing 0.1 % DL-malate, 0.3 % yeast extract, 0.2 % casamino acids, and 0.05 % ammonium sulfate (pH 6.6–6.8) can be used as a simple medium for the purification of *Rhodoferax* (Hiraishi and Imhoff 2005). *Rhodoferax* can be lyophilized or stored frozen at −80 °C or in liquid nitrogen (Hiraishi and Imhoff 2005).

Simplicispira metamorpha (originally *Spirillum metamorphum*) was isolated from a putrid infusion of freshwater mussels (*Corbicula japonica* Prime) on a medium containing (per l) 5 g peptone, 3 g yeast extract, 1 g NaCl, and 200 ml shellfish extract (obtained by boiling 250 g of broken shellfish in 500 ml of water for 20 min followed by filtering) (pH 8.0–8.2). The inoculum consisted of broken putrid shellfish that had been incubated with a teaspoon of mud in 0.1 % NaCl solution at 27–28 °C in a Petri dish. Incubation temperature for isolation was 30 °C (Terasaki 1970). It is thought that the source of the bacteria may also have been mud that was incubated with the shellfish (Krieg 1976). *Simplicispira psychrophila* (originally *Spirillum psychrophilum*) was isolated from an infusion of a moss (*Ceratodon purpureus*) collected from Antarctica and can be maintained at 4° by 3-monthly transfers on a medium containing (per l) 5 g peptone, 3 g meat extract, and 15 g agar (Terasaki 1979). *Simplicispira limi* was isolated from activated sludge that performed enhanced biological phosphorus removal in a laboratory-scale sequencing batch reactor. A serially diluted sludge sample was plated on R2A agar plates and incubated at 20 °C for 5 days (Lu et al. 2007).

Tepidicella strains were isolated from a hot spring runoff (temperature 70 °C, pH 7.5). Water samples were passed over membrane filters, and filters were placed on agar-solidified *Thermus* medium (Williams and Da Costa 1992), wrapped in plastic bags, and incubated at 50 °C for up to 4 days for isolation. For cultivation, Degryse 162 medium (Degryse et al. 1978) results in higher growth yields (França et al. 2006).

Variovorax species have been isolated from soil using diverse procedures. *Variovorax paradoxus* can be isolated from soil, mud, and water by enrichment in mineral medium by selecting for typical properties. Facultatively chemolithotrophic hydrogen-oxidizing strains can be isolated incubated at 30 °C under an atmosphere of 50 % H_2, 4–20 % O_2, 5 % CO_2, and 25–41 % N_2 (Davis et al. 1970). Heterotrophic strains can be isolated from soil by enrichment with pantothenate (Davis et al. 1970), poly-3-hydroxybutyrate (Delafield et al. 1965), or various other chemicals given the large metabolic versatility of the species (Willems and Gillis 2005). *Variovorax soli* GH9-3^T was isolated from greenhouse soil cultivated with lettuce, Wanju, Korea. It was obtained from a serial dilution series on R2A (Reasoner and Geldreich 1985) after 3 days incubation at 30 °C. The isolate was maintained on R2A and stored at −80 °C with 15 % glycerol (v/v) (Kim et al. 2006). *Variovorax dokdonensis* DS-43^T was isolated from soil using standard dilution plating on nutrient agar and incubation at 25 °C. It can be routinely cultivated on tryptic soy agar at 30 °C (Yoon et al. 2006). *Variovorax boronicumulans* strain BAM-48^T was obtained from soil from an experimental field at the Yayoi campus of the University of Tokyo, Japan. Soil samples (5 g) were incubated in sterilized PBS (pH 7.0) at 30 °C for several days, after which the supernatant was streaked on tryptic soya agar (TSA; Difco) and incubated at 25 °C. The strain was purified and maintained on TSA and as glycerol (35 %, w/v) stocks at −80 °C (Miwa et al. 2008). *Variovorax ginsengisoli* strain Gsoil 3165^T was first isolated from soil from a ginseng field, Pocheon Province, South

Korea. A serial dilution of soil sample in 50 mM phosphate buffer (pH 7.0) was plated on one-fifth-strength modified R2A agar (per liter distilled water: 0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g casamino acids, 0.25 g soytone, 0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.5 g KNO_3, 0.3 g K_2HPO_4, 0.05 g $MgSO_4$, 0.05 g $CaCl_2$, and 15 g agar). After anaerobic incubation ($N_2/CO_2/H_2$ at a ratio 80/15/5) at 25 °C, single colonies were purified and grown on modified R2A agar or half-strength modified R2A agar. Strain Gsoil 3165^T was routinely cultured under aerobic conditions on R2A agar (Difco) at 30 °C. Preservation was as a glycerol suspension (20 %, w/v) at −70 °C (Im et al. 2010). *Variovorax defluvii* strains 2C1-b^T and 2C1-21 were isolated from sewage, South Korea, during screening for 2-chlorophenol-degrading bacteria. Sewage samples were stimulated with 100 p.p.m. 2-chlorophenol, and stimulated cultures were serially diluted and plated on R2A agar. Yellow colonies were isolated after 7 days incubation at 25 °C (Jin et al. 2012).

Verminephrobacter can be isolated from nephridia of earthworms using *Acidovorax* complex medium (ACM) containing l^{-1}: 0.5 g yeast extract, 1.0 g casamino acids, 2.0 g pyruvic acid, 2.0 g L-glutamine, 0.3 g KH2PO4, 0.3 g MgSO4, and 2.0 g 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 7.2–7.3, adjusted with 5 M KOH before autoclaving (Pinel et al. 2008). For plates, 1.5 % Phytagel was added. Crushed and diluted nephridia are plated and incubated, sealed with parafilm, at about 25 °C. Pinel et al. (2008) used fluorescence in situ hybridization (FISH) with an *Acidovorax*-specific probe to identify and purify desired bacteria. Colonies of *Verminephrobacter* were circular and convex and had a diameter of 0.5–2 mm and an entire or lightly erose margin and a light bisque to peach color after 15–20 days incubation at room temperature (Pinel et al. 2008). Lund et al. (2012) used a similar procedure and FISH verification; however, they used modified R2A medium (DSMZ medium 830 containing in g l^{-1}: 0.5 proteose peptone, 0.5 yeast extract, 0.5 casamino acids, 0.5 glucose, 0.5 soluble starch, 0.3 sodium pyruvate, 0.3 K2HPO4, 0.3 MgSO4 · 7H2O, 0.3; pH adjusted to 7.0 with 1 M HCl). Gelling agent was 1 % Phytagel (Lund et al. 2012).

Xenophilus azovorans was isolated from a soil inoculum by selection for usage of the azo-dye carboxy-Orange II as the sole source of carbon and energy (Kulla et al. 1984). The species grows well on various nutrient-rich media (Luria ± Bertani or nutrient broth), at 30 °C, but rapidly loses the ability to degrade Orange II in these conditions (Blümel et al. 2001). *Xenophilus aerolatus* was isolated from air in an outdoor region of Suwon City, Republic of Korea, using a MAS-100 air sampler (Merck; single-stage multiple-hole impactor) containing Petri dishes with R2A agar with cycloheximide (200 μg ml^{-1}). Plates were incubated at 30 °C for 5 days before isolation (Kim et al. 2010).

Xylophilus ampelinus can be isolated from diseased tissue of *Vitis vinifera* on which this bacterium causes necrosis and canker. It can be grown on nutrient agar, but growth is generally very slow and poor with yellow colonies attaining a diameter of 0.2–0.3 (6 days) to 0.6–0.8 mm (15 days). Better growth is obtained on GYCA medium (in wt/vol, 1 % glucose, 0.5 % yeast extract, 3 % CaC03, 2 % agar), and the best growth is observed on a medium containing 1 % yeast extract, 2 % D-galactose, 2 % $CaC0_3$, and 2 % agar at 24 °C. On this medium, colonies are also yellow, and a brown diffusible pigment is produced. Some strains may produce two colony types of similar appearance but differing in size due to a difference in growth rate (Willems et al. 1987).

Ecology

From the preceding sections, it is clear that members of the family *Comamonadaceae* are very diverse. They inhabit a wide range of habitats. Three broad types of can be recognized: (1) bacteria that are free-living in the natural and man-made environment (various clean or polluted soils and waters, industrial settings, water purification, and bioremediation sites), (2) bacteria that are plant associated or plant pathogenic, and (3) bacteria that are associated with animals or clinical samples. An overview of the genera and species in these broad categories is given in *Table 29.24*. Environmental habitats are by far the most common (89 species), with plant-associated or plant-pathogenic species (10 species) and animal-associated species and species from clinical samples (11 species) restricted to the genera *Acidovorax*, *Simplicispira*, and *Xylophilus* and *Acidovorax*, *Brachymonas*, *Comamonas*, *Delftia*, *Simplicispira*, and *Verminephrobacter*, respectively.

Of the plant-associated species, *Acidovorax radicis* strain N35^T was isolated from the roots of wheat plants and found to be truly able to colonize roots endophytically. It is nonpathogenic and has plant growth-promoting properties (Li et al. 2012a). *Simplicispira psychrophila* was obtained from an infusion of mosses, but its possible association with these plants is undocumented (Terasaki 1979).

Pseudacidovorax intermedius is a nitrogen-fixing bacterium isolated from agricultural soil in Korea (Kämpfer et al. 2008). It has also been reported as an endophyte (Thomas and Soly 2009) and more recently also from surface sediments from the South China Sea (Zhang and Chen 2012).

Among the aquatic environmental species, many originate from activated sludge or various aquatic sediments (*Table 29.24*). However, quite a number of species inhabit at more oligotrophic freshwater habitats. For example, *Curvibacter* species are found in distilled water and well water; *Delftia lacustris* and *Delftia licopenaei* were described from freshwater, although the latter species came from a shrimp cultivation pond which may therefore be rather nutrient rich; also *Hylemonella gracilis* and several *Giesbergeria* species were isolated from pond water and one from a sulfide spring; *Malikia spinosa*, *Hydrogenophaga palleronii*, and *Hydrogenophaga pseudoflava* were found in river water, and *Polaromonas aquatica* was reported from tap water, *Polaromonas vacuolata* from the Antarctic ocean; *Pseudorhodoferax aquiterrae* was described from ground water (*Table 29.24*). The importance of these organisms in their ecosystem remains largely unknown. Most have been isolated

Table 29.24

Overview of habitats of species of the *Comamonadaceae*

Species	Environmental habitats	Plant associated or plant pathogenic	Animal associated or clinical	Remarks	References
Acidovorax anthurii		*Anthurium* affected by bacterial leaf spot			Gardan et al. (2000)
Acidovorax avenae		Diseased grasses			Willems et al. (1992), Schaad et al. (2008)
Acidovorax caeni	Activated sludge				Heylen et al. (2008)
Acidovorax cattleyae		Diseased *Phalaenopsis* and *Cattleyae*			Willems et al. (1992)
Acidovorax citrulli		Diseased Cucurbitaceae			Willems et al. (1992)
Acidovorax defluvii	Activated sludge				Schulze et al. (1999)
Acidovorax delafieldii	Soil, water		Clinical samples	Autotrophic growth with hydrogen (some strains)	Willems et al. (1990)
Acidovorax facilis	Soil			Autotrophic growth with hydrogen	Willems et al. (1990)
Acidovorax konjaci		Diseased *Amorphophallus rivieri* cv. Konjac			Willems et al. (1992)
Acidovorax oryzae		Diseased rice plants			Schaad et al. (2008)
Acidovorax radicis		Wheat roots			Li et al. (2011)
Acidovorax soli					Choi et al. (2010)
Acidovorax temperans	Activated sludge		Clinical samples		Willems et al. (1990)
Acidovorax valerianellae		Diseased *Valerianella locusta*			Gardan et al. (2003)
Acidovorax wautersii	Environmental samples		Clinical samples		Vaneechoutte et al. (2013)
Albidiferax ferrireducens	Coastal aquifer sediments			Oxygen, Fe(III) nitrilotriacetic acid, Mn(IV) oxide, or fumarate as terminal electron acceptor	Finneran et al. (2003)
Alicycliphilus denitrificans	Municipal sewage plant			Oxygen, nitrate, or nitrite as terminal electron acceptor	Mechichi et al. (2003)
Brachymonas chironomi			Chironomid egg mass in a waste stabilization pond		Halpern et al. (2009)
Brachymonas denitrificans	Activated sludge			Facultative anaerobic growth with nitrate	Hiraishi et al. (1995)
Caenimonas koreensis	Activated sludge				Ryu et al. (2008)
Caenimonas terrae	Paddy soil sample				Kim et al. (2012)
Comamonas aquatica	Environmental samples		Clinical samples		Wauters et al. (2003), Willems et al. (1991c)

Table 29.24 (continued)

Species	Environmental habitats	Plant associated or plant pathogenic	Animal associated or clinical	Remarks	References
Comamonas badia	Activated sludge				Tago and Yokota (2004)
Comamonas composti	Food waste compost				Young et al. (2008)
Comamonas denitrificans	Activated sludge			Facultative anaerobic growth with nitrate	Gumaelius et al. (2001)
Comamonas granuli	Granules from a wastewater treatment bioreactor				Kim et al. (2008)
Comamonas guangdongensis	Subterranean forest sediment			Facultative anaerobic growth with Fe(III)	Zhang et al. (2013a)
Comamonas jiangduensis	Soil from rice field				Sun et al. (2013)
Comamonas kerstersii			Clinical samples		Wauters et al. (2003), Willems et al. (1991c)
Comamonas koreensis	Wetland sediment				Chang et al. (2002), Chou et al. (2007)
Comamonas nitrativorans	Denitrifying reactor from a landfill leachate treatment system			Facultative anaerobic growth with nitrate	Etchebehere et al. (2001)
Comamonas odontotermitis			Gut of termite *Odontotermes formosanus*		Chou et al. (2007)
Comamonas terrae	Arsenic-contaminated soil				Chitpirom et al. (2012)
Comamonas terrigena	Hay infusion				De Vos et al. (1985)
Comamonas testosteroni	Soil		Clinical samples		Tamaoka et al. (1987), Willems et al. (1991c)
Comamonas thiooxidans	Sulfur spring sediment				Narayan et al. (2010), Pandey et al. (2009)
Comamonas zonglianii	Phenol-contaminated soil				Yu et al. (2011)
Curvibacter delicatus	Distilled water				Hylemon et al. (1973)
Curvibacter fontanus	Well water			Prefers microaerophilic conditions	Ding and Yokota (2010)
Curvibacter gracilis	Well water				Ding and Yokota (2004)
Curvibacter lanceolatus	Distilled water				Leifson (1962b)
Delftia acidovorans	Soil, sediment, activated sludge, crude oil, oil brine, water		Various clinical samples		Wen et al. (1999), Willems et al. (1991c)

Table 29.24 (continued)

Species	Environmental habitats	Plant associated or plant pathogenic	Animal associated or clinical	Remarks	References
Delftia lacustris	Freshwater				Jørgensen et al. (2009)
Delftia litopenaei	Freshwater shrimp culture pond				Chen et al. (2012)
Delftia tsuruhatensis	Activated sludge				Shigematsu et al. (2003)
Diaphorobacter nitroreducens	Activated sludge from sewage treatment plant			Facultative anaerobic growth with nitrate	Khan and Hiraishi (2002)
Diaphorobacter oryzae	Paddy field sediment				Pham et al. (2009)
Extensimonas vulgaris	Industrial wastewater				Zhang et al. (2013b)
Giesbergeria anulus	Pond water				Grabovich et al. (2006), Hylemon et al. (1973)
Giesbergeria giesbergeri	Pond water				Grabovich et al. (2006), Hylemon et al. (1973)
Giesbergeria kuznetsovii	Sulfide spring				Grabovich et al. (2006)
Giesbergeria sinuosa	Pond water				Grabovich et al. (2006), Hylemon et al. (1973)
Giesbergeria voronezhensis	Wastewater				Grabovich et al. (2006)
Hydrogenophaga atypica	Activated sludge				Kämpfer et al. (2005)
Hydrogenophaga bisanensis	Wastewater of a textile dye works				Yoon et al. (2008)
Hydrogenophaga caeni	Activated sludge				Chung et al. (2007)
Hydrogenophaga defluvii	Activated sludge			Autotrophic growth with hydrogen	Kämpfer et al. (2005)
Hydrogenophaga flava	Mud from ditch			Autotrophic growth with hydrogen	Palleroni (1984), Willems et al. (1989)
Hydrogenophaga intermedia	Wastewater				Contzen et al. (2000)
Hydrogenophaga palleronii	Water			Autotrophic growth with hydrogen	Palleroni (1984), Willems et al. (1989)
Hydrogenophaga pseudoflava	Water river			Autotrophic growth with hydrogen	Palleroni (1984), Willems et al. (1989)

Table 29.24 (continued)

Species	Environmental habitats	Plant associated or plant pathogenic	Animal associated or clinical	Remarks	References
Hydrogenophaga taeniospiralis	Soil			Autotrophic growth with hydrogen	Lalucat et al. (1982)
Hylemonella gracilis	Pond water				Canale-Parola et al. (1966)
Lampropedia hyalina	Water rich with organic matter, wastewater, and rumen fluid			Grows in square sheets of cells	Lee et al. (2004)
Macromonas bipunctata	Freshwater environments with low oxygen and hydrogen sulfide concentrations				Dubinina et al. (2005)
Macromonas mobilis	Freshwater environments with low oxygen and hydrogen sulfide concentrations				Dubinina et al. (2005)
Malikia granosa	Activated sludge				Spring et al. (2005)
Malikia spinosa	River water				Leifson (1962a)
Ottowia pentelensis	Activated sludge for coke plant effluent			Mixotrophic growth with thiosulfate	Felföldi et al. (2011)
Ottowia thiooxydans	Activated sludge			Facultative anaerobic growth with nitrate; mixotrophic growth with thiosulfate	Spring et al. (2004)
Polaromonas aquatica	Tap water				Kämpfer et al. (2006)
Polaromonas cryoconiti	Glacier cryoconite			Psychrotrophic	Margesin et al. (2012)
Polaromonas glacialis	Glacier cryoconite			Psychrotrophic	Margesin et al. (2012)
Polaromonas hydrogenivorans	Forest soil Alaska			Autotrophic growth with hydrogen, psychrotrophic	Sizova and Panikov (2007)
Polaromonas jejuensis	Soil				Weon et al. (2008), Margesin et al. (2012)
Polaromonas naphthalenivorans	Coal-tar contaminated sediment			Autotrophic growth with hydrogen, psychrotrophic	Jeon et al. (2004)
Polaromonas vacuolata	Antarctic ocean			Psychrotrophic	Irgens et al. (1996)
Pseudacidovorax intermedius	Soil			Capable of nitrogen fixation	Kämpfer et al. (2008)
Pseudorhodoferax aquiterrae	Groundwater				Chen et al. (2013)
Pseudorhodoferax caeni	Activated sludge				Bruland et al. (2009)
Pseudorhodoferax soli	Soil				Bruland et al. (2009)
Ramlibacter ginsenosidimutans	Agricultural soil				Wang et al. (2012)

Table 29.24 (continued)

Species	Environmental habitats	Plant associated or plant pathogenic	Animal associated or clinical	Remarks	References
Ramlibacter ginsenosidimutans	Agricultural soil				Wang et al. (2012)
Ramlibacter henchirensis	Desert soil				Heulin et al. (2003)
Ramlibacter henchirensis	Desert soil			Cyst formation	Heulin et al. (2003)
Ramlibacter tataouinensis	Desert soil				Heulin et al. (2003)
Ramlibacter tataouinensis	Desert soil			Cyst formation	Heulin et al. (2003)
Rhodoferax antarcticus	Aquatic microbial mat, Antarctica			Photoautotrophic and photoheterotrophic, hydrogen oxidizer	Madigan et al. (2000)
Rhodoferax fermentans	Activated sludge			Photoheterotrophic	Hiraishi et al. (1991)
Simplicispira limi	Activated sludge			Facultative anaerobic growth with nitrate	Lu et al. (2007)
Simplicispira metamorpha	Infusion of freshwater mussels		Infusion of freshwater mussels		Hylemon et al. (1973)
Simplicispira psychrophila	Infusion of Antarctic mosses	Infusion of Antarctic mosses		Facultative anaerobic growth with nitrate	Terasaki (1979)
Tepidicella xavieri	Hot spring runoff			Grows up to 55 °C	França et al. (2006)
Variovorax boronicumulans	Soil				Miwa et al. (2008)
Variovorax defluvii	Sewage				Jin et al. (2012)
Variovorax dokdonensis	Soil				Yoon et al. (2006)
Variovorax ginsengisoli	Agricultural soil			Facultative anaerobic growth with nitrate	Im et al. (2010)
Variovorax paradoxus	Soil			Autotrophic growth with hydrogen (some strains)	Willems et al. (1991a)
Variovorax soli	Greenhouse soil				Kim et al. (2006)
Verminephrobacter aporrectodeae			Nephridia of the earthworm genus *Aporrectodea*		Lund et al. (2012)
Verminephrobacter eiseniae			Nephridia of the earthworm species *Eisenia foetida*		Pinel et al. (2008)
Xenophilus aerolatus	Air				Kim et al. (2010)
Xenophilus azovorans	Soil			Azo-dye carboxy-Orange II as the sole source of carbon and energy	Kulla et al. (1984)
Xylophilus ampelinus		Diseased grapevine			Willems et al. (1987)

through some selective or enrichment process indicating that there abundance is perhaps not very high (e.g., size selection for *Hylemonella gracilis* [Canale-Parola et al. 1966], enrichment through repeated dilution of cultures for *Giesbergeria* [Williams and Rittenberg 1957], enrichment for hydrogen oxidizers for *Hydrogenophaga* [Palleroni and Doudoroff 1972]).

Limnohabitans is an important planktonic representative of the Betaproteobacteria in many freshwater habitats. It belongs to the R-BT065 freshwater cluster that can be quite abundant as was shown through the application of a specific FISH probe for the group (Simek et al. 2001; Kasalicky et al. 2010; Newton et al. 2011). Despite its numerical importance, relatively little is known about the metabolic capacities of this taxon. Recent genome analyses reports hint at larger metabolic versatility with the presence of phototropic genes, genes for RuBisCO, CO dehydrogenase, ammonia monooxygenase, and sulfur-oxidizing genes (Zeng et al. 2012).

Macromonas is found in freshwater environments with low oxygen and hydrogen sulfide concentrations, for example, the surface sediment in aeration tanks of sewage treatment plants, the upper layers of mud in lakes and ponds, the hypolimnion, and the chemocline layers of freshwater lakes (Dubinina et al. 2005).

Studying the microbial community associated with hypoxic zones below a *Microcystis* bloom in Lake Taihu, *Comamonadaceae* were found to be a prominent group at all stages of the development of the hypoxia. One hypothesis for their role is that they may be involved in the decomposition of the *Microcystis* biomass (Li et al. 2012b).

Although a large majority of *Comamonas* species have been described from various environmental samples (*Table 29.24*), one species, *Comamonas odontotermitis*, has been isolated from the gut of the termite *Odontotermes formosanus* recovered from a decaying bamboo tree, although its role or persistence in the termite gut is not documented (Chou et al. 2007). More recently, an endosymbiont in the parasitic nematode *Spirocerca lupi* was found to be a novel (as yet undescribed) member of the genus *Comamonas* (Gottlieb et al. 2012).

Several *Polaromonas* species are psychrophilic, and this genus has been found as one of the dominant groups together with *Flavobacterium* and *Pseudomonas* in shallow freshwater lakes in northern Victoria Land, East Antarctica (Michaud et al. 2012). Analyses of all available long-read 16S rRNA gene sequences of *Polaromonas* phylotypes from glacial and periglacial environments worldwide revealed that *Polaromonas* phylotypes are globally distributed with weak isolation by distance patterns at global scales. Analyses of aerobiological and genomic data suggest that *Polaromonas* phylotypes may be globally distributed as dormant cells through high-elevation air currents. A glacial-ice metagenome and the two sequenced *Polaromonas* genomes contain the gene *hipA*, suggesting that *Polaromonas* can form dormant cells (Darcy et al. 2011).

Tepidicella is a moderately thermophilic and moderately alkaliphilic genus that was isolated from the runoff of a hot spring in the Furnas geothermal area on the Island of Sao Miguel in the Azores. The 16S rRNA genes of two strains were closely related to sequences of environmental clones from a deep terrestrial fracture system and a gold mine borehole. The ecological significance of these observations is at present unclear (França et al. 2006).

Malikia strains have been isolated from activated sludge. Depending on growth conditions (mainly C and N sources), reserve materials such as PHA or poly-P granules are accumulated in the cells in large numbers. This capacity for storage makes *Malikia* a potentially important player in the enhanced biological phosphorus removal (EBPR) process used in some wastewater treatment plants although this remains to be firmly proved (Spring et al. 2005).

Verminephrobacter strains were isolated from the nephridia of several lumbricid earthworm species and genera (*Oligochaeta*, *Lumbricidae*). They are aerobic, though with a preference for low oxygen concentrations, and can grow on a range of sugars, fatty acids, and amino acids, at temperatures of 10 °C to about 30 °C. These characteristics are in agreement with the conditions reported or inferred for the nephridial environment (Lund et al. 2012). These microorganisms are thought to be vertically transmitted to the next generation of worms (Davidson and Stahl 2006, 2008), and their 16S rRNA gene sequence type is rarely detected in surveys of soils or worm culture bedding (Pinel et al. 2008). This seems to suggest the bacteria do not survive in the soil and have a close symbiotic relation with the earthworms. Although it is clear that the bacteria can utilize many fatty acids, sugars, and amino acids that are to be present in the host, the potential benefit for the earthworm remains unclear (Lund et al. 2012).

Pathogenicity and Clinical Relevance

The family *Comamonadaceae* comprises two genera with plant-pathogenic species, *Xylophilus* and *Acidovorax*, although not all species of the latter genus are pathogens.

Xylophilus ampelinus is the causal agent of bacterial necrosis and canker on *Vitis vinifera*, mainly of the woody parts. It has been reported from many of the wine-producing areas of the world (Willems et al. 1987). A real-time PCR assay has been developed for the detection of this pathogen (Dreo et al. 2007).

The plant-pathogenic species of *Acidovorax* can cause considerable damage to their hosts that include some important food, feed, and ornamental species: *Acidovorax anthurii* (bacterial leaf spot of *Anthurium*), *Acidovorax avenae* (pathogenic for various *Poaceae* including oats, corn, wheat, barley, rye, sorghum, sugarcane (red stripe disease), rice seedlings (bacterial stripe disease), Italian millet, and proso millet), *Acidovorax cattleyae* (leaf spot and bud rot on *Cattleya*, *Dendrolobium*, and *Phalaenopsis* orchids and their hybrids), *Acidovorax citrulli* (pathogenic for various species of the *Cucurbitaceae*, including watermelon, cantaloupe, cucumber, and squash), *Acidovorax konjaci* (bacterial leaf blight on *Amorphophallus rivieri* cv. Konjac), *Acidovorax oryzae* (pathogenic on rice), and *Acidovorax valerianellae* (bacterial spot disease of the salad

vegetable *Valerianella locusta*) (Gardan et al. 2000, 2003; Willems et al. 1992; Schaad et al. 2008). Recently, *Acidovorax valerianellae* was also reported to infect watermelon in Korea and cause bacterial black spot disease (Han et al. 2012). An ELISA-based system was developed for the detection of *Acidovorax citrulli* in leaves and seed of Cucurbitaceae (Himananto et al. 2011). Also for *Acidovorax valerianellae*, an ELISA test for its detection in seeds was recently developed (Thiele et al. 2012).

Some species of the genera *Acidovorax*, *Comamonas*, and *Delftia* have been isolated from various clinical samples. Both *Acidovorax delafieldii* and *Acidovorax temperans* include strains that were first described as members of E. Falsen (EF) groups 13 and 16. These groups comprised clinical isolates that were received for identification at the Culture Collection of the University of Goteborg, Sweden, examined using immunological methods and provisionally placed in two unnamed groups, EF13 and EF 16 (Willems et al. 1990). No pathogenic effects are documented, and the close relationship with free-living environmental strains would suggest the presence of these strains in clinical samples is most probably opportunistic (Willems et al. 1990). Recently a further species from clinical and environmental samples, *Acidovorax wautersii*, was described (Vaneechoutte et al. 2013). These authors report that the clinical isolation of *Acidovorax* strains is fairly rare and provide a recent inventory of human *Acidovorax* isolates from urine, nasopharynx, wound secretion, tibia puncture, and foot, classified as *Acidovorax delafieldii*, *Acidovorax temperans*, or *Acidovorax avenae* without a clear description of the clinical context (Malkan et al. 2009; Willems et al. 1990; Shetty et al. 2005).

A similar observation of close relatedness of environmental and clinical strains can be made for several *Comamonas* species and *Delftia acidovorans*. *Comamonas terrigena*, *Comamonas testosterone*, *Comamonas aquatica*, and *Comamonas kerstersii* comprise strains that were first placed in the unnamed clinical group EF10. Clinical significance of these strains was undocumented (Willems et al. 1991c). However, in a recent study, Vaneechoutte et al. (2013) provide for a literature overview of case reports of *Comamonas acidovorans* and *Comamonas testosteroni* (and once *Comamonas terrigena*) involving bacteremia (Gul et al. 2007; Lair et al. 1996; Nseir et al. 2011; Siebor et al. 2007; Smith and Gradon 2003; Tsui et al. 2011), endocarditis (Cooper et al. 2005; Horowitz et al. 1990), pneumonia (Franzetti et al. 1992), catheter-related bloodstream infections (Castagnola et al. 1997; Ender et al. 1996; Le Moal et al. 2001), and ocular infections (Lee et al. 2008; Lema et al. 2001; Miño de Kaspar et al. 2000; Reddy et al. 2009; Stonecipher et al. 1991).

Application

Several species belonging to at least 12 different genera of the family *Comamonadaceae* have been isolated from activated sludge (*Table 29.24*), and several of these are able to denitrify (e.g., *Acidovorax caeni*, *Acidovorax temperans*, *Brachymonas denitrificans*, *Diaphorobacter nitroreducens*, *Hydrogenophaga caeni*, *Ottowia thiooxydans*, *Simplicispira limi*). For the removal of nutrients from wastewater, denitrification is an important process that requires sufficient organic material as electron donor. The use of polyhydroxyalkanoates (PHA) and related biodegradable polymers as substrates for a solid-phase denitrification process has been studied as an alternative to liquid carbon sources, and PHA-degrading, denitrifying bacteria were found to be predominantly members of the *Comamonadaceae* (Hiraishi and Kahn 2003). In a study of the solid-phase denitrification of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), denitrifiers were isolated and identified by 16S rRNA gene sequencing as well as by characterization of *nirS* and *nosZ* genes. The results confirm that members of *Acidovorax*, *Brachymonas*, *Comamonas*, *Diaphorobacter*, and *Simplicispira* are important active denitrifiers in this system (Kahn et al. 2007). Similarly, in a denitrifying packed-bed bioreactor using another biodegradable polymer, poly-(butanediol succinate) (PBS), as a carbon source and biofilm support, pyrosequencing showed that most of the PBS-degrading bacteria belong to the *Comamonadaceae* (Wu et al. 2013).

Brachymonas denitrificans is an active denitrifier in activated sludge (Hiraishi et al. 1995) and tannery wastewater (Leta et al. 2004). It was found to be tolerant to relatively high concentrations of chromium(III) and sulfide (Leta et al. 2004). It is studied for use as part of denitrifying biofilms for wastewater treatment. Its activity was found to be influenced by interspecies interactions (Andersson et al. 2011). A PHB-accumulating isolate of *Brachymonas denitrificans* was shown to protect *Artemia* nauplii from pathogenic *Vibrio campbellii* (Halet et al. 2007).

Diaphorobacter strains are regularly reported to be present in bioremediation-related habitats. They have been reported to be able to perform nitrification and denitrification simultaneously in wastewater (Khardenavis et al. 2007). Some isolates are capable of nitrate-dependent arsenite (III) oxidation (Sun et al. 2009), others of pyrene degradation (Klankeo et al. 2009), degradation of several organophosphorus pesticides (Liang et al. 2011), and chloroaniline (Zhang et al. 2010).

Many other *Comamonadaceae* have been obtained from polluted soils or were obtained from soil or water by selecting for the degradation of particular pollutants. For example, *Alicycliphilus denitrificans* was isolated under denitrifying conditions using cyclohexanol as sole carbon source (Dangel et al. 1988), *Pseudorhodoferax caeni* was isolated using aniline-containing enrichment cultures (Bathe 2004), *Variovorax paradoxus* was obtained from soil by enrichment with pantothenate (Davis et al. 1970), and *Xenophilus azovorans* was isolated from soil using the azo-dye carboxy-Orange II as the sole carbon source (Kulla et al. 1984). The literature on the metabolic versatility and biochemical characterization of members of soil- and water-inhabiting genera, such as *Comamonas*, *Delftia*, *Acidovorax*, and *Variovorax*, is very extensive and too diverse to summarize in a short chapter. Some particular applications are listed below.

Pseudorhodoferax caeni has been used in bioaugmentation experiments. It was detected together with other bacteria in

2,4-dichlorophenoxyacetic acid (2,4-D) degrading aerobic granular sludge, obtained through plasmid pJP4 mediated bioaugmentation (Ma et al. 2012).

During studies using a pilot-scale bioreactor for the aerobic degradation of methyl tert-butyl ether (MtBE), a widely used fuel oxygenate and widespread environmental pollutant, *Hydrogenophaga* was found to be a member of the MtBE-degrading community (Zein et al. 2004). With a view to its application for bioaugmentation of contaminated aquifers, an adhesion-deficient variant of the MtBE-degrading wild-type strain *Hydrogenophaga flava* strain ENV735 was selected, which has improved mobility through sediments (Streger et al. 2002).

A novel strain of *Delftia tsuruhatensis*, isolated from the rhizoplane of rice, was found to have plant growth-promoting potential as a diazotroph and potential biocontrol agent against 14 fungal or bacterial plant pathogens of rice (Han et al. 2005).

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30 The Family *Gallionellaceae*

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Abstract
The family *Gallionellaceae* comprises the genus *Gallionella* with one established type species, *Gallionella ferruginea*. The phylogenetic position of *Gallionellaceae*, as determined by 16S-rDNA sequence comparisons, is among the *β-proteobacteria*. Its phylogenetic neighbors are *Methylophilaceae*, *Nitrosomonadaceae*, and *Spirillaceae*. The family contains gram-negative, chemolithoautotrophic, neutrophilic, and aerobic ferrous iron-oxidizing bacteria with the ability to secrete an extracellular twisted stalk composed of numerous fibers. *Gallionellaceae* can be found where anaerobic groundwater containing ferrous iron reaches an environment that contains oxygen. Large amounts of stalk material are usually produced; this material attracts iron hydroxides and many trace metals, giving it a brown, macroscopic appearance. The stalk and iron hydroxide masses formed may eventually cause severe clogging of ditches, drinking-water wells, and any other facilities utilizing iron-bearing, anaerobic groundwater. The family is relevant to biotechnological processes, as it can be used to remove ferrous iron when producing drinking water from groundwater.

Introduction

The family *Gallionellaceae*, with one established type species, *Gallionella ferruginea*, belongs to the microorganism group named "iron bacteria." Together with members of the sheath-forming genus *Leptothrix*, *Gallionella* is a signature organism for problems related to clogging by iron oxides and iron bacteria. *Gallionella* was first described in the early nineteenth century and is by that the earliest described bacterium, but nevertheless, its physiology and phylogeny are continuously discussed. The bacterium is easily recognized under the microscope by its twisted stalk, but it is a challenge to obtain, maintain, and study pure cultures of *Gallionella*. The carbon source for *Gallionella* is carbon dioxide, and the energy source is ferrous iron which is oxidized to ferric iron. The habitats of *Gallionella* are in gradients of ferrous iron and oxygen which can be iron seeps, drainage systems, drinking-water wells, i.e., when iron-rich groundwater comes in contact with air at neutral pH. In this environment, *Gallionella* produces a twisted stalk that can reach a length of 100 μm per cell. The analysis of environmental DNA libraries has revealed that iron bacteria, to which *Gallionella* belong, comprise a diverse group of unrelated species. Recent studies have shown that production of twisted stalks is not exclusively found in the family *Gallionellaceae* but also in another genus, i.e., the marine microorganism *Mariprofundus* (Emerson et al. 2007). In the future, DNA studies of pure cultures of iron-oxidizing and stalk-forming bacteria will give a better understanding how these characteristics are distributed in the microbial world.

Historical and Current Taxonomy

The family *Gallionellaceae* comprises the genus *Gallionella* with one established type species, *Gallionella ferruginea*. Historically, *Gallionella* has been described based on its production of a twisted stalk. The first report naming *Gallionella* was published in 1836 by Ehrenberg (1836), who examined ochre masses under the microscope. However, Ehrenberg described *G. ferruginea* as a fossil infusorian and called it "die Eisenochertierchen," i.e., the small iron ochre animal. In 1879, Zopf (1879) included *G. ferruginea* in Monera. For a long time, discussion of the morphology and physiology of *Gallionella* focused on the stalks (Van Iterson 1958). Winogradsky (1888, 1922) proposed that the so-called iron bacteria, including *G. ferruginea*, were lithotrophic. He suggested that these bacteria could grow using carbon dioxide as the sole carbon source and ferrous iron as the energy source and electron donor. Hanert (2006) proposed that the genus *Gallionella* could be differentiated into two species based on the stalk morphology, i.e., *G. ferruginea* (with stalks consisting of 40 or more filaments) and *G. filamenta* (with stalks consisting of 3–8 filaments).

The sequenced type strain of *G. ferruginea* (Hallbeck et al. 1993) was isolated from a drinking-water well drilled in granitic rock and was later denoted "Johan" (Hallbeck and Pedersen 2005). The phylogenetic position of *Gallionellaceae*, as determined by 16S-rDNA sequence comparisons for *G. ferruginea*, is

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Fig. 30.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequence of the *Gallionellaceae* type strain, *Gallionella ferruginea* strain Johan (Hallbeck et al. 1993). The tree was reconstructed using a subset of sequences representative of close related genera to stabilize the tree topology. In addition, a 40 % conservation filter for the whole bacterial domain was used to remove hypervariable positions. The number in the triangle denotes number of taxa included. The bar indicates 5 % sequence divergence

among the *β-proteobacteria* (Fig. 30.1). Its phylogenetic neighbors are Methylophilaceae, Nitrosomonadaceae, and Spirillaceae. The sequence of strain Johan was obtained in 1993, and it is still the only available type strain sequence obtained from a pure culture of a *Gallionella* species. Recently, the full genome of a strain denoted ES-2 was obtained (Emerson et al. 2013), and the 16S rDNA gene of this strain indicates close relationship (98.6 %) to *G. ferruginea*.

Phenotypic Analyses

The cell of *G. ferruginea* has a curved rod shape, usually 1.6–2.5 × 0.5–0.8 μm. Cells of strain Johan were 2.3 (SD ± 0.2) × 0.7 (SD ±0.1) μm in the exponential growth phase culture and 1.7 (SD ± 0.1) × 0.6 (SD ± 0.07) μm in the stationary phase culture (Hallbeck and Pedersen 1990). The cells are gram-negative (Teichmann 1935) with an outer membrane and a thin peptidoglycan cell wall (Hallbeck and Pedersen 2005). The cell has one polar flagellum and contains carboxysome-like structures, polyhydroxybutyrate granules, and glycogen as carbon storage compounds (Lutters and Hanert 1989). The mol% G+C was reported to be 51–54.6 % for strain BD (Hanert 1989), which agrees well with the G+C content of 53 % reported for strain ES-2 with a full genome size of 3.0 Mb (Emerson et al. 2013). As indicated by its assimilation of carbon from hydrogen [^{14}C]carbonate under inorganic cultivation conditions (Hallbeck and Pedersen 1991), *G. ferruginea* is a chemolithotroph using ferrous iron as its energy source and electron donor and carbon dioxide as its sole carbon source. Mixotrophic organic carbon assimilation was found for glucose, fructose, and sucrose concomitant with carbon dioxide fixation. The autotrophic character was recently confirmed by the presence of the form II RubisCO gene cluster in strain ES-2 (Emerson et al. 2013). The ferrous iron requirement is compulsory, and growth does not occur with organic carbon as the sole electron donor. Both nitrate and ammonium are used as sources of nitrogen.

Table 30.1 compares strain Johan from a drinking-water well in Sweden, strain BD isolated from groundwater in Braunschweig, Germany (Hanert 1989), as previously presented (Hallbeck and Pedersen 2005), and strain ES-2 isolated from groundwater in Michigan, USA (Emerson and Moyer 1997), with distantly related stalk-forming bacteria denoted R-1 (Krepski et al. 2012) and *Mariprofundus ferrooxydans* (Emerson et al. 2007). Strains Johan and ES-2 grow in a defined ring on the walls of glass culture tubes with opposing gradients of oxygen and ferrous iron, while strains BD and R-1 grow in small dot-like colonies on the glass walls of the tubes. Strains Johan and ES-2 are closely related, judging from their respective 16S rRNA sequences, even though strain ES-2 reportedly lacks stalks. Hallbeck and Pedersen (1995) have demonstrated that the ability to form stalks can be lost in laboratory cultures. The absence of stalk production is the only characteristic that currently distinguishes ES-2 from *G. ferruginea*. The presence of large clusters of genes involved in the production of exopolymers in the genome of strain ES-2 (Emerson et al. 2013) therefore suggests that strain ES-2 represents a *G. ferruginea* strain that has lost its stalk-forming ability due to weak selective pressure for stalk formation during enrichment of this strain in agarose-stabilized tubes, as suggested by Krepski et al. (2012).

G. ferruginea strain Johan grows to 1–5 × 10^6 cells mL^{-1} in a generation time of approximately 8 h at the optimum temperature of 20 °C (Table 30.1). It grows in the temperature range of 5–25 °C and in the pH range of 5.0–6.5. Temperature and pH values for growth have previously been reported for *G. ferruginea*, but they have been correlated to stalk formation and not to cell growth (Kucera and Wolfe 1957; Hanert 1973). Stalk production is initiated when the pH rises above 6, which coincides with cultures' reaching the stationary growth phase. When the pH rises along with increasing oxygen tension in the tubes, ferrous iron oxidizes and precipitates as hydroxides. This chemical iron oxidation both reduces the availability of ferrous iron for growth and probably induces stalk formation.

Table 30.1

Diagnostic properties in which the three described strains differ from each other and two other stalk-forming bacteria

	Gallionella ferruginea strain BD	*Gallionella ferruginea* strain Johan	*Gallionella* sp. strain ES-2	Strain R-1	*Mariprofundus ferrooxydans*
Enrichment culture conditions	Liquid	Liquid	Agarose	Liquid	Agarose
Morphology	Curved rod	Curved rod	Curved rod	Curved rod	Curved rod
Colony form in vitro	Circular colonies	Ring on the tube wall	Ring on the tube wall	Circular colonies	X
Stalk formation in culture	+	+ or −	−	+	+
Cell dimensions, width × length (μm)	0.5–0.7 × 0.8–1.8	0.5–0.8 × 1.6–2.5	0.7 × X	1.8 × 2.1	0.8 × 4.7
Average stalk length per cell (μm)	400	60 or 0	0	X	31
Temperature range for growth (°C)	X	5–25	X	10–35	X
Optimum temperature (°C)	17	20	X	22	X
pH range for optimal growth	X	5.0–6.5	X	5.6–7.0	6.0–6.5
Generation time (h)	X	8.3	X	15	12
Motility (without stalks)	+	+	+	X	X
Chemolithotrophy	+	+	+	+	+
Autotrophy	+	+	+	+	+
Mixotrophy	X	+	X	X	X
16S rDNA identity with type strain L07897 (%)	X	100	98.6	93.6	82

X = not reported

Cholodny (1924) conducted microscopic studies of cover slips submerged in habitats containing *G. ferruginea*. He sketched detailed images of cells attached to the ends of stalks and demonstrated that the stalk was excreted by the cell and was not a living part of it. Teichmann (1935) made cultures according to Lieske (1911) and found a great number of curved rods in the fluid. This observation prompted Pringsheim (1949) to suggest that "it is not impossible that motile cells are formed under certain conditions," a conclusion later confirmed with pure cultures of strain Johan (Hallbeck and Pedersen 1990). Detailed descriptions of stalk elongation rates were obtained using single cells and measurements of iron oxidation in both natural samples and lab cultures (Hanert 1968, 1970). Other early analyses suggested various functions of the stalk of *G. ferruginea* that were later found to be irrelevant. In 1953, Beger and Bringmann (1953) compared older drawings of the stalk of *G. ferruginea* with their own electron microscopy studies and proposed that the genus *Gallionella* consisted of five species. Excellent electron microscopy images of *G. ferruginea* were presented in a thesis by Van Iterson (1958), who suggested that the stalk was a living part of the organism with sporangia in the form of membrane sacs on the stalk. Balashova (1967a, b, 1968) and Balashova and Cherni (1970) concluded that the stalk might have zoogloeal forms and budding cells on the stalks, judging from electron microscopy observations.

In the laboratory, the cells produce a twisted stalk when the population enters the stationary phase, as demonstrated by simultaneous measurement of cell growth (using fluorescent staining of living cells) and of stalk length (Hallbeck and Pedersen 1990). The stalk consists of many definite, linear, electron-opaque strands, as first demonstrated by Vatter and Wolfe (1956) and later by van Iterson (1958). The cells produce an extracellular stalk 0.3–0.5 μm wide and up to 400 μm long, composed of numerous 2-nm-wide fibers protruding from the concave side of the cell, (*Table 30.1*). The chemical composition of the stalk is still unclear, but in vitro studies of the carbon/nitrogen (C:N) ratio found that a stalk-forming strain had a C:N ratio of 6.8, while a strain that lost its ability to produce stalks had a C:N ratio of 4.3, which suggests that the stalk is made of polysaccharides and contains significantly less nitrogen than does the cell (Hallbeck and Pedersen 1995). Studies of natural samples of extracellular *Gallionella*-like stalks have confirmed that the stalk is organic in character with numerous carboxylic groups (Martinez et al. 2003).

Enrichment, Isolation, and Maintenance

There are numerous reports of successful enrichment cultures of stalk-forming *Gallionella* strains, but few have been completed as pure cultures. Adler (1904) reported small amounts of

G. ferruginea in freshwater from the iron well of Karlspader, but when the water was left in bottles for several days, they "*ausserordentlich stark vermehrt*," i.e., they had grown or, more correctly, the stalks had become elongated. Lieske (1911) succeeded in cultivating *G. ferruginea* in carbonic water with metallic iron as the ferrous iron source. In 1957, Kucera and Wolfe (1957) introduced an excellent growth medium containing iron sulfide as a source of reduced iron and demonstrated that *G. ferruginea* can be cultivated in opposing ferrous iron and oxygen gradients using carbon dioxide as the sole carbon source. The source of ferrous iron was a solid phase such as ferrous sulfide, which was later demonstrated to be replaceable with ferrous carbonate (Hallbeck and Pedersen 1995).

Various media for the enrichment and cultivation of *Gallionella* strains have been reported. To create proper growth conditions, ferrous iron and carbon dioxide must be in the medium. Kucera and Wolfe's (1957) growth medium, which consists of a salt solution and iron sulfide, made it possible to obtain pure cultures of *Gallionella*. The salt solution was initially prepared with tap water, because the medium lacked a crucial component, later found to be calcium. The ferrous iron medium has since been widely used with some minor modifications. Kucera and Wolfe's modified medium is made as follows: Screw-capped tubes (180 × 16 mm) are filled with 10 mL of salt medium consisting of 1.0 g of NH_4Cl, 0.4 g of $MgSO_4 \times 7 H_2O$, 0.1 g of $CaCl_2 \times 2 H_2O$, 0.05 g of K_2HPO_4 and 1 L of double-distilled water. The salt medium is autoclaved, chilled to 5 °C and infused with sterile, and filtered CO_2 to pH 4.6–4.8. A ferrous sulfide or ferrous carbonate precipitate (0.5 mL) is added slowly to the bottom of the tubes using a Pasteur pipette, and the tubes are left for 4–6 h to allow gradient conditions to become established before inoculation. The ferrous sulfide and ferrous carbonate must be prepared in the laboratory. Ferrous sulfide is prepared by dissolving 7.8 g of $FeSO_4(NH_4)_2SO_4$ (Mohr's salt) and 4.8 g of sodium sulfide each in 200 mL of boiling, double-distilled water, and subsequently pouring the ferrous solution into the sulfide solution. Use two 500-mL beakers and stir with a glass rod. Fill the receiving beaker to the top and seal it with a rubber stopper to prevent oxidation of the ferrous iron. Let the iron sulfide settle for at least 4 h; decant and wash the iron sulfide with boiling double-distilled water five times. After centrifuging at high velocity, collect the iron sulfide in small bottles, fill the bottles with water, and seal them with airtight lids. Sterilize the bottles at 121 °C for 20 min. Store the bottles at a cool temperature in airtight vials. Ferrous carbonate is prepared by dissolving 3.9 g of $FeSO_4(NH_4)_2SO_4$ and 1.0 g of anhydrous Na_2CO_3 each in 100 mL of boiling double-distilled water and subsequently pouring the carbonate solution into the ferrous solution, preferably under a nitrogen atmosphere. The precipitated ferrous carbonate is then washed five times with boiling double-distilled water and sterilized in closed vials at 121 °C for 20 min. Store the precipitate at a cool temperature in airtight vials. It is recommended that all solutions used for preparing the medium and ferrous iron source be filter sterilized (0.2 μm) to remove any cells or particles that may give an elevated background during microscopic counts.

The best sources for *Gallionella* enrichment are places where groundwater containing ferrous iron meets the air. These could be natural springs, drainage systems, drinking-water wells, ditches, or drilled boreholes yielding groundwater. The closer to the anaerobic environment the sample is taken, the lower the oxygen concentration and the better the chance of obtaining *Gallionella* enrichment. It is not necessary to obtain stalk material with iron oxides for the successful enrichment of *Gallionella*; rather, the opposite is the case, because the bacteria are more active in their stalkless, exponential growth condition. Since most *Gallionella* strains are sensitive to temperatures above 20 °C, water samples should be kept cool, at approximately 5 °C, until inoculation.

The growth medium must be kept cool and inoculated culture tubes should be stored at temperatures below 22 °C. The optimum temperatures for strains BD and Johan were 17 °C and 20 °C, respectively (*Table 30.1*). The iron–oxygen gradient in culture tubes is easily disturbed by temperature-imposed convection, so tubes should be protected from temperature variation and air turbulence in culture chambers. *Gallionella* growth is normally visible after 4–7 days as a ring or as colonies of whitish material on the tube wall that, with time, turn brownish-orange due to the precipitation of oxidized iron on the stalks.

Pure cultures can be obtained by serial dilution of a successful enrichment culture. The medium, together with cells and stalk material, is withdrawn using a sterile Pasteur pipette to an empty tube and shaken. This material is serially diluted 10 times to a dilution of 10^{-8}. From each dilution, five growth tubes are inoculated and the tubes are incubated until growth is visible. The culture isolation is continued using one tube from the highest dilution series that displayed growth. The dilution step is repeated at least six times before a pure culture can be obtained. Purity is checked microscopically and by using a variety of heterotrophic and autotrophic media, i.e., yeast extract bouillon, nutrient agar, and media for species of *Nitrosomonas* and *Thiobacillus*. The retention of a pure culture requires subculturing with serial dilution transfers every 4–8 weeks.

Ecology

The environments where stalk-forming *Gallionella* can be found, commonly attached to surfaces, are in slow-flowing groundwater that is rich in ferrous iron but has low organic carbon content and low oxygen tension. Typical places to search for *Gallionella* are the insides of drainpipes, storage cisterns for groundwater from deep wells, as well as inside tunnels and on rock walls subject to groundwater seepage. A common feature of these environments is that cold (i.e., below 20 °C), reduced, anaerobic, and ferrous iron-bearing groundwater reaches an atmosphere that contains oxygen. Such environments are suitable for chemolithotrophic growth with ferrous iron as the energy source and electron donor and with oxygen as the electron acceptor. A flow appears to be an absolute prerequisite for

the growth of *Gallionella*, as it continuously supplies ferrous iron and possibly also carbon dioxide from the groundwater to the attached cells. Under such conditions, the stalk may act as a holdfast and prevent the cells from being washed out to a more oxidized environment that lacks ferrous iron.

Hallbeck and Pedersen (1995) have demonstrated an additional function of the stalk. The iron oxidation that occurs in a typical *Gallionella* environment can be divided into two processes: (a) respiratory iron oxidation performed by the cells in their energy metabolism and (b) non-metabolic iron oxidation induced by the increasing oxygen tension as the anoxic groundwater reaches the atmosphere. Ferrous iron reacting with oxygen participates in a chain of reactions yielding highly reactive oxygen species such as perhydroxyl (HO_2), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO). The survival of a stalk-forming (Sta^+) *Gallionella* variant of strain Johan in media with a low or high potential for oxygen radical formation was compared with that of a variant of strain Johan that had irreversibly lost its stalk-forming ability (Sta^-). *Gallionella* Sta^+ was found to survive longer (i.e., 9 weeks) than did Sta^- (i.e., 6 weeks) in cultures with a high potential for oxygen radical formation. It was therefore suggested that the stalk of *Gallionella* protects the cells against the toxic oxygen species discussed above by directing the oxidation of iron to the stalk. This phenomenon could be compared to the action of the protein ferritin, thought to perform iron oxidation in both prokaryotic and eukaryotic cells (Artymiuk et al. 1991). Whether the iron oxidation on the stalk is enzymatic or whether the stalk acts as a surface catalyst for the oxidation reaction is not known.

In conclusion, the stalk acts as a holdfast and gives *Gallionella* the unique ability to colonize and survive in an ecological niche with high contents of ferrous iron and with some oxygen, a niche inaccessible to bacteria without a defense system against the oxygen radicals formed during the inorganic oxidation of ferrous iron.

The stalks of *Gallionella* attract large amounts of iron hydroxides and other metals. Results from well-developed in situ biofilms suggest that *Gallionella* can concentrate metals at levels up to 1×10^3-fold higher than found in the host rock and more than 1×10^6 times the levels found in the groundwater (Anderson and Pedersen 2003). The processes governing the coating of the stalks with iron hydroxides and metals are not well established, although several intriguing theories exist. It is possible that the enzymatic ferrous iron oxidation occurring when *Gallionella* is extracting electrons for energy production takes part in the buildup of metal oxides on the stalks. In this process, oxidized, ferric iron from this metabolic process would be expelled and adsorbed on the stalk. In addition, as suggested above, the stalk may act as a protective agent against the creation of toxic levels of oxygen radicals. The stalk would in this case have a ferrous iron oxidation capability and a sorption capacity for ferric iron hydroxides and other metals. An interesting function of the stalk, in line with these assumptions, has been suggested by Banfield et al. (2000). This group discovered ferrihydrite nanocrystalline growth in samples containing *Gallionella* stalks and suggested that the stalks attract or flocculate 2–3-nm ferrihydrite particles formed by the cells' enzymatic oxidation of ferrous iron. The stalks then govern an aggregate-based growth of polycrystalline materials. Surfaces of nanocrystalline iron oxyhydroxides, such as goethite formed from ferrihydrite, are active adsorption sites for metals at neutral to alkaline pH levels. *Gallionella* may, consequently, exert an important influence on the fate of metals dissolved in natural groundwater.

Applications

Gallionella, together with other iron bacteria, are mostly known for the problems they cause by clogging filters and drainage systems. However, iron oxidation and biomass production can also have beneficial effects. This capability has been employed for the treatment of iron-rich groundwater to produce drinking water (de Vet et al. 2011a, b; Katsoyiannis and Zouboulis 2006). By the use of filters like sand or granulated carbon with biofilms of *Gallionella*, ferrous iron and trace metals are very efficiently removed from the water. The produced sludge is removed by back flushing and left to settle in sedimentation basins. The fact that the iron oxides adsorb anions like phosphate and arsenate very efficiently (Kappler and Straub 2005) makes it possible to remediate polluted groundwater by the use of iron-oxidating *Gallionella* biofilms (Katsoyiannis and Zouboulis 2006). Many metals coprecipitate with iron oxides and the bacterial structures, and thereby the metal concentration in the water phase is decreased (Ferris et al. 1999, 2000). One example presented by Ferris et al. (1999) shows how masses of iron oxides adsorbed metal ions as Co, Cu, Cr, and Zn.

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31 The Family *Hydrogenophilaceae*

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Abstract
Hydrogenophilaceae, a family within the order *Hydrogenophilales*, comprises the genera *Thiobacillus*, *Hydrogenophilus*, *Petrobacter*, *Tepidiphilus*, and *Sulfuricella*. Members of the family are all Gram negative, rod shaped, and non-sporulating; the reported biochemical characteristics of individual species are highly variable concerning various morphological and chemotaxonomic properties. Most members of the family are chemolithotrophic or mixotrophic using various inorganic electron donors such as reduced sulfuric compounds or hydrogen. Members of the family are either mesophilic or moderately thermophilic and have been isolated from various environments, e.g., freshwater, aerobic digesters on water treatment sludge, and hot springs. No strains have been reported as pathogens.

Taxonomy, Historical and Current

Short Description of the Family

Hy.dro.ge.no'phil.ce.ae. N.L. masc. n. *Hydrogenophilus*, type genus of the family; suff. *-aceae*, ending to denote family; N.L. fem. pl. n. *Hydrogenophilaceae*, the *Hydrogenophilus* family.

Hydrogenophilus is the type genus of the order *Hydrogenophilales* and of the family *Hydrogenophilaceae*, phylum Betaproteobacteria (Garrity et al. 2005). The family contains the type genus *Hydrogenophilus* (Hayashi et al. 1999), *Thiobacillus* (Bejerinck 1904), *Petrobacter* (Salinas et al. 2004), *Sulfuricella* (Kojima and Fukui 2010), and *Tepidiphilus* (Manaia et al. 2003). Species within all five genera are Gram negative and rod shaped and are motile by means of polar flagellum (*Table 31.1*). Most members of the family are chemolithotrophic using various reduced sulfur compounds or hydrogen as electron donors. Some species are mixotrophic, often using organic acids for growth. Members are typically aerobic but some are microaerophilic or facultative anaerobic using nitrate instead of oxygen as final electron acceptor. The predominant fatty acids, when analyzed, are straight chain C16, C18, and cyclo-C19. The dominant quinone is Q-8. Polar lipids, when analyzed, are typically phosphatidylethanolamine and phosphatidylglycerol, similar to other Betaproteobacteria. The G+C values of DNA range between 59 and 66 mol%. Mesophilic and psychrophilic species are found in anoxic lakes, sediments of sandy aquifers, soil, and industrial waste treatment lagoons. Moderately thermophilic species are found in aerobic water treatment digesters, terrestrial oil reservoirs or hot baths, and hot springs. No isolates have been demonstrated to be pathogenic.

Phylogenetic Structure of the Family and Its Genera

Figure 31.1 shows the position of the five genera within the family. The family is clearly not monophyletic, and *Thiobacillus* and *Sulfuricella* make up a subcluster together with bacteria that belong to other orders within the Betaproteobacteria, e.g., orders *Neisseriaceae*, *Methylophilaceae*, *Nitrosomonadaceae*, and *Spirillaceae*. The other three genera of the family make up another subcluster with the order *Rhodocyclaceae*.

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Table 31.1

Morphological and chemotaxonomic characteristics of genera of Hydrogenophilaceae

	Thiobacillus[a, b, c, d]	*Hydrogenophilus*[e, f, g, h, i]	*Sulfuricella*[j]	*Petrobacter*[k]	*Tepidiphilus*[l]
Origin of isolation	Freshwater, estuarine and marine sediments, aquifers, sulfur springs, soil	Hot springs	Freshwater lake (Japan)	Oil reservoir	Water treatment sludge aerobic digester
Temperature	Mesophilic, moderate thermophilic, max 55 °C	Thermophilic, max 65 °C	Mesophilic, max 28 °C	Thermophilic, max 55 °C	Thermophilic; max 61 °C
Morphology	Rods	Rods	Rods	Rods	Rods
Gram stain	Negative	Negative	Negative	Negative	Negative
Motility	+; polar	+; polar	+	+; polar	+; polar
Metabolism	Strict chemolithotrophic	Chemolithotrophic and mixotrophic	Microaerophilic	Aerobic	Aerobic and anaerobic with NO_3
	Aerobic and anaerobic respiration with N-compounds	Aerobic	Sulfur and thiosulfate oxidation	Nitrate respiration ($NO_3 \rightarrow N_2$)	
		Hydrogen oxidizers	Reduces nitrate to nitrite		
Major polar lipids	NA	NA	NA	NA	Phosphatidylethanolamine, phosphatidylglycerol
Major fatty acids	NA	C16:0, cyclic C17:0, C18:0 (ω7), C19:0	NA	NA	C16:0; C18:1; cyclo C19:0
Menaquinone	Ubiquinone 8	Ubiquinone 8	NA	NA	Ubiquinone 8
G + C content	61–68	61–65	59	58.6	64.8

Data taken from [a]Kellerman and Griebler (2009), [b]Wood and Kelly (1988), [c]Kelly and Wood (2000a), [d]Robertsson and Kuenen (2006), [e]Hayashi et al. (1999); [f]Stöhr et al. (2001); [g]Vesteinsdottir et al. (2011); [h]Goto et al. (1977), [i]Goto et al. (1978), [j]Kojima and Fukui (2010), [k]Salinas et al. (2004), [l]Manaia et al. (2003)

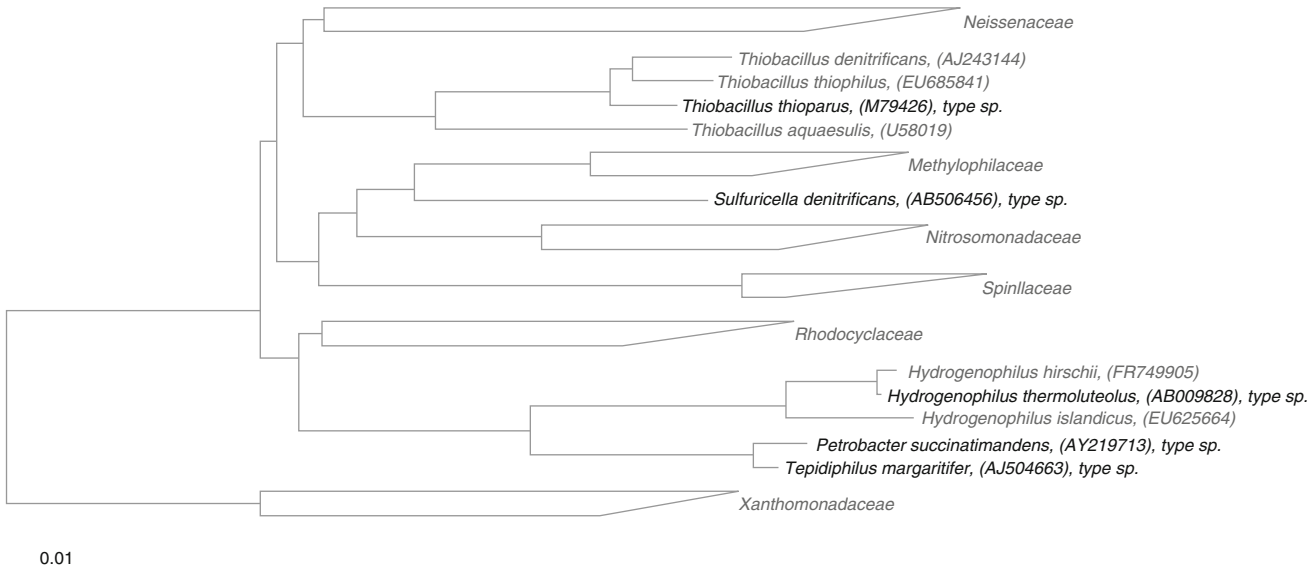

Fig. 31.1

Phylogenetic reconstruction of the family *Hydrogenophilaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum-frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

The family *Hydrogenophilaceae* was established by Garrity et al. (2005) and consists of five genera: *Hydrogenophilus*, *Petrobacter*, *Tepidiphilus*, *Thiobacillus*, *and Sulfuricella*. *Hydrogenophilus* is the type genus of the family and consists of three validly described species: *H. thermoluteolus* (type species), *H. hirshii*, and *H. islandicus*. All known species are thermophilic and were isolated from hot springs. Goto and coworkers (Goto et al. 1977, 1978) originally isolated strains that were assigned to two different genera, *Pseudomonas* and *Flavobacterium*. These strains were later reassigned to the genus *Hydrogenophilus* (with *H. thermoluteolus* as the type strain), based on further phylogenetic studies (Hayashi et al. 1999). Later, two other species were included in the family: *H. hirshii* (Stöhr et al. 2001) and *H. islandicus* (Vesteinsdottir et al. 2011). Together with *Hydrogenophilus*, *Petrobacter*, and *Tepidiphilus* form a subcluster within the family (*Fig. 31.1*) all of thermophilic origin and do not use reduced sulfur compounds (except for *H. thermoluteolus*). *Hydrogenophilus* species can oxidize hydrogen, but *Petrobacter* and *Tepidiphilus* do not grow chemolithotrophically but can use nitrate instead of oxygen as electron acceptors.

Today, the *Thiobacillus* genus has only four species but has in earlier volumes of *The Prokaryotes* received special attention and been described separately together with other "thiobacilli" and "colorless sulfur bacteria" (Robertsson and Kuenen 2006). The main reason for this reduction is based on the large number of species that were originally described as *Thiobacillus* that have now been moved to other genera. The genus was first described by Bejerinck (1904) where it was described as bacteria that could use reduced sulfur compounds as a source of energy. At this time, the genus comprised of only *T. thioparus* (type species) and *T. denitrificans*. This property of using reduced sulfur compounds, in addition to being rod shaped, non-phototrophic, and Gram negative, was used for placing new isolates within the genus for a long time. Modern taxonomic methods (16S rRNA gene sequencing and other molecular methods) have dramatically changed a number of existing or new genera, e.g., *Acidithiobacillus*, *Acidiphilium*, *Halothiobacillus*, *Paracoccus*, *Starkeya*, *Thiomonas*, and *Thermithiobacillus* (Battaglia-Brunet et al. 2011; Hirashi and Imhoff 2005; Katayama et al. 2006; Kelly and Wood 1998, 2000a; Kelly et al. 2000, 2005, 2007). In the last edition of *The Prokaryotes*, the genus consisted of three species: *T. thioparus* (type species), *T. denitrificans*, and *T. aquaesulis*. Since then, one new species has been identified, *T. thiophilus* (Kellerman and Griebler 2009), but two other have been accepted based on their 16S rRNA gene sequences, "*T. plumbophilus*" (Drobner et al. 1992) and "*T. sajanensis*" (Dul'tseva et al. 2006) but await full acceptance. *Sulfuricella denitrificans* is most closely related to *Thiobacillus plumbophilus*, showing 93 % homology, and was thus proposed as a new genus. All four *Thiobacillus* species, together with *Sulfuricella denitrificans*, are strictly chemolithoautotrophic and use reduced sulfur compounds as an energy source; all are of mesophilic or psychrophilic origin (although *C. aquaesulis* has temperature optimum at 43 °C).

Of the four validated *Thiobacillus* species, only *T. thioparus* and *T. denitrificans* have been retained through all the editions of *Bergey's Manual*, but the original isolates of these two species were lost. The type strain of the type species of the genus, *T. thioparus*, was originally isolated by Starkey (1934). A recent phylogenetic assessment of various culture collection strains of *T. thioparus* together with *T. denitrificans* has been done by Boden et al. (2012). Sequences of four examples of the Starkey type strain were identical, confirming their sustained identity after passage through different laboratories. Comparison of the 16S rRNA gene sequences of *T. denitrificans* indicates the importance of using the sequence for the type strain (NCIMB 9548 T) as a reference.

Molecular Analyses

DNA-DNA Hybridization Studies

Very few reports are on DNA-DNA hybridization analysis on bacteria within the *Hydrogenophilaceae family*. This is not surprising since three of the five genera in the family include only one species, and 16S rRNA analysis of the three *Hydrogenophilus* species shows less than 97 % similarity (Vesteinsdottir et al. 2011). The newest *Thiobacillus* species *B. thiophilus* was, according to 16S rRNA analysis, with 97.6 % similarity with *T. denitrificans* (Kellerman and Griebler 2009). To determine the genomic relatedness between these two strains, DNA-DNA hybridization was performed and showed relatedness value of 24.8 % indicating a clear distinction between the two strains.

Genome Comparison

Of the ten species that are presently members within the family, only one, *T. denitrificans* (ATCC 25259), has been sequenced for its complete genome (Beller et al. 2006a). The genome is 2,9 Mbp long and contains 2,827 protein-encoding genes, and the mol% G+C of DNA is 66.1 %. The genome contains two identical copies of the 16S rRNA gene, but these show only 97.6 % sequence identity to the type strain (NCIMB 9548 T) which is comparable to the difference between two distinct species.

Genes encoding for c-type cytochrome represent 1–2 % of the genome, and two genes were found to encode for NiFe hydrogenases which is interesting since there is no information on hydrogenases for the bacterium. More than 50 genes associated for sulfur compound oxidation were detected, and a relatively large number of genes are associated with inorganic ion transport, and heavy metal resistance is present. Finally, genes encoding for transporters of organic compounds were detected, which is not surprising since the bacterium is a strict chemolithotroph. Further studies (Beller et al. 2006b) on the expression of genes are involved in major chemolithotrophic metabolism (e.g., oxidation of sulfur compounds and fixation of carbon dioxide) under both aerobic and denitrifying conditions by using the whole genome data obtained. This further increases our knowledge of the basic regulation of genes involved during these processes

Phenotypic Analyses

The main features of *Hydrogenophilus* and *Thiobacillus* are listed in ❯ *Tables 31.2* and *31.3*, respectively.

Hydrogenophilus

Hy.dro.ge.no'phi.lus. Gr. n. hydro water; Gr. v. *genein* to produce; M.L. neut. n. *hydrogenum* hydrogen (that which produces water); Gr. adj. *philo* loving, friendly to; M.L. masc. n. *Hydrogenophilus* hydrogen lover (Hayashi et al. 1999).

The type strain of the genus is *H. thermoluteolus* TH-1^T (Hayashi et al. 1999). Gram-negative cells are slender rods ranging between 0.4 and 0.8 µm in diameter. The rods vary considerably in length (*H. hirshii*, 1.5 µm; *H. thermoluteolus*, 3.0 µm) and occur singly. Little or no data is available on pleomorphic structure depending on culture age or conditions. *H. hirshii* has polar flagella and is motile as is *H. islandicus* (Stöhr et al. 2001; Vesteinsdottir et al. 2011); *H. thermoluteolus* is not motile (Hayashi et al. 1999) although it was originally reported to be so (Goto et al. 1978). Colonies of *H. islandicus* are grayish in color and are about 1 mm in diameter, but *H. thermoluteolus* produces a dull yellow pigment (Goto et al. 1978). Optimum growth occurs at moderate thermophilic range (50–65 °C). *H. thermoluteolus* can grow at 35 °C, and *H. hirshii* has a maximum temperature of 65 °C. No spores have been detected in any species. All strains grow chemolithotrophically on hydrogen and carbon dioxide under aerobic conditions, but only *H. thermoluteolus* can use thiosulfate as electron donor (Miykae et al. 2007). The strains are also heterotrophic and grow on various organic substrates, although the extent and substrate spectrum varies to a great extent (❯ *Table 31.2*). The strains use the Calvin-Benson-Bassham cycle for carbon fixation as shown for, e.g., *H. thermoluteolus* (Hayashi and Igarashi 2002). *H. islandicus* is mixotrophic as well and able to grow on both hydrogen and butyrate as energy source (Vesteinsdottir et al. 2011).

The major cellular fatty acids in all strains are C16:0 and C18:0, but species that can be distinguished as both *H. islandicus* and *H. hirshii* also have considerable amount of cyclo C17:0, whereas *H. thermoluteolus* has higher amounts of 2-OH C10. The major quinone in all three species is Q-8.

Thiobacillus

Thi.o.ba.cill.us. Gr. n. *theion* (Latin transliteration *thium*), sulfur; L. masc. n. *bacillus*, a small rod; N.L. masc. n. *Thiobacillus*, sulfur rodlet (Bejerinck 1904).

The type strain of the genus is *T. thioparus* ATCC 8158^T (Starkey type). Cells are Gram-negative, motile rods and vary in length from 0.9 to 3.0 µm with typical diameters from 0.3 to 0.8 µm (❯ *Table 31.3*). Anaerobically grown colonies of *T. thiophilus* are circular, smooth, shiny, convex, and yellow in color with a lighter-colored fringe after two weeks of incubation (Kellerman and Griebler 2009). Cells of *T. denitrificans* are clear or weakly opalescent during anaerobic growth on thiosulfate nitrate agar but may become white with time with sulfur (Kelly and Wood 2000b). Cells of *T. aquaesulis* are small (1–2 mm), circular, convex, and smooth, becoming white or yellow with the formation of precipitated sulfur (Wood and Kelly 1988). Species of the genus are oxidase and catalase positive. Three of the strains are mesophilic, growing optimally at 25–30 °C, whereas *T. aquaesulis* is a moderate thermophile, with a T_{opt} of 43 °C. All strains grow in the presence of oxygen and reduced sulfur compounds (thiosulfate, trithionate, and tetrathionate) and produce sulfur or sulfate during aerobic growth conditions. All strains are facultative anaerobes, capable of using nitrate as an electron acceptor instead of oxygen-producing nitrite or, in the case of *T. denitrificans*, molecular nitrogen.

The dominant quinone for all four species is Q-8, and no information has been reported on the most common fatty acids in *Thiobacillus*.

Sulfuricella

Sul.fu.ri.cel'la. L. neut. n. *sulfur* sulfur; L. fem. n. *cella* a small room and, in biology, a cell; N.L. fem. n. *Sulfuricella* sulfur (−oxidizing) cell (Kojima and Fukui 2010).

The type strain, *Sulfuricella denitrificans* skB26^T, was isolated from anoxic water of a freshwater lake in Japan (Kojima and Fukui 2010); it is oxidase positive and catalase negative. Cells are 0.8–2.0 µm long and 0.4–0.6 µm wide and motile, but does not produce spores. Growth occurs up to 28 °C (optimum at 22 °C) and pH 6.0–9.0 (optimum at pH 7.5–8.0). Optimum growth was observed in medium without NaCl, and no growth occurred in medium containing more than 1.28 % NaCl.

The bacterium is strictly chemolithotrophic and oxidizes thiosulfate and sulfur to sulfuric acid in the presence of oxygen; nitrate (but not nitrite) can be utilized under anaerobic conditions with nitrogen as the end product. *S. denitrificans* skB26^T is negative for chemolithotrophic growth on sulfide, sulfite, and tetrathionate and does not utilize hydrogen and $FeSO_4$. Growth under 20 % oxygen concentration is considerably slower compared to concentrations of 2 % and 10 %. Carbon fixation occurs using the Calvin cycle. Growth of the strain was inhibited by kanamycin and ampicillin.

Tepidiphilus

Te.pi.di'phi.lus. L. adj. *tepidus* lukewarm; Gr. adj. *philos* friendly to; N.L. masc. n. *Tepidiphilus* liker of lukewarm conditions (Manaia et al. 2003).

The type species of *Tepidiphilus margaritifer* N2-214^T was isolated from an enrichment culture, growing on caprolactone, obtained from water treatment sludge aerobic digester at 60 °C; it is oxidase and catalase positive (Manaia et al. 2003). Cells are rod shaped, 2.0 µm long and 0.7 µm wide, motile by means of polar flagellum, and non-sporulating. Colonies are nacre-like and 1–2 mm in diameter after 36–48 h of growth. Growth occurs

Table 31.2

Comparison of selected characteristics of *Hydrogenophilus* species

Characteristic	*H. hirshii*	*H. thermoluteolus*	*H. islandicus*
Morphology	Rods	Rods	Rods
Motility	+	−	+
Catalase	ND	+	ND
Oxidase	ND	ND	ND
Temperature °C range (opt)	50–67	35–55	40–60
	(60–65)	(50–52)	(55)
pH range (opt)	5.5–8.0	5.5–8.0	ND
	(6.5)	(7.0)	(7.0)
Heterotrophic growth			
Acetate	+*	+	+
L-Alanine	−	−	ND
Aspartate	−	ND	ND
Beef extract	+	+	ND
Butyrate	+	−	+
Crotonate	w	ND	ND
Formate	−	−	−
Fructose	+*	−	−
D-Galactose	−	−	−
D-Glucose	+*	−	−
Glutamate	−	+	ND
Glycine	−	−	ND
Histidine	−	−	ND
α-Ketoglutarate	−	−	+
Lactate	+	+	+
Malate	+*	+	+
Oxalate	−	ND	−
Peptone	w	+	ND
Propionate	+	ND	+
L-Serine	−	ND	ND
Sorbitol	−	ND	−
Sucrose	−	−	−
Succinate	−	+	+
Threonine	−	ND	ND
Tryptone	+	+	ND
D-Xylose	−	−	−
Yeast extract	+	+	ND
Mixotrophic growth	ND	ND	+
Chemolithotrophic growth			
Hydrogen	+	+	+
Thiosulfate	−	+	−
Nitrate reduction	+	ND	ND

Data taken from Hayashi et al. (1999); Stöhr et al. (2001); Vesteinsdottir et al. (2011); Goto et al. (1977, 1978)

Symbols and abbreviations: + positive, − negative, w weakly positive, +* positive in the presence of yeast extract, *ND* not determined

Table 31.3
Comparison of selected characteristics of members of the genus *Thiobacillus*

Characteristic	*T. aquaesulis*	*T. thiophilus*	*T. denitrificans*	*T. thioparus*
Morphology	Rods	Rods	Rods	Short rods
Motility	+	ND	+	+
Catalase	ND	+	+	ND
Oxidase	+	+	+	+
Carboxysomes	ND	ND	–	+
NaCl	ND	0–2 %	ND	ND
Temperature °C range (opt)	30-55	–2–30	25–32	ND
	(43)	(25–30)	(28–32)	(25–30)
pH range (opt)	7.0–9.0	6.3–8.7	ND	5.0–9.0
	(7.5)	(7.5–8.3)	(6.8–7.4)	(6.0–8.0)
Heterotrophic growth	+*	–	–	–
Chemolithotrophic growth				
Hydrogen	ND	–	ND	ND
Thiosulfate	+	+	+	+
Trithionate	+	ND	ND	ND
Tetrathionate	+	+	+	+
Sulfur	ND	–	+	–
Ammonium	ND	–	ND	ND
Sulfide	ND	–	+	+
FeS	ND	–	+	ND
Thiocyanate	ND	–	+	+
Product of denitrification	NO_2^-	NO_2^-	NO_2^- and N_2	NO_2^-

Data taken from Kellerman and Griebler (2009), Wood and Kelly (1988), Kelly and Wood (2000), Robertsson and Kuenen (2006)
Symbols and abbreviations: + positive, – negative, * Nutrient broth and yeast extract

at above 25–61 °C (optimum at 50 °C) and pH 6.0–8.0. The strain produces polyhydroxybutyrate and can grow in the presence of 3 % NaCl.

The bacterium is aerobic, heterotrophic, and capable of growing on organic acids, some alcohols, and amino acids but is asaccharyolytic. The strain can grow anaerobically and reduce nitrate to compounds more reduced than nitrite. The strain could not grow autotrophically in medium supplemented with hydrogen carbonate on hydrogen, sulfur, or thiosulfate. However, it can reduce triphenyltetrazolium in the presence of hydrogen indicating a presence of hydrogenase activity.

According to API 50CH system, the strain shows positive growth on acetate, malate, caproate, benzoate, phenylacetate, ethanol, proline, L-asparagine, and L-glutamic acid. Strains do not utilize glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-xyloside, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, 5-ketogluconate, hydroxyproline, L-alanine, L-serine, L-glycine, L-histidine, DL-methionine, L-arginine, toluene, and caprolactone.

According to API ZYM system, the strain is positive for the following enzymes: alkaline phosphatase, esterase (C_4), esterase lipase (C_8), lipase (C_{14}), leucine acrylamidase, valine acrylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and β-glucosidase. The strain was negative for cystine acrylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-fucosidase, α-mannosidase, tweenase (Tween 80), amylase, and gelatinase and did not grow in the presence of ampicillin (10 μg) and penicillin G (10 U).

Petrobacter

Pe.tro.bac'ter. Gr. fem. n. *petra* rock, stone; M.L. masc.n. *bacter* equivalent of Gr. neut. n. *bacterion* rod, staff; N.L. masc. n. *Petrobacter* the stone bacterium (Salinas et al. 2004).

The *Petrobacter succinatimandens*-type strain, 4BON^T, was isolated from a non-water-flooded Australian terrestrial oil reservoir; it is oxidase and catalase positive (Salinas et al. 2004).

Cells are 2 μm long but only 0.3–0.4 μm wide, motile by means of one polar flagellum, and non-sporulating. Colonies are round (1–2 mm) after 1-week incubation. Growth occurs at 35–60 °C (optimum at 55 °C) and between pH 5.5 and 8.0 (optimum at pH 6.9). The strain grows in the presence of 0–3 % NaCl (optimum at 0.5 %) concentrations.

The bacterium is aerobic and heterotrophic on organic acids and ethanol. Nitrate could be used instead of oxygen as electron acceptor, being reduced to nitrous oxide with nitrite as an intermediate. Elemental sulfur, sulfate, thiosulfate, and nitrite are not used as electron acceptors.

The strain is positive for growth on fumarate, pyruvate, formate, ethanol, succinate, and yeast extract, but growth was poor in the absence of yeast extract. The strain did not growth on acetate, propionate, butyrate, valerate, isovalerate, lactate, adipate, alanine, L-glutamate, L-proline, L-serine, L-valine, fructose, glucose, lactose, maltose, ribose, xylose, vanillate, benzoate, 4-hydroxygbenzoate, 3-methylbenzoate, phenylacetate, 4-aminobenzoate, and benzaldehyde.

Isolation, Enrichment, and Maintenance Procedures

Most members of the family *Hydrogenophilaceae* are chemolithotrophic, and most species have been isolated and enriched on simple mineral medium with reduced sulfur compounds or hydrogen as the electron donors in the presence of either oxygen or nitrate. The main problem in isolation of strictly chemolithotrophic bacteria is the persistence of heterotrophic contamination because, in most cases, no carbon source other than carbon dioxide is available and cell debris that arise in growing cultures will be used as a substrate for heterotrophic contaminants. Therefore, pure cultures have to be isolated on agar as colonies repeatedly and rechecked for contaminants by cultivating them under heterotrophic conditions.

As earlier mentioned, the genus *Thiobacillus* has been dramatically reclassified in recent years and now consists of only four species (*T. thioparus*, *T. denitrificans*, *T. aquaesulis*, and *T. thiophilus*) based on the analysis of the 16S rRNA gene. These colorless sulfur bacteria have, in most cases, been isolated on mineral medium with reduced sulfur compounds as the energy source and carbon dioxide as carbon source. Most isolations have been in the presence of oxygen and/or nitrogen; the isolates have been described as aerobic or facultative anaerobes. The type species, *T. thioparus*, can be isolated from freshwater, estuarine, and marine sediments (Vishniac and Santer 1957). *T. denitrificans* can be easily isolated from similar environments, and selection can be made by using anaerobic conditions with nitrate as the electron acceptor (Kelly and Wood 2000b; Robertsson and Kuenen 2006). *T. aquaesulis* is the only moderate thermophile within the genus and was isolated from thermal sulfur spring on thiosulfate as electron donor in simple mineral medium (Wood and Kelly 1988). Finally, *T. thiophilus* was isolated from sediment collected from a tar oil-contaminated aquifer at a former gaswork site in Germany (Kellerman and Griebler 2009). In that case, initial enrichments were made on "Widdel freshwater medium" with sodium thiosulfate as the electron donor and sodium nitrate as the electron acceptor. The isolation procedure was actually performed anaerobically, and the enrichment was allowed to grow at 16 °C for 3–4 months, wherefrom dilution series led to the isolation of the bacterium on agar.

Sulfuricella denitrificans was isolated from the hypolimnion of a meromictic freshwater lake in Japan (Kojima and Fukui 2010) by using a carbonate-buffered defined medium modified from the medium for sulfate-reducing bacteria (Widdel and Bak 1992), where sulfate was eliminated and thiosulfate was used instead of sulfide as an alternative reductant and sulfur source. Enrichment was performed under anaerobic conditions (N_2/CO_2; 80/20, v/v) at 22 °C. Repeated enrichments followed by the use of agar shake dilutions resulted in a pure culture of a facultative anaerobic chemolithotrophic bacterium.

The three *Hydrogenophilus* species have all been isolated in mineral medium with hydrogen as the electron donor, oxygen as the electron acceptor, and carbon dioxide as the carbon source (Goto et al. 1977; Stöhr et al. 2001; Vesteinsdottir et al. 2011). The three species are all of thermophilic origin (from soil or water around hot spring or hot spring liquid-mud samples). Pure cultures were obtained by reinoculating positive samples several times into fresh medium, and individual colonies were selected from agar plates following end point dilutions.

Tepidiphilus margaritifer was isolated from a caprolactone enrichment culture obtained from a thermophilic aerobic digester of a domestic wastewater treatment plant in Portugal (Manaia et al. 2003). The decanted sludge was subjected to a mesobiotic anaerobic digestion followed by a thermophilic aerobic digestion (maximum temperature was 60 °C). The sludge was enriched (in air) on mineral medium at 50 °C (Manaia and Moore 2002) supplemented with polycaprolactone diol. The original enrichment resulted in a mixed culture dominated by *Bacillus* species, but *T. margaritifer* was isolated through purification of cultivable organisms in the mixed culture by using Luria-Bertani agar medium. Surprisingly, the purified isolate could not use caprolactones, but acetate served as good carbon donor which may be explained by the presence of yeast extract (0.2 g/L) in the mineral medium, tryptone (10 g/L), and yeast extract (5 g/L) in the agar medium, indicating that the organisms was using other carbon sources than caprolactones both during enrichment and isolation.

Petrobacter succinatimandens was isolated from a non-waterflooded Australian oil reservoir on anaerobically prepared *mineral medium containing acetate* (10 mM) and yeast extract (1 g/L) under a hydrogen and carbon dioxide gas phase. After three enrichment series, strains were isolated by repeated use of the Hungate roll-tube technique (Hungate 1969). Oil reservoirs are mainly thought to be anaerobic ecosystems (Magot et al. 2000), but the occurrence of aerobic and microaerophilic bacteria may be explained due to water flooding during oil extraction (Gevertz et al. 2000; Voodouw et al. 1996). Despite the presence of hydrogen as energy source in the enrichment medium, the isolate, *P. succinatimandens*, is a heterotrophic bacterium, capable of using organic acids both aerobically and anaerobically, but

not hydrogen. This is most likely due to the presence of yeast extract in the enrichment medium.

For the short-term preservation of strict chemolithotrophic bacteria, large inoculum volumes (10 % v/v) *should be used and serial transfers*. Transfers should be done with a 4-week interval and cultures kept at 4 °C. The medium-term storage of cells should be done in appropriate medium containing glycerol (20 % w/v) at −20 °C or −80 °C. Long-term preservation methods include freeze-drying and maintenance in liquid nitrogen at −196 °C.

Ecology

Habitat

The common trait among most *Hydrogenophilaceae* bacteria is their chemolithotrophic capacity. The strict chemolithotrophs, *Thiobacillus* and *Sulfuricella*, have been found in aquifers, hot springs, soil or mud, and anoxic water. A crucial factor for presence and growth is the presence of sulfides as electron donor and either molecular oxygen or nitrogen oxides as electron acceptors. Many of the "colorless sulfur bacteria" grow in narrow zones and gradients where sulfide and oxygen coexist (Nelson and Jannasch 1983), often in stratified lakes and interfaces between aerobic and anaerobic environments, sediments, and wet soils. The thiobacilli, together with mixotrophic bacteria, have been viewed as very important organisms for transformation of sulfur compounds in soil and sediments. The other members of the family, the three *Hydrogenophilus* species, *Petrobacter succinatimandens*, and *Tepidiphilus margaritifer* are all of thermophilic origin, isolated from hot springs, thermophilic digesters, and terrestrial oil reservoir. Little data is available on other habitats although the presence of both *Petrobacter* and *Tepidiphilus* species has been documented in microbial consortium with the ability to degrade cellulose, cotton, and rice straw (Wang et al. 2011) as well as acetate in anaerobic chemostat with synthetic wastewater (Tang et al. 2008).

Pathogenicity, Clinical Relevance

No species within any of the five genera that belong to the family *Hydrogenophilaceae* are known to be pathogenic.

Application

Removal of H_2S from Wastewater and Gas

The members of the *Hydrogenophilaceae* have, in general, not been used in many biotechnological applications. However, based on their chemolithotrophic ability, especially the oxidation of sulfur compounds by the thiobacilli, some members have been used for removal of contaminating sulfides, both from industrial wastewater and gaseous systems (Syed et al. 2006). The highly acidophilic thiobacilli (not discussed here) have been used extensively in mineral leaching for a long time. Leaching of uranium, gold, nickel, and zinc is an example where the acidophilic thiobacilli have been used (Brandl 2008; Chen et al. 2008; Ehrlich and Brierley 1990; Kelly 1985; Rawlings 2002).

Thiobacillus denitrificans and *T. thioparus* are those members of the neutrophilic thiobacilli that have primarily been investigated for industrial uses (Syed et al. 2006; Sublette et al. 1998). *T. thioparus* has been used for removal of H_2S from gas streams by using immobilized cells in biofilters (Kim et al. 2002; Oyarzún et al. 2003; Sublette and Sylvester 1987; Ramirez et al. 2009). The breakdown and detoxification of organic thiol compounds, such as thiocyanate and methyl sulfide, from contaminated streams and gas by *Thiobacillus thioparus* has also been studied (Aroca et al. 2007; Kanagawa and Kelly 1986; Kanagawa and Mikami 1989; Katayama and Kuraishi 1978). *T. denitrificans* has been studied for use in oxidative removal of H_2S from gas and wastewater aerobically and anaerobically through the reduction of nitrate to nitrogen gas (Kleerebezem and Mendez 2002; Soreanu et al. 2005; Zhang et al. 2009).

Corrosion of Concrete

The oxidation of sulfides and sulfur by thiobacilli results in accumulation of sulfuric acid which is known to corrode concrete structures. This is a particular problem for concrete sewer pipes used in many cities. The biologically mediated corrosion of sewer pipes is therefore a considerable cost to many municipalities, especially if the sewer water contains high concentrations of sulfates (Zhang et al. 2008). High sulfate concentrations promote the growth of sulfate-reducing bacteria, followed by sulfide oxidation and sulfuric acid formation.

Other Uses and Applications

Some hydrogen-oxidizing bacteria, in particular *Wautersia eutropha*, have attracted interest for the purpose of producing microbial biomass from hydrogen and carbon dioxide. Studies have shown that high growth yields and density up to 60 g dry cell weight (DCW) per liter have been reached by growing hydrogen-oxidizing bacteria under chemolithotrophic conditions (Taga et al. 1997). Even more interesting has been the use of autotrophic bacteria such as *Wautersia eutropha* and the *Hydrogenophilus* members for the production of polyhydroxyalkanoate-based bioplastics (Loo and Sudesh 2007). None of the *Hydrogenophilus* species discussed here have, however, been investigated for such applications.

No members of the *Hydrogenophilaceae* discussed here are known to be used as sources of any industrial enzymes. However, the presence of important enzymes of the sulfur metabolism has been demonstrated in *Thiobacillus thioparus* (Matin and Rittenberg 1971), as well as enzymes of the CO_2 fixation pathway in *T. thiophilus* (Kellermann et al. 2012). A cytochrome c from

Hydrogenophilus thermoluteolus has been isolated and its thermostability compared to related proteins (Nakamura et al. 2006).

Bacteria with similarities to *Tepidiphilus margaritifer* and *Petrobacter succinatimandens* have been identified in a microbial consortium with the ability to degrade cellulose, cotton, and rice straw (Wang et al. 2011) as well as acetate in anaerobic chemostat with synthetic wastewater (Tang et al. 2008).

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32 The Family *Methylophilaceae*

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Abstract
Methylophilaceae, a family within the order *Methylophilales*, embraces the genera *Methylophilus* (type genus), *Methylobacillus*, *Methylovorus*, and *Methylotenera*. Betaproteobacterial obligate and restricted facultative methylotrophs capable of utilizing methanol or methylamine as a sole source of carbon and energy. Do not use methane (methylobacteria). Gram-negative rods, multiply by binary fission. Assimilate C_1 compounds via the ribulose monophosphate (Quayle) cycle. Major fatty acids are $C_{16:1\omega7c}$ and $C_{16:0}$. However, obligate methylobacteria possess similar morphology and metabolic organization. Thus, the main criteria used to clarify obligate methylobacteria into separate genera and species are their genomic and phylogenetic characteristics. On the other hand, members of the family are defined by some chemotaxonomic and biochemical properties, such as specific phospholipids and enzymes which are used for the delineation of genera. Members of the family are mainly found in activated sludge, mud, river, lake and pond waters, and plants.

Taxonomy: Historical and Current

Short Description of the Family

Me. thy. lo. phi. la' ce. ae. M.L. masc. n. *Methylophilus* type genus of the family, *-aceae* ending to denote family, M.L. fem. pl. n. *Methylophilaceae* the *Methylophilus* family.

Phylogenetically a member of the order *Methylophilales*, class *Betaproteobacteia* (Garrity et al. 2005). The family contains the type genus *Methylophilus* (Jenkins et al. 1987), *Methylobacillus* (Yordy and Weawer 1977: emended by Urakami and Komagata 1986), *Methylovorus* (Govorukhina and Trotsenko 1991: emended by Doronina et al. 2005a), *Methylotenera* (Kalyuzhnaya et al. 2006).

Members of the family are not halophilic obligate or restricted facultative methylotrophs, assimilate one-carbon compounds via the 2-keto-3-deoxy-6-phospogluconate (KDPG) variant of the ribulose monophosphate (RuMP) pathway. Gram-negative rods, multiply by binary fission. Motile by means of one or several polar or subpolar flagella or nonmotile. Do not form resting bodies. Do not grow in TGY, LB, and Nutrient media. Methane is not used. Aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Major cellular fatty acids are $C_{16:1\omega7c}$ and $C_{16:0}$. The major phospholipid is phosphatidylethanolamine. Ubiquinone Q-8 is the predominant isoprenoid quinone. The phylogenetic distance between the four genera is about 93–96 % 16S rRNA gene sequence similarity.

Phylogenetic Structure of the Family and Its Genera

Analysis of the nearly complete sequences of the 16S rRNA genes indicate that methylobacteria of the family *Methylophilaceae* are separated from sequences of species representing the genera *Methylophilus*, *Methylobacillus*, *Methylovorus*, and *Methylotenera* (Fig. 32.1).

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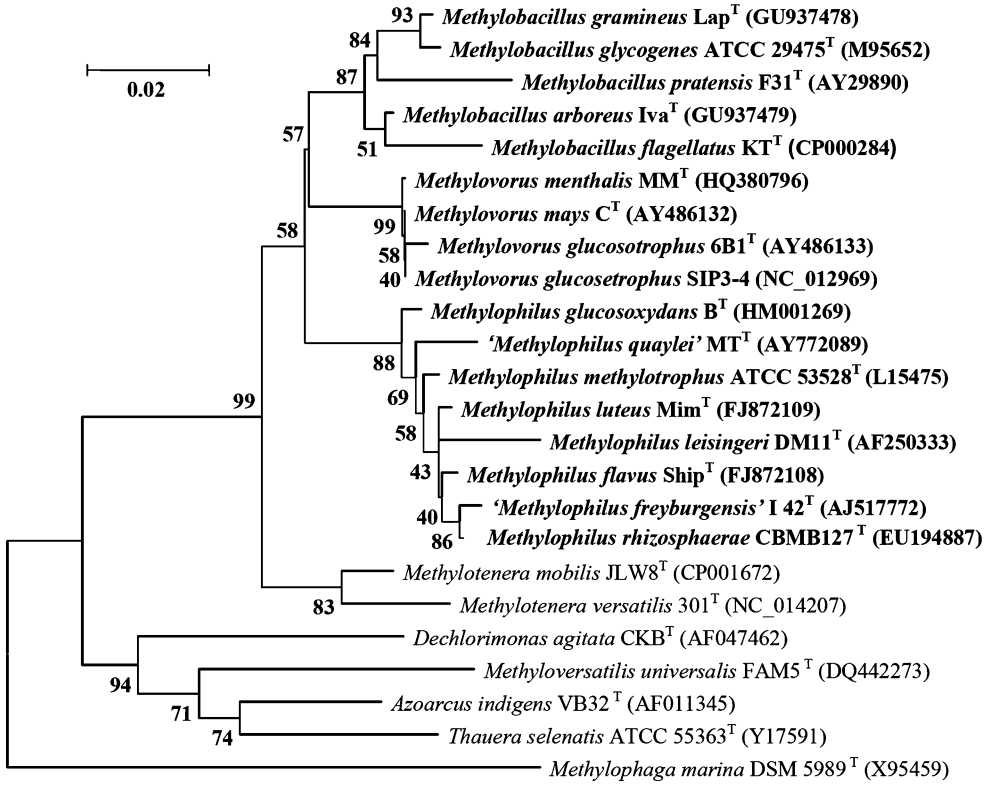

Fig. 32.1

***Maximum likelihood* phylogenetic tree showing the relationship of representatives of the *Methylophilaceae* based on 16S rRNA gene sequences. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011)**

Functional gene—the *mxaF* gene encodes the large subunit of the classical pyrroloquinoline quinone–linked methanol dehydrogenase which is found in majority of extant Gram-negative methylobacteria (Anthony and Williams 2003). Phylogenetic tree based on mxaF amino acid sequences show high levels of similarity between the members of the family *Methylophilaceae* (Fig. 32.2).

Molecular Analyses

DNA-DNA Hybridization Studies

Almost all descriptions of *Methylophilus*, *Methylobacillus*, and *Methylovorus* species include results of DNA-DNA hybridization (DDH) studies. Levels of DNA-DNA relatedness between *Methylophilus* strains—*M. methylotrophus* NCIMB 10515^T, *M. leisingeri* DSM 6813^T, *M. quaylei* VKM B-2338^T, *M. rhizosphaerae* CBMB127^T, *M. flavus* VKM B-2547^T, *M. luteus* VKM B-2548^T, and *M. glucosoxydans* VKM B-1607^T were 28–46 % (Madhaiyan et al. 2009; Doronina et al. 2012).

DNA-DNA relatedness between the reference strains of the genus *Methylobacillus* (*M. glycogens* ATCC 29475^T, *M. flagellatus* DSM 6875^T, *M. pratensis* NCIMB 13994^T, *M. gramineus* VKM B-2591^T, *M. arboreus* VKM B-2590^T) were in the range 38–45 % (Gogleva et al. 2011).

DDH relatedness between *Methylovorus glucosetrophus* VKM B-1745^T and *Methylovorus mays* VKM B-2221 was 56–58 % (Doronina et al. 2000). The levels of DNA-DNA homology between *Methylovorus menthalis* VKM B-2663^T and *M. glucosetrophus* VKM B-1745^T and *M. mays* VKM B-2221^T were 40 and 58 %, respectively (Doronina et al. 2011).

Moderate DDH relatedness of 12–18 % between type strains of the genera *Methylophilus*, *Methylobacillus*, and *Methylovorus* is a proof for intrageneric membership (Govorukhina and Trotsenko 1991; Doronina and Trotsenko 1994).

Genome Comparison

The complete genome sequence of five species branching within the 16S rRNA gene tree of *Methylophilaceae* have been released: the type strain of *Methylobacillus flagellatus* KT^T (Chistoserdova et al. 2007), *Methylotenera mobilis* JLW8^T, *Methylotenera versatilis* 301^T, and *Methylovorus glucosetrophus* SIP3-4 (Lapidus et al. 2011) and unclassified *Methylophilales* strain HTCC 2181^T

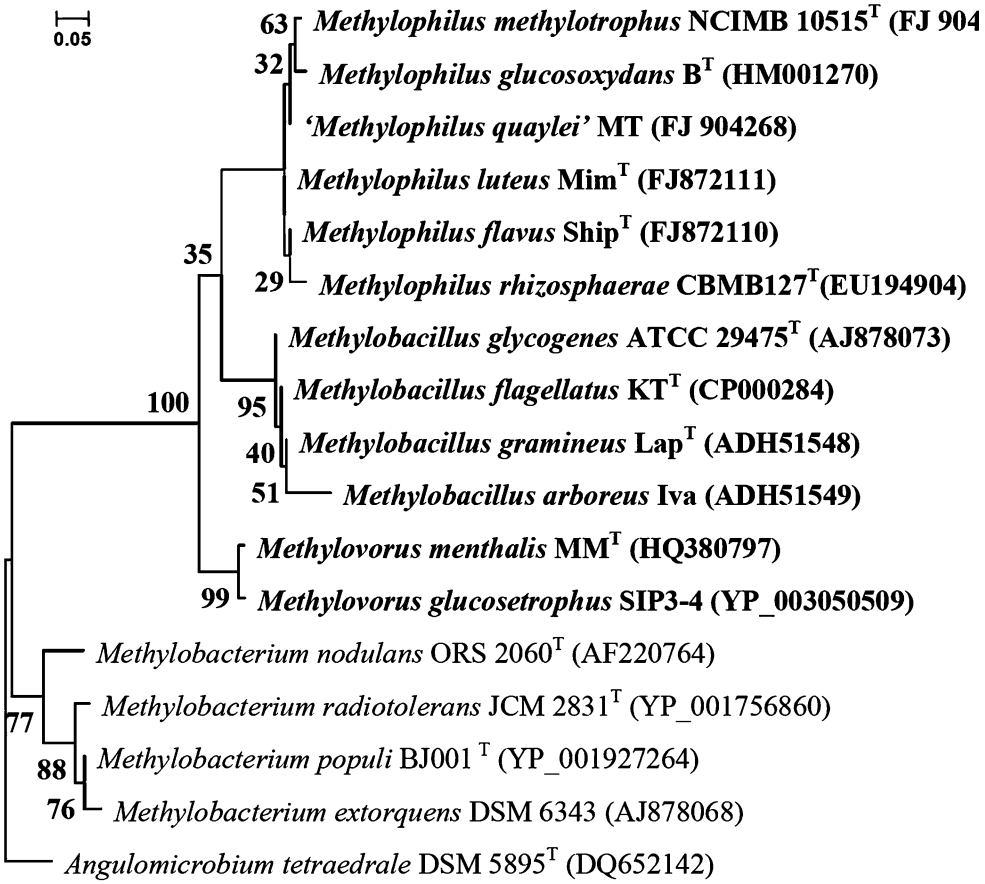

Fig. 32.2

Maximum likelihood tree based on *mxa*F amino acid sequences showing the phylogenetic position members of *Methylophilaceae* among methylotrophic bacteria. The numbers at the branch points are bootstrap values from 1,000 replicates. Bar, 5 % of evolutionary distance (5 amino acid substitutions per 100 amino acids). The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (see previous *Fig. 32.1*)

(Giovannoni et al. 2008) (*Tables 32.1–32.3*). The genome of *Methylobacillus flagellatus* KT^T represented by a single circular chromosome of approximately 3 Mbp, potentially enoding a total of 2,766 proteins.

Based on genome analysis as well as the results of mutational analyses, their methylotrophy is enabled by methanol and methylamine dehydrogenases and their specific electron transport chain components, the tetrahydromethanopterin (H_4MPT)-linked formaldehyde oxidation pathway and the assimilatory and dissimilatory RuMP cycles, and by a formate dehydrogenase. Some of the methylotrophy genes are present in more than one (identical or nonidentical copy). The obligate dependence on single-carbon compounds appears to be due to the incomplete tricarboxylic acid cycle (TCA), as no genes potentially encoding alpha-ketoglutarate, malate or succinate dehydrogenases are identifiable. The genome of *M. flagellatus* was compared in terms of methylotrophy functions to the previously sequenced genomes of three methylotrophs, *Methylobacterium extorquens* (an alphaproteobacterium, 7 Mbp) (Chistoserdova et al. 2003), *Methylibium petroleiphilum* (a betaproteobacterium, 4 Mbp) (Kane et al. 2007), and *Methylococcus capsulatus* Bath (a gammaproteobacterium, 3.3 Mbp) (Ward et al. 2004). Strikingly, metabolically and/or phylogenetically, the methylotrophy functions in *M. flagellatus* were more similar to those in *M. capsulatus* and *M. extorquens* than to the ones in the more closely related *Methylibium petroleiphilum* species, providing the first genomic evidence for the polyphyletic origin of methylotrophy in *Betaproteobacteria*.

Comprehensive proteomics to assess the expressed portion of the genome of *Methylobacillus flagellatus* was implemented (Chistoserdova et al. 2009; Chistoserdova 2011; Hendrickson et al. 2010). A total of 1,671 proteins (64 % of the inferred proteome) were detected, including all the predicted essential proteins. Nonrandom patterns observed with the nondetectable proteins appeared to the corresponding silent genomic islands, as inferred through the functional profiling and genome localization. The protein contents in methylamine- and methanol-grown cells showed a significant overlap, confirming the commonality of methylotrophic metabolism downstream of the primary oxidation reactions. The new insights into methylotrophy include detection of proteins for the *N*-methylglutamate pathway of methylamine

Table 32.1
Genome statistics and general features of *Methylophilaceae* species

Strain	Genome size (bp)	%GC DNA	No. of:				Mean coding sequence length (bp)	% Coding regions	Reference
			Proteins encoded	rRNA operons	$tRNA_s$	Replicons			
Methylotenera mobilis JLW8	2,547,570	45.51	2,348	2	46	1	975.63	89.96	Lapidus et al. (2011)
Methylotenera versatilis 301	3,059,871	42.64	2,800	3	47	1	993.59	90.26	Lapidus et al. (2011)
Methylovorus glucosetrophus SIP3-4	3,082,007	54.61	2,922	2	48	3	966.10	91.51	Lapidus et al. (2011)
Methylobacillus flagellatus KT	2,971,517	55.72	2,759	2	46	1	973.73	90.61	Chistoserdova et al. (2007)
Methylophilales strain HTCC2181	1,304,428	37.93	1,338	1	36	1	923.45	95.00	Giovannoni et al. (2008)

Table 32.2
Relationships between the five *Methylophilaceae* strains compared on the basis of 16S rRNA gene identity and on percentage of common proteins (Lapidus et al. 2011)

Strain	Gene or protein identity[a] with:				
	Mt. mobilis JLW8	*Mt. versatilis* 301	*Mv. glucosetrophus* SIP3-4	*Mb. flagellatus* KT	*Methylophilales* strain HTCC2181
Mt. mobilis JLW8		96.6	94.3	93.8	94.6
Mt. versatilis 301	51.6		93.5	93.6	94.3
Mv. glucosetrophus SIP3-4	33.6	29.6		96.5	93.9
Mb. flagellatus KT	29.6	25.9	38.7		92.9
Methylophilales strain HTCC2181	17.0	16.9	15.7	15.2	

[a]16S rRNA identity is shown in the upper right and percentage of common proteins (at 70 % identity) in the lower left

oxidation that appears to be auxiliary. Two alternative enzymes for the 6-phosphogluconate dehydrogenase reaction (GndA and GndB) and the formate dehydrogenase reaction (FDH1 and FDH4) were detected. Mutant analysis revealed that GndA and FDH4 are crucial for the organism's fitness, while GndB and FDH1 are auxiliary.

Comparative analysis revealed that the core genome of *Methylophilaceae* may be as small as approximately 600 genes, while the pangenome may be as large as approximately 6,000 genes. Significant divergence between the genomes in terms both gene content and gene and protein conservation was uncovered, including the varied presence of certain genes involved in methylotrophy. Overall, data demonstrate that metabolic potentials can vary significantly between different species of *Methylophilaceae*, including organisms inhabiting the very same environment. These data suggest that genetic divergence among the members of the family may be responsible for their specialized and nonredundant functions in C1 cycling, which in turn suggests means for their successful coexistence in the specific ecological niches.

Phenotypic Analyses

The main features of members of *Methylophilaceae* are listed in *Tables 32.4–32.7*.

Methylophilus Jenkins et al. 1987, 447[VP]; Doronina et al. 2010, 2012

Me.thy.lo.phi'lus. Fr.n. *methyle* the methyl radical; Gr.adj. *philos* loving; M.L. masc.n. *Methylophilus* methyl radical loving.

Gram-negative, asporogenous, motile by polar flagella or nonmotile rods that are 0.2–0.6 × 1.0–2.5 μm in size. Some strains produce exopolysaccharide. Colonies on methanol—minimal salt

Table 32.3

Major pathways and enzymes for carbon and nitrogen metabolism predicted from genomes (Lapidus et al. 2011)

Enzyme or pathway[a]	*Mt. mobilis* JLW8	*Mt. versatilis* 301	*Mv. glucosetrophus* SIP3-4	*Mb. flagellatus* KT	*Methylophilales* strain HTCC2181
RuMP cycle	+	+	+	+	+
Gnd enzymes	GndB	GndB	GndA	GndA, GndB	GndA
MADH	+	–	–	+	–
NMG pathway	–	+	+	+	–
H_4MPT pathway	+	+	+	+	–
H_4F pathway	+	+	+	+	+
Fae homologs	Fae2	Fae2, Fae3	Fae2, Fae3	Fae2, Fae3	–
FDH2	+	+	+	+	+
FDH4	+	–	+	+	–
MDH (MxaFJGI)	–	–	+	+	–
PQQ synthesis	+	+	+	+	+
pqqA (gene copies)	5	4	5	3	3
MxaRSACKL copies	2	2	3	3	2
XoxF (copies)	2	3	4	4	1
NapA/NirBD	+	+	+	+[b]	–
AniA/Nor	+	–	–	–	–
Urea metabolism	–	+	+	+	–
Choline degradation	–	+	–	–	–
MCA cycle	+	+	–	–	+

[a]*RuMP* ribulose monophosphate, *Gnd* 6-phosphogluconate dehydrogenase, *H_4MPT* tetrahydromethanopterin, *FDH* formate dehydrogenase, *MDH* methanol dehydrogenase, *MADH* methylamine dehydrogenase, *NMG N*-methylglutamate, *XoxF* homolog of the large subunit of MDH, *FDH* formate dehydrogenase, *NapA/NirBD* assimilatory nitrate reduction pathway, *AniA/Nor* denitrification pathway, *MCA* methylcitric acid

[b]Nonorthologous

agar plates incubated for 2 days at 29 °C—are pigmented or nonpigmented, 1–4 mm in diameter, with entire edge, convex, translucent to opaque, smooth. No or extremely poor growth on nutrient or blood agar; no hemolysis. Optimal temperature 24–37 °C; no growth occurs at 4 °C and 45 °C. Optimal pH 6.5–7.8. Aerobic. Metabolism respiratory; very little or no acid is produced from glucose. Methanol is oxidized as the sole carbon and energy source by all the strains. In addition, a very limited range of other carbon compounds such as methylated amines, formate, glucose, and fructose may be utilized as sole carbon and energy sources. Methane is not used. No vitamins or other growth factors are required. Catalase- and oxidase positive. Nitrate and ammonium salts are used as nitrogen sources. Produce indole from tryptophan on medium with nitrate as nitrogen source. The predominant cellular fatty acids are $C_{16:0}$ and $C_{16:1}$. The major isoprenoid quinone component is ubiquinone with eight isoprene units (Q-8). The predominant phospholipids are phosphatidylethanolamine and phosphatidylglycerol; diphosphatidylglycerol (cardiolipin) is absent.

Formaldehyde is assimilated via the RuMP pathway. Assimilate NH_4^+ via the glutamate cycle. The tricarboxylic acid cycle is incomplete due to the absence of α-ketoglutarate dehydrogenase. Glyoxylate shunt enzymes are absent. The mol% G + C of DNA is 48–54.

The type species is *Methylophilus methylotrophus* Jenkins, Byron, and Jones 1987. The type strain is AS1 (ATCC 53528^T = DSM 46235^T = LMG 6787^T = NCIMB 10515^T = VKM B-1623^T).

Comparison of selected characteristics of members of the genus *Methylophilus* are listed in ❯ *Table 32.4*.

Methylobacillus Yordy and Weawer, 1977, 254^{VP}, Emend. Urakami and Komagata 1986, 509

Meth.yl.o.ba.cil'lus. Fr. *methyle* the methyl radical; L.dim.n. *bacillus* a small rod; M.L. masc.n *Methylobacillus* methyl rodlet.

Cells are Gram-negative, asporogenous rods 0.3–0.6 × 0.8–2.0 μm motile or nonmotile, multiplying by binary fission, mesophilic and neutrophilic. Most strains are obligate methylotrophs; however, some strains can also use fructose. Colonies are shiny, smooth, raised, entire, white to light yellow, 1–4 mm in diameter on methanol-containing agar. Methanol is oxidized as the sole carbon and energy source by all strains. Aerobic, having a strictly respiratory type of metabolism with

Table 32.4
Comparison of selected characteristics of members of the genus *Methylophilus*

Characteristic	1	2	3	4	5	6	7
Flagellation	+	–	–	+	–	–	–
Colony pigmentation	White	Yellow	Yellow	Grayish white	Pale pink	White	White
Type of methylotrophy	Restricted facultative	Obligate	Restricted facultative	Restricted facultative	Restricted facultative	Obligate	Restricted facultative
Growth substrates	–						+
Methylamine		–	–	+	–	–	
Dimethylamine	–	–	–	+	–	–	–
Trimethylamine	–	–	–	+	–	–	+
Dichloromethane	–	–	–	–	+	–	+
Glucose	+	–	+	+	+/–[a]	–	–
Fructose	–	–	–	–	–	–	+
Urease	–	–	–	+	+	+	+
Acetoine production	–	+	+	–/+	–	ND	ND
Nitrate reduction	+	–	–	ND	ND	+	+
Fructose-1,6-bisphosphate aldolase	–	+	+	–	–	–	ND
Optimum temperature (°C)	28–30	19–24	24–26	30–37	30–35	25–29	28
Optimum pH	7.0–7.6	7.2–7.8	7.2–7.8	6.5–7.2	6.8–7.2	6.5–7.5	6.8
G + C content (*T*m) (mol%)	52.5	50.7	54.5	50.3	50.2	54.0	47.9
Isolation source	Rhizosphere of rice (*Oryza sativa* L.)	Phyllosphere of *Rosa cinnamomea* L.	Phyllosphere of *Tussilago farfara* L.	Activated sludge	Wastewater	Contaminant of *Methylocystis methanolicus*	Rhizosphere of rice (*Oryza sativa* L.)

Strains 1 *M. glucosoxydans* VKM B-1607^T (Doronina et al. 2012), 2 *M. flavus* VKM B-2547^T (Gogleva et al. 2010), 3 *M. luteus* VKM B-2548^T (Gogleva et al. 2010), 4 *M. methylotrophus* NCIMB 10515^T (Jenkins et al. 1987), 5 *M. leisingeri* DSM 6813^T (Doronina and Trotsenko 1994), 6 *M. quaylei* VKM B-2338 (Doronina et al. 2005b), 7 *M. rhizosphaerae* CBMB127^T (Madhaiyan et al. 2009). No glutamate and NADP + -specific isocitrate dehydrogenases activities were observed for all strains, and diphosphatidylglycerol was absent in the phospholipid profile. These characteristics were not determined for *M. rhizosphaerae* CBMB127^T. All strains were able to grow on methanol. Data were obtained under the same cultural conditions and by using standardized methodology except for *M. rhizosphaerae* CBMB127^T
ND Not determined
[a]Dichloromethane dehalogenase locates on a plasmid

oxygen as the terminal electron acceptor. No vitamins or other growth factors are required. Nitrate and ammonia are used as the nitrogen sources. The prevailing cellular fatty acids are straight–chain saturated $C_{16:0}$ and unsaturated $C_{16:1}$ acids. The major ubiquinone is Q-8. The predominant phospholipids are phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin (diphosphatidylglycerol). Ammonia is assimilated by glutamate dehydrogenase. The tricarboxylic acid cycle is incomplete due to the absence of α-ketoglutarate dehydrogenase. Glyoxylate shunt enzymes are absent. The mol. G + C of DNA is 50–61. The type species is *Methylobacillus glycogenes* Yordy and Weaver 1977. The type strain TK 0113 = Yordy and Weaver T-11 (= ATCC 29475^T = DSM 5685^T = JCM 2850^T = LMG 6082^T = NCIMB 11375^T = VKM B-2060^T).

Table 32.5 lists the differentiating properties of *Methylobacillus* species.

Methylovorus Govorukhina and Trotsenko 1991, 161^{VP}, Emend Doronina, Ivanova, and Trotsenko, 2005, 903

Me.thy.lo.vo'rus. N.L. *methyl* the methyl radical; N.L. masc.n adj. *vorus* consuming; N.L. masc.n. *Methylovorus* the methyl consumer.

Gram-negative rods, 0.4–0.6 × 1.0–1.4 μm. Motile by a single polar flagellum. Do not form endospores or complex intracellular membranes, either sheath or prosthecae. Some strains

Table 32.5

Major characteristics that allow differentiation among members of the genus *Methylobacillus* (Gogleva et al. 2011)

Characteristic	*M. arboreus* Iva^T VKM B-2590	*M. gramineus* Lap^T VKM B-2591	*M. flagellatus* DSM 6875^T	*M. glycogenes* ATCC 29475^T	*M. pratensis* F31^T VKM B-2224
Flagellation	1	1–4	1–4	–	1
Utilization of methylamine	–	–	+	+	+
Isocitrate dehydrogenase $NADP^+$	+	+	–	+	+
Urease	–	–	+	+	+
Acetoine production	+	+	–	–	–
Starch hydrolysis	+	+	+	–	+
Nitrate reduction	–	–	+	+	+
Growth at:					
37 °C	+	+	+	+	+
42 °C	–	–	+	–	–
3 % (w/v) NaCl	+	–	–	–	–
pH optimum	7.9–8.5	7.2–7.8	7.2–7.3	6.0–8.0	6.5–7.5
DNA G + C content (mol%)	54.0	50.5	55.5	53.2	61.5

Table 32.6

Characteristics of the type strains of the genus *Methylovorus* (Doronina et al. 2011)

Character	*M. menthalis* VKM B-2663	*M. mays* VKM B-2221^T	*M. glucosotrophus* VKM B-1745^T
Flagella	1	1	1
Methylotrophy type	Obligate	Obligate	Restricted facultative
Growth substrates:			
Methanol	+	+	+
Methylamine	–	–	–
Dimethylamine	–	–	–
Trimethylamine	–	–	–
Glucose	–	–	+
Fructose	–	–	–
Ammonium assimilation	Glutamate cycle (glutamate synthase/glutamine synthetase)		
6-phosphogluconate dehydrogenase ($NADP^+$)	–	–	–
Optimal growth temperature (°C)	24–26	35–40	35–37
pH optimum	8.5–9.0	7.0–7.5	7.2
G + C content (*Tm*), mol%	54.5	57.2	55.8
Source of isolation	*Mentha arvensis* L.	*Zea mays* L.	Wastewater

produce slime. Multiply by binary fission. No aggeregation or pigmentation in liquid medium. Colonies on methanol mineral salt agar incubated for 2 days at 30 °C are circular, 0.5–2.0 mm in diameter, with entire edges, convex and translucent to opaque, creamy or milky in color. No growth under an atmosphere of $CH_4 + O_2$ or $H_2 + CO_2 + O_2$. No growth in the presence of 3 % NaCl. Strictly aerobic with respiratory metabolism. Obligate or restricted facultative methylotrophs. Utilize methanol as the carbon and energy source. Some strains can grow poorly on glucose. Nitrates, ammonium salts, and glutamate serve as the nitrogen sources. Acetoine, H_2S, and NH_3 are not produced in test medium. Urease-, catalase-, and oxidase-positive. Peroxidase is variable. Do not degrade cellulose, gelatin, Tween 80. Indole is formed from L-tryptophan in mineral medium with methanol as the sole carbon and energy source with KNO_3 as a nitrogen source. Ammonium ions inhibit tryptophan

Table 32.7
Characteristics of the type strains of the genus *Methylotenera* (Kalyuzhnaya et al. 2012)

Character	*M. mobilis* JLW8^T	*M. versatilis* 301^T
Genome size (Mb)	2.55	3.06
DNA G + C content (mol%)	45.5	42.6
Plasmids	–	–
Copies of rRNA gene cluster	2	3
Methylamine dehydrogenase (*mau*FBEDACJG)	+	–
NMG pathway (*mgd*ABCD*gmas*A*mgs*ABC)	–	+
Methanol dehydrogenase (*mxa*FI)	–	–
Complete TCA cycle	–	–
Growth using:		
Methanol	–	+
Methylamine	+	+
Glucose	–	–
Fructose	–	+
Pyruvate	–	+
Ethanol	–	+
Nitrate as source of nitrogen	–	+

deamination. Assimilate C_1 compounds through the RuMP pathway (Entner-Doudoroff variant) and ammonia via the glutamate cycle (glutamate synthase and glutamine synthetase). Neither α-ketoglutarate dehydrogenase nor the glyoxylate shunt enzymes are present. 6-phosphogluconate dehydrogenase is specific for NAD^+ (not $NADP^+$). The prevailing cellular fatty acids are $C_{16:0}$ $C_{16:1\omega7}$. The major ubiquinone is Q-8. The predominant phospholipids are phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylcholine. Cardiolipin is present.

The G + C content of DNA is 54–58 mol%. The type species is *Methylovorus glucosotrophus* Govorukhina and Trotsenko 1991. The type strain is 6B1 (VKM B-1745^T = ATCC 49758^T = DSM 6874^T = NCIMB 13222^T = UCM B-1745^T). *Table 32.5* lists characteristics of the type strains of the genus *Methylovorus*.

Methylotenera Kalyuzhnaya, Bowerman, Lara, Lidstrom, and Chistoserdova 2006, 289^{VP}, Emend. Kalyuzhnaya, Beck, Vorob'ev, Smalley, Kunkell, Lidstrom, Chistoserdova, 2012

Me.thy.lo.te'ner.a. N.L. *methylum* (from French *me´thyle*, back-formation from French *méthylène*, coined from Gr. n. *methu* wine and Gr. n. *hulê* wood) the methyl group radical; N.L. pref. *methylo-* pertaining to the methyl radical; L. fem. adj. *tenera* delicate; N.L. fem. n. *Methylotenera* a methyl group-oxidizing delicate bacterium.

Gram-negative rods. Some strains are motile. Do not form resting bodies and multiply by binary fission. Utilize methylamine. In addition, may utilize methanol, betaine, fructose, ethanol, and pyruvate as the sole sources of carbon and energy. Some strains are positive for urease and nitrate reduction. Oxidize methylamine by methylamine dehydrogenase or via *N*-methylglutamate pathway and assimilate C_1 compounds via the RuMP pathway. The major cellular fatty acids are $C_{16:1\omega7c}$ and $C_{16:0}$. The DNA G + C content is in the range 42.6–45.5 mol%.

The type species is *Methylotenera mobilis* Kalyuzhnaya et al. 2006. The type strain is JLW8 (= ATCC BAA-1282^T = DSM 17540^T).

Comparison of selected characteristics of the type strains of the genus *Methylotenera* are listed in *Tables 32.7* and *32.8*.

Colonies of *Methylotenera mobilis* JLW8^T (= ATCC BAA-1282^T = DSM 17540 ^{T}) were cream to light brown and 1–2 mm in diameter when grown at 30 °C for 4–7 days. No pigmentation was observed when cells were grown in liquid culture (Kalyuzhnaya et al. 2006).

Colonies of *Methylotenera versatilis* 301^T were white (slightly yellowish in old cultures), mucoid, undulate, circular, convex, viscous, and up to 5 mm in diameter. The isolate grew well on solid media but not in a liquid culture. Only cultures incubated without shaking showed some growth (Kalyuzhnaya et al. 2012).

Isolation, Enrichment, and Maintenance Procedures

Isolation, Enrichment

Strains of *Methylophilus methylotrophus* have been isolated from activated sludge, mud, river, and pond water (MacLenman et al., British patent 1370892). The type strain AS1^T was isolated from activated sludge.

Methylophilus leisingeri DM11^T was isolated in Switzerland from groundwater contaminated with dichloromethane (Gaelli and Leisinger 1985; Doronina and Trotsenko 1994). 20 ml groundwater was passed through a 0.2 µm pore diameter membrane filter, and the filter was incubated in 50 ml mineral medium containing 10 mM dichloromethane. The medium contained (g/l): $K_2HPO_4 \cdot 3H_2O$, 4.1; KH_2PO_4, 1.4; $(NH_4)_2SO_4$, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2. Prior autoclaving, the pH of the medium was adjusted to 7.2. After sterilization dichloromethane was added, and 1 l of the medium was supplemented with 1 ml trace element solution (g/l): $FeSO_4 \cdot 7H_2O$, 1.0; $MnSO_4 \cdot H_2O$, 1.0; $(NH_4)_6Mo_7O_{24}$ $4H_2O$, 0.25; H_3BO_3, 1.0; $CuCl_2 \cdot 2H_2O$, 0.25; $ZnCl_2$, 0.25; NH_4VO_3, 0.1; $Co(NO_3)_2 \cdot 6H_2O$, 0.25; and $Ca(NO_3)_2$, 0.25. The bacteria were grown at 29 °C in 750-ml Erlenmeyer flasks containing 100 ml of the medium on a rotary shaker at 180 rpm. The flasks were tightly closed with rubber stoppers. Since the pH of the medium decreased when dichloromethane was used, dichloromethane was added in three portions to a final concentration of 10 mM (0.85 g/l) each after the pH of the medium had been adjusted to 7.2 by NaOH. Pure culture was isolated by the method of

Table 32.8
Differential characteristics of methylobacteria of the family *Methylophilaceae*

Characteristic	1	2	3	4
Flagellation	+/−	+/−	+	+/−
Colony pigmentation	White, yellow, grayish white, pale pink	White, light yellow	White	Cream, light brown, white, slightly yellowish
Growth substrates:				
Methanol	+	+	+	+/−
Methylamine	+/−	+/−	+/−	+
Glucose	+/−	+/−	+/−	−
Fructose	+/−	+/−	−	+/−
Methylamine dehydrogenase	+/−	+/−	−	+/−
N-methylglutamate pathway	+/−	+/−	−	+/−
Methanol dehydrogenase	+	+	+	−
Ammonia assimilation:				
Glutamate cycle	+	−	+	ND
Glutamate dehydrogenase	−	+	−	ND
Presence of 6-phosphogluconate dehydrogease ($NADP^+$ linked)	+	+	−	ND
Presence of diphosphatidylglycerol	−	+	+	ND
DNA G + C content (mol%)	48–54	50–61	54–57	42–46

Taxa: 1 *Methylophilus* (data from Doronina et al. 2012), 2 *Methylobacillus* (Gogleva et al. 2011), 3 *Methylovorus* (Doronina et al. 2011), 4 *Methylotenera* (Kalyuzhnaya et al. 2012)
"+" Positive "+/−" variable "−" negative *ND* not determined

exhausting plating of enrichment onto the same medium, containing 2.0 % purified agar Difco and 0.1 g/l of Bromothymol Blue. Petri plates were incubated in a 2.5 l desiccator, 0.2 ml portions of dichloromethane being added after 24 h. Colonies of bacteria that decompose dichloromethane are surrounded by yellow zones due to formation of HCl.

Methylophilus quaylei MT^T was isolated as an airborne contaminant during cultivation of methanotroph *Methylocystis methanolicus* on methanol (Doronina et al. 2005b). The sample of mixed liquid culture grown on medium "K" containing gl^{-1}: KH_2PO_4—2.0; $(NH_4)_2SO_4$—2.0; $MgSO_4 \cdot 7H_2O$—0.025; NaCl—0.5; $FeSO_4 \cdot 7H_2O$—0.002, pH 7.2 in Petri dish ($h = 5$ mm) was UV incubated for 10 min. After UV treatment, the culture was serially diluted and grown on "K" agar (Difco 2.0 % w/v) with 2 % (v/v) CH_3OH. A successive isolation of a single colonies resulted in the isolation of a pure culture of obligately methylotrophic strain MT^T, which could not grow on Difco nutrient agar or in atmosphere of methane: air (1:1).

Methylophilus rhizosphaerae $CBMB127^T$ was isolated from rhizosphere soils of rice cultivars (*Oryza sativa* L. cv O-dae and Nam-pyeoung, respectively) on selective ammonium mineral salt (AMS) medium (Whittenbury et al. 1970) with 0.5 % methanol. Cells were maintained on nutrient agar (NA, Difco) with 1 % (v/v) methanol or on AMS medium (Madhaiyan et al. 2009).

Strains *Methylophilus flavus* $Ship^T$ and *Methylophilus luteus* Mim^T were isolated from the phyllosphere of dog rose (*Rosa cinnamomea* L.) and coltsfoot (*Tussilago farfara* L.), respectively, sampled from the city park in Pushchino (Moscow region, Russia) (Gogleva et al. 2010). The strains were grown on medium "K." Solidified medium "K" was prepared by adding 2.0 % (w/v) Difco agar.

Methylophilus glucosoxydans B^T was isolated from rhizosphere rice (*Oryza sativa* L.) sampled from Vietnam on "K" agar with 2.0 % methanol (Doronina et al. 2012).

An enrichment culture of *Methylobacillus glycogenes* $T\text{-}11^T$ was prepared by adding a small amount of partially decayed tomato to a liquid mineral salt medium containing 2 % methanol (v/v) (Yordy and Weaver 1977). The bacterium was isolated from the enrichment culture by streaking for isolated colonies on a mineral salt agar medium containing 2 % methanol. A colony was picked and cultured at 30 °C with shaking (200 rpm) in a liquid mineral salt medium (pH 7.0), containing 2 % methanol. The procedure was repeated until a pure culture was obtained.

The strain *Methylobacillus flagellatus* KT^T was isolated by the same procedure from sewage (Govorukhina et al. 1987). *Methylobacillus pratensis* $F31^T$ was isolated on mineral salt medium, containing 0.5 % (v/v) methanol from meadow grass (*Poa trivialis* L.) sampled from the city park in Helsinki (Finland) (Doronina et al. 2004).

Stains *Methylobacillus arboreus* Iva^T and *Methylobacillus gramineus* Lap^T were isolated from willow buds (*Salix fragilis* L.) and phyllosphere of silverweed (*Potentilla anserina* L.), respectively, sampled from the city park in Pushchino (Moscow Region, Russia) (Gogleva et al. 2011).

Strain of the genus *Methylovorus* were isolated from activated sludge, mud, soil, pond water, and plants (Govorukhina and Trotsenko 1991; Seo and Kim 1993; Doronina et al. 2000, 2005a, 2011).

Methylovorus glucosotrophus $6B1^T$ was isolated from pond water (Govorukhina and Trotsenko 1991). *Methylovorus mays* C^T was isolated from maize phyllosphere (*Zea mays* L.) (Doronina et al. 2000). *Methylovorus menthalis* MM^T was isolated from the root of corn mint (*Mentha arvensis* L.) (Doronina et al. 2011).

The root was washed three times with sterile distilled water and placed into an Erlenmeyer flask (750 ml) with 200 ml of K medium and 0.5 % (v/v) methanol. After three transfers for 2 days on a rotary shaker (180 rpm) at 29 °C, the suspension of the methylobacterial enrichment culture was plated to obtaining

single colonies (exhaustive inoculation) onto agarized "K" medium with methanol. Isolated methylobacterial colonies were reinoculated on agar slants, transferred into liquid medium, and then again on solid medium for exhaustive inoculation. Reisolated methylobacterial colonies were reinoculated on slant agar. The purity of the isolated culture was controlled by light and electron microscopy, as well as by the uniformity of colonies on the agarized medium with methanol.

An obligate methylamine utilizer, *Methylotenera mobilis* JLW8^T, was isolated from Lake Washington sediment (Washington State, USA) after enrichment in a basal salt medium (Harder et al. 1973) diluted fivefold and supplemented with 0.1 % methylamine (Kalyuzhnaya et al. 2006). Also the restricted facultative methylotroph *Methylotenera versatilis* 301^T was isolated from this ecosystem by a dilution-plating approach (Kalyuzhnaya et al. 2012). To isolate strain 301^T, 1 ml aliquot of sediment samples was mixed with 9 ml filtered lake water, and serial dilutions were plated onto plates containing lake water-based medium solidified with agar (2 %; Difco) supplemented with 5 mM methylamine. After 2 weeks incubation at room temperature, individual colonies were restreaked onto fresh agar plates.

Maintenance Procedures

Generally, strains of the family *Methylophilaceae* are maintained on basal salts media as agar slants at 4 °C for 1 month. Some members of the genus *Methylophilus* must be subculturing every 10 days. For long-term storage at −20 °C or −80 °C, cells are suspended in the basal salts media and supplemented with 10 % DMSO or 20 % glycerol. Long-term preservation methods include freeze-drying in skim milk and maintenance in liquid nitrogen at −196 °C.

Ecology

Strains of the family *Methylophilaceae* are obligate or restricted facultative methylotrophs (methylobacteria) capable of growth on single-carbon compounds (methanol, methylamines, dichloromethane) and play an important role for the aerobic conversion of C1 compounds in different ecological niches. They have been isolated from activated sludge, mud, river, lake and pond waters, plant rhizosphere and phyllosphere. Association of aerobic methylobacteria with plants are permanent and results from the fact that methylobacteria consume methanol released by plant into the environment through leaf stomata (Nemecek-Marshall et al. 1995; Fedorov et al. 2011). Methanol is formed during demethylation of cell wall pectin under active growth of plant cells. Plants are therefore the main source of methanol in the biosphere (Galbally and Kirstine 2002). Association between plants and methylotrophs is mutually advantageous, because methylobacteria stimulate plant growth and development due to production of bioactive substances: phytohormones (auxins, cytokinins) and vitamins (Fedorov et al. 2011).

Methylophilus rhizosphaerae (Madhaiyan et al. 2009), *Methylophilus flavus* and *Methylophilus luteus* (Gogleva et al. 2010), *Methylophilus glucosoxydans* (Doronina et al. 2012), *Methylobacillus pratensis* (Doronina et al. 2004), *Methylobacillus arboreus* and *Methylobacillus gramineus* (Gogleva et al. 2011), *Methylovorus mays* (Doronina et al. 2000), and *Methylovorus menthalis* (Doronina et al. 2011) were isolated from plants.

Pathogenicity

No reports of the family *Methylophilaceae* causing overt or opportunistic infections in humans, animals, or insects have been published. The members of this family are obligate or restricted facultative methylotrophs and unable to grow on blood agar or other complicated media.

The type strain *Methylophilus methylotrophus* AS1 is resistant to penicillin, oleandomycin, and susceptible to nalidixic acid and streptomycin (Jenkins et al. 1987). *Methylophilus leisingeri* DM11^T (VKM B-2013^T = DSM 6813^T) is resistant to erythromycin and sensitive to nalidixic acid, novobiocin, and kanamycin (Doronina and Trotsenko 1994). *Methylophilus flavus* Ship^T (VKM B-2547^T = DSM 23073^T = CCUG 58411^T) is resistant to streptomycin and oxacillin and sensitive to ampicillin, novobiocin, nalidixic acid, gentamicin, neomycin, and lincomycin (Gogleva et al. 2010). *Methylobacillus flagellatus* KT^T (ATCC 51484 = DSM 6875 = VKM B-1610) is resistant to streptomycin, erythromycin, ampicillin, neomycin, and lincomycin and sensitive to novobiocin, nalidixic acid, kanamycin, and gentamicin (Govorukhina et al. 1987). *Methylobacillus arboreus* Iva^T (VKM B-2590^T = CCUG 59684^T = DSM 23628^T) is resistant to ampicillin, oxacillin, novobiocin, streptomycin, and neomycin and sensitive to nalidixic acid, gentamicin, kanamycin, and lincomycin (Gogleva et al. 2011). *Methylobacillus gramineus* Lap^T (=VKM B-2590^T= =CCUG 59683^T = DSM 23629^T) is resistant to novobiocin, nalidixic acid, and neomycin and sensitive to ampicillin, oxacillin, gentamicin, streptomycin, kanamycin, and lincomycin (Gogleva et al. 2011).

Mehylovorus glucosetrophus 6B1^T (=ATCC 49758^T = VKM B-1745^T = NCIMB 13222^T) is resistant to neomycin, lincomycin, erythromycin, and ampicillin, but sensitive to novobiocin, nalidixic acid, kanamycin, and streptomycin (Govorukhina and Trotsenko 1991).

Methylovorus mays C^T (=VKM B-2221^T = NCIMB 13922^T) is resistant to ampicillin and lincomycin and sensitive to gentamicin, kanamycin, nalidixic acid, neomycin, novobiocin, streptomycin, and erythromycin (Doronina et al. 2000).

Methylotenera mobilis JLW8^T (=ATCC BAA-1285^T = DSM17540^T) is sensitive to gramicidin, kanamycin, and tetracycline (Kalyuzhnaya et al. 2006). *Methylotenera versatilis* 301^T (=JCM 17579^T = VKM B-2679^T) is resistant to kanamycin, ampicillin, streptomycin, neomycin, and erythromycin (Kalyuzhnaya et al. 2012).

Application

Forage protein methylobacteria of the genera *Methylophilus* and *Methylobacillus* are characterized by higher values of specific growth rate, economic coefficient, protein and lysine content, and favorable composition of elements and fatty acids (MacLennan et al. 1974; Doronina and Trotsenko 1986; Large and Bamforth 1988; Trotsenko et al. 2005). For all the reasons above, they are preferable for large-scale cultivation and forage protein production.

Exopolysaccharides (EPSs)

Methylobacteria of the genera *Methylobacillus*, *Methylophilus*, and *Methylovorus* synthesize EPSs from methanol; of note, EPSs yield may be regulated by varying the composition of the medium and growth conditions (Doronina et al. 2005b; Gogleva et al. 2010).

Phytosymbiosis

Aerobic methylobacteria are ubiquitous in the phyllosphere and rhizosphere of plants and often colonize their seeds (Trotsenko et al. 2001; Fedorov et al. 2011). Biological testing (using *Amarantus caudatus* L. seedlings), TLC, HPLC, and solid-phase enzyme immunoassays established the presence of zeatin and zeatin riboside in the culture liquid of *Methylovorus mays* (Ivanova et al. 2000). It was also demonstrated that representatives of the genera *Methylophilus*, *Methylobacillus*, and *Methylovorus* synthesize indole compounds, particularly indoleacetic acid (IAA) when grown in media containing methanol or methylamine in the presence of 5 mM L-tryptophan (Doronina et al. 2002; Gogleva et al. 2010, 2011).

The synthesis of auxins in methylobacteria depended on the composition of the medium: when $(NH_4)_2SO_4$ was replaced by KNO_3, the amount of IAA synthesized increased three- to fivefold.

Methylovorus mays exerted a beneficial effect on the growth and morphogenesis of tobacco, potato, and fiber flax grown in vitro (Kalyaeva et al. 2001). This obligate methylobaterium stimulated morphogenesis and antifungal resistance of Chinese cabbage *Brassica chinensis* L. (Doronina et al. 2009). The ability of methylobacteria to stimulate the growth and morphogenesis of plants indicates their promise in experimental plant physiology and agrobiotechnology.

Enzymes

Enzymological studies demonstrated that a number of enzymes of methylobacteria exhibit very high activity. These results provided a basis for isolating pure preparations of various dehydrogenases and other enzymes, which have a potential as research reagent and analytical tools: glucose-6-phosphate dehydrogenase (EC 1.1.1.49; 250U/mg protein) (Sokolov et al. 1980); $NADP^+$ glutamate:dehydrogenase (EC 1.4.1.4; 180 U/mg protein) (Sokolov and Trotsenko 1987).

Biodegradation of Toxic Compounds

Methylobacteria of the family *Methylophilaceae* degrade a broad spectrum of highly toxic compounds: methanol, formaldehyde, methylated amines, and dichloromethane. The culture of *Methylobacillus* sp. is appropriate for elimination of methanol from industrial sewage (cellulose sulfate manufacturing facilities) (Trotsenko et al. 2005).

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33 The Genus *Neisseria*

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Abstract

The genus *Neisseria* comprises a number of closely related Gram-negative organisms isolated from humans and animals. Their interrelationships are poorly resolved by phenotypic approaches, and the classification of the species groups by molecular techniques has been confused by a combination of their genetic similarity and extensive shared sequence polymorphism as a consequence of shared ancestry, horizontal genetic exchange, or both. Whole genome sequence analysis, especially of large numbers of draft genome sequences, has enabled the comparison of core genes across the genus, and this has proved to be an effective means of defining species groups within the genus. This redefinition is largely consistent with previous species designations with relatively few adjustments necessary. Most members of the genus are not, or are very rarely, pathogenic, but the genus contains the globally significant pathogens *Neisseria meningitidis*, the meningococcus, and *Neisseria gonorrhoeae*, the gonococcus. The meningococcus is an "accidental pathogen": predominantly existing as a harmless commensal, with very few infections resulting in pathology. Pathology does not appear to play a role in the transmission of this organism, although some genotypes have a greater propensity to cause disease than others. The majority of work on the genus concentrates on the two pathogenic species, with attempts to develop a comprehensive vaccine against the meningococcus a major driver for research. The gonococcus is antigenically highly diverse but genetically quite uniform, and probably emerged from a single clone that changed its niche. Emerging antibiotic resistance of the gonococcus currently represents the most significant global health challenge presented by this genus.

Taxonomy: Historical and Current

Short Description of the Genus

The genus *Neisseria* is a group of Gram-negative, oxidase positive, aerobic β-Proteobacteria bacteria within the family *Neisseriaceae* (*Fig. 33.1*, *Table 33.1*) and are mostly diplococci (Tonjum 2005). The members of the genus are all very similar phenotypically and genetically and are poorly resolved from each other with 16S rDNA phylogenies (Harmsen et al. 2001; Bennett et al. 2012). They have conventionally been distinguished with a range of phenotypic tests, especially sugar utilization characteristics (Tonjum 2005), but genomic analyses have been required to fully characterize the members of the genus (Bennett et al. 2012).

The genus represents an excellent model system for studying the genetic traits of closely related organisms that exhibit very different phenotypes (Maiden 2008; Falush 2009; Bratcher et al. 2012), and members of the genus, especially the meningococcus and gonococcus, have been in the forefront of work on the population biology of bacteria in general and pathogens in particular (Spratt and Maiden 1999). The meningococcus was among the first organisms to be investigated by multilocus enzyme electrophoresis (MLEE) (Caugant et al. 1986) and the first to be studied with multilocus sequence typing (MLST) (Maiden et al. 1998). Population genetic studies indicate that most invasive disease cases are caused by a limited number of hyperinvasive lineages, whereas carriage isolates belong to many different lineages, many of which have never been associated with disease (Claus et al. 2005). In the context of its close relatives, comparative studies have provided valuable insights into meningococcal biology and understanding its pathogenicity (Maiden 2008). The gonococcus appears to be the descendant of a single clone of the ancestral population that gave rise to present-day meningococci and gonococci, that changed its primary niche from the oral cavity to the urogenital tract (Bennett et al. 2007).

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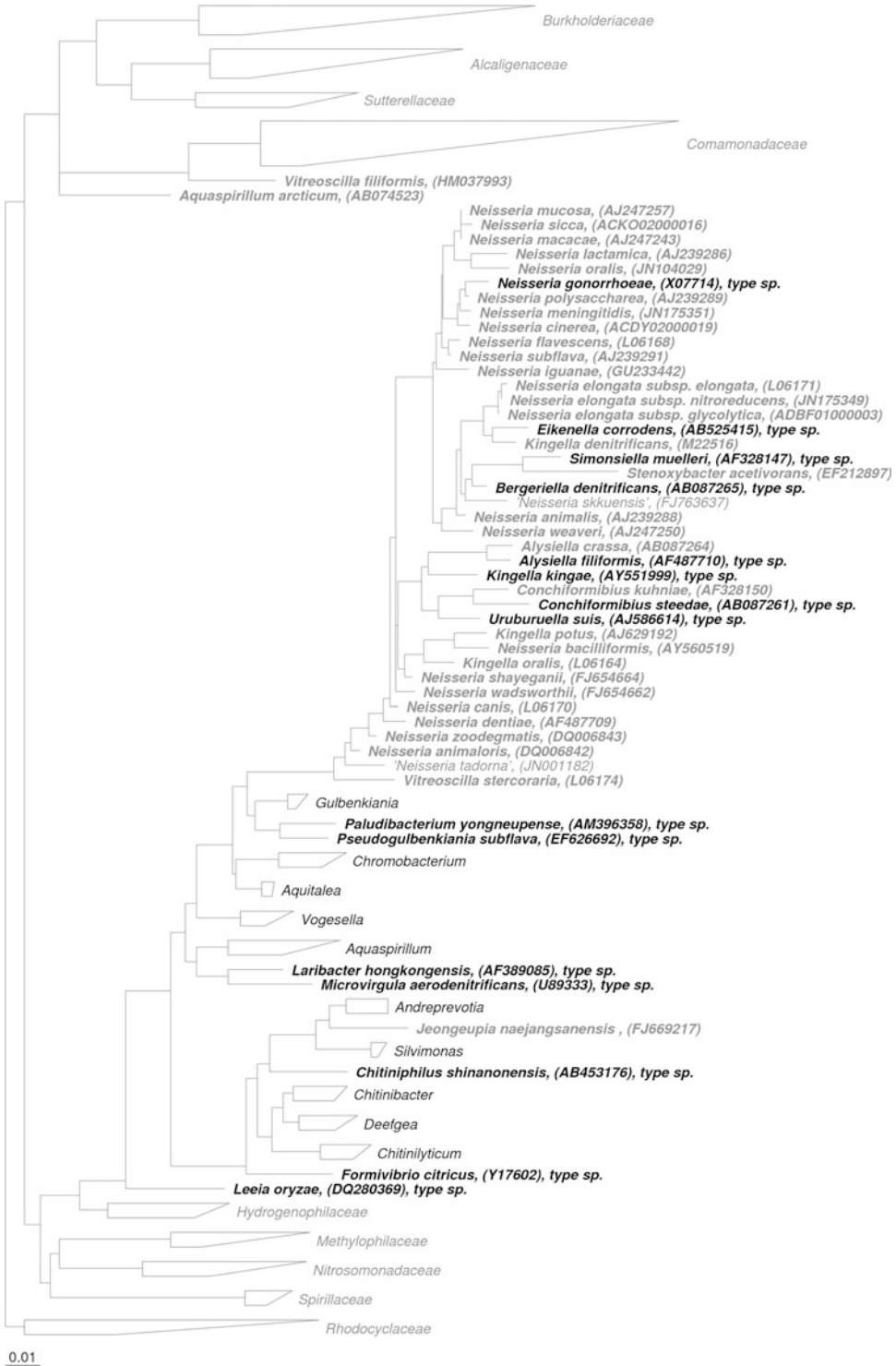

Fig. 33.1

Phylogenetic reconstruction of the position of the genus *Neisseria* within the family *Neisseriaceae* based on the maximum likelihood algorithm RAxML (Stamatakis 2006). Sequence dataset and alignments according to the All-Species Living Tree Project, release LTPs108 (Yarza et al. 2010). Representative sequences from close relative genera were used to stabilize the tree topology. In addition, a 40 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence. The family *Neisseriaceae* comprises the genera *Alysiella*, *Amantichitinum*, *Andreprevotia*, *Aquaspirillum*, *Aquitalea*, *Bergeriella*, *Chitinibacter*, *Chitinilyticum*, *Chitiniphilus*, *Chromobacterium*, *Conchiformibius*, *Deefgea*, *Eikenella*, *Formivibrio*, *Gulbenkiania*, *Iodobacter*, *Jeongeupia*, *Kingella*, *Laribacter*, *Leeia*, *Microvirgula*, *Morococcus*, *Neisseria*, *Paludibacterium*, *Prolinoborus*, *Pseudogulbenkiania*, *Silvimonas*, *Simonsiella*, *Snodgrassella*, *Stenoxybacter*, *Uruburuella*, *Vitreoscilla*, and *Vogesella*

Table 33.1

Named members of the genus *Neisseria*. Names in quotation marks have not been published in the *International Journal of Systematic and Evolutionary Microbiology* and are therefore not considered to have valid species names

Species	Notes	Reference strain	Reference
N. meningitidis		ATCC 13077	Weichselbaum 1887; Bergey and Buchanan 1974
N. gonorrhoeae		ATCC 19424	Trevisan 1885; Bergey and Buchanan 1974
N. lactamica		ATCC 23970	Hollis et al. 1969
N. polysaccharea		ATCC 43768	Riou and Guibourdenche 1987
"*N. bergeri*"	Originally designated *N. polysaccharea*, but ribosomal multilocus sequence typing (rMLST) data suggest the organism described is a separate species	CCUG 18032	Berger 1985; Bennett et al. 2012
N. cinerea		ATCC 14685	Murray 1939
N. flavescens	Species by rMLST: *N. subflava*	ATCC 13120	Branham 1930
N. subflava	*Includes previous species N. perflava and N. flava*	ATCC 49275	Bergey and Buchanan 1974
N. mucosa		ATCC 19696	Véron et al. 1959
"*N. mucosa* subsp. *heidelbergensis*"	See *N. oralis*	ATCC 25999	Berger 1971
N. sicca	rMLST data suggest that this is the same species as *N. mucosa*	ATCC 29256	Bergey and Buchanan 1974
N. macacae	rMLST data show that this is the same species as *N. mucosa*	ATCC 33926	Vedros et al. 1983
N. oralis	rMLST data show that this is the same species as "*N. mucosa* subsp. *heidelbergensis*"	LMG 26725	Wolfgang et al. 2012
N. elongata subsp. *elongata*		ATCC 25295	Bovre and Holten 1970
N. elongata subsp. *glycolytica*		ATCC 29315	Henriksen and Holten 1976
N. elongata subsp. *nitroreducens*		ATCC 49377	Anonymous 1991
"*N. elongata* subsp. *intermedia*"		CCUG 4554	Barrett and Sneath 1994
N. bacilliformis		ATCC BAA-1200	Han et al. 2006
N. wadsworthii		DSM 22247	Wolfgang et al. 2011
N. shayeganii		DSM 22246	Wolfgang et al. 2011
N. weaveri		ATCC 51410	Holmes et al. 1993
N. zoodegmatis		NCTC 12230	Vandamme et al. 2006
N. animaloris		NCTC 12228	Vandamme et al. 2006
N. dentiae		ATCC 700276	Anonymous 1997
N. canis		ATCC 14687	Berger 1962
N. animalis		ATCC 14678	Berger 1960
N. iguanae		ATCC 51483	Anonymous 1994
"*N. skkuensis*"		SMC-A9199	Lee et al. 2010
"*N. tadorna*"		20101216Y2	Yanhong et al. 2011

Molecular Analyses

Genome Structure

High-quality finished and annotated genome sequences for three meningococcal isolates, MC58 (Tettelin et al. 2000), Z2491 (Parkhill et al. 2000), and FAM18 (Bentley et al. 2007), and one *Neisseria lactamica* isolate (Bennett et al. 2010), demonstrate a genome size of about 2.2 Mbp, containing approximately 2,000 protein coding sequences, with an average G+C in the region of 51.5 %. These data are consistent with other published genomes, mostly from the meningococcus and, at the time of writing, high-quality draft genomes were available for over 100 *Neisseria* isolates, including at least one representative for type strains of each of the named species in *Table 33.1*, with the exception of *Neisseria zoodegmatis*, *Neisseria animaloris*, *Neisseria iguanae*, "*Neisseria skkuensis*," and "*Neisseria tadorna*" (Schoen et al. 2008; Marri et al. 2010; Budroni et al. 2011; Bennett et al. 2012). About 80 % of the genomes contain protein coding sequences and the finished genomes are largely co-linear; however, inversions around the origin of replication are seen in a number of these isolates (Bennett et al. 2010; Bratcher et al. 2012) and there is also extensive evidence for frequent inter- and intra-genomic genetic exchange (Bentley et al. 2007). Members of the genus have many associated non-chromosomal genetic elements, plasmids, prophages, and genetic islands (Snyder et al. 2009). At least one independent genetic element, thought to be a bacteriophage, has been associated with virulence in the meningococcus (Bille et al. 2005, 2008) and the gonococcus and some meningococci contain a 57 kb genomic island, also thought to be mobile among strains (Snyder et al. 2005; Dominguez et al. 2011). Plasmids are common in *N. gonorrhoeae* and have been observed in *N. lactamica*, but have been rarely reported in the meningococcus. Members of the genus are also characterized by large numbers of restriction and modification systems (Lau et al. 1994; Stein et al. 1995; Claus et al. 2000; Budroni et al. 2011). A large number of genes are phase variable (Jordan et al. 2005; Bentley et al. 2007).

Molecular Phylogeny

Genetic taxonomy in *Neisseria* was pioneered by Catlin and Cunningham in 1961 (Catlin and Cunningham 1961), who demonstrated close relationships between most *Neisseria* species. Members of the genus *Neisseria* are poorly resolved by techniques for bacterial speciation such as DNA-DNA hybridization (Guibourdenche et al. 1986) and *16S rRNA* sequencing (Harmsen et al. 2001; Bennett et al. 2012). Phylogenies produced from concatenated MLST fragments (MLSA) (Gevers et al. 2005) can be used to distinguish *N. meningitidis*, *N. gonorrhoeae*, and *N. lactamica* (Bennett et al. 2007), but are unsatisfactory for examination of the genus as a whole, as robust species-specific groups are not generated (Bennett et al. 2012) and some genes are absent from some species. Shared ancestry among conserved genes or horizontal genetic exchange among *Neisseria* contributes to difficulties in speciation (Bennett et al. 2012), but horizontal genetic exchange among species, at least in the core genome, is not as frequent as has been reported (Unemo and Dillon 2011). Ribosomal multilocus sequence typing (rMLST) (Jolley et al. 2012a) overcomes these problems, as it indexes variation in the 53 complete ribosomal protein subunit genes that encode the bacterial ribosomal proteins, which are present in all *Neisseria* (Bennett et al. 2012).

A phylogeny produced from the concatenated ribosomal protein subunit genes from 21 named species, generates distinct species groups, with *N. gonorrhoeae* most closely related to *N. meningitidis* (*Fig. 33.2*). The phylogeny supports previous deoxyribonucleic acid relatedness studies (Guibourdenche et al. 1986), which showed that *N. meningitidis*, *Neisseria*

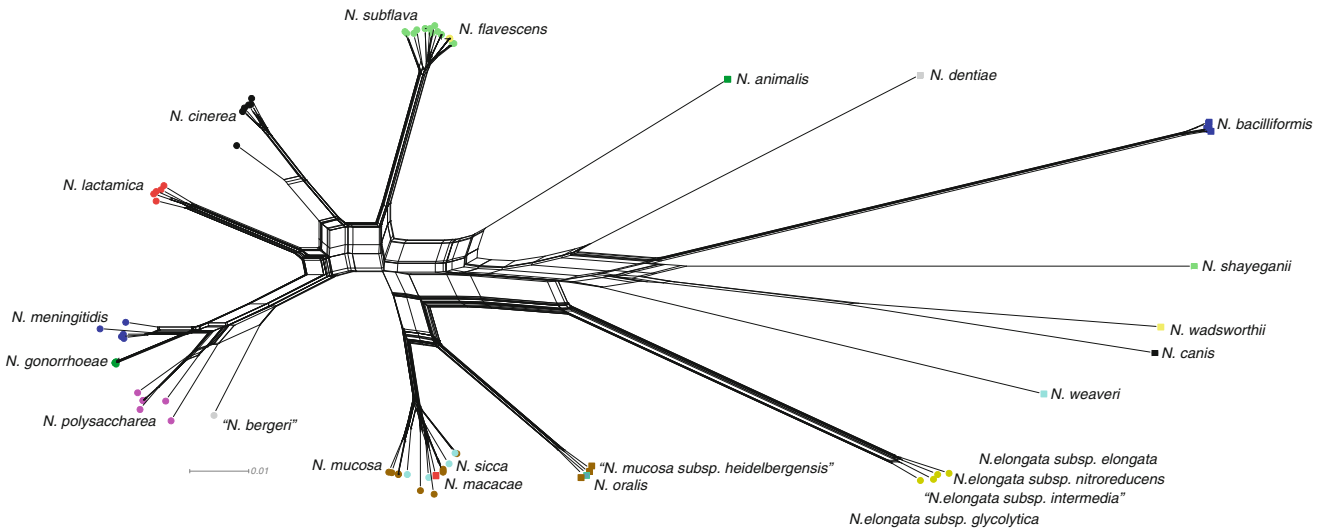

Fig. 33.2
Phylogeny of the genus *Neisseria* reconstructed with 53 concatenated *rps* genes (rMLST)

polysaccharea, *N. gonorrhoeae*, and *N. lactamica* were closely related. Another putative species is also very closely related genetically to *N. meningitidis* (Bennett et al. 2012). The proposed name for this species is "*Neisseria bergeri*," after the author who first described the strain as a phenotypically distinct strain of *N. polysaccharea* (Fig. 33.2; Berger 1985). This phylogeny is supported by one constructed from the concatenated sequences of 246 genes present in all members of the genus (Bennett et al. 2012).

The rMLST phylogeny refines the relationships among members of the genus, resolving a number of issues. Importantly, despite evidence for frequent recombination within and among currently recognized species groups in the genus (Spratt et al. 1991), the phylogenies reconstructed from the 53 ribosomal protein subunit genes, and indeed other "core" genes, demonstrate a coherent structure, with all isolates examined so far by this means being members of well-resolved clusters. These clusters are largely, but not entirely, congruent with species designations arrived at with more conventional techniques, and suggest that a number of reassignments of bacterial species names might be usefully made within the group (Bennett et al. 2012). This coherence is consistent with most interspecies recombination mostly involving the "accessory genome," those genes that are not universally present even within one species group (Hotopp et al. 2006; Bennett et al. 2010; Marri et al. 2010).

Within the rMLST phylogeny (Fig. 33.2), the *Neisseria cinerea* isolates form a species-specific group, although a subspecies may be present. *Neisseria subflava* and *Neisseria flavescens* isolates are present in a single cluster, and it has been suggested that *N. flavescens* should be reclassified as *N. subflava*, due to its genetic relatedness (Bennett et al. 2012). Three previously defined species, *Neisseria mucosa*, *Neisseria macacae*, and *Neisseria sicca*, cluster within a single distinct group, and these should be considered a single species (*N. mucosa*) based on their genetic relatedness (Bennett et al. 2012). Strains originally defined as "*Neisseria mucosa* subsp. *heidelbergensis*" (Berger 1971), a subspecies not officially validated, are very closely related to the recently defined species *Neisseria oralis* (Wolfgang et al. 2012): they also have up to 100 % *16S rRNA* and 99.5 % *23S rRNA* identity to this species (Harmsen et al. 2002). "*N. mucosa* subsp. *heidelbergensis*" strains should therefore be considered to be *N. oralis*. Eight other species were distantly related to *N. meningitidis*, including the *Neisseria elongata* variants: *Neisseria elongata* subsp. *elongata*, *Neisseria elongata* subsp. *glycolytica*, *Neisseria elongata* subsp. *nitroreducens*, *and* "*Neisseria elongata* subsp. *intermedia*." Two newly defined species: *Neisseria shayeganii* and *Neisseria wadsworthii* (Wolfgang et al. 2011) were more distantly related to *N. meningitidis* than many nonhuman strains.

Phenotypic Analyses

Cells of most *Neisseria* spp. are cocci except for *N. elongata*, *Neisseria weaveri*, *Neisseria bacilliformis*, and *N. shayeganii* which are rods (Table 33.1) normally occurring in pairs or quartets. They are Gram-negative and oxidase positive, with optimal growth at 35–37 °C in a 5–10 % CO_2 atmosphere. *Neisseria* can be differentiated from similar organisms using biochemical or other tests although definitive identification can prove difficult. Test results for carbohydrate utilization (Table 33.2), enzymatic activities, and cellular fatty acid composition may vary within a species (Wolfgang et al. 2012) and therefore cannot reliably be used alone to speciate *Neisseria*. Two enzyme substrate tests can be used to aid identification of *N. meningitidis* (gamma-glutamylaminopeptidase-positive) and *N. lactamica* (beta-galactosidase-positive), and gonococcal-specific antibodies can help to identify *N. gonorrhoeae*. As many species display similar phenotypic properties, it is recommended that at least two methods should be used to identify *Neisseria* in the laboratory, although the increasing availability of molecular techniques, especially sequencing approaches such as rMLST (Jolley et al. 2012a), suggests that these should form the basis of future isolate characterization and species definition (Bennett et al. 2012).

Isolation, Enrichment, and Maintenance Procedures

The pathogenic species may be grown on a selective medium, such as Thayer-Martin (Thayer and Martin 1966), which suppresses the growth of some commensal species. Species such as *N. mucosa*, *N. sicca*, *N. subflava*, *N. flavescens*, *N. meningitidis*, *N. cinerea* and *N. lactamica* can be grown on LBVT.SNR medium which is selective for commensal *Neisseria* (Knapp and Hook 1988). The meningococcus grows best on enriched media, such as blood agar (BAP) or chocolate agar (CAP), at 37 °C and in an atmosphere of 5 % carbon dioxide and fails to grow under strictly anaerobic conditions; however, under oxygen limitation, the bacterium expresses a denitrification pathway which does supplement growth (Rock et al. 2005). The gonococcus grows best on Thayer-Martin or Modified Thayer-Martin media (MTM) or chocolate agar (CAP) in a high carbon dioxide atmosphere (5–10 %) incubated at 35–37 °C. Colonies appear pinkish-brown and translucent, with smooth consistency and defined margins 0.5–1 mm in diameter. Fastidious strains may produce small, ~0.25 mm "pinpoint" colonies.

Ecology

Habitat

The majority of *Neisseria* have been isolated from the mucosal and dental surfaces of animals. Members of the genus have been shown to be consistent components of the commensal oral microbiota of humans (Dewhirst et al. 1993, 2010) and while many have only been isolated from humans, some species have been isolated from other sources including monkeys, dogs, cows, and even lizards (Vedros et al. 1983; Barrett et al. 1994; Sneath and Barrett 1996; Vandamme et al. 2006). The biology of many

Table 33.2
Phenotypic properties of members of the genus *Neisseria*

Species	Morphology	Acid from						Nitrate reduction	Polysaccharide from sucrose	DNAase	Colistin resistance
		Glucose	Maltose	Sucrose	Fructose	Mannitol	Lactose				
N. meningitidis	Cocci	+	+	–	–	–	–	+/–	–	–	R
N. gonorrhoeae	Cocci	+	–	–	–	–	–	+/–	–	–	R
N. lactamica	Cocci	+	+	–	–	–	+	+	–	–	R
N. polysaccharea	Cocci	+	+	–	–	–	–	–	+	–	S/R
"*N. bergeri*"	Cocci	+	+	+	+			–	+		S/R
N. cinerea	Cocci	–	–	–	–	–	–	+	–	–	S/R
N. flavescens	Cocci	–	–	–	–	–	–	+	+	–	S
N. subflava	Cocci	+	+	d	d	–	–	+	+/–	–	S/R
N. mucosa	Cocci	+	+	+	+	–	–	+	+	–	S
N. sicca	Cocci	+	+	+	+	–	–	+	+	–	S
N. macacae	Cocci	+	+	+	+	–	–	–	+	+	
N. oralis	Cocci	+/–	+/–	+/–		–	+/–	+			
N. elongata	Rod	–	–	–	–	–	–	+	–	+	S
N. bacilliformis	Rod	–	–	–			–	+			S
N. wadsworthii	Cocci	+/–	–	–	+/–	–	–	+			
N. shayeganii	Rod	+/–	–	–	–	–	–	+			
N. weaveri	Rod	–	–	–	–	–	–	–			
N. zoodegmatis	Cocci	+/–	–	–		–	–	+/–		–	
N. animaloris	Cocci	+/–	–	–	–	–	–	+		–	
N. dentiae	Cocci	+		+	+	+		–			
N. canis	Cocci	–	–	–	–	–	–	+	–	–	
N. animalis	Cocci	–	–	–	–	–	–	–	+		
N. iguanae	Cocci	+/–	–	+/–	–		–	+/–			
"*N. skkuensis*"	Cocci	+	–	+	+	+	–				
"*N. tadorna*"	Cocci	+	+	+			–	+			

Symbols: + most strains positive, – most strains negative, +/– strain dependent (some strains positive; some strains negative). Abbreviations: *R* resistance, *S* susceptibility, *S/R* some strains are resistant and some are sensitive, *d* delayed reaction

of these species is poorly understood, but the genus plays an important part in the normal oral microbiome of humans (Zaura et al. 2009).

Although a number of different species have been involved in various pathologies, by far the most important diseases are caused by the meningococcus, which can be a cause of devastating meningitis and septicemia (Stephens 2007) and the gonococcus which causes one of the most important sexually transmitted diseases (Morse and Knapp 1992; Tapsall et al. 2009). The principal interest in this genus has been these two pathogens, which are both of global significance and many studies of other *Neisseria* species have been with reference to these two organisms, with the aim of identifying the genetic determinants of virulence. This has, however, proved to be complex as with a few exceptions, most of the genetic elements thought to confer increased virulence are also found in the essentially non-pathogenic species, most meningococcal infections cause no overt pathology and even many gonococcal infections are asymptomatic (Hotopp et al. 2006; Snyder and Saunders 2006).

Pathogenicity and Clinical Relevance

Meningococcal Disease

Historical evidence suggests that epidemic meningococcal disease emerged at the beginning of the nineteenth century, with the first definitive description made by the Swiss physician Gaspard Vieusseux in 1805 (Vieusseux 1806), of an outbreak in Geneva which lasted three months, affecting children and young people, resulting in 33 deaths. The second recorded description was reported a year later in Medfield, Massachusetts, in the United States: in March 1806, nine fatal cases occurred over a period of 23 days. The youngest patient was just 15 months old and the oldest 10 years of age (Danielson and Mann 1806). Many outbreaks followed with the first epidemics of meningococcal disease in Africa described in 1905, with major epidemics occurring in the sub-Saharan "meningitis belt" for much of the twentieth century (Greenwood 1999).

The meningococcus was isolated from human cerebrospinal fluid in 1887 (Weichselbaum 1887), and in 1890, it was acknowledged that the organism could be found in an asymptomatic carrier state, in addition to being isolated from cases of invasive disease (Kiefer 1896). Fulminant meningococcal septicemia, or Waterhouse–Friderichsen syndrome, was first described in an 8-month-old child in 1911 (Waterhouse 1911). Bacterial meningitis remains a major threat to global health, accounting for an estimated annual 500,000 cases worldwide with at least 50,000 deaths (WHO.org). It is believed that 5–20 % of the population may be asymptomatic carriers of *N. meningitidis* at any given time and the rate may be higher in epidemic situations, although in diseased individuals, as long as an appropriate antimicrobial is administered promptly, prognosis is extremely good (Cartwright et al. 1992).

Meningococcal disease occurs with a range of different epidemiologies (Schwartz et al. 1989): sporadic cases of disease occur globally, with increases in disease incidence occurring from time to time. These can take the form of: (i) small scale localized outbreaks focused on a single family or contact group such as a university campus or a military recruit camp, (ii) hyperendemics, where a particular geographic region experiences increased rates of disease over a prolonged period of time, often lasting many months or years, and (iii) large-scale epidemic or pandemic outbreaks (Rosenstein et al. 2001). It is now known that each of these increases in incidence is related to the spread in asymptomatic carriage of particular genotypes of the meningococcus which are associated with the ability to cause invasive disease (Caugant and Maiden 2009).

Meningococcal disease comprises two distinct syndromes which can occur either separately or together. Meningitis, the syndrome most frequently associated with *N. meningitidis*, is a consequence of invasion of the cerebrospinal fluid and meninges, the membranes that surround the brain. If untreated, this is frequently fatal and surviving patients can exhibit serious sequelae such as brain damage, loss of coordination, and hearing loss. Meningococcal septicemia is a consequence of the invasion and uncontrolled rapid growth of the meningococcus in the blood stream; in many ways, this is even more serious than the meningitis syndrome. The release of endotoxins causes extensive tissue damage and shock, as a consequence of capillary leak and overstimulation of the innate immune system due to the release of meningococcal lipooligosaccharide: one of the most potent endotoxins known (Brandtzaeg and van Deuren 2012). In addition to being frequently fatal, meningococcal septicemia frequently results in extensive tissue damage, often resulting in digit or limb loss (Pace and Pollard 2012).

Neisseria meningitidis

N. meningitidis was first described by Weichselbaum (1887) and the first studies of intra-species meningococcal variation employed serological analyses, focusing initially on the serogroups, which are defined by the capsular polysaccharide (Vedros 1987). Further serological studies identified additional antigenic variation in the outer membrane proteins and short-chain lipopolysaccharides, leading to the development of meningococcal serotypes, serosubtypes, and immunotypes (Frasch et al. 1985). Sequence-based typing has become the method of choice for characterizing the highly variable surface proteins of this organism (Jolley et al. 2007). The capsule is of particular significance as it is both a virulence determinant, as only encapsulated meningococci regularly cause disease (Brandtzaeg and van Deuren 2012), and it is a major component of meningococcal vaccines (Tan et al. 2010).

Neisseria meningitidis Serogroups

The serogroup is the single most important characteristic of the meningococcus from a clinical perspective as it relates to the expression of a capsular polysaccharide, which is a prerequisite

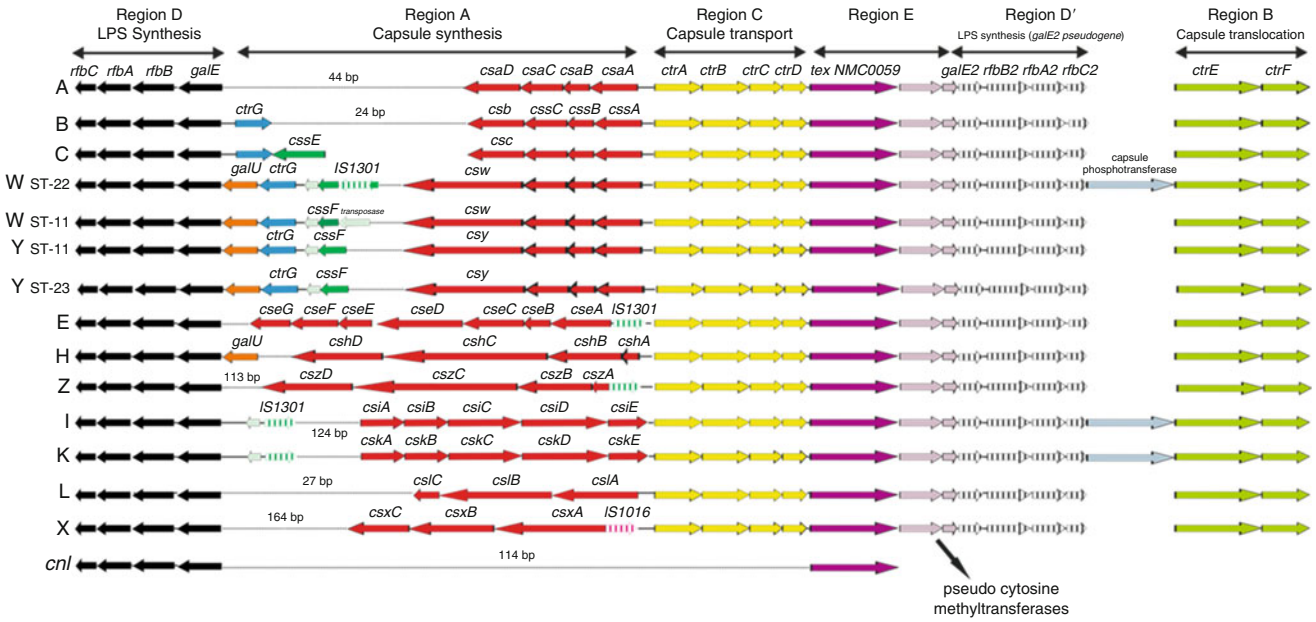

Fig. 33.3
Alternative genetic structures that occupy the *cps* region of the meningococcal genome

for meningococcal disease. Each meningococcus is genetically capable of expressing one type of capsule, known as a serogroup, although a significant proportion of meningococcal isolates from the oropharynx lack the genes required for capsule synthesis (Claus et al. 2002) and these can, very rarely, cause disease (Findlow et al. 2007). These meningococci have a short sequence known as the capsule null locus (*cnl*), which is also found in other *Neisseria* species. There are 12 serogroups, defined on the basis of biochemical properties and nucleotide sequences of the region of the genome encoding capsule synthesis (*cps*) (*Fig. 33.3*). Of these, six serogroups (A, B, C, W, X, and Y) cause the majority of meningococcal disease. Some *N. meningitidis* isolates express serogroup H, E, L, I, K, and Z polysaccharide capsules, but these are rarely associated with invasive disease and are more commonly found in asymptomatic carriage.

Within the genus, the expression of a polysaccharide capsule is unique to the meningococcus, with isolates from the related *N. lactamica* and *N. gonorrhoeae* species only containing regions D and E of the *cps* region, which are not implicated in capsule synthesis (*Fig. 33.3*; Petering et al. 1996). However, many meningococcal isolates recovered from carriage are unencapsulated either through the absence of capsule genes or through phase variation of the capsule biosynthesis genes (Hammerschmidt et al. 1996a, b; Claus et al. 2002). Since the pathogenicity of *N. meningitidis* is significantly dependent on the structure and expression of polysaccharide capsules, acquisition of the genes necessary for capsule expression may have been a critical step in the evolution of pathogenic *N. meningitidis* strains.

The capsule expressed by *N. meningitidis* is categorized as a group II capsule based on the similar chemical and physical properties of capsular polymers, with the genes encoding group II capsules from different bacterial species including *Haemophilus influenzae*, *Salmonella typhi Vi*, and *Escherichia coli* K5 containing a comparable genetic organization (Frosch et al. 1991; Roberts 1996; Frosch and Vogel 2006). The increasing number of bacterial genomes becoming available necessitates a more unified approach to the nomenclature of genes including those implicated in capsule biosynthesis. For example, there are in the annotated genomes belonging to FAM18, MC58 and Z2491, genes defined as *lipA* and *lipB* occurring twice, once in the capsule locus and then further on in the genome. Consequently, a nomenclature for the *N. meningitidis cps* locus has been proposed and accepted by the *Neisseria* community with the genes *lipA* and *lipB* renamed as *ctrE* and *ctrF* and a revised nomenclature for all of the remaining genes found in the *cps* locus. (*Table 33.3* and *33.4*; Harrison et al. 2013). The serogroup A capsule is composed of repeating units of *O*-acetylated (α1 → 6)-linked *N*-acetyl-D-mannosamine-1-phosphate (*Table 33.3*; Liu et al. 1971b). The capsule biosynthesis genes are contained within region A, the first gene *csaA*, encoding the UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc) 2-epimerase which converts UDP-GlcNAc into UDP-*N*-acetyl-D-mannosamine (UDP-ManNAc), *csaB* is the polymerase linking ManNAc-phosphate monomers together with *csaC* encoding an *O*-acetyltransferase transferring acetyl groups to ManNAc (Swartley et al. 1998; Gudlavalleti et al. 2004; Harrison et al. 2013). The fourth gene, *csaD*, is involved in either capsule transport or in cross-linking of the capsule to the meningococcal cell surface.

Capsular polysaccharides from serogroups B, C, W, and Y are composed of sialic acid derivatives with serogroups B and C expressing (α2 → 8)- and (α2 → 9)-linked sialic acid

Table 33.3

Genetic nomenclature for meningococcal capsule encoding genes

	New nomenclature	Previous nomenclature	Protein function	Size (bp)	G+C content (%)	References
Region A						
Serogroup A	*csaA*	*mynA/sacA*	UDP-*N*-acetyl-D-glucosamine 2-epimerase	1,119	25	(Swartley et al. 1998)
	csaB	*mynB/sacB*	Polymerase linking *N*-acetyl-D-mannosamine-1-phosphate monomers	1,638	28	(Swartley et al. 1998)
	csaC	*mynC/sacC*	*O*-acetyltransferase	744	30	(Gudlavalleti et al. 2004)
	csaD	*mynD/sacD*	Capsule transport	864	35	
Serogroups B, C, W and Y	*cssA*	*siaA/synX/synA/neuC*	*N*-acetylglucosamine-6-P 2-epimerase	1,134	31	(Claus et al. 1997)
	cssB	*siaB/synB/neuA*	CMP-*N*-acetylneuraminic acid synthase	687	41	
	cssC	*siaC/synC/neuB*	*N*-acetylneuraminic acid synthetase	1,050	40	
	csb	$siaD_B$/*synD*	Polysialyltransferase	1,488	28	
	csc	$siaD_C$/*synE*	Polysialyltransferase	1,479	31	
	csy	$siaD_Y$/*synF*	Polymerase linking glucose and *N*-acetylneuraminic acid	3,114	31	
	csw	$siaD_W$/*synG*	Polymerase linking galactose and *N*-acetylneuraminic acid	3,114	31	
	cssE	*oatC*	*O*-acetyltransferase	1,383	29	(Claus et al. 2004)
	cssF	*oatWY*		636	33	
	ctrG	*ctrG/NMB0065*	Putative role in surface expression of sialic acid capsules	957	33	(Hobb et al. 2010)
Serogroup E	*cseA*	*cap29Ea*	Unknown	1,197	33	
	cseB	*cap29Eb*	Unknown	639	38	
	cseC	*cap29eC*	Glycosyltransferase	1,461	40	
	cseD	Fusion of *cap29eD* and *cap29Ee*	Glycosyltransferase	2,198	39	
	cseE	*cap29Ef*	3-deoxy-D-manno-octulosonic acid 8-phosphate synthase	852	28	
	cseF	*cap29eG*	CMP-2-keto-3-deoxyoctulosonic acid synthetase and 3-deoxy-D-manno-octulosonate 8-phosphatase	1,290	26	
	cseG	*cap29Eh*	D-arabinose 5-phosphate isomerase	947	28	
Serogroup H	*cshA*	–	Glycerol-3-phosphate cytidylyltransferase	399	29	
	cshB	–	Putative phosphotransferase with LicD domain (involved in phosphorylcholine decoration of teichoic acid)	1,269	33	

Table 33.3 (continued)

	New nomenclature	Previous nomenclature	Protein function	Size (bp)	G+C content (%)	References
	cshC	–	Teichoic acid synthase	3,453	34	
	cshD	–	Unknown no putative conserved domains	1,035	38	
Serogroup I	*csiA*	–	UDP-*N*-acetylglucosamine 2-epimerase	1,125	36	
	csiB	–	UDP-*N*-acetyl-D-mannosamine dehydrogenase	1,269	45	
	csiC	–	Glycosyltransferase group 1	2,289	36	
	csiD	–	Glycosyltransferase group 2	2,520	34	
	csiE	–	Putative glycosyl transferase group 1	972	42	
Serogroup K	*cskA*	–	UDP-*N*-acetylglucosamine 2-epimerase	1,125	36	
	cskB	–	UDP-*N*-acetyl-D-mannosamine dehydrogenase	1,269	45	
	cskC	–	Glycosyltransferase group 1	2,289	36	
	cskD	–	Glycosyltransferase group 2	2,520	34	
	cskE	–	Putative glycosyl transferase group 1	972	42	
Serogroup L	*cslA*	*lcbA*	Capsule phosphotransferase	1,101	30	
	cslB	*lcbB*	Capsule polymerase	2,634	28	
	cslC	*lcbC*		651	39	
Serogroup X	*csxA*	*xcbA*	Capsule polymerase	1,461	39	(Tzeng et al. 2003)
	csxB	*xcbB*	Unknown	1,053	39	
	csxC	*xcbC*	Unknown	771	35	
Serogroup Z	*cszA*	*capZA*	Glycerol-3-phosphate cytidylyltransferase	399	43	
	cszB	*capZB*	Putative phosphotransferase with LicD domain (involved in phosphorylcholine decoration of teichoic acid)	1,290	45	
	cszC	*capZC*	Teichoic acid synthase	3,825	36	
	cszD	*capZD*	Unknown, no conserved domains detected	1,626	38	
Region B	*ctrE*	*lipA/ctrE*	Capsule translocation	2,115	51	(Kahler et al. 2005)
	ctrF	*lipB/ctrF*	Capsule translocation	1,260	49	
Region C	*ctrA*	*ctrA*	Capsule polysaccharide export outer membrane protein	1,179	47	(Frosch et al. 1991)
	ctrB	*ctrB*	Capsule polysaccharide export inner membrane protein	1,164	46	
	ctrC	*ctrC*	Capsule polysaccharide export inner membrane protein	726	43	
	ctrD	*ctrD*	Capsule polysaccharide export ATP-binding protein	651	46	

Table 33.4
Capsule biochemical structures

Serogroup	Structural repeating unit
A (homopolymer)	ManNAc-(1-P$\overset{\alpha}{\rightarrow}$6)- 3 | OAc
B (homopolymer)	NeuNAc-(2$\overset{\alpha}{\rightarrow}$8)-
C (homopolymer)	NeuNAc-(2$\overset{\alpha}{\rightarrow}$9)- 7 8 | | OAc OAc
E (disaccharide repeating)	D-GalNAc(1$\overset{\beta}{\rightarrow}$7)- KDO(2$\overset{\alpha}{\rightarrow}$3)- 4.5 | OAc
H (monosaccharide glycerol repeating unit)	→4) α-D-Gal-(1→2)-Gro-(3-P→
Z (monosaccharide glycerol repeating unit)	D-GalNAc(1$\overset{\alpha}{\rightarrow}$1)-Gro-(3'-P$\overset{\alpha}{\rightarrow}$4)
I (disaccharide repeating unit)	→4) α-L-GulNAcA-(1→3)β-D-ManNAcA(→ | 4-OAc
K (disaccharide repeating unit)	→3) β-D-ManNAcA-(1→4)β-D-ManNAcA-(1→ | 4-OAc
L (trisaccharide repeating unit)	→3)-β-D-GlcNAc-(1→3)β-D-GlcNAc-(1→3) α-D- GlcNAc-(1-P-
W (disaccharide repeating unit)	6-D-Gal(1$\overset{\alpha}{\rightarrow}$4)-NeuNAc-(2$\overset{\alpha}{\rightarrow}$6) | OAc

Table 33.4 (continued)

Serogroup	Structural repeating unit
Y (disaccharide repeating unit)	6-D-Glc-(1$\overset{\alpha}{\rightarrow}$4)-NeuNAc-(2$\overset{\alpha}{\rightarrow}$6) | OAc
X (homopolymer)	GlcNAc(1-P→4)-

Table adapted from Frosch and Vogel (2006)
ManNAc N-acetylmannosamine, *NeuNAc N*-acetylneuraminic acid (sialic acid), *OAc O*-acetylated, *Gro* glycerol, *GulNAc A* 2-acetamido-2-deoxy-L-guluronic acid, *Gal* galactose, *Glc* glucose, *P* phosphate, *GlcNAc N*-acetylglucosamine

homo-polymers respectively, while alternating sequences of D-galactose or D-glucose and sialic acid are expressed by serogroups W and Y (*Table 33.3*; Liu et al. 1971a; Bhattacharjee et al. 1975, 1976). All four serogroups contain the conserved *cssABC* genes for cytidine-5′-monophosphate-*N*-acetylneuraminic acid (CMP-NANA) synthesis (other designations include *siaABC*, *synABC* and *neuABC*). These are followed by *csb*, *csc*, *csw*, or *csy* genes (*siaD* or *synDEFG* respectively) which are the capsule polymerases and determine the functional and nucleotide specificity for the four serogroups. Serogroups C, W, and Y contain *O*-acetyltransferase genes termed *cssE* (*oatC*) or *cssF* (*oatWY*), respectively (Claus et al. 2004). The serogroup E capsule consists of alternating D-galactosamine and 2-keto-3-deoxyoctulosonate (KDO) residues (Bhattacharjee et al. 1978). Region A contains seven genes *cseA-G* (formerly 29eABCDEFGH) with genes including *cseE* encoding a putative 3-deoxy-phosphooctulonate synthase, *cseF* a putative 3-deoxy-D-manno-octulosonate cytidylyltransferase and *cseG* a D-arabinose 5-phosphate isomerase protein.

The biochemical structures of serogroups H and Z contain monosaccharide glycerol-3-phosphate repeat units and share similarities with teichoic acid polymers (Jennings et al. 1979; van der Kaaden et al. 1984). BLAST searches of region A genes encoding the capsule biosynthesis genes revealed that these were homologous to biosynthesis genes belonging to *Actinobacillus pleuropneumoniae* (Dubreuil et al. 2000) (Xu et al. 2010). These genes are predicted to encode a glycerol-3-phosphate cytidylyltransferase, and a hypothetical protein containing a LicD domain for a role in phosphorylcholine incorporation into teichoic acid polymers has been suggested. Serogroup L capsule polysaccharides contain 2-acetamido-2-deoxy-D-glucosyl residues and phosphate groups (Ding et al. 1981; Ashton et al. 1983), while the serogroup X capsular polysaccharide is composed of (α-1 → 4)-linked *N*-acetylglucosamine 1-phosphate (Bundle et al. 1974). Serogroup I capsules consist of *O*-acetylated alternating *N*-acetyl-guluronic acid and *N*-acetylmannosaminuronic acid (ManNAcA) units, while serogroup K capsules are composed of *O*-acetylated disaccharide repeat units containing *N*-acetylmannosaminuronic acid (Michon et al. 1985; Van der Kaaden et al. 1985).

Genes within the capsule locus are divided into six regions: A, B, C, D, D′, and E (*Fig. 33.3*), with genes in region A encoding enzymes for the biosynthesis of the capsular polysaccharide and regions B and C implicated in the translocation of the high molecular weight polysaccharides to the cell surface. Genes in regions D and D′ are involved in LPS biosynthesis, while genes in region E are not implicated in capsule biosynthesis or transport and are thought to encode a methyltransferase and a gene homologous to the *tex* gene belonging to *Bordetella pertussis* (Fuchs et al. 1996). All 12 serogroups have comparable *cps* loci with regions occurring in the following order D-A-C-E-D′-B, revealing a conserved gene synteny and a replication of the genomic rearrangements (*Fig. 33.3*). Genes in regions B, C, D, D′, and E are conserved, whereas genes in region A are diverse, in line with the distinct biochemical composition found within each serogroup.

Neisseria meningitidis Subcapsular Antigens

The meningococcal subcapsular antigens, which are components of the outer membrane of these Gram-negative organisms, have also been used for typing and as possible vaccine components. As with the capsule, the subcapsular antigens are also associated with particular clonal complexes. The major outer membrane proteins (OMPs) PorA and PorB are porins which have highly diverse surface regions (Urwin et al. 2004; Russell et al. 2008) and were first recognized as serotyping and serosubtyping antigens (Frasch et al. 1985). PorA in particular has also attracted interest as a vaccine component as it has been shown to elicit bactericidal immune responses in humans, although the diversity of this protein is a challenge in the formulation of vaccines which contain it (Granoff 2010).

The iron regulated surface protein FetA (also known as FrpB) (Thompson et al. 2003) has attracted similar interest and together with the serogroup, these antigens form the basis of the meningococcal strain typing nomenclature which is currently adopted by the European Centres for Disease Control (Jolley et al. 2007). It has the form: serogroup: PorA type: FetA

type: sequence type (clonal complex), thus: B: P1.19, 15: F5-1: ST-33 (cc32). Such typing schemes can be applied to surveillance, population biology, and epidemiological studies. For example, finetype data (serogroup: PorA: FetA) of invasive disease isolates collected in Germany over 42 months were used to retrospectively detect clusters of disease cases using automated scan statistics (Elias et al. 2006). This has been further extended to real-time GIS surveillance at the national level in Germany with EPIScanGIS which can potentially detect emerging clusters of disease using finetype data (Reinhardt et al. 2008). Unified and Web-accessible nomenclature schemes have greatly aided the study of the meningococcus. For example, a harmonized scheme was developed for the vaccine candidate fHbp which previously had two separate classification systems. The established database facilitates querying of sequences and submission of new allele sequences and cross-referencing with the other schemes, thereby enabling the monitoring of circulating variants (Brehony et al. 2007).

Examination of strain types from large collections of isolates has shown that the protein surface antigens occur in non-overlapping structure, such that strains that occur at high frequencies and persist do not share the same variants (Caugant 1998; Urwin et al. 2004; Russell et al. 2008; Watkins and Maiden 2012). This structuring is consistent with models that invoke immune selection as a driver of bacteria population structure and has important implications for the design of vaccines based on these proteins (Gupta et al. 1996; Buckee et al. 2008, 2010).

Neisseria meningitidis Population Structure

The meningococcus exhibits significant population structure, notwithstanding the fact that there is extensive evidence for horizontal genetic exchange within meningococcal populations. This structuring was first identified with MLEE (Caugant et al. 1987), and has since been confirmed with MLST (Maiden et al. 1998) and rMLST (Jolley et al. 2012b). MLST characterizes organisms by assigning each isolate an ST, a unique combination of alleles at the housekeeping loci (there are seven in the meningococcal scheme). Some of these STs are much more common in population samples than others, and these tend to be persistent over time and geographic spread. Analysis of these data with heuristic approaches, such as SPLITSTREE (Huson and Bryant 2006) and EBURST (Feil et al. 2004), shows that there are many variants of these abundant STs, which have up to three loci with different alleles (Maiden 2006). The central genotypes are used to define "clonal complexes" of related organisms, which have been shown to have a genealogical basis. The biological significance of these clonal complexes is that they are associated with particular phenotypes, particularly the expression of given serogroups or outer membrane protein repertoires (Urwin et al. 2004). Further, clonal complexes are associated with different epidemiologies and the likelihood of causing disease (Yazdankhah et al. 2004; Caugant and Maiden 2009).

Serogroup associations among hyper-virulent clonal complexes have been documented with, for instance, ST-1, ST-4, and ST-5 lineages almost exclusively possessing serogroup A capsules, while most invasive ST-32 meningococci express serogroup B capsules (Wang et al. 19921992; Scholten et al. 1994; Guibourdenche et al. 1996; Baker et al. 2001; Harrison et al. 2009). The majority of ST-11 clonal complex meningococcal disease has been caused by serogroup C isolates although there have been instances of invasive serogroup W, ST-11 isolates. The predominant clonal complex associated with serogroup Y meningococci is the ST-23 lineage. Serogroup X outbreaks have been reported in Niger and Ghana, Burkina Faso, and Togo (Gagneux et al. 2002; Boisier et al. 2007; Delrieu et al. 2011), with sporadic cases found in industrialized countries, a significant proportion of these isolates belonging to the ST-181 complex.

Gonococcal Disease

Gonococcal disease is one of the most important sexually transmitted infections (STIs) globally, causing both symptomatic and asymptomatic genital and extragenital tract infections. Disseminated infections do occur although they are less common, and rates vary greatly in both the developing and developed countries, with the highest rates in southern and southeast Asia, Sub-Saharan Africa, and Latin America, although reliable incidence data are often incomplete or inaccurate and are usually prevalence based rather than incidence based (Gerbase et al. 1998). In Western countries, gonorrhea is second only to chlamydia as the most common bacterial STI, with rates increasing in some countries since the mid-2000s presumably a result of emerging antimicrobial resistance.

Given the relatively recent history of meningococcal disease, it is interesting that gonococcal disease is one of the oldest syndromes known to man, with clinical descriptions dating from the earliest civilizations. The Ebers papyrus, for example, which dates from at least 1550 BCE, includes a reference to gonorrhea. However this compilation of therapeutic, diagnostic and theoretical considerations for the problems of everyday life, health and disease, when cross referenced with other papyri, suggests an origin closer to c. 3000 BCE. The name "gonorrhea" is believed to have been coined by the Greek physician Galen in the second century; it literally translates as "flow of seed" – one of the disease symptoms in males is urethral discharge.

N. gonorrhoeae is regarded as an obligate human pathogen with colonization which follows on from infection usually resulting in a localized inflammatory response. More rarely, this results in disseminated infection, which can lead to dermatitis, endocarditis, meningitis septic arthritis, and pelvic inflammatory disease (PID) (Morse and Knapp 1992). Symptoms of uncomplicated infection include discharge, dysuria, and inflammation of the urethral and cervical tissues. A serious complication of gonorrhea in women, particularly if left untreated, is PID. Once established, it is difficult to treat and can lead to chronic abdominal pain and infertility. Gonorrhea is usually contracted

from a sex partner who is either asymptomatic or only has minimal symptoms. While 85–90 % of men will develop symptoms within five days of infection, in women, the incubation period is longer, with 30–60 % of infected women remaining asymptomatic (van Duynhoven 1999). Women with asymptomatic infections are thus more at risk of developing PID and disseminated infection. Those with asymptomatic infections are important contributors to disease prevalence. Gonorrhea is unlike many other communicable diseases in that reinfection can occur as it does not appear to induce protective immunity. This means that incidence rates often do not account for reinfection and therefore, the true disease burden is distorted.

Neisseria gonorrhoeae

The German physician Albert Neisser first identified the gonococcus as the causative agent of gonorrhea in 1879, and the genus was thus named after him. Like the meningococcus, *N. gonorrhoeae* is naturally competent for DNA uptake and is therefore able to evade the immune system by frequent antigenic alteration. Unlike the meningococcus however, it does not possess a capsule. This along with highly diverse OMPs is a reason for the difficulty in developing a vaccine against the organism. Typing methods used to discriminate gonococcal isolates include phenotypic techniques such as auxotyping, serotyping, and antimicrobial susceptibility testing and genotypic methods such as restriction fragment length polymorphism (RFLP), ribotyping, pulsed field gel electrophoresis (PFGE), Opa-typing, PorB typing, *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), and MLST (Unemo and Dillon 2011). DNA-based typing methods are now considered the "gold standard" due to their discriminatory power, reproducibility, and ease of use relative to phenotypic methods.

Virulence Factors, Iron Acquisition

For successful proliferation within the body or on mucosal surfaces, the natural habitat of the *Neisseria*, microorganisms must possess the ability to acquire iron from their hosts. The latter contain an iron-withholding defense system that, while allowing host cells access to iron, inhibits potential pathogens from acquiring it. A variety of mechanisms are employed to suppress free iron in the human body (Weinberg 1978, 2009). These include: transferrin, which sequesters iron found in plasma, lymph, and cerebrospinal fluids (Evans 1999); lactoferrin, which is found in most mucosal secretions (Levay and Viljoen 1995); and hemoglobin and haptohemoglobin complexes (Stojilijkovic et al. 1995; Lewis et al. 1997, 1998, 1999).

N. meningitidis and *N. gonorrhoeae* are able to acquire iron from human transferrin and lactoferrin via distinct but parallel pathways through the expression of high-affinity receptors specific for each glycoprotein. These receptors consist of two iron-repressible outer membrane proteins: the transferrin or lactoferrin binding proteins A (TbpA or LbpA) and B (TbpB or LbpB). In addition, iron may be acquired from hemoglobin using the hemoglobin receptor HmbR and from haptohemoglobin complexes using the bipartite receptor HpuAB (Stojilijkovic et al. 1995; Lewis et al. 1997). Both receptors are phase variable with isolates variably expressing these such that for example, meningococci may express: (i) HmbR only; (ii) HpuAB only; (iii) both receptors simultaneously or (iv) neither (Tauseef et al. 2011). Furthermore, there is a significantly higher prevalence of meningococci associated with disease containing the *hmbR* gene and an under-representation of a *hpuAB* only phenotype (Harrison et al. 2009; Tauseef et al. 2011). All of these receptors have elicited some interest in vaccine research as they possess attributes making them ideal vaccine candidates. For example, they are surface-exposed and they are expressed in vivo. However, phylogenetic analysis of, for example, the lactoferrin and transferrin binding proteins has revealed them to be highly diverse and indeed, studies have shown for the transferrin and lactoferrin binding proteins, that although these were able to induce bactericidal antibodies, the cross-reactivity was limited (Rokbi et al. 1995, 1997a, b; Pettersson et al. 1998, 1999). Congruence was observed among meningococci expressing the hemoglobin receptor, *hmbR*; however, as this receptor is not universally expressed by all meningococci and gonococci (the latter persistently containing nonfunctional *hmbR* genes), it is not a suitable vaccine candidate (Evans et al. 2010).

Applications

The major applications of the study of the *Neisseria* are the monitoring of infection and the development of effective treatments and vaccines against meningococcal and gonococcal disease. Despite many years of research and development, these remain very active areas with significant problems yet to be resolved: there is no vaccine against the gonococcus and, despite major successes, no comprehensive meningococcal vaccine, and antimicrobial resistance is emerging as a significant problem in the gonococcus.

Vaccine Development

There has been considerable interest in the development of vaccines against both the meningococcus and the gonococcus, with appreciably more success in the former. For many years, the main component of meningococcal vaccines has been the polysaccharide capsule. This is an ideal component as there are relatively few capsular serogroups associated with disease (six, with most disease caused by three, serogroups A, B, and C). As well as raising protective bactericidal responses (Gotschlich et al. 1969), the capsule is also the major virulence determinant and there have been major successes with using capsular polysaccharide vaccines, especially since the development of protein conjugate vaccines (Tan et al. 2010).

The first capsular polysaccharide vaccines were developed in the 1960s and were specifically designed to target the serogroups A and C which had been responsible for various epidemics worldwide since World War 2. The vaccines proved effective in prevention of serogroup A and C disease in US military recruits and in epidemic control in Africa (Makela et al. 1975; Greenwood and Wali 1980; Hassan-King et al. 1988). However, as polysaccharide vaccines are T-cell independent, they do not induce immunological memory; so, the period of protection is short (3–5 years) and as they do not prevent mucosal colonization, there is no protection provided through herd immunity. These limitations and the success of the *Haemophilus influenzae* type b vaccine (Heath and McVernon 2002) spurred on the development of conjugate polysaccharide vaccines.

Conjugate meningococcal vaccines have been introduced successfully in several countries. The meningococcal C conjugate (MCC) vaccine was introduced by the Department of Health in the UK in October 1999 in response to increasing levels of serogroup C disease in the country. It was the first country to include the meningococcal conjugate vaccine in a routine infant immunization program (Miller et al. 2001). Infants were immunized at 2, 3, and 4 months of age, and all children up to 18 years of age were targeted in a catch-up campaign from November 1999 to December 2000. The impact of the campaign has been a dramatic reduction in serogroup C disease of over 80 % within 18 months of onset of the program (Miller et al. 2001). There was also a reduction of 66 % in carriage of serogroup C in adolescents a year after the program began (Maiden et al. 2002). The effects of herd immunity, i.e., protection of the unvaccinated population, have been shown with a reduction of disease by 67 % in this group, thanks to the high vaccine coverage rate and the reduction in carriage (Ramsay et al. 2003). A quadrivalent vaccine targeting serogroups A, C, W and Y was licensed in the USA in 2005 for adolescents aged 11–12, those who have not been vaccinated before high-school entry at 15 years, college freshmen, and various individuals at risk (Bilukha and Rosenstein 2005). The Meningitis Vaccine Project (MVP), a collaboration with WHO, PATH (Program for Appropriate Technology in Health), and various worldwide parties was established with the aim to produce conjugate vaccines for Africa at an affordable price and eventually eliminate epidemic meningococcal disease in Africa (Jodar et al. 2003; LaForce et al. 2007). The mass vaccination campaign began in 2010 with 19.5 million people vaccinated in Burkina Faso, Mali, and Niger and then to be rolled out across meningitis belt countries.

Vaccines against serogroup B are, however, a problem, as this polysaccharide is poorly immunogenic in humans due to identity to host antigens (Finne et al. 1983). Consequently, there have been many efforts to develop subcapsular, mainly outer membrane protein based serogroup B substitute vaccines. A major problem is the extensive diversity of these antigens, although this is ameliorated somewhat by the antigenic structuring of these antigen variants by clonal complex (Urwin et al. 2004). Attempts to overcome this problem have included the development of vaccines against particular clonal complexes and their associated antigens (Bjune et al. 1991; Rodriguez et al. 1999; O'Hallahan et al. 2005), the use of cocktails of antigenic variants (Rouppe van der Voort et al. 2000), or by attempts to identify "conserved antigens" (Pizza et al. 2000; Bernfield et al. 2002). To date none of these have been entirely successful, although some novel vaccines are in clinical development at the time of writing (Tan et al. 2010; Scarselli et al. 2011).

Gonococcal vaccines face many of the challenges seen for the meningococcus, except that if anything the outer membrane antigens are more variable for this pathogen. There is also limited understanding of the gonococcal immune response in humans. Infection stimulates an inflammatory response and the appearance of polymorphonuclear neutrophils. Antibodies against the major surface antigens pili, lipooligosaccharide, Opa and Por are produced in the serum (IgG, IgM), and at the mucosal surface (IgA) (Zhu et al. 2011). IgA is present in mucosal secretions and is thought to prevent bacterial adherence. However, in spite of the presence of antibody response to infection, there is no evidence of a protective immune response to gonococcal antigens and, as a result, individuals may be reinfected with no evident reduction in disease severity or duration (Fox et al. 1999). Relative to the meningococcus, there has been little research into potential gonococcal vaccines, with only two making it to clinical trial (Zhu et al. 2011). Current research is focused on developing correlates of human protection including the mouse model (Gu et al. 2010) and DNA and recombinant vaccines based on OMPs such as PorB and TbpB (Zhu et al. 2004; Price et al. 2005).

Antimicrobial Resistance

Despite their genetic similarity, the situation with antibiotic resistance, as with vaccines, differs markedly between the meningococcus and the gonococcus. While antimicrobial resistance is very widespread among gonococci, leading to serious concerns as to the future treatment options against this important pathogen, reduced susceptibility to antimicrobial agents is yet to become a major concern for the treatment of meningococcal disease. Resistance to antimicrobials in the gonococcus includes target-mediated resistance, such as the recruitment of gene fragments of penicillin binding protein genes, efflux pumps, such as the Mtr efflux pump, and the possession of plasmids encoding resistance elements such as the *tetM* gene and β-lactamases (Unemo and Shafer 2011).

With the meningococcus, the major problem with antimicrobial resistance is a historic one arising from the use of sulphonamides as a prophylactic against carriage (Perez-Trallero et al. 1989; Kristiansen et al. 1990; Tzanakaki et al. 1992) as well as treatment during and after World War 2. This led to widespread resistance to these antimicrobials, as a consequence of both mutation or horizontal genetic exchange involving the *folP* (*dhps*) gene which encodes dihydropterate synthase. This resulted in treatment failure and large-scale outbreaks of meningococcal disease in the US military recruits training for the Vietnam War (Millar et al. 1963). By 1972, 6 % of UK strains

were resistant and by the 1990s ~30 % were resistant (Jones and Kaczmarski 1994), so they are no longer used to treat meningococcal infection or carriage (Oppenheim 1997).

The differences in the biology of antibiotic resistance in the meningococcus and gonococcus underline a major feature of the biology of the genus as a whole: despite their close genetic similarity, the organisms belonging to the different species within the genus exhibit dramatic differences in the phenotypes which they express, and to a large extent, this can be explained by their responses to the different selection pressures which they are exposed to.

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34 The Family *Nitrosomonadaceae*

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Abstract
The *Nitrosomonadaceae* comprise a monophyletic phylogenetic group within the betaproteobacteria, all of whose cultivated representatives are lithoautotrophic ammonia oxidizers. Ammonia oxidizers generally exert control over nitrification by oxidizing ammonia to nitrite, which is subsequently oxidized by bacterial nitrite oxidizers to nitrate. They therefore play major roles in control of the nitrogen cycle in terrestrial, freshwater, and marine environments and in wastewater treatment processes. They are also of significant economic and environmental importance, leading to loss of ammonium-based fertilizers, nitrous oxide production, and nitrate pollution. Ammonia oxidation is also carried out by thaumarchaea, gammaproteobacteria, and anammox organisms.

Phylogeny of the *Nitrosomonadaceae* is now based on analysis of 16S rRNA genes and *amoA* genes that encode subunit A of ammonia monooxygenase, which catalyzes the first step in ammonia oxidation. Sequences of cultivated strains and those obtained directly from environmental DNA and RNA suggest two genera, *Nitrosomonas* and *Nitrosospira*, each containing seven lineages, although support for these lineages in *Nitrosospira* is weaker. Two of these lineages are not represented by a laboratory isolate. Genomic analyses are providing insights into the evolution of the *Nitrosomonadaceae* and into metabolic processes of relevance to ecological studies.

Taxonomy, Historical and Current

Nitrification, the oxidation of ammonia to nitrite and its subsequent oxidation to nitrate, are performed by two functional groups, ammonia oxidizers and nitrite oxidizers. Their combined role in this important step in the global nitrogen cycle led to their initial grouping within the *Nitrobacteraceae* (Buchanan 1917, 1918), with subdivisions based solely on their source of energy, ammonia or nitrite. The family *Nitrobacteraceae* was substantially revised and rationalized by Watson (1971) with description of three genera of autotrophic ammonia-oxidizing bacteria (*Nitrosomonas*, *Nitrosococcus*, and *Nitrosospira*) and three genera of autotrophic nitrite-oxidizing bacteria (*Nitrobacter*, *Nitrococcus*, and *Nitrospina*) and type and neotype strains designated for each genus (*Nitrosomonas europaea* ATCC25978; *Nitrosococcus oceanus* ATCC 19707; *Nitrosospira briensis* ATCC 25961; *Nitrobacter winogradskyi* ATCC 25391; *Nitrococcus mobilis* ATCC25380; *Nitrospina gracilis* ATCC25379). The advent of 16S rRNA gene-based phylogeny (Woese et al. 1984a, b, 1985) demonstrated ammonia and nitrite-oxidizing bacteria to be phylogenetically distinct, with ammonia oxidizers falling within the betaproteobacteria and gammaproteobacteria. The latter contains only two validly named species, *Nitrosococcus nitrosus* and *Nitrosococcus oceani* (formerly *Nitrosocystis oceanus* and *Nitrosococcus oceanus*; Watson 1971). Two other species *Nitrosococcus halophilus* and *Nitrosococcus watsonii* have also been described, but these have not yet been validly named in an IJSEM validation list (Campbell et al. 2011).

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The majority of known bacterial ammonia oxidizers therefore belong to the Betaproteobacteria, where they form the *Nitrosomonadaceae*. The *Nitrosomonadaceae* contains two genera, *Nitrosomonas* and *Nitrosospira*, while the genus *Nitrosospira* includes organisms that were previously assigned to the genera *Nitrosospira, Nitrosolobus*, and *Nitrosovibrio* (Watson 1971; Watson et al. 1971; Harms et al. 1976; Head et al. 1993). The known genera of nitrite-oxidizing bacteria fall within a range of phylogenetic groups: *Nitrobacter* (Alphaproteobacteria), *Nitrococcus* (Gammaproteobacteria), *Nitrospira* (Nitrospirae), *Nitrospina* (Deltaproteobacteria), *Nitrolancetus* (Chloroflexi; Sorokin et al. 2012), and Candidatus "Nitrotoga" (Betaproteobacteria; Alawi et al. 2007). In addition ammonia-oxidizing archaea also exist and belong to the Thaumarchaeota (see Chap. 26, "The Phylum Thaumarchaeota", in Volume 11, *The Prokaryotes – Other Major Lineages of Bacteria and the Archaea*).

Early classification of genera and species within the *Nitrosomonadaceae* was based on a limited number of morphological and physiological characteristics: cell size and shape, presence and organization of intracellular membranes, motility, urease activity, ammonia affinity and sensitivity, and salt requirement and tolerance (Koops et al. 2006). Again, taxonomy has been revised following phylogenetic analysis of both 16S rRNA sequences and *amoA*, encoding subunit A of ammonia monooxygenase, which catalyzes the initial oxidation of ammonia to hydroxylamine. This analysis includes genes from cultivated ammonia oxidizers but also sequences obtained by amplification of nucleic acids extracted from a wide range of environments (Koops et al. 2006). This analysis generates seven well-supported lineages within the genus *Nitrosomonas* and a number of less well-supported lineages within the genus *Nitrosospira*, including the previously described genera *Nitrosolobus* and *Nitrosovibrio* (Stephen et al. 1996; Purkhold et al. 2000).

Molecular Phylogeny of Autotrophic Ammonia-Oxidizing Bacteria

The phylogenetic position of ammonia-oxidizing bacteria was initially determined on the basis of 16S rRNA cataloguing (Woese et al. 1984b, 1985; Fox and Stackebrandt 1988) and subsequently refined using near full-length, PCR-amplified 16S rRNA gene sequences (Head et al. 1993; Teske et al. 1994). Comparative analysis of genes encoding different components of ammonia monooxygenase largely confirmed and extended the relationships revealed on the basis of comparative 16S rRNA gene analysis (Purkhold et al. 2000; Aakra et al. 2001a, b; Calvó et al. 2005) as did analysis of intergenic transcribed spacer (ITS) sequences (Aakra et al. 2001a). However, the validity of the relationships inferred from ITS sequences has been questioned due to some incongruences between 16S rRNA-derived trees and ITS trees attributed to the high sequence variation of ITS and difficulties in reliable alignment of such variable sequences (Koops et al. 2006).

In broad terms these analyses have shown that cultivated ammonia-oxidizing bacteria fall within three main lineages; two distinct, but sister lineages, representing the genera *Nitrosomonas* and *Nitrosospira*, fall within the Betaproteobacteria and collectively form the *Nitrosomonadaceae*, while those from the genus *Nitrosococcus* fall within the *Chromatiaceae* in the Gammaproteobacteria.

Nitrosococcus

The genus *Nitrosococcus* does not belong in the *Nitrosomonadaceae* but for historical reasons and completeness is included here. There are two validly named species of *Nitrosococcus*: *N. nitrosus* and *N. oceani* (formerly *N. oceanus* and before that *Nitrosocystis oceanus*), but *Nitrosococcus halophilus*, a salt lake isolate, has been described and the marine isolate C-113 has been proposed as a new species, *N. watsonii* (Campbell et al. 2011). A further species *Nitrosococcus mobilis* has been described, but 16S rRNA and *amoA* sequence analysis revealed this to be a misnamed *Nitrosomonas* sp., and it has been suggested that this be renamed *Nitrosomonas mobilis* (Campbell et al. 2011).

Nitrosomonas

Stephen et al. (1996) and Kowalchuk et al. (1997) delineated three clusters within the *Nitrosomonas* lineage (clusters 5, 6, and 7; *Fig. 34.1*) by comparative analysis of 16S rRNA gene sequences from cultured lithoautotrophic ammonia-oxidizing bacteria. Increasing numbers of 16S rRNA and *amoA* sequences led to recognition of seven lineages within the genus *Nitrosomonas* (clusters 5, 6a, 6b, 7, 8, *N. cryotolerans*, and cluster 9/Nm143 lineage) with cluster 5 currently represented only by sequences recovered from environmental samples (Purkhold et al. 2000, 2003; Koops et al. 2006; *Fig. 34.1*—note that cluster 5 is not presented).

- *N. europaea*/*Nc. mobilis* lineage (cluster 7 of Kowalchuk et al. 1997)
- *N. communis* lineage including *N. nitrosa* (cluster 8)
- *N. marina* lineage including *N. aestuarii* (cluster 6b after Kowalchuk et al. 1997)
- *N. oligotropha* lineage including *N. ureae* (cluster 6a after Kowalchuk et al. 1997)
- *N. cryotolerans* lineage
- *Nitrosomonas* sp. Nm143 lineage (cluster 9)
- Cluster 5 of Stephen et al. (1996) and Kowalchuk et al. (1997)

These lineages contain nine validly named species of *Nitrosomonas*: *N. aestuarii, N. communis, N. europaea, N. eutropha, N. halophila, N. marina, N. nitrosa, N. oligotropha*, and *N. ureae* (*Fig. 34.1*). In addition several species have been described that are not validly named or are misnamed including *N. cryotolerans* and *Nitrosococcus mobilis*. The second of these is a misnamed *Nitrosomonas* sp. It has been suggested that cultivated *Nitrosomonas* spp. represent 19 genospecies on the basis of DNA-DNA similarity $\leq$60 % and/or 16S rRNA sequence identity less than 97.5 % (Koops et al. 2006).

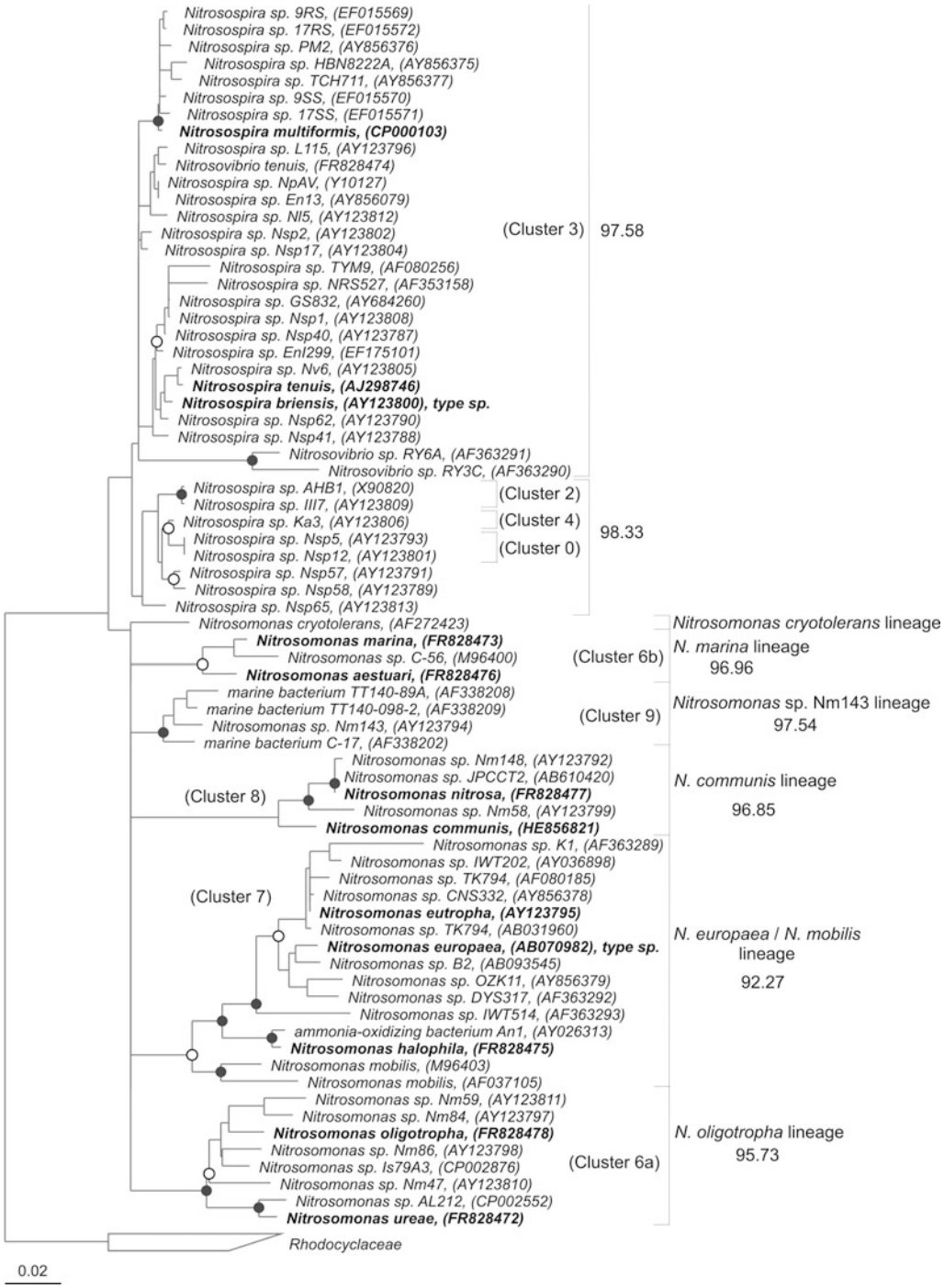

Fig. 34.1

Consensus phylogenetic tree of the family *Nitrosomonadaceae* based on the 16S rRNA gene. Final topology is based on a mix of reconstructions including maximum likelihood (RAxML, Stamatakis 2006), maximum parsimony (ARB, Ludwig 2004), and neighbor joining with the Jukes-Cantor correction. Initial trees were calculated using sequences longer than 1,300 nucleotides. Shorter sequences were added to the consensus tree using the ARB maximum parsimony tool. The sequence dataset and alignment were obtained from the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree) for validly published species (*bold* characters) and Silva SSU r114 database (http://www.arb-silva.de/search) for other sequences. Representative sequences from closely related taxa were used as out-groups. In addition, a bacterial homologues site filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Unstable topologies are shown as multifurcations. Filled and empty round nodes indicate neighbor-joining bootstrap values (100 re-samplings) above 90 % and 70 %, respectively. Minimum 16S rRNA sequence similarity among members of each cluster is depicted. Scale bar indicates estimated sequence divergence

Nitrosospira

Stephen et al. (1996) and Kowalchuk et al. (1997) delineated four clusters (clusters 1–4) within the *Nitrosospira* lineage, and more comprehensive analysis including a wider range of sequences from both cultures and environmental samples led to the discovery of three more clusters (cluster 0, a second cluster represented by the isolate *Nitrosospira* sp. Nsp65 and a third comprising *Nitrosospira* sp. Nsp57 and Nsp58; ❯ *Fig. 34.1*). However, these clusters are not consistently recovered with different treeing methods using either 16S rRNA or *amoA* sequences to infer relationships (Koops et al. 2006). This contrasts with the clear and distinct lineages that are recovered consistently within the genus *Nitrosomonas*. Moreover, one of the clusters identified by Stephen et al. (1996; cluster 1) is represented only by sequences recovered from environmental samples, and as with *Nitrosomonas* cluster 5 is not shown on ❯ *Fig. 34.1*.

Organisms now accommodated in the genus *Nitrosospira* were previously assigned to three different genera (*Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio*), but their 16S rRNA sequences have high identity (Woese et al. 1984a; Head et al. 1993), and even the most dissimilar sequences within the lineage exhibit 96.1 % 16S rRNA sequence identity (Koops et al. 2006). This compares with minimum 16S rRNA sequence identity values for individual lineages within the genus *Nitrosomonas* that range from 92.4 % to 97.5 % (Koops et al. 2006). Moreover, the differences in phenotypic characteristics of *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio* are minimal, and the primary distinguishing feature is their morphology, which is pleomorphic, as illustrated by the specific name of the only described *Nitrosolobus* sp., *Nitrosolobus multiformis* (Head et al. 1993). Consequently the three genera were combined with the generic epithet *Nitrosospira* having precedence (Head et al. 1993).

It was subsequently suggested that *Nitrosolobus* be reinstated as a separate genus (Teske et al. 1994; Aakra et al. 2001b), but a more comprehensive analysis has shown that different lineages within the *Nitrosospira* radiation may represent different species and the data currently available do not strongly support separation of different members of the *Nitrosospira* lineage into different genera (Koops et al. 2006). Currently there are three named *Nitrosospira* species: *N. briensis*, *N. multiformis*, and *N. tenuis* (❯ *Fig. 34.1*). However, based on DNA-DNA hybridization values of $\leq$60 % and/or 16S rRNA sequence identity $\leq$97.5 % for named *Nitrosospira* spp., cultivated *Nitrosospira* sp. may comprise 15 genospecies (Koops et al. 2006). It is clear that much is yet to be learned of the evolutionary relationships within the genus *Nitrosospira* from ongoing genome sequencing projects. The genome sequence of *Nitrosospira multiformis* ATCC 25196 is available (Norton et al. 2008), and sequencing of three further *Nitrosospira* genomes is currently underway (see section below on "❯ Genome Sequences").

Genome Sequences

At present, there are five published, closed, genome sequences of ammonia-oxidizing bacteria (AOB) within the *Nitrosomonadaceae*: *Nitrosomonas europaea* ATCC 19718 (Chain et al. 2003), *Nitrosomonas eutropha* C91^T (Stein et al. 2007), *Nitrosomonas* sp. AL212 (Suwa et al. 2011), *Nitrosomonas* sp. Is79 (Bollmann et al. 2013), and *Nitrosospira multiformis* ATCC 25196^T (Norton et al. 2008). As of this writing, known yet unpublished draft genome sequences of *Nitrosomonadaceae* isolates include *Nitrosomonas marina* C-113a, *Nitrosospira briensis* C128, *Nitrosospira tenuis* Nv1^T, *Nitrosospira* sp. B6 (cluster 2), *Nitrosomonas ureae* Nm10^T, *Nitrosomonas oligotropha* Nm45, *Nitrosomonas cryotolerans* Nm55^T, and *Nitrosomonas communis* Nm2^T. A published draft sequence of Nitrosospira sp. APG3, a cluster 0 isolate, is also available (Garcia et al. 2013). Genome sequences from four gammaproteobacterial ammonia-oxidizing bacteria have also been determined (*N. oceani* ATCC19707^T (CP000127; plasmid: CP000126), *Nitrosococcus* sp. strain C-113 (proposed as *N. watsonii*; one chromosome and two plasmids), *N. oceani* strain AFC-27(ABSG00000000.1), and *N. halophilus* strain Nc4; Campbell et al. 2011), but not all of these are closed. It is anticipated that many more isolates will be genome sequenced and publicly released within the next few years as genome sequencing costs continue to decline. Annotation and analysis of the five presently published AOB genomes reported information and statistics from the discontinued portal at the US Department of Energy Oak Ridge National Laboratories (ORNL); the information presented herein is reported from the Integrated Microbial Genomes (IMG) database, which is continuously maintained and updated by the US Department of Energy Joint Genome Institute (https://img.jgi.doe.gov/).

Each of the five closed AOB genome sequences contains a single circular chromosome and three contain plasmids of variable size (❯ *Table 34.1*). In addition, all of the genome sequences contain one complete ribosomal RNA operon and a full complement of tRNA genes. All of the *Nitrosomonadaceae* genomes encode multiple copies of the gene clusters encoding key genes of ammonia-oxidizing catabolism (❯ *Table 34.2*), a feature that is not shared by marine-dwelling gammaproteobacterial nitrosococci (Klotz et al. 2006). The presence of pseudogenes (❯ *Table 34.1*), numerous IS elements (Arp et al. 2007), large tandem repeats in the *N. europaea* ATCC 19716 genome (Chain et al. 2003), and a ca. 117 kb genomic island flanked by tRNA genes and phage-related integrase in the *N. eutropha* C91^T genome (Stein et al. 2007) indicate a high degree of recombinogenic activity among the AOB genomes. Comparison of highly conserved genes and neighborhoods in *N. europaea* ATCC 19718 and *N. eutropha* C91^T genome sequences (Stein et al. 2007) provides evidence of extensive replication and reshuffling. Prophage induction in *N. multiformis* ATCC 25196^T was stimulated by a variety of disturbances including changes in pH and exposure to chromium or cyanide (Choi et al. 2010).

Table 34.1

Properties of closed genome sequences of ammonia-oxidizing bacteria in the family *Nitrosomonadaceae* (Data were derived from the Integrated Microbial Genomes and Metagenomes annotation portal of the US Department of Energy Joint Genome Institute at: https://img.jgi.doe.gov/)

Strain	No. plasmids (sizes in kbp)	No. base pairs (Mbp)	G+C content	Total gene count (IMG)	No. predicted proteins with putative function	No. predicted proteins of unknown function	No. predicted tRNA coding genes	No. predicted rRNA operons	Misc. RNAs	Pseudogenes
Nitrosomonas europaea ATCC 19718	0	2.81	50.7	2,630	1,863	597	41	1	0	111
Nitrosomonas eutropha C-91	2 (65.1, 55.6)	2.78	48.5	2,695	2,001	442	41	1	3	89
Nitrosomonas sp. AL212	2 (92.7, 63.8)	3.18	44.7	3,238	2,536	447	38	1	0	211
Nitrosomonas sp. Is79A3	0	3.78	45.4	3,597	2,866	508	38	1	0	181
Nitrosospira multiformis ATCC 25196	3 (18.8, 17, 14.2)	3.23	53.9	2,885	2,026	801	43	1	3	22

Table 34.2

Selected functional inventory in closed genome sequences of *Nitrosomonadaceae*

Strain	Ammonia monooxygenase	Hydroxylamine oxidoreductase, cytochrome c_{554}, cytochrome c_{M552}	Nitrite reductase	Cytochrome *c* nitric oxide reductase	Cytochrome *c*′-beta (*cytS*)	Cytochrome P460 (*cytL*)	RuBisCO
Nitrosomonas europaea ATCC 19718	*amoCABED* (2) *amoC* (1)	*haoAB-cycAB* (2) *haoAB-cycA* (1)	*ncgABC-nirK*	*norCBQD* (1) *norSY-senC*- orf1 (1)	Present	Present	Form IA
Nitrosomonas eutropha C91	*amoCABED* (2) *amoC* (1)	*haoAB-cycAB* (2) *haoAB-cycA* (1)	*ncgABC-nirK*	*norCBQD* (1) *norSY-senC*- orf1 (1)	Present	Present	Form IA
Nitrosomonas sp. AL212	*amoCABED* (2) *amoCAB* (1) *amoC* (2)	*haoAB-cycAB* (3)	*nirK*	*norCBQD* (1)	Present	Present	Form IA Form IC
Nitrosomonas sp. Is79	*amoCABED* (2) *amoCAB* (1) *amoC* (2)	*haoAB-cycAB* (3)	*nirK*	Not present	Present	Present	Form IA Form IC
Nitrosospira multiformis ATCC 25196	*amoCABED* (1) *amoCAB* (1) *amoC* (1)	*haoAB-cycAB* (3)	*nirK*	*norCBQD* (1) *norSY-senC*- orf1 (1)	Present	Absent	Form IC

Numbers following gene inventory indicate the number of gene or operon copies present within the particular genome sequence. Inventory implicated in producing a functional enzyme or enzyme complex for ammonia monooxygenase, hydroxylamine oxidoreductase with electron transport partners, nitrite reductase, and cytochrome *c* nitric oxide reductase are listed as well as uncharacterized inventory encoded within the same operon. Because all of the strains encode a complete Calvin cycle for carbon fixation, only the type of RuBisCO is indicated. Details of these enzyme complexes and encoding gene inventory can be found in Sayavedra-Soto and Arp (2011) and references therein

Correlation coefficients from genome inventories based on four different gene-calling models (COG, Pfam, KEGG Orthology, and TIGRfam) show variable relationships between the genome sequences (*Table 34.3*). The most closely related genomes, as determined by the average correlation coefficient across the four models, are *Nitrosomonas* sp. AL212 and *Nitrosomonas* sp. Is79. Both of these are oligotrophic strains isolated from environments with low ammonium concentration, even though *Nitrosomonas* sp. AL212 was isolated from activated sludge and *Nitrosomonas* sp. Is79 was isolated from a freshwater lake. The most distantly related strains are *N. europaea* ATCC 19718, which is adapted to relatively high ammonium concentration and salinity, and *Nitrosomonas* sp. Is79. The moderate degree of similarity between the genome of *Nitrosospira multiformis* ATCC 25916^T, a soil isolate, to all of the *Nitrosomonas* strains is perhaps to be expected given that it falls within a different genus. Additional genome sequences of *Nitrosospira* isolates will allow for a much clearer evolutionary comparison between the two *Nitrosomonadaceae* genera. All five *Nitrosomonadaceae* genome sequences maintain multiple copies of operons encoding the central components of the ammonia oxidation pathway (*Table 34.2*): ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), and the two cytochromes (c_{554} an c_{M552}) that relay electrons from hydroxylamine oxidation to the quinone pool for production of a proton motive force and continued oxidation of ammonia by the reductant-requiring AMO (Sayavedra-Soto and Arp 2011). The *haoAB*, *cycA*, and *cycB* genes encoding HAO and cytochrome c_{554} and c_{M552} form the hydroxylamine redox module (HURM) that enables lithotrophic growth of bacteria on ammonium (Klotz and Stein 2008). The numbers and component genes of *amo* operons are variable among the genomes and, except for *Nitrosomonas* sp. Is79, encode the copper resistance genes (*copCD*) immediately following the *amoCABED* operons (Arp et al. 2007). All five of the *Nitrosomonadaceae* genomes encode three copies of the HURM operon, although both *N. europaea* ATCC 19718 and *N. eutropha* C91^T lack the *cycB* gene in one of these operon copies (*Table 34.2*). One copy of the operons encoding AMO and HURM is near each other in the genomes of *N. europaea* ATCC 19718 and *N. eutropha* C91^T, while the *amoCAB* operon in the *N. multiformis* ATCC 25196^T genome forms a contiguous supercluster with one of the *haoAB-cycAB* operons (Arp et al. 2007).

The *N. multiformis* ATCC 25196^T genome encodes all of the inventory for functional urease and hydrogenase (Norton et al. 2008). Urease genes and function have been detected in a number of other *Nitrosomonadaceae* and *Nitrosococcus* (gammaproteobacterial ammonia oxidizer) isolates (Koper et al. 2004), including *Nitrosomonas* sp. AL212 (Suwa et al. 2011). Although *Nitrosomonas* sp. Is79 encodes the biotin-containing urea carboxylase and putative allophanate hydrolase, its growth on urea has not been detected (Bollmann et al. 2013).

Table 34.3

Pearson correlation coefficients below the yellow boxes are based on COG (top left), Pfam (top right), KEGG Orthology (bottom left), and TIGRfam (bottom right) categorizations of genome inventories from IMG annotation. Average correlation coefficients and standard deviations of the four models are presented above the yellow boxes. Higher values indicate closer relationships than lower values

	Nitrosomonas europaea ATCC 19718	*Nitrosomonas eutropha C91*	*Nitrosomonas* sp. AL212	*Nitrosomonas* sp. Is79	*Nitrosospira multiformis* ATCC 25916
Nitrosomonas europaea ATCC 19718	1.00 1.00 1.00 1.00	0.80 (0.09)	0.70 (0.18)	0.65 (0.17)	0.74 (0.10)
Nitrosomonas eutropha C91	0.72 0.81 0.92 0.76	1.00 1.00 1.00 1.00	0.77 (0.12)	0.72 (0.14)	0.78 (0.09)
Nitrosomonas sp. AL212	0.47 0.71 0.92 0.71	0.60 0.80 0.90 0.77	1.00 1.00 1.00 1.00	0.85 (0.08)	0.75 (0.13)
Nitrosomonas sp. Is79	0.48 0.65 0.89 0.59	0.63 0.72 0.91 0.61	0.75 0.83 0.93 0.88	1.00 1.00 1.00 1.00	0.79 (0.13)
Nitrosospira multiformis ATCC 25916	0.71 0.72 0.87 0.64	0.80 0.77 0.89 0.67	0.58 0.71 0.86 0.84	0.61 0.77 0.86 0.91	1.00 1.00 1.00 1.00

In addition, all of the genes present in the genome of *N. multiformis* ATCC 25196^T, except for an ORF encoding a hypothetical protein for the NiFe hydrogenase, are also encoded in the *Nitrosomonas* sp. Is79 genome. However, the genes are not clustered together as in *N. multiformis* ATCC 25196^T, but are scattered throughout the genome (Bollmann et al. 2013). Neither hydrogenase activity nor its expression has yet been reported for any AOB isolate.

Sections of the nitrifier denitrification and nitrosative stress tolerance inventory, including copper-containing nitrite reductase (*nirK* with or without associated genes), cytochrome *c*- dependent nitric oxide reductase (*norCBQD* and *norSY-senC-orf1*), cytochrome P460 (*cytL*), and cytochrome c′-beta (*cytS*), are present in all *Nitrosomonadaceae* genomes, although some genes are apparently missing or have not yet been identified in the oligotrophic strains (*Table 34.2*). Both *Nitrosomonas* sp. AL212 and *Nitrosomonas* sp. Is79 lack the sNOR nitric oxide reductase *norSY-senC-orf* 1, and *Nitrosomonas* sp. Is79 also lacks the cNOR nitric oxide reductase (*norCBQD*). *Nitrosomonas* sp. Is79 is the first AOB examined that lacks both the *norCBQD* operon and the *ncyA* gene encoding the red copper protein, nitrosocyanin. Until now, nitrosocyanin was considered the only unique hallmark of the obligate chemolithotrophic AOB lifestyle and was hypothesized to be essential to ammonia lithotrophy (Arp et al. 2007). The physiological consequence of missing both cNOR and nitrosocyanin functions will be an important topic of research to define the core requirements for obligate ammonia chemolithotrophy and the correspondence of these genes and their translated products to the production of nitrogen oxide intermediates (Klotz and Stein 2008). All of the genomes encode the copper-containing nitrite reductase, *nirK*, which has been implicated in assisting hydroxylamine oxidation by *N. europaea* (Cantera and Stein 2007b) and in the nitrifier denitrification pathway for reduction of nitrite to nitric oxide (Stein 2011). Acquisition of *nirK* and *norCBQD* into AOB genomes was via lateral gene transfer from several origins (Cantera and Stein 2007a; Garbeva et al. 2007).

All the AOB examined to date use the Calvin cycle for fixation of carbon dioxide. Three of the five genomes encode a single variety of Type I RuBisCO, whereas *Nitrosomonas* sp. AL212 and *Nitrosomonas* sp. Is79 genomes encode both the "green" and "red" types, perhaps to retain flexibility in oligotrophic environments (*Table 34.2*). Only the *N. eutropha* C91^T genome encodes the full suite of genes for carboxysome biosynthesis, although carboxysomes have been observed microscopically in many other AOB strains whose genomes have yet to be sequenced (Koops et al. 1991).

Iron is an essential nutrient for AOB as they rely on several cytochromes and the iron-rich HAO enzyme for ammonia chemolithotrophy. The genomes of *N. multiformis* ATCC 25196^T, *N. eutropha* C91^T, and *N. europaea* ATCC 19718 have 29, 28, and 90 genes, respectively, with putative involvement in iron uptake. Only the genome of *N. europaea* ATCC 19718 has a remarkable diversity of putative iron siderophore uptake genes along with the absence of siderophore biosynthetic genes (Chain et al. 2003). The genome of *Nitrosomonas* sp. Is79 also encodes a low-affinity iron permease that may function under iron-replete conditions (Bollmann et al. 2013).

Other interesting, less central features of the five published genome sequences, including cell envelope, central carbon, biosynthetic, ion transport, and terminal oxidase inventories, can be found in the relevant reports for each genome sequence. In addition, several review articles comparing inventory and evolution of AOB and related genome sequences were published prior to publication of the two oligotrophic *Nitrosomonas* spp. genome sequences (Arp et al. 2007; Klotz and Stein 2008, 2011).

Phenotypic Analysis

By far the greatest knowledge of AOB and the process of chemolithotrophic ammonia oxidation derives from studies of the single model organism, *Nitrosomonas europaea* ATCC 19718. Several review articles have described in detail the enzymes and electron transport processes of ammonia oxidation based on studies of this strain (Whittaker et al. 2000; Arp and Stein 2003; Sayavedra-Soto and Arp 2011). Studies of other AOB isolates have revealed universally shared properties with *N. europaea* ATCC 19718, such as the apparent requirement for ammonia as the energy-deriving substrate. Other properties of AOB are not obligate, such as autotrophy and the requirement of O_2 by ammonia monooxygenase. Much focus has been placed on phenotypes that allow particular AOB phylotypes to occupy and sometimes dominate a niche, including ammonium and oxygen concentrations and tolerance to toxins. There has been extensive study of NO_x production by both the ammonia oxidation and nitrifier denitrification pathways among AOB phylotypes. The following descriptions focus on broad distinguishing phenotypic characteristics that inform niche preference of the seven phylogenetic lineages that contain cultivated members within the *Nitrosomonadaceae*.

Nitrosomonas europaea/Nitrosomonas (Nitrosococcus) mobilis Lineage

Members of this lineage include isolates with adaptation to relatively high concentrations of ammonium (tolerance to 400–600 mM; K_s for ammonia 30–61 μM) and salinity (maximum salt tolerance 400–900 mM NaCl). This lineage includes the cultured species *N. europaea*, *N. eutropha*, *N. halophila*, and *Nitrosomonas (Nitrosococcus) mobilis*. Molecular surveys have confirmed dominance of this lineage among AOB phylotypes in environments marked by high ammonium concentration, such as wastewater treatment plants and heavily fertilized soils (Koops and Pommerening-Röser 2001). *N. europaea* ATCC 19718 has been studied extensively as a model system to define the substrate range of ammonia monooxygenase for potential use of AOB in bioremediation strategies (Arp and Stein 2003). Members of this lineage also tend to be fairly tolerant to toxins including solvents and a variety of metals, yet their immediate stress responses have allowed process engineers to identify molecular markers indicative of early inhibition of ammonia oxidation (Radniecki and Lauchnor 2011).

Studies on the regulation of ammonia oxidation in *N. europaea* ATCC 19718 have shown that NH_3, the substrate of AMO, is a transcriptional activator of *amoA* and also enhances activity in whole cells by increasing expression of the AMO holoenzyme (Hyman and Arp 1995; Stein et al. 1997). In addition, nitrite is a specific inactivator of AMO activity in the absence of its substrates (Stein and Arp 1998), although nitrite can also enhance the recovery of *N. europaea* following starvation from ammonium (Laanbroek et al. 2002). Studies of anaerobic ammonia oxidation in both *N. europaea* ATCC 19718 (Schmidt et al. 2004) and *N. eutropha* N904 (Schmidt et al. 2001) revealed the use of NO_2 as an alternate oxidant for AMO while using nitrite as an electron acceptor. However, growth in continuous culture over several months showed that, while the presence of NO_2 can prevent loss of *N. eutropha* C91^T biomass, it does not allow sustainable anaerobic ammonia oxidation or cell growth (Kartal et al. 2012).

Nitrous oxide (N_2O)-producing pathways have been extensively characterized in *N. europaea* ATCC 19718 (Stein 2011). Two pathways produce N_2O: the oxidation of hydroxylamine produced during active ammonia oxidation and the reduction of nitrite and nitric oxide during nitrifier denitrification. The latter pathway is stimulated under low O_2 tension, while the former is more easily measured under high O_2 tension, but both processes can occur simultaneously. N_2O formed during ammonia oxidation is greatest under conditions of high ammonium and high oxygen, such as in certain wastewater treatment plants (Law et al. 2012) or during immediate recovery from anoxia (Yu and Chandran 2010). Nitrifier denitrification in *N. europaea* is positively regulated by nitrite. At high nitrite concentration, the NsrR transcriptional repressor releases the promoter of the nitrite reductase operon (*ncgABC-nirK*) and allows its expression (Beaumont et al. 2004). Mutagenized strains of *N. europaea* ATCC 19718 that do not express NirK show high levels of N_2O production from hydroxylamine under high oxygen, but low levels of N_2O production under hypoxia (Cantera and Stein 2007b). These results suggest that for *N. europaea* ATCC 19718, nitrite reduction by NirK under fully aerobic conditions is important to foster the complete conversion of hydroxylamine to nitrite. Under low oxygen, nitrifier denitrification may be important for siphoning electrons from the cytochrome pool

as re-oxidation by the terminal oxidase slows down and has the potential to create an electron flow bottleneck (Stein 2011). Under anoxic conditions, both NirK and the cytochrome *c* nitric oxide reductase, NorB, were necessary for the anaerobic oxidation of ammonium using NO_2 as an oxidant and nitrite as a terminal electron acceptor as described above (Schmidt et al. 2004).

N. europaea ATCC 19718 can use fructose or pyruvate as sole carbon sources, allowing it to grow chemolithoheterotrophically (Hommes et al. 2003). Under such growth conditions, ammonium was still required to support energy generation. Other members of this lineage have not been investigated for their ability to assimilate organic molecules. However, evidence from combined autoradiography and fluorescent in situ hybridization of ammonia-oxidizing bacterial populations in a wastewater treatment plant (Daims et al. 2001a) suggests that this ability may not be uncommon.

Nitrosomonas communis Lineage

Two named species are found in this lineage, *N. communis* (Nm2^T) and *N. nitrosa* (Nm90^T), and three further genospecies, represented by four isolates (*Nitrosomonas* sp. Nm33, Nm41 and Nm58, Nm148), are available in culture (Koops et al. 2006). Like the *N. europaea/Nc. mobilis* lineage, *N. communis* and *N. nitrosa* are adapted to relatively high ammonium concentration (up to 250 mM; K_s for ammonia 30–61 μM) and with the exception of *N. nitrosa* lack urease activity. It is rare to find sequences representative of this lineage in molecular diversity studies, perhaps due to poor recognition and hybridization to available primer sequences (Koops et al. 2006). The isolate *N. communis* YSNRA has significantly higher activity for HAO and lower activity for RuBisCO than *N. europaea* ATCC 25978^T or other AOB strains (Tokuyama et al. 2004), indicating physiological variation among species.

Nitrosomonas marina Lineage

Cultivated members of this lineage are all from marine environments, although related sequences have been detected in nonmarine ecosystems (Koops et al. 2006). They have a salt requirement, are urease positive, and are adapted to high concentrations of ammonium, 200–400 mM (Koops et al. 1991). This lineage is represented by two described species, *N. marina* (Nm22^T) and *N. aestuarii* (Nm36^T), and one undescribed genospecies (represented by isolatesNm51 and Nm63). *N. marina* C-113a produces nitrous oxide from both the ammonia oxidation and nitrifier denitrification pathways depending on cell density, nitrite concentration, and oxygen tension (Frame and Casciotti 2010). Members of this lineage may be important contributors of nitrous oxide to the atmosphere from marine ecosystems.

Nitrosomonas oligotropha Lineage

This lineage comprises two named species *N. oligotropha* (Nm45^T) and *N. ureae* (Nm10^T). A further four genospecies are represented in culture by seven isolates (Nm47, Nm59, Nm84, Nm86, AL212, Is79, and JL21), and culture-independent studies indicate that more species exist within this radiation (Speksnijder et al. 1998). Most isolates tested are urease positive. Molecular surveys indicate broad distribution of this phylotype from freshwater rivers and lakes to wastewater treatments plants to soils (Koops et al. 2006). *Nitrosomonas oligotropha* Nm45^T and *Nitrosomonas ureae* Nm10^T are the type strains of the two named species from this lineage, and their genomes are currently being sequenced. Several strains representing different species exist in current culture collections and two have published genome sequences (AL212 and Is79; see above). Strains in this lineage are adapted to moderate (*N. ureae*) to very low concentrations of ammonium (maximum tolerance 50–200 mM, but some grow in medium containing 1–5 mM ammonium) with the highest affinity to ammonium (K_s for ammonia 1.9–4.2 μM) of all AOB yet measured (Koops et al. 1991). The type strains of the two named species (Nm10^T and Nm45^T) and *Nitrosomonas* sp. AL212 are urease positive, but *Nitrosomonas* sp. Is79 is urease negative (Bollmann et al. 2013). It is surprising to find an oligotrophic AOB strain that lacks urease activity, as ammonium concentration is generally low in environments dominated by these strains. Along with adaptation to low ammonium, members of this phylotype tend to exist in biofilms and have been noted to produce ample amounts of EPS (Stehr et al. 1995).

Nitrosomonas cryotolerans Lineage

Only one isolate of this lineage, *N. cryotolerans* ATCC 49181^T (=Nm55^T) is in current culture collections. It is the only strain in the *Nitrosomonadaceae* that grows near 0 °C, although it is not a psychrophile (Jones et al. 1988). The lowest temperature for growth by other members of this family is around 5 °C (Koops et al. 1991). *N. cryotolerans* ATCC 49181^T was isolated from a marine environment; hence it has a requirement for salt. It is also urease positive and is adapted to relatively high ammonium concentrations (up to 400 mM; K_s for ammonia 42–59 μM). Nitrous oxide production by *N. cryotolerans* ATCC 49181^T is optimal during active ammonia oxidation at high cell density, high nitrite concentration, and low oxygen tension (Miteva et al. 2007) and continues in frozen cultures at temperatures as low as −32 °C.

Nitrosomonas sp. Nm143 Lineage

There is no named type species for this lineage but four representatives are available in culture (*Nitrosomonas* sp. Nm143, C-17, TT140-89A, TT140-098-2). Members of this lineage are associated with marine and estuarine environments and as such

are considered highly salt tolerant. Sequences related to *Nitrosomonas* sp. Nm143 and *Nitrosomonas cryotolerans* dominated active estuarine AOB, as assessed by stable isotope probing (Bernhard and Bollmann 2010). There have been no physiological studies on members of this lineage as the original isolates were lost from culture collections. Recently, further members of this lineage have been isolated and await further characterization.

Uncultured *Nitrosomonas* spp.

One lineage within the *Nitrosomonas* radiation is comprised solely of sequences recovered in culture-independent analyses. These have been termed environmental lineage or cluster 5, and members of this group have been detected in freshwater and marine systems and in coastal sand dunes (Koops et al. 2006).

Nitrosospira

Members of this lineage are considered to be the most dominant of the AOB in terrestrial environments based on frequency and abundance of their molecular markers in ecological surveys. Gene sequences related to *Nitrosospira* have been identified in other ecosystems including marine, wastewater, freshwater, and sand dunes (Koops et al. 2006). Interestingly, although *Nitrosospira* has been inferred to be prevalent in many marine systems (McCaig et al. 1994; Stephen et al. 1996; Phillips et al. 1999; Bano and Hollibaugh 2000; Horz et al. 2000; Hollibaugh et al. 2002; Nicolaisen and Ramsing 2002), no marine *Nitrosospira* isolates have yet been obtained. The *Nitrosospira* lineage was formed from the merging of three genera (*Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio*) and is more diverse phenotypically than the other lineages of the *Nitrosomonadaceae*, though they do not seem to form groups that are both phylogenetically and phenotypically consistent (Koops et al. 2006). *Nitrosospira* spp. are adapted to a wide range of ammonium concentrations (maximum tolerance 50–200 mM) and have a range of cellular morphologies, and only some are urease positive (Koops et al. 2006). Cultivated members from phylogenetic clusters 0, 2, 3, and 4 are maintained in current collections. *N. multiformis* ATCC 25196^T, currently the only member of this lineage with a published genome sequence, is from cluster 3. Although not yet publicly available, genome sequences of a cluster 2 isolate is undergoing completion, and a draft sequence from a cluster 0 isolate is publicly available (Garcia et al. 2013). Cultured isolates may be delineated into 15 genospecies, and interestingly it has been suggested, on the basis of DNA-DNA hybridization data, that *Nitrosospira* clusters 0, 2, and 4 might each represent single species (Koops et al. 2006) with cluster 3 being substantially more diverse. In addition to these main clusters, a fifth cluster (cluster 1) defined only from environmental sequences exists, as do two further lineages defined by isolates Nsp65, and Nsp58 and Nsp57 (Koops et al. 2006).

Nitrous oxide production in several *Nitrosospira* species results from both the ammonia oxidation and nitrifier denitrification pathways (Shaw et al. 2006; Sutka et al. 2006). However, for some *Nitrosospira* species, production of nitrous oxide is favored more during ammonia oxidation than under the low oxygen conditions that favor nitrifier denitrification (Dundee and Hopkins 2001). Expression of the *nirK* gene in *N. multiformis* ATCC 25196^T was constitutive and not influenced by the presence of nitrite as in *N. europaea* ATCC 19718 (Cua and Stein 2008). However, expression of *amoA* decreased in *N. multiformis* ATCC 25196^T in response to nitrite, indicating a different response to that of *N. europaea* ATCC 19718 or *N. eutropha* C91^T to the terminal product of ammonia oxidation.

Isolation, Enrichment, and Maintenance Procedures

Enrichment of members of the *Nitrosomonadaceae* from environments in which they have relatively high abundance is not difficult and is routinely achieved in selective inorganic media, which are also used for isolation of pure cultures. Media vary in composition (Koops et al. 2006), but all contain ammonium chloride or sulfate, mineral salts, and buffers of different strengths. Buffering is required because ammonia oxidation to nitrite produces acid equivalents. All cultivated AOB grow optimally at neutral or moderately alkaline pH and the majority cannot grow below pH 7, with the minimum for growth of 6.5 in liquid batch culture.

Isolation of pure cultures is difficult for two reasons. First, maximum specific growth rates of autotrophic ammonia oxidizers are low and much lower than many of the heterotrophs present in environmental samples that can grow on organic contaminants in solid media, glassware, volatile compounds entering liquid medium, and by-products of ammonia oxidizer growth. The second problem is low growth yield on ammonia and inhibition from acidity and nitrite. This prevents growth of readily visible colonies on solid medium, even when supplied with relatively high ammonium concentration, and reduces dominance over heterotrophs in liquid enrichment cultures. As a consequence, re-streaking of isolated colonies and inoculation of liquid media, as used to isolate typical heterotrophs, is difficult and continued subculture in liquid medium does not eliminate heterotrophs.

Despite these difficulties, pure cultures have been obtained using both approaches and many early attempts at purification involved isolation of colonies on solid medium (Kingma-Boltjes 1935; Meiklejohn 1950). Colonies were detected by binocular microscopy and were removed using micromanipulators and Pasteur pipettes, breaking the pipette tip into liquid medium. This is often necessary because colonies can be difficult to collect and disperse because of extensive EPS production. Colony detection can be increased by inclusion of neutral red, which stains acid-producing ammonia oxidizer colonies red (Macdonald and Spokes 1980), and heterotrophic growth can be reduced

by inclusion of antibiotics. Contamination of media with organic carbon can be reduced by use of gelatin, silica gel, or purified agar (Gould and Lees 1960; Soriano and Walker 1968). More recently, single colonies of variant *Nitrosomonas europaea* ATCC 19718 and *Nitrosospira multiformis* ATCC 25196^T were grown on nitrocellulose filters overlaying solidified mineral medium in order to cultivate cell lines containing introduced mutations (Sayavedra-Soto and Stein 2011).

Pure cultures are more frequently obtained by dilution of heterotrophs to extinction in liquid culture (e.g., Gibbs 1920; Lewis and Pramer 1958; Watson et al. 1971). This is possible if ammonia oxidizers are more abundant than heterotrophs and involves successive dilution to a level that eliminates heterotrophs but not ammonia oxidizers, followed by inoculation into many tubes of liquid medium. The approach was used to isolate the first ammonia oxidizer (Frankland and Frankland 1890), probably a *Nitrosomonas* sp. (Macdonald 1986). Contamination of liquid medium with organic carbon can be reduced by oven-heating of glassware and growth in sealed vessels, to reduce contamination with volatile compounds, although this requires sufficient headspace to avoid oxygen limitation (Aakra et al. 1999).

These methods are tedious and technically challenging but, like all cultivation-based methods, are selective and may not yield organisms that are representative of dominant or active organisms in the environment under study (Smith et al. 2001), which may be important for ecological studies. Traditionally this was addressed by inoculation of liquid medium from higher dilutions of probable number (MPN) tubes exhibiting growth (Belser and Schmidt 1978). This increases the diversity of cultures, including faster growing organisms dominating lower dilutions and those with high relative abundance, which are found in the higher dilutions (Watson et al. 1981). Enrichment and isolation are now accompanied by molecular analysis of source environments, to identify target phylotypes, and enrichment cultures, to identify cultures in which they are growing. This approach is, however, limited by current knowledge and will not detect organisms outside the range of current primer sets.

Growth conditions have also been modified to reflect source environments, e.g., through modification of salt concentration, pH, and incubation temperature. Traditional selective media contain ammonium at concentrations much greater than those in most natural environments. Reduction in initial ammonium concentration and continuous and semicontinuous culture have therefore been used to select strains that prefer low ammonia concentration, and continuous culture methods also increase selection of slower growing organisms (MacFarlane and Herbert 1984; Watson 1971; Bollmann and Laanbroek 2001; Bollmann et al. 2011). Ammonia oxidizers in many environments will be attached to particulate material, and biofilm growth is known to influence their ecophysiological characteristics (Armstrong and Prosser 1988; Powell and Prosser 1992; Allison and Prosser 1993). In fact, early ammonia oxidizer enrichment media contained insoluble carbonate, to which cells attached, and dispersed growth of cell suspensions was not considered possible. Enrichment and isolation in systems enabling growth on surfaces or as aggregates may increase the probability of enriching organisms of environmental relevance and increase the diversity of cultivated strains.

Although AOB were traditionally considered to be strict autotrophs, they are now known to contain organisms with the potential for mixotrophic and heterotrophic growth. Current enrichment methods, however, are designed to select for chemolithoautotrophs. Inclusion of organic carbon in media may increase selection for mixotrophic ammonia oxidizers, but will also increase growth of highly competitive heterotrophic non-ammonia oxidizers, and new approaches may therefore be required.

Ammonia oxidizers can be maintained by continued subculture, although this increases the probability of selection of strains that are specifically adapted to the cultivation conditions, potentially reducing their value for ecological studies. A further, practical disadvantage of this approach is the risk of contamination by heterotrophs and difficulty in subsequent re-isolation. Ammonia oxidizers may also be maintained at −80 °C in glycerol or by freeze-drying. The success of these methods varies between strains and resuscitation often requires 1–2 months. When successful, however, these approaches are more practical and avoid problems of strain selection. More recently, techniques have been developed for long-term freezer storage and resuscitation of AOB that, if adopted, may be extremely useful for safeguarding collections (Heylen et al. 2012).

Ecology

Soil

Both nitrosomonads and nitrosospiras have been isolated from soil and were considered to be the major contributors to soil ammonia oxidation before the discovery of abundant thaumarchaeal *amoA* genes in soil (Leininger et al. 2006). Reports of ratios of thaumarchaeal:bacterial *amoA* abundance >1 suggested greater contributions of thaumarchaea to soil nitrification in many soils. The limitations in relating functional gene (*amoA*) abundance to activity are discussed in Prosser and Nicol (2008, 2012), and the lower specific cell activity of cultivated thaumarchaea and other factors suggest reassessment of their relative contributions (Prosser and Nicol 2012). Other, more direct assessments of growth and activity (e.g., stable isotope probing, microcosm studies) provide evidence for dominance of thaumarchaea (Offre et al. 2009; Zhang et al. 2010) and bacterial ammonia oxidizers (Jia and Conrad 2009) in different soils, although the mechanisms determining their different contributions are currently not clear.

The apparent greater ease of isolation from soil of nitrosomonads, including *N. europaea*, suggested dominance over nitrosospiras in soil bacterial ammonia oxidizer communities. The first molecular analysis of soil demonstrated considerable diversity within both nitrosomonads and nitrosospiras and suggested dominance by the latter (Stephen et al. 1996). Subsequent studies, analyzing both 16S rRNA and *amoA* gene

sequences amplified from soil, provide further evidence for this (Norton 2011; Prosser 2011). A putative ammonia oxidizer group, *Nitrosomonas* cluster 5, has not yet been cultivated, and there is no reported evidence of gammaproteobacterial ammonia oxidizers in soil.

Nitrosomonadaceae can be detected using molecular techniques in virtually all soils, the exception being some acid soils (pH < 5.5), where current evidence suggests that thaumarchaea drive nitrification (Gubry-Rangin et al. 2011; Lehtovirta et al. 2011). Although some physiological characteristics of cultivated strains appear to be representative of particular lineages (see above) (Koops and Pommerening-Röser 2001), these relationships are based on relatively few strains and focus on nitrosomonads rather than nitrosospiras, which have been studied less. Physiological characteristics have also been implied from links between relative abundance of phylotypes and environmental characteristics or, more reliably, from experimental studies demonstrating differential growth of different phylotypes under different conditions (Webster et al. 2005). Generally, however, it is difficult to discern patterns between phylogeny and soil environmental characteristics (Norton 2011). The biogeography of soil ammonia oxidizers has been reviewed by Norton (2011), who presents evidence for spatial structure in nitrosospiras at fine-scale resolution of 16S rRNA gene sequences, and Prosser (2011), who considers the influence of soil characteristics and land management regimes on AOB community structure.

Marine

Nitrosomonas spp., *Nitrosospira* spp., and *Nitrosococcus* spp. have all been detected in marine environments, but only *Nitrosomonas* and *Nitrosococcus* spp. have been isolated in culture from marine systems (Koops et al. 2006). *Nitrosomonas* spp. from the *N. europaea*/*N. mobilis* lineage, *N. marina* lineage, and the *Nitrosomonas* sp. Nm143 lineage have all been detected and isolated from marine systems (Freitag et al. 2006). In addition the uncultured "cluster 5" *Nitrosomonas* spp. has been detected predominantly in marine systems. Members of the *N. marina* and *Nitrosomonas* sp. Nm143 lineages, however, seem to be the most prevalent of these (Koops et al. 2006). Members of the *N. oligotropha* and *N. communis* lineages do not appear to be major components of marine ammonia oxidizer communities.

It has been reported that *Nitrosomonas* spp. are primarily associated with marine particulates with higher ammonia concentration, while *Nitrosospira* spp. are apparently more prevalent in free-living planktonic state where ammonia concentration is likely to be lower (Phillips et al. 1999). Other studies suggest that salinity is the primary driver of AOB community composition (Freitag et al. 2006; Francis et al. 2003; Bollman and Laanbroek 2002; de Bie et al. 2001) with ammonium concentration being the second most significant factor (Francis et al. 2003; Ward et al. 2007) and oxygen levels also influencing AOB community composition (Ward et al. 2007; de Bie et al. 2001). Some studies have also demonstrated a shift in AOB communities in estuaries from terrestrial/freshwater communities dominated by *Nitrosomonas* spp. to marine communities dominated by *Nitrosospira* spp. (Freitag et al. 2006).

Although exclusively isolated from saline environments (marine waters and hypersaline lakes), detection of gammaproteobacterial AOB (*Nitrosococcus* spp.) in marine (or any other setting) using PCR-based approaches has rarely been successful (Nold et al. 2000; Ward and O'Mullan 2002). Despite their physiological adaptation to saline environments and detection using fluorescent antibody techniques (Ward 1982), it appears that they are not abundant or widespread in seawater (Ward et al. 2007).

Nitrosospira are the most frequently detected AOB in marine systems interrogated using betaproteobacterial AOB-specific 16S rRNA gene PCR or *amoA* PCR. Indeed these seem to be predominantly associated with cluster 1 *Nitrosospira*, which is currently known only from culture-independent AOB community inventories. The frequent detection of *Nitrosospira* spp. in marine environments and less frequently in marine nitrifying enrichments is in some ways paradoxical because none of the *Nitrosospira* spp. available in pure culture has an obligate salt requirement and their maximum salt tolerance is 100–250 mM NaCl (Koops et al. 2006), compared to typical seawater salt concentrations of approximately 600 mM.

Even though many studies based on PCR amplification of 16S rRNA or *amoA* genes and array-based analyses show *Nitrosospira* to be prevalent in marine environments (Phillips et al. 1999; Hollibaugh et al. 2002; O'Mullan and Ward 2005; Freitag et al. 2006; Ward et al. 2007), there is still no true consensus on the role of uncultured *Nitrosospira* in marine systems (Koops et al. 2006). In the absence of any cultured members of *Nitrosospira* cluster 1, evidence for their activity in the marine environment has come from stable isotope probing using ^{13}C-labelled bicarbonate (Freitag et al. 2006). Interestingly, in SIP experiments only DNA from AOB related to *Nitrosomonas* sp. Nm143 and *Nitrosomonas cryotolerans* and the nitrite oxidizer *Nitrospira marina* was labelled, and no *Nitrosospira* cluster 1 sequence was recovered, even when it was shown to be dominant in standard analysis of the same samples. The role of organisms from marine *Nitrosospira* cluster 1 therefore remains unclear, and conflicting data may result from the incubation conditions used for SIP analysis (high ammonia and high temperature), or *Nitrosospira* cluster 1 may be lithoheterotrophic ammonia oxidizers or indeed may not be ammonia oxidizers at all. The second of these possibilities is perhaps less likely given that *amoA* sequences from uncultured *Nitrosospira* have high sequence identity with genuine ammonia monooxygenase genes.

In addition to ammonia-oxidizing bacteria, members of the Thaumarchaeota have also been implicated in ammonia oxidation in marine environments (Francis et al. 2005; Könneke et al. 2005) and may even be the major ammonia oxidizers in some situations (Wuchter et al. 2006) with ammonia-oxidizing Thaumarchaeota predominating in oxygen minimum zones and in higher salinity reaches of estuaries. There was also a strong quantitative relationship between archaeal and bacterial ammonia oxidizer relative abundance with archaea

predominating at lower ammonia concentration (Bouskill et al. 2012). Anaerobic ammonia-oxidizing planctomycetes (anammox bacteria; see Chap. ?) may also be important players in the transformation of ammonia and nitrite in oxygen minimum zones and some anoxic sediments (Kuenen 2008).

Freshwater

Based on culture-independent analyses, members of the *Nitrosomonas oligotropha* lineage are typically dominant AOB in freshwater systems (Whitby et al. 2001; Speksnijder et al. 1998; Bollmann and Laanbroek 2001; Chen et al. 2009). This is supported by frequent isolation of organisms from this lineage from the highest positive dilutions of MPN cultures (Koops and Harms 1985; Stehr et al. 1995; Koops and Pommerening-Röser 2001) and in enrichment cultures (French et al. 2012). This group is also found in the upper (freshwater) reaches of estuaries (Freitag et al. 2006; de Bie et al. 2001). In addition, 16S rRNA sequences of *Nitrosospira* clusters 0, 2, 3, and 4 have been identified in freshwater habitats (Speksnijder et al. 1998; Whitby et al. 2001) and in a freshwater aquarium (Burrell et al. 2001). *N. europaea*, *N. eutropha*, and *N. nitrosa* strains have also been isolated from eutrophic freshwater environments (Koops and Pommerening-Röser 2001). Furthermore, 16S rRNA sequences from the *N. europaea/Nc. mobilis lineage*, *Nitrosomonas* environmental cluster 5, and the *N. marina* lineage have been detected in freshwater system (Speksnijder et al. 1998; Bollmann and Laanbroek 2001; Burrell et al. 2001).

As with marine and soil systems, AOB often share their environment with Thaumarchaeota that carry ammonia monooxygenase genes, and in a number of cases, these have been shown to be lithotrophic ammonia oxidizers (French et al. 2012; Hatzenpichler 2012). However, considerably less is known about freshwater ammonia-oxidizing archaea than those from marine and soil systems. One study has however shown that cultures of freshwater ammonia-oxidizing archaea are better adapted than AOB to low oxygen and low ammonia concentrations, and their growth rate was also less affected by changes in pH. Ammonia-oxidizing archaea are also more sensitive to continuous light and light cycles and recover from light exposure less well than AOB (Merbt et al. 2012; French et al. 2012). These factors may well dictate the relative significance of ammonia oxidizing bacteria and archaea in freshwater environments, though more systematic in situ studies will be required to test this fully (French et al. 2012). These factors may well dictate the relative significance of ammonia-oxidizing bacteria and archaea in freshwater environments, though more systematic in situ studies will be required to test this fully (French et al. 2012).

Wastewater Treatment Systems

Ammonia oxidation is a critical process for nitrogen removal in wastewater treatment plants, and the close physical and metabolic association of ammonia oxidizers and nitrite oxidizers is critical to its reliable operation (Mobarry et al. 1996; Schramm et al. 1996; Okabe et al. 1999; Graham et al. 2007). Nitrite produced from oxidized ammonia may, depending on prevailing conditions, be used as an electron acceptor by anaerobic ammonia-oxidizing planctomycetes leading to direct removal of nitrogen as nitrogen gas (Kuenen 2008) or as an electron donor for nitrite oxidizers forming nitrate that may be removed by classical denitrification. AOB are commonly considered to be strictly chemolithoautotrophic aerobic microbes that grow slowly, are rate limiting for nitrification, and are poor competitors for oxygen and ammonia compared to co-occurring heterotrophs (Verhagen and Laanbroek 1991; van Nielm et al. 1993; Laanbroek and Gerards 1993). Consequently, nitrifying systems are usually operated at solids retention time (SRT) of greater than 5 days and dissolved oxygen (DO) concentration above 2 mgl^{-1} to satisfy both carbon and nitrogen removal requirements and to overcome diffusional resistance to oxygen transfer to ammonia oxidizers in flocs. By virtue of urease production by many ammonia oxidizers, they are also central in conversion of urea to ammonia, an important process in wastewater treatment plants where urea is an important nitrogen species.

Nitrosomonas spp. appear to be the predominant AOB in wastewater treatment systems such as activated sludge plants (Mobarry et al. 1996; Schramm et al. 1996, 2000; Juretschko et al. 1998; Okabe et al. 1999; Daims et al. 2001a; Gieseke et al. 2001; Rowan et al. 2003a). This is typically recorded in analysis of activated sludge communities using FISH- or PCR-based analysis of AOB 16S rRNA genes or *amoA* to detect AOB (Koops et al. 2006). Nevertheless, *Nitrosospira* spp. have also been detected in wastewater treatment reactors though they are rarely found in full-scale plants (Schramm et al. 1998).

The most commonly identified ammonia oxidizers in municipal wastewater treatment plants come from the *Nitrosomonas europaea/Nitrosomonas (Nitrosococcus) mobilis* lineage (Juretschko et al. 1998; Koops and Pommerening-Röser 2001; Rowan et al. 2003a). *N. eutropha* appears to be the most commonly encountered isolate (Watson and Mandel 1971; Koops and Harms 1985). *Nitrosomonas (Nitrosococcus) mobilis* was once thought to be exclusively marine in origin, but it is the dominant ammonia oxidizer in some wastewater treatment plants, especially those treating industrial wastewaters, which often have higher salinity than domestic wastewater (Juretschko et al. 1998; Rowan et al. 2003a).

Almost all lineages in the *Nitrosomonas* radiation have been detected in wastewater treatment plants, and only organisms related to *Nitrosomonas cryotolerans* have not been detected. 16S rRNA gene sequences most closely related to the *Nitrosomonas halophila* lineage have been detected in lab-scale reactors treating industrial wastewater (Rowan et al. 2003b), and relatives of *Nitrosomonas* sp. Nm143 were detected in a full-scale reactor treating saline industrial wastewater (Rowan et al. 2003a). Interestingly, when this reactor ceased to be fed industrial wastewater and was instead fed domestic wastewater, the community composition altered and was dominated by *N. oligotropha*-like organisms (Head et al. unpublished data). Some nitrifying wastewater treatment plants are dominated by

a single AOB species (Juretschko et al. 1998; Rowan et al. 2003a), while other plants harbor a higher diversity of AOB (Rowan et al. 2003b; Purkhold et al. 2000; Daims et al. 2001b; Gieseke et al. 2001), and this in part relates to reactor configuration, with a full-scale biologically aerated filter exhibiting lower AOB diversity than a conventional trickling filter treating identical wastewater (Rowan et al. 2003a).

AOB in wastewater treatment reactors are typically found in large multicellular aggregates that have a close physical association with nitrite-oxidizing bacteria (Mobarry et al. 1996; Schramm et al. 1996, 1998; Juretschko et al. 1998; Okabe et al. 1999). Combining FISH or qPCR counts of AOB with measurements of ammonia oxidation in wastewater treatment plants has allowed estimation of cell-specific ammonia oxidation rates (CSAOR). These show a remarkable variation of over three orders of magnitude (from 43 to 0.03 fmol cell^{-1} h^{-1}) (Coskuner et al. 2005), and it appears that plants where AOB have higher CSAOR are more prone to failure (Coskuner et al. 2005).

As with other environments ammonia-oxidizing archaea may in some instances compete with AOB, though ammonia-oxidizing archaea are generally considered to be adapted to low ammonia concentration (French et al. 2012; Bouskill et al. 2012) not usually considered typical of wastewater. A number of studies have detected ammonia-oxidizing archaea in wastewater treatment plants (Park et al. 2006; Wells et al. 2009; Zhang et al. 2009, 2011; Jin et al. 2010; Sonthiphand and Limpiyakorn 2011; Kayee et al. 2011; Limpiyakorna et al. 2011; Bai et al. 2012), though in many, but not all cases, they are less abundant than co-occurring AOB. Mussman et al. (2011) surveyed 52 wastewater treatment plants from across Europe with only 6 giving positive detection by end-point PCR. Quantitative analysis however revealed that only two of these had high numbers of ammonia-oxidizing archaea (ca. 10^8 cells ml^{-1}; Mussman et al. 2011). Process modeling indicated that these population sizes were too great to be sustained from the levels of ammonia oxidation observed in the treatment plant. Subsequent analysis of inorganic carbon fixation by combined microautoradiography and FISH demonstrated that the AOB but not the archaea were capable of fixing inorganic carbon under the incubation conditions used. This coupled with no change in the expression of archaeal *amoA* in response to ammonia suggested that the archaea were not autotrophic ammonia oxidizers in this system. The debate therefore remains open regarding the relative importance of AOB and Thaumarchaeota in ammonia oxidation in wastewater treatment plants, though their low frequency of occurrence in these systems indicates that they are not commonly key players in nitrification in wastewater treatment plants.

Application

Nitrification is responsible for major losses of ammonium-based fertilizers. In some agricultural systems, as little as 30 % of fertilizer nitrogen is used by crops, the remainder being oxidized to nitrate or converted to nitrogen or the greenhouse gas nitrous oxide. Leaching of nitrate can lead to pollution of groundwaters, particularly in areas of intensive agriculture, while denitrification converts it to gaseous forms, nitrogen or nitrous oxide. Gruber and Galloway (2008) and Schlesinger (2009) estimated global nitrogen fertilizer input to soil at 100–150 Tg y^{-1}. Nitrogen fertilizer application will continue to increase with expanding global populations, and the economic and environmental significance of ammonia oxidizers will therefore increase. Strategies to reduce fertilizer loss include better informed management of fertilizer addition, application of inhibitors of ammonia oxidizers, and development of crops that produce natural inhibitors of ammonia oxidation. Persistent nitrification, through long-term fertilization, also acidifies soil and increases mobilization of toxic metals.

Ammonia-oxidizing bacteria also play a major role in cycling nitrogen in aquatic environments. Nitrogen budgets in soil and freshwater are closely coupled with most of the nitrogen in freshwater systems resulting from nitrogen run-off from land (Gruber and Galloway 2008). Of the estimated 295 Tg N y^{-1} input of fixed nitrogen to terrestrial environments from all sources (natural and driven by human activity), around 110 Tg N y^{-1} is lost to the atmosphere by denitrification (Schlesinger 2009), and close to 50 % of this budget is accounted for by denitrification in freshwater systems (Gruber and Galloway 2008). Although the contribution of ammonia conversion to nitrite and, indirectly, nitrate by ammonia-oxidizing bacteria and archaea relative to direct run-off of nitrate from other sources is poorly constrained, it is clear that AOB and AOA play an important role in this component of the global nitrogen cycle.

In marine settings AOB also play a crucial role in oxidizing the reduced nitrogen entering the world's oceans. The bulk of the nitrogen entering from rivers and atmospheric deposition (ca. 130 Tg y^{-1}) is in the form of nitrate, though atmospheric deposition of ammonia to the oceans does occur (Gruber and Galloway 2008). However, the 140 Tg N y^{-1} entering the ocean from nitrogen fixation (Gruber and Galloway 2008) must first be mineralized and oxidized by AOB and AOA before it can be removed by denitrification, which approximately balances marine nitrogen input (Gruber and Galloway 2008). Anaerobic ammonia-oxidizing planctomycetes also play an important role in driving nitrogen loss in some marine settings, and the interactions of anaerobic ammonia oxidation, ammonia oxidation, and denitrification and dissimilatory reduction of nitrate to ammonia and in marine settings are a hotly debated topic (Voss and Montoya 2009).

In engineered environments too, AOB play a vital role in nitrogen removal. In aerobic wastewater treatment systems such as trickling filters and activated sludge, it seems that AOB, rather than archaea, may be the principal drivers of ammonia oxidation (Mussman et al. 2011). A conservative underestimate (data are not available from all countries) indicates that around 250 billion m^3 of municipal wastewater is generated annually, of which 130 billion m^3 is treated (Mateo-Sagasta and Salian 2012). Municipal wastewater contains 20–85 mg Nl^{-1} (UN Food and Agriculture Organization; http://www.fao.org/docrep/t0551e/t0551e03.htm) equating to a total amount of

2.6–11.2 Tg of N in the municipal wastewater that is treated annually, worldwide. It should however be noted that only a proportion of treatment plants are operated for active nitrification but it is clear that AOB have a vital and large-scale role in nitrogen removal in wastewater treatment globally.

In all of the environments where the activity of ammonia-oxidizing bacteria has important practical consequences, their role in generation of the greenhouse gas nitrous oxide through ammonia oxidation or nitrifier denitrification must also be considered, and as discussed above, this can be a significant source of nitrogen oxide emissions under certain circumstances.

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35 The Family *Oxalobacteraceae*

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Abstract
Oxalobacteraceae is a family within the order *Burkholderiales* in the subclass of *Betaproteobacteria* which contains the 13 genera: *Collimonas*, *Duganella*, *Glaciimonas*, *Herbaspirillum*, *Herminiimonas*, *Janthinobacterium*, *Massilia*, *Noviherbaspirillum*, *Oxalicibacterium*, *Oxalobacter*, *Pseudoduganella*, *Telluria* and *Undibacterium*. According to the 16S rRNA gene sequence similarities of the type species, the genera *Herbaspirillum*, *Noviherbaspirillum*, *Collimonas*, *Glaciimonas*, *Oxalobacter*, and *Oxalicibacterium* as well as the genera *Telluria*, *Massilia*, *Duganella*, *Pseudoduganella*, *Janthinobacterium*, *Undibacterium*, and *Herminiimonas* appear more closely related within the family *Oxalobacteraceae*. The members of the family are heterotrophic and nonspore-forming gram-negative bacteria; they are mesophilic with the exception of some psychrophilic species.

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Their morphologies are rod, curved rod, vibrio- or spirillum-like. They represent a wide range of phenotypic and ecological properties. The general metabolism is mostly aerobic/microaerobic to facultative anaerobic in some species; only members of the genus *Oxalobacter* have a strictly anaerobic lifestyle. Members of the family *Oxalobacteraceae* are found in diverse environmental habitats like water, soil, and plant associated; some species/strains are mild plant pathogens or are claimed to be opportunistic human pathogens. Members of some genera (like *Collimonas* or *Glaciimonas*) are adapted to oligotrophic conditions, while, e.g., the genus *Herbaspirillum* harbors species of root-associated or even endophytic nitrogen-fixing bacteria, which are applied as plant growth-promoting bacterial inoculants in agriculture.

Taxonomy, Historical, and Current

Short Description of the Family

Oxalobacteraceae (Ox.al.o.bac.ter.a'ce.ae. M.L. masc. n. *Oxalobacter* type genus of the family; *-aceae* ending to denote family; M.L. fem. Pl. n. *Oxalobacteraceae* the *Oxalobacter* family; Garrity et al. 2005).

Phylogenetically, the family of *Oxalobacteraceae* is a member of the order *Burkholderiales* and class *Betaproteobacteria* in the phylum Proteobacteria (Stackebrandt et al. 1988; Garrity et al. 2005). According to the 16S ribosomal ribonucleic acid (rRNA) gene sequence comparison of the type species, the next related family within the *Burkholderiales* is the *Alcaligenaceae* (De Ley et al. 1986). The family *Oxalobacteraceae* contains the genera *Oxalobacter* (type genus) (Allison et al. 1985), *Herbaspirillum* (Baldani et al. 1986), *Noviherbaspirillum* (Lin et al. 2013), *Collimonas* (De Boer et al. 2004), *Glaciimonas* (Zhang et al. 2011), *Janthinobacterium* (De Ley et al. 1978; Lincoln et al. 1999), *Undibacterium* (Kämpfer et al. 2007), *Herminiimonas* (Fernandes et al. 2005), *Oxalicibacterium* (Tamer et al. 2002), *Duganella* (Hiraishi et al. 1997), *Pseudoduganella* (Kämpfer et al. 2012a), *Massilia* (La Scola et al. 1998), and *Telluria* (Bowman et al. 1993). The family is metabolically diverse and includes strict anaerobes, aerobes, and nitrogen-fixing organisms. The Gram staining of these bacteria is negative. The morphological forms vary from rods to curved rods, vibroid, or spirillum shapes. Most of the bacteria are motile by means of one or several polar or peritrichous flagella; some species are nonmotile.

The genus name *Oxalobacter* was first proposed by Allison and collaborators (1985). The description of the type species *Oxalobacter formigenes* was based on an anaerobic oxalate-degrading isolate (OxB^T) from sheep rumen (Dawson et al. 1980a). The type strain $WoOx3^T$ of the second species, *Oxalobacter vibrioformis*, was isolated from anoxic freshwater sediments (Dehning and Schink 1989).

First isolates of the genus *Herbaspirillum* were initially thought to be a new *Azospirillum* species due to its cell form, growth behavior, and habitat within grass roots (Baldani et al. 1984). However, RNA/RNA hybridization experiments showed no close relatedness with *Azospirillum* spp. or *Aquaspirillum itersonii* (Falk et al. 1986), and therefore the genus *Herbaspirillum* was defined with nitrogen-fixing endophytic bacterial isolates as the genus species *Herbaspirillum seropedicae* (Baldani et al. 1986). The genus was then expanded to embrace strains from the species [*Pseudomonas*] *rubrisubalbicans*, a mild pathogen causing mottled stripe disease in some susceptible sugarcane plants (Pimentel et al. 1991). Due to the high degree of DNA–DNA hybridization similarity (Gillis et al. 1990) and additional phenotypic characteristics to the genus *Herbaspirillum*, including the ability to fix nitrogen, it was reclassified as *H. rubrisubalbicans* (Baldani et al. 1996). A group of clinical isolates (EF group 1), non-nitrogen-fixing strains, had to be included into the genus *Herbaspirillum* as species 3 because of its molecular and overall physiological relatedness. However, the taxonomic position of the *Herbaspirillum* species 3 is uncertain and will not be treated here. New species were later described (DDH, 16S rRNA, and physiological characteristics), but few of them were found associated with plants (*H. frisingense*, *H. lusitanum*, *H. hiltneri*, and *H. rhizosphaerae*) and showed the ability to fix nitrogen (*H. frisingense*). The species *H. lusitanum* was described as nitrogen fixer, but this characteristic was not confirmed by genome sequencing of the type species (Weiss et al. 2012). The other species (*H. aquaticum*, *H. autotrophicum*, *H. huttiense* subsp. *huttiense*, *H. huttiense* subsp. *putei*) were isolated from water and lakes, while *H. chlorophenolicum* was originated from soil sediments. The species *H. canariense*, *H. aurantiacum*, *H. soli*, and *H. psychrotolerans*, isolated from soil, were reclassified based on polyphasic analysis and transferred to the genus *Noviherbaspirillum* that includes the type species *N. malthae* (Lin et al. 2013).

Bacteria belonging to the genus *Collimonas* were originally isolated from sandy dune soil in the Netherlands and were characterized by their mycophagous activity as *C. fungivorans* (De Boer et al. 2004). Different species (*C. arenae* and *C. pratensis*) were isolated from low-carbon sandy soil further on (Höppener-Ogawa et al. 2008a, b). An 18 bp stretch of the 16S rRNA gene of *Collimonas* spp. was found absent in a survey of the entire data set of 16S rRNA gene sequences. A fluorescence-labeled 18 bp probe (5′-CTCTTCGGGATTCTGTAC-3′) complementary to this region (998–1015) proofed to provide a quick discriminative test between *Collimonas* isolates and members of other species and genera (De Boer et al. 2004).

Glaciimonas immobilis was isolated from alpine glacier cryonite in Tyrol, Austria, by Zhang et al. (2011). It is nonmotile and metabolically adapted to oligotrophic conditions. Recently, phylogenetically related bacterium was obtained from water collected in a uranium mine in Portugal and named *G. singularis* (Chung et al. 2013).

Based on studies involving rRNA similarities of the *Chromobacterium* genus, De Ley et al. (1978) proposed a new genus named *Janthinobacterium* for the strains formerly classified as *Chromobacterium lividum*. The genus *Janthinobacterium* belong to the class *Betaproteobacteria*, order *Burkholderiales*, and *Oxalobacteraceae* family. The genus is composed by two validly published species. The G+C content range of the DNA for the

genus *Janthinobacterium* is 61–67 mol %. By quantitative comparison of rRNA cistron similarities studies with *Chromobacterium violaceum*, *Chromobacterium lividum*, and great variety of gram-negative bacteria with uncertain taxon position, De Ley et al. (1978) proposed a new genus named *Janthinobacterium* with one species *Janthinobacterium lividum* (syn. *C. lividum*) with *C. violaceum* nomenclature maintained. The previous *Chromobacterium* cluster with two species was constructed based on results from numerical analysis of phenotypic traits, and many authors pointed that the species of *Chromobacterium* were very different and should be separated at genus level (Leifson 1956). rRNA similarity maps clearly indicated separated clusters with all C. *lividum* strains and all *C. violaceum* strains tested forming tight separate clusters around their respective neotype strains (De Ley et al. 1978). More recently, Kämpfer et al. (2008b) using 16S rRNA gene sequence comparisons, chemotaxonomic approaches, and several biochemical characteristics reclassified *Pseudomonas mephitica* CCUG 2513^T as a later heterotypic synonym of *Janthinobacterium lividum*. Lincoln et al. (1999) isolated bacteria that caused a soft rot disease of *Agaricus bisporus* and based on nutritional, physiological, chemical, and molecular techniques had shown similarities with *Janthinobacterium* (*J. lividum*). These authors described a high degree of genotypic similarity between members of the genus *Janthinobacterium* and *Herbaspirillum* (DNA–RNA hybridization) and high degree of 16S rRNA sequence similarity between members of the genera *Janthinobacterium*, *Herbaspirillum*, *Oxalobacter*, and *Duganella*. However, members of the genus *Janthinobacterium* could be easily distinguished from these taxa by the use of whole-cell fatty acid analysis. Polyphasic data obtained for the mushroom pathogenic strains indicate a novel species within the genus *Janthinobacterium* named *Janthinobacterium agaricidamnosum* (Lincoln et al. 1999).

The genus *Undibacterium* was described based on two strains (CCUG 49009^T and CCUG 49012) that was isolated from drinking water (Kämpfer et al. 2007). Chemotaxonomic data (major ubiquinone, polyamines, and polar lipids plus fatty acids) coupled with 16S rRNA gene sequence similarities studies involving closely related genera allowed to propose a new genus named *Undibacterium* gen. nov., with one species, Undibacterium *pigrum* sp. nov., within the family *Oxalobacteraceae*. The type strain of *Undibacterium pigrum* is strain CCUG 49009^T (= CIP 109318^T). Four years later, Eder et al. (2011) described a new species, *Undibacterium oligocarboniphilum* sp. nov. (an isolate from purified water), as well as reclassified *Undibacterium pigrum* strain CCUG 49012 as the type strain of *Undibacterium parvum* sp. nov. and proposed an emended descriptions of the genus *Undibacterium* and the species *Undibacterium pigrum*. *Undibacterium oligocarboniphilum* sp. nov. was proposed based on similar chemotaxonomic profiles obtained for members of the family *Oxalobacteraceae*. The fourth species of the genus, *Undibacterium terreum*, was proposed based in one strain C3^T, isolated from permafrost soil in China. 16S rRNA gene sequences analysis revealed close relationship with *Undibacterium* genus and biochemical and physiological distinguishable characteristics allowed indicate this isolate as a new species from the genus. Quite recently, two new species emerged, *Undibacterium jejuense* and *Undibacterium seohonense*, based on two bacterial strains (JS4-4^T and SHS5-24^T) isolated from soil and freshwater, respectively (Kim et al. 2014).

The genus *Herminiimonas* was created to accommodate new bacterial isolates from water samples within the family *Oxalobacteraceae* (Fernandes et al. 2005). Currently, the genus comprises six validly published names species.

The genus *Oxalicibacterium* harbors as type strain a yellow-pigmented bacterial strain (TA17^T) which was isolated from litter of oxalate-rich plants in Turkey (Tamer et al. 2002). The strain was similar to the genus *Pseudomonas*, but differed from it with respect to 16S rRNA sequence and physiological and biochemical data, which led to the description of the genus *Oxalicibacterium*, with type species and strain *Oxalicibacterium flavum* (Tamer et al. 2002, 2003). Two additional species, *Oxalicibacterium horti* and *Oxalicibacterium faecigallinarum* (Sahin et al. 2009a, b) and a fourth species (*Oxalicibacterium solurbis*) were described later (Sahin et al. 2010a, b).

Duganella zoogloeoides was originally described as an isolate within the genus *Zoogloea* because of the formation of cell clumping and the formation of a zoogloeal matrix. Therefore, it was presented in the second edition of the Prokaryotes within the genus *Zoogloea* (Dugan et al. 1992). Due to its floc-forming ability, it was reasoned to have an important role in wastewater treatment. *Duganella violaceinigra* was recently renamed to *Pseudoduganella violaceinigra* by Kämpfer et al. (2012b). *Duganella* and *Pseudoduganella* are related to *Massilia*.

To date, the genus *Massilia* is the most species-rich genus of the family *Oxalobacteraceae*, currently composed of 22 described species with validly published names. The genus has a relatively recent history, being created in 1998 to harbor an isolate from human origin as a new species *Massilia timonae*, the type species, with UR/MT95^T as the type strain (La Scola et al. 1998, 2000). New species were incorporated to the genus only from 2006 to 2011. The genus was proposed based on a single fastidious, strictly aerobic, gram-negative isolate from a culture of blood of an immunocompromised patient with cerebellar lesions (La Scola et al. 1998), mainly based on sequence analysis of 16S rRNA gene that demonstrated its closer proximity to *Duganella zoogloeoides* and *Telluria mixta* and polyphasic characterization (La Scola et al. 1998, 2000). Three more strains were added to the species based on its 16S rRNA sequence and phenotypic similarities, and an emended description of the species was proposed (Lindquist et al. 2003). These new strains were also isolated from human patients' blood, cerebrospinal fluid, and bone samples (Lindquist et al. 2003). However, as La Scola et al. (1998) pointed out, although some evidences for the Timone isolate being the causal agent of the patient syndrome were observed, its close proximity to environmental species may also be a naturally niche for Timone isolate. Accordingly, new species added later confirmed this hypothesis, being isolated from environmental samples, such as water, air, and soil. The genus *Naxibacter* was proposed by Xu and collaborators (2005)

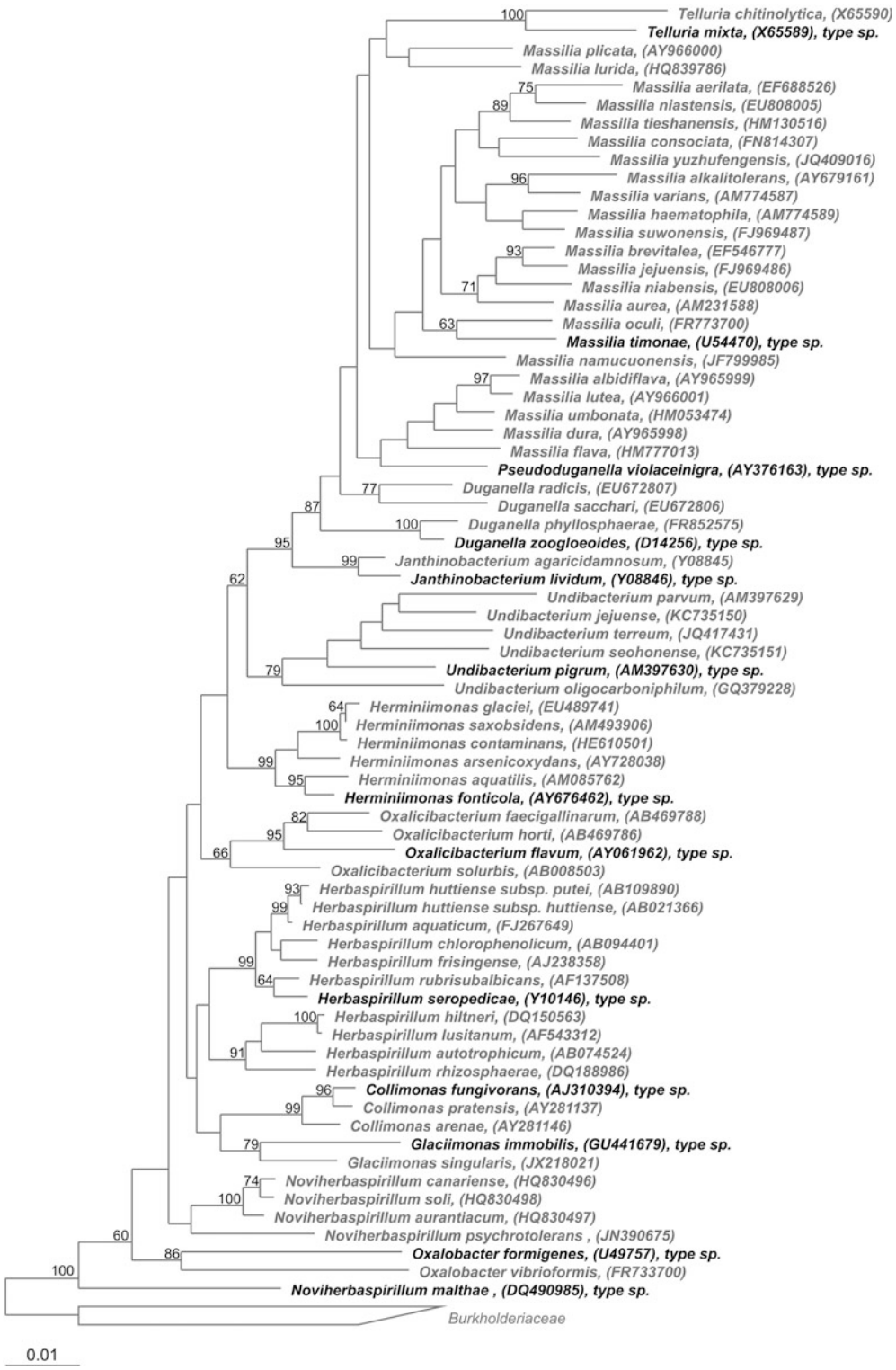

Fig. 35.1

Phylogenetic reconstruction of the family *Oxalobacteraceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). 1000 resampling boostrap values over 50 % shown. Scale bar indicates estimated sequence divergence

with the type species *Naxibacter alkalitolerans*. Phylogenetically, it was placed in the vicinity of *Massilia*, *Telluria*, *Duganella*, and *Janthinobacterium*. Later, two additional species, *Naxibacter varians* and *Naxibacter haematophilus*, were published together with an emended description of the genus *Naxibacter* (Kämpfer et al. 2008a). In the same year, the fourth species of the genus, *Naxibacter suwonensis*, was described (Weon et al. 2010). However, 16S rRNA sequence analyses and chemotaxonomic data justified the transfer of all *Naxibacter* species to the genus *Massilia*, as *Massilia alkalitolerans*, *Massilia varians*, *Massilia haematophilus*, and *Massilia suwonensis* (Kämpfer et al. 2011).

The history of the genus *Telluria* started with the characterization of a novel species of polysaccharide-degrading bacteria, which was named *Pseudomonas mixta* in 1988 (Bowman et al. 1988). Phenotypic, genotypic, and phylogenetic evidence showed that *Pseudomonas mixta* was highly similar to the chitin-degrading bacterium *Pseudomonas chitinolytica* and that both species differed from other Pseudomonads, leading to the description of the new genus *Telluria* (Bowman et al. 1993). The type species is *Telluria mixta*, and the second known species is *Telluria chitinolytica* (Bowman et al. 1993).

Phylogenetic Structure of the Family and Its Genera

According to the phylogenetic relationship based on 16S rRNA gene sequence analyses within the order *Burkholderiales*, the family *Oxalobacteraceae* is moderately affiliated to the family *Alcaligenaceae* (Garrity et al. 2005). Extensive 16S rRNA gene sequence analyses of type species and strains constitute the phylogenetic structure within the family *Oxalobacteraceae* (*Fig. 35.1*).

Herbaspirillum: The phylogenetic analysis based on the 16S rRNA sequences (performed using maximum-likelihood and maximum-parsimony methods) showed that the genus *Herbaspirillum* did not constitute a monophyletic group. The species *Herbaspirillum hiltneri*, *H. lusitanum*, *H. rhizosphaerae*, *and H. autotrophicum* formed a separated cluster (Carro et al. 2012). Similarly, the species *H. canariense*, *H. aurantiacum*, *H. soli*, and *H. psychrotolerans*, previously included in the genus *Herbaspirillum*, form a separate group and therefore were reclassified and transferred to the genus *Noviherbaspirillum* containing also the species *N. malthae* (genus species). Members of the genus *Herbaspirillum* have 94.1–95.2 % 16S rRNA gene sequence similarity to members of the genus *Noviherbaspirillum* (Lin et al. 2013) and 95.9–96.7 to the genus *Collimonas* (De Boer et al. 2004). Furthermore, members of *Herbaspirillum* have 96 % to genus *Herminiimonas*, 96.3–97.0 % to genus *Glaciimonas*, and lower than 96.2 % 16S rRNA gene sequence similarities to members of the genus *Oxalicibacterium* (Chung et al. 2013). Interestingly, the ten *Herbaspirillum* species exhibit very high degree of 16S rRNA gene sequence similarities with each other (97.0–99.9 %). Based on these 16S rRNA gene similarities, new species cannot be described. However, Rothballer et al. (2006) found only 14–32 % similarity in DNA–DNA hybridization experiments between the members of the new species *H. hiltneri* and *H. lusitanum*, although the 16S rRNA gene similarity was 98.9–99.0 % between the two species. A similar situation was found for *H. frisingense* (Kirchhof et al. 2001). On the other hand, the high level of the 16S and 23S rRNA gene sequence similarities between the type strains of *H. putei* and *H. huttiense* (99.9 % and 99.7 %) and DDH of 96 % with the *H. huttiense* DSM 10281T used as a probe excluded the possibility of both strains as representatives of different species (Dobritsa et al. 2010). However, the genome of *H. huttiense* type strain is 0.9 MB lower than that of *H. putei* type strain so that this difference reduced the DNA–DNA relatedness to 72 % which is very close to the threshold for delineating new bacterial species so two subspecies were described (*H. huttiense subsp. putei* and *H. huttiense subsp. huttiense*).

Noviherbaspirillum: Members of the genus *Noviherbaspirillum* have shown 94.1–95.2 % 16S rRNA gene sequence similarities to members of the genus *Herbaspirillum* but slightly lower for the closest genera within the family: *Oxalicibacterium* (93.4–94.4 %), *Duganella* (93.1–94.4 %), *Undibacterium* (93.2–94.3 %), *Herminiimonas* (93.5–94.2 %), *Collimonas* (93.8–94.0 %), *Janthinobacterium* (93.7 %), *Massilia* (92.5–93.2 %), *Oxalobacter* (93.1 %) and *Telluria* (91.1 %) (Lin et al. 2013). The type species *Noviherbaspirillum malthae* showed a high degree of similarity to *N. soli* (96.5 %), *N. aurantiacum* (96.3 %), *N. canariense* (96.0 %), and *N. psychrotolerans* (95.4 %) (Lin et al. 2013). However, these values were lower than that observed for the species *N. psychrotolerans* when compared to *N. canariense* (97.3 %), *N. aurantiacum* (97.2 %), and *N. soli* (97.2 %) (Bajerski et al. 2013). Higher degree of 16S rRNA gene sequence similarities (99.2–99.6) were observed during the phylogenetic tree construction involving the three species *N. canariense*, *N. aurantiacum*, and *N. soli* (Carro et al. 2012). However, all these species formed a cluster clearly divergent from the species of the genus *Herbaspirillum*.

Collimonas–Glaciimonas: *Collimonas* and *Glaciimonas* are closely related species; their nearest neighbors are *Herbaspirillum* and *Oxalicibacterium*. In all cases, full or almost full length 16S rRNA gene sequences are available which allowed calculating phylogenetic trees and 16S rRNA gene sequence similarities. Members of the genus *Glaciimonas* have 96.1–97.0 % 16S rRNA gene sequence similarity to members of the genera *Collimonas* and *Herbaspirillum* (Zhang et al. 2011; Chung et al. 2013). Furthermore, members of *Collimonas* have 95.9–96.7 % and 94.3–95.6 % 16S rRNA gene sequence similarities to members of the genera *Herbaspirillum* and *Janthinobacterium*, respectively (De Boer et al. 2004). *Pseudoduganella* has 96.8 % 16S rRNA gene sequence similarity to *Duganella* and also 96.5–97.1 % 16S rDNA gene sequence similarity to members of the genera *Duganella* and *Massilia*, respectively (Kämpfer et al. 2012b).

Janthinobacterium: Reference strains of species from *Janthinobacterium lividum* had shown values ranging from

99.0 % to 99.8 %. 16S rRNA gene sequence similarities of *Janthinobacterium* and closely related type species *Herbaspirillum seropedicae*, *Duganella zoogloeoides*, *Oxalobacter formigenes*, *Telluria mixta*, and *Telluria chitinolytica* were 90.6–96.4 %.

Undibacterium: The genus *Undibacterium* was described based on 16S rRNA sequence analysis of two strains (CCUG 49009^T and CCUG 49012) and emerged as a new member of *Oxalobacteraceae* family, not related to any other known most closely related genus (*Herminiimonas*, *Massilia*, *Duganella*, *Telluria*, *Herbaspirillum*, *Janthinobacterium*, *Naxibacter*, and *Paucimonas*) showing less than 96.5 % 16S rRNA gene sequence similarities (Kämpfer et al. 2007). Related to genospecies of *Undibacterium*, 16S rRNA gene sequence similarities were higher than 95.6 % with maximum value of 97.6 %, indicating that all species belong to the genus *Undibacterium*. In addition, genomic DNA–DNA hybridization showed low levels of relatedness (below 70 %), which support the proposition of six separated species.

Herminiimonas: Phylogenetic analysis of the 16S rRNA gene sequence between all the six reference strains of the genus *Herminiimonas* obtained similarity levels ranging from 97.7 % to 99.6 % which suggest their affiliation to the genus *Herminiimonas*. The separation into six separate species is supported by the low levels of genomic DNA–DNA hybridization (below 57 %). The genus *Herminiimonas* was described based on 16S rRNA sequence and RAPD analysis of four strains (S-94^T, S-97, S-99, and S-92) and emerged as clone of a new betaproteobacterium member of *Oxalobacteraceae* family, not closely related to any other known species (Fernandes et al. 2005). 16S rRNA gene sequence similarities of new isolates of *Herminiimonas* to the type species of nearest related genera from *Oxalobacteraceae* family such as *Herbaspirillum*, *Paucimonas*, *Janthinobacterium*, and *Duganella* are in the range 93.0–96.5 %. For species belonging to the *Herminiimonas* genus, 16S rRNA gene similarities were higher than 97.9–100 %.

Oxalicibacterium: Analysis of the 16S rRNA sequence of *Oxalicibacterium flavum* strain TA 17^T showed sequence similarity between 88 % and 93 % with the genera *Janthinobacterium*, *Duganella*, *Herbaspirillum*, and *Burkholderia* (Tamer et al. 2002, 2003). 16S rRNA gene sequences of *Oxalicibacterium horti* OD1^T and *Oxalicibacterium faecigallinarum* YOx^T showed 97.1 % and 97.44 % resp. sequence similarities with strain TA 17^T. In contrast, sequence similarities were lower than 96.2 % with all other established genera of the *Oxalobacteraceae* family (Sahin et al. 2009a, b). The 96.8 % 16S rRNA sequence similarity of strain MY14^T with *Oxalicibacterium flavum* was consistent with its phylogenetic characterization as *Oxalicibacterium solurbis*.

Duganella–Pseudoduganella: In a phylogenetic analysis, *Pseudoduganella violaceinigra* YIM 31327^T was clustered with the *Massilia* species using the neighbor-joining and maximum-likelihood methods, but it was grouped in another cluster containing *Duganella zoogloeoides* IAM 12670^T in the maximum-parsimony tree (Du et al. 2012). However, another phylogenetic tree based on the neighbor-joining method supported the reclassification of *D. violaceinigra* (Shen et al. 2013).

Massilia–Telluria: Phylogenetic 16S rRNA gene sequence analyses of *Massilia* and former *Naxibacter* species often grouped together and were found intermixed depending on the mode of sequence comparison (Kämpfer et al. 2008a; Weon et al. 2009, 2010). Even with the inclusion of *Naxibacter* species, phylogeny of the genus *Massilia* based on 16S rRNA gene sequences continues to be not well resolved. Kämpfer et al. (2011) observed that *Telluria* spp. fell together with a cluster of four *Massilia* spp. and suggests a detailed investigation of *Telluria* spp. However, topology and clusters in phylogenetic trees are very sensitive to the set of organisms used, choice of models of evolution and phylogenetic methods, alignment reliability, and sequence lengths/quality, to cite a few. Further, many of the clusters within *Massilia* genus are not well supported by bootstrap analysis in the phylogenetic trees reported so far. Accordingly, species from other genera of the family can also be seen clustering with *Massilia* (i.e., *Duganella* spp., *Pseudoduganella* spp., *Janthinobacterium* spp., and *Telluria* spp.) based on 16S rRNA gene sequence phylogeny (Monteiro et al. 2014). For example, *Telluria* species fell together with *M. albidiflava*, *M. dura*, *M. lutea*, and *M. plicata*. [*Duganella*] *violaceinigra* was reclassified within a novel genus as *Pseudoduganella violaceinigra* (Kämpfer et al. 2012b). Kämpfer et al. (2011) suggested that the fatty acid $C_{14:0}$ 2-OH, found in the species *M. albidiflava*, *M. dura*, *M. lutea*, and *M. plicata*, which grouped together with the species of the genus *Telluria* in 16S rRNA gene sequence phylogenetic tree, may represent a separate line of variation. Although these species consistently clusterize in different phylogenetic trees, this fatty acid is also observed in *M. aerilata*, *M. aurea*, *M. flava*, and *M. lurida*, in proportional amounts, and in *M. umbonata* in less than 1 %. However, these species fell outside the cluster of *Massilia* species containing $C_{14:0}$ 2-OH (Monteiro et al. 2014; Rodríguez-Díaz et al. 2013). Although the distinct phylogenetic positions and differences in chemotaxonomic markers such as polar lipids and fatty acids are usually used to distinguish bacteria at the genus level, assigning any novel isolate to Massilia–Duganella–Telluria species needs particular care because of the absence of clear phylogenetic boundaries and genus-specific chemotaxonomic markers (Shen et al. 2013).

Molecular Analyses

DNA–DNA Hybridization Studies

DNA–DNA hybridization analyses support the phylogenetic structure of the family *Oxalobacteraceae* and the genera within this family.

Herbaspirillum: The DNA–DNA hybridization values between the type strains of the ten *Herbaspirillum* species, and the type species *H. seropedicae* are in the range of 1–34 %. Higher values were observed for the species *H. huttiense* subsp. *putei* and *H. huttiense* subsp. *huttiense* hybridized against *H. aquaticum* (59 %) (Dobritsa et al. 2010) and *H. huttiense* subsp. *huttiense* toward *H. rhizosphaerae* (56 %) (Jung et al. 2007).

Noviherbaspirillum: The genus *Noviherbaspirillum* with five species was created based on polyphasic analysis including the 16S rRNA gene sequence similarities. Concerning the reclassification of *Noviherbaspirillum* and *Herbaspirillum*, the determination of DNA–DNA hybridization values of the different type strains with the type species of *Herbaspirillum* was decisive. The species *N. canariense*, *N. aurantiacum*, and *N. soli* presented DNA–DNA hybridization values of 25 %, 12 %, and 11 %, respectively, with *H. seropedicae* Z67. These DNA relatedness values were below 60 % when the three species type strains of *Noviherbaspirillum* were compared (Carro et al. 2012). In the case of *N. psychrotolerans*, the DNA–DNA relatedness values were below 30 % for the type strain hybridized against the other three *Noviherbaspirillum* species (Bajerski et al. 2013).

Collimonas: Within the genus *Collimonas*, the DNA–DNA hybridization values are in the range of 31–64 %, while they are between 70–75 % and 75–87 % within the species *C. fungivorans* and *C. pratensis*, respectively (Höppener-Ogawa et al. 2008b).

Janthinobacterium: The *Janthinobacterium* genus was proposed by De Ley et al. (1978) based on comparisons of DNA–rRNA cistron similarities of hybrids between the ^{14}C-labeled 23S rRNA fraction of *Janthinobacterium* (*Chromobacterium*) *lividum* against DNA from a variety of bacteria and DNA–DNA hybridization assays. The rRNA cistron of both *Chromobacterium* species were quite distinct in nucleotide ($Tm_{(e)}$) and percent rRNA binding, and the low degree of DNA hybridization conducted the authors to conclude that the species of *Chromobacterium* were not species of the same genus. Genotypic data coming from DNA–RNA hybridization analysis had shown a close relationship among members of the genus *Janthinobacterium* and *Oxalobacter*, *Duganella*, and *Herbaspirillum*. DNA–DNA hybridization between *J. lividum* DSM 1522T and the *J. agaricidamnosum* strain Wlr3T gave a very low value (35 %) to consider them as the same species.

Undibacterium: Few results of DNA–DNA hybridization related to *Undibacterium* were reported. In one study, two strains (CCUG 49009^T and CCUG 49012) were shown to represent separate species (sharing only 20 % DNA–DNA relatedness) being positioned as different species (Kämpfer et al. 2007; Eder et al. 2011) value. Values of DNA–DNA relationship for new proposed species of *Undibacterium* against reference strains of *Undibacterium* ranged in 29 % ± 4 % and 39 % ± 5 %, confirming the six proposed genomic species in the genus *Undibacterium*.

Herminiimonas: Despite the high 16S rRNA gene sequence similarities, DNA–DNA hybridization values among proposed species of the genus *Herminiimonas* showed levels ranging from 3.0 % to 25.7 %. Higher DNA–DNA hybridization values (52–57 %) were obtained among three species of the genus (*H. saxobsidens*, *H. glacier*, and *H. arsenicoxydans*), but the values were below the cutoff (<70 %) to proposed species level affiliation. These low hybridization values confirm the affiliation of six genospecies within *Herminiimonas*. In only one case, strain ND5^T previously classified as *H. glaciei* based on a DNA–DNA hybridization value of 78 % was reclassified as the second strain of *H. contaminans* (Kämpfer et al. 2013).

Oxalicibacterium: DNA–DNA homology experiments with three phenotypically similar strains (TA17^T, TA23, and TA24) of *Oxalicibacterium flavum* showed between 90 % and 100 % similarity values. These values clearly separated these strains from other strains tested such as oxalate-oxidizing strain TA25 (43 % similarity) and *Ralstonia eutropha* (41 % similarity) (Tamer et al. 2002). The DNA relatedness of *Oxalicibacterium horti* strain OD1^T and *Oxalicibacterium faecigallinarum* strain YOx^T was investigated by measuring the divergence between the thermal denaturation midpoint of homoduplex DNA and heteroduplex DNA (ΔT_m) (González and Sáiz-Jiménez 2005). When comparing DNA from different strains, the DNA reassociation values of pairs of strains TA17^T–OD1^T, TA17^T–YOx^T, andYOx^T–OD1^T were 9.7 °C, 6 °C, and 8 °C, respectively, thus above the threshold value (5 °C) for species delineation (Sahin et al. 2010a, b). *Oxalicibacterium solurbis* strain MY14^T showed a DNA reassociation value of 5.2 °C when compared to strain *Oxalicibacterium flavum* TA17^T, which was most similar to MY14^T with respect to 16S rRNA and *cpn60* gene sequence similarity, thus confirming that it belongs to a different species.

Massilia: Results for DNA–DNA hybridization (DDH) were obtained for all type strains of the described species of the genus *Massilia* and showed a maximum of 60.6 %, a minimum of 13.0 %, and with a mean of 34.1 % in cross-species hybridization. The highest value (60.6 %) was observed between *M. oculi* CCUG 43427A^T versus *M. timonae* CCUG 45783^T (Kämpfer et al. 2012a). Elevated values (>50.0 %) were observed among *M. varians/M. consociata* and *M. oculi/M. timonae/M. aurea* (Kämpfer et al. 2011, 2012a). The lowest value (13.0 %) was observed for the hybridization between type strains of *M. aurea*, AP13^T, and *M. timonae*, DSM 16850^T (Gallego et al. 2006). An intergenus DDH of 20.0 % was observed between *M. flava* Y9^T and *Pseudoduganella violaceinigra* KCTC 12193^T (Wang et al. 2012). Interestingly, 62.0 % hybridization was observed between *M. alkalitolerans* CCUG 50882^T versus *M. varians*, non-type strain CCUG 48018. However, cross-hybridization between type strains of *M. alkalitolerans*, CCUG 50882^T, and *M. varians*, CCUG 35299^T, showed value of 43.0 %. Similar results (48.0 %) were obtained for DDH against two other non-type strains of *M. varians* CCUG 49054 and CCUG48700A (Kämpfer et al. 2008a). Intraspecific DDH experiments among five different *M. varians* strains (CCUG 35299^T, CCUG 48700A, CCUG 48018, CCUG 49054, CCUG 24677A) were performed by Kämpfer et al. (2008a). A maximum of 100 % were obtained, and most of comparisons reached values more than 84.0 %, except for strain CCUG 48700A, with values between 64.0 % and 76.0 %.

Telluria: DNA–DNA hybridization experiments conducted by Bowman and collaborators (1993) revealed 14 % DNA homology between the two known *Telluria* species (*T. mixta* strain ACM 1762^T and *T. chitinolytica* strain ACM 3522^T), confirming that they represent distinct species. *T. mixta* ACM

1762^T was also distinct from *Burkholderia* (former *Pseudomonas*) *gladioli* pv. *gladioli* ACM 1770^T (13 %), *Burkholderia* (former *Pseudomonas*) *cepacia* ACM 1771^T (13 %), and *Stenotrophomonas* (former *Xanthomonas* and *Pseudomonas*) *maltophilia* ACM 497^T (22 %) (Bowman et al. 1993).

MALDI-TOF Analysis

Collimonas spp. isolates were examined by MALDI-TOF analysis as well as Box-PCR-fingerprinting (Höppener-Ogawa et al. 2008b). They obtained consistent grouping of four clusters, which confirmed exactly the 16S rRNA gene similarity as well as phenotypic analyses.

Herbaspirillum lusitanum strains presented identical patterns when RFLP-16S rDNA fragments were digested with *Dde*I and *Cfo*I, while a different TP-RAPD profile was observed for each *Herbaspirillum* species (Valverde et al. 2003). A MALDI-TOF analysis has been applied to identify successfully different bacteria, including *Herbaspirillum* species, colonizing the human gut (Lagier et al. 2012a).

Ribotyping

Ribotype (*Eco*RI) patterns of *Oxalicibacterium flavum* strains TA 17^T and NS13 were highly similar, whereas *Oxalicibacterium horti* strain OD1^T and *Oxalicibacterium faecigallinarum* strain YOx^T differed from the two *O. flavum* strains and from each other, confirming the species delineation (Sahin et al. 2009a, b).

Genome Analysis

Despite the fact that many genomes have been sequenced, either completed or draft, the majority of the sequenced genomes within the *Oxalobacteraceae* family is from the genus *Herbaspirillum*. At least seven strains of different species and origin are reported.

Herbaspirillum and Noviherbaspirillum: The first complete *Herbaspirillum* genome sequenced was *H. seropedicae* strain SmR1/Z78 (Pedrosa et al. 2011), isolated from surface-sterilized roots of sorghum grown in Brazil (Baldani et al. 1986). The genome is composed of a circular chromosome of approximately 5.5 Mbp and a total of 4,804 genes. The genome sequence revealed that strain Z78 has the capacity to metabolize a wide range of carbon and nitrogen sources using four distinct terminal oxidases for energy production. It contains the *nif* genes involved in the nitrogen fixation process, NO_3^- and NO_2^- assimilation, and NO oxidation, but no denitrification genes were detected. The type I, type II, type III, type V, and type VI secretion systems and type IV *pili* were present suggesting a high potential to interact with host plants. In addition, genes coding for auxins and siderophore production, ACC deaminase activity and for hemagglutinins/hemolysins/adhesins were found which were proposed to play a role in plant growth promotion. The strain also contains genes coding for degradation of benzoate, benzamide, benzonitrile, hydroxybenzoate, and vanillate aromatic compounds that may confer both metabolic flexibility and defense against plant-derived toxic chemicals (Pedrosa et al. 2011).

Draft genome sequences from other *Herbaspirillum* species have been published recently. For example, a draft genome from *H. lusitanum* strain P6-16, isolated from common bean nodule, has an estimated size of 4.9 Mb (37 scaffolds) with 5,240 open reading frames covering 84 % of the chromosome, 38 tRNAs, and a single 16S-23S-5S rRNA operon (Weiss et al. 2012). The authors identified many genes similar to the *H. seropedicae* strain Z67 but the type III secretion system and the nitrogen fixation (*nif*) genes were absent, although the ability to fix nitrogen in nitrogen-free semisolid medium was mentioned in the species description (Valverde et al. 2003). An interesting finding was the presence of a gene coding for the ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO), although the exactly gene function in this strain remains unknown.

Another draft genome was recently published for *H. frisingense* GSF30 by Straub et al. (2013a), a diazotrophic endophyte isolated from the energy plant *Miscanthus sinensis*, grown in Freising, Germany (Kirchhof et al. 2001). 93 contigs were assembled with a total length of 5.4 Mb, which is in the lower range for an endophyte genome. The *H. frisingense* GSF30 genome encodes, for example, the type I, type II, type IV, type VI, Sec-SRP, and the Tat-secretion system, similar as in, e.g., in *H. seropedicae* SmR1. Like many other beneficial endophytic bacteria, but in contrast to other sequenced *Herbaspirillum* genomes, it lacks the type III secretion system.

A report related to one-scaffold draft genome from the *H. huttiense* subsp. *putei* strain 7-2T (IAM 15032), isolated from well water, was published recently (Souza et al. 2013). The estimated genome size is 5.7 Mb with 5,317 open reading frames (covering 86 % chromosome), 49tRNAs, and 2 16S-23S-5S operons. No nitrogen fixation genes were found although genes for nitrate/nitrite metabolism were observed. Genes coding for the Embden–Meyerhof–Parnas, Entner–Doudoroff, and TCA pathways were present. Only one potential pathway for trehalose biosynthesis (*otsA* and *otsB*) was detected. No type III secretion system was observed in contrast to the presence of type I, II, and IV secretion systems. Similar to *H. rubrisubalbicans*, a gene cluster for cellulose biosynthesis and degradation was detected in this strain. Two strains (Os34 and Os45) belonging to *H. seropedicae* and considered pathogenic due to their ability to inhibit growth of rice seedlings and induce a hypersensitive response in tobacco leaves had their draft genome announced (Ye et al. 2012; Zhu et al. 2012). The strain Os34 contains 6.15 Mb and a total of 5,309 coding sequences, while the genome of the strain Os45 was estimated to 5.63 Mb with a total of 4,978 CDs. Both strains have 3 rRNA operons and 73 and 75 tRNAs, respectively. Similar to *H. seropedicae* strain Z78 both strains present genes involved in nitrogen fixation, IAA and siderophore production, and ACC deaminase activity. In addition, they also contain genes for the type III, type VI, and type IV pili. A draft

genome from a *Herbaspirillum* sp. strain GW103, isolated from the rhizosphere soil of the reed *Phragmites australis*, was also announced in 2012 (Lee et al. 2012). It contains approximately 5.04 Mb size and a total of 4,665 CDs, 56 tRNAs, and 6 rRNAs (two 5S, two 16S, and two 23S). No plasmids were detected. The strain also presents genes coding for the IAA and siderophore production and cellulolytic enzymes as well as hemagglutinins and hemolysins like those found for the *H. seropedicae* Z78. Other two draft genomes of *Herbaspirillum* sp. strains (CF444 and YR522), isolated from the endosphere of *Populus deltoids*, were announced and presents 5.59 Mbs (5,004 CDs) and 5.13 Mbs (4,949 CDs), respectively (Brown et al. 2012). A draft genome sequence of a *Herbaspirillum massiliense* (species validated after preparing the chapter and therefore was not included in the Table) strain isolated from a fecal flora of a healthy Senegalese patient contains approximately 4.18 Mb chromosome size and a total of 3,847 protein-coding sequences and 54 RNA genes including 3 rRNA genes (Lagier et al. 2012b). No sequenced genomes for the *Noviherbaspirillum* species are available at present.

Collimonas: The genome of *Collimonas fungivorans* Ter331 is fully sequenced and annotated (http://www.ncbi.nlm.nih.gov/genome/1392). The genome size is 5.19 Mb, and coding sequences for 4,432 proteins and 91 RNA genes were identified. There is also evidence for one plasmid. The genome contents of four *Collimonas* strains (Ter14, Ter6, Ter91, and Ter10) from three *Collimonas* species, *C. fungivorans*, *C. pratensis*, and *C. arenae*, were analyzed by hybridization to an microarray based on the reference genome of *C. fungivorans* Ter331 (Mela et al. 2012). The unique genes of strain Ter331 were in part on the plasmid. In addition, 136 genes could be identified as common in all tested *Collimonas* strains, but absent from the genomes of other members of the family *Oxalobacteraceae*. Predicted products of these "*Collimonas* core" genes include lytic, secreted enzymes such as chitinases, peptidases, nucleases, and phosphatases with a putative role in mycophagy and weathering (Mela et al. 2012).

Duganella–Pseudoduganella: There are two draft genome sequences available for *Duganella zoogloeoides* and *Pseudoduganella violaceinigra*. The draft assembly of the whole genome shotgun sequences and automatic prokaryotic genome annotation of *Duganella zoogloeoides* ATCC25935 by NCBI was released in 2013 (http://www.ncbi.nlm.nih.gov/genome/15561). The draft genome assembly of the *Pseudoduganella violaceinigra* DSM 15887 by the DOE Joint Genome Institute has after the primary assembly at present 41 scaffolds and 48 contigs (http://www.ncbi.nlm.nih.gov/assembly/60191).

Janthinobacterium: Four *Janthinobacterium* genome projects are available (under assembly and as complete genome). Antimicrobial effects related with water-insoluble pigment violacein, water-living gene, UV stress survival environments, and cold-adaptive properties are among the phenotypic trait of great interest in these genome projects. The general features of the genomes are *Janthinobacterium* sp. Marseille (4.11 Mb size, 3,697 predicted genes, 2,510 assigned functions, 6 rRNA operons, 46 tRNA.), *Janthinobacterium* sp. strain CG3 (6.26 Mb size, 5,352 predicted genes, 4,356 assigned functions, 15 rRNA operons, 81 tRNA), *Janthinobacterium lividum* strain PAMC 25724 (4.98 Mb, a total of 4,332 predicted genes, 3,538 assigned functions, 80 tRNA, 21 rRNA), and *Janthinobacterium* sp. strain HH01 (7.11 Mb size, a total of 5,098 predicted genes, 4,877 assigned functions, 84 tRNA, 20 rRNA). For more details, see (Audic et al. 2007; Smith et al. 2013; Kim et al. 2012; Hornung et al. 2013).

Herminiimonas: The only species of the genus *Herminiimonas* with whole genome sequenced is *H. saxobsidens* strain ASM2612v1 that is able to perform detoxification reaction, converting arsenite (As^{III}) in less toxic and less mobile form arsenate (As^{V}). The strain ASM2612v1 contains 3,424,307 bp, a total of 3,333 predicted genes, 6 rRNA operons, and 45 tRNA. As expected, the genome had shown biochemical machinery to arsenic-related stress adaptation involved in coupled redox reactions, efflux, chemotaxis, oxidative stress, DNA recombination, and repair and motility toward arsenic, biofilm expression, and metalloid scavenging by EPS (Muller et al. 2007).

Massilia: Only four genomes of the genus *Massilia* are available with draft status, three of them are genome sequences from strains of well-characterized species (*M. timonae*, *M. alkalitolerans*, and *M. niastensis*), while the fourth draft genome sequence is of *Massilia* sp. strain B08, an *Arabidopsis thaliana* rhizosphere/endosphere isolate. For *M. timonae*, a draft genome sequence is available for an isolate of the Human Microbiome Project. Interestingly, two other projects are included in NCBI BioProject: one for a plant-associated isolate (BioProject accession PRJNA187940) and other for an isolate from an immunocompromised patient with cerebellar lesions (BioProject accessions PRJNA187940); in both projects, no sequence data are available yet. The genome sequences for *M. alkalitolerans* (PRJNA163031) and *M. niastensis* (PRJNA200436) are part of the Genomic Encyclopedia of Bacteria and Archaea (GEBA), an initiative to fill phylogenetic gaps, generating reference genomes for every major and minor group of bacteria and archaea (www.jgi.doe.gov/programs/GEBA), reinforcing the need for an effort to fill the gap in this field for *Massilia* genus.

Oxalobacter: *Oxalobacter formigenes* strains can be divided into two main groups (Allison et al. 1985). Almost complete genome sequences of one strain of each group, OXCC13 and HOxBLS for groups 1 and 2, respectively, have been determined as part of the Human Microbiome Project (http://www.hmpdacc.org). Both strains have relatively small genomes containing between 2.4 and 2.5 Mb and 2,121 (for OXCC13) and 2,329 (for HOxBLS) genes. The functional comparison of the two genomes revealed few differences between strains OXCC13 and HOxBLS, both carrying genes involved in several signaling pathways, stress response, and phages (Knight et al. 2013). Nevertheless, the OXCC13 genome contains an apparent operon for the production of type 1 pili, which might be involved in adhesion to surfaces or to other cells. These genes are absent in the HOxBLS genome (Knight et al. 2013). Strain OXCC13 harbors a 5-nitroimidazole antibiotic resistance gene and is resistant to nitrofurantoin, whereas strain HOxBLS is sensitive to this antibiotic and lacks this gene (Knight et al. 2013).

Phenotypic Analyses

The main features of members of *Oxalobacteraceae* are listed in Table 35.1. The morphological and physiological characteristics within some genera present only very small differences making molecular analysis absolutely required to identify the species, while in other cases, only few aspects are sufficient to identify the species.

Herbaspirillum, Baldani et al. 1986. Baldani et al. 1996 Emend. Carro et al. 2012 Emend. Lin et al. 2013

Herbaspirillum (Her.ba.spi'ri.llum. L. fem. n. *herba*, an herb, grass, seed-bearing plant that does not produce persistent woody tissue; N.L. dim. neut. n. *spirillum* (from L. n. *spira*, a coil, spiral), small spiral; N.L. neut. n. *Herbaspirillum*, small, spiral-shaped bacteria from herbaceous, seed-bearing plants).

Cells are gram-negative; they are aerobic, curved, or slightly curved rods, sometimes spiral with a diameter varying from 0.3 to 0.8 μm. Cells lengths vary with the species and culture medium from 1.4 to 5.0 μm. They are motile, using one to five flagella at one or both poles. Oxidase and catalase activity is present. Nitrate is reduced by some species but no denitrification was detected. The species have a strictly respiratory type of metabolism; however, one species possesses a facultative hydrogen autotrophic system. Sugars are oxidized but not fermented. Optimal growth temperature is between 20 °C and 37 °C, although 40 °C is tolerated by some species. Optimum pH for growth is between 5 and 8. Nitrogenase activity occurs only in three species (*H. seropedicae*, *H. rubrisubalbicans*, and *H. frisingense*) only under microaerobic conditions, although the nifH/nifD genes, required for nitrogen fixation, have been detected in two non-nitrogen-fixing species (*H. lusitanum* and *H. huttiense subsp. putei*). The favored carbon sources are organic salts such as malate and succinate. Other carbon sources like glycerol, mannitol, D-glucose, and D-fructose are used by the majority of the species, except for *H. autotrophicum* and *H. chlorophenolicum*. *H. chlorophenolicum* is the only species able to grow in phenol and 4-chlorophenol, while *H. hiltneri* uses L-phenylalanine as a carbon source. Sucrose cannot be used as a carbon source by any of the species. Other carbon sources summarized in Table 35.2 have been used to separate species; but with few exceptions, the identification based only on phenotypic characteristics may not be sufficient and requires additional molecular methods. The use of the nitrogen-free JNFb semisolid medium allows the enrichment and isolation of the nitrogen-fixing species. Major cellular fatty acids are C16:0, C16:1 ω7c, and C18:1 ω7c. Ubiquinone 8 is the sole respiratory quinone. DNA G+C content varied from 57.9 to 65 mol%. This genus is most closely related to the genera *Glaciimonas*, *Collimonas*, *Herminiimonas*, and *Oxalicibacterium* of the family *Oxalobacteraceae*, order *Burkholderiales*, subclass *Betaproteobacteria*. The type species is *H. seropedicae*, and the type strain is Z67^T (=ATCC 35892 = CCUG 24564 = DSM 6445 = JCM 21448 = LMG 6513 = NBRC 102524). The genus contains 10 other species: *H. rubrisubalbicans* (red-whitish, referring to the symptoms of mottled stripe disease), *H. frisingense* (pertaining to Frisinga, now known as Freising, Germany, town where the species was first isolated), *H. lusitanum* (Lusitania, the Roman name of Portugal, where the strains reported in this study were isolated), *H. chlorophenolicum* (relating to chlorophenols), *H. autotrophicum* (self-nursing), *H. hiltneri* (*in honor of Professor Hiltner, who coined the rhizosphere concept in 1904*), *H. rhizosphaerae* (of the rhizosphere), *H. aquaticum* (living in water, aquatic.), *H. huttiense subsp. huttiense* (pertaining to Lower Hutt, New Zealand), and *H. huttiense subsp. putei* (of a well). The type strains and additional characteristics for these species are listed in Table 35.2.

Noviherbaspirillum, Lin et al. 2013

Noviherbaspirillum (No.vi.her.ba.spi'ril.lum. L. adj. novus, new; N.L. neut. n. *Herbaspirillum*, a bacterial genus name; N.L. neut. n. *Noviherbaspirillum*, new genus separated from *Herbaspirillum*).

Cells are gram-negative, slightly or short curved rods, 1.0–1.3 μm in width and 1.4–4.5 μm in length. They are motile using one polar flagellum. Catalase activity is positive although oxidase is variable. Nitrate is reduced to nitrogen but not to nitrite. The species have a respiratory type of metabolism; however, one species shows a facultative anaerobic system. Optimal growth temperature is between 14 °C and 30 °C, although few species tolerate the temperature of −5 °C to 40 °C. Optimum pH for growth is between 6 and 8. The species tolerate between 1.5 % and 2.5 % NaCl. Cells are unable to fix atmospheric N_2 under microaerobic conditions. The use of carbon sources is variable. Most of the species use malate, citrate, adipate, and gluconate. Glucose, L-arabinose, mannitol, mannose, *N*-acetylglucosamine, maltose, caprate, and phenylacetate are not used for growth. The species *N. malthae* uses Tween 80, β-hydroxybutyric acid, and L-proline (API 20NE system), while the *N. psychrotolerans* uses glycerol, DL-arabinose, D-xylose, D-galactose, D-fructose, D-lyxose, and D-fucose as sole carbon sources. Other carbon sources are summarized in Table 35.1 and have been used to separate species. With few exceptions, the identification based only on phenotypic characteristics may not be sufficient and requires additional chemotaxonomic and molecular methods. The polyamines putrescine (Put) and 2-hydroxylputrescine were predominant in the species. Major cellular fatty acids are C16:0, C16:1ω7c/C16:ω6c, and C18:1ω7c/C18:1ω6c. The polar lipid profile constitutes diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), one unidentified glycolipid (GL), one unidentified aminophospholipid (APL), and five unidentified lipids (L1–L5). The predominant quinone is ubiquinone (Q-8). DNA G+C content varied from 60.4 to 63.4 mol%. This genus is most closely related to the genera *Herbaspirillum*, *Oxalicibacterium*,

Table 35.1

Morphological, physiological, and molecular differentiating characteristics among the genera of the family *Oxalobacteraceae*

	Collimonas	*Glaciimonas*	*Duganella*	*Pseudoduganella*	*Herbaspirillum*	*Noviherbaspirillum*	*Herminiimonas*	*Janthinobacterium*	*Massilia*	*Oxalicibacterium*	*Oxalobacter*	*Telluria*	*Undibacterium*
Morphology	Straight or slightly curved rods	Rods	Rods	Rods	Straight or slightly curved rods	Short or slightly curved rods	Thin or short rods	Rods	Rods	Small rods	Rods	Rods	Rods
Gram stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Motility	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Variable	Positive	Positive
Metabolism	Aero- and microaerobic	Aero- and microaerobic	Aerobic	Aerobic	Aero- and microaerobic/ one species facultative hydrogen autotrophic	Aerobic or facultative anaerobic (one species)	Aerobic	Aerobic	Aerobic	Aerobic	Anaerobic	Aerobic	Aerobic
Nitrogen fixation	Negative	Negative	Negative	Negative	Variable	Negative	na	na	na	Negative or unknown	Negative or unknown	Negative or unknown	na
Nitrate reduction	Variable	Negative	Negative	Negative	Variable	Negative	Variable	Variable	Variable	Negative	Negative	Variable	Variable
Range for growth													
Temperature (°C)	20–30	1–25	15–35	28–30	5–40 (optimal 20–37)	−5 to 40 (optimal 14–30)	4–35 °C	25–30	5–55 (optimal 28–30)	4–37	14–45	20–45	4–37 °C
pH	6.5	6–8	7.0	7.2	5–8	4.5–10 (optimal 6–8)	6–9.5	5–8	4–10.5 (optimal 6.5–9.0)	6.9–8	na	7	5–11 (optimal 6–8)
Major fatty acids	$C_{16:0}$, $C_{18:1}$ ω7c	$C_{16:0}$, $C_{18:1}$ ω7c	$C_{16:0}$	$C_{16:0}$, $C_{16:1}$ ω7c	C16:0, C16:1 ω7c, and C18:1 ω7c	C16:1ω7c/C16:ω6c-summed feature 3, C18:1ω6c/ C18:1ω7c-summed feature 8, and C16:0	$C_{16:1}$ ω7c; $C_{16:0}$	$C_{10:0}$ 3-OH, $C_{12:0}$, $C_{12:0}$ 2-OH, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ω7c, $C_{17:0}$ cyclo, and $C_{18:1}$ω7c	Summed 3 (*iso*-$C_{15:0}$ 2-OH and/ or $C_{16:1}$ ω7c) and $C_{16:0}$	C16:1 ω7c and/or iso-C15:0 2-OH, C16:0, C17:0 cyclo, C18:1ω6c C18:1ω7c, C19:0 cyclo ω8c, C10:0 3-OH	$C_{17:0}$ cyclo, $C_{16:0}$, $C_{19:0}$ cyclo	$C_{16:0}$, $C_{16:1}$ω7c, and/or $C_{15:0}$ iso 2-OH, $C_{17:0}$ cyclo, $C_{18:1}$ω7c (Kämpfer et al. 2007)	C16:0, summed feature 3 (C16:1ω7c/C15:0 iso 2-OH) and C18:1ω7c. C10:0 3-OH

Table 35.1 (continued)

	Collimonas	*Glaciimonas*	*Duganella*	*Pseudoduganella*	*Herbaspirillum*	*Noviherbaspirillum*	*Herminiimonas*	*Janthinobacterium*	*Massilia*	*Oxalicibacterium*	*Oxalobacter*	*Telluria*	*Undibacterium*
Ubiquinone type	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8 and unknown	Unknown	Q-8	Q-8
Genome size (Mb)	n.d.	n.d.	n.d.	n.d.	3–6.15	na	3.4	4.11–7.11	6.0–6.5	Unknown	2.4–2.5	Unknown	Unknown
G+C content	57.0–62.0	51.0–55.0	54.9–64.0	62.8	57.9–65.0	62.5–63.6	52.0–59.0	61.0–67.0	62.4–68.9	55.6–63.3	48.0–51.6	67–72	50.6–57.4
Habitat	Soil	Oligotrophic soil and water	Soil, water, and plant	Soil, rhizosphere	Water, soil, and plant	Volcanic or glacier soil	Water, lichen–rock interface	Soil and soft rot pathogen of A. bisporus	Air, soil, water, deep ice, and human	Soil, oxalate-rich plant litter, chicken dung	Gastrointestinal tract of man and animals and from anoxic freshwater sediments	Soil	Aquatic environments or solid
Other important characteristics of the genus	Mycophagous growth	Oligotrophic growth	Formation of extracellular matrix	Production of violet pigment			No use of amino acids; Low ability to use carbohydrates			Uses oxalate and few other organic acids, but no sugars, alcohols and C1 compounds	Requires oxalate as carbon and energy source. A small amount of acetate is also required for growth		$C_{17:0}$ cyclo is absent

na not available

Table 35.2

Morphological, physiological, and molecular characteristics differentiating species within genus *Herbaspirillum*

	Herbaspirillum aquaticum ATCC BAA-1628T	*Herbaspirillum autotrophicum IAM14942* ^{T}	*Herbaspirillum chlorophenolicum NBRC 102525T*	*Herbaspirillum frisingense* DSM 13128 ^{T}	*Herbaspirillum hiltneri LMG 23131*^{T}	*Herbaspirillum huttiense subsp. huttiense ATCC 14670* ^{T}	*Herbaspirillum huttiense subsp. putei* ATCC BAA-806^{T}	*Herbaspirillum lusitanum* LMG 21710^{T}	*Herbaspirillum rhizosphaerae* KCTC 12558^{T}	*Herbaspirillum rubrisubalbicans* ATCC 19308^{T}	*Herbaspirillum seropedicae* ATCC 35892^{T}
Morphology	Curved rods	Curved to spiral (one or more complete turn or twist)	Slightly curved rods	Slightly curved, spiral rods	Slightly curved rods	Slightly curved rods	Curved rods or spirilla	Curved	Slightly curved rods	Slightly curved rods	Rod, sometimes spiral
Cell size (μm)	0.5 × 2	0.6–0.8 × 2–5	0.7 × 2.3	0.5–0.7 × 1.4–1.8	0.5–0.6 × 1.6–2	0.4 × 1.8	0.5–0.7 × 2.1–3.4	0.5 × 1.6	0.3–0.4 × 1.8–2.2	0.6–0.7 × 1.4–1.8	0.6–0.7 × 1.5–5.0
Flagellation	1–4 polar	1–5, bipolar tufts	1, unipolar	1–3, unipolar	2, unipolar	1–3, bipolar	1–4	1–2, polar	Bipolar	Several, unipolar	1–3, bipolar
Motility	+	+	+	+	+	+	+	+	+	+	+
Swarming on soft NB agar	nd	nd	nd	+	nd	nd	nd	nd	nd	+	+
PHB	–	+	nd	nd	nd	nd	nd	nd	nd	+	nd
N_2 fixation semisolid medium	–	–	–	+	–	–	–	+	–	+	+
Detection nifD and/or nifH	–	–	–	+	–	–	+	+	–	+	+
Temperature for growth (°C):											
Range	10–35	10–35	nd	30–37	26–34	25–37	25–37	20–40	4–34	Up to 40	22–38
Optimum	25–35	28	30	30–37	26–34	25–37	25–37	20–35	25–30	30	34
pH for growth:											
Range	5–8	5–8	6–8	nd	nd	nd	nd	nd	nd	5.7–6.8	5.3–8.0
Optimum	nd	nd	6–7	6–7	6–8	nd	6–7	5–8	6.5–7.5	5.8	5.8
Oxidase	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+
Urease hydrolysis	+	+	nd	+	nd	+	+	+	+	nd	+
Nitrate reduction	–	–	+	+	nd	–	–	–	–	+	+

Table 35.2 (continued)

	Herbaspirillum aquaticum ATCC BAA-1628T	*Herbaspirillum autotrophicum IAM14942* T	*Herbaspirillum chlorophenolicum NBRC 102525T*	*Herbaspirillum frisingense* DSM 13128 T	*Herbaspirillum hiltneri LMG 23131*T	*Herbaspirillum huttiense subsp. huttiense ATCC 14670* T	*Herbaspirillum huttiense subsp. putei* ATCC BAA-806T	*Herbaspirillum lusitanum* LMG 21710T	*Herbaspirillum rhizosphaerae* KCTC 12558T	*Herbaspirillum rubrisubalbicans* ATCC 19308T	*Herbaspirillum seropedicae* ATCC 35892T
Nitrite reduction	nd	+	–	–	nd	nd	nd	nd	nd	–	–
NaCl concentration for growth (%)	nd	–3 %	1.5 %	nd	nd	nd	nd	nd	Less 3 %	nd	nd
Utilization of:											
N-acetyl-D-glucosamine	–	+	+	+	+	+	+	+	nd	–	+
meso-inositol	–	–	–	–	–	nd	nd	–	nd	–	+
L-rhamnose	–	–	–	–	–	–	–	+	–	–	+
meso-erythritol	–	–	–	–	–	+	+	–	nd	+	–
Adonitol	nd	nd	–	+	–		nd	nd	–	+	+
D-arabinose	nd	+	–	+	+	+	–	+	–	+	+
Malate	nd	nd	+	+	+	nd	nd	+	+	+	+
Sucrose	–	nd	–	nd	–	–	–	nd	–	–	–
D-glucose	+	–	–	+	+	+	+	+	+	+	+
D-fructose	+	nd	–	+	–	+	+	+	+	+	+
Mannitol	+	nd	–	+	+	+	+	+	nd	+	+
Glycerol	+	nd	–	+	+	+	+	nd	+	+	+
L-phenylalanine	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd
Adipate	nd	nd	+	–	nd	+	+	–	nd	–	–
Itaconic acid	+	nd	nd	+	+	–	+	nd	nd	nd	nd
Azelaic acid	+	nd	+	nd	nd	–	–	nd	nd	–	–
Sebacic acid	+	nd	+	nd	–	–	–	nd	nd	–	–
Phenol	nd	nd	+	–	nd	nd	nd	nd	nd	nd	nd

4-chlorophenol	nd	nd	+	–	nd	nd	nd	nd	nd	nd	nd
Cellulose	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Casein	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Predominant ubiquinone	nd	Q-8	Q-8	Q-8 a	nd	Q-8	Q-8	nd	Q-8	Q-8	Q-8
Predominant cellular fatty acids	C16:1 ω7c and/or iso-C15:0 2-OH, C18:1 ω7c, and C 16:0	C16:0, C16:1, and C18:1 ω7c	C16:0, C17:0, and C18:1 ω7c	C16:0, C18:1 ω7c a	C16:1 ω7c, C16:0, and C18:1 ω7c	C16:1 ω7c and/or iso-C15:0 2-OH, C18:1 ω7c, and C 16:0	C16:1 ω7c and/or iso-C15:0 2-OH, C18:1 ω7c, and C 16:0	nd	C 16:0, cyclo C17:0, and C16:1 ω7c and/or iso-C15:0 2-OH	C16:1 ω7c and/or iso-C15:0 2-OH, C 16:0, and C18:1 ω7c	C16:1 ω7c and/or iso-C15:0 2-OH, C 16:0, and C18:1 ω7c
Indole production	nd	nd	nd	nd	nd	–	–	–	nd	+	+
DNA G+C (mol%)	62.1	60–62	61.3	61–65	60.9–61.5	62.5	62–62.1	57.9–61.9	59.8–60	62–63	64–65
Genome size	4 Mb	nd	nd	~5.4 b	nd	3 Mb	3.9–5.7 Mb	4.9	nd	nd	5.3–6.15 Mb
Habitat	Water	Eutrophic lake	Soil sediment – stream	Roots, leaves, and stems – pennisetum and miscanthus	Roots – wheat	Distilled water	Well water	Nodules – P. vulgaris	Rhizosphere soil – wild edible green	Roots, stems, and leaves – several plants	Roots, stems, and leaves – several plants
Additional characteristic		Facultatively autotrophic, hydrogen oxidizing									

Nd not determined, positive (+), negative (–)

Duganella, *Undibacterium*, *and Collimonas* of the family *Oxalobacteraceae*, order *Burkholderiales*, and subclass *Betaproteobacteria*. The type species is *Noviherbaspirillum malthae* (mal.tha'e. L. fem. n. maltha, a kind of thick petroleum; L. gen. n. malthae, of petroleum because the type strain was isolated from an oil-contaminated site). The type strain of the *N. malthae* is CC-AFH3^T (=BCRC 80516^T = JCM 18414^T) isolated from the oil-contaminated site at Kaohsiung city in Taiwan. The genus contains four other species reclassified from the genus *Herbaspirillum*: *N. canariense* (isolated from Canary Islands), *N. aurantiacum* (orange-colored), *N. soli* (from soil), and *N. psychrotolerans* (cold-tolerating strain). The type strains and additional characteristics for these species are listed in Table 35.3.

Collimonas, De Boer et al. 2004

Collimonas (Col.li.mo'nas. L. masc. n. *collis* hill; Gr. n. *monas* a unit, monad; N.L. fem. n. *Collimonas* cell from the hill).

Cells are gram-negative; they are strictly aerobic, straight, or slightly curved 0.3–0.5 × 1.0–2.0 μm in size. They occur singly and possess flagella (mostly one to three polar, but in some cases several lateral ones). When cultured in liquid media, they harbor pili. Oxidase reaction is positive, and the catalase activity is negative to weakly positive. Major cellular fatty acids are $C_{16:0}$ and $C_{16:1}$ ω7c. DNA G + C content is 57–62 mol%. This genus is most closely related to the genera *Glaciimonas*, *Herbaspirillum*, and *Janthinobacterium* in the family *Oxalobacteraceae*, order *Burkholderiales*, and subclass *Betaproteobacteria*. Collimonads are characterized by their special feature of mycophagous activity (De Boer et al. 2001). The mycophagous phenotype of collimonads was explained as an adaptation to life under conditions of limited nutrient availability (Leveau et al. 2010). Optimal growth temperature is between 20 °C and 30 °C; 35 °C are tolerated. Optimum pH for growth is 6.5. Cells are able to hydrolyze colloidal chitin and milk proteins, but not lichenan or cellulose. Halo formation of colloidal chitin is completely inhibited in agar containing glucose or TSB. The *nif* genes required for nitrogen fixation are not present. After 3 days incubation at 20 °C on tenfold diluted TSB agar, the type strain forms flat, glossy, turbid, and whitish colonies of 3–7 mm diameter with a layered structure (colony type I; De Boer et al. 2004). In purified sand, cells proliferate upon introduction of living fungal hyphae of various species of soil fungi. A wide range of sugars, alcohols, organic acids, and amino acids can be metabolized. DNA G + C content is 57–59 mol%. The type species is *Collimonas fungivorans* (fun.gi.vo'rans. L. n. *fungus* mold, mushroom, fungus; L. part. adj. *vorans* devouring, eating; N.L. part adj. *fungivorans* fungus-eating). The type strain is Ter6^T (=NCCB 100033^T = LMG 21973^T). Further reference strains are Ter166, Ter300, and Ter330. *Collimonas arenae* (isolated from sandy soil) and *Collimonas pratensis* (isolated from grassland) are described as further species in this genus (Höppener-Ogawa et al. 2008b). Details of the described species are given in Table 35.4.

Glaciimonas, Zhang et al. 2011

Glaciimonas (Gla.ci.i.mo'nas. L. fem. n. *glacies* ice: L. n. *monas* a unit, monad; N.L. fem. n. *Glaciimonas* a cell from the glacier).

The cells are staining gram-negative; they are rod-shaped and nonmotile. They are able to grow under aerobic and microaerobic conditions and unable to grow under anaerobic conditions. The cells are oxidase- and catalase-positive and negative for nitrate reduction. The predominant cellular fatty acids are $C_{16:0}$ and $C_{18:1}$ ω7c and summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH). The lipid profile showed the presence of phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), and phosphatidylglycerol (PG). The major respiratory quinone is ubiquinone 8 (UQ-8). The most closely related genus phylogenetically is *Collimonas*. The type species is *Glaciimonas immobilis* (im.mo'bi.lis. L. fem. adj. *immobilis* motionless). The type strain is Cr9-30^T (=DSM 23240^T = LMG 25547^T), isolated from alpine glacier cryonite collected from the Tiefenbachferner glacier in the Oetztaler Alps, Tyrol, Austria (Zhang et al. 2011). The DNA G+C content of the strain Cr9-30^T is 51.0 mol%. Strain Cr9-12 (=DSM 22814^T), isolated from the same source, is a second isolate. The following properties are observed. Cells are 0.9–1.0 × 1.1–1.9 μm in size. Flagella or pili are absent. Colonies on R2A agar are white, round, convex, and smooth with entire margins; in addition, slime is produced. Growth is good in R2A medium and on R2A agar plates at 1–20 °C. There is no growth at 25 °C on agar plates and weak in liquid culture. Growth on R2A agar plates is optimal in the presence of 0–1 % (w/v) NaCl and at pH 6–8. Cells are negative for indole and H_2S production, hydrolysis of urease, aesculin, gelatin milk proteins, and starch. Cells are also negative for arginine dihydrolase, lysine dihydrolase, and ornithine dihydrolase. The other species of the genus is *G. singularis* (*sin.gu.la'ris. L. fem. adj. singularis* alone of this kind, singular, referring to the different characteristics of this organism in relation to those of *G. immobilis*). It was isolated from water collected in uranium mine of Poco das Cobras (Urgeirica mine), central Portugal (Chung et al. 2013). For phenotypic details, see Table 35.5.

Janthinobacterium, De Ley et al. 1978

Janthinobacterium (*Jan.thin.o.bac.te'ri.um.* L. adj. *janthinus*, violet–blue, violet; L. neut. n. *bacterium*, rod or staff; N.L. neut. n. *Janthinobacterium*, a violet-colored rod).

Bacteria of the genus are rod-shaped, gram-negative motile bacteria measuring 0.8–1.5 μm (width) and to 1.8–6 μm (length). Colonies produce violet color by the presence of violacein pigment. Non-pigmented colonies on PAF medium

Table 35.3

Morphological, physiological, and molecular characteristics differentiating species within genus *Noviherbaspirillum*

	Noviherbaspirillum aurantiacum LMG 26150T	*Noviherbaspirillum canariense* LMG 26151T	*Noviherbaspirillum psychrotolerans LMG 27282T*	*Noviherbaspirillum malthae* BCRC 80516T	*Noviherbaspirillum soli* LMG 26149T
Morphology	Slightly curved rods	Slightly curved rods	Curved rods	Short curved rods	Slightly curved rods
Cell size (μm)	0.8–1.1 × 1.7–1.9	1–1.3 × 1.6–1.9	0.8–1.2 × 3.5–4.5	1–1.2 × 1.4–1.6	1.2 × 1.9
Flagellation	1, polar	1, polar	nd	1, polar	1, polar
Motility	+	+	+	+	+
N_2 fixation	−	−	−	−	−
Relation to oxygen	Aerobic	Aerobic	Facultatively anaerobic	Aerobic	Aerobic
Temperature for growth (°C):					
Range	20–37	20–30	−5–30	20–40	20–37
Optimum	28	28	14-20	30	28
pH for growth:					
Range	5–10	5–10	4.5–8	5–10	5–10
Optimum	6–8	6–8	6.5–7	6.0	6–8
Oxidase	+	+	−	+	+
Catalase	+	+	+	+	+
Urease hydrolysis	−	−	Low	−	−
Nitrate to nitrite reduction	−	−	−	−	−
Nitrate to nitrogen reduction	+	+	+	+	+
NaCl concentration for growth (%)	1.5	1.5	2.5	2	1.5
Utilization of:					
N-acetyl-D-glucosamine	−	−	−	−	−
Potassium gluconate	+	+	+	−	+
Tween 40	+	−	−	+	+
L-arabinose API 20 NE	−	−	+		−
Malate API 20NE	+	+	nd	nd	+
Sucrose API ID32GN	−	+	nd	nd	−
D-glucose API 20NE	−	+	−	−	−
Mannitol API ID 32GN	−	−	−	−	−
Glycerol	nd	nd	+	−	nd
Adipate API 20NE	+	+	+	+	+
Suberate API ID32GN	+	+	nd	nd	+
3-hydroxybenzoate API ID 32GN	+	+	nd	nd	+

Table 35.3 (continued)

	Noviherbaspirillum aurantiacum LMG 26150T	*Noviherbaspirillum canariense* LMG 26151T	*Noviherbaspirillum psychrotolerans LMG 27282T*	*Noviherbaspirillum malthae* BCRC 80516T	*Noviherbaspirillum soli* LMG 26149T
L-proline API ID32GN	+	−	−	+	−
Predominant ubiquinone	Q-8	Q-8	Q-8	Q-8	Q-8
Predominant cellular fatty acids	C16:1ω7c/C16:ω6c, summed feature 3; C18:1ω6c/C18:1ω7c, summed feature 8; and C16:0	C16:1ω7c/C16:1ω6c, summed feature 3; C16:0; and C18:1ω6c/C18:1ω7c, summed feature 8	C16:1ω7cis, C16:0, and C18:1ω7cis	C16:1ω7c/C16:1 ω6c, summed feature 3; C16:0; and C18:1ω7c/C18:1ω6c, summed feature 8	C16:1ω7c/C16:ω6c, summed feature 3; C18:1ω6c/C18:1ω7c, summed feature 8; and C16:0
Indole production					
DNA G+C (mol%)	60.4	61.6	62.5	63.4	61.9
Genome size					
Habitat	Old volcanic soil	Old volcanic soil	Glacier forefield soil	Oil-contaminated site	Old volcanic soil
G + C % (data from Lin et al. 2013)	63.6	62.8	62.5	63.4	63.4

nd not determined, positive (+), negative (−)

are 3–4 mm in diameter, translucent buff color, and circular, slightly convex, smooth, and slightly mucoid. The bacteria grow strictly aerobic, chemoorganotrophic, with optimum growth temperature at 25–30 °C; they do not grow at 2 or 37 °C. No growth occurs below pH 5 or in media containing 2–9 % NaCl. Negative for production of gas from nitrate, production of indole from tryptophan, fermentation of glucose, presence of arginine dihydrolase, presence of urease, β-galactosidase activity, assimilation of glucose, gelatin hydrolysis and assimilation of gluconate, and positive for assimilation of glucose, malate, mannose, and mannitol. Related to carbon use, the genus was positive for glycerol, ribose, D-glucose, D-fructose, D-mannose, inositol, mannitol, saccharose, and D-arabitol and negative for erythritol, L-xylose, adonitol, methyl P-xyloside, L-sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, aesculin, lactose, melibiose, melezitose, amidon, glycogen, D-turanose, D-tagatose, D-fucose, L-arabitol, gluconate, and 5-ketogluconate. *Janthinobacterium* major phospholipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol, as well as Q-8 as respiratory lipoquinone. Characteristic fatty acid profile consists of $C_{10:0}$ 3-OH, $C_{12:0}$, $C_{12:0}$ 2-OH, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ω7c, $C_{17:0}$ cyclo, and $C_{18:1}$ω7c. The polyamine major compounds are 2-hydroxyputrescine and putrescine. The G+C range (mol %) is 64.2–66.1. The type species of the genus is *Janthinobacterium lividum*, and type strain is ATCC 12473^T (= CCUG 2344 = CIP 103349 = DSM 1522 = HAMBI 1919 = JCM 9043 = LMG 2892 = NCTC 9796 = VKM B-1223) (De Ley et al. 1978). The second species of the genus is *J. agaricidamnosum*, the causal agent of *A. bisporus* disease, type strain W1r3 (= CCUG 43140 = CIP 106332 = DSM 9628 = JCM 21444 = NBRC 102515 = NCPPB 3945) (Lincoln et al. 1999). The type strains and additional characteristics for these species are listed in *Table 35.6*.

Undibacterium, Kämpfer et al. 2007 Emend. Eder et al. 2011

Undibacterium (Un'di.bac.te'ri.um. L. n. *unda*, water; L. neut. n. *bacterium*, rod; N.L. neut. n. *Undibacterium*, a rod of water).

Common phenotypic traits shared by members of the genus are nonspore-forming, gram-negative, aerobic metabolism, and mesophilic growth. Cells are motile by one or more polar flagella, and they have pili. Temperature for growth ranged mainly between 10 °C and 30 °C, with few species growing at 4 °C and 37 °C. The bacteria of the genus are predominantly isolated from aquatic environments; they produce circular, entire, convex, and creamy- to yellow-colored colonies which can reach the size of 2.0 mm on R_2A agar. The bacteria are positive for oxidase, leucine arylamidase, acid phosphatase, alkaline phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for indole production, glucose fermentation, arginine dihydrolase, β-galactosidase (PNG), lipase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. None of the strains assimilated L-arabinose, D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, inositol, itaconic acid, sodium malonate, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid,

Table 35.4
Morphological, physiological, and molecular characteristics differentiating species within genus *Collimonas*

	Collimonas fungivorans LMG 21973T	*Collimonas arenae LMG 23964T*	*Collimonas pratensis LMG 23965T*
Morphology	Straight or slightly curved rods	Straight or slightly curved rods	Straight or slightly curved rods
Cell size (μm)	0.3–0.5 × 1.0–2.0	0.3–0.5 × 1.0–2.0	0.3–0.5 × 1.0–2.0
Flagellation	1 polar flagellum	1 polar flagellum	1 polar flagellum
Motility	+	+	+
N_2 fixation	–	–	–
PHB accumulation or other charact	nd	nd	nd
Detection nifD and/or nifH	nd	nd	nd
Temperature for growth (°C):			
Range	20–30	20–300	20–30
Optimum	30	30	30
pH for growth:			
Range	5.0–8.0	5.0–8.0	5.0–8.0
Optimum	6.5	6.5	6.5
Oxidase	+	+	+
Catalase	–	Variable	Variable
Urease hydrolysis	–	–	+
Nitrate reduction	+	–	–
Nitrite reduction	nd	nd	nd
NaCl concentration for growth (%)	nd	0–2	0–2
Utilization of:			
N-acetyl-D-glucosamine	+	Variable	+
meso-inositol	+	+	+
L-rhamnose	–	–	–
meso-erythritol	–	–	–
Adonitol	–	–	–
D-arabinose	nd	nd	nd
Malate	+	+	+
Sucrose	–	–	–
D-glucose	+	+	+
D-fructose	+	+	+
Mannitol	+	+	+
Glycerol	+	+	+
Adipic acid	–	–	–
Azelaic acid	nd	nd	nd
Sebacic acid	–	–	–
Phenol	nd	nd	nd
4-chlorophenol	nd	nd	nd
Predominant ubiquinone	Q-8	Q-8	Q-8
Predominant cellular fatty acids	C16:0, C16:1 ω7c	C16:0, C16:1 ω7c	C16:0, C16:1 ω7c
Chitinolysis	+	+	+
DNA G+C (mol %)	59	57	59
Genome size (Mb)	5.19	nd	nd
Habitat	Slightly acidic dune soils	Seminatural grassland soil	Seminatural grassland soil

nd not determined, positive (+), negative (–)

L-serine, salicin, melibiose, L-fucose, D-sorbitol, potassium 2-ketogluconate, or 4-hydroxybenzoic acid. Chemotaxonomic common data include putrescine and 2-hydroxyputrescine as major polyamines and ubiquinone Q-8 as the major quinone. The polar lipid profile consists of the predominant compound phosphatidylethanolamine with moderate amounts of diphosphatidylglycerol and phosphatidylglycerol. The major fatty acids are $C_{16:0}$, summed feature 3 ($C_{16:1}\omega7c/C_{15:0}$ iso 2-OH), and $C_{18:1}\omega7c$. $C_{10:0}$ 3-OH is the only hydroxylated fatty acid detected; $C_{17:0}$ cyclo is absent. Bacterial from the genus *Undibacterium* are sensitive to standard laboratory salt concentration being nonreactive in API or Biolog tests. Reduction of the salt or substrate concentration allows positive reaction and phenotypic pattern analysis. The G+C content for the genus ranges from 50.6 to 57.4 mol%. The type species of the genus is *Undibacterium pigrum*, type strain CCUG 49009 (= CIP 109318 = DSM 19792) (Kämpfer et al. 2007). The genus comprises other five species: *U. oligocarboniphilum* (Eder et al. 2011), *U. parvum* (Eder et al. 2011), *U. terreum* (Liu et al. 2013), *U. seohonense* (Kim et al. 2014), and *U. jejuense* (Kim et al. 2014). The type strains and additional characteristics for these species are listed in *Table 35.7*.

Herminiimonas, Fernandes et al. 2005

Herminiimonas (Her. mi. ni. i. mo' nas, L. masc. n. Mons Herminius, a mountain range of Lusitania; L. fem. n. monas,

Table 35.5
Morphological, physiological, and molecular characteristics differentiating species within genus *Glaciimonas*

	Glaciimonas immobilis LMG 25547T	*Glaciimonas singularis LMG 27070T*
Morphology	Rods	Rods
Cell size (μm)	0.9–1.0 × 1.1–1.5	0.6–0.8 × 1.0–2.0
Flagellation	–	–
Motility	–	–
N_2 fixation	–	–
PHB accumulation or other charact	nd	nd
Detection nifD and/or nifH	nd	nd
Temperature for growth (°C):		
Range	1–20	10–30
Optimum	20	25
pH for growth:		
Range	6.0–8.0	6.0–7.0
Optimum	7.0	7.0
Oxidase	+	+
Catalase	+	+
Urease hydrolysis	+	+
Nitrate reduction	–	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	0–1	0
Utilization of:		
N-acetyl-D-glucosamine	–	+
meso-inositol	+	–
L-rhamnose	–	–
meso-erythritol	nd	nd
Adonitol	–	–
D-arabinose	nd	nd
Malate	–	nd
Sucrose	nd	nd
D-glucose	+	+
D-fructose	–	+
Mannitol	–	+
Glycerol	–	+
Adipic acid	nd	nd
Azelaic acid	nd	nd
Sebacic acid	nd	nd
Phenol	nd	nd
4-chlorophenol	nd	nd
Predominant ubiquinone	Q-8	Q-8
Predominant cellular fatty acids	C16:0, summed feature 3, C18:1 ω7c, C17:0 cyclo	C16:0, summed feature 3, C18:1 ω7c, C17:0 cyclo
Chitinolysis	nd	nd
DNA G+C (mol%)	51	55
Genome size (Mb)	nd	nd
Habitat	Alpine glacier cryonite	Water, uranium mine

nd not determined, positive (+), negative (–)

a unit, monad; N. L. fem. n. *Herminiimonas*, a monad isolated from mineral water coming from the Portuguese mountain *Mons Herminius* that is now known as the Serra da Estrela).

Bacteria of this genus form translucent or pale white, cream, or yellowish punctiformes to 3 mm diameter colonies. Common phenotypic traits shared by members of the genus are the rod shape, negative gram staining, motile, and nonspore-forming. The bacteria have an aerobic metabolism, mesophylic growth, and are oxidase and catalase-positive. They utilize short-chain organic acids and a few amino acids as carbon and energy sources and have a low ability to utilize carbohydrates. The optimum growth temperature ranged from 25 °C to 30 °C and optimum growth pH ranged from 7.0 to 8.5 among the species. *Herminiimonas* chemotaxonomic features include putrescine and 2-hydroxyputrescine as major polyamines; fatty acids straight-chained with major phospholipids as phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol; and ubiquinone Q-8 as the major respiratory quinone. All type strains of the genus *Herminiimonas* harbor fatty acid profiles composed of $C_{17:0}$ cyclo, summed feature 3 ($C_{16:1}$ω7c and/or iso-$C_{15:0}$ 2-OH), and $C_{16:0}$ as the major components. They are characterized by the absence of dodecanoic fatty acids that differ notably from those of other members of the *Oxalobacteraceae* family. The G+C content of the DNA is 52.2. Currently, the genus comprises six validly published species. The type species of the genus is *Herminiimonas fonticola*, and the type strain is S-94^T (= CCUG 52513 = CIP 108398 = LMG 22527) which was isolated from mineral water. The other species are *H. aquatilis* (Kämpfer et al. 2006), *H. arsenicoxydans* (Muller et al. 2006), *H. saxobsidens* (Lang et al. 2007), *H. glaciei* (Loveland-Curtze et al. 2009), and *H. contaminans* (Kämpfer et al. 2013). Most of these isolates were obtained directly from aquatic habitat, exception for lichen–rock interface (*H. saxobsidens*). Details for these species are presented in *Table 35.8*.

Oxalicibacterium, Tamer et al. 2002

Oxalicibacterium (*O*.xa.li.ci.bac.te'ri.um. N.L. *acidum* oxalicum, oxalic acid; gr. N. Bakterion, a small rod; N.L. neut. N. *Oxalicibacterium*, oxalic acid utilizing rod).

Bacteria from the genus *Oxalicibacterium* are small gram-negative, non-sporulating chemoorganoheterotrophic rods, which are motile by 1–3 polar flagella. They have a strictly

Table 35.6
Morphological, physiological, and molecular characteristics differentiating species within genus *Janthinobacterium*

	Janthinobacterium lividum strain ATCC 12473^T	*Janthinobacterium agaricidamnosum* CCUG 43140^T
Morphology	Rods	Rods, single or pairs
Cell size (μm)	0.8–1.2 × 2.5–6	1.0–1.5 × 1.8–2.5
Flagellation	Motile	Motile
Motility	+	+
N_2 fixation	nd	nd
PHB accumulation or other charact	nd	nd
Detection nifD and/or nifH	nd	nd
Temperature for growth (°C):		
Range	nd	2–30
Optimum	25	25
pH for growth:		
Range	nd	nd
Optimum	nd	nd
Oxidase	+	+
Catalase	+	+
Urease hydrolysis	+	+
Nitrate reduction	+	–
Nitrite reduction	–	–
Aesculin hydrolysis	+	–
NaCl concentration for growth (%)	nd	Less than 2 %
Utilization of:		
D-trehalose	–	+
Dextrin	+	–
N-acetyl-D-galactosamine	+	–
D-mannose	–	+
Maltose	+	–
Xylitol	+	–
DL-lactic acid	+	–
Succinic acid	+	–
Succinamic acid	+	–
D-alanine	+	–
L-alanine	+	–
L-asparagine	+	–
L-aspartic acid	+	–
L-glutamic acid	+	–
L-proline	+	–
Inosine	+	–
Sorbitol	+	–
Arbutin	+	–
Salicin	+	–
Cellobiose	+	–
L-fucose	+	–
2-ketogluconate	+	–
Predominant ubiquinone	Q-8	Q-8
Predominant cellular fatty acids	$C_{10:0}$ 3-OH, $C_{12:0}$, $C_{12:0}$ 2-OH, $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7c$, $C_{17:0}$ cyclo, and $C_{18:1}\omega7c$	C10 : 0 3-OH, C12 : 0, C12 : 0 2-OH, C14 : 0, C16 : 0, C16 : 1ω7c, C17 : 0 cyclo, and C18 : 1ω7c
Indole production	–	–
DNA G+C (mol %)	65.1–66.1	64.2
Genome size	4.98	x
Habitat	Soil, water, spoilage milk, and butter	Causal organism of this soft rot disease of *Agaricus bisporus*
Other main characteristics of the species/genus	Usually with purple pigment	Purple pigment is not produced

References: (De Ley et al. 1978; Lincoln et al. 1999)
Use of C-source with API 20NE, API 50CH, and BIOLOG Kit
nd not determined, positive (+), negative (–)

aerobic metabolism and use a limited number of organic acids. No autotrophic growth occurs with either H_2 or formate as an electron donor. The bacteria are catalase- and oxidase-positive. Poly-β-hydroxybutyrate is accumulated. No growth occurs on phenol, sugars, alcohols, and C_1 compounds. The main fatty acids are $C_{16:0}$ (14–24 %), summed feature 3 ($C_{16:1}\omega7c/C_{15:0}$ iso 2OH; 11.9–37.3 %), and moderate amounts of $C_{18:0}\omega7c$ (4.2–17 %). $C_{10:0}$ 3OH was the only hydroxylated fatty acid detected (Sahin et al. 2009a, b). The G+C content of the DNA varied from 55.6 to 64 mol%. The type species of the genus is *Oxalicibacterium flavum* (strain TA17^T = NEU98^T = LMG 21571^T), isolated by enrichment in synthetic medium (Aragno and Schlegel 1981) containing potassium oxalate as sole carbon and energy source, inoculated with litter of oxalate-producing plants from the genus *Mesembryanthemum* in the region of Izmir in Turkey. Two additional species, *Oxalicibacterium horti* and *O. faecigallinarum* were described based on two isolates, obtained by enrichment in oxalate-containing minimal medium, respectively, from garden soil (strain OD1^T = DSM

Table 35.7

Morphological, physiological, and molecular characteristics differentiating species within genus *Undibacterium*

	U. pigrum strain CCUG 49009^T	*Undibacterium oligocarboniphilum* strain CCUG 57265^T	*Undibacterium parvum* strain CCUG 49012^T	*Undibacterium terreum strain CGMCC 1.10998T*	*Undibacterium jejuense* strain NBRC 108922T	*Undibacterium seohonense NBRC 108929T*
Morphology	Rods	Rods	Rods	Rods	Rods	Rods
Cell size (μm)	0.5 × 1.5–2.0	0.5 × 1.5–2.0	0.5 × 1.5–2.0	0.3–0.4 × 1.0–2.6	0.6–0.6 × 1.8–3.4	0.7–1.0 × 1.5–2.5
Flagellation	One or more flagella	One or more flagella	One or more flagella	One or more flagella	One or more flagella	One or more flagella
Motility	+	+	+	+	+	+
N_2 fixation	nd	nd	nd	nd	nd	nd
PHB accumulation or other charact	nd	nd	nd	nd	nd	nd
Detection nifD and/or nifH	nd	nd	nd	nd	nd	nd
Temperature for growth (°C):						
Range	4–30	10–35	5–27	15–37	10–33	10–30
Optimum	25	28–35	21	25	28	28
pH for growth:						
Range	nd	nd	nd	5.0–9.0	5.0–9.0	6.0–11.0
Optimum	7.0–8.0	nd	7.0–8.0	6.0–7.0	7.0	7.0
Oxidase	+	+	+	+	+	+
Catalase	+	+	+	+	–	–
Urease hydrolysis	–	–	–	+	–	–
Nitrate reduction	+	+	+	–	–	+
Nitrite reduction	–	–	–	–	–	–
NaCl concentration for growth (%)	Less than 0.8 %	Less than 0.8 %	Less than 0.8 %	Less than 1 %	0.0 %	Less than 0.8 %

Utilization of:						
D-glucose	−	−	+	+	+	−
D-mannose	+	−	−	+	+	−
N-acetylglucosamine	+	−	−	−	−	−
Maltose	+	+	+	−	+	+
Potassium gluconate	−	+	−	+	−	−
D-ribose	−	−	+	−	+	−
Sucrose	−	−	−	−	−	−
Suberic acid	+	−	−	+	−	+
Sodium acetate	−	−	−	+	+	−
Lactic acid	+	−	+	+	+	−
L-alanine	−	−	−	−	+	−
Propionic acid	−	−	−	−	+	−
Valeric acid	+	+	+	−	+	−
L-histidine	−	−	+	−	+	−
3-hydroxybutyric acid	+	−	+	−	−	−
L-proline	−	−	+	−	+	+
Predominant ubiquinone	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8
Predominant cellular fatty acids	C16:0, summed feature 3 (C16:1ω7c/C15:0 iso 2-OH) and C18:1ω7c. C10:0 3-OH	Summed feature 3 (including unsaturated C16 : 1ω7c), unsaturated C18 : 1ω7c, and straight-chain C16 : 0	Summed feature 3 (including unsaturated C16 : 1ω7c), straight-chain C16 : 0, and unsaturated C18 : 1ω7c and C10: 0 3-OH	Summed feature 3 (C16 : 1ω7c/C16 : 1ω6c), C17 : 0 cyclo, straight-chain C16 : 0, C12 : 0, and C10 : 0, unsaturated C18 : 1w7c and hydroxylated fatty acids C10 : 0 3-OH and C12 : 0 2-OH.	Summed feature 3 (C16 : 1ω7c and/or C16 : 1ω6c) and C16 : 0	Summed feature 3 (C16 : 1ω7c and/or C16 : 1ω6c) and C16 : 0
Indole production	−	−	−	−	−	−
DNA G+C (mol%)	52.3	52.4	50.6	57.4	nd	nd
Genome size	x	x	x	x	x	x
Habitat	Drinking water	Purified water system	Drinking water	Permafrost soil	Forest soil	Freshwater

References: Kämpfer et al. (2007), Eder et al. (2011), Liu et al. (2013), Kim et al. (2014)
Use of C-source with API 20NE, API 50CH, and BIOLOG Kit
nd not determined, positive (+), negative (−)

Table 35.8

Morphological, physiological, and molecular characteristics differentiating species within genus *Herminiimonas*

	Herminiimonas fonticola LMG 22527^T	*Herminiimonas aquatilis* CCUG 36956^T	*Herminiimonas arsenicoxydans* LMG 22961^T	*Herminiimonas saxobsidens* DSM 18748^T	*Herminiimonas glaciei* ATCC BAA-1623^T	*Herminiimonas contaminans* CCUG 53591^T
Morphology	Shot rods	Shot rods	Curved rods	Ovoid rods	Thin rods	Thin rods
Cell size (μm)	1–4 × 0.7	0.8–1.2 × 1.0–1.2	1–2 × 0.5–0.7	0.8 × 0.4	0.5–0.9 × 0.3–0.4	0.5–1.0 × 0.5–0.6
Flagellation	1 polar	Motile	1 polar	Polar flagella	1–3, bipolar	Motile
Motility	+	+	+	+	+	+
N_2 fixation	nd	nd	nd	nd	nd	nd
PHB accumulation or other charact	nd	nd	nd	nd	nd	nd
Detection nifD and/or nifH	nd	nd	nd	nd	nd	nd
Temperature for growth (°C):						
Range	10–35	nd	4–30	4–37	1–35	4–30
Optimum	30	25	25	nd	30	28
pH for growth:						
Range	6.0–9.5	nd	nd	nd	nd	nd
Optimum	7.5–8.0	nd	7–8.5	7–7.5	nd	nd
Oxidase	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Urease hydrolysis	−	−	−	−	−	v
Nitrate reduction	−	−	+	+	+	−
Nitrite reduction	−	−	−	−	−	−
NaCl concentration for growth (%)	nd	nd	nd	Weak at 2 %	nd	nd
Utilization of:						
Citrate	+	−	−	−	+	−
Malate	+	−	−	+	+	+
Succinate	+	−	nd	+	+	nd
Lactate	+	+	+	+	+	+
Alanine	+	−	−	+	+	−
Predominant ubiquinone	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8
Predominant cellular fatty acids	16:1 ω7c and 16:0, C17:0 cyclo, and C14:0	$C_{16:1}$ω7c and C16:0	C16:0 and C17:0 cyclo, C14:0	C16:0, C17:0 cyclo, and C16:1ω7c. C10:0 3-OH and C18:1ω7c	C16:0, C17:0 cyclo, and C16:1ω7c	$C_{16:1}$w7c, $C_{16:0}$, $C_{19:0}$ cyclo w8c C17 : 0 cyclo
Indole production	−	−	−	−	−	−
DNA G+C (mol%)	52	nd	54	nd	59	nd
Genome size	nd	nd	3.4	nd	nd	nd
Habitat	Bottled mineral water	Drinking water	Activated sludge contaminated with heavy metals	Lichen–rock interface of a limestone bedrock	3,042 m deep Greenland ice core	Contaminant of a biopharmaceutical production process

References: Fernandes et al. (2005), Kämpfer et al. (2006), Muller et al. (2006), Lang et al. (2007), Loveland-Curtze et al. (2009), Kämpfer et al. (2013)
Use of C-source with API 20NE, API 50CH, and BIOLOG Kit
nd not determined, *v* variable, positive (+), negative (−)

21640^T = NBRC 13594^T) and from chicken dung (YOx^T = DSM 21641^T = CCM 2767^T) (Sahin et al. 2009a, b). A fourth species of the genus named *Oxalicibacterium solurbis* (MY14^T = NBRC 102665^T = CCM 7664^T) was isolated from urban soil using a membrane-filter enrichment technique (Sahin et al. 2010a, b). *O. horti* can be distinguished from other *Oxalicibacterium* species by the presence of $C_{13:0}$ (0.5 %), *O. faecigallinarum* by the presence of $C_{18:0}\omega6c$ (11.1 %), and *O. solurbis* (MY14^T) by the lack of $C_{14:0}$ and summed feature 7 ($C_{18:1}\omega7c$, 12t/9t) from the fatty acid profiles. The type strains and additional characteristics for these species are listed in *Table 35.9*.

Oxalobacter, Allison et al. 1985

Oxalobacter (Ox.al.o.bac'ter. Gr. N. *oxal*-pertaining to oxalate; M.L.n. *bacter* the masculine form of the Gr. neut. n. *bactrum* a rod).

Oxalobacter spp. are anaerobic bacteria that have been isolated from the gastrointestinal tract of man and animals and from anoxic freshwater sediments. *Oxalobacter* cells are rod-shaped, gram-negative bacteria (0.4–0.6 × 1.2–2.5 μm) with rounded ends, occurring as single cells, in pairs or in chains. Often, the cells are curved. No endospores are formed. The motility is variable with 0–2 polar flagella. G+C content of DNA is 48.0–51.6 mol%. The bacteria are strictly anaerobic, chemoorganotrophic growth. Oxalate is decarboxylated to formate and used as major carbon and energy source. A small amount of acetate (0.5–2 mM) is also required for growth (Cornick and Allison 1996; Dehning and Schink 1989). Anaerobic growth occurs under a gas phase of CO_2 in a defined carbonate–bicarbonate-buffered medium that contains minerals, oxalate, and acetate. Growth is improved by the addition of yeast extract to the medium. Growth of some, and possibly all, intestinal and sediment strains requires acetate. Growth occurs at temperatures in the range of 14–45 °C. None of a wide variety of substances can replace oxalate as growth substrate. The type species is *Oxalobacter formigenes*. The type strain DSM 4420^T (= ATCC 35274^T, = BA-2^T, = CCUG 52237^T, = CIP 106513^T, = DSM 4420^T, = OxB^T) was isolated from sheep rumen by enrichment culture techniques (Dawson et al. 1980a, b; Allison et al. 1985). *O. formigenes* has an optimal growth temperature of 37 °C. Cells are rod-shaped and often curved, and occasionally chains are observed as spiral filaments. No flagella were observed (Allison et al. 1985). *O. formigenes* plays a role in the destruction of oxalate in the gastrointestinal tract, where it decarboxylates oxalate, generating formate and CO_2 (Anantharam et al. 1989). An oxalate^{2-}/formate$^-$ antiporter mediates simultaneous substrate import and product export accompanied by the net-extrusion of a proton (Anantharam et al. 1989). A lack of colonization by this species is believed to be a risk factor for the development of calcium oxalate disease (Knight et al. 2013). Based on cellular fatty acid composition, *O. formigenes* strains can be divided in two main groups. Group 1 strains are characterized by a predominant cyclic 17 carbon fatty acid, while in group 2 strains, a cyclic 19 carbon fatty acid is predominant (Allison et al. 1985). The second species of this genus is *Oxalobacter vibrioformis*, and the type strain (DSM 5502^T = WoOx3^T) was isolated from anoxic freshwater sediments. It is able to use oxamate as a carbon and energy source after deamination (Dehning and Schink 1989, 1990). The type strains and additional characteristics for these species are listed in *Table 35.10*.

Duganella, Hiraishi et al. 1997

Duganella (Du.ga.nel'la. M.L. dim. ending *-ella*; M.L. fem. n. *Duganella*, named after P. R. Dugan, an American microbiologist, who isolated the organism).

Cells are gram-negative, nonspore-forming, motile rods. The cells are aerobic chemoorganotrophs having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. No chemolithotrophic growth occurs with molecular hydrogen. Amorphous or fingerlike flocculent growth occurs in liquid media. The organism is mesophilic and prefers neutral pH. Growth is good on ordinary nutrient media and also occurs in mineral media supplemented with simple organic compounds as carbon and energy sources. Catalase and oxidase are present. Acid from glucose and other carbohydrates is produced oxidatively. The cells harbor amylase, gelatinase, and urease. Major components of the cellular fatty acids are $C_{16:0}$ and 3-OH-$C_{10:0}$, and 3-OH-$C_{10:0}$ is the major hydroxyl-fatty acid. The predominant polar lipids are phosphatidylglycerol (PG) and phosphatidylethanolamine (PE). Ubiquinone 8 is the sole respiratory quinone. Putrescine and hydroxyputrescine are intracellular polyamines. The G+C content of the genomic DNA is 54.9–64 mol%. Members of the genera *Massilia* and *Telluria* are the closest phylogenetic neighbors. The type species is *Duganella zoogloeoides* (zo.o.gloe.o'i.des. M.L. bacterial genus name *Zoogloea*; Gr. suf. *-oides*, similar to; M.L. adj. *zoogloeoides*, similar to *Zoogloea*). The characters are the same as described for the genus. Cells are straight or slightly curved rods, 0.6–0.8 um wide, and 1.8–3.0 um long and motile by means of single polar flagella. The type strain is IAM 12670^T (=ATCC 25935^T = P. R. Dugan 115) (Hiraishi et al. 1997). The genus is constituted by three other species detailed in *Table 35.11*: *Duganella phyllosphaerae* (of the phyllosphere), *D. sacchari* (isolated from sugarcane), and *D. radicis* (of a root, isolated from sugarcane) (Kämpfer et al. 2012b; Madhaiyan et al. 2013). The type strains and additional characteristics for these species are listed in *Table 35.11*.

Pseudoduganella, Kämpfer et al. 2012

Pseudoduganella (Pseu.do.du.ga.nel'la. Gr. adj. *pseudes* false; N. L. fem. n. *Duganella* a bacterial genus name; N.L. fem. n. *Pseudoduganella* false *Duganella*).

Table 35.9

Morphological, physiological, and molecular characteristics differentiating species within genus *Oxalicibacterium*

	Oxalicibacterium flavum LMG 21571^T	*Oxalicibacterium horti* DSM 21640^T	*Oxalicibacterium fecal gallinarum DSM 21641^T*	*Oxalicibacterium solurbis NBRC 102665^T*
Morphology	Small rods	Small rods	Small rods	Small rods
Cell size (μm)	0.4 × 0.9–1.4	0.5 × 1.5	0.75 × 1.5	0.4–0.5 × 0.8–1.2
Flagellation	1–3 polar flagella	Polar flagella	Polar flagella	Polar flagella
Motility	+	+	+	+
N_2 fixation	- (1)	nd	nd	nd
PHB accumulation or other charact	+	+	nd	nd
Detection nifD and/or nifH	nd	nd	nd	nd
Temperature for growth (°C):				
Range	25–37	25–37	28–37	4–37
Optimum	25	25	nd	37
pH for growth:				
Range	nd	nd	nd	nd
Optimum	6.9	7	7–7.5	8
Oxidase	+	+	+	nd
Catalase	+	+	+	nd
Urease hydrolysis	nd	−		−
Nitrate reduction	−	−	−	−
Nitrite reduction	nd	nd	nd	nd
NaCl concentration for growth (%)	Up to 4 %, no growth at 7 %	nd	Up to 3 %	Up to 5 %
Utilization of:				
N-acetyl-D-glucosamine	−	−		
meso-inositol	−	−	−	−
Formate	−(1)/+(2, 3)	+	+	−
Succinate	−	+(2)/w(3)	w	−
L-alanine	−(3)	+	+(2)/w(3)	−
D-alanine	+(3)	+	+	−
alpha-ketobutyrate	−	−	w(2)/−(3)	+
alpha-ketoglutarate	+(1)/w(2,3)	+	+	−
alpha-OH-butyrate	−	+	w(3)/+(2)	+
beta-OH-butyrate	−	−	−	+
Propionate	−	−	−	−
Malate	+	+	+	+
Citrate	−	−	−	−
D-glucose	−	−	−	−
Oxalate	+	+	+	+
Glycolate	+	+	+	+
DL-lactate	+	+	+	+
DL-glycerate	+	−	−	−
Acetate	−	−	−	−
Pyruvate	+	+	+	+
L-rhamnose	−	−	−	−
meso-erythritol	−	−	−	−

Table 35.9 (continued)

	Oxalicibacterium flavum LMG 21571^T	*Oxalicibacterium horti* DSM 21640^T	*Oxalicibacterium fecal gallinarum DSM 21641^T*	*Oxalicibacterium solurbis NBRC 102665^T*
Adonitol	–	–	–	–
D-arabinose	–	–	–	–
Malate	+	+	+	+
Sucrose	–	–	–	–
D-glucose	–	–	–	–
D-fructose	–	–	–	–
Mannitol	–	–	–	–
Glycerol	–	–	–	–
Adipate	–	–	–	–
Azelaic acid	–	–	–	–
Sebacic acid	–	–	–	–
Phenol	–	–	–	–
4-chlorophenol	–	–	–	–
Predominant ubiquinone	nd	nd	nd	Q-8
Predominant cellular fatty acids	C17:0 cyclo, C16:0, C19:0 cyclo omega8c, summed feature 3 (2,3)	C17:0 cyclo, summed feature 3, C19:0 cyclo ω8c, C16:0, C18:1 ω7c (2, 3)	summed feature 3, C16:0, C18:1ω7c, C18:1ω6c (2, 3)	C16:0, C17:0 cyclo (3)
Indole production	–	–	–	–
DNA G+C (mol%)	63	59.7	55.6	63.3
Genome size	nd	nd	nd	nd
Habitat	Oxalate-rich soil and plant litter	Soil	Chicken dung	Soil

References: (1) Tamer et al. (2002), (2) Sahin et al. (2009a), (3) Sahin et al. (2010a)
nd not determined, *w* weak, positive (+), negative (–); when information is ambiguous or where gathered from many sources, references are indicated in parentheses (numbered according to the field "reference")

Cells are gram-negative, obligate aerobic, and nonspore-forming. They are rod-shaped with flagella, about 0.4–0.6 × 0.8–1.0 μm in size, and occur singly. Violet-pigmented colonies are formed on agar media, like on R_2A agar or TSA. The major ubiquinone is Q-8, and the predominant polar lipids are phosphatidylglycerol and phosphatidylethanolamine. In addition, minor amounts of phosphatidylserine, an unidentified aminolipid and diphosphatidylglycerol are found. Major cellular fatty acids are $C_{16:0}$, $C_{16:1}$ ω7c, and $C_{15:0}$ iso 2OH (detected as summed feature). Hydroxylated fatty acids are $C_{12:0}$ 3-OH, $C_{10:0}$ 3-OH, and $C_{12:0}$ 2-OH. The optimum growth temperature is 28–30 °C, and the optimum pH for growth is 7.2. The DNA G + C content is 62.8 mol%. The type species is *Pseudoduganella violaceinigra* (vio.la.ce.i.ni'gra. L. adj. *violaceus* violet; L. adj. *niger-gra-grum*, black; N. L. fem. adj. *violaceinigra* violet-black, after the color of the colonies). Basonym: *Duganella violaceinigra* Li et al. (2004). The type strain is YIM 31327^T (=CIP 108077^T = KCTC 12193^T = CCUG 50881^T = DSM 15887^T) (Kämpfer et al. 2012b). For further details of phenotypic features, see *Table 35.12*.

Massilia, La Scola et al. 2000 Emend. Kämpfer et al. 2011

Massilia (*Mas.sil' i.a.* L. fem. n. *Massilia*, Latin name of Marseille, France. According to Kämpfer et al. 2011, *Massilia* La Scola et al. 2000 is an earlier heterotypic synonym of *Naxibacter* Xu et al. 2005).

The members of this genus are aerobic, gram-negative, motile, nonspore-forming rods, and are catalase-positive. Chemotaxonomically, ubiquinone Q-8 is the predominant isoprenoid quinone. The major compounds in the polar lipid profile are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Oxidase reaction may be variable. Acid production occurs during utilization of carbohydrates. Arginine dihydrolase, urease, and aesculin reactions as well as gelatin hydrolysis may be variable. The G+C content of the DNA ranges from 62.4 to 68.9 mol% with an average of 66.2 mol%. The bacteria of the genus contain fatty acid profiles composed of summed features 3 (*iso*-$C_{15:0}$ 2-OH and/or $C_{16:1}$ ω7c) and $C_{16:0}$ as the major components, as well as $C_{18:1}$ ω7c, $C_{10:0}$ 3-OH, and

Table 35.10
Morphological, physiological, and molecular characteristics differentiating species within genus *Oxalobacter*

	Oxalobacter formigenes ATCC 35274^T	*Oxalobacter vibrioformis DSM 5502T*
Morphology	Rods, often curved, sometimes forming coiled filaments	Vibrioid rods
Cell size (μm)	0.4–0.6 × 1.2–2.5	1.8–2.4 × 0.4
Flagellation	–	1–2 polar flagella
Motility	–	+
N_2 fixation	nd	nd
PHB accumulation or other charact	nd	nd
Detection nifD and/or nifH	nd	nd
Temperature for growth (°C):		
Range	14–45	18–35
Optimum	37	30–32
pH for growth:		
Range	Around 7	5.6–8.3
Optimum	Around 7	6.8–7
Oxidase	nd	nd
Catalase	nd	–
Urease hydrolysis	nd	–
Nitrate reduction	–	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	nd	nd
Utilization of:		
N-acetyl-D-glucosamine	–	–
meso-inositol	–	–
Oxalate	+ (main carbon source)	+ (main carbon source)
Glycolate	–	–
DL-lactate	–	–
DL-glycerate	–	–
Acetate	+ (required for growth)	+ (required for growth)
Pyruvate	–	–
L-rhamnose	–	–
meso-erythritol	–	–
Adonitol	–	–
D-arabinose	–	–
Malate	–	–
Sucrose	–	–
D-glucose	–	–
D-fructose	–	–
Mannitol	–	–
Glycerol	–	–
Adipate	–	–
Azelaic acid	–	–
Sebacic acid	–	–
Phenol	–	–
4-chlorophenol	–	–
Predominant ubiquinone	nd	nd
Predominant cellular fatty acids	$C_{17:0}$ cyclo, $C_{16:0}$, $C_{19:0}$ cyclo	nd
Indole production	–	–
DNA G+C (mol %)	48–51	51.6 +/− 0.6
Genome size	2.4–2.5 Mb	nd
Habitat	Anaerobic environments such as the rumen and colon of man and mammals and lake sediments	Anoxic freshwater and marine sediments

nd not determined, *w* weak, positive (+), negative (–)

$C_{12:0}$ in moderate amounts. Polar lipid profile contains the major compounds phosphatidylglycerol, phosphatidylethanolamine, and diphosphatidylglycerol. The type species is *Massilia timonae*. The type strain is UR/MT95^T (=CCUG 45783^T = CIP105350^T) (La Scola et al. 1998). The genus embraces 23 species detailed in ❯ *Table 35.13*. Most of the species have names related to the colony morphology.

Naxibacter, Xu et al. 2005 Abolished Kämpfer et al. 2011

Naxibacter (Na.xi.bac'ter. N.L. n. *Naxi* referring to the Naxi nationality, who lived in Lijiang, Yunnan Province, China, from where the organism was isolated; n. *bacter* from Gr. n. *baktron* rod; N.L. masc. n. *Naxibacter* rod-shaped microbe from the place in which the Naxi nationality lived).

Bacteria from the genus *Naxibacter* form beige/ivory-colored, shiny convex colonies with entire edges. Cells are

Table 35.11

Morphological, physiological, and molecular characteristics differentiating species within genus *Duganella*

	Duganella zoogloeoides ATCC 25935T	*Duganella sacchari KCTC 22381T*	*Duganella radicis KCTC 22382T*	*Duganella phyllosphaerae LMG 25995T*
Morphology	Rods	Rods	Rods	Rods
Cell size (μm)	0.6–0.8 × 1.8–3.0	0.3–0.5 × 1.2–1.7	0.3–0.4 × 1.1–1.2	1.0–2.0 × 2.0–3.0
Flagellation	+	+	+	+
Motility	+	+	+	+
N_2 fixation	–	–	–	–
PHB accumulation or other charact	nd	nd	nd	nd
Detection nifD and/or nifH	nd	nd	nd	nd
Temperature for growth (°C):				
Range	25–30	20–37	15–37	15–37
Optimum	30	28	30	28
pH for growth:				
Range	6.0–8.0	6.0–8.0	4.0–10.0	4.5–9.5
Optimum	7.0	7.2	7.2	7.0
Oxidase	+	w	–	+
Catalase	+	+	+	–
Urease hydrolysis	+	–	–	–
Nitrate reduction	–	–	+	–
Nitrite reduction	nd	–	nd	nd
NaCl concentration for growth (%)	nd	0–0.5	0–0.5	nd
Utilization of:				
N-acetyl-D-glucosamine	nd	nd	nd	–
meso-inositol	–	nd	nd	nd
L-rhamnose	–	nd	nd	–
meso-erythritol	nd	nd	nd	nd
Adonitol	nd	nd	nd	–
D-arabinose	nd	nd	nd	nd
Malate	+	nd	nd	–
Sucrose	+	–	+	+
D-glucose	+	+	+	+
D-fructose	+	–	+	+
Mannitol	–	+	–	–
Glycerol	–	nd	nd	–
Adipic acid	nd	nd	nd	–
Azelaic acid	nd	nd	nd	–
Sebacic acid	nd	nd	nd	nd
Phenol	nd	nd	nd	nd
4-chlorophenol	nd	nd	nd	nd
Predominant ubiquinone	Q-8	Q-8	Q-8	Q-8
Predominant cellular fatty acids	C16:0, C16:1, 3-OH C10:0	C12:0, C16:1, C17:0 cyclo, summed feature 3	C12:0, C16:0, summed feature 3	C16:0, C18:1 ω7c, summed feature 3
Chitinolysis	nd	nd	nd	nd
DNA G+C (mol%)	63–64	56.4	54.9	nd
Genome size (Mb)	nd	nd	nd	nd
Habitat	Soil, water	Rhizosphere, sugarcane	Rhizoplane, sugarcane	Leaf, phyllosphere

nd not determined, *w* weak, positive (+), negative (–)

Table 35.12
Morphological, physiological, and molecular characteristics differentiating species within genus *Pseudoduganella*

	Pseudoduganella violaceinigra KCTC 12193T
Morphology	Rods
Cell size (μm)	0.4–0.8 × 0.8–1.0
Flagellation	+
Motility	+
N_2 fixation	–
PHB accumulation or other charact	nd
Detection nifD and/or nifH	nd
Temperature for growth (°C):	
Range	28–30
Optimum	30
pH for growth:	
Range	nd
Optimum	7.2
Oxidase	–
catalase	+
Urease hydrolysis	–
Nitrate reduction	–
Nitrite reduction	–
NaCl concentration for growth (%)	nd
Utilization of:	
N-acetyl-D-glucosamine	+
meso-inositol	–
L-rhamnose	–
meso-erythritol	–
Adonitol	nd
D-arabinose	nd
Malate	+
Sucrose	–
D-glucose	Variable
D-fructose	–
Mannitol	–
Glycerol	–
Adipic acid	–
Azelaic acid	–
Sebacic acid	nd
Phenol	nd
4-chlorophenol	nd
Predominant ubiquinone	Q-8
Predominant cellular fatty acids	C16:0. C12:0, summed feature 3
Chitinolysis	nd
DNA G+C (mol%)	62.8
Genome size (Mb)	nd
Habitat	Rhizosphere soil

nd not determined, positive (+), negative (–)

gram-negative, rod-shaped, nonspore-forming, and variably motile with one or more polar flagella. The predominant respiratory quinone is Q-8, although minor amounts of Q-7 are also detected. The G+C content of the DNA of is 62–63 mol %. The predominant fatty acids are $C_{16:1}\omega7c$ and $C_{16:0}$ (Xu et al. 2005; Kämpfer et al. 2008a). The first representant of the genus was isolated in a screening program for new antibiotics. This strain,YIM31775^T, which was found to contain genes for type I and type II polyketide biosynthesis, led to the description of the novel genus *Naxibacter* and species *N. alkalitolerans* (Xu et al. 2005). As by Xu and colleagues (2005), *N. alkalitolerans* are gram-negative nonspore-forming rods with rounded ends and which are motile with one or more polar flagella. The cells are oxidase-negative, catalase-positive, aerobic, and chemoorganotrophic. Colonies of strain YIM 31775^T are 1.1–1.4 mm in diameter, circular, entire, convex, glistening, butyraceous, and opaque with a pale white-yellow color on nutrient agar plates (Xu et al. 2005). Later, the description of the genus *Naxibacter* was emended by Kämpfer and colleagues (2008a), who described two additional species of the genus: *N. varians* and *N. haematophilus*. The description of *N. varians* is based on five isolates from water samples and from clinical samples in Scandinavia. The type strain CCUG 35299^T was isolated from the eye of a 90-year-old man in Norway. The same study describes a second new species, based on a single isolate from the blood of a 23-year-old man with multiple health problems (type strain CCUG 38318^T), which was named *N. haematophilus* (Kämpfer et al. 2008a). Both species presented nonmotile, nonspore-forming rods, gram-negative, and oxidase-positive with an oxidative metabolism. Both species produce the polyamines putrescine, 2-hydroxyputrescine, spermidine, and spermine. Good growth at 25–30 °C. After 24 h, colonies have a diameter of approximately 2 mm and are beige, translucent, and shiny with entire edges. Although the strains CCUG 35299^T and CCUG 38318^T showed 99 % 16S rRNA gene sequence similarity, DNA–DNA hybridization experiments led the authors to the description of these two novel species (Kämpfer et al. 2008a). These authors affirmed that Xu and colleagues (2005) had erroneously detected the glycolipid phosphatidylinositol mannoside in the polar lipid profile of strain YIM 31775^T. This absence of phosphatidylinositol mannoside was later also confirmed by Weon and colleagues (2010) when these authors described the fourth species of the genus, *N. suwonensis*. According to the emended description of the genus, its species share phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol as the major polar lipids. Also, the presence of 2-hydroxyputrescine and putrescine in the polyamine pattern was added as a common trait (Kämpfer et al. 2008a). One determining trait that justified the description of the novel genus *Naxibacter* was the erroneous detection of the glycolipid phosphatidylinositol mannoside by Xu and colleagues (2005). Also, 16S rRNA gene phylogenetic analyses and phenotypic traits do not permit a clear separation between the closely related genera *Naxibacter* and *Massilia* (Kämpfer et al. 2011; La Scola et al. 1998). Therefore, Kämpfer and colleagues (2011)

revised the description of the genus *Massilia*, by the reclassification of the species *N. alkalitolerans*, *N. varians*, *N. haematophilus*, and *N. suwonensis* as *Massilia alkalitolerans*, *M. varians*, *M. haematophila*, and *M. suwonensis*. No detailed table showing the differential characteristics among the Naxibacter species are provided considering that the genus was eliminated, and the species were transferred to the genus *Massilia*.

Telluria, Bowman et al. 1993

Telluria (Tel.lu'.ri.a. L. fem. N. *Tellus*, a Roman goddess of the earth, also the ground or earth; M. L. fem. N. *Telluria*, from the earth).

Bacteria from the genus *Telluria* – former [*Pseudomonas*] – are gram-negative rods of 0.5–1.0 × 2.0–3.0 μm, which occur singly, in pairs, or in short chains. In liquid media, single polar flagella are formed, while on solid media, lateral flagella are also observed. They accumulate β-hydroxybutyrate, have a strictly aerobic chemoheterotrophic metabolism, and are unable to chemolithotrophically derive energy from H_2. Denitrification does not occur. They are sensitive to NaCl. Good growth occurs on media containing carbohydrates and a combined nitrogen source; in media lacking carbohydrates and on nutrient agar, growth is poor. Complex carbohydrates, including starch and xylan, are used; cellulose is not hydrolyzed. The bacteria are able to hydrolyze gelatin, esculin, casein, DNA, Tween 40, Tween 60, and Tween 80. Optimal growth occurs between 30 °C-35 °C and at pH 7.0. The major ubiquinone is Q-8. The G+C content of DNA is between 67 and 72 mol%. The only known habitat is (rhizosphere) soil. The description of the type species *Telluria mixta* (former [*Pseudomonas*] *mixta*, Bowman et al. 1988) is based on dextranolytic strains, isolated from soil and from the rhizosphere of sugarcane plants in Queensland, Australia, in the 1970s. The type strain is ACM 1762 (= UQM 1762 = ATCC 49108). The G+C content of the DNA is determined as 69 mol%. The major fatty acids are $C_{16:0}$, $C_{16:1}\omega7c$, and/or $C_{15:0}$ iso 2-OH, $C_{17:0}$ cyclo, and $C_{18:1}\omega7c$ (Kämpfer et al. 2007). The second known species of the genus is *Telluria chitinolytica* (former *Pseudomonas chitinolytica*), which was described based on a single strain. The type strain (ACM 3522^T = CNCM I-804^T = $20M^T$) was isolated from a loamy soil from Israel amended with crustacean shell (Spiegel et al. 1991). The G + C content of the DNA is 72 mol%. The type strains and additional characteristics for these species are listed in *Table 35.14*.

Isolation, Enrichment, and Maintenance Procedures

Member of the family *Oxalobacteraceae* grow in a wide range of media, although specific media such as the nitrogen-free semisolid or specifically enriched media have been used successfully to isolate bacteria of some genera.

Herbaspirillum

H. aquaticum – isolated from deionized water in USA (Dobritsa et al. 2010) and can be cultivated in tryptic soy agar at 28 °C. Although it was not mentioned, it can be maintained in the same conditions applied to *H. huttiense* subspecies.

H. autotrophicum – isolated from a small eutrophic lake in Switzerland and the procedure was that essentially described by Aragno and Schlegel (1978). Water samples were filtered on membrane filters, and the filters were deposited on mineral agar medium: incubation was under an atmosphere of 60 % H_2–30 % air–10 % CO_2 at 30 °C. Pure cultures were obtained by repeated subculturing on mineral agar plates incubated under a hydrogen–air–carbon dioxide mixture. Stock cultures are maintained autotrophically on mineral agar slants and transferred to fresh medium every 2 months.

H. chlorophenolicum – isolated from soil sediment collected in an industrial stream sites using liquid MSM (minimal salt medium) containing 4-chlorophenol (50 mg/l) as described by Bae et al. (1996). Flasks are incubated up to 7 days at 30 °C until an increase in turbidity is observed. Single colonies are obtained by plating 0.1 ml suspension on 4-CP agar plates.

H. frisingense – isolated from washed roots or aerial part (stems and leaves) of *Miscanthus* and *Pennisetum* plants grown in Germany and in Brazil, respectively (Kirchhof et al. 2001). The N-free JNFb semisolid medium described for isolation of other nitrogen-fixing *Herbaspirillum* species (Döbereiner 1995) was applied to count and obtain single colonies. After 4 ± 6 days of incubation at 30 °C, characteristic subsurface pellicle-forming bacteria were subjected to further purification steps by streaking on JNFb agar plates containing an additional 20–50 mg yeast extract, and single colonies were again transferred for cultivation in fresh vials containing the same semisolid nitrogen-free medium. Pure isolates are obtained by streaking the veil-like pellicles on potato agar or BSA medium (Baldani et al. 1986). Stock cultures are maintained on JNFb supplemented with yeast extract or on potato agar slants under mineral oil, lyophilized, or cryopreserved at −70 °C.

H. hiltneri – isolated from surface-sterilized (chloramine T-1 %) wheat roots (4- to 8-week-old) grown in agricultural soil in Germany after roots were crushed in 1xPBS, diluted, and plated on NB solidified (16 g) agar. Single colonies were picked and streaked onto new NB agar plates. Cultures are maintained on NB slants under mineral oil or lyophilized according to the culture collection procedures.

H. huttiense subsp. huttiense – isolated from distilled water samples from New Zealand using simple peptone or yeast extract media as described by Leifson (1962). Medium B1 composed of 0.5 % polypeptone (Difco), 0.3 % yeast extract (Difco), 3 % NaCl, and 1.5 % agar are used to cultivate the species (Ding and Yokota 2004). The organism also grows readily on the strains were maintained in the laboratory for 3 years without changes (Leifson 1962), but no information about the maintenance method was indicated.

Table 35.13

Morphological, physiological, and molecular characteristics differentiating species within genus *Massilia*

	M. aerilata 5516S-11^T	*M. albidiflava 45^T*	*M. alkalitolerans YIM 31775^T*	*M. aurea AP13^T*	*M. brevitalea byr23-80^T*	*M. consociata CCUG 58010^T*	*M. dura 16^T*	*M. flava Y9^T*	*M. haematophila CCUG 38318^T*	*M. jejuensis 5317J-18^T*	*M. lurida D5^T*
Morphology	Rods	Short rods	Short rods	Rods	Short rods	Rods	Straight rods	Rods	na	Rods	Rods
Cell size (μm)	0.7–10 × 1.5–3.0	1.8–2.0 × 3.0–3.5	0.5–0.8 × 1.35–2.0	na	1.5–2.0 × 0.7–1.0	2.0 × 1.0	0.6–0.8 × 1.8–2.2[1, 4]	0.6–0.8 × 1.5–2.5	2.0 × 1.0	1.1–1.5 × 1.3–3.0	0.6–0.9 × 1.4–2.8
Flagellation	1, polar	Lateral, peritrichous	One or more, polar	na	1, polar; occasionally, cells with 2–3 polar or lateral flagella were observed	na	One or more, lateral[1, 4]	na	na	1	na
Motility	+	+	+	+	+	w	+	na	na	+	+
N_2 fixation	na	na	na	na	na	na	na	na	na	na	na
PHB accumulation or other charact	+	+	na	+	+	na	+	na	na	na	na
Detection nifD and/or nifH	na	na	na	na	na	na	na	na	na	na	na
Temperature for growth (°C):											
Range	5–35	10–45	4–55	4–40	4–30	15–37	10–45[1, 4]	10–45[1, 2]	15–37	5–35	10–37
Optimum	28	28–30	28–37	28[1, 3]–30[3]	15	25–30	28–30	28–30[1, 2]	25–30	30	28–30
pH for growth:											
Range	5.0–9.0	6.5–8.5	6.5–12.0	4.0–9.0	6.0–10.0	5.5–10.5	6.5–8.5[1, 4]	6.5–8.5[1, 2]	5.5–10.5	6.0–9.0	6.0–9.0
Optimum	6.0–8.0	7.0–7.5	7.0–9.0	7.0–8.0[1]/ 6.0–7.0[3]	7.0–7.5	na	7.0–7.5	7.0–7.5[1, 2]	na	7.0	7.0
Oxidase	+	+[1, 3, 4]	+	w[1]/+[3]	–	–[1, 4]/+[1]	+[1, 4]	+[1, 2]	+	+	+
Catalase	+	+[1, 2, 3]	na	+[2, 3]	w	na	+[2]	+[1, 2]	na	+	+
Urease hydrolysis	+	+[1]/–[2, 4]	–	–[1, 2]	–	–[1, 2, 3, 4]	+[1, 2, 4]/–[2]	–	–[2, 3]	–[1, 2]	+
Nitrate reduction	+	+[1, 2, 3, 4]	–	–[1, 2, 3, 4]	+	–[1, 2, 3, 4]	+[1, 4]/–[2]	+[1, 2]	–[2, 3]	–[1, 2]	–
Nitrite reduction	na	na	na	na	na	na	na	na	na	na	na
NaCl concentration for growth (%)[c]	0–1	ng >1[1, 3]	ng =3	0–5[1]; ng >1[3]	ng >2	na	ng >1[1, 4]	ng >1[1, 2]	na	0.01	ng >0.5
Utilization of:											
N-acetyl-D-glucosamine	–	+[2, 3]	na	+[2, 3]	+	–[2]	–[2]	–[2]	–[3]	na	na
Inositol	–	-	+[1]/–[1]	na	–	na	–	–	na	na	–
meso-inositol	na	na	na	na	na	na	na	na	na	na	na
L-rhamnose	–	na	+[2]	+[4]	–	–[1, 2, 4]	+[3]	+	–[2, 3]	–	+
meso-erythritol	na	na	na	na	–	na	na	na	na	na	–
Adonitol	na	–	–	na	–	na	–	na	na	na	–
D-arabinose	ND; +	ND; –[1]/+[1, 3]	ND; +[2, 3]	ND; –[2, 3, 4]	–; –	+; +[3, 4]	ND; –[1]/+[2, 3]	–; +[1, 2]	ND; –[1, 2]	ND; –[1, 2]	+; +
Malate	na	na	+[3]	na	–/+	na	na	na	+	na	na
Sucrose	–	+	–[1, 3]	na	–	na	+	+	–	–	–
D-glucose	+	+[2, 3]	+[2, 3]	+[2, 4]/–[3]	na	+[1, 2, 3, 4]	+[2, 3]	+[1, 2]	–[1, 2, 3]	–[1, 2]	+
D-fructose	na	na	–	na	–	na	na	w	na	na	+
Mannitol	–	–[2, 3]	na	–[2, 3]	–	na	–[2]	–[1, 2]	na	na	na
Glycerol	na	na	na	na	na	na	na	na	na	na	na
Adipate adipic acid	–	+[2]/–[3]	–[2, 3]	+[2, 4]/–[3]	+	–[3]	–[2, 3]	–[2]	+[2]	–[1, 2]	–
Azelaic acid	na	na	na	na	na	na	na	na	na	na	na
Sebacic acid	na	na	na	na	na	na	na	na	na	na	na
Phenol	na	-	na	na	na	na	–	na	na	na	na
4-chlorophenol	na	na	na	na	na	na	na	na	na	na	na
Predominant ubiquinone	Q-8	Q-8	Q-8	na	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8

M. lutea 101^T	*M. namucuonensis 333-1-0411^T*	*M. niabensis 5420S-26^T*	*M. niastensis 5516S-1^T*	*M. oculi CCUG 43427A^T*	*M. plicata 76^T*	*M. suwonensis 5414S-25^T*	*M. tieshanensis TS3^T*	*M. timonae^T UR/MT95^T*	*M. umbonata LP01^T*	*M. varians CCUG 35299^T*	*M. yuzhufengensis Y1243-1^T*
Short rods	Rods	Rods	Rods	Rods	Straight rods	Rods	Rods	Straight rods	Rods	Rods	Rods
na	0.3–0.5 × 0.5–1.5	0.6–0.9 × 1.4–4.0	0.6–0.8 × 1.5–5.0	2.0 × 1.0	0.6–0.7 × 2.0–2.5	0.6–0.8 × 1.2–3.0	0.3–0.6 × 1.2–2.0	1.0 × 3.0	0.6–0.8 × 2.0–2.5	2.0 × 1.0	0.7–1.0 × 2.3–2.7
Peritrichous, lateral	na	1, polar	1, polar	na	One or more, lateral	1	Present	Present	1, polar	na	Polar
na	na	na	na	na	na	+	+	+	+	no[a]	+
na	na	na	na	na	na	na	na	na	na	na	na
+	na	na	na	na	+	na	na	na	+	na	na
na	na	na	na	na	na	na	na	na	na	na	na
10–45[1, 3]	4–37	5–35[1]/ 4–37[2]	5–40	15–37	10–45[1, 4]	5–40	10–40	4–37[3]	4–37	15–37	2–35
28–30	28–30	28[1, 2]	28	na	28–30[1, 4]	30	28	28–30[3]	28–30	25–30	25
6.5–8.5[1, 3]	5.5–9.5	7.0–9.0[1, 2]	6.0–8.0	5.5–10.5	6.5–8.5[1, 4]	6.0–9.0	5.0–9.0	5.5–10.0[3]	7.0–8.0	5.5–10.5	5.0–8.0
7.0–7.5	6.5–7.5	7.0[1, 2]–7.5[2]	7.0	na	7.0–7.5[1, 4]	7.0	7.0	7.0–7.5[3]	7.5–8.0	na	7.0
+[1, 3]	+	+[1, 2]	+	+/−[b]	−[1, 4]	+	+	−[1, 3]	+/−[b]	+[a]	−
+[1, 2]	+	+[1, 2]	+	na	+[1, 2, 4]	+	+	+[1, 2, 3]	+	na	+
−[1, 2, 3]	−	−	−[1, 2, 3]	−	+[1]/−[2, 3]	−[1, 2]	−	−[1, 2, 4]	−	−[2, 3]	−
−[1, 2]/w[3]	−	+[1]/−[2]	−[1, 2, 3]	−	+[1]/−[2, 4]	n[1, 2]	+	−[2, 4, 4]	−	n[2, 3]	−
na	na	na	na	na	na	na	na	na	na	na	na
ng >1[1]/ <=1[3]	ng >1	0–1[1]/ng >1[2]	0–1	na	ng >1[1, 4]	0.01	0.01	ng >1; ng =3[3]	0–0.3	na	0–3
+[2]	−	−[1, 2]	+[1, 2]	na	−[2, 4]	−	−	−[2, 3]	w	−[3]	−
−	na	na	−	na	−	−	−	na	−	na	na
na	na	na	na	na	na	na	na	na	na	na	na
na	na	na	+[1, 2]	−/+ (both are in the text)	+[3]	−	−	+[4]	−	+[2, 3]	na
na	na	na	na	na	na	na	na	na	na	na	na
−	na	na	na	na	−	na	na	na	na	na	na
ND; +[2]	ND; +	ND; −[1]/+[2]	ND; +[1, 3]	ND; +	ND; +[2, 3, 4]	ND; −[1, 2]	ND; +	ND; +[1, 2, 3, 4]	na	ND; −[1]/+[2]	ND; +
na	na	na	na	na	na	na	−	+	na	+	na
−	−	−	−	−	−	−	+	−	na	−	na
−[2]	+	−[1]/+[2]	+[1, 2, 3]	+	+[2, 3, 4]	−[1, 2]	+	−[1, 3]/+[2, 4]	na	−[1, 2]/+[3]	−
na	na	na	na	na	na	na	+	−	+	na	na
−[2]	+	−[1, 2]	−	na	−[2, 4]	−	−	−[2, 3]	na	−	−
na	na	na	na	na	na	na	na	na	na	na	na
+[2]	−	−[1, 2]	−[1, 3]	−	−[2, 3, 4]	+[1, 2]	−	−[2, 3, 4]	− (acid production from)	−[2]	−
na	na	na	na	na	na	na	na	na	na	na	na
na	na	na	na	na	na	na	na	na	na	na	na
−	na	na	na	na	−	na	na	na	na	na	na
na	na	na	na	na	na	na	na	na	na	na	na
Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	na	Q-8	Q-8	Q-8

Table 35.13 (continued)

	M. aerilata 5516S-11^T	*M. albidiflava 45^T*	*M. alkalitolerans YIM 31775^T*	*M. aurea AP13^T*	*M. brevitalea byr23-80^T*	*M. consociata CCUG 58010^T*	*M. dura 16^T*	*M. flava Y9^T*	*M. haematophila CCUG 38318^T*	*M. jejuensis 5317J-18^T*	*M. lurida D5^T*
Predominant cellular fatty acids	Summed features 3 and C16:0 predominant/ C18:1 omega7c and C17:0 cyclo moderate	Summed features 3 and C16:0 predominant/ C12:0, C10:0 3-OH, and C18:1 omega7c moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C17:0 cyclo moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, C10:0 3-OH, C14:0, and C17:0 cyclo moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate
Indole production	–	–[(1, 2, 3)]	–	–[(2, 3)]	–	na	–[(1, 2)]	–[(1, 2)]	na	–	–
DNA G+C (mol %)	68.9	65.3	62.4	66.0	65.3	68.9[(3)]	65.9[(1, 4)]	68.7	na	66.1	65.9
Genome size	na	na	na	na	na	na	na	na	na	na	na
Habitat	Air	Heavy-metal-polluted farm soil	Soil	Drinking water distribution system	Lysimeter soil in association with higher plants (at Botanical Garden at the University of Bayreuth)	Blood of a 48-year-old man	Heavy-metal-polluted farm soil	Soil	Human blood of a 23-year-old man with multiple health problems in 1997	Air sample	Soil sample
Reference	Weon et al. 2008	(1) Zhang et al. 2006; (2) Weon et al. 2008; (3) Kong et al. 2013; (4) Rodríguez-Díaz et al. 2013	(1) Xu et al. 2005; (2) Weon et al. 2010; (3) Kämpfer et al. 2012	(1) Gallego et al. 2006; (2) Weon et al. 2008; (3) Kong et al. 2013; (4) Kämpfer et al. 2013	Zul et al. 2008	(1) Kämpfer et al. 2011; (2) Du et al. 2012; (3) Shen et al. 2013; (4) Kämpfer et al. 2012	(1) Zhang et al. 2006; (2) Weon et al. 2008; (3) Luo et al. 2013; (4) Rodríguez-Díaz et al. 2013	(1) Wang et al. 2012; (2) Kong et al. 2013	(1) Kämpfer et al. 2008a, b; (2) Weon et al. 2010; (3) Du et al. 2012	(1) Weon et al. 2010; (2) Shen et al. 2013	Luo et al. 2013
Biochemical properties, carbohydrate utilization, and enzymatic activities	API ID 32GN; API 20NE; API ZYM	Li et al. 2004	O'Brien and Colwell 1987; API ID32 E; API 20NE	API ID 32E; API 20NE; API ZYM	Gich and Overmann 2006; API ZYM	Kämpfer et al. 1991	Li et al. 2004	API 20NE; API ZYM	Kämpfer et al. 1991	API ID 32GN; API 20NE	API 50CH; API 20NE; API ZYM

w weak, positive (+), negative (−)

[a]general description, without specifying the strain; [b]both appear in the same work; [c]ng, means that no growth were observed at a given concentration or that the strain cannot tolerate more than specified concentration; otherwise, growth at a particular or range of concentration is showed; *na*, not available; when information is ambiguous or was gathered from many sources, references are indicated in parentheses (numbered according to the field "reference"); otherwise, information was taken from the paper describing the species; genome sizes are estimates from draft genome sequence data

M. lutea 101[T]	*M. namucuonensis 333-1-0411*[T]	*M. niabensis 5420S-26*[T]	*M. niastensis 5516S-1*[T]	*M. oculi CCUG 43427A*[T]	*M. plicata 76*[T]	*M. suwonensis 5414S-25*[T]	*M. tieshanensis TS3*[T]	*M. timonae*[T] *UR/MT95*[T]	*M. umbonata LP01*[T]	*M. varians CCUG 35299*[T]	*M. yuzhufengensis Y1243-1*[T]
Summed features 3 and C16:0 predominant/ C18:1 omega7c and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0 and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, C10:0 3-OH, and C14:0 moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate	Summed features 3 and C16:0 predominant/ C12:0, C18:1 omega7c, and C10:0 3-OH moderate
–[(1, 2)]	–	–[(1, 2)]	–	na	–[(1, 2, 4)]	–	–	–[(2, 3)]	–	na	na
63.3	66.7	67.8	66.6[(1, 2)]	na	65.1	67.8	65.9	64.61	66.0	na	65.7
na	na	na	6.59	na	na	na	na	6.04	na	na	na
Heavy-metal-polluted farm soil	Soil	Air samples	Air samples	From the eye of a patient suffering from endophthalmitis in 2000	Heavy-metal-polluted farm soil	na	Subsurface soil of a metal mine	Human blood (febrile patient with common variable immuno-deficiency)	Lab-scale microcosm packed with a mixture of soil and sewage sludge compost designed to study the evolution of microbial biodiversity through the time	Eye of a 90-year-old man in 1996	119.6 m deep ice core section
(1) Zhang et al. 2006; (2) Weon et al. 2008; (3) Rodríguez-Díaz et al. 2013	Kong et al. 2013	(1) Weon et al. 2009; (2) Kong et al. 2013	(1) Weon et al. 2009; (2) Du et al. 2012; (3) Shen et al. 2013	Kämpfer et al. 2012	(1) Zhang et al. 2006; (2) Weon et al. 2008; (3) Luo et al. 2013; (4) Kong et al. 2013	(1) Weon et al. 2010; (2) Shen et al. 2013	Du et al. 2012	(1) La Scola et al. 1998; (2) Weon et al. 2008; (3) Kämpfer et al. 2008; (4) Kong et al. 2013; (5) Kämpfer et al. 2012	Rodríguez-Díaz et al. 2013	(1) Kämpfer et al. 2008; (2) Weon et al. 2010; (3) Du et al. 2012	Shen et al. 2013
Li et al. 2004	API 20NE; API ZYM	API ID 32GN; API 20NE; API ZYM	API ID 32GN; API 20NE; API ZYM	Kämpfer et al. 1991	Li et al. 2004	API ID 32GN; API 20NE	API ID 32GN; API 20NE; API ZYM	API 20A; API 20NE	API 32E; API 50CH; API 20E; API 20NE	Kämpfer et al. 1991	API 20E; API 20NE; API ZYM

Table 35.14

Morphological, physiological, and molecular characteristics differentiating species within genus *Telluria*

	Telluria mixta ATCC 49108^T	*Telluria chitinolytica* ACM 3522^T
Morphology	Rods	Rods
Cell size (μm)	0.5–1 × 2–3	0.5–1 × 2–3
Flagellation	Mixed	Mixed
Motility	+	+
N_2 fixation	nd	nd
PHB accumulation or other charact	+	+
Detection nifD and/or nifH	nd	nd
Temperature for growth (°C):		
Range	20–45	20–46
Optimum	30–35	30–36
pH for growth:		
Range	nd	nd
Optimum	7	7
Oxidase	+	+
Catalase	+	+
Urease hydrolysis	+	–
Starch hydrolysis	+	+
Cellulose hydrolysis	–	–
Nitrate reduction	Variable	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	Poor growth at 0.5 %; no growth at 1.5 %	Poor growth at 0.5 %; no growth at 1.5 %
Utilization of:		
DL-arabinose	+	–
Lactose	+	–
Dextran	+	–
Butyrate	+	–
Benzoate	+	–
p-hydroxybenzoate	+	–
DL-serine	+	–
DL-threonine	+	–
L-arginine	+	–
L-citrulline	+	–
L-ornithine	+	–
L-phenylalanine	+	–
meso-inositol	–	–
Oxalate	–	–
Glycolate	+	–
DL-lactate	+	+
DL-glycerate	+	–
Acetate	-	–
Pyruvate	+	+
L-rhamnose	+	+
meso-erythritol	–	–
Adonitol	–	–
D-arabinose	+	–
Malate	+	+

Table 35.14 (continued)

	Telluria mixta ATCC 49108^T	*Telluria chitinolytica* ACM 3522^T
Sucrose	+	+
D-glucose	+	+
D-fructose	+	+
Mannitol	Variable	–
Glycerol	–	+
Adipate adipic acid	–	–
Azelaic acid	–	–
Sebacic acid	–	–
Phenol	Variable	–
4-chlorophenol	nd	nd
Predominant ubiquinone	Q-8	Q-8
Predominant cellular fatty acids	Summed feature 3, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}\omega7c$ (1)	nd
Indole production	nd	nd
DNA G+C (mol%)	69	72
Genome size	nd	nd
Habitat	Isolated from soils in Australia	Isolated from loamy soil in Israel

Reference: (1) Kämpfer et al. (2007)
nd not determined, positive (+), negative (–)

H. huttiense subsp. putei – isolated from well water in Japan. Cultivation and maintenance (stab) are carried out in medium B104 (IAM 1998) containing (per liter distilled water): 10.0 g polypeptone, 2.0 g yeast extract, and 1.0 g MgSO4.7H2O, pH 7.0.

H. lusitanum – isolated from young root nodules of common bean plants grown in soil in Portugal according to Vincent (1970) and using YMA medium (Bergersen 1961). Single colonies appeared after growth in YMA plates for 10 days at 28 °C. All strains were able to grow and form a pellicle in N-free JNFb semisolid medium (Döbereiner 1995). However, this characteristic was not confirmed during the genome sequencing study carried out with the *H. lusitanum* type strain P6-12 (Weiss et al. 2012).

H. rhizosphaerae – isolated from the rhizosphere soil sample of *Allium victorialis* var. *platyphyllum* using the standard dilution plating technique at 30 °C on nutrient agar (NA, beef extract 3.0 g, peptone 5.0 g, agar 15.0 g, and distilled water added to 1.0 L). Strains are routinely cultivated at 30 °C on NA. No maintenance procedure was provided.

H. rubrisubalbicans – initially, the bacteria were isolated from diseased leaves of the sugarcane variety D-74 grown in Louisiana, USA, using the methodology described for *Phytomonas rubrisubalbicans*, causing the mottled stripe disease (Christopher and Edgerton 1930), later renamed as *Pseudomonas rubrisubalbicans*. For that, the authors used small sections of stripe tissue sterilized in 1–1,000 mercuric chloride solution for 30 s, washed three times in sterile water, and then macerated in a small amount of Bacto-dextrose broth. Dilution cultures were then made with Bacto-dextrose agar and pure culture isolations obtained. Later on, during the identification of nitrogen-fixing strains among these *Pseudomonas rubrisubalbicans* strains (Pimentel et al. 1991), a new N-free semisolid medium (named JNFb) was developed (Baldani et al. 1992) and applied to isolate either *H. rubrisubalbicans* or *H. seropedicae* from different plants, including sugarcane, grown in Brazil (Baldani et al. 1996). The recipe and procedures to count, isolate, and maintain *H. rubrisubalbicans* are exactly the same as described below for *H. seropedicae*, the first species described for the genus *Herbaspirillum* (Baldani et al. 1986).

H. seropedicae – the species was initially isolated from roots of sorghum, maize, and rice plants using the semisolid NFb medium (used for isolation of *Azospirillum lipoferum* and *A. brasilense-Rhodospirillaceae*). As mentioned above, during the identification of the *H. rubrisubalbicans* species, the semisolid JNFb medium was established and becomes used in routine to count and isolate *H. seropedicae* in association with graminaceous plants. The JNFb medium contains (g L^{-1}) malic acid, 5.0; K_2HPO_4, 0.6; KH_2PO_4, 1.8; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $CaCl_2.2H_2O$, 0.02; micronutrient solution ($CuSO4.5H_2O$, 0.04; $ZnSO4.7H_2O$, 0.12; H_3BO_3, 1.40; $Na_2MoO4.2H_2O$, 1.0; $MnSO4.H_2O$, 1.175. Complete volume to 1,000 mL with distilled water), 2 mL; Bromothymol blue (5 g L^{-1} in 0.2 N KOH), 2 mL; FeEDTA (16.4 g L^{-1}), 4 mL; vitamin solution (biotin, 10 mg; pyridoxal-HCl, 20 mg; dissolve in hot water bath; and complete to 100 mL adding distilled water), 1 mL; and KOH, pH 4.5. Add distilled water to bring total solution to 1 l. Adjust the pH to 5.8 with KOH. To semisolid and solid medium, add 1.8 and 17 g agar L^{-1}, respectively. The isolation procedure is essentially as described by

Baldani et al. (1986) and consists on the inoculation of the JNFb semisolid medium (10 ml vials containing 5 ml medium) with aliquot (0.1 ml) from serial dilutions of smashed soil, root, stem, and leaf samples and incubation for 1 week at 32 °C. Fine white pellicles, very similar to *Azospirillum* spp. are observed. Cells from these white pellicles move rapidly close to air bubbles when examined under the microscope. These pellicles are transferred to fresh JNFb semisolid medium, incubated for 24–48 h, and streaked out on solid NFb medium containing yeast extract (20–50 mg/L) and 3× the Bromothymol blue concentration of the medium. The grown colonies are small, moist with blue centers which are again transferred to fresh JNFb semisolid medium and further purified on potato agar medium or BSA. The cultures are maintained at −80 °C in Dygs medium (Rodrigues Neto et al. 1986 – composition (g L-1): glucose, 2.0; malic acid 2.0; peptone, 1.5; yeast extract, 2.0; K_2HPO_4, 0.5; $MgSO_4.7H_2O$), 0.5; glutamic acid, 1.5; and complete with distilled water up to 1,000 mL, containing 10 % (v/v) glycerol. Alternative preservation is by lyophilization after growth of the culture on slant tubes containing solid Dygs medium followed by resuspension on peptone – sucrose (10 %) preservative solution.

Noviherbaspirillum

N. canariense, *N. aurantiacum*, and *Noviherbaspirillum soli* – the TSA medium (Difco) containing 1 % soil extract was used for isolation of the three species. The soil extract is prepared by suspending 10 g of the original soil in 90 ml sterile water that is maintained under shaking conditions for 2 h. Ten ml of this suspension is added to 90 ml water to which the corresponding amount of TSA powder is added. The medium is then sterilized by autoclaving at 121 °C for 20 min and dispensed into Petri dishes. Decimal dilutions of the native soil extract are obtained, and 1 ml from each dilution is spread on these plates. Individual colonies are transferred to Petri dishes containing M9-CAS agar (Alexander and Zuberer 1991) and colonies surrounded by an orange halo indicative of siderophore production were transferred to TSA plates for identification.

N. psychrotolerans – the R_2A medium [0.05 % proteose peptone, 0.05 % casamino acids, 0.05 % yeast extract, 0.05 % glucose, 0.05 % soluble starch, 0.03 % dipotassium phosphate, 0.005 % magnesium sulfate heptahydrate, 0.03 % sodium pyruvate, 1.5 % agar for solid media (w/v), Reasoner and Geldreich 1985] is inoculated with a soil suspension diluted in sterile saline solution (0.9 % NaCl) and incubated at 10 °C for 3 weeks. The isolated bacteria are cultivated at 18 °C on R_2A and nutrient medium (w/v: 0.5 % tryptone, 0.3 % meat extract, for solid media 1.5 % agar, pH 7.2).

N. malthae – the M457 mineral basal medium (Meyer et al. 1999) containing heavily crude oil is inoculated with oil-contaminated soil samples for enrichment and incubated at 30 °C under darkness. After 1 week, samples are serially diluted with sterile saline solution (0.85 % NaCl) and plated on nutrient agar (NA, Hi-Media). Individual colonies are isolated and maintained on the nutrient agar after incubating at 32 °C for 3 days. The colonies are straw-colored, circular, and smooth. The species is preserved in glycerol suspension at −80 °C.

Collimonas

C. fungivorans was originally isolated on chitin agar plates of samples of slightly acidic dune soils located on the Wadden island Terschelling as described by De Boer et al. (1998). *C. arenae* and *C. pratensis* were also isolated from chitin agar plates of soil samples of (semi-) natural grasslands and a heathland in the Netherlands as described by Höppener-Ogawa et al. (2007). The ability of bacterial colonies to produce chitinases is indicated by the formation of clear zones (halos) on the colloidal chitin-containing agar plates. *Collimonas* spp. bacteria form translucent colonies of little biomass on minimal chitin agar. All isolates are stored at −80 °C and maintained on 10-fold diluted tryptone soy broth (TSB) agar for routine culturing.

Glaciimonas

G. immobilis was isolated from alpine glacier cryonite from an alpine glacier at an altitude of 2.900 m above sea level at 10 °C on R_2A-agar plates (Zhang et al. 2011). The cryonite sample had been shaken with 9 ml sterile 1 % sodium pyrophosphate for 20 min at 150 r.p.m. to desorb the bacteria. The other member of this genus, *G. singularis*, was recovered from a water sample from Poco das Cobras in the uranium mining area of Urgeirica, Portugal, on R_2A-agar after incubation at 30 °C for 7 days. The cultures are maintained at −80 °C in nutrient broth containing 15 % (v/v) glycerol.

Janthinobacterium

J. lividum and *J. agaricidamnosum* – *Janthinobacterium lividum* grows on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at optimum temperature of 25 °C. The procedure for isolation of *J. agaricidamnosum* was described by Lincoln et al. (1999) as followed: samples of diseased mushroom tissue were transferred aseptically to sterile quarter-strength Ringer's solution, and a tenfold dilution series was inoculated onto *Pseudomonas* agar F (PAF; Merck) and nutrient agar (Oxoid) and incubated at 25 °C for 24 h. All the strains can grow on nutrient agar for 24 h at 28 °C shaken at 100 r.p.m. on an orbital shaker. There are reports indicating the need for culturing *J. agaricidamnosum* every 10 d when maintained on agar.

Undibacterium

U. pigrum – the type strain, *U. pigrum* strain CCUG 49009^T, was isolated from a drinking water in Göteborg (Sweden) on R_2A agar. It forms beige-colored colonies after incubation at 25 °C for 7 days. The bacteria can also be cultivated on tryptone soy agar (TSA), nutrient agar, and MacConkey agar (Kämpfer et al. 2007).

U. oligocarboniphilum – strain EM 1^T (CCUG 57265^T) was isolated from purified water system at Roche, Penzberg (Germany) on R_2A agar (Oxoid) plates incubated for 7 days at 30 °C. Colonies on R_2A agar were tiny, cream-colored, and translucent. Strain EM 1^T was not able to grow on tryptone soy agar and was unable to grow at 8 g.L^{-1} NaCl (Eder et al. 2011). Such sensibility to salt concentration could explain the absence of reactivity of strain EM 1 on API 20NE or API 20E strips (BioMérieux).

U. parvum – strain CCUG 49012^T was isolated from drinking water forming yellowish and translucent, shiny, and convex colonies, 0.5 mm in diameter, with a narrow and delicate lacerated rim after 3 days. The bacteria were not able to grow on TSA, PYE, and grew only slowly on nutrient agar. A typical phenotypic trait of the species was the narrow temperature range for growth (optimum at 21 °C). A new medium to cultivate the strain CCUG 49012^T was proposed by Kämpfer et al. (2007), which consisted of the carbon sources of PYE medium and the salts of R_2A (designated R2-PYE) (l^{-1}: 0.75 g peptone from casein, 0.75 g yeast extract, 0.3 g K_2HPO_4, 0.024 g$MgSO_4$, pH 7.2), in which the bacteria presented flocculent growth.

U. terreum – strain C3^T (CGMCC 1.10998^T) was isolated from permafrost soil in Beijicun (Liu et al. 2013). The isolation procedure was described by Liu et al. (2013) as followed: soil samples from surface collected at a depth of 20–30 cm (not frozen at the time of sampling). Strain C3^T was isolated from dilution after plating on R_2A agar plates and incubation at 15 °C for 1 week. Colonies on R_2A agar appear after 3 days at 25 °C. They are circular, entire, and creamy colored with 2.2 mm diameter. Modified R_2A medium was used to grow strain C3^T as follows (g.l^{-1}): 0.5 protease peptone, 0.5 calamine acids, 0.5 yeast extract, 1.0 glucose, 1.0 soluble starch, 0.5 K_2HPO_4. $3H_2O$, 0.5 KH_2PO_4, 0.3 sodium pyruvate, 0.2 $MgSO_4$. $7H_2O$, and modified R_2A agar contains 15 g agar l^{-1}. The pH was adjusted to 7.0.

Herminiimonas

H. fonticola – the type species of the genus, *H. fonticola* strains S-94T, S-97, S-99, and S-92 were isolated occasionally from a borehole used as source for a mineral water bottling plant located in the Serra da Estrela in Eastern Portugal (Fernandes et al. 2005). Briefly, the isolation procedure involves water samples filtration through membrane filters (pore size 0.2 µm) and filters placement on R_2A agar (Difco) plates followed by incubation at 22 °C for 15 days. Purification steps were performed on R_3A agar, and maintenance procedures involve storage at 70 °C in nutrient broth (Difco) with 15 % (w/v) glycerol. Fernandes et al. (2005) stated that the bacterial growth was improved in the heterotrophic medium H3P (= DSMZ medium 428).

H. aquatilis – the strain CCUG 36956T was obtained from a municipal drinking water from Uppsala (Sweden) at routinely industrial hygiene control (Kämpfer et al. 2006). Cultivation and maintenance of the bacteria are on nutrient agar (Oxoid) at 25 °C. The strain grows slowly on nutrient-rich media, like TSA (Oxoid), R_2A agar (Oxoid), and nutrient agar at 30 °C.

H. arsenicoxydans – the strain ULPAs1T was isolated by Muller et al. (2006) from activated sludge of an industrial treatment plant contaminated with heavy metals after aerobic enrichment on chemically defined medium (CDM) supplemented with 1.33 µM As[III]. The medium is prepared as follows: 100 ml solution A [81.2 mM $MgSO_4.7H_2O$ (Sigma), 187 mM NH_4Cl (99.8 % purity; Merck), 70 mM Na_2SO_4 (99 %; Prolabo), 0.574 mM K_2HPO_4 (97 %; Prolabo), 4.57 mM $CaCl_2.2H_2O$ (99.5 %; Merck), 446 mM sodium lactate (98 %; Sigma)], 2.5 ml solution B [4.8 mM $Fe_2SO_4.7H_2O$ (99 %; Prolabo)], and 10 ml solution C [950 mM $NaHCO_3$ (99.5 %; Prolabo)] were mixed and made up to 1 l with water. The final pH of the medium was about 7.2 (Weeger et al. 1999).

H. saxobsidens – the strain NS11T was isolated from the lichen–rock interface of a limestone bedrock colonized by lichen at Mugla (Turkey), from enrichment cultures with potassium oxalate as the sole source of carbon and energy by Sahin et al. (2002). The bacterium grows routinely on nutrient agar (per liter: 5 g peptone, 3 g beef extract, 15 g agar; Difco) and R_2A medium (Difco; Reasoner and Geldreich 1985) at 28 °C. Culture enrichments can be achieved with addition of 4 g potassium oxalate per liter as the sole source of carbon and energy in mineral medium designed by Aragno and Schlegel (1992).

H. glaciei – strain UMB49T was isolated from 3,042 m deep Greenland ice core by a selective filtration enrichment procedure and long-term incubations at low temperatures according to Miteva and Brenchley (2005). The isolation was performed under anaerobic liquid culture in R_2B (Reasoner and Geldreich 1985), started from a 0.2 µm filtrate of a melted ice core sample and incubated for 7 months at 2 °C. Subsequently, the culture was plated on tryptic soy agar without glucose in a presence of oxyrase additive (Oxyrase) and incubated at 5 °C for 4.5 months, where two small brown-purplish colonies appeared. After that, under aerobic recultivation on TSA and R_2A (Becton Dickinson) at 18 °C, strain UMB49T formed small, convex, translucent colonies (Loveland-Curtze et al. 2009). Resuscitated strain UMB49T formed colonies on TSA and R2A within 3–4 days at 25 °C and grown in M9 mineral salts medium (Miller 1992) with a suitable carbon source, without supplementation with vitamins or other growth factors.

H. contaminans – the strain CCUG 53591T was isolated in Sweden as a contaminant of a biopharmaceutical production process (Kämpfer et al. 2013). Ordinary cultivation is performed on tryptone soy agar (TSA; Oxoid) at 28 °C for 48 h. Peptone yeast extract (PYE) broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at 28 °C is recommended for biomass cell production.

Oxalobacter

O. formigenes – strain OxB^T was obtained from sheep rumen contents using previously described enrichment procedures (Dawson et al. 1980a, b), based on previously described methods developed by Hungate and modified as described by Bryant (1972) and Holdeman and colleagues (1977). The primary enrichment occurred in a fermentor culture established with

sheep rumen contents which was maintained by feeding of ground alfalfa. Anaerobic conditions were maintained by flushing with CO_2. Tenfold dilutions from this system were inoculated into rumen fluid-oxalate (RFO) enrichment medium containing Na_2SO_4 (0.2 g), KH_2PO_4 (0.09 g), NaCl (0.09 g), $(NH_4)_2SO_4$ (0.09 g), $MnSO_4.H_20$ (0.001 g), $CaSO_4$ $7H_20$ (0.0001 g), $MgSO_4$. $7H_20$ (0.0005 g), yeast extract (0.1 g), clarified rumen fluid (10.0 ml), resazurin (0.0001 g), L-cysteine-$HCl.H_20$ (0.025 g), Na_2CO_3 (0.4 g), and sodium oxalate (0.6 g) completed to 100 ml with distilled water. The final pH of the medium was 6.8. After enrichment in RFO medium, a mixed population of oxalate-degrading bacteria and coliform non-oxalate-degrading bacteria appeared. To further enrich the oxalate degraders, a nutrient-depleted medium was developed by the cultivation of coliform contaminants in RFO medium and the resultant coliform-depleted (CD) medium (Dawson et al. 1980a). All cultures were kept under anaerobiosis by incubating under a CO_2 gas phase. Ten and 100× dilutions of the enrichment cultures were inoculated in roll tube cultures with RFO medium containing 2 % agar in 7 ml volumes, and single colonies were isolated (Dawson et al. 1980b). Later, another culture medium, medium A (Allison et al. 1985), was developed to facilitate the isolation of other oxalate-degrading anaerobes. Manipulation of the medium occurs under a CO_2 atmosphere to guarantee anaerobic conditions. This somewhat opaque medium contains calcium oxalate on which colonies of oxalate-degrading bacteria are detected by the formation of cleared zones. Another medium, designated as "medium B" was used for studies with DNA, lipids, and antigens by Allison and colleagues (1985). Manipulation of "medium A" and "medium B" should be under a CO_2 atmosphere to guarantee anaerobic conditions (Allison et al. 1985).

O. vibrioformis – strain WoOx3^T was isolated from anoxic freshwater sediments from polluted creeks by enrichment using a carbon-buffered, sulfide-reduced mineral medium described by Widdel and Pfennig (1981) and which contained a 7-vitamin solution (Widdel and Pfennig 1981), selenite–tungstate solution, and the trace element solution SL10 (Widdel et al. 1983). The pH was between 6.8 and 7.0, and the growth temperature was 30 °C. Enrichment cultures were inoculated with 50 mL freshwater and contained 20 mM diammonium oxalate and 3 mM acetate. The headspace of the enrichment cultures was composed of an N_2/CO_2 (90 %/10 %) gas mixture (Dehning and Schink 1989). Pure cultures were obtained by repeated application of the agar shake culture technique (Pfennig and Trüper 1981).

Oxalicibacterium

O. flavum – was isolated using a mineral medium described by Aragno and Schlegel (1981). Liquid enrichment cultures with this medium were amended with 0.4 % (w/v) potassium oxalate, inoculated with samples of 1 g, and incubated under air at 28 °C on a shaker for 5 days; the enrichment procedure was repeated three times by transferring 1 mL portions to new medium. Single colonies were obtained by spreading this enrichment culture on the same medium solidified with 1.2 % agar (Tamer et al. 2002).

Duganella

D. zoogloeoides was originally isolated by P. R. Dugan from a zoogloeal matrix from wastewater bacteria and misnamed as *Zoogloea ramigera*. The bacteria form characteristic cell aggregates surrounded by gelatinous matrices – the so-called zoogloeal matrix. The strain IAM12670 was later reclassified according the chemotaxonomic markers and 16S rDNA similarity as *D. zoogloeoides* (Hiraishi et al. 1997). Colonies on nutrient agar are glistening, convex with entire margins, viscous, and pale yellow to straw-colored. *D. phyllosphaerae* was isolated on tryptone soy (TS) agar from a leaf (phyllosphere) of *Trifolium repens* collected in the Hainich-Dün region, Germany (Kämpfer et al. 2012b). On TS agar, the colonies have a yellow color, and the morphology is circular, low convex with an entire margin and a smooth appearance. *Duganella sacchari* and *Duganella radicis* were isolated from rhizosphere soil and rhizoplane of sugarcane (*Saccharum officinarum* L., clone Co86032) on selective ammonium mineral salts (AMS) medium (Whittenbury et al. 1970). The rhizosphere sample was collected 90 days after planting from an experimental sugarcane field at the Sugarcane Breeding Institute, Coimbatore, Tamil Nadu, India. The colonies on AMS/R2A agar are white, tough, dry, viscous, and convex with entire margins and a smooth appearance.

Pseudoduganella

Duganella violaceinigra was recovered on HV agar medium (Hayakawa and Nonomura 1987) from a forest soil sample in Yunnan, China (Li et al. 2004). The strain is maintained on YM (yeast extract/malt extract) agar at 4 °C and as 20 % (w/v) glycerol suspension at −20 °C. This isolate is now reclassified based on striking phenotypic differences and refined 16S rDNA similarity to P*seudoduganella violaceinigra* (Kämpfer et al. 2012b).

Massilia

M. timonae – the type strain was isolated from blood of a 25-year-old male whose diagnosis was common variable immunodeficiency and meningoencephalitis in 2000 from a hospital in Marseille, France. Other strains were isolated from femur of a 25-year-old male patient diagnosed with osteomyelitis in 1985, cerebrospinal fluid (CSF) of a 49-year-old female patient diagnosed with pseudotumor in 1996, blood of a 41-year-old male in end stage renal disease in 1997, blood of a 39-year-old female with sepsis in 1999, and surgical wound infection in an immunocompetent 36-year-old male who had undergone orthopedic surgery. Cell shape is straight rods, and, occasionally, filamentous cells are formed in old cultures and are especially formed at 37 °C; no growth is observed at 4 °C. Cell size of 1.0 µm wide and 3.0 µm long with monotrichous or

polytrichous flagellation. Grow well in MacConkey agar and tend to form flocs and films in liquid medium without fingerlike projections. Growth does not occur at 42 °C, on SS agar, or in nutrient broth with 6 % NaCl. The species is sensitive to polymyxin B. A straw-colored growth pigment is produced on HI agar (Zhang et al. 2006; Lindquist et al. 2003, 2005; Sintchenko et al. 2000; La Scola et al. 1998).

M. umbonata – includes a single isolate from a lab-scale microcosm packed with a mixture of soil collected nearby Guadalimar (Jaén, Spain) and sewage sludge compost from Granada (Spain), designed to study the evolution of microbial biodiversity through the time. The rod-shaped cell is 0.6–0.8 μm wide and 2.0–2.5 μm long harboring a single polar flagellum. They form intracytoplasmic granules when grown on ISP2 medium amended with soluble starch. Colonies grown on TSG for 3 days are yellowish pigmented and glossy, with an umbo surrounded by bosses, and round, entire edges that grow outwardly in decreasing waves (Rodríguez-Díaz et al. 2013).

M. yuzhufengensis – includes a single isolate from 119.6 m deep ice core section from Yuzhufeng Glacier, Tibetan Plateau, China. Cell shape is rodlike with 0.7–1.0 μm width and 2.3–2.7 μm length containing a polar flagellum. Colonies produced on R_2A agar after incubation at 28 °C for 2–3 days are yellow, round, smooth, convex, and opaque (Shen et al. 2013).

M. tieshanensis – includes a single isolate from subsurface soil of a metal mine located at Tienshan, China. The rod-shaped cells are 0.3–0.6 μm wide and 1.2–2.0 μm long with monotrichous and polytrichous flagella present. Colonies are circular, convex, viscous, translucent, straw yellow, and 1.0–2.0 mm in diameter with entire edges on nutrient agar plates after 36 h of incubation. Some differences are observed using R_2A medium, on which colonies are opaque, yellowish, and rough. Growth occurs on nutrient agar, R_2A- and trypticase soy agar, but does not occur on MacConkey agar.

M. varians – former [*Naxibacter*] *varians* includes many isolates. The type strain was isolated from an eye of a male patient of 90-year-old in 1996 at Tromsö (Norway). Other strains were isolated from water in Sweden and from a human blood in 1989 in Stockholm (Sweden). Cell shape is rod with 1.0 μm wide and 2.0 μm long. Beige, translucent, and shiny colonies with entire edges and with a diameter of approximately 2 mm are formed within 24 h. Good growth occurs on R_2A agar, TSA, PYE agar, nutrient agar, and MacConkey agar at 25–30 °C.

M. haematophila – former [*Naxibacter*] *haematophilus* includes one isolate from blood of a 23-year-old man with multiple health problems. Good growth occurs on R_2A agar, TSA agar, PYE agar, and nutrient agar at 25–30 °C. The cells are rod-shaped and are 1.0 μm wide and 2.0 μm long colonies formed within 24 h are beige, translucent, and shiny with entire edges and a diameter of approximately 2 mm. Moderate amounts of an unknown aminolipid and an unknown aminophospholipid and minor trace amounts of one unknown aminolipid and three unknown phospholipids are present.

M. aerilata – includes one isolate from an air sample collected at Suwon, Republic of Korea. The cell shape is rodlike with 0.7–1.0 μm width and 1.5–3.0 μm lengths containing a single polar flagellum. Colonies are light yellow, round, and convex with clear margins. Grows well on R_2A agar, nutrient agar, and trypticase soy agar, but does not grow on MacConkey agar (Weon et al. 2008).

M. albidiflava – includes one isolate from heavy-metal-polluted farm soil in Nanjing, Jiangsu province, China. The strain was isolated by means of dilution plating method using yeast extract/malt extract agar medium (ISP 2 medium – 4.0 % yeast extract, 10.0 % malt extract, 4.0 % glucose, 2.0 % agar). After incubation at 28 °C on nutrient agar plates for 2 weeks, the colonies are 1.0–1.5 mm in diameter, circular, entire, convex, desiccated, opaque, and pale white to yellow on nutrient agar plates. The cells are short rods with 1.8–2.0 μm width and 3.0–3.5 μm length having peritrichous flagella. It cannot tolerate NaCl concentration higher than 1 % (Zhang et al. 2006).

M. plicata – includes one isolate from heavy-metal-polluted farm soil in Nanjing, Jiangsu province, China. The strain was isolated by means of dilution plating method using ISP 2 medium; incubation was at 28 °C for 2 weeks. Colonies on nutrient agar plates are 2.0–3.0 mm in diameter, circular, entire, convex, viscous, opaque, and yellow to pale brown. Cell shape is straight rod with 0.6–0.7 μm width and 1.8–2.5 μm length and contains one or more flagella. Soluble pigment is produced on ISP2 medium and some other tested media. It cannot tolerate > 1 % NaCl (Zhang et al. 2006).

M. dura – includes one isolate from heavy-metal-polluted farm soil in Nanjing, Jiangsu province, China. The strain was isolated by means of dilution plating method using ISP 2 medium; incubation was at 28 °C for 2 weeks. Colonies on nutrient agar plates are 0.9–1.2 mm in diameter, circular, entire, convex, opaque, hard, compact, and pale white to yellow. Cells shape is straight rod with 0.6–0.8 μm width and 1.8–2.5 μm length and one or more flagella. It cannot tolerate > 1 % NaCl (Zhang et al. 2006).

M. lutea – includes one isolate from heavy-metal-polluted farm soil in Nanjing, Jiangsu province, China. The strain was isolated by means of dilution plating method using ISP2 medium; incubation was at 28 °C for 2 weeks. Colonies are 2.0–3.0 mm in diameter, circular, entire, convex, viscous, opaque, and yellow on nutrient agar plates. Cell shape is short rods which are 1.8–2.0 μm wide and 3.0–3.5 μm long and shows peritrichous flagella. It cannot tolerate > 1 % NaCl (Zhang et al. 2006).

M. alkalitolerans – former [*Naxibacter*] *alkalitolerans* – includes one isolate from soil, collected at Lijiang, Yunnan Province, China, after incubation for 2 weeks at 28 °C on water proline agar (1 % proline/tap water). Colonies developing on nutrient agar plates are 1.1–1.4 mm in diameter, circular, entire, convex, glistening, butyraceous, opaque, and with pale white-yellow to straw color. The cell shape is rodlike with 0.45–0.8 μm width and 1.35–2.0 μm length and one or more flagella. The bacteria cannot grow in the presence of 3 % sodium chloride. The type strain, YIM 3175^T, is a soil bacterium with no apparent pathogenic or symbiotic relationship with mushrooms; it contains genes encoding both type I and type II polyketide biosynthesis pathways (Xu et al. 2005).

M. aurea – includes one isolate from drinking water distribution system of Seville, Spain. Samples (25 l) of drinking

water were concentrated by using a tangential flow filtration system, plated on plate count agar, and incubated at 28 °C for 7 days. Colonies on PCA agar plates after 2 days incubation are circular, translucent, yellow-pigmented, and 0.6–1.0 mm in diameter. Good growth occurs on TSA, R_2A, and nutrient agar medium. No growth occurs on MacConkey agar. Cells have a tendency to form pellicles on the surface of static liquid cultures, and they do not grow in the presence of 2 % NaCl. Cell shape is straight rod with 1.0 µm width and 1.6–3.0 µm length and occurs singly or in pairs on PCA medium at 28 °C after 48 h.

M. brevitalea – includes one isolate from lysimeter soil in association with higher plants from the Botanical Garden at the University of Bayreuth, Germany. The high-throughput MicroDrop technique was employed to inoculate bacterial cells into microtiter plates containing soil solution equivalent, buffered at pH of 7.0, and supplemented with artificial root exudates, yeast extract (0.01 %, w/v), and inducers. Cultures were incubated at 15 °C for 6 weeks. Bacterial strains were isolated by streaking liquid cultures onto the medium described above, solidified with gellan gum (8 g l^{-1}; Sigma-Aldrich Chemie). Colonies are circular, entire, convex, opaque, and pale white to yellow. Cell shape is short rod with 0.7–1.0 µm width and 1.5–2.0 µm length harboring a single polar flagellum. Occasionally, cells with two or three polar or lateral flagella are observed. Cells occur singly or in pairs (on 1:10-diluted HD medium at 15 °C after 36 h). A striking feature of the cells was the presence of numerous protuberances of various sizes, formed by the outer membrane, over the entire cell surface.

M. consociata – includes one isolate from blood of a 48-year-old man at Göteborg, Sweden. Beige, translucent, and shiny colonies with entire edges are formed within 24 h, with a diameter of approximately 2 mm. Cell shape is rod with 1.0 µm width and 2.0 µm length. Good growth occurs on R_2A agar, TSA, PYE agar, nutrient agar, and MacConkey agar at 25–30 °C. Growth occurs on TSA at temperature ranging from 15 °C to 37 °C and at pH values between 5.5 and 10.5. No growth occurs below 10 °C and above 45 °C. Moderate amounts of three unknown phospholipids and of one unknown aminolipid are present (Kämpfer et al. 2011).

M. flava – includes one isolate from a soil sample collected from Ningxia Province, China. For isolation, 1 g of soil was suspended in 100 ml of distilled water and spread on plates of ISP2 (containing 4 g yeast extract, 10 g malt extract, 4 g glucose, and 20 g agar; pH 7.5) medium after serial dilution. Plates were incubated at 28 °C for 3 days. Colonies are circular to irregular, convex, yellow, and 1–3 mm in diameter after 3 days cultivation at 28 °C on ISP2 medium. Cells are rods with 0.6–0.8 µm width and 1.5–2.5 µm length (Wang et al. 2012).

M. jejuensis – includes one isolate from an air sample from the Suwon region, Republic of Korea. The air samples were collected by using a MAS-100 air sampler that contained Petri dishes with R_2A medium (BBL) supplemented with cycloheximide at 200 mg/ml. Colonies are light orange, round, and convex with clear margins. Cells are rods with 1.1–1.5 µm width and 1.3–3.0 µm length and a single polar flagellum. Grow on R_2A and nutrient agar, but not on tryptic soy agar or MacConkey agar (Weon et al. 2010).

M. suwonensis – former [*Naxibacter*] *suwonensis* – includes one isolate from an air sample from the Suwon region, Republic of Korea. The air samples were collected by using a MAS-100 air sampler that contained Petri dishes with R_2A medium (BBL) supplemented with cycloheximide at 200 mg/ml. Colonies are ivory-colored, round, and convex with clear margins. Cells are rod-shaped with 0.6–0.8 µm width and 1.2–3.0 µm length and have a single flagellum (Weon et al. 2010).

M. lurida – includes one isolate from a soil sample collected from an experimental sunflower field in Wuyuan County, Inner Mongolia Autonomous Region, China. The soil sample (1.0 g) was suspended in 100 ml of sterilized water, and diluted solutions were spread on ISP2 medium at 28 °C and incubated for 3 days. Colonies are circular to irregular, convex, yellow, and 1–3 mm in diameter after 72 h cultivation at 28 °C on ISP2 medium. Cells shape is rodlike which are 0.6–0.9 µm wide and 1.4–2.8 µm long; cells are motile by means of flagella (Luo et al. 2013).

M. namucuonensis – includes one isolate from a soil sample collected from Namucuo in Tibet Autonomous Region, China. Soil samples were collected from sites located 25 m from the Namucuo Saltwater Lake (30° 46′ 27″ N, 90° 52′ 00″ E and 4,598 m in altitude). For isolation, 1 g soil sample was suspended in 50 ml distilled water and spread on plates of R_2A medium after serial dilution. After 3 days cultivation at 28 °C on R_2A-agar, colonies are circular to irregular, convex, opaque, and white to yellow in color having a diameter of 1.0–3.0 mm. No growth was observed on TSA and MacConkey agar. Cells are rod-shaped and 0.3–0.5 µm wide and 0.5–1.5 µm long.

M. niabensis – includes one isolate from an air sample collected from Suwon, Republic of Korea. Air samples were collected in the Suwon region of Korea (37° 16′ 46″ N 126° 59′ 10″ E) by using a MAS-100 air sampler that contained Petri dishes with R2A medium supplemented with cycloheximide at 200 µg/ml. Bacteria were isolated after incubation at 28 °C for 5 days. Colonies are yellowish white, round, and convex with clear margins. Grows well on R_2A and nutrient agar, but does not grow on trypticase soy agar or MacConkey agar. Cell shape is rodlike with 0.6–0.9 µm width and 1.4–4.0 µm length; cells are motile by means of a single polar flagellum (Weon et al. 2009).

M. niastensis – includes one isolate from an air sample collected from Suwon, Republic of Korea. Air samples were collected in the Suwon region of Korea (37° 16′ 46″ N 126° 59′ 10″ E) by using a MAS-100 air sampler that contained Petri dishes with R_2A medium supplemented with cycloheximide at 200 µg/ml. Bacteria were isolated after incubation at 28 °C for 5 days. Colonies are ivory-colored, round, and convex with clear margins. Grows well on R_2A and nutrient agar, but does not grow on trypticase soy agar or MacConkey agar. The cells are rods with a width of 0.6–0.8 µm and a length of 1.5–5.0 µm. They are motile by means of single polar flagella (Weon et al. 2009).

M. oculi – includes one isolate from the eye of a patient suffering from endophthalmitis at Linköping, Sweden. Within 24 h of incubation, colonies have a diameter of approximately 2 mm and are beige, translucent, and shiny. Cell shape is rodlike with 1.0 μm width and 2.0 μm length. Good growth occurs on R_2A agar, TSA, peptone yeast extract agar, nutrient agar, and MacConkey agar at 25–30 °C (Kämpfer et al. 2012a).

Telluria

T. mixta – former [*Pseudomonas*] *mixta* – the type species of the genus *Telluria* was isolated from the rhizosphere of sugarcane plants in Australia (Bowman et al. 1988), via enrichment using the method described by Blackall et al. (1985). The isolates formed semiopaque halos on dextran-containing mineral media, indicative of dextran hydrolysis.

T. *chitinolytica* – former *Pseudomonas chitinolytica* – the type species (strain ACM 3522^T) was isolated from a natural sandy loam soil from Bet Dagan in Israel, which was mixed with 1 % (wt/wt) crustacean shells (rich in chitin) and incubated up to 45 days in a glass house. Aliquots of this soil were suspended in sterile water, and this water was diluted and spread on agar plates containing 0.2 % (wt/vol) colloidal chitin as the sole carbon source and minerals. Colonies producing a cleared zone were isolated (Bowman et al. 1993).

Ecology

Habitat

The species belonging to the family *Oxalobacteraceae* present a very wide range of habitats. For example, members of the genus *Herbaspirillum* were first described as endophytic bacteria colonizing plant tissues, but more recently has been reported in a broad range of niches, such as contaminated soil, water, antarctic soil, and clinical samples. Species from the other genera such as *Massilia* have soil and water (rivers and lakes) as common habitats, although isolations from the phyllosphere and rhizosphere of plants and from clinical specimen have also been reported. Anaerobic environments are common habitats for species of the genus *Oxalobacter*, while members of other genera, e.g., feed on living fungal hyphae (*Collimonas*) or colonize environments with extremely low levels of available carbon, such as the alpine glacier cryoconite or groundwater.

Herbaspirillum: The genus *Herbaspirillum* was created in 1986 and included exclusively nitrogen-fixing strains belonging to *H. seropedicae* species isolated from *Poaceae* plants (Baldani et al. 1986). These characteristics were confirmed for the *H. rubrisubalbicans* and *H. frisingense* species but not for the other *Herbaspirillum* species despite an original report indicating the nitrogen fixation ability for *H. lusitanum*, bacteria isolated from nodules of common bean plants (Valverde et al. 2003). With the exception of *H. hiltneri* and *H. rhizosphaerae* that were also found associated with plants, the other species (*H. aquaticum*, *H. autotrophicum*, *H. huttiense subsp. putei*, and subsp. huttiense) were detected in water samples. On the other hand, H. *chlorophenolicum*, previously classified as *Comamonas testosteroni*, was the only species isolated from soil sediment. The ecological distribution of *H. seropedicae* has been more deeply investigated, and besides the colonizing roots of maize, sorghum, and rice plants grown in Seropédica, RJ, Brazil, it was later detected associated with poaceae plants grown in other regions of Brazil: roots, stems, and leaves of sugarcane varieties (Reis Junior et al. 2000), roots and stems of rice (Rodrigues et al. 2006), roots of forage grasses (Brasil et al. 2005), roots of wheat (Sala et al. 2005), and roots of sorghum (Bergamaschi et al. 2007). More recently, the species was detected colonizing rice plants grown in China (Zhu et al. 2012). The species are also associated with dicotyledonous plants such as pineapple and banana cropped in Brazil (Weber et al. 1999; Cruz et al. 2001). In addition, *Herbaspirillum* spp. were isolated from the endosphere of *Populus deltoids* grown along the Caney Fork River in central Tennessee and at the Yadkin River in North Carolina, USA (Brown et al. 2012). The species *H. rubrisubalbicans*, originally isolated as mild-pathogenic bacteria from leaves of sensitive sugarcane varieties grown in different countries (the USA, Mauritius, Jamaica, etc.), was later found colonizing resistant sugarcane (roots and stems) and weed (roots) plants grown in Brazil (Baldani et al. 1992) and sugarcane variety NIF-8 grown in Japan (Asis et al. 2000). More recently, it was isolated from a sensitive sugarcane variety grown in China (Tan et al. 2010). *H. frisingense* was found colonizing the C4-energy plants *Miscanthus sinensis* and *Pennisetum purpureum* grown in Germany and Brazil (Kirchhof et al. 2001). *H. lusitanum* was found cohabiting young nodules of common bean plants (*Phaseolus vulgaris*) grown in the northeast of Portugal (Valverde et al. 2003), while the species *H. hiltneri* was associated with wheat plants grown in Germany (Rothballer et al. 2006). *H. rhizosphaerae* was detected in the rhizosphere of *Allium victorialis* var. *platyphyllum* plants grown in South Korea (Jung et al. 2007). The species H. *aquaticum*, H. *autotrophicum*, *H. huttiense* subsp. *putei*, and subsp. *huttiense* were isolated from water samples collected in the USA, Switzerland, New Zealand, and Japan, respectively. As mentioned before, *H. chlorophenolicum* was isolated from soil sediment collected in a stream near the industrial region of Cheongju, Korea (Im et al. 2004). Other unclassified *Herbaspirillum* species have been isolated from rhizosphere soil of the reed *Phragmites australis* cultivated on reclaimed land (South Korea) (Lee et al. 2012) and wild and traditional rice plants grown in Japan (Elbeltagy et al. 2000).

An interesting characteristic related to the nitrogen-fixing *Herbaspirillum* spp. associated with plants is the ability of the bacteria to colonize plant tissues endophytically (Döbereiner 1992). Many studies have shown the endophytic colonization of the xylem of sugarcane roots by *H. seropedicae* and *H. rubrisubalbicans* strains (Olivares et al. 1997; James and Olivares 1998) as well as the intercellular spaces of root and stem tissues of two Japanese micropropagated sugarcane varieties (Njoloma et al. 2006). Infection and colonization of the rice root intercellular spaces were also demonstrated by

James et al. (2002). The colonization process seems to involve bacterial envelope structures such as LPS, EPS, adhesins, and the T3SS secretion system (Monteiro et al. 2012). Even for *H. rubrisubalbicans*, no pathogenic symptoms are observed during the colonization process of commercial-resistant sugarcane varieties cultivated in Brazil (Olivares et al. 1997). Similarly, the *H. frisingense* species are localized in intercellular spaces of the root cortex and the root vascular tissues of *Miscanthus sinensis* as well as in the central cylinder and xylem vessels of barley root tissues (Rothballer et al. 2008). Endophytic colonization of common bean plants has also been observed after inoculation with *H. seropedicae*, but no pathogenic symptoms or nodule formation was observed (Schmidt et al. 2011). The low number of strains available for most of the described *Herbaspirillum* species suggests that, with the exception of the nitrogen-fixing species, the other species are limited to their specific environmental local of isolation. The use of culture-independent method to search for the presence of *Herbaspirillum* associated with sugarcane and *Pennisetum* cultivated in Seropédica, Brazil, did not detect the presence of *Herbaspirillum* species (Fischer et al. 2012; Videira et al. 2013). On the other hand, assessment of the diazotrophic bacterial community within the rhizosphere, roots, and stems of maize grown in the South Brazil region showed the presence of bacteria from the genus *Herbaspirillum* dominating the interior of the plants but not the soil (Roesch et al. 2008). It is already known that *H. seropedicae* does not survive in soils and requires the seed germination for recovering the bacteria that may stay in viable but not culturable conditions in soil (Olivares et al. 1996).

Noviherbaspirillum: The genus *Noviherbaspirillum* was created quite recently (Lin et al. 2013) and includes species isolated from very specific niches. The three species *N. aurantiacum*, *N. canariense*, *and N. soli* occurred in an old volcanic mountain soil in northeast of Tenerife, Canary Islands (Carro et al. 2012). The vegetation at this site is dominated by herbaceous nitrophilous communities with species such as *Galactites tomentosa*, *Calendula arvensis*, *Oxalis pes-caprae*, *Sinapis arvensis*, and *Brachypodium distachyon* and also plantations of *Eucalyptus globulus*. The species *N. psychrotolerans* was detected in a soil sample of the Larsemann Hills, East Antarctica (69° 24′ S 76° 20′E) (Bajerski et al. 2013), while the type species of the genus, *N. malthae*, occurred in oil-contaminated soils in the vicinity of a refinery located at Kaohsiung city, Taiwan (Lin et al. 2013). No additional occurrence study has been reported up to date.

Collimonas and ***Glaciimonas***: *Collimonas* strains have the remarkable ability to grow on living fungal hyphae based on their chitinolytic activity (De Boer et al. 2001, 2004). This mycophagy was demonstrated in a gnotobiotic sand system, where bacterial numbers increased after invasion of common soil fungi like *Mucor hiemalis* and *Chaetomium globosum* (De Boer et al. 2001). It was shown that mycophagy also occurred under natural carbohydrate poor conditions in soil (Höppener-Ogawa et al. 2009). *Glaciimonas* seems also to specialize on very nutrient poor environments because its members colonize obviously also nutrient poor environments such as the alpine glacier cryoconite or from wastewater samples from a uranium mine (Zhang et al. 2011; Chung et al. 2013).

Janthinobacterium: The type species, *Janthinobacterium lividum*, have been predominantly isolated in temperate regions from soils and water bodies such as rivers, lakes, and springs (Johnson et al. 1990). There are reports for isolations associated with spoilage of pasteurized milk (Eneroth et al. 2000), and *J. lividum* syn. *P. mephitica* was isolated from butter (Claydon and Hammer 1939). *Janthinobacterium agaricidamnosum*, the causal organism of soft rot disease of *Agaricus bisporus*, was isolated on a farm in southern England. Lincoln et al. (1999) showed that the disease is most active under very moist conditions and thus may have been introduced to mushroom farms through the water supply or casing peat as *J. lividum* have been found in soil and water.

Undibacterium: The predominant habitat of the *Undibacterium* genus is the water and soil. The genus contains six validly published species, originally isolated from drinking water in Göteborg (Sweden), from purified water system at Roche, Penzberg (Germany), from a permafrost soil sample (China) and two new reports from soil and freshwater from South Korea (Kämpfer et al. 2007; Eder et al. 2011; Liu et al. 2013; Kim et al. 2014). Most of the species of *Undibacterium* are sensitive to elevated salt concentrations. This is the case for *Undibacterium oligocarboniphilum* strain EM 1^T isolated from pure water that is adapted to low NaCl concentration. Its notable predominance of environmental clones closely related to the 16S rRNA genes of *Undibacterium* was retrieved from aquatic environments in different studies – for example, in an ecological survey of a bacterial population recovered from arsenic-contaminated groundwater of North East India (Assam) reported by Ghosh and Sar (2013) as *Undibacterium* sp. VA1-17 16S ribosomal RNA gene and *Undibacterium* sp. A1-2 16S ribosomal RNA gene (both partial sequences). Other studies about the bacterial diversity in groundwaters and bottled natural mineral waters (*Undibacterium* sp. B6.09-36 16S rRNA gene, complete sequence), in subglacial Himalayan lakes (*Undibacterium* sp. NBGD29 16S ribosomal RNA gene, partial sequence), in the water of Powai Lake (uncultured *Undibacterium* sp. isolate DGGE gel band PW-14 16S ribosomal RNA gene, partial sequence), in biofilms formed on glass surfaces exposed to continuously flowing drinking water, and in bottled mineral water (*Undibacterium pigrum* strain A2RO6 16S ribosomal RNA gene, partial sequence), all demonstrate sequences resembling *Undibacterium* spp. Other habitats include cultivable isolates from soils, deep argillite geological formation (uncultured *Undibacterium* sp. clone 19186_CC1M241_B03 16S ribosomal RNA gene, partial sequence), excavation areas in Korea (*Undibacterium* sp. CMFN9 16S ribosomal RNA gene, partial sequence), and plant rhizosphere and roots of *Stellera chamaejasme* L. (uncultured *Undibacterium* sp. clone OTUrs17 16S ribosomal RNA gene, partial sequence) (Liu et al. 2013; Ghosh and Sar 2013; Sahay et al. 2013; Kim et al. 2014).

Herminiimonas: The predominant habitat of the quite recent described *Herminiimonas* genus (Fernandes et al. 2005) seems to be water bodies. The genus contains at present six validly published species. Members of these species were

originally isolated from bottled mineral water, drinking water (Sweden), arsenic-contaminated industrial wastewater sludge (East Germany), high depth Greenland ice (Antarctica), contaminant of a biopharmaceutical production process (Sweden and Japan), and from lichen-colonized rock (Fernandes et al. 2005; Kämpfer et al. 2006, 2013; Muller et al. 2006; Lang et al. 2007; Loveland-Curtze et al. 2009). More recently, *H. contaminans* (Kämpfer et al. 2013) was isolated from urban soil in Japan (Iizuka et al. 1998); this means that it can be isolated from environments other than water bodies. Reasonable evidences for a wide ecological occurrence of *Herminiimonas* would come from environmental clones obtained using culturable and non-culturable microbiological methods. Presumably, new habitats colonized by phylogenetically related *Herminiimonas* sp. could be detected through environmental sequences with 16S rRNA gene sequence similarities to cultured *Herminiimonas* spp. A 16S rRNA gene sequence closely related to *Herminiimonas* sequences was reported as uncultured *Herminiimonas* sp. (clone NJGS-34) and obtained from a Tibetan ice core. *Herminiimonas* sp. (clone ATA91) retrieved from postglacial soils of Ecology Glacier, King George Island, Antarctica, and *Herminiimonas* sp. (SP-B) from permanently cold arctic fjord sediments, resulted from studies conducted by Xiong et al. (2012), Zdanowski et al. (2013), and Canion et al. (2013), respectively. These psychrophilic sites resemble in part the habitat for the original description of *H. glaciei* that by a very elegant recovery, isolation procedure was resuscitated from deep Greenland ice core with an estimated age of 120,000 year (Loveland-Curtze et al. 2009). The authors highlighted that the conditions at this environment may be similar to other planets and open up opportunities to understand key life-forms outside the earth. Interestingly, the bacterium is 10–50 times smaller than *Escherichia coli*, and its size would help more efficient nutrient exchange and protection from predators and survival in the liquid veins that exist between crystals of ice in over 3 km deep. Such extremely cold environments, like in the depth of the Greenland ice, best analogies may exist for certain extraterrestrial habitats. Besides the aquatic habitat, rocks, soils, and sediments and its interfaces seem to be proper habitats to *Herminiimonas* related species. In ecological studies with bacterial communities in soils and the associated Fe–Mn nodules, an uncultured *Herminiimonas* sp. (clone SDn1-56) was retrieved. A combination of water–soil dynamics under psychrophilic to mesophilic conditions that cover the most predominant habitats for the genus was investigated in a study related to diversity of culturable bacteria from freshly deglaciated granite sand in the Central Alps, from where *Herminiimonas* sp. related clones were recovered. *H. arsenicoxydans* closely related clones (acr 26, 27, and 28) represent part of the microbial community of heterotrophic bacteria on marble surfaces of Acropolis monuments. *H. arsenicoxydans* is a heterotrophic bacterium capable of reducing and oxidizing arsenic with the objective of detoxification. Arsenic is both a product from natural sources and of human activities and is widely distributed in the environment (Kämpfer et al. 2006). The association of *H. saxobsidens* in the lichen–rock interface zone is remarkable, where bacteria biomineralization activities are promoted by oxalic acid produced by the lichen communities, which accelerate weathering of the rock by solubilizing the cement between the rock grains (Johnston and Vestal 1993). A clone closely related to uncultured *Herminiimonas* sp. (8,204) have been retrieved from mice skin under laboratory conditions (Chehoud et al. 2013), being part of the microbial ecosystem of the skin that is colonized by a diversity of microbiota. Sequences closely related to *H. saxobsidens* have been recovered from (AV109) human oral microbiome (Dewhirst et al. 2010) demonstrating possible association with human being.

Oxalobacter: *Oxalobacter* spp. occur in anaerobic environments, such as the rumen of man and mammals and in freshwater sediments (Allison et al. 1985; Dehning and Schink 1989), where they play a role in the degradation of oxalate, which readily occurs in such environments (Morris and Garcia-Rivera 1955; Allison et al. 1977, 2005).

Oxalicibacterium: Species from the genus *Oxalicibacterium* are aerobic oxalate degraders that have been isolated from soil (*O. horti* and *O. solurbis*), from oxalate-rich plant litter in Turkey (*O. flavum*) and from chicken dung (*O. faecigallinarum*) (Tamer et al. 2002; Sahin et al. 2009b, 2010b). Apart from their description, little information is available on the ecology of these bacteria.

Duganella and ***Pseudoduganella***: These bacteria thrive in quite diverse ecological niches, in wastewater sewage systems, and soils, but also in plant habitats, like roots or leaves (Aranda et al. 2011) and leaves (Kämpfer et al. 2012b). Some members of the genus *Duganella* are characterized by their extracellular polysaccharide production (Hiraishi et al. 1997). *D. sacchari* and *D. radicis* isolates from the rhizosphere/rhizoplane of sugarcane are claimed to have plant growth-promoting abilities (Madhaiyan et al. 2013).

Massilia: *Massilia*, as well as other members of *Oxalobacteraceae*, has been described in a broad range of niches in recent years (Ofek et al. 2012). It was initially characterized as clinical isolates, but subsequently isolated and detected in many environments, such as air, aerosols, dust, freshwater, soil, phyllosphere, and more recently as an important component of the rhizosphere (Ofek et al. 2012). This ability to explore different niches seems to be a characteristic for the family *Oxalobacteraceae* with more and more genera and species being isolated and characterized in diverse ecological niches. Recently, the number of bacterial species and genera classified as root-associated bacteria increases due to technical advances enabling comprehensive cultivation-independent studies. *Massilia* is such a group of organisms, and it was detected inside the roots and in the rhizosphere of many plant species as one of major groups, and it seems to play an important role (Ofek et al. 2012). In another study, *Duganella* and *Massilia*, among other groups, were found at higher frequencies associated to the rhizosphere of diseased wheat plants affected by *Rhizoctonia* bare patch, related to non-affected plants (Yin et al. 2013). The first evidence of a plant-associated *Massilia* sp. was in stems of soybean or of sweet pepper (Grönemeyer et al. 2012; Ikeda et al. 2009; Rasche et al. 2006). Many other studies have reported plant-associated *Massilia* sp. with increasing importance of the

Table 35.15
Rhizosphere-associated *Massilia*

Niche	Plant	Observation	Reference
Stem	Soybean (*Glycine max*)	Bacterial cell enrichment method to minimize interference of plant DNA	Ikeda et al. 2009
Stem	Sweet pepper (*Capsicum annuum*)	Climate chamber experiment testing the effect of low temperature on bacteria diversity	Rasche et al. 2006
Rhizosphere	Sugarcane (*Saccharum* sp.)	Crop field	Pisa et al. 2011
Rhizosphere	Wheat (*Triticum aestivum*)	*Rhizoctonia solani* bare patch diseased plants	Yin et al. 2013
Rhizosphere	*Alyssum murale*	Metal hyperaccumulator plants	Abou-Shanab et al. 2003, 2007
Rhizosphere	*Alyssum murale*	Ni-resistant bacteria in metal hyperaccumulator plants	Abou-Shanab et al. 2010
Rhizosphere	*Sonchus asper*	Germinated and raised from seeds under ozone exposure (65 days)	Dohrmann and Tebbe 2005
Rhizosphere	Willows (*Salix viminalis*)		Hrynkiewicz et al. 2010
Rhizosphere	Potato (*Ipomoea batatas*)	Crop field	Weinert et al. 2010
Surface-sterilized leaves/green branch segments	*Salix caprea*	Heavy-metal-accumulating plant	Kuffner et al. 2010
Surface-sterilized roots	Maize (*Zea mays*)		Grönemeyer et al. 2012
Root	Cucumber (*Cucumis sativus*)	Sown in three peat-based potting mixes amended with composts	Green et al. 2007

rhizosphere environment (Table 35.15). The successful recovery of *Massilia* isolates from soil under hyphae-exclusion bags (i.e., bags of 0.5 μm mesh which excludes hyphae of ectomycorrhizal fungi incubated in a forest soil) is also worth to mention (Brooks et al. 2011). Strain Y1243-1^T of *M. yuzhufengensis* is able to grow at 2 °C, whereas other species of this genus cannot and had a relatively higher proportion of $C_{18:0}$ (6.95 %) compared with recognized species of the genus *Massilia* (Shen et al. 2013).

Telluria: *Telluria mixta* and *T. chitinolytica* were isolated from soil in Australia and Israel, respectively. *T. chitinolytica* demonstrated to have nematicidal activity and potential for the control of the root-knot nematode *Meloidogyne javanica* (Spiegel et al. 1991). *T. chitinolytica* is able to degrade chitin, but not dextran or pectate that is performed by *T. mixta* (Sly and Fegan 2005).

Pathogenicity, Clinical Relevance

Analysis of the species belonging to the family *Oxalobacteraceae* indicates the presence of mild phytopathogenic species and opportunistic human pathogenic species/strains. Despite the large spectrum of antibiotic resistance among the species, the majority of them are not pathogenic to plants or human. No information is available so far for the genera *Collimonas*, *Glaciimonas*, *Duganella/Pseudoduganella*, *Herminiimonas*, and *Undibacterium*.

Plant-Related Pathogenicity

Herbaspirillum: The reclassification of few strains of *[Pseudomonas] rubrisubalbicans*, causative agent of the mottled strip disease in some older susceptible sugarcane varieties, to *Herbaspirillum rubrisubalbicans* (Gillis et al. 1990; Baldani et al. 1996) raised the question about the phytopathogenicity of *Herbaspirillum* strains colonizing plants. Olivares et al. (1997) showed that neither *H. rubrisubalbicans* nor *H. seropedicae* strains produced any characteristic symptoms when artificially inoculated into leaves of commercial-resistant varieties of sugarcane grown in Brazil. However, these species caused a red stripe disease in *Pennisetum* and *Sorghum bicolor* although the symptoms were very mild in *Sorghum* leaves inoculated artificially (Pimentel et al. 1991). It was also shown that a particular *H. rubrisubalbicans* strain caused a mottled stripe disease in a sugarcane cv Taiwan cultivated in China (Tan et al. 2010). More recently, it was claimed that the *H. seropedicae* strains Os45 and Os34, isolated from roots of the rice cultivar C2 grown in China, are pathogenic since they inhibited growth of rice seedlings

and induced a hypersensitive response in tobacco leaves (Zhu et al. 2012; Ye et al. 2012). On the other hand, no phytopathogenic symptoms have been observed for endophytic *H. frisingense* strains inoculated into barley and *Miscanthus* plants (Rothballer et al. 2008). There are also no reports showing the pathogenicity caused by the other *Herbaspirillum* species in plants.

Janthinobacterium: *Janthinobacterium agaricidamnosum* was reported to be pathogenic to mushroom crops, especially brown Agaricus strains. Reduction of relative humidity in crop house below 85 % can reduce the disease progress.

Telluria: *Telluria chitinolytica* demonstrated nematicidal activity and potential for control of the root-knot nematode *Meloidogyne javanica* (Spiegel et al. 1991).

Clinical Relevance

Herbaspirillum: Few reports associating the presence of *Herbaspirillum* species with opportunistic pathogenicity in human comes mainly from patients with cystic fibrosis, published in the last decade. For example, two *Herbaspirillum* strains (AU2210A and AU2339) showing high 16S rRNA sequence similarity to *Herbaspirillum huttiense* (99.5 %) and *H. frisingense* (99.6 %), respectively were identified among 51 bacterial isolates recovered from respiratory secretions of cystic fibrosis patients (Coenye et al. 2002). A report suggested that a bacteremia and cellulitis detected in a homeless man patient with cirrhosis was due to *H. seropedicae* (Tan and Oehler 2005). Another study involving the 16S rRNA sequence analysis of 28 bacteria isolated from persons with cystic fibrosis indicated that all strains belonged to the *Herbaspirillum* genus (Spilker et al. 2008). Three strains were identified (>99.5 % identity) as *H. huttiense subsp. huttiense*, three as *H. frisingense*, two as *H. seropedicae* and two as *H. huttiense subsp. putei*. The rest of the strains could not be assigned to any described species, but 14 of these strains shared 99 % similarity to *H. huttiense* subsp. *huttiense* and subsp. *putei*, while the other strains may constitute novel *Herbaspirillum* species. Despite the recovery of *Herbaspirillum* species from persons with cystic fibrosis, the authors argued that the frequency of occurrence was low, since *Herbaspirillum* sp. was recovered from only 3 % of all patients analyzed (1,100); it was speculated that isolation of *Herbaspirillum* sp. may be influenced by other species present in the samples which might overgrow *Herbaspirillum* during the isolation procedure. According to Morovic et al. (2010), the majority of the reported patients presenting chronic respiratory infection and bacteremia had only transient respiratory colonization by *Herbaspirillum*.

Janthinobacterium: The genus *Janthinobacterium* is probably not pathogenic. However, according to Patjanasoontorn et al. (1992), the species *Janthinobacterium lividum* caused opportunistic infections in hospital environment and fatal septicemia.

Massilia: *Massilia timonae* was first described by La Scola et al. in 1998 based on a single isolate from the blood of an immune-compromised patient with meningoencephalitis (La Scola et al. 1998). Since then, other isolates have been identified infecting humans as *M. timonae*, mainly based on 16S rRNA sequence comparison. The diagnosis for the patients from whom the bacteria were isolated varies: meningoencephalitis, wound infection following elective orthopedic surgery, osteomyelitis, cerebral pseudotumor, sepsis, lymphadenopathy, and otitis (Park and Shin 2013; Van Craenenbroeck et al. 2011; Lindquist et al. 2003; Sintchenko et al. 2000; La Scola et al. 1998). In one case, the isolate was derived from the lymph node of a patient with generalized lymphadenopathy and was reported as a coinfection with malaria (Van Craenenbroeck et al. 2011). These reports show the wide range of clinical presentation of *M. timonae* infections, but the source of infection appears to be unclear in all cases. In one patient, abscessed teeth were suspected to be the source, suggesting that *M. timonae* may occur as part of the transient normal oral flora. In another case, the contamination with this organism occurred possibly in a lake (Van Craenenbroeck et al. 2011). Furthermore, an isolate from otitis media was identified as *Massilia* sp., although the authors pointed out some possibility of contamination (Park and Shin 2013). Bacteria associated to otitis media commonly are *Streptococcus pneumonia*, *Moraxella catarrhalis*, and *Haemophilus influenza*. The identification of an isolate belonging to the genus *Massilia* possibly constitutes to a novel genus (Park and Shin 2013).

Oxalobacter: *Oxalobacter formigenes* is found in the gastrointestinal tract of man and animals. It relies on the presence of oxalate as energy and carbon source and aids in the destruction of dietary oxalate, a toxic substance which absorption contributes to increases of urinary oxalate and the formation of renal stones. This also limits the absorption of calcium and other minerals in the gastrointestinal tract (Holmes and Assimos 2004). At birth, the human intestine is free of *O. formigenes*, but newborn children are quickly colonized by these bacteria. There is clinical evidence that their absence in the gastrointestinal tract is a risk factor in the development of renal stone formation (Mittal and Kumar 2004). The oral application of Oxalobacter formigenes as a probiotic agent was tested in a clinical study with 43 human subjects. The application had no significant effect on urinary oxalate of treated subjects as compared to placebo-treated sub-jects (Hoppe et al. 2011).

Antibiotic Sensitivity

The antibiotic sensitivity is variable among members of the *Oxalobacteraceae* family and is dependent on the method used to test the minimal inhibitory concentration (MIC). Details about the levels of sensitivity/resistance are found in the description of the species, when available, or in the bibliography listed here.

Herbaspirillum: The antibiotic sensitivity varied among the *Herbaspirillum* species, and most of the available information are related to the wild type strains. *H. seropedicae* is susceptible to chloramphenicol, tetracycline, gentamicin, kanamycin, erythromycin, and streptomycin and is resistant to penicillin. *H. rhizosphaerae* is susceptible to chloramphenicol, gentamicin, kanamycin, neomycin, oleandomycin, streptomycin, and tetracycline but not to ampicillin, carbenicillin, cephalothin,

lincomycin, novobiocin, penicillin G, or polymyxin B. *H. lusitanum* is susceptible to gentamicin, tobramycin, netilmicin, and amikacin and resistant to cefotaxime and ceftazidime. *H. aquaticum* is resistant to polymyxin B. A *Herbaspirillum* sp. isolate from a patient was found to be susceptible to the majority of antimicrobial agents tested (amikacin, aztreonam, colistin, doxycycline, cefepime, tigecycline, ciprofloxacin, meropenem, piperacillin–tazobactam, ticarcillin–clavulanate, tobramycin, and ceftazidime).

Noviherbaspirillum: The species of the genus *Noviherbaspirillum* (*N. canariense*, *N. aurantiacum*, *and N. soli*) are sensitive to the antibiotics cefuroxim, ciprofloxacin, erythromycin, gentamicin, neomycin, polymyxin B, and tetracycline. However, *N. canariense* is resistant to ampicillin, penicillin, and cloxacillin. *N. aurantiacum* is resistant to cloxacillin and N. soil to penicillin (Carro et al. 2012). *N. psychrotolerans* carries resistance against the antibiotics ampicillin, cephalosporin, erythromycin, metronidazole, novobiocin, penicillin, fosfomycin, and troleandomycin and is sensitive to gentamicin, kanamycin sulfate, oxytetracycline, and rifampicin (Bajerski et al. 2013). No antibiotic studies were presented for the *N. malthae*.

Glaciimonas: *Glaciimonas immobilis* Cr9-30^T are resistant to ampicillin, fusidinic acid, and erythromycin and sensitive to streptomycin, chloramphenicol, tetracycline, kanamycin, gentamicin, amikacin, rifampicin, and nalidixic acid (Zhang et al. 2011).

Janthinobacterium: *Janthinobacterium* strains (W1r3^T, Clb4, H5, NCIMB 9230, NCIMB 9414, and DSM 1522^T) are resistant to penicillin G and vancomycin, but sensitive to erythromycin, streptomycin, tetracycline, or nalidixic acid. Interestingly, *J. lividum* has active metallo-beta-lactamase, which is responsible for resistance to several beta-lactam antibiotics (Rossolini et al. 2001).

Herminiimonas: *Herminiimonas arsenicoxydans* is resistant to tetracycline or ampicillin, but inhibited by kanamycin, chloramphenicol, streptomycin, and trimethoprim–sulfamethoxazole (Kämpfer et al. 2006). This species is able to resist to multiple toxic elements, especially arsenic. *Herminiimonas glaciei* strain UMB49^T is resistant to ampicillin, bacitracin, chloramphenicol, ciprofloxacin, penicillin, nalidixic acid, rifampicin, streptomycin, and vancomycin. Inhibition was detected for gentamicin, neomycin, and tetracycline.

Massilia: *Massilia aurea* is resistant to penicillin, bacitracin, and cephalothin. It is sensitive to the following antibiotics: tetracycline, rifampicin, streptomycin, neomycin, erythromycin, kanamycin, vancomycin, nalidixic acid, novobiocin, and chloramphenicol (Gallego et al. 2006), while *Massilia brevitalea* is resistant to bacitracin, carbenicillin, chloramphenicol, erythromycin, gentamicin, novobiocin, and penicillin G but sensitive to amikacin, ampicillin, kanamycin monosulfate, rifampicin, streptomycin sulfate, trimethoprim–sulfamethoxazole, tetracycline, tobramycin, and vancomycin (Zul et al. 2008). *Massilia haematophila and Massilia varians* are sensitive toward polymyxin B, penicillin G, amoxicillin, amoxicillin/clovalanat, gentamicin, trimethoprim, ciprofloxacin, rifampicin, and colistin (Kämpfer et al. 2008a). *Massilia tieshanensis* is sensitive to amoxicillin, ampicillin, cefotaxime, cefoxitin, chloramphenicol, erythromycin, kanamycin, nalidixic acid, neomycin, nitrofurantoin, novobiocin, polymyxin B, streptomycin, penicillin, tobramycin, and trimethoprim (Du et al. 2012). *Massilia namucuonensis* is susceptible to azithromycin, kanamycin, ofloxacin, tetracycline, rifampicin, cefotaxime, and streptomycin; it shows intermediate susceptibility to chloramphenicol and is resistant to amphotericin B (Kong et al. 2013). No information is available for the other *Massilia* species.

Oxalobacter: Four *Oxalobacter formigenes* strains HC1, Va3, CC13, and OxK, which are found in humans, have been tested for antibiotic sensitivity (Lange et al. 2012). All strains were sensitive to azithromycin, ciprofloxacin, clarithromycin, clindamycin, doxycycline, gentamicin, levofloxacin, metronidazole, and tetracycline. All strains were resistant to amoxicillin, clavulanate, ceftriaxone, cephalexin, and vancomycin. Only strain CC13 was resistant to nitrofurantoin (Lange et al. 2012).

Oxalicibacterium: *O. flavum* strain TA17^T was found to be sensitive to erythromycin, streptomycin, and gentamicin, whereas it was resistant to bacitracin and ampicillin (Tamer et al. 2002). Although Tamer et al. (2002) reported sensitivity of strain TA17^T to chloramphenicol, the strain is found resistant to this antibiotic in another study (Sahin et al. 2009b). *Oxalicibacterium faecigallinarum* strain YOx^T was found sensitive to the following antibiotics: chloramphenicol, erythromycin, streptomycin, penicillin, and colistin, whereas *Oxalicibacterium horti* strain OD1^T was resistant to chloramphenicol and penicillin (Sahin et al. 2009b).

Application

The *Oxalobacteraceae* family is formed by bacterial species with very diversified metabolic functions, but so far only *Herbaspirillum* and *Collimonas* species have already been applied as biofertilizer or biocontrol agents in agriculture, while other genera have been exploited for industrial application. Many species have shown biotechnological potential as suggested by the genome sequencing analyses, while others have not been exploited yet, such as *Noviherbaspirillum* spp., *Glaciimonas* spp., and *Telluria* spp.

Agricultural Application

Herbaspirillum: Among the species of the genus *Herbaspirillum*, only the species *H. seropedicae*, *H. rubrisubalbicans*, and *H. frisingense* have shown both the ability to fix nitrogen and to associate with plants of agricultural importance as plant growth-promoting agents (Monteiro et al. 2012). In addition to fix nitrogen, strains from these species produce phytohormones such as gibberellin and auxin (Bastián et al. 1998; Lambrecht et al. 2000; Bottini et al. 2004) and siderophores (Rosconi et al. 2013). In addition, they have abilities to solubilize inorganic P (Estrada et al. 2013) to present ACC deaminase to lower inhibitory ethylene levels in stressed plant roots (Rothballer et al. 2008) and to exert biological control activities

(Weber et al. 2007; Ting et al. 2011). All these features have been suggested to contribute to plant growth promotion (Baldani and Baldani 2005), but most of these effects have been observed under axenic conditions such as rice inoculated with *H. seropedicae* strains (Baldani et al. 2000), micropropagated sugarcane plants inoculated with both *H. seropedicae* and *H. rubrisubalbicans* strains (Canuto et al. 2003), and micropropagated *Miscanthus giganteus* seedlings inoculated with *H. frisingense* strain Mb11 (Rothballer et al. 2008). For *H. frisingense*, it could further be shown in a soil system that root ethylene signaling is involved in growth promotion of young plants of *Miscanthus sinensis* inoculated with endophytic strain *H. frisingense* GSF30^T (Straub et al. 2013b). Pot and field inoculation studies have been intensified in the last decade, although already in 1988, a successful field study had demonstrated a significant sorghum yield increase upon inoculation with *H. seropedicae* (Pereira et al. 1988). Yield responses due to inoculation with strain *H. seropedicae* ZAE94 was observed for different rice varieties (commercial and traditional ones) grown at different locations in Brazil (Ferreira et al. 2010; Araújo et al. 2013). Also, certain aluminum-tolerant rice varieties were stimulated in growth and nitrogen accumulation due to inoculation with *Herbaspirillum seropedicae* (Gyaneshwar et al. 2002). Additional studies showed that inoculation of maize varieties (hybrids and varieties) with strain ZAE94 resulted in significantly increased yields and in addition indicated that about 30 % of the nitrogen accumulated in the plant was derived from the BNF (Alves 2007). Furthermore, the *H. seropedicae* strain ZAE94 was recommended as a biofertilizer for inoculation of maize (Reis et al. 2009). Another study showed that the application of *H. seropedicae* strain Z67 together with humic substances via foliar application, produced 65 % higher grain yield than uninoculated maize plants grown under field conditions (Canellas et al. 2013). The strains *H. seropedicae* HRC54 and *H. rubrisubalbicans* HCC 103 are being used as components of a consortium inoculant recommended by the Brazilian Agricultural Research Enterprise (Embrapa) for sugarcane (Oliveira et al. 2006). A significant yield response to inoculation with a mixture of these bacteria was demonstrated for many commercial sugarcane varieties grown in different regions of Brazil (Schultz et al. 2012).

Collimonas: The species *Collimonas fungivorans* was demonstrated to have efficient biocontrol activity in vivo to suppress tomato foot and root rot in greenhouse experiments (Kamilova et al. 2007). *C. fungivorans* has a strong ability to colonize roots and is competing successfully against the excellent root colonizer *Pseudomonas fluorescens* WCS365. Direct competition for colonization and efficient in situ chitinolytic attack of *Fusarium oxysporum f. sp. radicis-lycopersici* is discussed as mechanisms of efficient biocontrol (Kamilova et al. 2007). Therefore, a potential for practical application is present for *Collimonas* similar as to chitinolytic *Serratia plymuthic*a (Berg 2000). In addition, some *Duganella* isolates from roots are claimed to have biotechnological potential (Aranda et al. 2011; Madhaiyan et al. 2013).

Janthinobacterium: Recently, it was demonstrated that *Janthinobacterium lividum* also produces indole-3-carboxaldehyde and that this plays a role in a possible mutualistic relationship with the red-backed salamander *Plethodon cinereus*, acting as a chemical defense mechanism against the chytrid fungus *Batrachochytrium dendrobatidis*, causal agent of the chytridiomycosis disease (Brucker et al. 2008). Such biological properties of *J. lividum* could be potentially explored to provide antifungal biocontrol. In addition to its antifungal product, three antibiotics produced by *J. lividum* act against both gram-negative and gram-positive bacteria (under patent process).

Industrial Application

Janthinobacterium: Some strains of *Janthinobacterium*, especially *J. lividum* strains, produce the pigment violacein that is preferentially produced when glycerol is the carbon source. Pantanella et al. (2007) showed a relationship between violacein production, biofilm formation, and improved survival, suggesting that it may play a role in response to environmental stress. Although the role of this pigment in bacterial life and metabolism is at present not fully understood, different functions have been attributed to violacein such as regulation of tryptophan production, environmental competition with other microorganisms, an antiprotozoal, antibacterial, anti-candida, antiviral, and antitumoral activity (Momen and Hoshino 2000; Matz et al. 2004; Leon et al. 2001). An industrial application involves its use as dye for natural or synthetic fibers (Shirata et al. 2000).

Massilia: Six bacteria of the genus *Massilia*, isolated from plants at Mallorca Island, Spain, and phylogenetically related to *M. aurea*, *M. plicata*, *M. niabensis*, and weakly to *M. niastensis* and *M. aerilata* (two potentially new species) were found to produce poly-3-hydroxybutyrate (PHB) when cultured with glucose or glycerol as carbon source. Polyhydroxyalkanoate (PHA) yields varied considerably among strains, but reach up to 50 wt% of cell mass as polyhydroxybutyrate (PHB) when grown on glycerol (Bassas-Galia et al. 2012). Type strains of many nonpathogenic *Massilia* species (*M. plicata*, *M. dura*, *M. aurea*, *M. albidiflava*, *M. brevitalea*, *M. lutea*, *M. aerilata*, and *M. umbonata*) were also shown to produce PHB when grown on different carbon sources (Rodríguez-Díaz et al. 2013; Bassas-Galia et al. 2012; Cerrone et al. 2011). An isolate of *Massilia* sp., designated BS-1, was shown to produce violacein, a compound produced by various bacteria and presenting important biological activities, including broad-spectrum antibacterial activity against gram-positive bacteria and antifungal, antiprotozoan, antimalarial, antitumor, antiviral, antioxidant, and antidiarrheal activities (Agematu et al. 2011). Although the 16S rRNA gene sequence of BS-1 isolate was reported to be closer to *Massilia* sp. strains (Agematu et al. 2011), a more recently search using MegaBLAST (Zheng et al. 2000) against NCBI 16S ribosomal RNA sequences (Bacteria and Archaea) database showed the best hit with *Massilia brevitalea* byr23-80 with 98 % identity.

Environmental Application

Herbaspirillum: In addition to a plant growth-promoting effect, it was shown that *Herbaspirillum chlorophenolicum* strain FA1 was able to use fluoranthene as sole carbon and energy source. It was therefore suggested as potential bioremediation technology for treatment of fluoranthene-polluted soil (Xu et al. 2011). A Brazilian *H. seropedicae* isolate was able to degrade Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine) to an extent comparable to the only TFL degrader found in a commercial culture collection (Bellinaso et al. 2003).

Herminiimonas: Based on the predominant habitat and phenotypic traits, some potential applications of *Herminiimonas* genus can be raised. For example, the well-demonstrated ability of *H. arsenicoxydans* to perform the oxidation of As[III] to arsenate As[V] represents potential biotechnological relevance for detoxification, converting As[III] into the less toxic and less mobile form As[V] (Muller et al. 2006). With improved technological approaches and backed up by whole genome sequence information of this species (Muller et al. 2007), more efficient bioremediation process may be generated. The type strain of *H. arsenicoxydans* is resistant to numerous heavy metals such as Se [IV], Mn[II], Cr[III], Cd[II], Sb[III], and Ni[II], increasing its potential as a bioremediation resource. Delivering methods for microbial release to contaminated environments have been attempted using bacteria immobilized in a calcium alginate gel (Simeonova et al. 2005). As a common genus associated to water bodies, its potential for water reclamation should be tested. Other possible applications are related to the biomineralization abilities of *Herminiimonas saxobsidens*, which could be tested in agriculture systems to increase the nutrient availability of, for example, P, Zn, and K elements for plant production.

Massilia: Some species of the genus *Massilia* were reported to metabolize glucose (Padmanabhan et al. 2003) and to have the ability to degrade phenanthrene (Bodour et al. 2003) or other aromatic compounds (Khammar et al. 2005). Additional yet unidentified members of the genus *Massilia* have shown their capacity to degrade aromatic compounds (Bodour et al. 2003; Khammar et al. 2005). *M. tieshanensis* TS3^T was isolated from a mining soil as an arsenic-resistant bacterium. Test have shown its high tolerance to Cu^{2+} (4.0 mM), which is about 10 times more than in other *Massilia* species (*M. niastensis*, *M. aerilata*, *M. varians*, and *M. consociata*) (Du et al. 2012).

Undibacterium: There are no reports available yet about possible biotechnological application of *Undibacterium spp.* Its intimate relationship with water environment, including low resistance to salts, could raise interesting aspects about its dynamic role on water quality.

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36 The Family *Rhodocyclaceae*

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Abstract

The order *Rhodocyclales*, phylogenetically affiliated with the *Betaproteobacteria*, currently (April, 2012) consists of a single family, the *Rhodocyclaceae*, with 18 genera (*Rhodocyclus* [type genus], *Azoarcus*, *Azonexus*, *Azospira*, *Azovibrio*, *Dechloromonas*, *Dechlorobacter*, *Denitratisoma*, *Ferribacterium*, *Georgfuchsia*, *Methyloversatilis*, *Propionivibrio*, *Quatrionicoccus*, *Sterolibacterium*, *Sulfuritalea*, *Thauera*, *Uliginosibacterium*, and *Zoogloea*), and a total of 45 species. Members of the order display many different modes of living: There are anoxygenic photoheterotrophs; plant-associated nitrogen-fixing aerobes, species that degrade a wide range of carbon sources including many aromatic compounds, using oxygen, nitrate, chlorate, perchlorate, selenate, and other electron acceptors; and sulfur-oxidizing chemoautotrophs, methylotrophs, and anaerobes that perform propionic acid fermentation. These highly disparate organisms are classified within a single family solely on the basis of 16S rRNA phylogeny. Representatives have been isolated from diverse environments: soil; sewage treatment plants; polluted and unpolluted waters of ponds, rivers, and aquifers; and plant roots. Some aromatic compound-degrading members have considerable potential for biodegradation of organic waste material and bioremediation of polluted environments.

Taxonomy, Historical and Current

Order *Rhodocyclales* Garrity, Bell, and Lilburn 2006, 3^{VP} (Effective Publication: Garrity, Bell, and Lilburn 2005a, 887)

Rho.do.cy.cla'les. N.L. masc. n. *Rhodocyclus*, type genus of the order; suff. *–ales*, ending denoting an order; N.L. fem. pl. n. *Rhodocyclales*, the *Rhodocyclus* order.

The mol% G + C of the DNA varies between 59 and 71.

Type genus: *Rhodocyclus*.

The order *Rhodocyclales* was created in 2005, based on 16S rRNA sequence comparisons. Currently (April 2012) the order consists of a single family, the *Rhodocyclaceae*. Physiologically the group is very disparate. Some members are anoxygenic photoheterotrophs; others are plant-associated nitrogen-fixing bacteria; there are species that degrade a wide range of carbon sources, including many aromatic compounds, using oxygen, nitrate, nitrite, chlorate, perchlorate, or selenate as electron acceptors; one genus contains anaerobes that perform propionic acid fermentation, one species is a sulfur-oxidizing chemolithoautotroph, and one species is a versatile facultative methylotroph that grows on a wide range of one-carbon compounds and on larger organic compounds as well.

At the time of writing (April 2012), the family *Rhodocyclaceae* consisted of 18 genera (*Table 36.1*) with 45 species (*Tables 36.2–36.10*): *Rhodocyclus* (type genus) (2 species), *Azoarcus* (8 species) *Azonexus* (3 species), *Azospira* (2 species), *Azovibrio* (1 species), *Dechloromonas* (3 species), *Dechlorobacter* (1 species, thus far only effectively published), *Denitratisoma* (1 species), *Ferribacterium* (1 species), *Georgfuchsia* (1 species), *Methyloversatilis* (1 species), *Propionivibrio* (4 species, one of which was only effectively published), *Quatrionicoccus* (1 species), *Sterolibacterium* (1 species), *Sulfuritalea* (1 species), *Thauera* (9 species), *Uliginosibacterium* (1 species), and *Zoogloea* (4 species). All these genera were described in the last two decades, with two exceptions: *Zoogloea* and *Rhodocyclus*.

Zoogloea ramigera was first published as a taxon in 1868 (Itzigsohn 1868). The name *Zoogloea* was used earlier by Cohn (1854), who used it to describe a growth form and not as a nomenclatural genus (Zvirbulis and Hatt 1967). Strain ATCC 19544 was proposed as neotype of *Z. ramigera* by Unz (1971), and as such it appeared in the Approved Lists of Bacterial Names published in 1980. A taxonomic reevaluation of strains classified in the genus *Zoogloea* in the 1990s based on analysis of fatty acids, isoprenoid quinones, and DNA-DNA hybridization showed that the genus contained members that were unrelated (Hiraishi et al. 1992; Shin et al. 1993). Comparative analysis of 16S rRNA sequence data (Rosselló-Mora et al. 1993) led to the reclassification of a number of strains affiliated with the *Alphaproteobacteria* and containing ubiquinone-10 in the newly created genus *Shinella*, while the ubiquinone-8 and rhodoquinone-8 containing strains affiliated with the *Betaproteobacteria* remain classified in the genus *Zoogloea* (An et al. 2006).

The genus *Rhodocyclus* was created by Pfennig (1978) with the description of the red anoxygenic phototrophic *Rhodocyclus purpureus* showing unusual ring-shaped cells. It uses acetate, butyrate, caproate, pyruvate, malate, fumarate, benzoate, cyclohexane carboxylate, and H_2 + CO_2 anaerobically in the light when supplied with small amounts of yeast extract. Its photosynthetic pigments are bacteriochlorophyll *a* and carotenoids of the rhodopinal series. Originally it was classified within the family *Rhodospirillaceae*. The second red anoxygenic phototroph currently classified in the genus *Rhodocyclus* as *Rhodocyclus tenuis* was originally described as *Rhodospirillum tenue* (Pfennig 1969). The two isolates share a similar type of lipopolysaccharide in their outer membrane (Weckesser et al. 1983). A taxonomic rearrangement of the anoxygenic purple nonsulfur bacteria placed the two in the genus *Rhodocyclus*, while the former *Rhodocyclus gelatinosus* (Molisch 1907; Imhoff et al. 1984) comb. nov. was renamed *Rubrivivax gelatinosus* and classified within the family *Comamonadaceae* (*Betaproteobacteria*) (Imhoff 2005; Imhoff et al. 1984).

Further taxonomic rearrangements made within the family *Rhodocyclaceae* are the splitting of the genus *Azoarcus*, established by Reinhold-Hurek et al. (1993), into four genera: *Azoarcus*, *Azovibrio*, *Azospira*, and *Azonexus*. This rearrangement was necessary, as the genus *Azoarcus sensu lato* was not monophyletic, and was supported by analyses of electrophoresis patterns of proteins, genomic fingerprints, and morphological and nutritional features (Reinhold-Hurek and Hurek 2000). The organism previously described as *Dechlorosoma suillum* (Achenbach et al. 2001; Brenner et al. 2005e) was transferred to the genus *Azospira* as a later heterotypic synonym of *Azospira oryzae* (Tan and Reinhold-Hurek 2003).

The fermentative propionic acid producing isolate previously described as *Propionibacter dicarboxylicus*

Table 36.1

The genera classified within the order *Rhodocyclales*, family *Rhodocyclaceae*, as of April 2012

Genus	Number of species[a]	Type species	General properties
Rhodocyclus (type genus)	2	*Rhodocyclus purpureus*	Anoxygenic phototrophs containing bacteriochlorophyll *a* and carotenoids. Preferably grow photoheterotrophically under anoxic conditions in the light with different organic substrates as carbon and electron sources. Chemotrophic growth is also possible under microoxic to oxic conditions in the dark. Reduced sulfur compounds are not used as photosynthetic electron donors
Azoarcus	8	*Azoarcus indigens*	Chemoorganoheterotrophs, having a strictly respiratory metabolism with O_2 as the terminal electron acceptor (except for *A. anaerobius*). Under anaerobic conditions, nitrate can be used as an electron acceptor. Some species fix nitrogen under microaerobic conditions. Most species grow on salts of organic acids but not on mono- or disaccharides. Some soilborne species use aromatic substrates as sole carbon sources under denitrifying conditions
Azonexus	3	*Azonexus fungiphilus*	Chemoorganoheterotrophs, aerobic or microaerophilic when fixing nitrogen. O_2 is the only electron acceptor. Grow on salts of organic acids but not on mono- or disaccharides
Azospira	2	*Azospira oryzae*	Chemoorganoheterotrophs, aerobic or microaerophilic when fixing nitrogen. Some species use nitrate and perchlorate as electron acceptors. Growth does not occur on most amino acids or on mono- or disaccharides
Azovibrio	1	*Azovibrio restrictus*	Chemoorganoheterotrophs, aerobic or microaerophilic when fixing nitrogen. Some species use nitrate as electron acceptor. Grow on acetate, ethanol, fumarate, L-glutamate, DL-lactate, L-malate, propionate, and succinate. No growth occurs on most other organic acids or on any mono- or disaccharides
Dechloromonas	3	*Dechloromonas agitata*	Chemoorganoheterotrophs with a strictly respiratory metabolism. Oxidize acetate with O_2, chlorate, perchlorate, or nitrate
Dechlorobacter	1	*Dechlorobacter hydrogenophilus*	Chemoorganoheterotrophs with a strictly respiratory metabolism. Oxidize acetate with O_2, chlorate, perchlorate, Mn(IV), or nitrate as electron acceptors
Denitratisoma	1	*Denitratisoma oestradiolicum*	Chemoorganoheterotrophs with a strictly respiratory metabolism, using O_2, nitrate, nitrite, chlorate, or perchlorate as electron acceptors. Can grow anaerobically on 17β-oestradiol with nitrate as electron acceptor
Ferribacterium	1	*Ferribacterium limneticum*	Chemoorganoheterotrophs that oxidize acetate, benzoate, formate, and lactate using Fe(III) as the electron acceptor. Acetate is oxidized using fumarate or nitrate as electron acceptors
Georgfuchsia	1	*Georgfuchsia toluolica*	Chemoorganoheterotrophs with a strictly respiratory type of metabolism, capable of degrading aromatic compounds
Methyloversatilis	1	*Methyloversatilis universalis*	Versatile methylotrophs, able to use a variety of C_1 and multicarbon compounds. Assimilate C1 units via the serine pathway
Propionivibrio	4	*Propionivibrio dicarboxylicus*	Strictly anaerobe or aerotolerant chemoorganoheterotrophs that produce propionate and acetate as end products of fermentation of sugars, dicarboxylic acids, sugar alcohols, and aspartate. Succinate is decarboxylated to propionate. *Propionivibrio militaris* (a name not yet validated) cannot be considered to be a member of the genus as its metabolism is respiratory and not fermentative
Quatrionicoccus	1	*Quatrionicoccus australiensis*	Chemoorganoheterotrophic large aerobic cocci, occurring in tetrads
Sterolibacterium	1	*Sterolibacterium denitrificans*	Chemoorganoheterotrophs with a strictly respiratory type of metabolism with oxygen or nitrate as terminal electron acceptor. Does not grow on complex media. Cholesterol is completely oxidized to CO_2, coupled with nitrate reduction
Sulfuritalea	1	*Sulfuritalea hydrogenivorans*	Chemolithoautotroph growing under anoxic conditions by the oxidation of reduced sulfur compounds and hydrogen. Heterotrophic growth occurs on organic acids
Thauera	9	*Thauera selenatis*	Chemoorganoheterotrophs with a strictly respiratory metabolism. Molecular oxygen, nitrate, nitrite, and nitrous oxide are used as terminal electron acceptors. Selenate is used as the electron acceptor by some strains. Various organic acids, amino acids, and aromatic and aliphatic compounds are used as sole substrates. Only a few carbohydrates are utilized

Table 36.1 (continued)

Genus	Number of species[a]	Type species	General properties
Uliginosibacterium	1	*Uliginosibacter gangwonense*	Chemoorganoheterotrophs, strictly aerobic, that oxidize a range of simple sugars and organic acids
Zoogloea	4	*Zoogloea ramigera*	Chemoorganoheterotrophs. Aerobic, having a strictly respiratory type of metabolism with oxygen or nitrate as the terminal electron acceptor. Major carbon sources include salts of several organic acids, dicarboxylic amino acids, alcohols, and salts of certain aromatic acids. Form flocs and films in liquid media at late growth stages, when the cells become embedded in gelatinous matrices

[a]Including species whose names have been effectively but not yet validly published

Table 36.2
Comparison of selected characteristics of the members of the genus *Rhodocyclus*

Character	*R. purpureus*[a,b]	*R. tenuis*[b,c]
Basonym		*Rhodospirillum tenue*
Type strain	DSM 168	DSM 109
Cell shape and diameter	Half circle or circle; 0.6–0.7 μm	Curved rods, 0.3–0.5 μm
Motility	−	+
Slime production	−	+
Major carotenoids	Rhodopin, rhodopinal	Rhodopsin, rhodopinal, lycopene
Requirement for growth factors	B_{12}, *p*-aminobenzoic acid, biotin	−
N_2 fixation	−	+
Use of acetate, butyrate, caproate, fumarate, malate, pyruvate, hydrogen	+	+
Use of arginine, aspartate, citrate, formate, fructose, glucose, glutamate, glycerol, glycolate, malonate, mannitol, mannose, methanol, tartrate, sulfide, sulfur, thiosulfate	−	−
Use of benzoate	+	−
Use of lactate, pelargonate, succinate, valerate	−	+
Use of caprylate, ethanol, propionate	−	+/−
G + C content of DNA (mol%)	65.1–67.7	64.1–67.2
Sample source and site	Swine wastewater lagoon, Iowa	Freshwater ponds, Grünenplan, Germany

Data taken from: [a]Pfennig (1978), [b]Imhoff (2005), [c]Pfennig (1969)
+ positive in most strains, − negative in most strains, +/− variable among strains

(Tanaka et al. 1991) was found to be phylogenetically affiliated with *Rhodocyclus* (Hippe et al. 1999). *Propionibacter pelophilus*, another Gram-negative propionigenic bacterium (Meijer et al. 1999), was reclassified in the genus *Propionivibrio* as *Propionivibrio pelophilus* comb. nov. (Brune et al. 2002). A further rearrangement within the genus *Propionivibrio* may be necessary in the future when the name *Propionivibrio miltaris*, which currently is only effectively published (Thrash et al. 2010), will be validated. The genus description circumscribes the members of *Propionivibrio* as strictly anaerobic or aerotolerant fermentative organisms that produce propionate and acetate. The organism described as *Propionivibrio miltaris* is not fermentative but lives as a facultative anaerobe, oxidizing acetate or propionate using O_2, perchlorate, chlorate, nitrate, or nitrite as electron acceptors.

Phylogenetic Structure of the Family and Its Genera

Figure 36.1 shows a neighbor-joining tree of the type strains of the 45 species of the order *Rhodocyclales*. The family is associated with the *Betaproteobacteria*, the closest relatives being the families *Nitrosomonadaceae* and *Methylophilaceae*.

Generally, the 18 genera currently recognized within the family are well separated in the tree. One notable exception is the position of *Propionivibrio militaris* (a name not yet validly published) which does not cluster closely with the type strains of the two other genera of *Propionivibrio*: *P. decarboxylicus* and *P. pelophilus*. As stated above, also the mode of life of *P. militaris* (respiration using a range of electron acceptors) differs greatly from that of the other two species (fermentation). The monospecific genera *Ferribacterium* and *Quatrionicoccus* form a cluster within the genus *Dechloromonas*, separating the type species *D. agitata* from *D. hortensis* and *D. denitrificans*. This topology is supported by at least one other tree reconstruction algorithm (RAxML).

Genome Analysis

In view of the possible application of some representatives of the *Rhodocyclales* in biodegradation and bioremediation operations,

Table 36.3

Comparison of selected characteristics of the members of the genus *Azoarcus*

Character	*A. indigens* [a,b,c]	*A. anaerobius*[a,b,d]	*A. buckelii*[b,e]	*A. communis*[a,b,c]	*A. evansii*[a,b,f]	*A. toluclasticus*[a,b,g]	*A. tolulyticus*[a,b,h]	*A. toluvorans*[a,b,i]
Type strain	LMG 9092	DSM 12081	DSM 14744	LMG 9095	DSM 6898	ATCC 700605	ATCC 51758	ATCC 700604
Cell size (μm)	0.5–0.7 × 2.0–4.0	1.5 × 2.7–3.3	1.0–2.0 × 1.0–2.0	0.8–1.0 × 1.5–3.0	0.4–0.8 × 1.5–3.0	0.6–0.8 × 1.7–4.0	0.8–1.0 × 1.4–2.8	0.8–1.0 × 1.4–2.8
O_2 terminal electron acceptor	+	–	+	+	+	+	+	+
Catalase	+	–	+	+	+	–	d	+
N_2 fixation	+	–	–	+	+[i]	NR	+	NR
Growth at 40 °C	+	–	+	+	–	–	–	–
Requirement for *p*-aminobenzoic acid	+	–	–	–	–	–	–	–
Growth on adipate, D-ribose	–	NR	–	–	–	+	+	+
Growth on L-arabinose, D-xylose	–	–	NR	–	–	+	+	+
Growth on D-fructose	–	–	NR	–	+	d	+	–
Growth on 3-hydroxybenzoate	+	+	+	+	+	–	d	–
Growth on isovalerate	–	+	NR	+	+	–	+	ND
Growth on D-tartrate	+	NR	NR	d	+	–	–	–
G + C content of DNA (mol%)	66.6	65.5	66	62.4	67.5	67.3	66.9	67.8
Sample source and site	Roots and stem bases of *Leptochloa fusca*, Pakistan; rice soil, rice roots, Pakistan and Nepal	Sewage sludge	Soil, Ulm, Germany	Roots and stem bases of *Leptochloa fusca*, Pakistan; refinery oil sludge, France	Industrial wastewater, Korea	Aquifer sediment, USA	Aquifer sediment, petroleum-contaminated soil, USA	Soil from industrial area, Brazil; soil, USA

Additional data on growth substrates are given by Reinhold-Hurek et al. (2005), Reinhold-Hurek and Hurek (2006) and are given in the original species descriptions

NR not reported, *d* different reactions in different isolates of the species

Data taken from: [a]Reinhold-Hurek et al. (2005), [b]Reinhold-Hurek and Hurek (2006), [c]Reinhold-Hurek et al. (1993), [d]Springer et al. (1998), [e]Mechichi et al. (2002), [f]Braun and Gibson (1984), Anders et al. (1995), [g]Song et al. (1999), [h]Zhou et al. (1995)

[i]No PCR-amplifiable *nifH* fragment or hybridization with a *nifH* probe could be demonstrated (Hurek et al. 1997); however, the species was reported to fix nitrogen (Mechichi et al. 2002; see also Reinhold-Hurek and Hurek 2006)

Table 36.4

Comparison of selected characteristics of the members of the genera *Azonexus*, *Azospira*, and *Azovibrio*

Character	*Azonexus fungiphilus*[a,b,c]	*Azonexus caeni*[d]	*Azonexus hydrophilus*[e]	*Azospira oryzae*[a,b,c]	*Azospira restricta*[f]	*Azovibrio restrictus*[a,b,c]
Type strain	LMG 19178	DSM 17719	LMG 24005	LMG 9096	DSM 18626	LMG 9099
Cell size (μm)	0.6–0.8 × 1.5–4.0	0.7–1.1 × 1.6–2.3	0.3–0.5 × 1.1–2.2	0.4–0.6 × 1.1–2.5	0.5 × 1.2–2.5	0.6–0.8 × 1.5–3.5
Catalase	+	+	+	+	+	+
Use of nitrate as electron acceptor		+	+	+	–	
N_2 fixation	+	–[g]	– but *nifH* is present	+	+	+
Growth at 40 °C	+	+	+	+	+	+ (most strains)
Requirement for *p*-aminobenzoic acid	–	NR	NR	–	NR	–
Growth on L-arabinose, D-xylose	–	–	–	–	–	–
Growth on D-fructose	–	–	–	–	–	–
Growth on 3-hydroxybenzoate	–	NR	NR	–	NR	–
Growth on 3-hydroxybutyrate	+	+	+	+	+	–
Growth on isovalerate	–	–	–	–	NR	–
Growth on *n*-caproate	–	NR	NR	+	NR	–
Growth on D-tartrate	–	NR	NR	+	NR	–
G + C content of DNA (mol%)	NR	65.6	64.0–66.0	65.2	67.9	64.8
Sample source and site	Black sclerotia of a Basidiomycete, rice field, Pakistan	Sewage sludge, S. Korea	Freshwater spring, Korea	Kallar grass roots, rice, Pakistan	Groundwater, Louisiana	Kallar grass roots, Pakistan

Additional data on growth substrates are given by Brenner et al. (2005a–c) and are given in the original species descriptions

NR not reported, *d* different reactions in different isolates of the species

Data taken from: [a]Brenner et al. (2005a), [b]Reinhold-Hurek and Hurek (2006, [c]2000), [d]Quan et al. (2006), [e]Chou et al. (2008), [f]Bae et al. (2007)

[g]A nitrogenase gene was detected, but growth on N_2 as sole nitrogen source could not be demonstrated

it is not surprising that a number of genome sequencing projects have targeted isolates belonging to the group. ❯ *Table 36.11* summarizes the data available at the time of writing (April 2012). The seven investigated genomes are 3.8–5.1 Mbp in length. Only one of the sequencing projects dealt with a type strain (*Methyloversatilis universalis*) (Kittichotirat et al. 2011). Further, there is genomic information for three *Azoarcus* strains (BH72, EBnI, and KH32C) (Krause et al. 2006; Nishizawa et al. 2012; Rabus et al. 2005), the strain earlier known as *Dechlorosoma suillum* PS and now reclassified as a strain of *Azospira oryzae*, a strain known as "Dechloromonas aromatica RCB" that still awaits taxonomic evaluation and description (Coates et al. 2001; Kellaris Salinero et al. 2009), and *Thauera* strain MZ1T.

The ~4.2 Mbp genome of *Methyloversatilis universalis* FAM5^T, the only methylotrophic member of the family, is as yet not completely closed, and is available as three scaffolds (Kittichotirat et al. 2011). Publication of the genome of the mutualistic, N_2-fixing grass endophyte *Azoarcus* strain BH72 (Krause et al. 2006) was preceded by the publication of a physical map based on a bacterial artificial chromosome library as a platform for genome sequencing and functional analysis (Battistoni et al. 2005). The genome of the denitrifying and N_2O-reducing bacterium *Azoarcus* sp. strain KH32C contains genes for plant-microbe interactions and gene clusters for aromatic compound degradation, and consists of a circular chromosome (5.1 Mbp) and a megaplasmid (0.74 Mbp) (Nishizawa et al. 2012).

Table 36.5

Comparison of selected characteristics of the members of the genus *Thauera*

Character	*T. selenatis*[a,b]	*T. aminoaromatica*[c]	*T. aromatica*[a,d]	*T. butanivorans*[e]	*T. chlorobenzoica*[a,f]
Earlier name				"Pseudomonas butanovora"[g]	
Type strain	ATCC 55363	DSM 14742	DSM 6984	ATCC 43655	ATCC 700723
Cell size (μm)	0.6 × 1.4	0.5–0.75 × 2–3	0.5–1.5 × 1.0–2.5	0.6–0.8 × 1.1–2.4	0.85–1.0 × 1.2–2.7
Flagellation	Monotrichous	Motile; mode of flagellation not reported	Peritrichous	Monotrichous	Peritrichous
Optimal pH	8	7.5–8.6	7.0–7.4	NR	7.5–8.0
Optimal temperature (°C)	28	28	28	NR	30–37
Selenate used as electron acceptor	+	NR	–	NR	–
Growth on benzoate + O_2	+	+	+	–	+
Growth on benzoate + nitrate	–	+	+	–	+
Growth on vanillate + O_2	NR	NR	+	NR	+
Growth on vanillate + nitrate	NR	NR	+	NR	+
Growth on terpenes (menthol, eucalyptol) + O_2	NR	NR	NR	NR	NR
Growth on terpenes (menthol, eucalyptol) + nitrate	NR	NR	NR	–	NR
Growth on toluene + O_2	NR	+	–	NR	–
Growth on toluene + nitrate	NR	+	+	–	–
Growth on phenylacetate + O_2	+	Poor	+	NR	NR
Growth on phenylacetate + nitrate	NR	Poor	+	NR	NR
Growth on propionate + O_2	+	NR	+	NR	–
Growth on pronionate + nitrate	NR	NR	+	NR	–
Growth on glucose + O_2	+	Poor	–	NR	NR
Growth on glucose + nitrate	NR	NR	–	NR	NR
G + C content of DNA (mol%)	66	68.4	67	67.3	69
Sample source and site	Agricultural drainage water, San Joaquin Valley, California	Anoxic ditch sludge, Konstanz, Germany	Creek sediment, USA	Activated sludge, oil refining plant, Japan	Sediment, Hudson River, Albany, NY, USA

Character	*T. linaloolentis*[a,h]	*T. mechernichensis*[a,i]	*T. phenylacetica*[c]	*T. terpenica*[a,h]
Type strain	DSM 12138	DSM 12266	DSM 14743	DSM 12139
Cell size	0.5–0.8 × 1.4–2.7	0.75 × 1.5–2.0	0.75–1 × 1.5–2	0.8–1.0 × 1.3–2.2
Flagellation	Motile; mode of flagellation not reported	Monotrichous	Motile; mode of flagellation not reported	Monotrichous
Optimal pH	7.0–7.3	NR	7.0–7.5	7.9–8.8
Optimal temperature	32	40	28	32
Selenate used as electron acceptor	–	–	NR	–
Growth on benzoate + O_2	NR	+	+	NR
Growth on benzoate + nitrate	–	+	+	–
Growth on vanillate + O_2	NR	NR	NR	NR

Table 36.5 (continued)

Character	*T. linaloolentis*[a,h]	*T. mechernichensis*[a,i]	*T. phenylacetica*[c]	*T. terpenica*[a,h]
Growth on vanillate + nitrate	NR	NR	NR	NR
Growth on terpenes (menthol, eucalyptol) + O_2	NR	NR	NR	NR
Growth on terpenes (menthol, eucalyptol) + nitrate	+	NR	NR	+
Growth on toluene + O_2	NR	NR	Poor	NR
Growth on toluene + nitrate	–	NR	Poor	–
Growth on phenylacetate + O_2	NR	+	Poor	NR
Growth on phenylacetate + nitrate	–	+	Poor	NR
Growth on propionate + O_2	NR	NR	NR	NR
Growth on pronionate + nitrate	+	+	NR	+
Growth on glucose + O_2	–	NR	Poor	NR
Growth on glucose + nitrate	–	NR	–	–
G + C content of DNA (mol%)	66	64.9	70.6	64.2
Sample source and site	Sewage sludge	Leachate treatment plant, Mechernich, Germany	Activated sludge, municipal sewage plant, Ulm, Germany	Forest ditch

Additional data on growth substrates are given by Heider and Fuchs (2005) and are given in the original species descriptions
NR not reported
Data taken from: [a]Heider and Fuchs (2005), [b]Macy et al. (1993), [c]Mechichi et al. (2002), [d]Tschech and Fuchs 1987, Evans et al. 1991, Anders et al. (1995), [e]Dubbels et al. (2009), [f]Song et al. (2001), [g]Takahashi et al. 1980, [h]Foss and Harder (1998), [i]Scholten et al. (1999)

Analysis of the genome of the soil microbe "*Dechloromonas aromatica* RCB" gives indications for a surprisingly complex lifestyle and cryptic anaerobic pathways for degradation of aromatic compounds such as chlorobenzoate, toluene, and xylene, using perchlorate as electron acceptor. Classic pathways such as the benzylsuccinate synthase genes (responsible for fumarate addition to toluene) and the central benzyl-CoA pathway for degradation of monoaromatic compounds appear to be missing. Unexpectedly, genes coding for enzymes of the Calvin cycle enzymes and sulfur oxidation are present as well (Kellaris Salinero et al. 2009).

Phages

No phages active on strains of *Rhodocyclales* have yet been described.

Phenotypic Analyses

The Properties of the Genera and Species of *Rhodocyclaceae*

As the genera and species of the order *Rhodocyclales*, family *Rhodocyclaceae* are physiologically so diverse, there are very few common properties beyond the fact that all show a Gram-negative type of cell wall, as expected on the basis of their affiliation with the *Proteobacteria*. Information on the phenotypic properties of the genera and species as is summarized below and in *Tables 36.2–36.10*.

Family *Rhodocyclaceae* Garrity, Bell, and Lilburn 2006, 3[VP] (Effective Publication: Garrity, Bell, and Lilburn 2005b, 887)

Rho.do.cy.cla'ce.ae. N.L. masc. n. *Rhodocyclus*, type genus of the family; suff. *–aceae*, ending to denote a family; N.L. fem. pl. n. *Rhodocyclaceae*, the *Rhodocyclus* family.

The family *Rhodocyclaceae* was circumscribed on the basis of phylogenetic analysis of 16S rRNA sequences. The family is phenotypically, metabolically, and ecologically diverse. It includes photoheterotrophs, aerobes, anaerobes, and facultative anaerobes utilizing a number of electron acceptors, fermentative organisms, and nitrogen-fixing organisms.

Type genus: *Rhodocyclus*.

The representatives of the family are further discussed in the following order: (1) the anoxygenic photoheterotrophs of the type genus *Rhodocyclus*; (2) the nitrogen fixers *Azoarcus*, *Azonexus*, *Azospira*, and *Azovibrio*; (3) the genus *Thauera* consisting of facultatively anaerobic degraders of aromatic compounds; (4) the floc-forming aerobes of the genus *Zoogloea*; (5) the chlorate reducers of the genera *Dechloromonas* and *Dechlorobacter*; (6) the sterol degraders of the monospecific

Table 36.6
Comparison of selected characteristics of the members of the genus *Zoogloea*

Character	*Z. ramigera*[a,b]	*Z. caeni*[c]	*Z. oryzae*[a,c,d]	*Z. resiniphila*[a,c,e]
Type strain	ATCC 19544	DSM 19389	IAM 15218	ATCC 700687
Cell size (μm)	1.0–1.3 × 2.1–3.6	0.6–0.9 × 1.1–2.0	0.5–0.7 × 1.5–2.0	1.0 × 1.5–2.8
Flagellation	Polar flagellum	Polar flagellum	Polar flagellum	NR
Optimal pH	NR	6.5–7.5	7.0–7.5	NR
Optimal temperature (°C)	NR	25–30	NR	NR
Catalase	+	+	+	–
Urease	+	–	+	–
Denitrification	+	+ (slow)	+	–
Hydrolysis of gelatin	+	+	–	+
Hydrolysis of casein	+	–	–	+
Acetate as carbon source	+	+	–	+
Citrate as carbon source	+	–	–	–
Glucose as carbon source	–	+	–	+
Mannitol as carbon source	+	–	–	+
Abietic acid and dehydroabietic acid as carbon source	NR	NR	NR	+
G + C content of DNA (mol%)	67.3–69.0	64.9–65.0	65.1	NR
Sample source and site	Sewage sludge	Wastewater treatment plant, Korea	Rice paddy soil	Laboratory sequencing batch reactor, Vancouver, Canada

Additional data on growth substrates are given by Unz (2005) and are given in the original species descriptions
NR not reported
Data taken from: [a]Unz (2005), [b]Shin et al. (1993), [c]Shao et al. (2009), [d]Xie and Yokota (2006), [e]Mohn et al. (1999)

genera *Sterolibacterium* and *Denitratisoma*; (7) the disparate monospecific aerobic genera *Methyloversatilis*, *Quatrionicoccus*, *Uliginosibacterium*, and *Sulfuritalea*; (8) the genera *Propionivibrio*, *Ferribacterium*, and *Georgfuchsia*, showing different types of anaerobic metabolism.

The Anoxygenic Photoheterotrophs of the Type Genus *Rhodocyclus*

Genus *Rhodocyclus* Pfennig 1978 (Approved Lists 1980)

Rho.do.cy'clus Gr. n. *rhodon*, the rose; L. masc. n. *cyclus*, a circle; N.L. masc. n. *Rhodocyclus*, red circle.

Cells are slender, curved, or straight thin rods. Motile by means of polar flagella or nonmotile. Multiply by binary fission. Gram negative. Internal photosynthetic membranes may appear as small, single, finger-like intrusions of the cytoplasmic membrane, or may be absent. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids. Respiratory quinones are ubiquinone-8 and menaquinone-8. The major cellular fatty acids are $C_{16:1}$ and $C_{16:0}$; $C_{10:0\ 3\text{-OH}}$ is present. The major phospholipids are cardiolipin, phosphatidylethanolamine, and phosphatidylglycerol. Preferably grow photoheterotrophically under anoxic conditions in the light with different organic substrates as carbon and electron sources. Benzoate and cyclohexane are both used. Photoautotrophic growth with molecular hydrogen may be possible if growth factors are supplied. Chemotrophic growth is also possible under microoxic to oxic conditions in the dark. Reduced sulfur compounds are not used as photosynthetic electron donors. Assimilatory sulfate reduction is possible. Growth factors may be required. Mesophilic and neutrophilic freshwater bacteria. Habitat: freshwater ponds, sewage ditches, swine waste lagoons.

The mol% G + C of the DNA is 64.1–65.1.

Type species: *Rhodocyclus purpureus*.

The genus currently contains two species: *R. purpureus* and *R. tenuis* (Table 36.2). *Rhodocyclus gelatinosus* (Molisch 1907; Imhoff et al. 1984), comb. nov. has been transferred to the genus *Rubrivivax* (*Comamonadaceae*, *Betaproteobacteria*) (Imhoff 2005; Imhoff et al. 1984).

The type species *R. purpureus* was isolated only once and was never observed in nature again. Some strains of *R. tenuis*, including the type strain, are colored brownish-red, as they transform rhodopin further to spirilloxanthin. Other strains are purple-violet and do not form anhydrorhodovibrin and spirilloxanthin, but accumulate major amounts of rhodopinal, rhodopinol, and lycopenal (Imhoff 2005).

Table 36.7
Comparison of selected characteristics of the members of the genera *Dechloromonas* and *Dechlorobacter*

Character	*Dechloromonas agitata*[a]	*Dechloromonas denitrificans*[b]	*Dechloromonas hortensis*[c]	*Dechlorobacter hydrogenophilus*[d,e]
Type strain	ATCC 700666	ATCC BAA-841	ATCC BAA-776	ATCC BAA-1869
Cell size (μm)	0.5 × 2.0	0.5 × 1.7	NR	0.3 × 0.8–1.6
Colony color	NR	Yellowish	Yellow	NR
Motility	One polar flagellum	One polar flagellum	Motile, mode of flagellation NR	Motile, mode of flagellation NR
Growth on acetate	+	+	+	+
Growth on glucose or ethanol	–	–	–	–
Use of hydrogen as electron donor	NR	–	NR	+
Use of Fe(II) as electron donor	+	NR	NR	–
Use of sulfide as electron donor	+	NR	–	NR
Use of perchlorate and chlorate as electron acceptor	+	+	+	+
Use of nitrate and oxygen as electron acceptor	+	+	+	+
Use of Mn(IV) as electron acceptor	NR	NR	NR	–
G + C content of DNA (mol%)	63.5	61.2	63.3	NR
Sample source and site	Paper plant pulp waste sludge, Pennsylvania	Gut of the earthworm *Aporrectodea caliginosa*	Garden soil	Soil from an army ammunition plant, Texas

Additional data on growth substrates are given in Brenner et al. (2005d) and are given in the original species descriptions
NR not reported
Data taken from: [a]Achenbach et al. (2001), [b]Horn et al. (2005), [c]Wolterink et al. (2005), [d]Thrash et al. (2010)
[e]Name yet to be validly published

The Nitrogen Fixers *Azoarcus*, *Azonexus*, *Azospira*, and *Azovibrio*

Genus *Azoarcus* Reinhold-Hurek, Hurek, Gillis, Hoste, Vancanneyt, Kersters, and De Ley 1993, 582[VP]

A.zo.ar'cus. N.L. n. *azotum* (from Fr. n. *azote* [from Gr. prep. *a*, not; Gr. n. *zôê*, life; N.Gr. *azôê*, not sustaining life]), nitrogen; N.L. pref. *azo-*, pertaining to nitrogen; L. masc. n. *arcus*, arch, bow; N.L. masc. n. *Azoarcus*, nitrogen (–fixing) bow.

Straight to slightly curved rods, 0.4–1.5 × 1.1–4.0 μm. Cell pairs often appear slightly S shaped. In most species, some elongated cells (8–12 μm) occur in late-exponential or stationary-phase cultures. Highly motile by means of one polar flagellum; rarely there are two flagella. Accumulate poly-β-hydroxybutyrate granules. Gram negative. Some species are nitrogen fixers; these require microaerobic conditions for growth on N_2. On semisolid nitrogen-free media, microaerophilic growth can be observed as veil-like pellicles developing several millimeters below the surface and moving to the medium surface during growth. In most species, colonies on VM agar (Reinhold-Hurek et al. 2005) supplemented with ethanol develop a nondiffusible yellowish pigment. Optimal temperature for growth 30–40 °C; no growth occurs at 45 °C. Chemoorganoheterotrophic. Bacteria have a strictly respiratory metabolism with O_2 as the terminal electron acceptor (except for *A. anaerobius*). Alternatively, under anaerobic conditions nitrate can be used for dissimilatory nitrate reduction. Oxidase positive. Grow well on salts of organic acids such as L-malate, succinate, fumarate, DL-lactate; also grow well on ethanol, on L-glutamate, but not on mono- or disaccharides except for species that are not plant associated. These soilborne species utilize a variety of aromatic substrates as sole carbon sources under denitrifying conditions. Nitrate can be used as a nitrogen source (assimilatory nitrate reduction). Growth factor requirements vary: Some strains depend on *p*-aminobenzoic acid or on cobalamin. All investigated species have $C_{16:1}$ as major cellular fatty acid; all species except one have $C_{16:1\ \omega7c}$ and $C_{18:1}$ as the major cellular fatty acids.

The mol% G + C of the DNA is 62–68.

Type species: *Azoarcus indigens*.

The genus currently contains eight species: the plant-associated *A. indigens* and *A. communis* (Reinhold-Hurek and Hurek 1998) and the species *A. evansii*, *A. anaerobius*, *A. buckelii*,

Table 36.8
Comparison of selected characteristics of the members of the monospecific genera *Sterolibacterium* and *Denitratisoma*

Character	*Sterolibacterium denitrificans*[a]	*Denitratisoma oestradiolicum*[b]
Type strain	DSM 13999	DSM 16959
Cell size (μm)	0.5–0.6 × 1.0–1.3	0.4–0.8 × 0.8–2.0
Motility	Polar flagellum	Polar flagellum
Catalase	+	–
Anaerobic growth in the presence of nitrate on:		
17β-Oestradiol	–	+
Oestrone	–	+
4-Androstene-3,17-dione	+	–
Cholesterol	+	–
Acetate, propionate, butyrate	+	+
Palmitate, stearate	+	–
Pyruvate, fumarate, succinate	–	+
Electron acceptors:		
Oxygen	+	+
Nitrate	–	+
Chlorate, perchlorate	–	+
G + C content of DNA (mol%)	65.3	61.4
Sample source and site	Upflow sludge bed anoxic reactor, Montevideo, Uruguay	Municipal waste treatment plant, Aachen, Germany

Data taken from: [a]Tarlera and Denner (2003), [b]Fahrbach et al. (2006)
Additional data on growth substrates are given in the original species descriptions

A. toluclasticus, *A. tolulyticus*, and *A. toluvorans*, isolated from soils, aquifers, or wastewater (Reinhold-Hurek et al. 2005) (*Table 36.3*). All *Azoarcus* spp. are strictly respiratory and fermentative metabolism was never demonstrated. All species except *A. anaerobius* can use oxygen as electron acceptor. Carbohydrates are poorly metabolized if at all; none of the plant-associated species is able to utilize any out of 50 mono- and disaccharides tested (Reinhold-Hurek et al. 1993). Nitrogen fixation is widespread, but the property can no longer be considered universal in the genus. Attempts to amplify a *nifH* gene fragment from *A. evansii* failed and no hybridization with a *nifH* probe was obtained (Hurek et al. 1997); however, recently the species was reported to fix nitrogen (Mechichi et al. 2002). *Azoarcus* sp. strain BH72, an endophyte isolated from Kallar grass, produces stacks of intracellular membranes ("diazozomes") when grown on N_2 as sole nitrogen source (Hurek et al. 1995).

Genus *Azonexus* Reinhold-Hurek and Hurek 2000, 658[VP]

A.zo.nex.us N.L. n. *azotum* (from Fr. n. *azote* [from Gr. prep. *a*, not; Gr. n. *zôê*, life; N.Gr. *azôê*, not sustaining life]), nitrogen; N.L. pref. *azo-*, pertaining to nitrogen; L. masc. n. *nexus*, coil; N.L. masc. n. *Azonexus*, nitrogen-fixing coil.

Gram-negative, non-sporeforming, slightly curved motile rods, 0.6–0.8 × 1.5–4.0 μm. Elongated straight to coiled cells of up to 50 μm occur in stationary phase, alkalinized, nitrogen-fixing cultures. Motile by means of a single polar flagellum. Microaerophilic growth occurs as veil-like pellicles which move up to the surface in semisolid N-free media. Chemoorganoheterotrophic and aerobic; microaerophilic when fixing nitrogen. Strictly respiratory, O_2 being the only electron acceptor. Oxidase and catalase positive. Fix N_2. Require cobalamin. Grow on acetate, ethanol, fumarate, DL-lactate, L-malate, 2-oxoglutarate, L-proline, and succinate. None of the tested mono- or disaccharides serves as sole carbon source. Grow well at 37 °C, can grow at 40 °C. Neutral pH optimal for growth.

The mol% G + C of the DNA is 64–66 (but no information is available on the G + C content of the DNA of the type species).

Type species: *Azonexus fungiphilus*.

Currently, the genus contains three species: *A. fungiphilus*, *A. caeni*, and *A. hydrophilus* (*Table 36.4*).

Genus *Azospira* Reinhold-Hurek and Hurek 2000, 658[VP], Emend. Bae, Rash, Rainey, Nobre, Tiago, da Costa, and Moe 2007, 1525

A.zo.spi'ra N.L. n. *azotum* (from Fr. n. *azote* [from Gr. prep. *a*, not; Gr. n. *zôê*, life; N.Gr. *azôê*, not sustaining life]), nitrogen; N.L. pref. *azo-*, pertaining to nitrogen; L. fem. n. *spira*, coil, spire; N.L. fem. n. *Azospira*, nitrogen-fixing spiral.

Gram-negative, non-sporeforming curved rods, 0.4–0.6 × 1.1–2.5 μm. Elongated cells with one to several spiral windings of up to 8 μm cell length occur rarely in late-stationary-phase, alkalinized, nitrogen-fixing cultures. Motile by means of one polar flagellum. Microaerophilic growth as veil-like pellicles which move up to the surface in semisolid N-free media. Chemoorganoheterotrophic and aerobic; microaerophilic when fixing N_2. Strictly respiratory; variable with respect to use of nitrate and perchlorate as electron acceptors. Oxidase and catalase positive. Fix N_2. Growth does not occur on most amino acids or on mono- or disaccharides. Grow on acetate, *n*-caproate, ethanol, fumarate, L-glutamate, DL-lactate, L-malate, 2-oxoglutarate, propionate, and succinate. Growth factor requirement varies. Grows well at 37 °C. Produces poly-β-hydroxybutyrate storage granules. Neutral pH optimal for growth. Major cellular fatty acids are $C_{16:1\ \omega7c}$, $C_{16:0}$, and $C_{18:1\omega7c}$.

The mol% G + C of the DNA is 65–68.

Type species: *Azospira oryzae*.

The genus *Azospira* currently consists of two species: *A. oryzae* and *A. restricta* (Brenner et al. 2005b) (*Table 36.4*). It includes

Table 36.9

Comparison of selected characteristics of the members of the monospecific aerobic or facultatively anaerobic genera *Methyloversatilis*, *Quatrionicoccus*, *Sulfuritalea*, and *Uliginosibacterium*

Character	*Methyloversatilis universalis*[a]	*Quatrionicoccus australiensis*[b]	*Sulfuritalea hydrogenivorans*[c]	*Uliginosibacterium gangwonense*[d]
Previous name		*Quadricoccus autraliensis (illegitimate synonym)*		
Type strain	JCM 13912	NCIMB 13738	DSM 22779	DSM 18521
Cell size (μm)	0.3–0.5 × 0.8–1.6	2.2–4.5 × 2.2–4.5	0.3–0.5 × 1.0–3.5	0.6–1.0 × 3.0–7.0
Colony color	White; older colonies brown	NR	NR	Yellow
Motility	–	–	Motile; mode of flagellation NR	Single polar flagellum
Temperature optimum (range) (°C)	30–37	25–30 (15–30)	25 (8–32)	25–30 (4–35)
pH optimum (range)	7.5–8.0 (6.0–9.0)	7.5–8.5 (6.0–8.5)	6.7–6.9 (6.4–7.6)	6.0–7.0 (4.0–8.0)
Relation to molecular oxygen	Strict aerobe	Strict aerobe	Facultative aerobe	Strict aerobe
Metabolism	Oxidizes C1 compounds (methanol, methylamines, formaldehyde, formate) and multicarbon compounds	Oxidizes a range of simple sugars and organic acids	Anaerobic autotrophic growth with S^0 or H_2 as electron donor and nitrate as electron acceptor; aerobic heterotrophic growth on organic acids	Oxidizes a range of simple sugars and organic acids
G + C content of DNA (mol%)	64–65	67	67	59.3
Sample source and site	Lake Washington, WA; contaminated soil, Portugal	Activated sludge, Australia	Freshwater lake, Japan	Wetland, Korea

Data taken from: [a]Kalyuzhnaya et al. (2006), [b]Maszenan et al. (2002), Tindall and Euzéby (2006), [c]Kojima and Fukui (2011), [d]Weon et al. (2008)
Additional data on growth substrates are given in the original species descriptions

Dechlorosoma suillum Achenbach et al. 2001, considered a later heterotypic synonym of *Azospira oryzae* Reinhold-Hurek and Hurek 2000 emend. Tan and Reinhold-Hurek 2003. *Dechlorosoma suillum* was reported to oxidize Fe(II) aerobically using oxygen as electron acceptor or anaerobically in the presence of nitrate, generating orange ferric oxyhydroxide or ferrihydrite (Lack et al. 2002).

Genus *Azovibrio* Reinhold-Hurek and Hurek 2000, 657[VP]

A.zo.vi'bri.o N.L. n. *azotum* (from Fr. n. *azote* [from Gr. prep. *a*, not; Gr. n. *zôê*, life; N.Gr. *azôê*, not sustaining life]), nitrogen; N.L. pref. *azo-*, pertaining to nitrogen; L. v. *vibrio*, to set in tremulous motion, move to and fro, vibrate; N.L. masc. n. *vibrio*, that which vibrates, and also a bacterial genus name of bacteria possessing a curved rod shape (*Vibrio*); N.L. masc. n. *Azovibrio*, nitrogen-fixing organism that vibrates.

Gram-negative, non-sporeforming slightly curved motile rods, 0.6–0.8 × 1.5–3.6 μm. Elongated cells occur very rarely in stationary, alkalinized cultures. One polar flagellum. Microaerophilic growth as veil-like pellicles which move up to the surface in semisolid N-free media. Chemoorganoheterotrophic and microaerophilic. Strictly respiratory; O_2 and nitrate are electron acceptors. Oxidase positive. Catalase weak. Fix N_2. Grow on acetate, ethanol, fumarate, L-glutamate, DL-lactate, L-malate, propionate, and succinate. No growth occurs on most other organic acids or on any mono- or disaccharides. Grow well at 37 °C. Neutral pH optimal for growth. Major cellular fatty acids are $C_{16:1\ cis\text{-}9}$, $C_{16:0}$, $C_{18:1}$, $C_{14:0}$, and $C_{10:1\ 3\text{-}OH}$.

The mol% G + C of the DNA is 64–65.

Type species: *Azovibrio restrictus*.

Currently the genus *Azovibrio* is represented by a single species, *A. restrictus* (Brenner et al. 2005c) (*Table 36.4*).

The Genus *Thauera* Consisting of Facultatively Anaerobic Degraders of Aromatic Compounds

Genus *Thauera* Macy, Rech, Auling, Dorsch, Stackebrandt, and Sly 1993, 139[VP] (Emend. Anders, Kaetzke, Kämpfer, Ludwig, and Fuchs 1995, 331; Emend. Song, Young, and Palleroni 1998, 893)

Thau'e.ra. N.L. fem. n. *Thauera*, named after R.K. Thauer, a German microbiologist.

Table 36.10

Comparison of selected characteristics of the members of the genera *Propionivibrio*, *Ferribacterium*, and *Georgfuchsia*

Character	*Propionivibrio* dicarboxylicus[a,b]	*Propionivibrio limicola*[b,c]	*Propionivibrio* pelophilus[c,d]	*Propionivibrio militaris*[e,h]	*Ferribacterium limneticum*[f,h]	*Georgfuchsia toluolica*[g]
Basonym			*Propionibacter pelophilus*			
Type strain	DSM 5885	DSM 6832	DSM 12018	DSM 21683	ATCC 700589	DSM 19032
Cell size (μm)	0.5–0.6 × 1.0–2.0	0.6–0.7 × 1.5–2.5	0.5–0.6 × 0.9–1.1	0.3 × 2.0	0.3–0.5 × 1.4–2.0	0.5–0.6 × 0.8–1.2
Colony color	White	NR	White	NR	NR	Brown-reddish
Motility	Tuft of flagella	Single polar flagellum	Single polar flagellum	Motile; mode of flagellation NR	Motile; mode of flagellation NR	Motile; mode of flagellation NR
Temperature optimum (°C)	30	37	27–30	30	NR	25–30
pH optimum	6.0–7.0	7.0–7.5	7.5–8.0	6.8	NR	7.3
Metabolism	Ferments maleate, fumarate, and L-malate to propionate and acetate. Succinate is decarboxylated to propionate	Sole substrates for fermentation are quinic acid and shikimic acid; products are propionate and acetate	Ferments sugars, dicarboxylic acids, sugar alcohols, and aspartate to propionate and acetate	Non-fermentative	Oxidizes acetate and other organic acids with Fe(III), nitrate, or fumarate as electron acceptor. No growth on sugars and alcohols	Only grows by oxidation of aromatic compounds (toluene, ethylbenzene, phenol, *p*-cresol, *m*-cresol, and others) with Fe(III), Mn(IV), or nitrate as electron acceptor
			Reduces nitrate to nitrite. Fixes N_2. Aerotolerant	Respires acetate, propionate, and anthraquinone-2,6-disulfonate using O_2, perchlorate, chlorate, nitrate, or nitrite as electron acceptors		
G + C content of DNA (mol%)	61	61.6	60.8	NR	NR	NR
Sample source and site	Anaerobic pond and lake sediment, Japan	Freshwater pond sediment, Tübingen, Germany	North Sea estuary, the Netherlands	Perchlorate-reducing bioelectrical reactor, Berkeley, CA	Lake sediment, Idaho	Polluted aquifer, the Netherlands

Additional data on growth substrates are given in the original species descriptions

Data taken from: [a]Tanaka et al. (1990), [b]Brenner et al. (2005g), [c]Brune et al. (2002), [d]Brune and Schink (1992), Hansen (2005), Hansen et al. (1990), Meijer et al. (1999), [e]Thrash et al. (2010), [f]Cummings et al. (2000), Brenner et al. (2005f), [g]Weelink et al. (2011)

[h]Name yet to be validly published

Rods, 0.5–1.4 × 1.4–3.7 μm, usually occurring singly. Some species exhibit great variability of cell form, from rods to coccoid forms of different sizes. Gram negative. Most species are motile by a single polar flagellum. No resting stages known. Oxidase and catalase positive. Colonies on minimal medium are nonpigmented. Aerobic, having a strictly respiratory metabolism; never fermentative. Molecular oxygen, nitrate, nitrite, and nitrous oxide are used as terminal electron acceptors, and the nitrogen oxides are reduced to N_2O or N_2; selenate is used as the electron acceptor by some strains and reduced to elemental selenium. Contains the lipoquinone Q-8. Optimal temperature 28–40 °C. All species grow at pH 7.5; pH optima of individual species range from 7.2 to 8.4. Chemoorganotrophic, using various organic acids, amino acids, and aromatic and aliphatic compounds as sole substrates. Only a few carbohydrates are utilized. Ammonium and nitrate salts can be used as sole nitrogen sources. No N_2 fixation known. Vitamins are required for growth by some strains. Not proteolytic on casein or gelatin. Starch, cellulose, chitin, and agar are not hydrolyzed. The major polyamines are putrescine and 2-hydroxyputrescine. Occur in polluted freshwater and wet soil environments and wastewater treatment plants.

The mol% G + C of the DNA is 64–71.

Type species: *Thauera selenatis*.

Table 36.11

Properties of the sequenced genomes of members of the *Rhodocyclales* (as of April 2012)

Property	*Methyloversatilis universalis* FAM5[Ta]	*Azoarcus* sp. BH72[b,f]	*Azoarcus* sp. EbN1[c]	*Azoarcus* sp. KH32C[d]	"Dechloromonas aromatica" RCB[e]	*Dechlorosoma suillum* PS (=*Azospira oryzae*)	*Thauera* sp. MZ1T
Accession number	AFHG00000000	AM406670	CR555306	AP012304	C000089	NC_016616	CP001281
			CR555307	AP012305			
			CR555308				
Genome length (bp)	~4,200,000	4,376,040	4,296,230	5,081,166	4,501,104	3,806,980	4,496,212
G + C content	67.0	67.9	65.1	65.1	60	NR	NR
Extrachromosomal elements	0	0	2 (207,355 bp; 57.6 mol% G + C and 223,670 bp, 63.1 mol% G + C)	1 (737,589 bp; 64.5 mol% G + C)	NR	NR	NR
% of coding bases	NR	91.2 %	90.9 %	NR	NR	NR	NR
Number of predicted genes	NR	3,992	4,133 + 274 + 196	NR	NR	NR	NR
Predicted protein-coding genes	NR	NR	NR	4.531 + 657	4,204	3,443	NR
% of proteins with putative function	NR	3,418	2,560	NR	NR	NR	NR
Number of 16S rRNA genes	NR	4	4	5	NR	NR	NR

NR not reported

Data taken from: [a]Kittichotirat et al. (2011), [b]Krause et al. (2006), [c]Rabus et al. (2005), [d]Nishizawa et al. (2012), [e]Kellaris Salinero et al. (2009)

[f]Also known as "Aromatoleum aromaticum"

The genus *Thauera* currently contains nine species: *T. selenatis*, *T. aminoaromatica*, *T. aromatica*, *T. butanivorans*, *T. chlorobenzoica*, *T. linaloolentis*, *T. mechernichensis*, *T. phenylacetica*, and *T. terpenica* (Table 36.5).

The Floc-Forming Aerobes of the Genus *Zoogloea*

Genus *Zoogloea* Itzigsohn 1868, 39 (Approved Lists 1980); Emend. Shin, Hiraishi, and Sugiyama 1993, 830

Zo.o.gloe'a. Gr. adj. *zôos*, alive, living; Gr. fem. n. *gloia*, glue; N.L. fem. n. *Zoogloea*, living glue.

The following description is representative of the genus based on well-characterized isolates recovered directly from naturally occurring, typical, fingered zoogloeae: Straight to slightly curved, plump rods, 1.0–1.3 × 2.1–3.6 μm, with rounded ends; sometimes tapered to a blunt point at one or both poles. Non-sporeforming and non-cystforming. Cells in older cultures are demonstrably encapsulated. Gram negative. Actively motile, especially in young cultures, by means of a single polar flagellum. Intracellular granules of poly-β-hydroxybutyrate are formed on media containing the salts of organic acids. Cultures enter into formation of flocs and films in liquid media at late growth stages; the cells become embedded in gelatinous matrices to form zoogloeae, which may be distinguished by a "tree-like" or "finger-like" morphology. Young colonies on solid media under a normal air atmosphere are translucent and punctiform, but may increase to 1–2 mm in diameter and exhibit opaque centers. Nonpigmented. Aerobic, having a strictly respiratory type of metabolism with oxygen or nitrate as the terminal electron acceptor. Denitrification occurs with formation of N_2. Optimal temperature for growth, 28–37 °C. Optimal pH 7.0–7.5. Oxidase positive. Weakly catalase positive. Chemoorganotrophic. Acid is not formed from carbohydrates except from xylose, glycerol, and ethanol, which are attacked oxidatively by a few strains. Proteolytic on gelatin. Most strains are urease positive. Litmus milk is unchanged. Hydrogen sulfide is usually not produced from cysteine. Major carbon sources include salts of several organic acids (e.g., lactate, pyruvate, and fumarate), dicarboxylic amino acids (e.g., aspartate, glutamate, and asparagine), alcohols, and

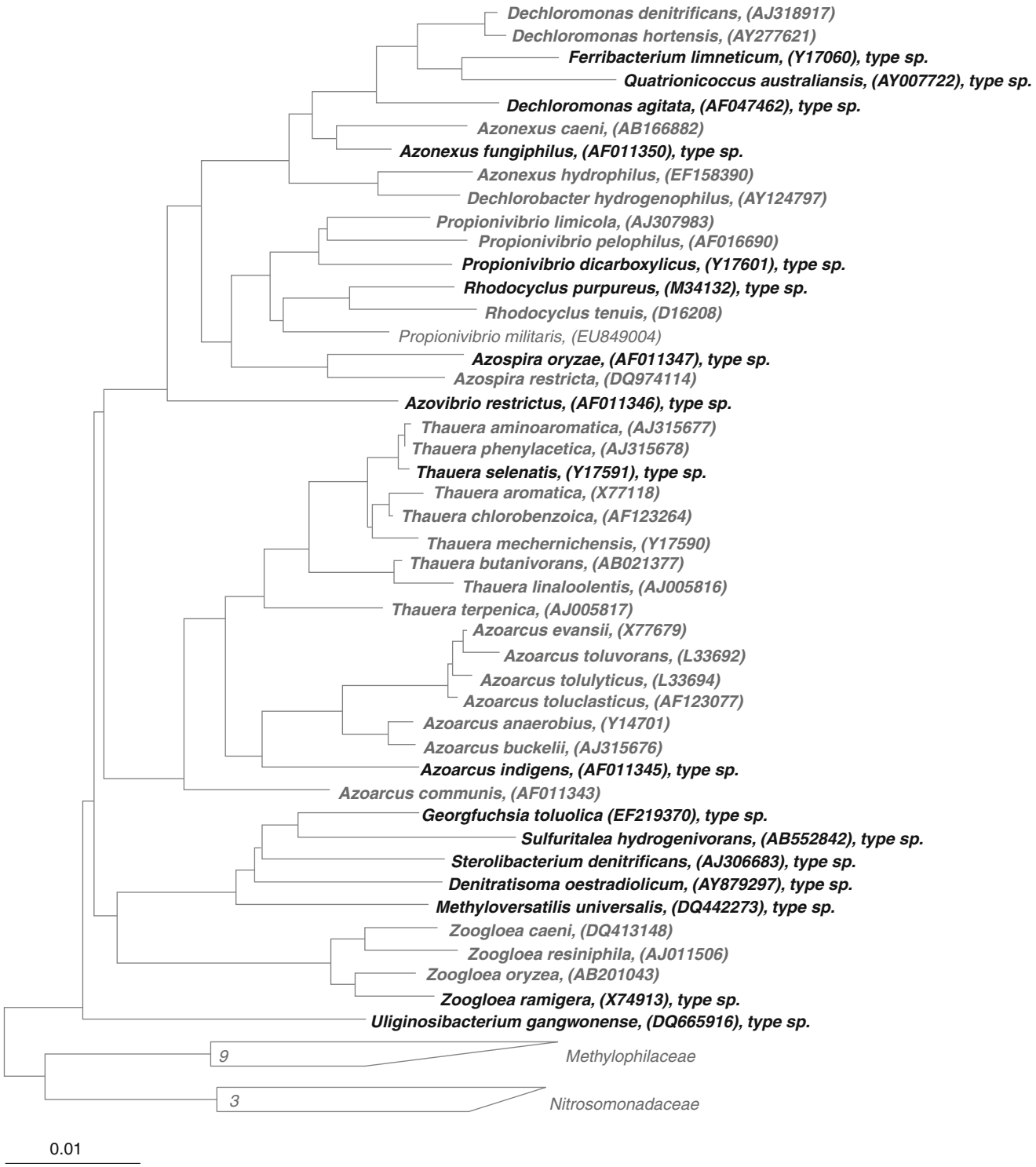

Fig. 36.1

Neighbor-joining genealogy reconstruction of the 45 species of the order *Rhodocyclales* present in the LTP_106 (Yarza et al. 2010). The tree was reconstructed by using a subset of sequences 767 type strains of Bacteria and Archaea to stabilize the tree topology. In addition, a 40 % conservational filter for the whole bacterial domain was used to remove hypervariable positions. Numbers in triangles denote number of taxa included. The bar indicates 1 % sequence divergence

salts of certain aromatic acids (e.g., benzoate and *m*-toluate). Benzene derivatives are attacked by *meta*-cleavage of the ring structure. Organic nitrogen compounds (e.g., dicarboxylic amino acids) and ammonia serve as nitrogen sources; nitrate is unsuitable. Specific growth factor requirements, if any, are unknown. Major cellular fatty acids are $C_{16:1}$ and $C_{10:0\ 3\text{-OH}}$ with smaller amounts of $C_{12:0\ 3\text{-OH}}$. Major respiratory quinones are Q-8 and RQ-8. The major polyamine is 2-hydroxyputrescine. Occur free-living in organically polluted waters and in wastewaters at all stages of treatment.

The mol% G + C of the DNA is 65.1–69.0.

Type species: *Zoogloea ramigera*.

Four species are currently recognized within the genus: *Z. ramigera*, *Z.caeni*, *Z. oryzae*, and *Z. resiniphila* (Table 36.6).

The Chlorate Reducers of the Genera *Dechloromonas* and *Dechlorobacter*

Genus *Dechloromonas* Achenbach, Michaelidou, Bruce, Fryman, and Coates 2001, 531[VP]

De.chlo.ro.mo'nas L. pref. *de*, from; N.L. n. *chlorinum* (from Gr. adj. *chlorôs*, green) chlorine; N.L. pref. *chloro-* pertaining to chlorine; L. fem. n. *monas*, unit, monad; N.L. fem. n. *Dechloromonas*, a dechlorinating monad.

Gram-negative, non-sporeforming, facultatively anaerobic, rods, 0.5 × 2 μm, motile by a single polar flagellum. Occur singly or in chains of two or three cells. Strictly respiratory metabolism. Oxidizes acetate with O_2, chlorate, perchlorate, or nitrate. Chlorate and perchlorate are reduced to chloride; O_2 also serves as an electron acceptor; some strains use nitrate as an electron acceptor.

The mol% G + C of the DNA is 61.2–63.5.

Type species: *Dechloromonas agitata*.

Currently the genus contains three species: *D. agitata*, *D. denitrificans*, and *D. hortensis* (Table 36.7).

Genus *Dechlorobacter* (Effective Publication: Thrash, Pollock, Torok, and Coates, 2010, 340)

De.chlo.ro.bac'ter. L. pref. *de*, from; N.L. n. *chlorinum* (from Gr. adj. *chlorôs*, green) chlorine; N.L. pref. *chloro-* pertaining to chlorine; masc. n. *bacter* a short rod; N.L. masc. n. *Dechlorobacter*, a dechlorinating rod.

Rod-shaped cells, 0.3 × 0.8–1.6 μm, non-sporeforming, non-fermentative, facultative anaerobe. Cells are motile and occur singly or in chains. Gram negative. Strictly respiring, complete oxidizer that oxidizes acetate with O_2, perchlorate, chlorate, Mn(IV), or nitrate as electron acceptors. Perchlorate and chlorate are completely reduced to chloride.

The mol% G + C of the DNA of the type strain of the type species was not reported.

Type species: *Dechlorobacter hydrogenophilus*.

At the time of writing (April 2012), the names *Dechlorobacter* and *Dechlorobacter hydrogenophilus*, the only species yet assigned to the genus, were not yet validly published. Further information about its properties is given in Table 36.7.

The Sterol Degraders of the Monospecific Genera *Sterolibacterium* and *Denitratisoma*

Genus *Sterolibacterium* Tarlera and Denner 2003, 1089[VP]

Ste.ro.li.bac.te'ri.um. N.L. neut. n. *sterolum*, sterol; L. neut. n. *bacterium*, small rod; N.L. neut. n. *Sterolibacterium*, sterol-utilizing small rod.

Straight or slightly curved, small, rod-shaped, Gram-negative cells. Mesophilic. Strictly respiratory type of metabolism with oxygen or nitrate as terminal electron acceptor. Nitrate is reduced to N_2. Oxidase- and catalase positive. Chemoorganoheterotrophic. Does not grow on complex media. Cholesterol is completely oxidized to CO_2; this reaction is coupled to nitrate reduction. Q-8 is the sole lipoquinone. Phosphatidylethanolamine is the predominant polar lipid. Major fatty acids are $C_{16:0}$, summed feature 4 ($C_{16:1\ \omega7c}$ and/or iso-$C_{15:0\ 2\text{-OH}}$), $C_{18:1\ \omega7c}$ and $C_{10:0\ 3\text{-OH}}$; minor amounts of $C_{8:0\ 3\text{-OH}}$ and $C_{16:0\ 3\text{-OH}}$ are present.

The mol% G + C of the DNA of the type species, thus far the sole species of the genus, is 65.3.

Type species: *Sterolibacterium denitrificans*.

Genus *Denitratisoma* Fahrbach, Kuever, Meinke, Kämpfer, and Hollender 2006, 1549

De.ni.tra.ti.so'ma L. pref. *de-*, away, from; N.L. n. *nitras –atis*, nitrate; Gr. neut. n. *soma* body; N.L. neut. n. *Denitratisoma*, a body that reduces nitrate.

Cells are Gram negative, non-sporeforming, motile, curved rods (0.4–0.8 × 0.8–2.0 μm in size) with rounded ends, and occur singly or in pairs. In cultures grown on sodium acetate and nitrate, more elongated cells with light-refracting inclusions predominate (length up to 2.0 μm). Metabolism is strictly oxidative. Nitrate is reduced to a mixture of N_2O and N_2; nitrite accumulates intermediately. Oxidase positive and catalase negative. Anaerobic oxidation of 17β-oestradiol to CO_2 with nitrate as electron acceptor, but no growth with cholesterol or C19 steroids such as testosterone or 4-androstene-3,17-dione. Electron acceptors nitrate, nitrite, or (per)chlorate are used; sulfate or sulfite is not reduced. Optimal growth occurs at 28–30 °C and pH 7.0–7.2. Salinity range for growth is 0–1.0 % NaCl. Ubiquinone-8 is the only quinone. Major fatty acids are $C_{16:1\omega7c}$/iso-$C_{15:0\ 2\text{-OH}}$ and $C_{16:0}$. Minor components are $C_{18:1\ \omega7c}$ and $C_{8:0\ 3\text{-OH}}$ (as the only hydroxylated fatty acid).

The mol% G + C of the DNA of the type strain is 61.4.

Type species: *Denitratisoma oestradiolicum*.

The properties of *Sterolibacterium denitrificans* and *Denitratisoma oestradiolicum* are summarized in Table 36.8.

The Disparate Monospecific Aerobic Genera *Methyloversatilis*, *Quatrionicoccus*, *Uliginosibacterium*, and *Sulfuritalea*

Genus *Methyloversatilis* Kalyuzhnaya, De Marco, Bowerman, Pacheco, Lara, Lidstrom, and Chistoserdova 2006, 2521[VP]

Me.thy.lo.ver.sa'ti.ils N.Gr. n. *methyl* (from Gr. n. *methu*, wine and Gr. n. *hulê*, wood), methyl radical; L. adj. *versatilis*, versatile; N.L. fem. n. *Methyloversatilis*, versatile methyl (utilizer), reflecting the versatile trophic abilities of the first isolates.

Cells are Gram negative, nonmotile rods that multiply by binary fission. Able to utilize a variety of C_1 and multicarbon compounds. Assimilate C1 units via the serine pathway. The major fatty acids are $C_{16:1\ \omega7c}$ and $C_{16:0}$. The major quinone is Q8.

The mol% G + C of the DNA is 64–65.

Type species: *Methyloversatilis universalis*.

Genus *Quatrionicoccus* Tindall and Euzéby 2006, 2712[VP]

Qua.tri.o.ni.coc'cus. L. masc. n. *quatrio -onis*, the number four; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos*, grain, seed), coccus; N.L. masc. n. *Quatrionicoccus*, four spherical cells.

Large Gram-negative, non-sporeforming cocci, 2.2–4.5 μm in diameter, occurring in tetrads. Cells are aerobic, nonmotile, and no flagella are observed. Both polyphosphate and poly-β-hydroxyalkanoate granules are synthesized. Oxidase negative. Catalase positive.

The mol% G + C of the DNA of the type species, thus far the sole species of the genus, is 67.

Type species: *Quatrionicoccus australiensis*.

Note: The name *Quatrionicoccus* (Tindall and Euzéby 2006) was proposed to replace the name *Quadricoccus* (Maszenan et al. 2002) which was illegitimate as it was earlier used to name an algal genus.

Genus *Sulfuritalea* Kojima and Fukui 2011, 1654[VP]

Sul.fu.ri.ta'le.a L. n. *sulfur*, sulfur; L. fem. n. *talea*, a rod; N.L. fem. n. *Sulfuritalea*, sulfur-oxidizing rod.

Grows chemolithoautotrophically under anoxic conditions by the oxidation of reduced sulfur compounds and hydrogen. Heterotrophic growth occurs on organic acids. Major fatty acids are $C_{16:0}$ and summed feature 3 ($C_{16:1\ \omega7c}$ and/or iso-$C_{15:0\ 2\text{-}OH}$).

The mol% G + C of the DNA of the type species, thus far the sole species of the genus, is 67.

Type species: *Sulfuritalea hydrogenivorans*.

Genus *Uliginosibacterium* Weon, Kim, Yoo, Kwon, Go, and Stackebrandt 2008, 134[VP]

U.li.gi.no.si.bac.te'ri.um. L. adj. *uliginosus*, wet, moist, marshy; L. neut. n. *bacterium*, a rod; N.L. neut. n. *Uliginosibacterium*, a rod isolated from peat.

Gram-negative, strictly aerobic rods. Motile by means of a single polar flagellum. The major isoprenoid quinone is Q-8. The major fatty acids are $C_{16:0}$ and summed feature 3 ($C_{16:1\ \omega7c}$ and/or iso-$C_{15:0\ 2\text{-}OH}$). $C_{18:1\ \omega7c}$, $C_{17:0\ cyclo}$, $C_{14:0}$, and $C_{12:0\ 3\text{-}OH}$ are present as minor components.

The mol% G + C of the DNA of the type strain of the single species is 59.3.

Type species: *Uliginosibacterium gangwonense*.

Table 36.9 summarizes the properties of *Methyloversatilis universalis*, *Quatrionicoccus australiensis*, *Sulfuritalea hydrogenivorans*, and *Uliginosibacterium gangwonense*.

The Genera *Propionivibrio*, *Ferribacterium*, and *Georgfuchsia* Showing Different Types of Anaerobic Metabolism

Genus *Propionivibrio* Tanaka, Nakamura, and Mikami 1991, 331[VP], Emend. Brune, Ludwig, and Schink 2002, 443 (Effective Publication: Tanaka, Nakamura, and Mikami 1990, 327)

Pro.pi.o.ni.vi'bri.o. N.L. n. *acidum propionicum*, propionic acid; L. v. *vibrio*, to set in tremulous motion, move to and fro, vibrate; N.L. masc. n. *vibrio*, that which vibrates, and also a bacterial genus name of bacteria possessing a curved rod shape (*Vibrio*); N.L. masc. n. *Propionivibrio*, the propionic acid vibrio.

Gram-negative, curved or straight rods, may be motile by means of a single polar flagellum. Does not form spores. Strict anaerobe or aerotolerant. Chemoorganoheterotrophic. Propionate and acetate formed as end products of fermentation. Some species grow on sugars, dicarboxylic acids, sugar alcohols, and aspartate. Succinate is decarboxylated to propionate. Some species may reduce nitrate to nitrite. Mesophilic.

The mol% G + C of the DNA is 60.8–61.6.

Type species: *Propionivibrio dicarboxylicus*.

Currently the genus contains four species: *P. dicarboxylicus*, *P. limicola*, *P. pelophilus*, and *P. militaris*. The name of the last species was not yet validly published. The species was classified within the genus *Propionivibrio* only on account of its 16S rRNA gene sequence, without taking its further properties into account. The mode of life of this species (respiratory rather than fermentative) is very different from those given in the genus protologue, and therefore the organism cannot be considered to belong to the genus *Propionivibrio*. *P. militaris* completely oxidizes acetate using O_2, perchlorate, chlorate, nitrate, or nitrite as electron acceptors. It can also grow on propionate (Thrash et al. 2010).

Propionibacter pelophilus (Meijer et al. 1999) has been reclassified as *Propionivibrio pelophilus* comb. nov. (Brune et al. 2002).

Genus *Ferribacterium* Cummings, Caccavo, Spring, and Rosenzweig 2000, 953T (Validation List no. 77); Effective Publication Cummings, Caccavo, Spring, and Rosenzweig 1999, 187

Fer.ri.bac.te'ri.um. L. n. *ferrum*, iron; L. neut. n. *bacterium*, a small rod; N.L. neut. n. *Ferribacterium*, rod-shaped iron bacterium.

Strictly anaerobic straight or curved motile rods, 0.3–0.5 × 1.4–2.0 μm. Contain poly-β-hydroxybutyrate granules. Non-sporeforming. Oxidize acetate, benzoate, formate, and lactate using Fe(III) as the electron acceptor; also oxidize acetate using fumarate or nitrate as electron acceptors. Hydrogen and alcohols are not used as electron donors.

The mol% G + C of the DNA of the type species and only species described thus far has not been reported.

Type species: *Ferribacterium limneticum*.

Genus *Georgfuchsia* Weelink, van Doesburg, Saia, Rijpstra, Röling, Smidt, and Stams 2011, 1011^{VP} (Validation List no. 139); (Effective Publication: Weelink, van Doesburg, Saia, Rijpstra, Röling, Smidt, and Stams 2009, 583)

Ge.org.fuch'si.a N.L. fem. n. *Georgfuchsia*, named after Georg Fuchs, for his contribution to our present understanding of anaerobic microbial growth on aromatic hydrocarbons.

Straight or slightly curved, small, rod-shaped Gram-negative cells. Mesophilic. Strictly respiratory type of metabolism. Chemoorganoheterotrophic. Capable of degrading aromatic compounds.

The mol% G + C of the DNA of the type species and only species described thus far has not been reported.

Type species: *Georgfuchsia toluolica*.

The main features of the members of the genus *Propionivibrio*, *Ferribacterium*, and *Georgfuchsia* are summarized in Table 36.10.

Isolation, Enrichment, and Maintenance Procedures

There are relatively few procedures for the selective enrichment and isolation of members of the different genera and species of the *Rhodocyclaceae*. The type species of the type genus, *Rhodocyclus purpureus*, has been isolated only once. Theoretically, a suitable enrichment procedure could be based on incubation in the light using media with benzoate and vitamin B_{12} and without added reduced sulfur compounds. Benzoate is not a common substrate for purple nonsulfur bacteria, but is used by *R. purpureus* (Imhoff 2005).

The growth potentials of the different species within the family, as defined in the species descriptions and highlighted in Tables 36.2–36.10, provide strategies for the isolation of novel types belonging to the group. Especially the ability of some genera and species to metabolize aromatic compounds, sterols, and other organic substrates that are degraded by a few types of organisms only under anaerobic conditions, using nitrate, chlorate, or perchlorate as electron acceptors, may be exploited for the enrichment and isolation of novel members of *Azoarcus*, *Thauera*, *Georgfuchsia*, and other genera. *Ferribacterium limneticum* can be enriched using media containing acetate and Fe(III)-oxyhydroxide (Cummings et al. 1999). Maleate was suggested to be a substrate for the selective enrichment of *Propionivibrio dicarboxylicus* (Tanaka et al. 1990). The ability of some species to fix nitrogen can also be exploited when designing specific enrichment and isolation protocols for selected members of the family.

Maintenance

Long-term preservation is by lyophilization, or by freezing cell suspensions in liquid nitrogen or at −80 °C in the presence of a cryoprotectant (Heider and Fuchs 2005; Reinhold-Hurek et al. 2005; Unz 2005). For *Azoarcus* and for *Thauera*, 5 % DMSO was recommended as cryoprotectant (Heider and Fuchs 2005; Reinhold-Hurek and Hurek 2006; Reinhold-Hurek et al. 2005). Preservation in liquid nitrogen with 8 % glycerol was suggested for *Propionivibrio pelophilus* (Hansen 2005). For the anaerobes within the family, anaerobic conditions must be maintained during the preparation of cell suspensions for lyophilization or cryopreservation.

Physiological and Biochemical Features

A feature of special interest shown by many representatives of the *Rhodocyclales* is their ability to degrade a wide range of aromatic and other difficult-to-metabolize organic compounds under anaerobic conditions, and sometimes under aerobic conditions as well. Compounds degraded by representatives of the genera *Azoarcus*, *Azospira*, *Dechloromonas*, *Thauera*, *Sterolibacterium*, *Denitratisoma*, and *Georgfuchsia* include benzene; toluene; catechol (1,2-dihydroxybenzene); ethylbenzene; *o*-, *m*-, and *p*-xylene *m*- and *p*-cresol; benzoate; 3-chlorobenzoate; 3-bromobenzoate; 2-fluorobenzoate; 4-fluorobenzoate; 3-hydroxybenzoate; 4-hydroxybenzoate; 2-aminobenzoate (anthranilic acid) 3-aminobenzoate; protocatechuate; and pyridine; monoterpenes such as linalool, menthol, and eucalyptol, cholesterol, testosterone, and 4-androstene-3,17-dione (Beller and Spormann 1997; Braun and Gibson 1984; Chakraborty et al. 2005; Chiang et al. 2007, 2008; Ding et al. 2008; Fahrbach et al. 2006; Heider and Fuchs 2005; Kniemeyer and Heider 2001; Rhee et al. 1997; Song et al. 2000). The herbicide benazolin-ethyl (4-chloro-2-oxobenzothiazolin-3-yl-acetic acid) was found to be degraded by a strain of *Methyloversatilis* isolated from activated sludge from a wastewater treatment pool (Cai et al. 2011).

The biochemical pathways used in the anaerobic breakdown (and sometimes in the aerobic degradation as well) of aromatic compounds are often highly unusual. Therefore, the biochemistry of some of these pathways has been investigated in depth in recent years. One well-established anaerobic pathway of activation of aromatic rings (toluene, xylenes) operative in some members of the family is by the addition of fumarate to form benzylsuccinate (Beller and Spormann 1997), catalyzed by benzylsuccinate synthase via a glycyl radical (Krieger et al. 2001). This pathway was characterized in *Thauera* sp. strain DNT-1 (Shinoda et al. 2004). An isolate related to *Denitratisoma oestradiolicum* obtained from a mixed inoculum of freshwater pond sediment and wastewater treatment plant sludge completely oxidized *p*-xylene to CO_2 with nitrate as electron acceptor. 4-Methylbenzene(succinate) and (4-methylphenyl) itaconate were identified as metabolites, suggesting addition of fumarate as an initial activation reaction (Rotaru et al. 2010).

Anaerobic degradation of catechol (1,2-dihydroxybenzene) by *Thauera aromatica* with CO_2 as cosubstrate and nitrate as electron acceptor proceeds via catechol-phosphate followed by carboxylation in the *para* position by phenylphosphate carboxylase. The product protocatechuate (3,4-dihydroxybenzoate) is converted to protocatechuyl-CoA which is then reductively dehydroxylated to 3-hydroxybenzoyl-CoA, followed by reduction of the aromatic ring (Ding et al. 2008).

Hydroxyhydroquinone (1,2,4-trihydroxybenzene) was identified as intermediate in the degradation of resorcinol (1,3-dihydroxybenzene) by *Azoarcus anaerobius* (Philipp and Schink 2000). Denitrifying degradation of ethylbenzene by *Azoarcus* sp. strain EbN1 proceed via two sequential dehydrogenation reactions forming (*S*)-1-phenylethanol and then acetophenone (Kniemeyer and Heider 2001).

Degradation of cholesterol under denitrifying conditions by *Sterolibacterium denitrificans* starts with the oxidation of ring A to form cholest-4-en-3-one, followed by an oxygen-independent hydroxylation of the terminal C-25 of the side chain by a cholest-4-en-3-one-Δ^1-dehydrogenase. The anaerobic hydroxylation of this tertiary carbon using water as oxygen donor is unprecedented and may be catalyzed by a novel molybdenum-containing enzyme (Chiang et al. 2007, 2008).

Aerobic degradation of aromatic ring compounds by *Thauera aromatica* and other *Thauera* strains uses common oxygenases (Heider and Fuchs 2005; Shinoda et al. 2004). An unusual mode of aerobic metabolism of benzoate was found in *Azoarcus evansii*, an organism that metabolizes benzoate and 3-hydroxybenzoate both under aerobic and anaerobic conditions. None of the previously known aerobic pathways, including the conversion of benzoate to catechol or protocatechuate, is operative in this organism. The first step is the activation of benzoate to benzoyl-CoA by a benzoate-CoA ligase. It was first assumed that the following hydroxylation of benzoyl-CoA is catalyzed by a benzoyl-CoA oxygenase (Mohamed et al. 2001); however, it was recently shown that the reaction proceeds via formation of the 2,3-epoxide (Rather et al. 2010).

Metabolism of methylamine by *Methyloversatilis universalis* involves an unusual glutamate-mediated pathway in which *N*-methylglutamate and γ-glutamylmethylamide are intermediates. Cells grown on methylamine displayed high activities of *N*-methylglutamate synthetase, *N*-methylglutamate dehydrogenase, and γ-glutamylmethylamide synthetase (Latypova et al. 2010).

Ecology

The range of habitats inhabited by members of the *Rhodocyclales* is as diverse as the metabolic properties displayed by the genera and the species of the order.

Anoxygenic phototrophs of the genus *Rhodocyclus* have been isolated both from polluted environments (*R. purpureus* was obtained from a swine wastewater lagoon) and from unpolluted freshwater ponds (*R. tenuis*). They have not been frequently encountered, and little is known about their distribution worldwide (Imhoff 2005; Pfennig 1969, 1978).

The genera *Azoarcus*, *Azonexus*, and *Azospira* consist of two types of organisms: those that grow epi- or endophyically on grass roots (rice, Kallar grass), and soilborne strains. The soilborne organisms that can degrade aromatic hydrocarbons under denitrifying conditions have been isolated from a wide range of environments, polluted as well as uncontaminated soils, aquifers, activated sludge, and oil refinery sludge. Most soilborne species grow on aromatic compounds such as toluene or phenol, benzoate or resorcinol (Anders et al. 1995; Bae et al. 2007; Braun and Gibson 1984; Chou et al. 2008; Fries et al. 1994, 1997; Quan et al. 2006; Reinhold-Hurek and Hurek 2006; Song et al. 1999; Springer et al. 1998; Zhou et al. 1995). The recent discovery of *Azoarcus* strains that oxidize As(III) using nitrate, nitrite, chlorate, and oxygen as electron acceptors in sediments or sludge from pristine environments (Rodríguez-Freire et al. 2012; Sun et al. 2009) shows that the group may be more diverse than assumed thus far.

Thauera strains appear to be common saprophytic inhabitants of polluted freshwater and soil environments. The type species *T. selenatis* was encountered in agricultural drainage water in California (Macy et al. 1989, 1993). *Thauera* strains occur in wastewater treatment plants, aquifers, and contaminated lake and pond sediment compounds where they may be involved in mineralization and detoxification of toxic aromatic compounds (Foss and Harder 1998; Heider and Fuchs 2005; Liu et al. 2006; Scholten et al. 1999).

Zoogloea, with its typical extracellular polysaccharide slime and floc formation displayed by most species, is known for a century and a half as a characteristic inhabitant of polluted waters and aerobic wastewater purification plants (Itzigsohn 1868; Mohn et al. 1999; Shao et al. 2009; Unz 2005), but representatives have also been isolated from rice paddy soils (Xie and Yokota 2006).

Chlorate- and perchlorate-reducing bacteria of the genera *Dechloromonas* and *Dechlorobacter* have been isolated both from pristine sites such as garden soil and from chlorate-polluted sites such as paper mill waste and soil from an army ammunition plant (Achenbach et al. 2001;

Bruce et al. 1999; Thrash et al. 2010; Wolterink et al. 2005) and even from the gut of an earthworm (Horn et al. 2005).

The sterol-degrading *Sterolibacterium denitrificans* and *Denitratisoma oestradiolicum* were both isolated from water treatment plants. Little information is available about their geographic distribution (Fahrbach et al. 2006; Tarlera and Denner 2003), but 16S rRNA gene sequences similar to that of *D. oestradiolicum* were found in anaerobic enrichment cultures with *p*-xylene and nitrate using a mixed inoculum of freshwater pond sediment and wastewater treatment plant sludge (Rotaru et al. 2010).

Methyloversatilis strains were recovered from different environments: Lake Washington, USA, and contaminated soil in Portugal (Kalyuzhnaya et al. 2006). Strain *Methyloversatilis* cd-1, which uses the herbicide benazolin-ethyl (4-chloro-2-oxobenzothiazolin-3-yl-acetic acid) as sole carbon source, was isolated from a wastewater treatment pool (Cai et al. 2011).

Little is known about the distribution of the genera *Quatrionicoccus* (isolated once from activated sludge biomass in Australia) (Maszenan et al. 2002), the facultative autotroph *Sulfuritalea* (obtained once from a freshwater lake in Japan) (Kojima and Fukui 2011), and *Uliginosibacterium*, reported once from a Korean wetland (Weon et al. 2008).

The obligatory anaerobic representatives of the genus *Propionibacter* were isolated from a marine estuary (Hansen et al. 1990; Meijer et al. 1999) or from freshwater ponds in Germany and Japan (Brune and Schink 1992; Tanaka et al. 1990). The iron-reducing *Ferribacterium limneticum* was recovered from the sediments of a lake in Idaho contaminated with tailings of mining activities (Cummings et al. 1999). The strictly anaerobic *Georgfuchsia toluolica*, degrading aromatic compounds with Fe(III), Mn(IV), or nitrate as an electron acceptor, was obtained from a polluted aquifer in the Netherlands (Weelink et al. 2009). No further information is available about the geographic distribution of these genera.

For molecular, culture-independent ecological studies of the *Rhodocyclales*, a 16S rRNA gene-based oligonucleotide microarray (RHC-PhyloChip) was developed. This PhyloChip, designed for environmental monitoring, consists of 79 probes based on sequences of cultured and as yet uncultured species. It was successfully applied to analyze the microbial diversity of activated sludge of an industrial wastewater (Loy et al. 2005). Use of stable isotope probing has shed some light on the presence of *Methyloversatilis* in a denitrifying sequencing batch reactor fed with ^{13}C-methanol. Analysis of 16S rRNA clone libraries prepared from the ^{13}C-enriched DNA fraction showed the dominant methylotrophs to be affiliated with the genera *Methyloversatilis* and *Hyphomicrobium* (Baytshtok et al. 2008). A study of activated sludge of a wastewater treatment plant in Denmark was performed using an isotope array approach, allowing for parallel functional probing of different phylogenetic groups. The PhyloChip microarray identified 27 operational taxonomic units that grouped in at least seven main *Rhodocyclales* lineages. The substrate utilization patterns of probe-defined populations were determined by radioactive isotope array analysis and microautoradiography—fluorescence in situ hybridization of samples that were briefly exposed to different radiolabeled substrates under oxic and anoxic nitrate-reducing conditions. Most detected *Rhodocyclales* groups were actively involved in nitrogen transformation, but varied in their consumption of propionate, butyrate, or toluene (Hesselsoe et al. 2009).

Pathogenicity, Clinical Relevance

No pathogenic bacteria are known within the *Rhodocyclales*. The plant-associated species of the genus *Azoarcus* that colonize rice roots and stems mainly live intracellularly within the cortex and they rarely enter xylem cells. No symptoms of plant disease were ever attributed to *Azoarcus* and related organisms. These plant-associated bacteria may contribute fixed nitrogen to the plant, but the existence of a truly mutualistic relationship was never proven (Reinhold-Hurek et al. 2005).

Sensitivity to antibiotics and other antibacterial compounds was reported only for a very few members of the order. *Azoarcus indigens*, *Azoarcus communis*, *Azoarcus* strain BH72, *Azonexus hydrophilus*, *Azonexus caeni*, and *Azonexus fungiphilus* and are all sensitive to ampicillin, kanamycin, streptomycin, and tetracycline. *Azonexus hydrophilus*, *Azonexus caeni*, and *Azonexus fungiphilus* are in addition sensitive to chloramphenicol, erythromycin, gentamycin, nalidixic acid, novobiocin, and penicillin (Chou et al. 2008; Reinhold-Hurek and Hurek 2006; Reinhold-Hurek et al. 2005). *Methyloversatilis universalis* proved resistant to ampicillin, erythromycin, chloramphenicol, trimethoprim/sulfamethoxazole, vancomycin, and penicillin, but sensitive to amoxicillin, cephalothin, kanamycin, nalidixic acid, tetracycline, streptomycin, neomycin, and gentamycin (Kalyuzhnaya et al. 2006).

Application

The floc-forming *Zoogloea* is always associated with activated sludge water purification plants and probably contributes much to the oxidation of organic matter.

Many representatives of the *Rhodocyclales* degrade aromatic compounds aerobically and/or anaerobically. Many of the aromatic compound-degrading species were isolated from wastewater treatment plants (*Tables 36.3–36.5*, *36.7*, *36.8*, and *36.11*), and they can be assumed to contribute there to the breakdown of aromatic compounds. Because of the versatile nature of species of, e.g., *Thauera*, *Dechloromonas*, and *Azoarcus* spp. able to degrade a wide range of aromatic compounds under different conditions, they have been suggested as suitable candidates for bioremediation operations for the removal of aromatic chemicals (Chakraborty et al. 2005; Mao et al. 2010; Reinhold-Hurek et al. 2005).

Thauera selenatis was isolated from selenium-rich agricultural drainage water in California, and its possible use for selenium bioremediation has been proposed (Macy et al. 1993).

A second anaerobic/microaerophilic isolate that reduces selenate and selenite to elemental red selenium is an *Azospira oryzae* (*Dechlorosoma suillum*) strain that developed in a laboratory reactor setup to remove selenate from groundwater (Hunter 2007). Another suggested way to remediate selenium-contaminated agricultural drainage with relatively high nitrate levels is by using a combination of a selenate-reducing isolate known as *Bacillus* sp. RS1 and *Dechloromonas* sp. strain HZ. In model experiments, the *Bacillus* reduced part of the Se(VI) to Se(IV). Addition of the *Dechloromonas* enhanced the Se reduction while simultaneously removing nitrate (Zhang and Frankenberger 2007).

The finding of perchlorate-reducing organisms such as *Dechlorobacter hydrogenophilus* in soil collected at an army ammunition plant (Thrash et al. 2010) shows that perchlorate reducers (*Dechloromonas*, *Dechlorobacter*, *Propionivibrio militaris*) may find applications in the bioremediation of soil and groundwater near ammunition factories and other perchlorate-polluted industrial areas.

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37 The Family *Spirillaceae*

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Abstract

Spirillaceae, a family with the order *Nitrosomonadales*, class of *Betaproteobacteria*, is monogeneric and contains only one genus *Spirillum*. Phylogenetic neighbors are the families *Nitrosomonadaceae*, *Methylophilaceae*, and species *Sulfuricella denitrificans*, which belong to the family *Hydrogenophilaceae*. Members of the family *Spirillaceae* (genus *Spirillum*) are a monophyletic branch of closely related organisms with similar phenotypic and genotypic characteristics. The genus is represented by three species (*S. volutans, S. winogradskyi*, and *S. kriegii*). All three species are morphologically close to each other and present themselves as large spiral cells. They are facultative microaerophiles with low level G + C (38–38.9 mol %) content and characterized by similar habitat environments. Preferably, they develop in stagnated water such as ponds, and industrial and domestic liquid-waste drains with microaerobic conditions and a high concentration of organic substrates.

Taxonomy: Historic and Current

Short Description of the Family and the Genus

Spirillaceae Migula 1894, 237[AL], Garritty, Bell and Lilburn, 2005

Spi.ril.la'ce.ae. ML.dim. neut.n. *Spirillum* type genus of the family; *aceae* ending to denote family; ML fem. pl.n. *Spirillaceae* – the *Spirillum* family.

The description is modified and an emended version of previous family description given on *Bergey's Manual*, 2nd edition (Garrity et al. 2005; Podkopaeva et al. 2006, 2005, 2009).

Based on phylogenetic analysis of the set of 16S rRNA gene sequence, the family *Spirillaceae* was placed in the phylum Betaproteobacteria of the order *Nitrosomonadales* (Garrity et al. 2005). The family *Spirillaceae* Migula 1894 comprises only genus *Spirillum* Ehrenberg 1832 (Krieg 2005) (type genus).

The genus *Spirillum* Ehrenberg 1832, 38[AL] is one of the oldest established bacterial genera. Prior to 1973, the genus *Spirillum* included several aerobic/microaerophilic chemoheterotrophic spirilla. Hylemon et al. (1973a) divided the genus into the three genera: *Spirillum*, *Aquaspirillum*, and *Oceanospirillum* on the basis of their DNA base composition and certain physiological characteristics. Currently, the genus *Spirillum* is reserved for large, microaerophilic, freshwater spirilla having DNA base composition of 38–38.9 mol % G + C (tm).

The 16SrRNA gene signature of the family *Spirillaceae* consists of: 121(C), 126–235 (A–U), 131–231 (U:A), 241–285 (C–G), 407–435 (G:C), 443–491 (G–C), 598–640 (C–G), 658–748 (U–A), 870 (A), 990 (U), 1124-1149 (G–C), 1131 (C).

Genus *Spirillum* Ehrenberg 1832 includes three species: *S. volutans*, *S. winogradskyi*, and *S. kriegii*.

Phylogenetic Structure of the Family and the Genus

According to the phylogenetic branching of proteobacterial type strains in the 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010), the family *Spirillaceae* is moderately related to the families *Nitrosomonadaceae*, *Methylophilaceae*, and *the* recently described type strain of *Sulfuricella denitrificans* (Kojima and Fukui 2010) from the family *Hydrogenophilaceae* (◗ *Figs. 37.1*, ◗ *37.2*). At present, the family *Spirillaceae* comprises a very narrow group of organisms. Only with the availability of sequences of newly described type strains within the family *Spirillaceae* will the phylogenetic boundary with close generic and suprageneric taxa be clarified.

After a loss of vital strains of type species of genus *Spirillum*, *S. volutans*, in international collections in 2009, it was recommended to place the name *Spirillum volutans* on the list of obsolete names, and to approve *S. winogradskyi* (Podkopaeva et al. 2009) as a neotype species of the genus. The vital type strain of the species *S. kriegii* was also lost, and is no longer maintained in any international collections.

E. Rosenberg et al. (eds.), *The Prokaryotes – Alphaproteobacteria and Betaproteobacteria*, DOI 10.1007/978-3-642-30197-1_242,

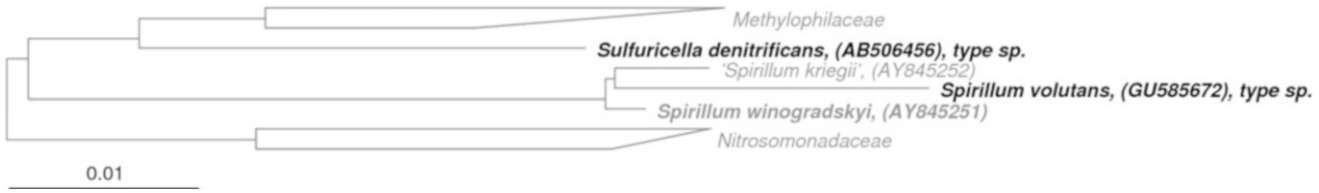

Fig. 37.1
Dendrogram of *Spirillaceae* and related families and species *Sulfuricella denitrificans* of the family *Hydrogenophilaceae* based upon 16S rRNA gene sequence analysis

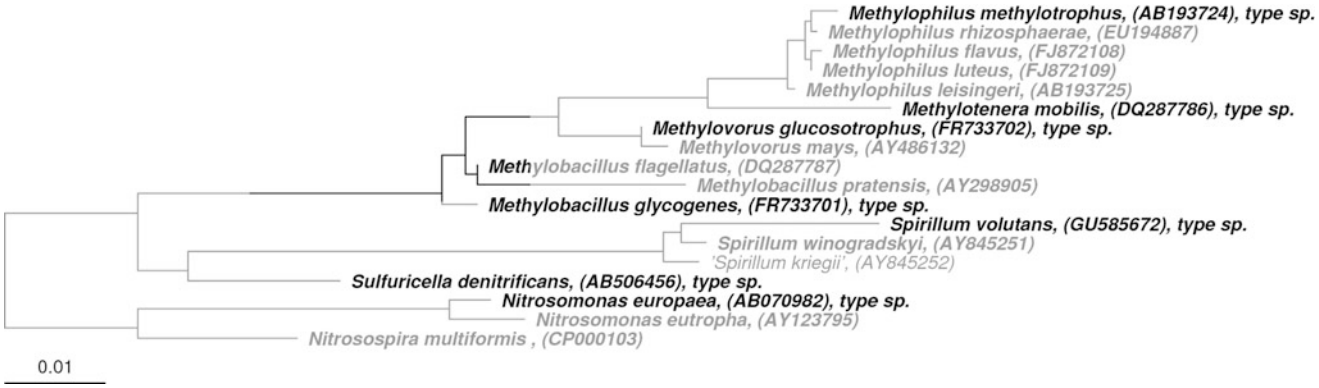

Fig. 37.2
Dendrogram of the type and the closest species of the genera *Spirillaceae* and related families

Phylogenetic reconstruction of the family *Spirillaceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, a 40% maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence.

Phenotypical Analyses

Main phenotypic features of the members of genus *Spirillum* (Family *Spirillaceae*) are given in *Table 37.1*.

Rigid helical cells, 1.4–2.1 * 10.5–60 μm motile by bipolar tufts of large flagella which are composed of 60–75 flagella and are visible in phase contrast microscope like one thick flagellum. Both cells and flagella of *S. winogradskyi* and *S. kriegii* are covered with sheath of polysaccharides (Podkopaeva et al. 2006).

Coccoid bodies with diameters up to 7–10 μm appear in *S. winogradskyi* and *S. kriegii* at the phase of stationary growth. In electron microscopy of ultrathin section reveals of the coccoid cells, with destruction of peptidoglycan layer of the cell wall and retention of the cytoplasmic membrane was evident. After passage in fresh medium under microaerobic conditions, coccoid cells revert to helical, allowing them to be considered as spheroplasts.

Intracellular poly-ß-hydroxybutyrate and polyphosphate granules are formed.

Some species (*S. winogradskyi* and *S. kriegii*) are capable of abundant accumulation of elemental sulfur inside the cells when cultured in the medium with sulfide.

Colonies of *S. volutans* are able to grow in a bilayer agar medium using Alban and Krieg's method (1996) which calls for pouring semisolid agar medium onto the surface of a Petri dish, and introducing 0.1–ml of inoculum added to 10 ml of semi-solid medium at 45 °C, poured onto the plates as an overlay, and incubated at 30 °C in the atmosphere of 6 % O_2 and 94 % N_2 for 3–4 days. Very small colonies with irregular form and uneven edges were grown in the upper agar strata. In contrast to *S. volutans, S. winogradskyi* and *S. kriegii* do not need bilayer agar for Petri dish cultivation, and they create small flat irregular form colonies with uneven edges 0.5–1 μm in diameter in the mass of agar medium. Their viability is about 3–5 days. *S. winogradskyi* is also able to grow on the surface of agar medium in the presence of reduced sulfur compound. Development of *S. kriegii* takes place within agar medium without reduced sulfur compound while exposing Petri dishes to air. This indicates a higher degree of microaerophily of *S. kriegii* in comparison with *S. winogradskyi*.

They have a strictly respiratory type of metabolism with oxygen as a terminal electron acceptor. They are facultative

Table 37.1
Phenotypic characteristics of representatives of the genus *Spirillum*

Properties	*S. volutans* ATCC 19553	*S. winogradskyi* DSM 12756	*S. kriegii*
Morphology			
Cell size, μm:	1.4–1.7	1.7–2.1	0.7–2.1
Width of cell	14.0–60.0	23.0–49.0	10.5–34.0
Length of helix	16.0–28.0	15.0–23.0	6.0–14.0
Height of wave Helix diameter	5.0–8.0	6.1–10.3	4.2–8.3
Bipolar tufts of flagella	+	+	+
Presence of sheathed flagella	ND	+	+
Coccoid bodies (spheroplasts)	–	+	+
Gram-stain	Negative	Negative	Negative
Cytoplasmic inclusions			
Poly-ß-hydroxybutyrate granules	+	+	+
Polyphosphates	+	+	+
Globule S^o			
Sulfide oxidation	ND	+	+
Thiosulfate oxidation	ND	–	–
Facultative microaerophils	+	+	+
Anaerobic growth in presence of			
Nitrate	–	–	–
Sulfate	ND	–	–
Fumarate	ND	–	–
Thiosulfate	ND	–	–
Elemental sulfur	ND	–	–
Aerobic reduction NO_3^-	–	–	–
Oxidase	+	+	+
H_2S from cysteine	+	+	+
Hydrolysis of casein	–	–	–
Hydrolysis of starch	–	–	–
Used substrata:			
2-oxoglutarate	±	+	+
Succinate	+	+	+
Fumarate	+	+	+
Malate	+	+	+
Oxaloacetate	+	+	+
Pyruvate	+	+	+
Acetate	–	+	+
Citrate	±	–[a]	–[a]
Aconitate	±	–[a]	–[a]
Isocitrate	±	–[a]	–[a]
Lactate	+	–	–[a]
Aminoacids (histidine, tyrosine, phenylalanine, alanine, glutamate, serine, aspartate, asparagine, glutamine, proline, valine, hydroxyproline, glycine, ornithine, arginine, lysine, methionine, cystine, cysteine, leucine, threonine, tryptophan)	–	–	–
Alcohols (propanol, mannite, glycerol, ethanol, butanol)	–	–	–
Carbohydrates (glucose, fructose, maltose, arabinose, galactose, saccharose)	–	–	–
Peptone	–	–	–
Starch	–	–	–
Casein hydrolysate	–	–	–
Resistance to NaCl, %	0.02	0.5	0.3
Range and optimum of O_2 concentration in gas phase, %	1–12 (6,0)	1–20 (2.0)	0.5–20 (0.8)
Catalase activity	+[b]	+[b]	+[b]
Requirement of vitamins	–	+	+
Range and optimum of pH	7.0–8.2	6.5–8.5 (7.5–7.8)	6.5–8.5 (7.0–7.5)
Range and optimum of temperature, °C	(30–36)	4–39 (28)	4–39 (33)
Mol. % G + C of DNA (Tm)	38	38	38,9

[a]Use in a presence of yeast extract
[b]Weak activity
+ positive
– negative
± use weakly
ND no data

microaerophils. Growth does not occur anaerobically with nitrate, sulfate, thiosulfate, elemental sulfur, or fumarate as electron acceptors. They also have weak catalase activity, and are oxidase-positive. Optimal temperature ranges from 27 °C to 33 °C for different species.

All three species use a limited number of organic substrates, mainly intermediate substances of the tricarboxylic acid cycle, such as succinate, fumarate, malate, oxaloacetate, and pyruvate. Additionally, in the presence of yeast extract, *S. winogradskyi*

Table 37.2
Cellular fatty acid composition of *S. winogradskyi* D-427^T. Values are percentages of total fatty acids. *a* Anteiso-branched, *i* iso-branched

Fatty acid	Proportion (%)
14:0	2.2
i-15:0	0.1
a-15:0	0.5
15:0	1.9
16:1	36.4
16:0	32.1
a-17:0	0.6
17:1	2.5
17:0	1.8
18:1Δ11	17.9
18:0	3.8
20:0	0.2

uses citrate, aconitate, and isocitrate, and *S. kriegii* uses lactate. While *S. volutans* is able to grow on lactate, *S. winogradskyi* and *S. kriegii* can grow on acetate and 2-oxoglutarate in the absence of yeast extract. Representatives of the genus *Spirillum* do not use as a source of carbon: aminoacids (histidine, tyrosine, phenylalanine, alanine, glutamate, serine, aspartate, asparagine, glutamine, proline, valine, hydroxyproline, glycine, ornithine, arginine, lysine, methionine, cystine, cysteine, leucine, threonine, and tryptophan); alcohols (propanol, mannite, glycerol, ethanol, and butanol); carbohydrates (glucose, fructose, maltose, arabinose, galactose, and saccharose); and polymer compounds (peptone, starch, and casein hydrolysate).

As a source of nitrogen, the bacteria use ammonium salts, peptone, and casein hydrolysate. They are unable to utilize nitrate, nitrite, and individual amino acids. The nitrates are not reduced to nitrites, and the bacteria do not grow in a presence of NaCI higher than 0.5 % concentration.

In the presence of organic growth substrate, *S. winogradskyi* and *S. κriegii* are capable of oxidizing sulfide and polysulfide to elemental sulfur with its accumulation in the cells, and of quantitatively oxidizing thiosulfate to tetrathionate, which accumulates in the medium (Podkopaeva et al. 2005). Enzymes of sulfur oxidative metabolism were not found in these bacteria, and oxidation of inorganic sulfur compounds is not associated with energetic metabolism, but takes place as a result of a chemical interaction of reduced sulfur with reactive oxygen species (ROS) (Podkopaeva et al. 2003, 2005).

Analysis of the composition of fatty acids in cells was done for *S. winogradskyi* only. Major cellular fatty acids were 16:0, 16:1, and 18:1. The detailed fatty acid profile of *S. winogradskyi* D-427^T is shown in *Table 37.2*.

Several physiological and biochemical tests were carried out for *S. volutans* only. *S. volutans* is relatively inert biochemically in most routine characterization tests, but is phosphatase-positive (Hylemon et al. 1973b). It is negative for: enzymes DNase, RNase, urease, and arylsulfatase; gelatin liquefaction; hydrolysis of casein, starch, and esculin; indole production; reduction of selenite; and growth in the presence of 1 % bile or 1 % glycine. The response of *S. volutans* ATCC 19554 to 44 antibiotics and other chemotherapeutic agents was determined by Friedman (1987). The organism was inhibited by each of the agents tested except clindamycin, cloxacillin, lincomycin, and oxacillin. It is interesting that, although *S. volutans* respires with oxygen, its growth is completely inhibited by as little as 0.05 mkg/ml of metronidazole, a compound that is selectively toxic toward anaerobes. As indicated by Padgett and Krieg (1986), this provides circumstantial evidence for the occurrence of ferredoxins or flavodoxins in *S. volutans*, because metronidazole reduction is preferentially linked to these electron carriers.

The nucleotide DNA composition of all species of the genus *Spirillum* is close to each other: Content of G + C in DNA is 38–38.9 mol % (tm). DNA-DNA hybridization between *S. winogradskyi* and *S. volutans* has a degree of homology 12 %, and 28 % – with *S. kriegii* (Podkopaeva et al. 2006, 2009).

Influence of the Oxygen Regime of Cultivation on Growth and Metabolism

Though the known species of genus *Spirillum* prefer microaerobic growth conditions and traditionally belong to microaerophils, stationary growth is possible under specific conditions and on exposure to atmospheric air, particularly with the introduction of components which block toxic impact of H_2O_2 and other ROS to the medium: catalase (1–230 U/ml), SOD (4–30 U/ml), peptone (2 g/l), casein hydrolysate (2 g/l), pyruvate (1 g/l), norepinephrine (0.002 %), potassium metabisulfite (0.002–0.005 %), thiosulfate (1 g/l) correspond to these components (Bowdre et al. 1976; Padgett et al. 1982; Podkopaeva et al. 2003). The introduction of thiosulfate to the medium under aerobic conditions as a result of removing ROS by chemical interaction prevents cell lysis, doubles the cell harvest, and stabilizes the cultures (Podkopaeva et al. 2005).

Besides preventing cell lysis, augmentation of the cell harvest by thiosulfate oxidation is associated with decreasing expenditures on biosynthesis of exopolysaccharides as a way of protecting from abundance oxygen on the medium. Oxidation of thiosulfate in *S. winogradskyi* and *S. kriegii* takes place only during consumption of organic substrates and plays no part in energetic metabolism as inorganic donors of electrons.

Isolation, Enrichment, and Maintenance Procedures

Cultures of *S. volutans* occurring in hay infusions or other natural media can be enriched by inoculating Pringsheim's soil medium (Rittenberg and Rittenberg 1962). To date, the only

successful method for isolating *S. volutans* is the capillary method first described by Giesberger (1936) and used successfully with *S. volutans* by Rittenberg and Rittenberg (1962). Transfers from the capillary should be made directly to the test tubes of a suitable medium such as semisolid modified peptone-succinate-salts (MPSS) or casein hydrolysate-succinate-salts (CHSS). Media for *S. volutans*: MPSS broth (Caraway and Krieg 1972): Succinic acid (free acid) 1.0 g, $MgSO_4 \cdot 7H_2O$ 1.0 g, Bacto Peptone (Difco) 5.0 g, $(NH_4)_2SO_4$ 1.0 g, $FeCl_3 \cdot 6H_2O$ (0.2 % aqueous solution) 1.0 ml, $MnSO_4 \cdot H_2O$ (0.2 % aqueous solution) 1.0 ml.

The first four ingredients are dissolved in 1 l of distilled water and the pH is adjusted to 7.0 with 2 N KOH. The $FeCl_3$ and $MnSO_4$ solutions are added and the medium is sterilized by autoclaving. The medium is stored in the dark to avoid photochemical production of hydrogen peroxide. Semisolid MPSS medium is prepared by adding 0.15 % agar to the liquid medium. MPSS agar is prepared by adding 1.5 % agar to the liquid medium.

CHSS Broth (Padgett and Krieg 1986): Succinic acid (free acid) 1.0 g, $MgSO_4 \cdot 7H_2O$ 1.0 g, acid-hydrolyzed casein, "vitamin-free, salt-free" 2.5 g, $(NH_4)_2SO_4$ 1.0 g, NaCl 0.1 g, KH_2PO_4 0.14 g, $FeCl_3 \cdot 6H_2O$ (0.2 % aqueous solution) 1.0 ml, $MnSO_4 \cdot H_2O$ (0.2 % aqueous solution) 1.0 ml, potassium metabisulfite 0.05 g.

The first eight ingredients are dissolved in 1 l of distilled water and the pH is adjusted to 7.0 with 2 N KOH. The potassium metabisulfite is added, and the pH readjusted to 7.0. The medium is sterilized by autoclaving and stored in the dark.

Semisolid CHSS medium is prepared by adding 0.15 % agar to the medium after addition of the potassium metabisulfite, boiling to dissolve the agar, and then autoclaving. CHSS agar is prepared by adding 1.5 % agar to the liquid medium. Due to the difficulty of cultivating *S. volutans*, a detailed description of the method of medium preparation is given below. Goatcher et al. (1984) reported that Bactocasitone (Difco) could be substituted for the indicated casein hydrolysate, and that $MnSO_4$ could be omitted under these conditions. Bowdre and Krieg (1974) did not include NaCl in their original formulation of CHSS broth. However, Padgett et al. (1982) found that the casein hydrolysate used by Bowdre and Krieg was not completely salt-free. When salt-free batches of casein hydrolysate were used, a low level of NaCl was required for growth. Levels of NaCl greater than 0.02 % inhibited growth. Bowdre and Krieg (1974) did not include KH_2PO_4 in their original formulation of CHSS broth. However, its addition to the medium has since been found to increase the growth response of *S. volutans* (Friedman 1987). For *S. volutans*, optimal growth occurs between 30 °C and 36 °C and at pH values of 7.0–8.2 (Moore 1984). Although the organism is a microaerophile, cultures in semisolid media can be incubated in an air atmosphere due to stratification of the medium. Growth begins as a thin disk some distance below the surface at a point where the respiratory rate of the cells matches the rate of diffusion of oxygen to the cells. As the cell numbers increase, the disk becomes denser and migrates toward the surface. After 48 h, dense growth occurs just beneath the surface. Cultures grown in MPSS broth (no agar added) or on solid media for colony development should be incubated under an atmosphere of 6 % O_2 and 94 % N_2.

To isolate microaerophilic spirilla *S. winogradskyi* and *S. kriegii* from the sludge of municipal purification plants, samples were taken from aeration tanks of a wastewater treatment plant containing domestic wastewater, large spirilla, H_2S at a range between 1 and 2 mgl-1 and oxygen at 2–3.5 mgl-1 were used as passage material for the inoculation. A modified semisolid MPSS medium (Caraway and Krieg 1974) was used with a freshly prepared FeS suspension (Kucera and Wolfe 1957) with the following composition (l-1): 1 g $(NH_4)_2SO_4$; 1 g $MgSO_4$; 0.03 g $CaCl_2 \cdot 2H_2O$; 0.002 g $FeCl_3$ $6H_2O$; 0.002 g $MnSO_4$; 1 g sodium succinate; 1 g casein hydrolysate; 1 g agar (Difco). The pH of the medium was adjusted to 7.5. Vitamins and trace elements (Pfennig and Lippert 1966) were added before inoculation. Test tubes filled with medium at 2/3 volume were incubated at 28 °C after the passage, corresponding to the temperature in aeration tanks. After 3 days of incubation, thin white bands consisting of large motile spirilla with intracellular inclusions of elemental sulfur appeared in the medium at about 0.5–1.0 cm below the surface. These cells were collected with a capillary tube and transferred to a test tube. Enrichment cultures were obtained by using a serial 10-fold dilution technique in test tubes. The last positive tube was used for isolation on the same medium solidified with 1.5 % agar (Difco) in Petri dishes. After 5 days of incubation at 28 °C, small semitransparent colonies of large spiral cells appeared only below the agar surface. A single colony was transferred into liquid MPSS medium without FeS. Routine cultivation was carried out in liquid MPSS medium without FeS, casein hydrolysate being replaced by peptone at a concentration of 5 gl-1.

The members of the genus *Spirillum* may be maintained in semisolid MPSS of CHSS medium in test tubes exposed to air atmosphere at 28–30 °C, with transfer every 4–5 days for *S. volutans* and *S. kriegii*, and 14 days for *S. winogradskyi*.

Preservation

Preservation by lyophilization has not yet been possible for known species of the genus *Spirillum*, as well as preservation under −80 °C. Long-term preservation is possible in liquid nitrogen (Pauley and Krieg 1974) with 10 % dimethyl sulfoxide (DMSO) as cryoprotectant.

Ecology

Habitat

Representatives of the genus *Spirillum* are widespread in many stagnant fresh water reservoirs (Wells and Krieg 1965), polluted with organic substances, waste waters of treatment facilities, the cooling water of sugar refinery (Rittenberg and Rittenberg), and

aeration tanks of municipal sewage water treatment plants (Podkopaeva et al. 2006, 2009). As a rule, development of spirilla takes place in the water layer at a lower margin of oxygen distribution in microaerobic conditions. Species *S. winogradskyi* and *S. kriegii* were picked out from domestic waste water contained up to 1–2 mg/l of sulfide and 2.0–3.5 mg/l of oxygen.

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38 The Family *Sutterellaceae*

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Abstract

Sutterellaceae, a family within the order *Burkholderiales* in the lineage of phylum Proteobacteria (class *Betaproteobacteria*), includes the genera *Sutterella* and *Parasutterella*. The genus *Sutterella* contains three validly described species: *Sutterella wadsworthensis* (the type species), *Sutterella parvirubra*, and *Sutterella stercoricanis*. In contrast, the genus *Parasutterella* comprises the species *Parasutterella excrementihominis* (the type species) and *Parasutterella secunda*. Members of the family are mainly found in the intestinal tract of humans and some animals as members of the indigenous intestinal microbiota, and can be isolated from both the intestinal tract and from infections of gastrointestinal origin (*S. wadsworthensis*). The cells are Gram-negative rods or coccobacilli, and grow under anaerobic conditions or in a microaerophilic atmosphere. They are asaccharolytic and negative for oxidase and catalase activities. The main isoprenoid quinone is methylmenaquinone-5 (MMK-5) or MMK-6. The type genus is *Sutterella* (Wexler et al. Int J Syst Bacteriol 46:252–258, 1996).

Taxonomy, Historical and Current

Sutterellaceae (Sut.te.rel.la'ce.ae. N.L. fem. n. *Sutterella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Sutterellaceae*, the *Sutterella* family).

The genus *Sutterella* was first described by Wexler et al. (1996) and was placed within the family *Alcaligenaceae* in the second edition of *Bergey's Manual of Systematic Bacteriology* (Wexler 2005). The genus *Parasutterella* was described by Nagai et al. (2009) as the closest neighbor of genus *Sutterella*. Morotomi et al. (2011) proposed the new family *Sutterellaceae* to accommodate these two genera after finding that the genera *Sutterella* and *Parasutterella* formed a separate line of descent within the order *Burkholderiales*. In their research, this lineage could not be associated with any of the four known families (*Alcaligenaceae*, *Burkholderiaceae*, *Comamonadaceae*, and *Oxalobacteraceae*) in the order *Burkholderiales* (◗ *Fig. 38.1*). The rationale for the new family was based both on the distinct phylogenetic positions and on the biological and biochemical differences of the genera *Sutterella* and *Parasutterella* from known genera in the family *Alcaligenaceae*, which is phylogenetically the nearest neighboring family of the *Sutterellaceae*. All known members of the genera *Sutterella* and *Parasutterella* are oxidase- and catalase negative, and no aerobic growth has been observed, although some species may grow in a microaerophilic atmosphere. In contrast, species in the *Alcaligenaceae* are oxidase- and catalase positive, and can generally grow aerobically (◗ *Table 38.1*). All type strains of the *Sutterellaceae* contain $C_{18:1}\omega9c$ (32–68 % of the total) and $C_{16:0}$ (9–23 %) as the predominant fatty acids (◗ *Tables 38.1* and ◗ *38.2*). However, $C_{18:1}\omega9c$ has not been reported to be a major component in any species of the family *Alcaligenaceae* (◗ *Table 38.1*). The major respiratory quinone of the members of the *Sutterellaceae* is methylmenaquinone-5 (MMK-5) or MMK-6, whereas ubiqinones have not been detected (◗ *Tables 38.1* and ◗ *38.3*). In contrast, species of the family *Alcaligenaceae* have, in general, been characterized by the presence of ubiquinone-8 (Q-8) as the major isoprenoid quinone (◗ *Table 38.1*; Fletcher et al. 1987; Oyaizu-Masuchi and Komagata 1988).

Molecular Analyses

Phylogeny

On the basis of 16S rRNA gene sequence similarities, members of the family *Sutterellaceae* are members of the order *Burkholderiales*, with the nearest neighboring family being the *Alcaligenaceae* (◗ *Fig. 38.1*). The phylogenetic distance between type species of the family is relatively high. The 16S rRNA gene sequence of the type strain of *Sutterella wadsworthensis* shares 94.6 % similarities with the sequences of *Sutterella parvirubra* and *Sutterella stercoricanis*. The type strains of *S. parvirubra* and

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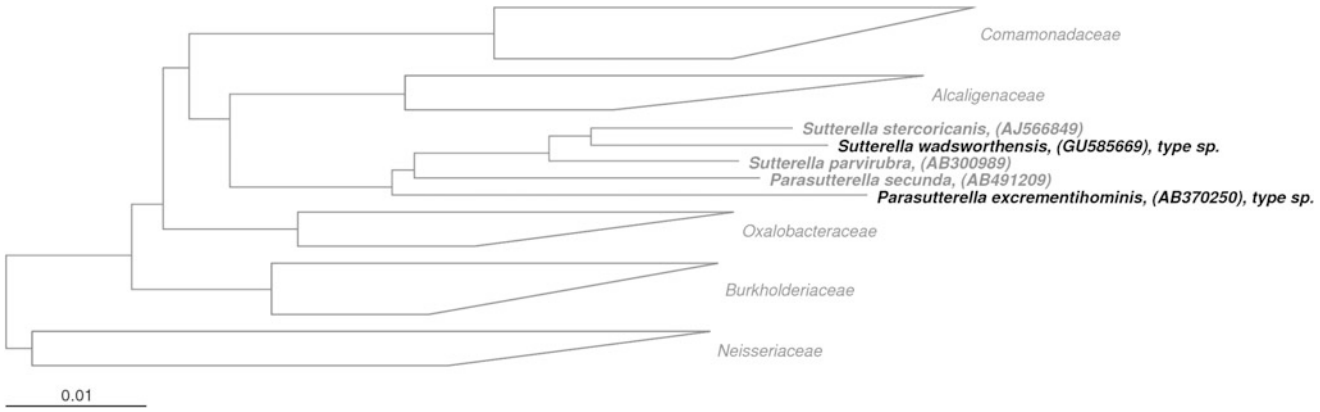

Fig. 38.1

Phylogenetic reconstruction of the family *Sutterellaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al., 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type-strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40% maximum-frequency filter was applied in order to remove hyper variable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence. This tree was provided by Dr. Raul Muñoz of the Instituto Mediterráneo de Estudios Avanzados

S. stercoricanis share 94.7 % similarity. The type strains of the two *Parasutterella* species, *Parasutterella excrementihominis* and *Parasutterella secunda*, share 90.0 % similarity. In contrast, similarities between members of the *Sutterellaceae* and type species of the neighboring family *Alcaligenaceae* range between 88.3 % and 90.9 %. Thus, all species described so far can be unambiguously identified by their 16S rRNA gene sequence (Morotomi et al. 2011).

Genome

Sutterella parvirubra YIT 11816^T, *P. excrementihominis* YIT 11859^T, and four strains of *S. wadsworthensis* (HGA0223, HGP1, 3_1_45B, and 2_1_59BFAA) were selected for inclusion in the catalog of reference genomes by the Human Microbiome Project (http://commonfund.nih.gov/hmp/) and, at the time of writing, the assembled and annotated genomic sequences of three strains (*S. parvirubra* YIT 11816^T, *P. excrementihominis* YIT 11859^T, and *S. wadsworthensis* 3_1_45B) have been submitted to the GenBank/EMBL/DDBJ databases. The genome size, G+C content, number of predicted protein-encoding genes, and number of predicted rRNA and tRNA genes of these strains are listed in *Table 38.4*. Details are available from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/).

DNA–DNA Hybridization

Sutterella wadsworthensis WAL 9799^T did not undergo DNA–DNA hybridization with DNA from either *Campylobacter gracilis* or other *Campylobacter* species (*Campylobacter rectus*, *Campylobacter curvus*, *Campylobacter consisus*, *Campylobacter sputorum*, and *Campylobacter showae*), which cannot be differentiated phenotypically (Wexler et al. 1996). The degree of the hybridization between *S. wadsworthensis* CCUG 42229^T (= WAL 9799^T) and *S. stercoricanis* 5BAC4^T was 35 %, which is low enough to be consistent with separate species status (Greetham et al. 2004). No hybridization studies have been done for the *Parasutterella* species, as the 16S rRNA gene sequence similarity between the type strains of the respective species was only 90.0 % that indicates separate genospecies (Morotomi et al. 2011).

Matrix-associated laser desorption/ionization—time of flight mass spectrometry (MALDI-TOF MS).

Eleven strains of *S. wadsworthensis*, including the type strain, were analyzed by MALDI-TOF MS, and the results included a dominant peak at approximately 9,400 Da that was common to all *S. wadsworthensis* strains (Mukhopadhya et al. 2011). No mass spectrometry data is available for any other species in the *Sutterellaceae*.

Neither riboprinting nor ribotyping analyses are available for any member of the *Sutterellaceae*.

Phenotypic Analyses

Members of the *Sutterellaceae* are strictly anaerobic or microaerophilic, non-motile, non-spore-forming, Gram-negative rods or coccobacilli. Biochemically, they are largely unreactive and asaccharolytic.

Differential characteristics of members of the family *Sutterellaceae* and those of the phylogenetically nearest family, the *Alcaligenaceae*, are listed in *Table 38.1*. All type strains of the genera *Sutterella* and *Parasutterella* cannot grow under aerobic conditions, and are oxidase- and catalase-negative

Table 38.1

Differential characteristics of members of the family *Sutterellaceae* and those of the phylogenetically nearest (Fig. 38.1) family, the *Alcaligenaceae*

		Aerobic growth	Oxidase	Catalase	Major fatty acids[a]	Major quinone	Reference
Sutterellaceae	1	–	–	–	$C_{18:1}\omega9c$, $C_{16:0}$, SF10	MMK-5	Morotomi et al. (2011)
	2	–	–	–	$C_{18:1}\omega9c$, $C_{16:0}$, $C_{16:1}\omega7c$	MK-5, MMK-5	Morotomi et al. (2011)
	3	–	–	–	$C_{18:1}\omega9c$, $C_{16:0}$	MK-5, MMK-5	Morotomi et al. (2011)
	4	–	–	–	$C_{18:1}\omega9c$	MK-6, MMK-6	Morotomi et al. (2011)
	5	–	–	–	$C_{18:1}\omega9c$, $C_{16:0}$, $C_{14:0}$	MK-5, MMK-5	Morotomi et al. (2011)
Alcaligenaceae	6	+	+	+	$C_{16:0}$, $C_{17:0}$cyclo, SF2	Q-8	Coenye et al. (2003), Busse and Aulling (2004b)
	7	+	+	+	$C_{18:1}\omega7C$, SF3, C16:0, SF2	ND	Coenye et al. (2005)
	8	+	+	+	$C_{16:0}$, $C_{17:0}$cyclo, $C_{14:0}$-3OH	Q-8	Lipski et al. (1992), Busse and Aulling (2004a)
	9	+	+	ND	$C_{16:1}\omega7C$, $C_{16:0}$	Q-8	Vancanneyt et al. (1995), von Wintzingerode et al. (2001), Sanden and Weyant (2004)
	10	+	+	+	$C_{18:1}\omega7C$, $C_{16:0}$, $C_{19:0}$cyclo$\omega8C$	ND	Willems et al. (2002)
	11	–	ND	ND	$C_{16:0}$, $C_{16:1}\omega7C$, SF7	Q-8	Kämpfer et al. (2006)
	12	+	+	–	$C_{18:1}\omega7C$, $C_{16:1}\omega7C$, $C_{16:0}$	Q-8	Xie and Yokota (2004)
	13	+	–	+	$C_{16:0}$, $C_{17:0}$cyclo, SF2, $C_{18:1}\omega7C$	ND	Coenye et al. (2003)
	14	+	+	+	$C_{18:1}\omega7C$, $C_{16:0}$	ND	Rossau et al. (1987)
	15	+	+	+	SF7, $C_{16:1}\omega7C$, $C_{16:0}$, SF2[b]	ND	Vandamme et al. (1998)
	16	+	+	+	$C_{16:0}$, $C_{17:0}$cyclo, $C_{19:0}$cyclo$\omega8C$	Q-8	Blümel et al. (2001)
	17	+	+	ND	$C_{17:0}$cyclo, $C_{19:1}$cyclo$\omega8C$, C16:0	Q-8	Stolz et al. (2005)
	18	–	+	+	SF7, $C_{16:0}$, SF2[b]	ND	Vandamme et al. (1998), Bleumink-Pluym and van der Zeijst (2005)
	19	+	+	+	$C_{18:1}\omega7C$, SF3, $C_{16:0}$, SF2	ND	Ghosh et al. (2005)

Taxa: 1 *Sutterella parvirubra* YIT 11816^T, 2 *Sutterella stercoricanis* DSM 17807^T, 3 *Sutterella wadsworthensis* DSM 14016^T, 4 *Parasutterella excrementihominis* YIT 11859^T, 5 *Parasutterella secunda* YIT 12071^T, 6 *Achromobacter xylosoxidans* (*n* = 10 strains), 7 *Advenella incenata* (*n* = 8), 8 *Alcaligenes faecalis* DSM 30033^T, 9 *Bordetella pertussis* (*n* = 12), 10 *Brackiella oedipodis* LMG 19451^T, 11 *Castellaniella defragrans* DSM 12141^T, 12 *Derxia gummosa* IAM 13946^T (=ATCC 15594^T), 13 *Kerstersia gyiorum* (*n* = 6), 14 *Oligella urethralis* (*n* = 8), 15 *Pelistega europaea* (*n* = 13), 16 *Pigmentiphaga kullae* K24^T, 17 *Pusillimonas noertemannii* BN9^T, 18 *Taylorella equigenitalis* LMG 6222^T, 19 *Tetrathiobacter kashmirensis* WT001^T

Symbols: + positive, – negative, *ND* no data available, *Q* ubiquinone

[a]Includes fatty acids that account for >10 % of the total, listed in descending order. Summed feature 2 (SF2) comprises 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with an equivalent chain length (ECL) of 10.928 or 12:0 aldehyde. SF3 comprises 16:1$\omega7c$ or 15:0 iso 2-OH. SF7 comprises $C_{17:1}\omega9c$ or an unknown fatty acid of ECL 16.760. SF10 comprises $C_{18:1}\omega7c$ or an unknown fatty acid of ECL 17.834. SF12 contains iso-$C_{19:0}$ or an unknown fatty acid of ECL 18.622.

[b]Referred to as SF3 by Vandamme et al. (1998)

(Table 38.1). In addition, they are negative for urease and indole production and for hydrolysis of gelatin and aesculin (Greetham et al. 2004; Sakon et al. 2008; Nagai et al. 2009; Morotomi et al. 2011 and unpublished). In contrast, species of the family *Alcaligenaceae* have, in general, the opposite characteristics (Table 38.1). All type strains of the *Sutterellaceae* contain $C_{18:1}\omega9c$ (32–68 %) and $C_{16:0}$ (9–23 %) as the predominant fatty acids, and MMK-5 or MMK-6 as the major respiratory quinone (Tables 38.1 and 38.2). The typical fragmentation of a ubiquinone ring nucleus at mole peak of $m/z = 197$ was not detected in these strains, indicating that ubiquinones are not present in the known strains of the genera *Sutterella* and *Parasutterella* (Morotomi et al. 2011). In contrast, species of the family *Alcaligenaceae* are characterized by the presence of ubiquinone-8 (Q-8) as the major isoprenoid quinone, and fatty acids other than $C_{18:1}\omega9c$ are the major cellular fatty acids (Table 38.1).

All type strains of the genera *Sutterella* and *Parasutterella* do not utilize the following API 20A substrates: L-arabinose, D-cellobiose, glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, D-raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, D-trehalose, and D-xylose (Greetham et al. 2004;

Sakon et al. 2008; Nagai et al. 2009; Morotomi et al. 2011 and unpublished). They are negative for the following API ZYM and API rapid ID 32 A reactions: N-acetyl-β-glucosaminidase, α-arabinosidase, chymotrypsin, cystine arylamidase, fermentation of mannose, fermentation of raffinose, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, glutamyl glutamic acid arylamidase, histidine arylamidase, indole production, lipase (C14), α-mannosidase, 6-phosphate β-galactosidase, proline arylamidase, pyroglutamic acid arylamidase, trypsin, urease, and valine arylamidase (Greetham et al. 2004; Sakon et al. 2008; Nagai et al. 2009; Morotomi et al. 2011 and unpublished). Diagnostic phenotypic differences for the fatty acid compositions of the type strains of the genera *Sutterella* and *Parasutterella* are listed in *Table 38.2*.

The fatty acid and isoprenoid quinone compositions of the type strains of the genera *Sutterella* and *Parasutterella* are listed in *Tables 38.2* and *38.3*, respectively. *Table 38.5* provides additional phenotypic details.

Table 38.2
Fatty acid compositions of the type strains of the genera *Sutterella* and *Parasutterella*

	% of total fatty acids				
Fatty acid	**1**	**2[a]**	**3**	**4**	**5**
Saturated straight-chain					
$C_{12:0}$	–	–	–	–	2.82
$C_{14:0}$	4.25	2.25	8.54	5.96	10.78
$C_{15:0}$	1.36	1.10	1.01	–	–
$C_{16:0}$	21.13	23.19	15.68	8.66	14.09
$C_{18:0}$	3.25	1.38	1.04	1.46	2.82
Unsaturated straight-chain					
$C_{16:1}\omega7c$	6.13	23.10	1.69	–	1.29
$C_{18:2}\omega6,9c$	1.27	–	–	–	–
$C_{18:1}\omega9c$	43.36	31.77	56.37	68.10	39.95
Hydroxy acids					
$C_{16:0}$ 3-OH	–	–	–	6.26	–
Dimethyl acetal (DMA)					
$C_{16:0}$ DMA	–	–	–	–	1.63
$C_{18:1}\omega9c$ DMA	–	–	–	–	6.95
Summed features[b]					
1	–	–	–	2.37	–
2	2.43	2.81	2.07	–	4.17
5	2.19	1.86	3.79	–	3.68
7	–	–	–	–	1.05
10	12.98	7.78	5.06	4.43	6.60
12	–	–	1.11	–	2.57

Taxa: 1 *Sutterella parvirubra* YIT 11816^T, 2 *Sutterella stercoricanis* DSM 17807^T, 3 *Sutterella wadsworthensis* DSM 14016^T, 4 *Parasutterella excrementihominis* YIT 11859^T, 5 *Parasutterella secunda* YIT 12071^T. Values are percentages of total fatty acids; only those fatty acids that make up more than 1 % of the total are shown. Data are from Morotomi et al. (2011)

[a]Data for *Sutterella stercoricanis* DSM 17807^T are slightly different from those of Greetham et al. (2004); the difference may have resulted from the different culture conditions

[b]Summed feature 1 (SF1) contained $C_{13:1}\omega12c$ or $C_{14:0}$ aldehyde. SF2 contained $C_{12:0}$ 3-OH or $C_{13:0}$ DMA. SF5 contained $C_{15:0}$ DMA or $C_{14:0}$ 3-OH. SF7 contained $C_{17:1}\omega9c$ or an unknown fatty acid of equivalent chain length (ECL) 16.760. SF10 contained $C_{18:1}\omega7c$ or an unknown fatty acid of ECL 17.834. SF12 contained iso-$C_{19:0}$ or an unknown fatty acid of ECL 18.622

Sutterella Wexler, Reeves, Summanen, Molitoris, Mcteague, Duncan, Wilson, and Finegold 1996a, 257[VP]

Sut.ter.el'la. M.L. dim. Fem. n. *Sutterella* named in memory of Vera Sutter, respected colleague and director of the Wadsworth Anaerobe Laboratory for 20 years.

The data described for *Sutterella* species in this section are from Sakon et al. (2008), Greetham et al. (2004), Wexler et al. (1996), Wexler (2005).

Cells of *Sutterella* species are straight rods (*S. wadsworthensis* and *S. stercoricanis*), 0.5–1.0 μm × 1.0–3.0 μm, or are coccoid to coccobacillary (*S. parvirubra*), approximately 0.4–1.0 μm × 0.4–2.0 μm. They grow under anaerobic conditions or in a microaerophilic atmosphere. Colonies of *S. wadsworthensis* on *Brucella* blood agar with 5 % lysed sheep blood, 1 μg/mL Vitamin K1, 1 μg/mL hemin, and 1 % w/w formate/fumarate after 48 h at 37 °C in anaerobic chamber are circular, entire, convex, yellow to brown, translucent to opaque, 1–1.5 mm in diameter. Colonies of *S. parvirubra* on GAM agar after 48 h anaerobic incubation are 0.2–1.1 mm in diameter, circular, flat, and translucent. No culture data has been reported for *S. stercoricanis* in the literature. *Sutterella wadsworthensis* and *S. stercoricanis* are resistant to 20 % (v/v) bile; no data are available for *S. parvirubra*. Other biological and biochemical characteristics of the type strains of the genus *Sutterella* are listed in *Tables 38.1–38.3* and *38.5*.

The G + C values for DNA of the species determined by HPLC for *S. parvirubra* YIT 11816^T and *S. stercoricanis* 5BAC4^T are 64.4 and 60.0 mol%, respectively. This value for *S. parvirubra* YIT 11816^T (64.4 mol%) is slightly lower than that determined by the genome analysis (65.3 mol%, *Table 38.4*). The value for *S. wadsworthensis* 3_1_45B determined by genome analysis is 55.1 mol% (*Table 38.4*), and that for the type strain has not been reported.

The type species is *S. wadsworthensis*.

The type strains are *S. parvirubra* YIT 11816^T (= DSM 19354^T = JCM 14724^T); *S. stercoricanis* 5BAC4^T (= CCUG 47620^T = CIP 108024^T); *S. wadsworthensis* WAL 9799^T (= ATCC 51579^T = CCUG 42229^T = CIP 104799^T = DSM 14016^T).

Parasutterella Nagai, Morotomi, Sakon, and Tanaka 2009, 1795[VP]

Pa.ra.sut.te.rel'la. Gr. prep. *para* besides, next to; N.L. fem. n. *Sutterella* name of a bacterial genus; N.L. fem. n. *Parasutterella* a genus similar to *Sutterella*.

Table 38.3
Isoprenoid quinone compositions of the type strains of the genera *Sutterella* and *Parasutterella*

	% of total isoprenoid quinones				
Isoprenoid quinone	**1**	**2**	**3**	**4**	**5**
		–	–	–	–
MK-4	–	–	–	1.58	–
MK-5	–	10.19	5.59	4.35	9.01
MK-6	–	–	–	13.40	–
MMK-5	100	89.75	94.41	3.16	90.99
MMK-6	–	–	–	77.51	–

Taxa: 1 *Sutterella parvirubra* YIT 11816^T, 2 *Sutterella stercoricanis* DSM 17807^T, 3 *Sutterella wadsworthensis* DSM 14016^T, 4 *Parasutterella excrementihominis* YIT 11859^T, 5 *Parasutterella secunda* YIT 12071^T. Values are percentages of total isoprenoid quinones; only those isoprenoid quinones that make up more than 1 % of the total are shown. MK-4, MK-5, and MK-6 are menaquinones with four, five, or six isoprene units, respectively; MMK-5 and MMK-6 are methylmenaquinones with five or six isoprene units, respectively. Data are from Morotomi et al. (2011)

The data described for *Parasutterella* species in this section are from Nagai et al. (2009) and Morotomi et al. (2011).

Cells of *Parasutterella* species are cocci to coccobacilli, 0.4–1.3 μm × 0.6–2.6 μm. Colonies on modified GAM agar after 4 days of anaerobic incubation at 37 °C are translucent to beige, circular, convex, and pinpoint in size. Growth in peptone-yeast extract broth (Holdeman et al. 1977) is weak, producing no visible turbidity, and no short-chain fatty acids are detected as an end product of metabolism. Addition of glucose, lactate, or succinate does not enhance growth or result in the production of short-chain fatty acids. Other biological and biochemical characteristics of the type strains of the genus *Parasutterella* are listed in *Tables 38.1–38.3* and *38.5*.

The G+C of DNA of the species determined by HPLC for *P. excrementihominis* YIT 11859^T and *P. secunda* YIT 12071^T are 49.8 and 48.2 mol%, respectively. The value for *P. excrementihominis* YIT 11859^T (49.8 mol%) is slightly higher than that determined by genome analysis (48.1 mol%, *Table 38.4*).

The type species is *P. excrementihominis*.

The type strains are *P. excrementihominis* YIT 11859^T (= DSM 21040^T = JCM 15078^T) and *P. secunda* YIT 12071^T (= DSM 22575^T = JCM 16078^T).

Isolation, Enrichment, and Maintenance Procedures

Sutterella wadsworthensis grows under anaerobic conditions or in a microaerophilic atmosphere of 2 % or 6 % oxygen. The type strain of *S. stercoricanis* grows in a microaerophilic atmosphere of 2 % oxygen but not at 6 % oxygen, or under anaerobic conditions. Growth of the type strains of *S. parvirubra*, *P. excrementihominis*, and *P. secunda* was only observed under strict anaerobic conditions (Wexler et al. 1996; Wexler 2005; Greetham et al. 2004; Sakon et al. 2008; Nagai et al. 2009; Morotomi et al. 2011).

Members of the family *Sutterellaceae* are asaccharolytic and their colonies on agar plates are very small, ranging from pinpoint in size to 1.5 mm in diameter. Therefore, the main problem in isolating these organisms from samples of feces or intestinal contents is the exclusion of the dominant intestinal microbiota, which cover large areas of the isolation plates.

Sutterella wadsworthensis is isolated on *Brucella* blood agar with 5 % lysed sheep blood, 1 μg/mL Vitamin K1, 1 μg/mL hemin, and 1 % w/w formate/fumarate, and is mainly obtained from the intestinal tract and from infections of gastrointestinal origin (Wexler 2005).

Sutterella parvirubra YIT 11816^T was isolated from the feces of a healthy human adult on a medium 10 (Caldwell and Bryant 1966) agar plate supplemented with 40 mM succinic acid as the sole carbon source, from which the other basal carbon sources (a mixture of glucose, cellobiose, soluble starch, and volatile fatty acids) had been excluded (Sakon et al. 2008).

Sutterella stercoricanis 5BAC4^T was isolated from the feces of a healthy male Labrador Retriever dog on bacteroides agar (Holdeman et al. 1977) by Greetham et al. (2004).

Parasutterella excrementihominis YIT 11859^T was isolated from the feces of a healthy human adult on anaerobe basal agar (Oxoid), pH 6.0 (Nagai et al. 2009).

Parasutterella secunda YIT 12071^T was isolated from the feces of a healthy human adult on modified Gifu anaerobic agar (GAM; Nissui Pharmaceutical) supplemented with oxacillin (4 μg/mL; Sigma) (Morotomi et al. 2011).

Some growth media suitable for cultivation of strains of the family *Sutterellaceae* and their compositions are shown in the websites of Leibniz-Institut DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (http://www.dsmz.de/) and Japan Collection of Microorganisms (http://www.jcm.riken.jp/JCM/JCM_Home_J.shtm).

The strains are generally maintained in anaerobic medium as broths or agar slants at 4 °C for a few days. Medium-term maintenance is as suspensions in 20 % v/v glycerol, 20 % w/v skim milk, or 10 % w/v skim milk supplemented with 1 % w/v sodium glutamate at −70 °C. Long-term preservation is by lyophilization.

Ecology

Sutterella wadsworthensis was first reported when performing biochemical characterization and susceptibility testing of *Campylobacter gracilis*–like clinical isolates from patients with diverse infections of the gastrointestinal tract (Wexler et al. 1996). Although a potential role of *S. wadsworthensis* in human gastrointestinal diseases has been documented in the past (Wexler et al. 1996; Molitoris et al. 1997), recent evaluation of the colonic mucosal isolates of this species from patients with inflammatory bowel disease has led to the conclusion that this species is probably a commensal; *S. wadsworthensis* was detected in

Table 38.4

Basic genome statistics for strains of the family *Sutterellaceae*

	Genome size (bp)	G+C content (mol%)	Number of predicted genes		
			protein	rRNA	tRNA
Sutterella parvirubra YIT 11816^T	2,374,505	65.3	2,483	2	63
Sutterella wadsworthensis 3_1_45B	2,965,437	55.1	2,373	7	64
Parasutterella excrementihominis YIT 11859^T	2,831,696	48.1	2,751	1	54

Data are from the GenBank/EMBL/DDBJ databases

Table 38.5

Diagnostic phenotypic differences for the type strains of the genera *Sutterella* and *Parasutterella*

Characteristic	1	2	3	4	5
Microaerophilic growth					
2 % oxygen	–	+	+[a]	–	–
6 % oxygen	–	–	+[a]	–	–
Nitrate reduction	–	+	+[a]	–	–
API ZYM and API rapid ID 32 A reaction					
Alkaline phosphatase	–	–	+	w	w
Acid phosphatase	–	–	+	w	–
Alanine arylamidase	–	–	+	w	–
Alkaline phosphatase	–	–	+	w	+
Arginine arylamidase	w	–	+	+	–
Arginine dihydrolase	–	–	+	–	+
Esterase lipase (C8)	w	–	+	w	+
Esterase (C4)	–	–	+	+	+
Glutamic acid decarboxylase	–	–	+	–	–
Glycine arylamidase	–	w	+	w	–
Leucine arylamidase	–	–	+	+	–
Leucyl glycine arylamidase	–	–	+	–	–
Naphthol-AS-BI-phosphohydrolase	w	–	–	w	+
Phenylalanine arylamidase	–	–	+	–	–
Serine arylamidase	–	–	+	–	–
Tyrosine arylamidase	–	–	+	–	–

Taxa: 1 *Sutterella parvirubra* YIT 11816^T (Data from Sakon et al. 2008), 2 *Sutterella stercoricanis* DSM 17807^T (Greetham et al. 2004), 3 *Sutterella wadsworthensis* DSM 14016^T (Morotomi et al. 2011), 4 *Parasutterella excrementihominis* YIT 11859^T (Nagai et al. 2009), 5 *Parasutterella secunda* YIT 12071^T (Morotomi et al. 2011)

+positive, – negative, *w* weakly positive

[a]Data from Wexler et al. (1996)

83.8 % of adult patients with ulcerative colitis as opposed to 86.1 % of the control subjects (Mukhopadhya et al. 2011). This study also indicated that *S. wadsworthensis* adheres closely to the mucosal lining and is thus more likely to be detected in biopsy samples than in feces.

Recently improved sequencing technology has led to the deposition of a large number of uncultured bacterial clones in the GenBank/EMBL/DDBJ public databases. There is evidence that *S. wadsworthensis* occurs in human feces as a common member of the human indigenous microflora, because many uncultured bacteria with highly similar 16S rRNA gene sequences (>98.7 % identity, the threshold proposed for distinguishing species by Stackebrandt and Ebers (2006) have been reported in these databases. These uncultured bacteria have been identified in fecal samples and intestinal biopsy samples from apparently healthy subjects from different countries, suggesting that *S. wadsworthensis* is a normal inhabitant of the human intestinal microbiota.

As described above, *S. wadsworthensis* is not associated with inflammatory bowel disease, but its presence has been reported in ileal mucosal biopsy samples from children with autism and gastrointestinal dysfunction (AUT-GI) by Williams et al. (2012). They reported that the 16S rRNA gene sequences of either *S. wadsworthensis* or *S. stercoricanis* were found in 12 of 23 AUT-GI children but in none of 9 control children with GI but not autism. Further investigations of the microbiome are needed in larger cohorts of patients with AUT-GI compared to the control GI groups, as well as in patients with AUT but without GI manifestations and in normally developing children with no GI disturbances.

The two other *Sutterella* species, *S. parvirubra* and *S. stercoricanis*, were isolated as novel species of the genus from healthy human feces (Sakon et al. 2008) and from the feces of a healthy Labrador Retriever dog (Greetham et al. 2004), respectively. Although there are no subsequent reports of the isolation of these species, a number of uncultured bacteria with highly similar 16S rRNA gene sequences have been deposited in the GenBank/EMBL/DDBJ databases. For example, the most similar 16S rRNA gene sequences (99.8–99.9 % similarity) to the type strain of *S. parvirubra* were derived from studies of uncultured bacteria from human intestinal mucosal biopsies (accession nos. FJ507106, FJ507078, and FJ506786; Walker et al. 2011). In contrast, the most similar 16S rRNA gene sequence for *S. stercoricanis* (99.2 % similarity) was detected in the feces of dhole (*Cuon alpinus*, a species of canid native to southern and southeastern Asia). This sequence (accession no. JN559525) is a direct submission by Zhang et al. (Unpublished). Williams et al. (2012) reported detecting *S. stercoricanis* 16S rRNA gene sequences in ileal mucosal biopsy specimens from patients diagnosed with AUT-GI symptoms, although it remains unclear whether this species contributes to the disease or is simply a normal component of the human intestinal microbiota.

The genus *Parasutterella* contains two species, *P. excrementihominis* (Nagai et al. 2009) and *P. secunda* (Morotomi et al. 2011). These species were isolated from the feces of healthy human subjects and each was described based on a single strain. There are no subsequent reports of isolation of these species, but a number of uncultured bacteria with highly similar 16S rRNA gene sequences have been deposited in the GenBank/EMBL/DDBJ databases. For *P. excrementihominis*, 200 clones (as of August 2012) with similar 16S rRNA gene sequence (>98.7 % similarity) have been derived from feces, intestinal contents, and mucosal biopsies of healthy human subjects and of patients with gastrointestinal diseases (e.g., ulcerative colitis, Crohn's disease, and *Clostridium difficile*–associated diarrhea); from feces of the black lemur (*Eulemur macaco*), the brown rat (*Rattus norvegicus*), the wolf (*Canis lupus*), and cattle (*Bos taurus*); and from human skin samples and mattress dust. Based on these similar sources of isolation and the similar 16S rRNA gene sequences, *P. excrementihominis* is presumably common in the intestines of humans and other animals. For *P. secunda*, nine clones with a similar 16S rRNA gene sequence (>98.7 %) have been derived from human feces and the intestinal contents of turkeys and cattle.

Overall, all these data suggest that members of the family *Sutterellaceae* are common inhabitants of the intestines of humans and various animals.

Pathogenicity, Clinical Relevance

In addition to the details presented in the Ecology section, there is limited information on the antibiotic sensitivity of this family. Most strains of *S. wadsworthensis* (>95 %) are susceptible to amoxicillin/clavulanate, ticarcillin/clavulanate, cefoxitin, ceftriaxone, and clindamycin, and 85–95 % of the strains are susceptible to piperacillin, piperacillin/tazobactam, ceftizoxime, ciprofloxacin, trovafloxacin, azithromycin, clarithromycin, erythromycin, and roxithromycin (Wexler 2005). Strains of *S. wadsworthensis* (8 strains) were susceptible to kanamycin and colistin, but were resistant to vancomycin (Warren et al. 2005).

No information on antibiotic sensitivity and resistance is available for other species of the genera *Sutterella* and *Parasutterella*.

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Erratum to Chapters 5 and 19

5 The Family *Bradyrhizobiaceae*

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19 The Family *Rhizobiaceae*

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Owing to an unfortunate oversight the name of Lucia Maria Carareto Alves was initially published with an error.

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