Eugene Rosenberg
Editor-in-Chief

Edward F. DeLong
Stephen Lory
Erko Stackebrandt
Fabiano Thompson
Editors

The Prokaryotes

Deltaproteobacteria and Epsilonproteobacteria

Fourth Edition

Springer Reference

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Edward F. DeLong, Stephen Loy, Erko Stackebrandt and Fabiano Thompson (Eds.)

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Fourth Edition

With 111 Figures and 105 Tables

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Editor-in-Chief
Eugene Rosenberg
Department of Molecular Microbiology and Biotechnology
Tel Aviv University
Tel Aviv, Israel

Editors
Edward F. DeLong
Department of Biological Engineering
Massachusetts Institute of Technology
Cambridge, MA, USA

Center for Microbial Oceanography: Research and Education
University of Hawaii, Manoa
Honolulu, HI, USA

Stephen Lory
Department of Microbiology and Immunobiology
Harvard Medical School
Boston, MA, USA

Erko Stackebrandt
Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures
Braunschweig, Germany

Fabiano Thompson
Laboratory of Microbiology, Institute of Biology, Center for Health Sciences
Federal University of Rio de Janeiro (UFRJ)
Ilha do Fundão, Rio de Janeiro, Brazil

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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!

Ralph S. Wolfe
Department of Microbiology
University of Illinois at Urbana-Champaign

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.

Eugene Rosenberg
Editor-in-Chief

About the Editors

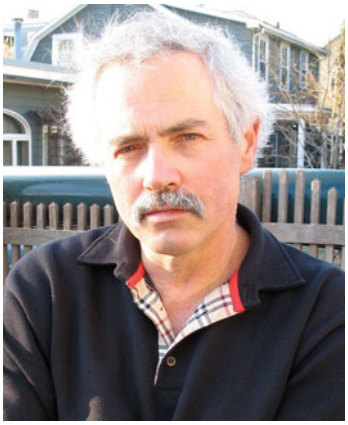
Eugene Rosenberg (Editor-in-Chief)
Department of Molecular Microbiology and Biotechnology
Tel Aviv University
Tel Aviv
Israel

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.

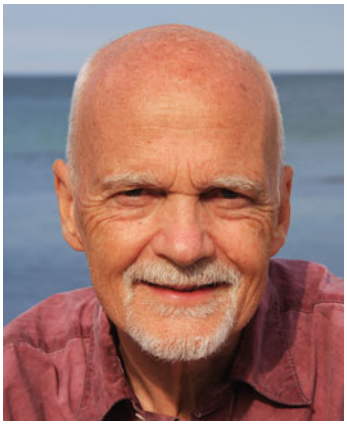
Edward F. DeLong
Department of Biological Engineering
Massachusetts Institute of Technology
Cambridge, MA
USA
and
Center for Microbial Oceanography: Research and Education
University of Hawaii, Manoa
Honolulu, HI
USA

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.



Stephen Lory
Department of Microbiology and Immunobiology
Harvard Medical School
Boston, MA
USA

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.



Erko Stackebrandt
Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures
Braunschweig
Germany

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.

Fabiano Thompson
Laboratory of Microbiology
Institute of Biology
Center for Health Sciences
Federal University of Rio de Janeiro (UFRJ)
Ilha do Fundão
Rio de Janeiro
Brazil

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).

Table of Contents

List of Contributors

Mercedes Maria da Cunha Bustamante
Department of Ecology
University of Brasilia (UnB)
Brasilia-DF
Brazil

Holger Daims
Department of Microbiology and Ecosystem Science
Division of Microbial Ecology
University of Vienna
Vienna
Austria

Tatiana Amabile De Campos
Department of Cellular Biology
University of Brasilia (UnB)
Brasilia-DF
Brazil

Alexander Galushko
Department of Plant Physiology and Biotechnology
Tomsk State University
Tomsk
Russia
and
Department of Microbial Ecology
Vienna University
Vienna
Austria

Ronald Garcia
Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland – Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology
Saarland University
Saarbrücken
Germany

Anthony C. Greene
School of Biomolecular and Physical Sciences
Griffith University
Brisbane, QLD
Australia

Edouard Jurkevitch
Department of Plant Pathology and Microbiology
Faculty of Agriculture, Food and Environmental Sciences
The Hebrew University of Jerusalem
Rehovot
Israel

Nadeem O. Kaakoush
School of Biotechnology and Biomolecular Sciences
The University of New South Wales
Sydney, NSW
Australia

Ricardo Kruger
Department of Cellular Biology
University of Brasilia (UnB)
Brasilia–DF
Brazil

Jan Kuever
Department of Microbiology
Bremen Institute for Materials Testing
Bremen
Germany

Cynthia Maria Kyaw
Department of Cellular Biology
University of Brasilia (UnB)
Brasilia-DF
Brazil

Elke Lang
Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
Braunschweig
Germany

Albert J. Lastovica
Extraordinary Professor (Food Microbiology)
Department of Biotechnology
University of the Western Cape
Bellville
South Africa

Sebastian Lücker
Department of Microbiology and Ecosystem Science
Division of Microbial Ecology
University of Vienna
Vienna
Austria

Pedro M. Meirelles
Instituto de Biologia, Federal University of Rio de Janeiro (UFRJ)
Rio de Janeiro, RJ
Brazil

Robert Neil Gerard Miller
Department of Cellular Biology
University of Brasilia (UnB)
Brasilia-DF
Brazil

Hazel M. Mitchell
School of Biotechnology and Biomolecular Sciences
The University of New South Wales
Sydney, NSW
Australia

Ana Paula B. Moreira
Instituto de Biologia, Federal University of Rio de Janeiro (UFRJ)
Rio de Janeiro, RJ
Brazil

Rolf Müller
Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland – Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology
Saarland University
Saarbrücken
Germany

Satoshi Nakagawa
Laboratory of Microbiology
Faculty of Fisheries Sciences
Hokkaido University
Hakodate, Hokkaido
Japan
and
Subsurface Geobiology Advanced Research (SUGAR) Project
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)
Yokosuka, Kanagawa
Japan

Eliane Ferreira Noronha
Department of Cellular Biology
University of Brasilia (UnB)
Brasilia-DF
Brazil

Stephen L. W. On
Leader of the Food Programme
Institute of Environmental Science and Research (ESR)
Christchurch Science Centre
Ilam, Christchurch
New Zealand

Jani L. O'Rourke
School of Biotechnology and Biomolecular Sciences
The University of New South Wales
Sydney, NSW
Australia

Zohar Pasternak
Department of Plant Pathology and Microbiology
Faculty of Agriculture, Food and Environmental Sciences
The Hebrew University of Jerusalem
Rehovot
Israel

Dulciene M. M. Queiroz
Faculdade de Medicina
Universidade Federal de Minas Gerais
Belo Horizonte, MG
Brazil

Gifone A. Rocha
Faculdade de Medicina
Universidade Federal de Minas Gerais
Belo Horizonte, MG
Brazil

Wilfred F. M. Röling
Faculty of Earth and Life Sciences
Molecular Cell Physiology
VU University Amsterdam
Amsterdam
The Netherlands

Or Rotem
Department of Plant Pathology and Microbiology
Faculty of Agriculture, Food and Environmental Sciences
The Hebrew University of Jerusalem
Rehovot
Israel

Débora Farage Knupp dos Santos
Department of Cellular Biology
University of Brasilia (UnB)
Brasilia-DF
Brazil

Ken Takai
Subsurface Geobiology Advanced Research (SUGAR) Project
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)
Yokosuka, Kanagawa
Japan

Fabiano Thompson
Instituto de Biologia, Federal University of Rio de Janeiro (UFRJ)
Rio de Janeiro, RJ
Brazil

Li Zhang
Senior Lecturer (Medical Microbiology and Immunology)
School of Biotechnology and Biomolecular Sciences
University of New South Wales
Sydney, NSW
Australia

Deltaproteobacteria

1 *Bdellovibrio* and Like Organisms

Or Rotem · Zohar Pasternak · Edouard Jurkevitch
Department of Plant Pathology and Microbiology, Faculty of Agriculture, Food and Environmental Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

Abstract
Bdellovibrio and like organisms (BALOs) are gram-negative, obligate predators of other gram-negative bacteria. These small bacteria interact with their prey as highly motile attack phase cells, attaching to the outer membrane and consuming the prey extracellularly (epibiotic predation) or penetrating their periplasm (periplasmic predation). The former divides in a binary fashion, while the latter grows as a polynucleotide filament to finally split as progeny attack cells. High-resolution microscopy, molecular genetics, genomics, and functional genomics have been applied to study the cell cycle of BALOs, revealing functions required for predation and for cellular organization. Until recently, *Bdellovibrio bacteriovorus* was the only recognized species of BALOs. Culture-dependent and culture-independent approaches have shown that these predators form diverse monophyletic groups, including the three families *Bdellovibrionaceae*, *Bacteriovoraceae*, and *Peridibacteraceae* in the δ-proteobacteria, and the genus *Micavibrio* in the α-proteobacteria. Based on this detailed taxonomical knowledge, it has become possible to track predator and prey interactions in natural systems, providing first evaluations of the impact of bacterial predation on community structure.

Introduction

Predation is an important factor affecting both the ecology and evolution of organisms. While predatory interactions are common and the subject of numerous investigations in the animal world, much less is known in the bacterial realm. A number of reasons may explain the paucity of knowledge on predatory bacteria: It is difficult and time consuming to search for predatory interactions between bacteria in vivo by examining natural samples; predatory bacteria vary in their prey range, and thus, their isolation is limited by the number of possible prey that can be experimentally manipulated – most potential prey in the environment, as other bacteria, may not be amenable to cultivation and thus their predators remain unknown; until recently (Pasternak et al. 2012) genome data could not be used to identify novel predators.

That said, the field of predatory bacterial interactions has had many significant contributions since serendipitous discovery of the first obligate predatory bacterium *Bdellovibrio* by Stolp and Petzold (1962). This was followed by numerous groundbreaking researches on the physiology, ecology, taxonomy, interactions with prey, and cell cycle of *Bdellovibrio* in the 1960s, 1970s, and into the 1980s, mainly by the groups of Conti, Diedrich, Hespell, Rittenberg, Ruby, Shilo, Stolp and Starr, Thomashow, Tudor, and Varon. More recently, the field has greatly benefited from the introduction of modern molecular biology into microbial ecology and genetics brought by the groups of Jurkevitch, Kadouri, Koval, Linscheid, Sockett, Strauch, and Williams. Most, if not all, of these works were performed on *Bdellovibrio* and like organisms (BALOs), the "taxonomical progeny" of the original single *Bdellovibrio bacteriovorus* taxon. These bacteria can be described as predators, parasites or symbionts (Starr 1975), or parasitoids, and the consumed bacterium as prey or host. In this review, the terms predator–prey and predator-host will be used interchangeably.

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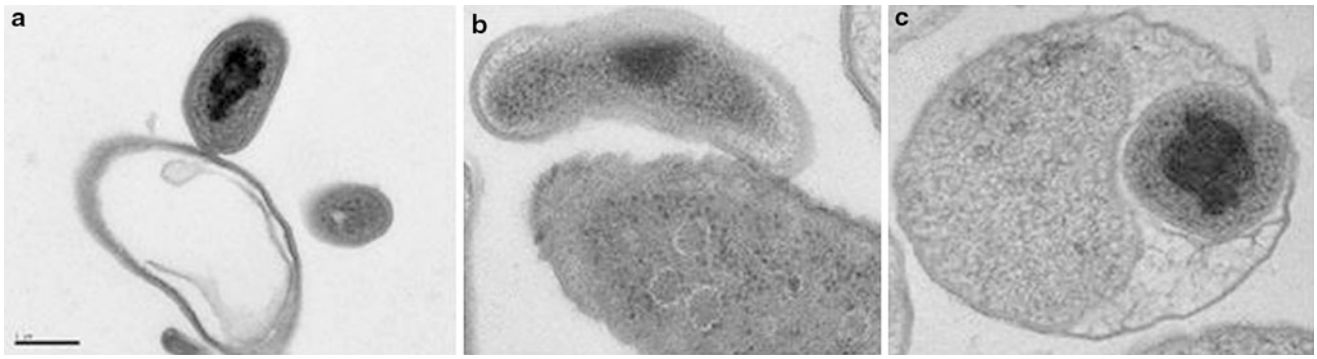

Fig. 1.1
Predatory strategies of *Bdellovibrio* and like organisms. *Bdellovibrio exovorus* (a) and *Micavibrio aeruginosavorus* (b) are epibiotic predators. *Bdellovibrio bacteriovorus* (c) is a periplasmic predator

The BALOs' Life Cycle

The life cycle of BALOs is concomitant to its cell cycle and is composed of two main and distinct phases, i.e., an attack phase (AP) and a growth and division phase (GP). Further subdivision of these stages depends upon the predatory strategy adopted: BALOs are whether epibiotic or periplasmic predators. Epibiotic predators like *Bdellovibrio exovorus* (Fig. 1.1*a*) and *Micavibrio aeruginosavorus* (Fig. 1.1*b*) (Davidov et al. 2006b; Koval et al. 2012) attach onto the prey cell, digesting its content while remaining extracellular to finally divide in a binary fashion. Most of the knowledge being on periplasmic predators (Fig. 1.1*c*), the description of BALOs' life cycle will center on this particular predatory strategy (Fig. 1.2), with an emphasis on the physiological features of each stage.

Attack Phase

I. *Motility and prey detection.* Attack phase (AP) cells are small (0.7–1.5 × 0.5 mm), mostly vibrioid, highly motile non-replicative cells (Fig. 1.2, stage 1). Cell shape is maintained by the cytoskeleton protein MreB2 (Butan et al. 2011; Fenton et al. 2010c;) with the coiled-coil-repeat protein CcrP probably acting as an underlying scaffold (Fenton et al. 2010a). The nucleus is tightly packed, and electron-dense granules resembling acidocalcisomes, enriched in phosphorus, calcium, and oxygen can be found in the cytoplasm (Borgnia et al. 2008).

The AP cell is endowed with a single sheathed flagellum composed of six different flagellin proteins (FliC1-6) that propels it to velocities as high as 160 μm s^{-1} (Iida et al. 2009; Lambert et al. 2006; Seidler and Starr 1968; Thomashow and Rittenberg 1985). None of the flagellin genes appears to be essential except for *fliC3* which is required for predation in suspension cultures (Lambert et al. 2006). Likewise, three pairs of MotAB flagellar motor proteins contribute unevenly to flagellar rotation, none being essential (Morehouse et al. 2011). Flagellar motility is crucial for encountering prey but is neither required for prey penetration nor for slow surface-associated gliding motility (15–20 μm h^{-1}). Gliding appears to be advantageous in predation of biofilms and in low-moisture environments (Abram et al. 1974; Lambert et al. 2006, 2011; Medina et al. 2008).

Multiple methyl-accepting chemotaxis proteins (MCP) sense various ligands, providing chemotactic cues towards inorganic ions, organic acids and amino acids, and oxygen (LaMarre et al. 1977; Sourjik and Wingreen 2012; Straley et al. 1979). Although attraction towards prey bacteria is only noticeable at high prey concentrations (Straley and Conti 1977), deletion of *mcp2* reduces predation, suggesting chemotaxis is involved in prey detection (Lambert et al. 2003).

II. *Attachment to prey.* Attachment of BALO to prey (Fig. 1.2, stage 2) is affected by many factors such as the composition and the pH of the medium, oxygen tension, and temperature (Varon and Shilo 1968). At first attachment is reversible – as seen when the predator encounters non-prey cells (Shemesh et al. 2003) – but within minutes, it becomes irreversible. The basis of prey recognition by BALOs is still obscure. Core oligosaccharides of the prey's lipopolysaccharide layer (LPS) are sensed by *B. bacteriovorus*, yet when they are depleted, attachment stalls but is not abolished. In contrast, attachment by *Bacteriolyticum stolpii* is indifferent to prey LPS composition but is reduced in the absence of particular porins (outer membrane proteins, OMPs) such as OmpF and OmpC in *E. coli* (Schelling and Conti 1986; Varon and Shilo 1969). BALOs synthesize unique membrane lipid structures that greatly vary between taxa: in *B. bacteriovorus*, the lipid A is completely devoid of negatively charged groups and possesses α-D-mannopyranose residues instead of phosphate groups; *B. stolpii* contains sphingophospholipids with unique 2-amino-3-phosphonopropanate heads; and *Peridibacter starrii*'s lipids include phosphatidylethanolamine structures with an additional *N*-glutamyl residue (Muller et al. 2011; Nguyen et al. 2008). The varied composition of lipids implies that predator's membrane fluidity and permeability

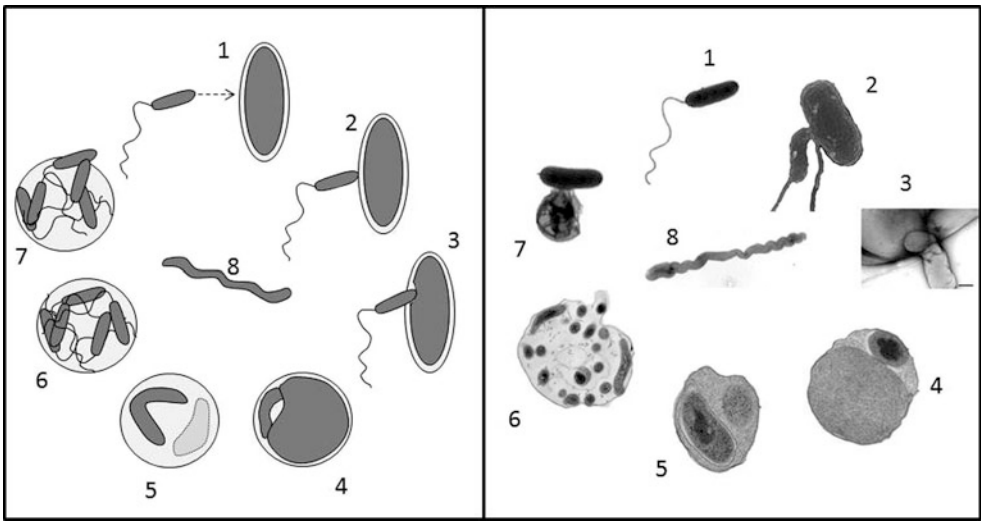

Fig. 1.2
The life cycle of *Bdellovibrio bacteriovorus*. Eight stages are depicted. *1* free swimming, attack phase, *2* attachment to prey cell, *3* penetration of the prey periplasm, *4* establishment and initiation of growth, *5* filamentous growth and depletion of prey cytoplasm, *6* division to progeny cells, *7* lysis of ghost prey cell and release of progeny, *8* host-independent mutant

are altered and, in turn, its interaction with the prey surface. However, the ability of the different predators to use the same prey (e.g., *E. coli* or pseudomonads) while bearing diverse lipid structures in their outer membranes suggests that these structures whether have no meaningful interaction with the prey, interact with different components of the prey's cell wall, or interact with similar components of the prey through different mechanisms.

Type IVa pili are present at the nonflagellated pole of AP cells and are essential for prey attachment and penetration of periplasmic and probably for attachment of *B. exovorus* to its prey as well (Evans et al. 2007; Mahmoud and Koval 2010). Anchoring of the pilus onto the prey envelope enables the invasion of the prey periplasm. This appendage is kept during GP, when it is found adhering to the prey cytoplasmic membrane. The machinery for its assembly is expressed throughout the entire growth cycle, suggesting that the pilus also plays a role during replication. In addition, a type IVb Flp pilus promotes *B. bacteriovorus* predation in biofilm (Medina et al. 2008).

III. *Prey invasion and bdelloplast formation.* Irreversible attachment triggers local lysis of the prey envelope (Abram et al. 1974). Penetration of the periplasm by *B. bacteriovorus* is achieved by the predator squeezing through a pore (Evans et al. 2007) (*Fig. 1.2*, stage 3), a process that may also involve the use of pili (Evans et al. 2007; Mahmoud and Koval 2010). The process is completed within 5–20 min after attachment and is sensitive to streptomycin (Varon and Shilo 1968), suggesting the production of enzymes in the formation of the pore. All the while, the prey peptidoglycan is modified, producing a bdelloplast. At that stage, damage to the prey's cytoplasmic membrane leads to a rapid drop in prey respiration (Rittenberg and Shilo, 1970).

Bdelloplast construction is brought about by the activity of glycanase(s) and the solubilization of 10–15 % of the cell wall's *N*-acetylglucosamine (Thomashow 1978a). To prevent premature prey cell lysis, *N*-deacetylase(s) controls glycanase activity immediately after penetration (Thomashow 1978b). Acylation of the prey peptidoglycan by long-chain fatty acids (Thomashow 1978c) and solubilization of 25 % of the LPS glucosamine by a lipopolysaccharidase activity (Thomashow 1978a) increase bdelloplast hydrophobicity, potentially stabilizing the outer membrane, which now acts as an osmotic barrier (Cover 1984). The growth chamber is further altered by the action of peptidases that actively cleave Braun's lipoprotein (Thomashow 1978b), the release of diaminopimelic acid (DAP) from the peptidoglycan during penetration and latter during prey cell lysis (Thomashow 1978a), and the re-incorporation of DAP into the prey peptidoglycan during penetration and bdelloplast stabilization (Araki 1988; Ruby 1984).

After predator invasion, a bdelloplast is immune to superinfection. This was initially thought to result from N-deacetylation of the peptidoglycan (Thomashow 1978c). However, (Tudor et al. 1990) observed that in *Bdellovibrio* sp. strain W both glycanase and *N*-deacetylase activities are lacking and the non-spherical bdelloplasts generated are not superinfected. This and other data suggested that peptidase and not glycanase activity enables prey penetration and that bdelloplast rounding is a by-product caused by host autolytic muramidases (Tudor et al. 1990). However, (Lerner et al. 2012) showed that a double mutation in two homologs of the PBP4 DD-endo/carboxypeptidase that are mostly expressed during invasion leads to non-spherical bdelloplasts. The mutants were also slower to penetrate the prey, demonstrating that peptidase activity is a non-exclusive enzymatic requirement

for invasion; further, single mutants in each of the encoding genes and more so the double mutant were sensitive to superinfection.

IV. *Growth and division.* The intraperiplasmic *B. bacteriovorus* predator grows in a filamentous manner (*Fig. 1.2*, stages 4 and 5) at the expanse of the prey cytoplasmic content. It incorporates up to 70 % of the prey's DNA material by sequentially digesting it with dedicated enzymes (Rosson and Rittenberg 1979). The predator also degrades 20–40 % of the prey's RNA ribonucleotides into the base and the ribose-L-phosphate moieties. The sugar phosphate is then used for energy production and for the biosynthesis of non-nucleic acid cell material (Hespell and Odelson 1978). Yet, BALOs encode the full complement of genes for purine and pyrimidine metabolism (Pasternak et al. 2012; Rendulic et al. 2004; Wang et al. 2011). In contrast, BALOs lack the ability to synthesize and degrade various amino acids and riboflavin, which should be acquired from the host (Pasternak et al. 2012; Rendulic et al. 2004). Other prey cell components were thought to be imported and utilized as building blocks by the predator, such as lipopolysaccharide moieties (Kuenen and Rittenberg 1975; Stein et al. 1992) and OMPs (Diedrich et al. 1984). It was shown that in fact, *B. bacteriovorus* synthesizes its own lipopolysaccharides (Schwudke et al. 2003) and does not import OMPs (Barel et al. 2005; Beck et al. 2004). Strikingly, and for hitherto unknown reasons, most BALOs use the mevalonate pathway instead of the common DOXP bacterial pathway (Pasternak et al. 2012).

The cytoskeleton is affected during GP: the MreB eukaryotic actin homologue MreB1 is essential, as hampering polymerization of the protein leads to arrested growth early in bdelloplast formation (Fenton et al. 2010c). Localization studies of MreB2-mTFP in AP cells showed it to be intimately connected to the spiral-shaped nucleoid. Also, the position of the nucleoid at approximately equal distances from the cell poles suggested that a parallel pattern of extension with cell length during cell division (Butan et al. 2011). Finally, the filament divides into progeny, the number of which is proportional to the size of the prey (Kessel and Shilo 1976). The number of progeny varies, so that odd and even numbers of *Bdellovibrio* are produced (Fenton et al. 2010b): an average of 5.7 progeny per prey in *E. coli* (Seidler and Starr 1969a) and up to 30 in *Aquaspirillum serpens* (Stolp 1967) (*Fig 1.2*, stage 6). Division exhibits particular features as septation events occur synchronously along the filamentous *Bdellovibrio* cell, even in doubly infected prey (Fenton et al. 2010b). The resulting attack phase cells escape from the bdelloplast through discrete pores (*Fig. 1.2*, stage 7). AP cells then mature and increase in length (Fenton et al. 2010b).

Marine BALOs were shown to produce stable bdelloplasts under nutrient deprivation, synchronous infection of stationary phase prey, and final low concentration of bdelloplasts. These structures remained viable for months, in contrast to attack phase cells that died rapidly but were as sensitive to environmental challenges (Sanchez Amat and Torrella 1990). They lysed in the presence of yeast extract, releasing AP cells.

V. *Bdellocysts.* A few *Bdellovibrio* strains have been reported to enter a cyst-like stage under low-nutrient conditions and multiplicity of infection per prey cell (Tudor and Conti 1977). Bdellocysts occur in an infected prey. The predatory cell enlarges into a kidney-shaped cell enclosed by a structureless, amorphous outer layer. A finely particulate inner layer surrounds the more particulate plasma membrane of the predatory cell. Structures resembling storage granules are present. Bdellocysts are more resistant than vegetative cells to desiccation, high temperatures, and sonication (Tudor and Conti 1977), and their germination is favored by L-glutamate, K^+, and NH_4^+ (Tudor and Conti 1978).

Host Independence

Soon after the discovery of *Bdellovibrio bacteriovorus*, saprophytic variants capable of growing in the absence of prey were isolated by plating concentrated suspensions of WT cells on a standard complete medium (Stolp and Petzold 1962; Stolp and Starr 1963). To date, all the periplasmatically growing *B. bacteriovorus* strains and *Bacteriovorax* species tested are able to generate saprophytic derivatives under laboratory conditions (Baer et al. 2000, 2004; Schwudke et al. 2001; Seidler and Starr 1969b). These variants, coined host-independent (H-I), manifest the archetypical dimorphic life cycle and retain a predatory potential (*Fig. 1.2*, stage 8). They are, hence, facultative predators. Yet, predation is less efficient than in the parental strain (Cotter and Thomashow 1992a, b). Additionally, sequential transfers on complete medium without prey result in the loss of predatory ability (Roschanski et al. 2011; Varon and Seijffers 1975; Wurtzel et al. 2010). H-I BALOs may also occur in the environment as such strains were isolated on several occasions (Diedrich et al. 1970; Doskina 1973; Hobley et al. 2012b). Unique characteristics of H-I variants, in comparison to wild-type (WT) progenitors, suggest that host independence might be a genuine stage of the BALOs' lifestyle. Unlike colorless WT cells, H-I isolates produce a yellowish pigment, protective against photooxidative damage (Friedberg 1977). H-I derivatives of *B. bacteriovorus* utilize a broader variety of carbon sources (Ishiguro 1974), synthesize different LPS structures (Schwudke et al. 2003), and, perhaps most intriguingly, form tenacious biofilms (Medina and Kadouri 2009).

H-I variants are isolated on a standard complete medium where the vast majority of the isolates form small colonies called "type I" (Seidler and Starr 1969b; Varon and Seijffers 1975). These colonies cannot be sub-cultured after initial development unless a large inoculum is streaked to form tight and small growing colonies or if the medium is supplemented with an extract of prey cells (Gray and Ruby 1990). Under such conditions, about 1 % of the isolates will forms large colonies

that can be regrown in a density-independent manner on standard, un-supplemented medium, forming "type II" mutants (Thomashow and Cotter 1992). "Type I" H-I mutants are cell-extract dependent (they are saprophytic) and result from a single mutation; "type II" H-I mutants do not require cell extract (they are axenic) and result from an additional mutation, i.e., they are double mutants. Type I mutants arise at a frequency of 10^{-6} to 10^{-7} (Seidler and Starr 1969b; Varon and Seijffers 1975); type II H-I mutants are selected from type I at a frequency of 10^{-2} to 10^{-3} (Thomashow and Cotter 1992). Strikingly, it has been reported that H-I derivatives (type undefined) can be obtained at a frequency of up to 10^{-2} (Dashiff and Kadouri 2009). These data suggest that at least part of the pathway leading to the axenic phenotype is mutation prone and not based on single, random events. Genetic studies in *B. bacteriovorus* addressed the genetic background for this gradual acquisition of host independence, identifying the genomic loci implicated in it (Cotter and Thomashow 1992a, b; Roschanski et al. 2011; Wurtzel et al. 2010). Deleterious mutations in *bd0108*, a gene with no known function, lead to the type I phenotype. Moreover, not all BALO forming H-I variants contain *bd0108* homologs in their genomes, and H-I mutants with a WT *bd0108* allele were isolated, thus indicating that other gene products may underlie this phenotype (Lambert et al. 2010a; Schwudke et al. 2001; Wurtzel et al. 2010). Type II H-I mutants result from alterations in *rhlB* (*bd3461*) or *pcnB* (*bd3464*). These two genes encode for distinct components of the degradosome machinery, which is a multiprotein complex involved in RNA turnover. A loss of function of each enables progression from a type I to a type II H-I mutant (Roschanski et al. 2011).

The identities of the prey molecules necessary for WT or saprophytic growth are still not known. It has been shown that prey extract is required for initiation of DNA synthesis in saprophytic H-I mutants and in WT cells released from bdelloplasts. In contrast, prey extract cannot promote de novo proliferation of WT AP cells (Gray and Ruby 1990; Ruby and Rittenberg 1983; Thomashow and Cotter 1992), suggesting that replication of WT cells relies on two cues from the prey: one leading to a physiological transition from AP to GP and another one activating DNA synthesis (Gray and Ruby 1991). To date, the nature of the first cue is not known. The second cue is soluble, heat stable, resistant to RNase or DNase treatments, and fractionated over a wide range of molecular masses (10 to >200 kDa) (Gray and Ruby 1990). Saprophytic mutants overcome the need to sense the first cue; axenic mutants surmount the requirement for the second cue as well (Roschanski et al. 2011; Thomashow and Cotter 1992).

Cell Cycle Genetics

I. *Phase transition.* Each phase is characterized by different gene and protein expression patterns and by characteristic activities (Karunker et al. 2013; Lambert et al. 2010a; McCann et al. 1998; Roschanski et al. 2011; Thomashow and Cotter 1992) Work on H-I mutants and prematurely released cells from bdelloplasts showed that the transition between AP to GP necessitates sensing of two prey cues (Gray and Ruby 1991; Roschanski et al. 2011; Thomashow and Cotter 1992): the first being activated during attachment (Thomashow and Cotter 1992), the second during growth (Gray and Ruby 1990). Such programming is most likely governed by distinct, master regulators whose modulations afford a swift transition between the phases (Lambert et al. 2010a; McCann et al. 1998). In *B. bacteriovorus* FliA (sigma 28) promoters are over represented upstream of AP-specific genes. *Flia* by itself is overexpressed during AP. It is thus reasonable to assume that FliA acts as an AP master regulator (Karunker et al. 2013), been shown that RpoE-like sigma factors in *B. bacteriovorus* are not essential but affect predatory efficiency or regulate chaperonin levels (Lambert et al. 2012). The heat shock response might be involved in phase transition, as heat shock promotes axenic growth (Gordon et al. 1993; Wang et al. 2011).

The prevalent second messenger molecule cyclic di-GMP is implicated in lifestyle determination in many bacteria (Mills et al. 2011) and BALOs are no exception. BALO genomes encode for a plethora of cyclic di-GMP synthesizing and degrading enzymes (diguanylate cyclases and phosphodiesterases, respectively) as well as cyclic di-GMP and a single cyclic di-GMP type I riboswitch (Karunker et al. 2013). Cyclic di-GMP signaling has been shown to be essential in determining WT and H-I phenotypes, as well as to affect flagellar and gliding motility in *B. bacteriovorus* (Hobley et al. 2012a). Such phenotypic differentiation is achieved by different sets of specific diguanylate cyclases (Hobley et al. 2012a) The cyclic di-GMP riboswitch is abnormally expressed during AP and is down regulated during GP. Its function is yet to be deciphered (Karunker et al. 2013).

II. *Genetics, genomics, and post-genomics.* High-throughput transcriptome analyses were performed in the periplasmic predator *B. bacteriovorus* and in the epibiotic predator *M. aeruginosavorus* (Karunker et al. 2013; Lambert et al. 2010a; Wang et al. 2011). In all, global transcriptional changes correlated with phase transition. Lambert et al. 2010a compared transcription profiles of AP, predatory (i.e., 30 min post-prey infection) and host-independent (H-I) *B. bacteriovorus*, and identified exclusively overexpressed genes in each state. This enabled them to confine subsets of genes (most of which unannotated) and functions to the AP, the invasion phase and the GP. Motility and taxis genes were overexpressed in the AP. Cell wall metabolism, activation of transport systems, and early replication functions were expressed during the early stages of predation. Macromolecule degradation, massive transport, biosynthesis pathways, and DNA replication were expressed in the H-I samples and inferred to be expressed in the GP. Clearly, H-I mutants represented a transcriptional mosaic, mixing profiles unique to the AP, to attachment and, probably, to the GP. These data thus support the hypothesis that the mutations underlying the H-I phenotype essentially lead to a cell cycle freed from regulatory constraints

(Dori-Bachash et al. 2008; Roschanski et al. 2011; Thomashow and Cotter 1992) Karunker et al. (2013) utilized whole transcriptome sequencing to find large subset of B. bacteriovorus genome exclusively expressed over AP and GP. Here again, genes encoding for motility, chemotaxis and cell surface proteins were upregulated in AP. Genes overexpressed during GP are related to cell growth, including ribosome biogenesis, cell division, DNA polymerase and chromosome partitioning proteins, and energy metabolism (Karunker et al. 2013). In the epibiotic a-proteobacterium M. aeruginosavorus expression profiles of AP and growth phase corresponded to those of the periplasmic, δ-proteobacterium *B. bacteriovorus*. Flagellar and chemotaxis genes were upregulated in the AP, while replication-associated genes and transport-related genes were upregulated during attachment. Surprisingly, hydrolase coding genes were found to be expressed constitutively (Wang et al. 2011).

Transport

Secretion by BALOs relies on type I and type II (sec) systems (not to be confounded with type I and type II H-I mutants) and include the twin arginine targeting protein translocation (Tat) system (Rendulic et al. 2004). Type III and type IV secretion systems are absent. Protein secretion into the prey cytoplasm is probably first accomplished via secretion into the prey periplasm and then by retrotranslocation into the host cytoplasm (Barabote et al. 2007). Another peculiar mechanism is the embedding by *B. bacteriovorus* of predator OMPs onto the prey cytoplasmic membrane, probably permeabilizing it to small hydrophilic molecules (Barel et al. 2005; Beck et al. 2004).

In *B. bacteriovorus* the Tat system is essential for growth of WT and of H-I strains. Some of the system's components are specifically expressed during particular phases of the cell cycle and appear to promote the transfer of proteins to the prey cytoplasm (Chang et al. 2011).

BALO genomes encode for unusually large inventories of transport systems (Barabote et al. 2007). The δ-proteobacteria *Bdellovibrionaceae* and *Bacteriovoracaceae* bear numerous uptake systems for amino acids and peptides; in contrast, none is detected in the epibiotic α-proteobacteria *Micavibrio aeruginosavorus* (Hobley et al. 2012b; Rendulic et al. 2004; Wang et al. 2011). BALOs have few sugar transporters and depend on noncarbohydrate metabolism for carbon and energy. *B. bacteriovorus* has three sugar permeases, enabling the import of ribose, glycosides, maltose, and malto-oligosaccharides (Hespell et al. 1973). In contrast, many efflux pumps are found (Barabote et al. 2007). The phosphoenolpyruvate-dependent sugar transporting phosphotransferase system (PTS) is absent. Nucleotide uptake is a rare trait in bacteria, mostly found in obligate parasites. *B. bacteriovorus* is able to take up nucleotides, possibly through two different systems (Barabote et al. 2007; Ruby and McCabe 1986; Ruby et al. 1985). The *M. aeruginosavorus* genome lacks nucleotide transporter coding genes (Wang et al. 2011).

Energy Metabolism

In culture, BALOs do not enter stationary phase, and they are continuously using energy at high rates, whether for high-speed swimming or for growth and replication. During both phases, endogenous and substrate respiration rates, which are seven times higher than in *E. coli* (Hespell et al. 1973), lead to the saturation of the functional capacity of either the tricarboxylic acid cycle or the electron-transport chain (Hespell et al. 1973; Rittenberg and Shilo 1970). BALOs are incapable of fermentation and de facto are unable to use sugars (Seidler and Starr 1969a). They obtain energy (ATP) from amino acids, some organic acids (acetate and α-ketoglutarate), and polyhydroxyalcanoates (PHA) (Hespell et al. 1973; Martinez et al. 2012). ATP turnover in *B. bacteriovorus* during endogenous respiration is five times higher than in *E. coli* but similar during substrate respiration, while P/O ratios under both conditions are similar to these of *E. coli* (Gadkari and Stolp 1976). The ability of BALOs to recycle prey material renders them extremely energy efficient: GP *B. bacteriovorus* displays a Y_{ATP} (biomass formed per ATP consumed) of 26, compared with 10.5 for bacteria cultivated in rich medium (Rittenberg and Hespell 1975).

The high respiration rates result in rapid energy depletion and a typically short half-life for BALOs, e.g., a 95 % loss in viability of a cell suspension of *B. bacteriovorus* in 20 h Hespell et al. 1973). This is due, at least in part, to BALOs' energetically costly vigorous swimming which uses 20–40 % of the total available energy (Hespell et al. 1974). *B. bacteriovorus* exhibits a peculiar mode of oscillatory energy production in the absence of an exogenous substrate, by degrading its own cellular materials (Gadkari and Stolp 1975). This pattern fits the observed pattern of varying intensity – in contrast to constant rate – of RNA degradation under starvation (Hespell et al. 1974).

Survival is extended by using respirable substrates like amino acids, some organic acids, and PHAs. Lately, it has been shown that *B. bacteriovorus* is able to depolymerize PHA made by its prey and to use it to produce ATP (Hespell et al. 1973; Martinez et al. 2012).

Some BALOs are able to overcome harsh environmental conditions by entering a cyst-like state or, as shown with marine BALOs, to transform the bdelloplast into a dormant state (see above).

Taxonomy

Bdellovibrio and like organisms form a polyphyletic taxon which is so defined for ease: the term describes all known obligate predatory bacteria, endowed with high motility, and having a basically two-phase life cycle composed of a search phase and of a growth and division phase. BALOs are found in the

α-proteobacteria, where they form the genus *Micavibrio* and in the δ-proteobacteria where they form three families: the *Bdellovibrionaceae*, the *Bacteriovoracaceae*, and the *Peridibacteriaceae* (Fig. 1.3).

The *Bdellovibrionaceae* contains one species, *Bdellovibrio*, and two defined species, *B. bacteriovorus* and *B. exovorus*. *B. bacteriovorus* HD100^T and *B. exovorus* JSS^T differ in % mol G+C content (50 % and 41 %, respectively) and diverge by 7 % (i.e., they are 93 % similar) in their 16S rRNA gene sequence (Koval et al. 2012). Yet, the major difference between the two is in their predatory strategy: *B. bacteriovorus* is a periplasmic predator, i.e., it penetrates and settles in the periplasmic space of its gram-negative prey, but *B. exovorus* is epibiotic, remaining attached to the prey's cell wall (for details see the section "The BALOs' Life Cycle"). The *Bdellovibrionaceae* was defined as encompassing nine clusters (Davidov and Jurkevitch 2004). The advent of large-scale sequencing of environmental samples revealed that the *Bdellovibrionaceae* tree splits into two clusters: one encompassing all the sequences from cultured strains as well as sequences from uncultured bacteria and another cluster without any cultured representative. This latter group may thus represent organisms that are different from the "classic" *Bdellovibrio*, maybe due to their inability to use the organisms used so far as prey to isolate the predators (Koval et al. 2012).

The *Bacteriovoracaceae* is composed of 10 phylogenetic clusters based on 96.5 % gene similarity in the 16S rRNA gene sequence of environmental isolates (Pineiro et al. 2007). These clusters are robust, as shown by further analysis of the *rpoB* gene that enables finer subdivisions but retains the same clusters (Pineiro et al. 2008). The family encompasses nine clusters of the saltwater *Bacteriovorax*, with the defined species *Bacteriovorax marinus* and *Bacteriovorax litoralis* (Baer et al. 2004), as well as one cluster of the freshwater/soil *Bacteriolyticum stolpii* (Pineiro et al. 2008). The former has a % mol G+C content of 37.7–38.3, and the latter of 41.8.

The *Peridibacteriaceae* were recently split as a monophyletic offshoot of the family *Bacteriovoracaceae* based on a % mol G+C content of 43.5 %, on its presence in freshwater and soil but not in saline environments, and in marked differences in 16S rRNA gene sequence with both *Bdellovibrionaceae* and *Bacteriovoracaceae* (Pineiro et al. 2008) (Fig. 1.3).

Few, yet striking, relationships between phylogeny and ecological parameters have been discovered: as mentioned above, *Bacteriovorax* are found in salt waters, while *Bdellovibrio*, *Bacteriolyticum*, and *Peridibacter* are freshwater and soil isolates. More specifically, *Bacteriovorax* clusters are widely distributed, but cluster V has been exclusively found in estuarine environments (Pineiro et al. 2007). Further, particular taxa appear to be differentially associated with prey specificity: in prey spiking experiments of natural water samples, it was shown that *Bacteriovorax* cluster IX is a versatile predator, able to prey as efficiently on various prey that other separate clusters specialize on (Chen et al. 2011). It was further discovered that cluster IV, consisting of predators that are predominantly isolated from low-salt waters, is selected for by the addition of prey bacteria originating from freshwater (Chen et al. 2012). In the *Bdellovibrionaceae*, predators isolated using *Agrobacterium tumefaciens* as prey, and originating from sources such as the tomato rhizosphere and soils in Israel and soils in Germany and India, formed discrete clusters, separated from clusters containing isolates from many sources and using both various enterobacteria and pseudomonads as prey (Davidov and Jurkevitch 2004). These data strongly suggest that both prey and environmental parameters shape BALO communities in the environment.

Micavibrio is defined as two species: *M. admirantus* (% mol G+C content 57.1 %), which only grows on *Stenotrophomonas* (*Pseudomonas*) *maltophilia* (Lambina et al. 1982), and *M. aeruginosavorus* (% GC 54.7 M) which grows on various enterobacteria and pseudomonads. *Micavibrio* forms a deep branching clade in the α-proteobacteria, sister to the *Rhodospirillales* (Davidov et al. 2006b; Wang et al. 2011). Very few isolates and environmental sequences of *Micavibrio* are available.

Habitat and Ecology

BALOs are widely distributed in marine, freshwater, and terrestrial ecosystems, including estuaries, seacoasts and oceans, rivers, sewage, fish ponds, runoff of irrigation water, and man-made water supplies (Davidov and Jurkevitch 2004; Framatico and Cooke 1996; Fry and Staples 1976; Pineiro et al. 2007; Schoeffield and Williams 1990; Snyder et al. 2002). BALOs have been isolated from the gills of crabs, sediments, submerged surfaces, soils, rice paddies, the rhizosphere of plants, and fish ponds (Chu and Zhu 2009; Kelley et al. 1997; Uematsu 1980; Williams et al. 1995) (Jurkevitch et al. 2000; Stolp and Starr 1963). They have been isolated from animal feces (Schwudke et al. 2003) and detected as forming dominant populations in the leech *Hirudo verbana* (Kikuchi et al. 2009). BALOs colonize biofilms that form in aquatic habitats (Kelley et al. 1997), and under lab conditions, they efficiently clear biofilms (Kadouri and O'Toole 2005; Kadouri et al. 2007). As mentioned above (see taxonomy section), salinity and, less so, prey range are ecological factors that can be used to characterize BALO clades.

The abundance and diversity of BALO populations as appraised by culture-based approaches appear to have been largely underestimated. Indeed, first estimates of population sizes using specific primers targeting the different BALO clades have shown their abundance to be more than 2.5 orders of magnitude higher than that detected by counting plaques (Van Essche et al. 2009b; Zheng et al. 2008). A limited evaluation of the relative abundance of BALOs, based on 16S rRNA gene sequence distributions in a wide range of environments, showed that the predators account in average for close to 0.2 % of total bacteria (Table 1.1). Similarly, culture-independent technologies reveal that BALO diversity is much larger than the one detected by the characterization of isolates (Davidov et al. 2006a).

BALOs are able to consume the majority of gram-negative cells present in natural water bodies (Rice et al. 1998). They were

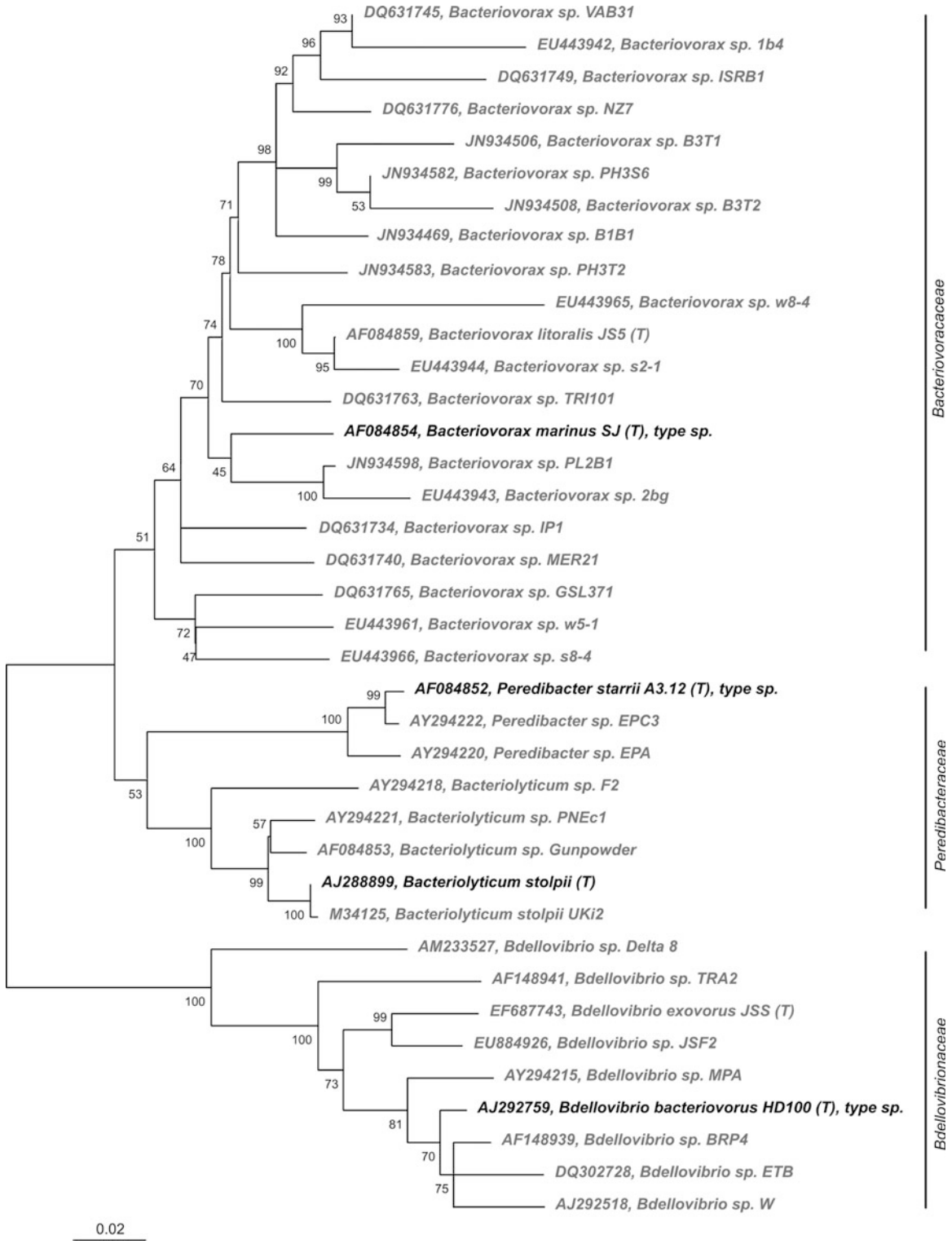

Fig. 1.3

All long (>1,200 bp), cultured rDNA 16S sequences of the order *Bdellovibrionales* were retrieved from the RDP-II database, and the 375 sequences were aligned using MUSCLE. The alignment was trimmed at both ends to eliminate artificial gaps and overhangs, resulting in 1,248 bp. Pairwise distances between the aligned sequences were calculated using MOTHUR (Schloss et al. 2009), with consequent clustering which resulted in 38 operational taxonomic units (OTUs) at >97 % similarity between all sequences within each OTU. A representative sequence was chosen for each OTU, either a type sequence or the non-type sequence which had the lowest average pairwise distance to all the other members of the OTU. A phylogenetic tree containing the 38 representatives was inferred by the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993) in MEGA5 (Tamura et al. 2011). Sequence names

Table 1.1

Relative abundance of *Bdellovibrio* and like organisms of the δ-proteobacteria – families *Bdellovibrionaceae*, *Bacteriovoracaceae*, and *Peredibacteraceae* – estimated by the distribution of 16S rRNA gene sequences in various environments. Metagenomic Sanger sequences from environmental samples (obtained using general bacterial primers) were retrieved from the ENV division of GenBank on August 2009. In silico recognition of the probes Bd529F, Bac676F, and Per676F (Davidov et al. 2006a) in the database was used to detect sequences belonging to the genus *Bdellovibrio*, *Bacteriovorax*, and *Peredibacter*, respectively. Therefore, BALO percentages are probably underestimated

Environment		Sequences (total, *N*)	Sequences (BALO, %)
Main	Sub		
Saline waters	Open sea	11,512	0.64
	Sediment	11,960	0.66
	Coastal	7,829	0.09
	Deep	2,949	0.12
Freshwaters	Sediment	4,612	0.20
	Wastewater	7,068	0.14
	River	4,182	0.19
	Lake	7,406	0.19
	Aquifer	1,820	0.14
	Wetlands	3,987	0.11
Oil	–	1,707	0.21
Air	–	3,938	0.11
Living host	–	9,942	0.15
Terrestrial	Rhizosphere	6,329	0.10
	Rocks	3,833	0.17
	Mines	5,476	0.12
	Plants	3,500	0.11
	Agricultural	16,333	0.09
	Forests	7,346	0.10
	Mangroves	3,446	0.11
	Other soils	13,837	0.13
Thermal	Hydrothermal	4,396	0.24
	Geothermal	5,163	0.23
Total		148,571	–
Mean ± standard deviation		6,460 ± 3,890	0.19 ± 0.15

shown to move to spots of concentrated native aquatic bacteria (Chauhan et al. 2009) and to respond to sudden increases or "spikes" in numbers of specific prey bacteria or consortia of native microbial communities (Chauhan et al. 2009; Chen et al. 2011, 2012).

BALOs are considered as obligate aerobes but they are commonly found in sediments (Williams 1988). Recently, Monnappa et al. (2013) showed that *B. bacteriovorus* successfully preyed under complete anoxic conditions as long as nitrate was supplemented. Nitrite reductase and nitrite oxide reductase but not *bona fide* nitrate reductase genes are found in *B. bacteriovorus* (Rendulic et al. 2004). In *Micavibrio*, *M. aeruginosavorus* EPB but not strain ARL-13 encodes for a nitrate reductase complex (Pasternak et al. 2012).

Prey acquisition of resistance to BALO predation was shown by Varon (1979), using a chemostat containing *B. bacteriovorus* with its prey *Photobacterium leiognathi* (Varon 1979). Also, Gallet et al. (2007, 2009) demonstrated that predation

Fig. 1.3 (continued) are comprised of GenBank accession number, species name, strain (if available), and (T) (if a type sequence). The bootstrap consensus tree inferred from 200 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (200 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of nucleotide substitutions per site

pressure can lead to the selection of resistant prey but that the type of resistance (moderate or extreme) depended upon the ecological conditions under which selection occurred. In addition to such genetic resistance, it appears that prey may also exhibit transient plastic responses in prey populations thereby eradication preventing eradiction. However, resistance falters as prey populations expend (Shemesh and Jurkevitch 2004). Defense mechanisms may be triggered in predatory cultures: Lambert et al. (2010b) reported a futile transcriptional "scream" of genes as a response to predation-induced osmotic stress in *E. coli* cultures, 15 min postinfection with *B. bacteriovorus*.

Applications

The BALOs' characteristic lifestyle makes them attractive candidates for a number of applications: BALOs have been proposed as living alternatives to chemical antibiotics(Sockett and Lambert 2004), as agents for improving water quality in aquaculture (Qi et al. 2009), as a means to control dental plaque bacteria (Dashiff and Kadouri 2011; Van Essche et al. 2009a), and as treatment of *Salmonella*-induced dysbiosis in chickens (Atterbury et al. 2011). This follows older attempts made to use BALOs in animal models: Nakamura (1972) effectively treated *Shigella flexneri*-induced keratoconjunctivitis in rabbit with *Bdellovibrio*, but BALO use against pathogens in the intestinal tract of rabbits was unsuccessful (Westergaard and Kramer 1977). Very few studies explored the potential of BALOs against phytopathogens: BALOs efficiently eradicated *Xanthomonas oryzae* from rice paddy field water and caused a rapid decline in populations of *Pectobacterium carotovorum* subp. *carotovorum* in soil (Uematsu 1980). BALO isolates from the rhizosphere of soybean were used to control bacterial blight caused by *Pseudomonas glycinea* (Scherff 1973). Reduction in disease severity and systemic symptoms was significant.

BALOs can also be employed as theoretical models for understanding the evolution of the eukaryotic cell (Davidov and Jurkevitch 2009; Guerrero et al. 1986); they also are convenient empirical models for testing hypotheses pertaining to ecological and evolutionary theories (Gallet et al. 2007, 2009; Wilkinson 2007).

Isolation

BALOs are isolated as bacteriophages are, using a prey as "bait." The sample, or serial dilutions of it, is mixed with a potential prey bacterium in melted soft agar and poured onto an agar plate containing a diluted growth medium. The bacterial predator forms lytic, transparent plaques that have to be differentiated from those formed by protozoa or bacteriophages. A drawback is that only BALOs able to prey on the proposed bacterium can be retrieved. As BALOs vary in host range, no single bacterial species can potentially support the growth of all isolates. However, *Vibrio parahaemolyticus* was shown to be an effective host for the retrieval of *Bacteriovoracaceae* from marine environments (Pineiro et al. 2008). Another limitation of the method stems from the presence of much higher levels of non-predatory bacteria in the sample that can grow on the plate and blur the detection of plaques.

Direct Isolation of *Bdellovibrio* from Environmental Samples

The most common approach is based on the use of one or more filtration steps with or without differential centrifugation of the sample analyzed.

Based on Stolp (1981), a water sample or 50-g soil in 500 mL in sterile buffer is shaken vigorously for 1 h and then centrifuged for 5 min at 2,000 g to remove gross particles. The supernatant is passed through a series of membrane filters of decreasing pore size (3.0, 1.2, 0.8, and 0.45 μm). Filtrates from the last two steps are serially diluted, and 100-μL aliquots are mixed with approximately 10^9 cells of the prey bacterium in molten soft top low-nutrient or buffer agar. The mixture is poured onto a low-nutrient or buffer agar plate and incubated at 28–32 °C. Rapidly developing lytic regions visible after 24 h are the result of bacteriophage multiplication. These plaques are usually small and do not grow further. They should be marked to differentiate them from the slower-growing BALOs. Plaques becoming visible within 2–3 days and showing further expansion for a few more days are potentially BALOs (*Fig. 1.4*). Small and highly motile BALO cells can be detected by examination of crushed plaque material in wet mounts with a phase contrast microscope.

Media. DNA: (diluted nutrient agar (Seidler and Starr 1969b)); 0.08 % Difco Nutrient Broth is supplemented with $CaCl_2 \cdot 2H_2O$, 2 mM, and $MgCl_2 \cdot 6H_2O$, 3 mM after autoclaving, and pH is adjusted to 7.2 with 0.1 N NaOH.

Bottom agar: 1.2–1.5 %. Top agar: 0.6 %.

Plating: Aliquots of 4-mL molten top agar are kept at 42 °C in a water bath prior to mixing with prey and sample suspensions.

Marine BALOs require salts to grow. Therefore, the medium used for isolating marine strains should contain at least 25 % sea water or appropriate salts (Marbach and Shilo 1978). A common method used to isolate marine BALOs is that of (Schoeffield and Williams 1990): 5 mL of the sample is added to 3.3 mL of molten top agar having 0.7 mL of the prey suspension for a final agar concentration of 0.65 %. The mixture is then poured onto large Petri dishes and incubated at 25 °C.

Media. Polypeptone (Pp 20) medium (Williams et al. 1982): Filtered ocean water, 1 L; Polypeptone, 1 g; agar, 15 g for bottom agar and 6.5 g for top agar; pH 7.7–7.8.

Synthetic marine salt solution (Marbach and Shilo 1978): NaCl, 500 mM; KCl, 10 mM; $MgSO_4$, 25 mM; $MgCl_2$, 25 mM; $CaCl_2$, 10 mM.

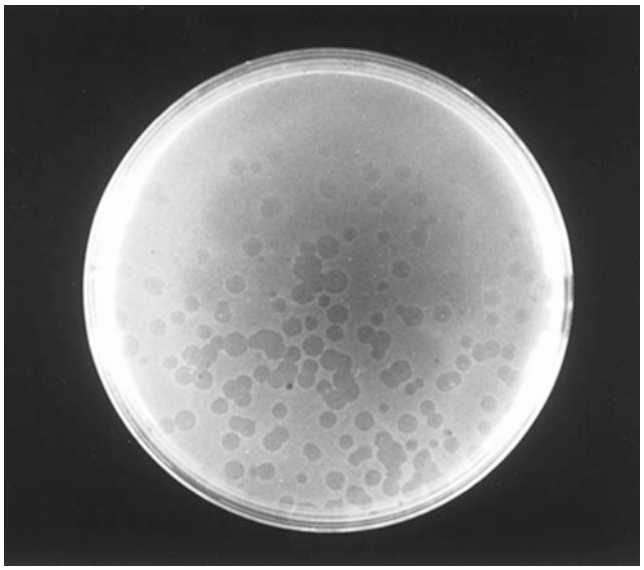

Fig. 1.4
Lytic plaques of *Bdellovibrio bacteriovorus* growing on a lawn of *Pseudomonas corrugata* prey cells

Specific Enrichment for *Bdellovibrio*

Stolp (1968) devised a method yielding large numbers of BALOs, thereby greatly facilitating the isolation of predators on a specific host bacterium whenever quantification is not needed. This approach has been applied to obtain BALOs lytic to *Rhizobium* and *Agrobacterium* (Parker and Grove 1970), *Legionella* (Richardson 1990), and *Azospirillum brasilense* from 2-year-stored air-dried soils (Germida 1987).

Based on Stolp, modified by Ruby (1991): One-hundred milliliter aliquots of a dense suspension of the prospective prey bacterium (10^{10} cells mL^{-1}) are prepared in DN medium or HM buffer in Erlenmeyer flasks. The sample (soil, 100 mg; sewage, 0.5 mL; river water, 1 mL) is added and the flasks are incubated on a rotary shaker. The suspension is examined daily over 2–4 days for lysis (reduction in optical density) and for the presence of small, highly motile presumptive BALO cells or bdelloplasts by phase contrast microscopy. If no BALOs are apparent, the incubation can be prolonged or a 1-ml aliquot can be transferred into a fresh suspension of substrate bacterium. When BALOs are detected, the enrichment culture is centrifuged for 5 min at 2,000 g (250 g) (Germida 1987)and the supernatant filtered through a 0.45-μm membrane. Serial dilutions are plated on the prospective prey bacterium to obtain plaques. Developing plaques are checked microscopically for small, highly motile cells.

HM buffer: Hydroxyethyl piperazine-N′-2-ethanesulfonic acid, 25 mM adjusted to pH 7.6 with NaOH and supplemented with $CaCl_2 \cdot 2H_2O$, 1 mM and $MgCl_2 \cdot 6H_2O$, 1 mM.

Isolation and Cultivation of H-I Mutants

H-I BALOs are isolated by introducing WT attack phase cells in a rich medium such as PYE (g L^{-1}-peptone, 10; yeast extract, 3; $MgCl_2$, 3 mM; $CaCl_2$, 2 mM; pH 7.4–7.6) without prey, selecting for growing isolates. A drawback of this approach is the spurious growth of residual prey cells in the medium. To overcome this shortcoming, lytic suspensions are filtered through a 0.45 μm membrane, efficiently separating BALO cells from prey bacteria but resulting in low recovery rates (Shilo and Bruff 1965). An efficient approach utilizes streptomycin-resistant (Sm^r) host-dependent isolates. After cultivation with a streptomycin-sensitive prey, a lytic suspension containing Sm^r AP cells is inoculated onto a complete medium amended with streptomycin, allowing the growth of H-I mutants but restricting that of the Sm-sensitive prey (Seidler and Starr 1969b). Recently, Dashiff and Kadouri (2009) used prey auxotrophic to diaminopimelic acid (DAP) that could not grow in a complete medium devoid of DAP. They obtained H-I mutants without filtration or selection for antibiotic resistance at a very high frequency (up to 10^{-2}) (Dashiff and Kadouri 2009).

Mutants can grow as a suspension or form yellowish colonies on a solid medium. Saprophytic (type I) H-I mutants are essentially cultured with buffered heat-killed prey bacteria (10^9 CFU mL^{-1}, $MgCl_2$, 3 mM; $CaCl_2$, 2 mM, HEPES or DNB, pH 7.4–7.6) or on PYE medium amended with cell extract (usually derived from prey bacteria, 0.3 mg mL^{-1} protein). Both media are suitable for liquid as well as for agar-based growth. Type I H-I mutants cultivated on heat-killed prey have to be laid over a semisolid medium using the double-layer agar plating (Schwudke et al. 2001). Colonies are then surrounded by clear halos (Roschanski et al. 2011).

Growth Requirements

With axenic mutants at hand, the minimal nutritional requirements of BALOs can be addressed. Ishiguro (1974) successfully developed a chemically defined medium for axenic *B. bacteriovorus* consisting of (g L^{-1}): Na_2HPO_4, 7; KH_2PO_4, 3; NaCl, 0.5; $MgSO_4$, 0.2; NH_4Cl. Mutants were capable of assimilating ammonium and required no additional vitamins. Various amino acids, organic acids, and glycerol – but no sugars – were utilized to different efficiencies.

Conclusions

The field of predatory interactions between bacteria, as represented by studies of the *Bdellovibrio* and like organisms, has been revived in the past decade by the interest of new research groups and the introduction of modern molecular biology, genetics, and genomics technologies.

These have enable to gain a much more detailed understanding of the taxonomy and phylogeny of this diverse group, to appreciate the intricate molecular mechanisms at play during the cell cycle of these fascinating bacteria as well as of their genomic particularities. Based on these significant advances, novel applications of predatory bacteria in medicine, agriculture, and environmental sciences may now become a reality.

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2 The Family *Cystobacteraceae*

Débora Farage Knupp dos Santos[1] · *Cynthia Maria Kyaw*[1] · *Tatiana Amabile De Campos*[1] · *Robert Neil Gerard Miller*[1] · *Eliane Ferreira Noronha*[1] · *Mercedes Maria da Cunha Bustamante*[2] · *Ricardo Kruger*[1]
[1]Department of Cellular Biology, University of Brasilia (UnB), Brasilia-DF, Brazil
[2]Department of Ecology, University of Brasilia (UnB), Brasilia-DF, Brazil

Abstract
Myxobacteria are a group of eubacteria classified in the order *Myxococcales*, further subdivided into two suborders and eight families. These unique microorganisms exhibit distinctive social behavior and morphogenetic characteristics, such as the ability to form myxospores and fruiting bodies. Cellular aggregation is an important feature that allows these microorganisms to adapt to almost every environment.

The genomic features of myxobacteria are also peculiar. Myxobacterial genomes are large, approximately 10 Mb, which may explain, in part, their unique morphophysiological behavior. Hence, myxobacteria are relevant targets for basic microbiological research, including the areas of ecology and morphogenesis. They are also interesting alternatives for the screening of new bioactive molecules, with production that may reach the levels of well-recognized bacterial producers such as *Actinomycetes* and *Bacillus* spp. Since the last decades of the twentieth century, a great effort has been made to isolate and characterize myxobacterial secondary metabolites. Antifungal and antibacterial agents are the most common compounds identified to date. However, other important metabolite activities, such as cytotoxicity to eukaryotic cell lines, suggest their potential applications in biotechnology and pharmaceutical industrial research.

Here, some important features of the genera and species of the *Cystobacteraceae* family, which was established in 1970, are described. The inclusion of new members and genera is still taking place as previously unknown myxobacteria are characterized. In addition, important ecological, genomic, phylogenetic, and morphophysiological questions are discussed and some of the genetic and physiological components that assure the ecological adaptability of these bacteria in highly variable habitats (both soil and water) are described. Finally, ongoing research and future perspectives are summarized.

Taxonomy

Cys.to.bac.ter.a' ce.ae M.L. masc. n. *Cystobacter* type genus of the family; -aceae ending to denote a family; M.L. fem. pl.n. *Cystobacteraceae* the *Cystobacter* family.

Type genus: *Cystobacter* (Schroeter 1886, p. 170).

Howard McCurdy established the *Cystobacteraceae* family in 1970 in a revised classification of cylindrical-shaped myxobacteria (McCurdy 1970). To better separate different cell morphologies, the *Cystobacteraceae* family was proposed to include tapered-ended myxobacteria, thus distinguishing this morphology from cylindrical cells of the *Polyangium* family. As such, the former *Cystobacter fuscus* (*Polyangium fuscum*) was re-established and became the type species of this new family. The type genus became *Cystobacter* (McCurdy 1970).

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Like all myxobacteria, the taxonomy is based primarily on the morphology of vegetative cells, swarms, fruiting bodies, and myxospores.

McCurdy (1970) describes the family as consisting of three genera based on sporangia characteristics: *Cystobacter* (sessile sporangia), *Podangium* (sporangia borne singly on an unbranched stalk), and *Stigmatella* (sporangia in clusters on branched or unbranched stalks). The type genus of the family, *Cystobacter*, was described by Schroeter in 1886 (Schroeter 1886) with two species: *Cystobacter fuscus* and *Cystobacter erectus*. However, in 1897, Thaxter (1897) described *Cystobacter fuscus* as a myxobacterium, later renamed *Polyangium fuscum*, because the shape and structure of its fruiting bodies are similar to those of *Polyangium vitellinum* Link 1809 (Reichenbach 2005c).

In 1970, McCurdy proposed the removal of species *Polyangium fuscum* from the *Polyangium* genus and redefinition of the family *Polyangiaceae* based on observations of the vegetative cells and myxospores. *Polyangiaceae* was changed to consist of myxobacteria with cylindrical cells, as described earlier.

One year later, McCurdy changed the name of the genus *Podangium* to *Melittangium*, based on an analysis performed with the type species of *Podangium*. In this study, he concluded that the type species of *Podangium* was a variant of *Stigmatella brunnea* and changed its name to *Stigmatella erecta*. McCurdy (1971a) proposed a new genus for the other species previously placed in the genus *Podangium*, in which *P. lichenicolum* would be considered the type species. As one of the species, *Podangium boletus*, was the type species of *Melittangium*, the name of the *Podangium* genus was changed to *Melittangium*.

The genus name *Archangium* was coined by Jahn (1924) The etymology (arch, primitive; angium, vessel) describes the primitive nature of *Archangium* fruiting bodies compared to the more elaborate structures found in other genera of fruiting myxobacteria (McDonald 1967). Jahn recognized five species. *Archangium gephyra* was a redesignation of another myxobacterial species, *Chondromyces serpens* Quehl (1906). This genus is considered problematic, because its definition rests on the morphology of its fruiting bodies, which are also produced by many other myxobacteria in place of the typical fruiting body (Reichenbach 2005c).

Until 1970, the genus *Stigmatella* consisted of a range of morphological types closely related to *Chondromyces*. The distinguishing features of its species were considered arbitrary (McCurdy 1971a) and not always consistent. In addition, there was no indication of their optimal growth conditions or whether they were grown and characterized in pure culture (Krzemieniewska and Krzemieniewski 1946). Thus, McCurdy proposed dividing *Chondromyces* into two genera: *Stigmatella* and *Chondromyces* (*Polyangiaceae*), with *Stigmatella* having tapered vegetative cells and encapsulated myxospores, and *Chondromyces* having cylindrical vegetative cells and similar myxospores (McCurdy 1971a).

Hyalangium was added to *Cystobacteraceae* in 2005 by Reichenbach (2005b). The members of this genus bear fruiting bodies with a distinct wall like *Cystobacter*. However, their vegetative cells, sporangioles, and swarm colonies differ morphologically from those of *Cystobacter*, which allowed the classification of *Hyalangium* as a new genus.

Anaeromyxobacter is described by Euzeby's list of bacterial names[1] as a genus within *Cystobacteraceae*. However, the genus belongs to the *Myxococcaceae* family according to the NCBI Taxonomy database and is a separate genus according to its describers (Sanford et al. 2002) and Shimkets et al. (2006). This genus does not appear in the last edition of *Prokaryotes*, because its characterization was not thorough enough for accurate placement (Shimkets et al. 2006). *Anaeromyxobacter* was the first anaerobic myxobacteria to be identified. Results of 16S sequence analysis revealed that it forms a deep branch in the *Myxococcales*, specifically related to the *Myxococcus* subgroup but outside any of the subgroup's three families (Sanford et al. 2002). Thus, its taxonomy is still controversial, and further studies are needed.

Short Descriptions of the Genera

The list of genera is presented according to Euzeby's list of bacterial names.

Anaeromyxobacter Sanford et al. 2002

An.aer.o.my.xo.bac.ter. Gr. pref. an, not or without; Gr. n. aer, air; Gr. n. myxa, slime; N.L. masc. n. bacter, rod; N.L. masc. n. Anaeromyxobacter, slime rod (living without air).

This genus consists of myxobacterial species capable of facultative anaerobic growth using terminal electron acceptors such as nitrate, fumarate, and chlorophenolic compounds. Sulfur compounds are not reduced, and oxygen is used at low concentrations. Cells are narrow rods (4–8-μm long, 0.25-μm wide) that exhibit gliding motility. Terminal ends of cells have pilus structures and form blebs periodically. Refractile cysts are visible in older cultures (Sanford et al. 2002).

Type species: *Anaeromyxobacter dehalogenans* (Sanford et al. 2002).

Archangium Jahn 1924

Ar. chan'. gium. Gr. Fem. n. arch. beginning, origin, primitive; Gr. neut. *angion* vessel, container.

Vegetative cells are long, slender, needle-shaped rods with tapered ends. Fruiting bodies without sporangioles are contorted strings of myxospores in hardened slime forming cushion-shaped masses; they vary in shape and size and may separate into packets under pressure. The myxospores are optically refractile and vary from fat rods with rounded ends to almost spherical in shape. Swarm colonies exhibit

[1] http://www.bacterio.cict.fr/

branched radial veins in a tough slime sheet. This genus cannot degrade chitin (Reichenbach 2005c).

Type species: *Archangium gephyra* (Jahn 1924).

Cystobacter Schroeter 1886

Gr. n. kustis, bladder; N.L. masc. n. bacter, rod; N.L. masc. n. Cystobacter, bladder-forming rod.

Vegetative cells are slender, tapered, flexible rods. Sporangia are sessile, occurring singly or in groups; they are rounded, elongate, or coiled, and surrounded by a definite slime envelope or membrane, either free or embedded in a second slimy layer. Microcysts are rod-shaped, phase-dense or refractile, and rigid. Vegetative colonies do not etch or erode agar media. Congo red is adsorbed. This genus hydrolyzes glycogen but does not utilize carbohydrates (McCurdy 1970).

Hyalangium Reichenbach 2005

*Hy. al. an'. gi. um.*Gr. fem. n. *hyalos* glass; Gr. neut. *angion* vessel, container; M.L. neut. n. *Hyalangium* glassy vessel.

Vegetative cells are delicate, slender rods with tapered ends. Fruiting bodies consist of small spherical sporangioles that are often empty; they appear glassy and transparent, and are arranged in extended dense sheets or in chains. Myxospores are short rods or exhibit an irregular spherical shape and are optical refractile. Swarm colonies form a thin but tough slime sheet with fine veins and adsorb Congo red to produce a purple red color. They are of the proteolytic-bacteriolytic nutritional type.

Type species: *Hyalangium minutum* (Reichenbach 2005b).

Melittangium (Jahn 1924)

r. n. melitta, bee; Gr. neut. n. angion, vessel; N.L. neut. n. *Melittangium*, a vessel resembling a honeycomb.

Vegetative cells are tapered rods. Sporangia are borne singly on a stalk. Microcysts are rod-shaped, and phase-dense or refractile. Vegetative colonies do not etch or erode agar media. Congo red is adsorbed (McCurdy 1971b).

Type species: *Melittangium boletus* (Jahn 1924).

Stigmatella (Berkeley and Curtis 1874)

Vegetative cells are rods with tapered ends. Sporangia are borne singly or in clusters on stalked fruiting bodies; the stalks often occur in groups arising from a common hypothallus. Myxospores are short, rigid, phase-dense, or refractile rods, surrounded by a slime capsule. Vegetative colonies do not etch, erode, or penetrate agar media. Congo red is adsorbed. Members of the genus are aerobic and most hydrolyze urea (McCurdy 1971a).

Type species: *Stigmatella aurantiaca* (Berkeley and Curtis 1874).

Phenetic Basis of the Taxonomy

Morphological characteristics of fruiting bodies, myxospores, and vegetative cells are used for the taxonomic classification of this family. Although aberrant fruiting bodies are common in cultures, members of *Cystobacteraceae* are characterized by fruiting bodies that consist of sporangioles with a distinct outer wall, often with stalks or naked masses of slime and myxospores (Reichenbach 2005c). Myxospores are short, fat rods. Vegetative cells are slender rods with tapered ends, either boat- or needle-shaped (Shimkets et al. 2006).

Genus taxonomy is determined by fruiting body and sporangiole morphology. Fruiting body morphology is used to differentiate *Archangium* from other genera. Members with fruiting bodies consisting of a naked mass of myxospores belong to the *Archangium* genus. Those with fruiting bodies composed of sporangioles with a distinct wall are differentiated by sporangia morphology: Members with sporangia borne on slime stalks belong to the *Melittangium* genus, whereas those with sessile sporangia belong to *Hyalangium* or *Cystobacter*. Finally, sporangiole morphology distinguishes *Hyalangium* and *Cystobacter*. *Cystobacter* has relatively large sporangioles, in more or less extended aggregates, often piled up, and sometimes surrounded by a translucent slime layer. However, sporangioles of *Hyalangium* are rather small, often glassy, translucent, and empty, and arranged in chains or sheets (Reichenbach 2005c).

The *Archangium* genus has a single species, *Archangium gephyra* (Jahn 1924). According to Reichenbach (2005c), several species have been described in the past; some are identical to *A. gephyra*, whereas others are clearly different organisms, and still others are not easily recognized, because they are poorly described. Part of the taxonomy problem is due to limited cultivation or comparative analysis with an insufficient number of strains.

As shown in Euzeby's list of bacterial names,[2] seven species comprise the *Cystobacter* genus: *C. fuscus* (type species), *C. armeniaca*, *C. badius*, *C. ferrugineus*, *C. minus*, *C. velatus*, *and C. violaceus*. However, *C. disciformis* and *C. gracilis* are also recognized by Reichenbach (2005c). Antibiotic sensitivity and enzymatic activities were used to differentiate species (McCurdy 1970), but species identification is currently based only on chitin degradation activity and morphological features of sporangioles and myxospores (Reichenbach 2005c).

Hyalangium minutum is the sole species of the genus *Hyalangium* (Reichenbach 2005c). Its glassy and translucent sporangioles are used for identification.

Fruiting body and sporangiole color and morphology are the phenotypic characteristics used to identify *Melittangium* species (McCurdy 1970; Reichenbach 2005, p. 117, 2005c). This genus comprises three species: (1) *M. boletus*, which produces yellow to brown spherical or flattened sporangia that resemble a mushroom pilus; (2) *M. lichenicola* which produces orange or red fruiting bodies that are single or arranged in an irregular

[2] Available at http://www.bacterio.cict.fr/c/cystobacter.html

mass; and (3) *M. alboraceum*, which produces a single irregular, pale orange, globe sporangium on a long, white, corkscrew-shaped sporangiophore (McCurdy 1970).

Three species are also recognized in the *Stigmatella* genus: *S. aurantiaca*, *S. erecta*, and *S. hybrida*. Sporangia of *S. aurantiaca* and *S. erecta* are similar, although fruiting bodies shows variable morphology in *S. aurantiaca*. However, vegetative colonies of *S. aurantiaca* and *S. erecta* exhibit different features. In *S. aurantiaca*, they are thin and flat with numerous radiating and concentric ridges, filamentous, and poorly defined. In *S. erecta*, vegetative colonies are at first thin and transparent, and later yellow or light flesh-colored with numerous radiating ridges and indefinite, thin edges.

Genomic Basis of the Taxonomy

The first 16S rRNA-based taxonomy of myxobacteria was reported by Shimkets and Woese, who used all cultivated genera described thus far (Shimkets and Woese 1992). The authors chose the molecular taxonomy approach in an attempt to solve some of the problems associated with phenotype-based taxonomy of myxobacteria.

At that time, fruiting body morphology was the primary basis of species classification, even though some genera form aberrant fruiting bodies or no fruiting bodies at all after continued cultivation (McCurdy 1971a, b; Reichenbach 2005c) Later, Spröer et al. (1999) used the same approach to determine the suitability of morphological criteria to affiliate myxobacteria strains to species. An analysis of 54 myxobacterial strains representing 21 morphological species demonstrated the phylogenetic coherence of myxobacteria, because strains classified on morphological grounds clustered next to their respective strains according to phylogenetic analysis.

Since the Shimkets and Woese analysis, several 16S rDNA molecular analyses of myxobacteria have been performed, including analyses of unculturable strains (Garcia et al. 2010, 2011; Jiang et al. 2007; Wu et al. 2005). Molecular taxonomy analysis performed by Garcia et al. (2010) showed the clustering of *Cystobacteraceae* species in a defined branch. However, *Anaeromyxobacter* strains were clustered in a separate branch within *Cystobacterinae*, constituting an anaerobic branch within Myxobacteria (branch XIII).

Two clusters of strains belonging to *Cystobacteraceae* were observed: the *Archangium-Cystobacter-Melittangium* cluster (branch XIV) and the *Hyalangium-Stigmatella* cluster (branch XV). In the first cluster (branch XIV), *Archangium* strains comprise a monophyletic group (bootstrap percentage = 99 %); however, *Cystobacter* strains comprise a polyphyletic cluster. Furthermore, many *Cystobacter* strains did not match to their corresponding type strains. Garcia et al. (2010) attributed these results to incorrect morphologic characterization. Fruiting body characteristics vary in different media and are often lost after several transfers in the same medium. *Melittangium* strains fall between polyphyletic branches of *Cystobacter*. The proposed neotype strain proposed by McCurdy, *M. lichenicola* (McCurdy 1971b), was evidently divergent from *M. alboraceum* and *M. boletus*. The current type strain of *M. lichenicola* was found in the *Corallococcus* cluster, suggesting a case of misclassification (Garcia et al. 2010).

According to Garcia et al. (2010), the *Hyalangium-Stigmatella* cluster (branch XV) doubtless represents a novel genus based on its 16S rDNA sequence, a unique branch in *Cystobacteraceae*, and unusual morphology. *Stigmatella* appears to be a homogenous group (bootstrap 100 %). All strains were matched with their corresponding types, indicating that the three species are distinct from each other. This cluster (branch XV) appears to represent a separate family, based on at least 4 % identity difference in 16S rDNA sequences and branching patterns with clusters XIV–XVI.

Similar to previous studies (Jiang et al. 2007; Sproer et al. 1999; Wu et al. 2005), the molecular taxonomy analysis of Garcia showed that myxobacteria are a phylogenetically coherent group. Most of the strains in the phylogenetic tree matched with the type or proposed neotype of *Cystobacteraceae* strains, except for members belonging to *Cystobacter* and *Melittangium*. The genus *Cystobacter* holds the most misclassified isolates, which may represent more than three genera.

To construct our 16S rRNA tree and compare it with the cited phylogenetic analysis, 17 sequences from *Cystobacteracea* family were used, including selected sequences from other suborders and families as well. *Table 2.1* shows the accession numbers used to construct the phylogenetic tree.

Figure 2.1 shows the clusterization of some species of the *Cystobacteraceae* family. The tree was constructed using Neighbor-joining algorithm and the type species are highlighted in bold letters. One of the species most recently included in this family, *Anaeromyxobacter dehalogenans*, is slightly divergent. However, the branching is consistent with the phylogenetic tree published in 2002 (Sanford et al. 2002). The deep branching of *H. minutum*, a new species characterized in 2005 (Reichenbach 2005b), can also be observed. *Stigmatella* and *Hyalangium* members are closely related to the *Archangium*, *Cystobacter*, and *Melittangium* members—this last branch is the most heterogeneous and includes *Cystobacter* and *Melittangium* species that were also misplaced in the phylogenetic tree performed by Garcia et al. (2010). It also shows the clusterization of species from *Cystobacter* and *Melittangium* genera, which is consistent with the *Cystobacter-Melittangium* branch previously described.

These discrepancies may be due to using partial 16S rRNA sequences, which can contribute to incorrect phylogenetic placement. Bootstrap indexes must be considered too, as some values are too low to be reliable for clusterization.

In general, morphology-based characterization alone is not conclusive for the classification of an isolate. Fruiting bodies, vegetative cells, and myxospore morphology are appropriate for genera identification but are frequently insufficient for species identification. Thus, it is highly recommended to combine phenotypic, chemo-physiological, and genetic characteristics for the classification of a strain.

Table 2.1
List of cataloged *Cystobacteraceae*[a] species

Genus	Species	Type strain	16S rRNA gene NCBI accession number	Genome sequencing
Cystobacter	*C. armeniaca*	CIP 109127; DSM 14710; JCM 12622	DQ768107	No
	C. badius	CIP 109126; DSM 14723; JCM 12623	DQ768108	No
	C. ferrugineus	DSM 14716; JCM 14717	AJ233901	No
	C. fuscus[b]	ATCC 25194; DSM 2262	DQ768109	Yes
	C. miniatus	CIP 109119; DSM 14712; JCM 12626	DQ768111	No
	C. minus	DSM 14751; JCM 12627	AJ233903	No
	C. velatus	CIP 109132; DSM 14718; JCM 12628	DQ768115	No
	C. violaceus	CIP 109131; DSM 14727; JCM 12629	DQ768114	No
Hyalangium	*H. minutum*[b]	CIP 109157; DSM 14724; JCM 12630	DQ768124	No
Melittangium	*M. alboraceum*	DSM 52894	AJ233907	No
	M. boletus[b]	DSM 14713; JCM 12633	AJ233908	No
	M. lichenicola	ATCC 25944	DQ768126	No
Stigmatella	*S. aurantiaca*[b]	ATCC 25190	DQ768127	Yes
	S. erecta	ATCC 25191; DSM 16858T	AJ970180	No
	S. hybrida	CIP 109130; DSM 14722; JCM 12640	DQ768129	No
Anaeromyxobacter	*A. dehalogenans*[b]	ATCC BAA-258; DSM 21875	AF382396	Yes
Archangium	*A. gephyra*[b]	ATCC 25201; DSM 2261	DQ768106	No
%				3/17 (17.64 %)

[a]According to the List of Prokaryotic names with Standing in Nomenclature (LPSN), available at: www.bacterio.cict.fr (accessed February 2013)
[b]Type species

Phenotypic Analysis

Cell and Colony Morphology

Members of the *Cystobacteraceae* family share many morphological and physiological features, the most important of which is the formation of fruiting bodies (and myxospores) under starvation conditions. Fruiting bodies are cell aggregations that enable the accumulation and dispersion of metabolites to ensure cell survival in stressful environments. This cooperative trait is one example of the exceptional social behavior of myxobacteria, which are also characterized by gliding motility and the production of a wide range of secondary metabolites.

As described in *Bergey's Manual of Determinative Bacteriology* (Reichenbach 2005c), vegetative cells are slender, flexible rods with tapering ends, often long and needle-shaped. The fruiting bodies can be naked masses of slime and myxospores that are of cartilaginous consistency, variable size, and irregular shape. However, in most cases, the fruiting bodies are made up of pale to deep brown sporangioles in various arrangements, with or without a slime stalk or pedicle. Myxospores are short, optically refractile rods, or spheroids. In the latter case, they are always slightly deformed and accompanied by short rods with rounded and tapered ends. Swarm colonies produce a firm slime layer with more or less conspicuous straight and often branched radial veins. The slime sheet, which may become tough, quickly adsorbs Congo red, giving the swarm a purple red color. All species utilize mono-, di-, and polysaccharides. Some species vigorously degrade chitin.

However, some differences can be seen between genera in the *Cystobacteraceae* family. A recent member, *Anaeromyxobacter dehalogenans*, was described in 2002 and designated to a new genus, *Anaeromyxobacter* (Sanford et al. 2002). It was the first myxobacteria identified as a facultative anaerobe; all other members are strict aerobes. The *Hyalangium* genus was proposed in 2005 (Reichenbach 2005b) and validated in 2007 (Euzeby 2007). Small delicate vegetative cells with glassy and transparent sporangioles characterize *Hyalangium minutum*, the only member of the genus so far.

Myxobacterial colonies exhibit a characteristic multicellular spreading behavior called swarming. Thus, their colonies are called swarms, and those of *Cystobacterineae* members are flat and thin-layered (Dawid 2000; Kaiser and Crosby 1983). Factors influencing the motility of myxobacteria include nutrient concentrations, temperature, and original cell density.

On nutrient-rich media, colonies may show a round, compact morphology, like those of common bacteria. However, on commonly used media with relatively low nutrient concentrations, colonies appear as a sheet of cells and excreted slime, revealing patterns of veins, rings, or even depressions in the agar. This is commonly observed in the swarms of *Cystobacter*, *Archangium*, *Melittangium*, and *Stigmatella*. Swarms of

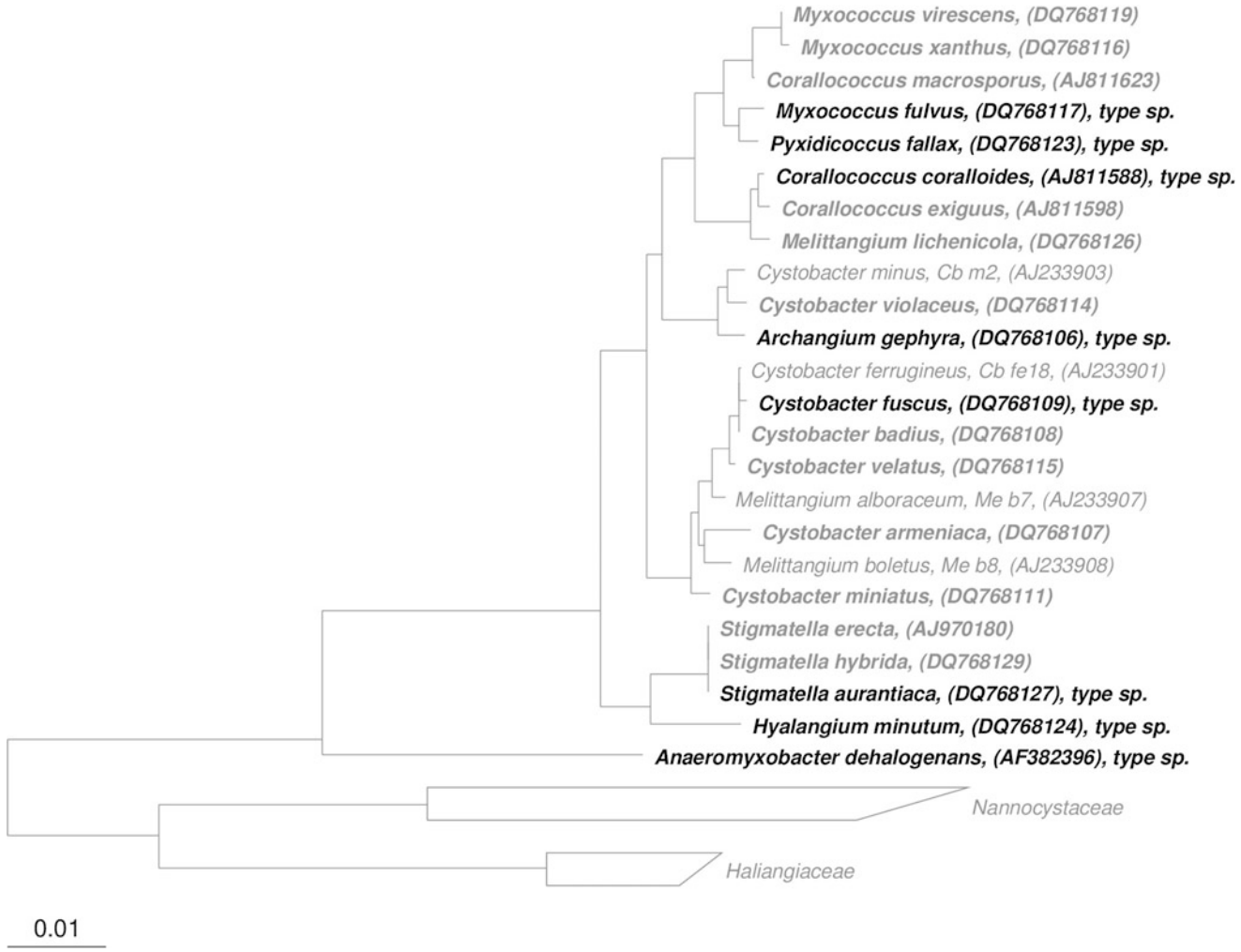


Fig. 2.1

Phylogenetic reconstruction of the family *Cystobacteraceae* 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

Archangium, *Cystobacter*, and *Melittangium* can become very tough and strongly adherent to the medium (Shimkets et al. 2006). On some media, colonies are pigmented, probably due to the presence of melanoid pigments. Depending on the specific medium, colonies of some *Cystobacter*, *Archangium*, and *Stigmatella* species may appear purple, red, or even black.

According to Dawid (2000), fruiting bodies are diverse, ranging from globular to unusual shapes, with hardened slimes like those of *Archangium*. In most species, spores are enclosed in sporangioles, which may be embedded in the substrate (as in *Cystobacter*) or associated with slime stems (as in *Melittangium* and *Stigmatella*). The fruiting body ranges from 10 to 1,000 µm, and its color may be white, brown, bright yellow, orange, red, brown, or lilac (Shimkets et al. 2006).

In *Stigmatella aurantiaca*, fruiting body development is enhanced by light, whereas in other genera, factors such as nutrient concentration, pH, cations, or temperature influence this process (Dworkin 1996). Fruiting bodies of *Cystobacteraceae* are briefly described below (adapted from Shimkets et al. 2006). Morphological changes that occur during the life cycle of myxobacteria are illustrated in the ❯ *Fig. 2.2*.

As previously mentioned, fruiting bodies have not been described in *Anaeromyxobacter*. Those of *Archangium* are naked masses of slime and myxospores, or hard and cartilaginous. In *Melittangium* and *Stigmatella erecta*, the fruiting body consists of a single sporangiole on top of a stalk, whereas in *Cystobacter*, it consists of a group of sporangioles on or in the substrate. In *Hyalangium*, the fruiting body consists of a group of small spherical sporangioles often empty, glassy, and

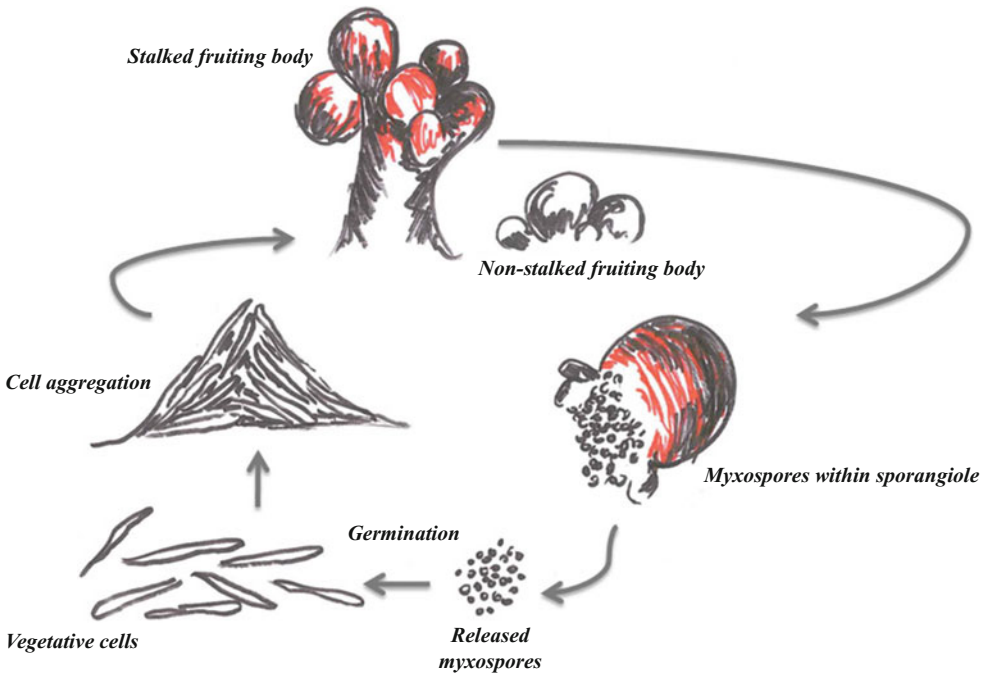


Fig. 2.2

Morphological changes of myxobacteria during their characteristic life cycle. Under stress conditions, vegetative cells aggregate to form multicellular structures known as fruiting bodies. The figure shows two morphologies: stalked (as seen in *Stigmatella aurantiaca*) and non-stalked fruiting bodies. Within these structures, cells continue to change to form myxospores, which can endure stressful circumstances (e.g., low nutrient levels) for long periods. When conditions improve, the myxospores germinate into vegetative cells

transparent, arranged in extended, dense sheets or in chains. In *Stigmatella aurantiaca*, it appears as a cluster of sporangioles on top of an unbranched stalk.

Thus, *Cystobacteraceae* family members exhibit Gram-negative, tapered-ended, needle-shaped vegetative cells with gliding motility, convertible to refractile or phase-dense myxospores. Fruiting bodies, when present, are of variable sizes and shapes, often brown to red-colored, and can be structured or not on pedicles or stalks (McCurdy 1970; Reichenbach 2005a). *Table 2.2* summarizes the information described above.

Isolation, Enrichment, and Maintenance Procedures

Members of *Cystobacteraceae* are commonly found in soils, especially near the base of stems or between roots, but are also found on decaying plant material, tree bark, and the dung of herbivorous animals. Attempts to isolate these organisms from those habitats often fail, in particular because of the slime matrix produced by the cells, which makes cell dispersion difficult (Shimkets et al. 2006). When nutrients are scarce, cells aggregate into fruiting bodies, within which some differentiate into spores. When nutrients are sufficient, cells grow actively on surfaces as a swarming colony. Among the *Cystobacteraceae* genera, only *Anaeromyxobacter*, a microaerophilic organism, does not produce fruiting bodies (Huntley et al. 2011). *Stigmatella aurantiaca* digests chitin, and its growth is enhanced by glucose (Reichenbach and Dworkin 1969). *Archangium gephyra* can be isolated from cold environments such as the Swedish arctic tundra, Alaska, Iceland, and soils of northern Canada. On the other hand, some species such as *Stigmatella aurantiaca*, *Cystobacter*, *Melittangium*, and *Archangium* are found in hot biotopes like desert soils. *Archangium* and *Cystobacter* have also been found in coastal samples from North and South America (Dawid 2000).

Oligotrophic media are better for characterizing an organism as a myxobacterium (some protocols are described in Supplement 1). On these types of media, colonies develop as delicate swarmings. On rich media, colonies appear as compact structures, not easily recognizable as myxobacteria (Shimkets et al. 2006).

It is possible to isolate members of *Cystobacteraceae* directly from their natural habitats by soaking the substrate (dung, leaves, or bark) in water containing up to 0.08 mg/ml cycloheximide for a few hours in Petri dishes lined with filter paper. This system should be wrapped in a sterile plastic bag or placed in an incubator to reduce the chance of contamination. The cultures should be moistened periodically and maintained at room temperature or higher (approximately 30 °C) to prevent fungal growth. Cultures should be inspected daily for the presence of fruiting bodies. Alternatively, pigmented colonies may be detected, especially on the surface of the filter paper (Shimkets et al. 2006). When using soils as natural samples, the general procedure is the same, except for the initial step, in which soils are incubated in the presence of sterile dung pellets.

Table 2.2
Morphophysiological features of *Cystobacteraceae* members according to type species

Genus	*Anaeromyxobacter*	*Archangium*	*Cystobacter*	*Hyalangium*	*Melittangium*	*Stigmatella*
Vegetative cell morphology	4–8 × 0.25 μm narrow rods; pilus at terminal ends	0.8 × 6–15 μm needle-shaped rods with tapered ends	0.6–0.8 × 3–20 μm needle-shaped rods	0.6–0.7 × 3–6 μm delicate rods with tapered ends	0.7–4.5 × 10.5 μm rods with tapered round ends	0.6–1.0 by 4–10 μm rods with tapered ends
Pigmentation	Red colonies in fumarate-grown cells	Reddish to violet	Diffuse dark brown to red	Brownish fruiting bodies	White to yellowish sporangiophores	Yellowish pigmentation on agar; reddish sporangia
Spores and multicellular morphology	Refractile microcysts (older cultures); fruiting bodies are not characteristic	No sporangioles, fruiting bodies' size varies, cushion-shaped myxospores	Sessile, oval sporangia in gelatinous matrix	Potato-like sporangioles, often translucent or glassy	Mushroom-like sporangia, brown-colored	Stalked fruiting bodies, ≤400 μm; unbranched; pedicles may be present
Growth	Facultative anaerobe	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Type species	*Anaeromyxobacter dehalogenans*	*Archangium gephyra*	*Cystobacter fuscus*	*Hyalangium minutum*	*Melittangium boletus*	*Stigmatella aurantiaca*
Type strain	ATCC BAA-258; DSM 21875	ATCC 25201; DSM 2261	ATCC 25194; DSM 2262	CIP 109157; DSM 14724; JCM 12630	DSM 14713; JCM 12633	ATCC 25190
pH	7.0 optimum	ND	6.8–8.2	ND	4.0–8.5	7.0–7.2
Temperature	30 °C optimum		18–40 °C, 30 °C optimum	ND	20–31 °C	18–37 °C, 30 °C optimum
Catalase	ND	ND	Positive	ND	Positive	Positive
Oxidase	ND	ND	Negative	ND	Negative	Negative
Nitrate reduction	Yes	ND	No	ND	No	No
G+C content	ND	68 mol%	68 mol%	ND	ND	68 mol%
Metabolism	2-chlorophenol best for halorespiration; does not reduce sulfur compounds	Cellulolytic, proteolytic; does not degrade chitin	Non-cellulolytic; hydrolyzes starch, nucleic acids	Does not degrade chitin	Non-cellulolytic; hydrolyzes starch, nucleic acids	Hydrolyzes starch, nucleic acids

- **Isolation from soils with bait**. Soil samples should be placed in a Petri dish, moistened with distilled water, and mixed with sterile dung pellets. Vegetative cells will colonize the dung pellets, resulting in the development of fruiting bodies. This method is useful for the isolation of *Cystobacter*, *Stigmatella*, *Archangium*, and *Melittangium* species.
- **Isolation from swarms**. This method is based on the bacteriolytic properties of *Cystobacteraceae*. Small samples, preferentially soil or vegetal debris, should be inoculated onto a plate of water agar (WAT) previously inoculated with an organism preyed upon by *Cystobacteraceae* (e.g., *Escherichia coli*) as parallel, cross, or circular streaks. The streaks should be made in the center (cross or circular) or ends of the plate (parallel). Moistening the sample facilitates inoculation, and the sample should not be scattered over the medium. To minimize fungal contamination, use WCX agar, which contains cycloheximide (25–100 μg/ml) (Brockman and Boyd 1963) (protocol described in Supplement 1).

According to a recent paper by Garcia et al. (2009), the isolation and cultivation of members of the suborder *Cystobacterineae*, to which the *Cystobacteraceae* family belongs, is likely to be more successful than that of other myxobacteria. Commonly used culture media include CY and VY/2 agar (Shimkets et al. 2006), mineral salts agar, and anaerobic medium. A buffered yeast agar (modified version of VY/2 agar) is a useful transfer medium, because it supports spore germination and promotes the spreading of the swarm colony and fruiting body formation. This is also a suitable medium to preserve the cultures (Garcia et al. 2009) (protocol described in Supplement 1).

Because the cultures must sometimes be incubated for long periods (e.g., 1–4 weeks), it may be wise to prepare thick agar

plates and incubate the organisms in humid chambers or incubators that have a source of humidity. Generally, the best culture conditions are:

- pH: 6.5–8.5
- Temperature: 30–36 °C, although some species of *Cystobacter* and *Archangium* tolerate higher temperatures, nearly 40 °C
- Oxygen: with the exception of the microaerophile *Anaeromyxobacter*, which is a microaerophile, all *Cystobacteraceae* are strict aerobes

Anaeromyxobacter dehalogenans is a motile facultative anaerobic/microaerophilic Gram-negative rod that uses acetate as an electron donor and chlorinated phenols as electron acceptors. Its colonies produce a red pigment, and to date, no fruiting bodies have been described (Sanford et al. 2002). Two of the media commonly used to grow this organism are anaerobic medium and mineral salts medium (protocol described in ❯ Supplement 2) (Loffler et al. 1996; Sanford and Tiedje 1996/1997). This organism is metabolically versatile and considered an important bioremediation agent. *Anaeromyxobacter dehalogenans* can reduce a variety of toxic compounds, such as soluble U(VI) to sparingly soluble, immobile U(IV) (Thomas et al. 2010) and Se(IV) to Se(0) (He and Yao 2011).

Ecology

Main Habitats

Previous studies have reviewed the ecological distribution of myxobacteria based upon enrichment and classical isolation (Dawid 2000; Parish 1984; Reichenbach 1993, 1999). Noted for the formation of multicellular fruiting bodies containing myxospores that resist desiccation and freezing, as well as temperatures up to 60 °C when suspended in liquids (Holt et al. 1994), myxobacteria are found in both moderate and extreme environments.

In terms of global distribution, Dawid (2000) reported the isolation of myxobacteria from numerous countries across all continents. Regarding climate, myxomycetes have been isolated from Antarctic soil, arctic tundra, steppes, deserts, bogs, a range of altitudes, warm and humid climates, warm and dry climates, tropical climates, and temperate climates (Shimkets 1990; Shimkets et al. 2006). The greatest abundance and diversity of myxobacteria are associated with warm semiarid zones such as the southwest United States, northern India, and Egypt (Reichenbach 1999).

A recent metagenomic survey from Cerrado soil in Middle-western Brazil showed that 16S rDNA sequences from *Myxococcales* order comprised 1.3 % of all rDNA sequences analyzed; however, no sequences from *Cystobacteraceae* were detected. Nevertheless, the metagenome sequence analysis identified *Cystobacteraceae*, *Kofleriaceae*, *Myxococcaceae*, *Nannocystaceae*, and *Polyangiaceae* families. With respect to function, these sequences appear to be closely related to RNA metabolism, as determined by a comparison with sequences in the SEED database (Castro de, Bustamante and Kruger, Unpublished results).

Most myxobacteria are aerobic mesophiles (Reichenbach 1999); however, psychrotrophs (Zhukova 1963) and psychrophiles have also been described (Dawid et al. 1988). Regarding pH, the most common range for growth in culture is 6.8–7.8, although isolation from acid soils (pH 2.5) (Dawid 1979) and alkaline lakes (pH 9.5) in east Africa (Reichenbach 1999) has been reported.

Observed predominantly in the topmost layers of aerated soils, myxobacteria are principally observed in neutral or slightly alkaline soils (McCurdy 1969), compost (Singh 1947), herbivore dung (especially that of rabbits, hares, deer, moose, sheep, and goats), rotting wood or tree bark (Shimkets et al. 2006), and decomposing lichens and insects (Reichenbach 1999). Specific species can adapt to different substrates according to climatic and edaphic characteristics (Shimkets et al. 2006). The attraction of myxobacteria to such habitats may reflect micropredation (Shimkets 1990), with dead and living microorganisms in these typically rich microbial communities serving as secondary substrates for degradation by host-secreted enzymes (Shimkets et al. 2006). Myxobacteria also include cellulose decomposers; thus, attraction to such habitats may reflect the abundance of degradable plant residues. The presence of myxobacteria on dung collected from snow suggests their ability to pass through the animal digestive system (Rückert 1975). This was confirmed under laboratory conditions by pipette feeding of myxobacterial fruiting bodies to white mice (Kühlwein 1950). The involvement of myxomycetes in soil microbial communities is limited, but their involvement in microbial predation and secondary metabolite antibiotic production is common (Reichenbach et al. 1988).

Myxobacteria also appear to settle in specific habitats through the movement of soil and dust particles by air currents, as suggested by reports of air contamination (Wu et al. 1968) and their presence on plant leaf surfaces (Ruckert 1981). Fresh-water habitats can also support myxobacteria (Hook 1977). Although their presence may simply be a result of run-off from soil, cultivation of strains in aquatic conditions has been dem-onstrated (Shimkets et al. 2006). The isolation of halotolerant myxobacteria from marine environments has also been described, but this is less common (Dawid 2000; Iizuka et al. 1998; Li et al. 2002).

It should be noted that culture-dependent identification methods for myxobacteria may be limited in their precision and sensitivity as a result of unsuitable growth media, slow growth of cells into colonies, and microbial contamination. In contrast, culture-independent methods (e.g., characterization of 16S rDNA genes) are promising alternatives, with potentially greater resolution. For example, the diversity of halotolerant myxobacteria in oceanic sediments has been shown to be considerable based upon this approach, with 69 highly diverse marine myxobacteria-related 16S rRNA gene sequences characterized from Japanese oceanic sediments (Wu et al. 2005).

Given their unique features and highly adaptable phenotypes (e.g., sporulation and gliding motility), myxobacteria are able to survive in nearly any environment, although the main habitat of this ubiquitous group of microorganisms appears to be soil. Their ability to grow on decaying plant matter, decomposed substrates, and living and dead microorganisms (Reichenbach 1999) depends on an extensive enzymatic supply, consisting of different types of molecules with a broad range in activities, from proteolytic to signaling.

The distribution of myxobacteria among marine habits remains unclear. A survey published in 2010 described a 16S rRNA myxobacteria-enriched library constructed with marine samples (Jiang et al. 2010). The results of phylogenetic analysis showed a distinct separation between marine and terrestrial myxobacteria, which is consistent with the previous hypothesis of environmental selection and adaptation of these microorganisms. However, some questions regarding marine myxobacteria remain. Are their nutritional requirements similar to those of soil myxobacteria? How do the two types react to saline concentration? Is saline concentration essential for morphogenetic changes? Are marine myxobacteria able to form fruiting bodies and myxospores, and do they use the same mechanism as soil myxobacteria? Isolation methods must be improved to compare morphological and physiological features of soil and marine myxobacteria and to correlate those features with phylogenetic characteristics.

With a specific focus on the family *Cystobacteraceae* (encompassing the genera *Anaeromyxobacter*, *Archangium*, *Cystobacter*, *Hyalangium*, *Melittangium*, and *Stigmatella*), a summary of common substrate sources for these myxobacteria is provided.

1. ***Anaeromyxobacter***
   Members of the *Anaeromyxobacter* genus are distributed throughout the environment (Sanford et al. 2002), but undisturbed and contaminated soils and sediments appear to be their principal habitat. Studies have reported isolates obtained from anaerobic enrichment cultures of soil samples in Michigan and rainforest soils of Cameroon (Sanford et al. 2002) and the presence of this genus in acidic subsurface sediments (North et al. 2004). *Anaeromyxobacter* spp. appear to use a variety of respiratory electron acceptors, including soluble hexavalent uranium U(VI), nitrate, ferric iron, and manganese oxide (He and Sanford 2003; Sanford et al. 2002; Wu et al. 2006). Given the capability of *A. dehalogenans* for metabolic reduction of soluble U(VI) to sparingly soluble, immobile U(IV) (Marshall et al. 2009; Sanford et al. 2007; Wu et al. 2006), which is important for containment at uranium-contaminated locations, 16 s rRNA gene sequence analyses were conducted to examine its abundance in uranium-contaminated soils at the U.S. Department of Energy Field-scale Subsurface Research Challenge (IFC) site near Oak Ridge, Tennessee (Cardenas et al. 2008; North et al. 2004; Petrie et al. 2003). Numerous *Anaeromyxobacter* spp. from the IFC site samples were characterized via sequencing of 16S rRNA gene clone libraries and quantitative PCR (Thomas et al. 2009).
2. ***Archangium***
   Members of the genus *Archangium* are commonly isolated from soil and herbivore dung (Holt et al. 1994; Shimkets et al. 2006). The presence of *A. gephyra* has been reported in grassland soils in Southern Chile (Ruckert 1978), rock, bark, rotting wood, dung (Dawid 1979), and plant leaf samples (Ruckert 1981). Isolation agar with 0.5 % NaCl stimulates the development of *A. gephyra* (Ruckert 1978), indicating some degree of salt tolerance, which most myxobacteria lack. *Archangium serpens* has also been detected in soil and herbivore dung (Shimkets et al. 2006). Culture-independent characterization by 16S rDNA gene analysis has also identified sequences phylogenetically close to *A. gephyra* in Chinese soils (Wu et al. 2005).
3. ***Cystobacter***
   Results of culture-based identification show that *C. fuscus*, *C. ferrugineus*, *C. gracilis*, *C. velatus*, *and C. violaceus* are common in soil and herbivore dung. *Cystobacter disciformis* is common in moderately acidic soils and alkaline peat bogs, and its presence was originally reported in muskrat and deer dung (Reichenbach 2005a). Phylogenetic analysis of 16S rDNA genes from genomic DNA extracted from soil samples from Jinan, China, has identified sequences homologous to *C. ferrugineus*, *C. fuscus*, *C. violaceus*, and *C. minus* (Wu et al. 2005).
4. ***Hyalangium***
   Hyalangium minutum is relatively common in typical myxobacterial habitats. The translucent fruiting bodies can be overlooked or suffer degradation; therefore, its abundance can be underestimated when based solely upon culture-based identification approaches. The type strain NOCB-2 originated from a soil sample obtained from the mountains of Izu and Manazuru peninsula, Japan, and the type strain Hy m4 originated from soil samples containing rotting wood from Iowa, USA (Reichenbach 2005b).
5. ***Melittangium***
   Melittangium boletus also occurs in habitats typically associated with myxobacteria, such as soil containing wood and rotting bark. The type strain Me b8 was isolated from such soil material in Uttar Pradesh, India (Reichenbach 2005a). Culture-independent 16S rDNA gene-based identification of *M. alboraceum* and *M. boletus* in soil samples in China has been reported (Wu et al. 2005).
6. ***Stigmatella***
   Bark, rotting wood, and herbivore dung are also typical substrates for members of the genus *Stigmatella*. The species *S. aurantiaca* has been isolated from bark and rotting wood; its occurrence is common in North America (McCurdy 1969; Nellis and Garner 1964; Reichenbach and Dworkin 1969) but rare in Europe (Krzemieniewska and Krzemieniewski 1946). The more frequent isolation from substrates in North America may reflect the more humid summers (Shimkets et al. 2006). Characterization of 16S rRNA gene sequences has identified sequences homologous to *S. aurantiaca* from soil samples in China (Wu et al. 2005).

The presence of *Stigmatella erecta* has been reported in herbivore dung (Shimkets et al. 2006) and soil (Wu et al. 2005).

Molecular Analysis

Genome Structure

Anaeromyxobacter, *Cystobacter*, *Melittangium*, and *Stigmatella* are the *Cystobacteraceae* genera that have at least one fully sequenced genome. Two strains of *Anaeromyxobacter dehalogenans* have been sequenced: 2CP-1 (GenBank NC_011891.1) and 2CP-C (GenBank NC_007760.1). Analysis of these genomes show that *A. dehalogenans* possesses one circular chromosome (approximately 5.00 Mb, 74.7 % GC) with approximately 4,500 genes and 4,400 protein-coding sequences. The two strains have two copies each of 16S, 5S, and 23S rRNA. Each copy of 16S rRNA is identical within the genome of each strain, and there is 99 % similarity between strains. The distribution of genes into clusters of orthologous groups (COG) functional categories showed that the highest number of genes are categorized as hypothetical (1,490, 34.3 %), followed by signal transduction (339, 7.8 %); energy production and conversion (296, 6.8 %); cell wall, membrane, and envelope biogenesis (257, 5.9 %); amino acid transport and metabolism (231, 5.3 %); transcription (214, 4.9 %); translation and ribosome structure (198, 4.6 %); posttranslational modification, protein turnover, and chaperones (187, 4.3 %); inorganic ion transport and metabolism (178, 4.1 %); replication, recombination, and DNA repair (169, 3.9 %); carbohydrate transport and metabolism (143, 3.3 %); lipid transport and metabolism (127, 2.9 %); coenzyme transport and metabolism (120, 2.8 %); secondary metabolite biosynthesis, transport, and catabolism (93, 2.1 %); nucleotide transport and metabolism (70, 1.6 %); defense mechanisms (70, 1.6 %); intracellular traffic, secretion, and vesicular transport (63, 1.4 %); cell motility (59, 1.4 %); cell cycle control, cell division, and chromosome partitioning (38, 0.9 %); chromatin structure and dynamics (2, <0.1 %); and RNA processing and modification (2, <0.1 %) (Huntley et al. 2011). Extrachromosomal elements were not related.

Assembly and annotation of *Cystobacter fuscus* (strain DSM2262) are in progress (GenBank ANAH00000000.1). Its 12.44-Mb genome (68.6 % GC) contains 10,372 genes and 10,372 protein-coding sequences. *Melittangium boletus* (strain DSM1473) sequences were submitted by the Max Planck Institute for Terrestrial Microbiology on February 15, 2013 (BioProject PRJNA189498). Data regarding genome size, GC content, genes, and protein-coding sequences are not yet available.

The genome analysis of *Stigmatella aurantiaca* strain DW4/3-1 revealed one circular chromosome (approximately 10.26 Mb; 67.5 % GC) with 8,500 genes and 8,500 protein-coding sequences, three 16S rRNA gene copies; two 5S rRNA gene copies; and one 23S rRNA gene (GenBank CP002271.1; AAMD00000000). The sequences of the three 16S rRNA and two 5S rRNA gene copies of strain DW4/3-1 are identical. No extrachromosomal elements were related. COG analysis categorized the highest number of genes as hypothetical (4,014; 41.1 %), followed by signal transduction (674, 8.1 %); transcription (352, 4.2 %); cell wall, membrane, and envelope biogenesis (329, 3.9 %); replication, recombination, and DNA repair (326, 3.9 %); amino acid transport and metabolism (324, 3.9 %); carbohydrate transport and metabolism (306, 3.7 %); posttranslational modification, protein turnover, and chaperones (272, 3.3 %); secondary metabolite biosynthesis, transport, and catabolism (261, 3.3 %); energy production and conversion (257, 3.1 %); inorganic ion transport and metabolism (254, 3.0 %); translation and ribosome structure (228, 2.7 %); lipid transport metabolism (188, 2.3 %); coenzyme transport and metabolism (168, 2.0 %); nucleotide transport and metabolism (102, 1.2 %); intracellular traffic, secretion, and vesicular transport (93, 1.1 %); defense mechanisms (89, 1.1 %); cell motility (58, 0.7 %); cell cycle control, cell division, chromosome partitioning (49, 0.6 %); RNA processing and modification (4, <0.1 %); chromatin structure and dynamics (3, <0.1 %); and cytoskeleton (1, <0.1 %) (Huntley et al. 2011).

Metabolism and Biotechnological Relevances

Overall and Secondary Metabolism

Soil is the most common habitat for members of the *Cystobacteraceae* family, but they are also frequently found in the dung of herbivorous animals and on decaying plant material, tree bark, and the surface of plant leaves. These bacteria are also adapted to extreme environments, including extremely acidic soil and conditions that are extremely hot and dry, or extremely cold and nutrient-poor (Dawid 2000). The family consists primarily of aerobic organisms. The exception is the genus *Anaeromyxobacter*, which consists of anaerobic or microaerophilic organisms (Wenzel and Muller 2009b). The metabolism of aerobic members is similar to that of other aerobic eubacteria; however, little is known regarding metabolic pathways of extremophile members.

The soil- and plant-associated species secrete exoenzymes that catalyze the hydrolysis of biological macromolecules and entire microorganisms (e.g., bacteria and yeasts) as sources of carbon, nitrogen, and energy. The most important enzymes secreted by this bacterial family under natural conditions are hydrolases such as proteases, nucleases, lipases, and glucanases. Chitinases, amylases, and xylanases have also been described. Regarding cellulose degradation and utilization, some myxobacteria have been categorized as cellulose degraders (Group I, represented by the suborder Sorangineae) or those unable to use cellulose (Group II), which represents most myxobacterial species, including the *Cystobacteraceae* family (Dawid 2000).

Whole genome sequencing projects have contributed to the description and discovery of metabolic pathways in microorganisms, including myxobacteria such as *S. aurantiaca* and *C. fuscus*. This approach enabled the identification of respiratory chain proteins, the enzyme NADH oxidase, cytochromes, and the complete tricarboxylic acid cycle of *Cystobacteraceae* aerobes, as well as the classic metabolic pathways of carbohydrate metabolism (glycolysis, gluconeogenesis, and pentose phosphate pathway), energy metabolism (oxidative phosphorylation), lipid metabolism, nucleotide metabolism, amino acid metabolism, glycan biosynthesis, and metabolism of cofactors and vitamins.

Most metabolism studies have focused on the remarkable secondary metabolism of these organisms (Gross 2007). *Cystobacteraceae* secondary metabolism is responsible for the production of a large number of bioactive molecules with antifungal, antibiotic, and antitumor activities (Wenzel and Muller 2005). Most secondary metabolites characterized to date are polyketides or nonribosomal peptides, which are synthesized by the two multifunctional enzyme systems polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS), respectively. A hybrid PKS-NRPS system synthesizes hybrid polyketide-nonribosomal peptide metabolites. The biosynthesis initiates through the coupling of simple precursors (i.e., acyl-coenzyme A thioesters for polyketide metabolites, and both proteinogenic and non-proteinogenic amino acids for nonribosomal peptide metabolites). Chain extension intermediates undergo various processing reactions during the assembly process and may be further modified after release from the multienzyme (Wenzel and Muller 2009b).

According to studies of *Bacillus* and actinomycete species, PKS and NRPS act as molecular assembly lines. A PKS consists of an acyl transferase for selection of the specific building block, a ketosynthase to catalyze carbon–carbon bond formation, and an acyl carrier protein, to which the chain is assembled. Other domains have been also described as a ketoreductase, a dehydratase, and an enoyl reductase. These enzymes together determine the final redox-state at the β-carbon of each chain extension unit, as well as C-, O-, and N-methyltransferases. The NRPS system involves adenylation, condensation (or heterocyclization) assembled in a peptidyl carrier protein, complemented by the processing enzymes epimerase, *N*- and *C*-methyltransferases, and an oxidase. The final products are released from the carrier proteins through the action of a thioesterase (Wenzel and Muller 2009b). During a cycle of chain extension, each module of the biosynthetic pathway is used only once before passing the nascent molecule to the downstream module; therefore, the number of modules and their order determine the structure of the secondary metabolite (*Fig. 2.3*). In the model organisms mentioned above, these genes are organized as clusters exhibiting colinearity of the genetic organization and the enzymatic transformations. This common type of genomic organization enables the reconstruction of the biosynthetic pathway by determining the gene sequence within the clusters (Gross 2007).

In contrast, secondary metabolite biosynthesis in myxobacteria differs from this model. It often begins with precursors other than acetate or propionate, such as benzoate, isovalerate, isobutyrate, 2-methylbutyrate, dehydro-isobutyrate, 3-hydroxyvalerate, pipecolic acid, or polyunsaturated fatty acids. Whole genome sequencing of myxobacteria has revealed that gene order within a cluster does not represent the order of enzymatic transformations, making it difficult to predict the structures of the secondary metabolites. Thus, understanding the biosynthesis of these secondary metabolites remains a challenge, and more studies are needed to characterize this process (Li and Muller 2009; Wenzel and Muller 2009b).

Secondary metabolism in the well-characterized species *Stigmatella aurantiaca* includes three main biosynthesis and degradation pathways: terpenoid and polyketide biosynthesis, antibiotic biosynthesis, and xenobiotic degradation (*Table 2.3*). This species is a potential candidate for bioremediation of environments contaminated by phenolic compounds. The *S. aurantiaca* strain Sg a 15 produces structurally different molecules that inhibit the mitochondrial respiratory chain (e.g., aurachins) (*Fig. 2.4*). Aurachins are quinoline alkaloids that target mitochondrial complexes I and II and the cytochrome B6/f complex. These compounds exhibit antifungal, antibacterial, and antiplasmodial activities (Pistorius et al. 2011b).

The biosynthetic pathway by which the aurachins are produced has been studied using a combination of approaches including genome analysis, transposon mutagenesis, and in vivo inactivation. Aurachin synthesis starts with the precursor anthranilate, which is activated and transferred to the acyl carrier protein (ACP). Subsequent condensation of anthraniloyl-ACP with two malonyl units produces one of the major aurachin types, aurachin D (Li and Muller 2009; Pistorius et al. 2011a) (*Fig. 2.5*).

At least three different loci on the *S. aurantiaca* chromosome have been identified as encoding proteins involved in the synthesis of the four aurachin types (locus I to III). Genes at locus I (*auaA–auaE*) are responsible for the synthesis of aurachin D. AuaA is a prenyltransferase with activity demonstrated in vitro. The other genes encode proteins with homology to ACP (AuaB), β-ketoacyl-ACP synthase II (AuaC and AuaD), and benzoate:coenzyme A ligase (AuaE). Thus, aurachin biosynthesis is a complex and intricate metabolic pathway that requires the action of a set of proteins encoded by different genetic loci (Pistorius et al. 2011a, b).

The species *A. dehalogenans* appears to be an unusual myxobacterium, because it grows under anaerobic or microaerophilic conditions, and its genome does not appear to contain typical secondary metabolite gene clusters (Thomas et al. 2008). This species has received considerable attention because of its ability to oxidize organic matter in both pristine and contaminated environments (e.g., uranium sediments). Another noteworthy feature of *A. dehalogenans* is the ability of to derive energy from reductive dechlorination of chlorophenols and its use of a broad range of electron acceptors including fumarate, nitrate, Fe (III), and U(VI) (Sanford et al. 2002).

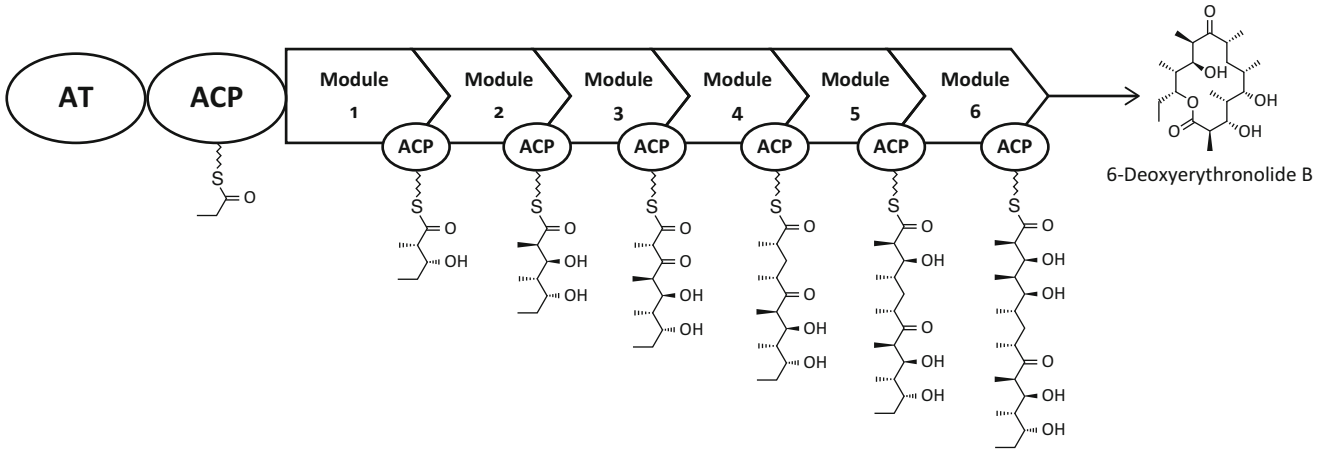


Fig. 2.3

Modular organization of polyketide biosynthesis enzymes showing the six successive steps of extension leading to erythromycin production by *Saccharopolyspora erythraea*. Individual domains include ketoreductase (KR); dehydratase (DH); enoyl reductase (ER); ketosynthase (KS), which catalyzes carbon–carbon bond formation; acyl carrier protein (ACP), to which the chain is assembled; and thioesterase (TE), which releases the final products from the carrier proteins. Module 1, KS/KT/KR; Module 2, KS/AT/KR; Module 3, KS/AT; Module 4, KS/AT/DH/ER/KR; Module 5, KS/AT/KR; and Module 6, KS/AT/KR/ACP/TE

However, little is known regarding its overall metabolism and physiology. Chao (Chao et al. 2010) used a proteomic approach to characterize 50 metabolic pathways of *A. dehalogenans* during its growth using fumarate as primary electron acceptor. The study revealed a range of enzymes involved in carbohydrate and amino acid metabolism, including the fermentation of lysine to butyrate. However, many issues must be addressed for a complete understanding of its metabolism.

Applications

Myxobacteria produce an extensive arsenal of secondary metabolites, similar to well-known bacterial producers such as actinomycetes, pseudomonads, *Bacillus spp.*, yeasts, and filamentous fungi. The core structures of these secondary metabolites are promising tools for the pharmaceutical and biotechnological industry, because they appear to have unique cellular targets (Weissman and Muller 2009). Many possess antifungal and antibacterial properties, and others are herbicidal, cytostatic to eukaryotic cells, or antineoplastic.

Most of the bioactive compounds described thus far are produced by *Myxococcus xanthus*, *Sorangium cellulosum*, or *Chondromyces* species, with *Sorangium spp.* being the most prominent (Wenzel and Muller 2009a). However, these species are all members of myxobacteria families other than *Cystobacteraceae*. In this chapter, the discussion of bioproducts and their pharmaceutical and biotechnological applications will focus on *Cystobacteraceae* members, particularly *Stigmatella aurantiaca*. Two strains are of great importance: *S. aurantiaca* DW4/3-1 and *S. aurantiaca* Sg a15. Some of these metabolites will be briefly discussed. Most are electron transport inhibitors that target microbial respiration.

A group of researchers from the German Research Center for Biotechnology (GBF) in Braunschweig, Germany, published several papers in the 1980s describing the isolation and characterization of novel molecules, mainly antibiotics, derived from myxobacteria. Of these primary references, three are of great value for understanding *S. aurantiaca* myxalamids (Gerth et al. 1983), stigmatellin (Kunze et al. 1984), and aurachins (Kunze et al. 1987). Two other compounds from members of the *Cystobacteraceae* family should also be mentioned: cystothiazole (Ojika et al. 1998) (from *Cystobacter fuscus*) and melithiazols (Sasse et al. 1999) (from *Melittangium lichenicola* and others).

Although the 1980s were extremely important for myxobacterial antibiotic screening and identification, the molecular techniques available today are much more efficient. Genetic screening for biosynthetic modules (such as PKS and NRPS) and gene clusters are the central theme of several works on secondary metabolite identification in microorganisms. Many of these studies relied on mutagenesis and/or gene inactivation strategies. Recent studies have focused on identifying hybrid biosynthetic clusters of secondary metabolites from myxobacteria, mainly PKS/NRPS, which may contribute to more complex and rare molecular structures.

Molecular approaches offer an additional advantage over genetic screening. Myxobacteria are slow growing, making chemical and functional screenings more difficult. Furthermore, molecular techniques enable culture-independent screening, even though only 15 myxobacterial genomes have been completely sequenced according to the NCBI Microbial Genome Database[3].

[3] Accession in 01.21.13, available at: www.ncbi.nlm.nih.gov/genomes/MICROBES/microbial_taxtree.html

Table 2.3
Secondary metabolism pathways of *Stigmatella aurantiaca*

Metabolism of terpenoids and polyketides
Terpenoid backbone biosynthesis
Sesquiterpenoid and triterpenoid biosynthesis
Carotenoid biosynthesis
Limonene and pinene degradation
Geraniol degradation
Type I polyketide structures
Polyketide sugar unit biosynthesis
Biosynthesis of siderophore group nonribosomal peptides
Biosynthesis of other secondary metabolites
Penicillin and cephalosporin biosynthesis
Streptomycin biosynthesis
Novobiocin biosynthesis
Xenobiotic biodegradation and metabolism
Benzoate degradation
Aminobenzoate degradation
Fluorobenzoate degradation
Chloroalkane and chloroalkene degradation
Chlorocyclohexane and chlorobenzene degradationToluene degradation
Xylene degradation
Nitrotoluene degradation
Ethylbenzene degradation
Styrene degradation
Caprolactam degradation
Bisphenol degradation
Dioxin degradation
Naphthalene degradation
Polycyclic aromatic hydrocarbon degradation
Steroid degradation

Myxalamids

Although myxalamids antibiotics were first isolated from *Myxococcus xanthus* Mx x12, their production was also observed in *Stigmatella aurantiaca* Sg a15. This antibiotic is actually a mixture of four compounds (myxalamid A–D). The main compound, myxalamid B, has a spectrum of activity that includes molds, yeasts, and Gram-positive bacteria (Gerth et al. 1983). However, this range was considered narrow in comparison to myxothiazol, an antifungal compound isolated in 1980 (Gerth et al. 1980).

Stigmatellin

This metabolite was identified in an antibiotic screening of *Stigmatella aurantiaca* Sg a15 in 1984. It is active against yeast, filamentous fungi, and many Gram-positive bacteria. Because it possessed a chemical structure that differs completely from other known metabolites of myxobacteria, it was considered a new compound and named stigmatellin (Kunze et al. 1984).

Aurachins

Aurachin antibiotics are the third structurally different family of metabolites produced by *Stigmatella aurantiaca* Sg a15. Similar to myxalamids, aurachins are a mixture consisting of 13 compounds; the three major components are aurachin A–C. At the time of the first isolation, they were classified as new quinoline alkaloids and named aurachins (Kunze et al. 1987).

Myxochromide S

More recently, the screening of secondary metabolites has included genomic and molecular processes. One example is the screening for hybrid gene clusters of secondary metabolites in *Stigmatella aurantiaca* DW4/3-1. As described above, most of the myxobacterial machinery of secondary metabolites relies on large enzymatic modules, mainly PKS and NRPS. In 2005, three metabolites of *Stigmatella aurantiaca*, named myxochromide $S_{1\text{-}3}$, were identified. The myxochromide biosynthetic pathway is encoded by a hybrid PKS/NRPS cluster in a three-gene operon (Wenzel et al. 2005).

Aurafurons

These compounds were initially isolated by high performance liquid chromatography-diode array detector analysis of cell extracts from *Stigmatella* and *Archangium* strains (Kunze et al. 2005). They were characterized as new polyketides and named aurafuron A and B. Aurafuron A was isolated from *Archangium gephyra* Ar 10844, and aurafuron B from *Stigmatella aurantiaca* DW4/3-1. Their spectra of activity differ: Aurafuron A is active against some filamentous fungi, and aurafuron B is weakly active against some Gram-positive bacteria. However, both were shown to be cytotoxic to a mouse cell line. This cytotoxic activity has been of great interest since the isolation and characterization of epothilones, a new class of microtubule inhibitors first extracted from *Sorangium cellulosum* So ce90, a myxobacterium from the *Sorangiaceae* family (Gerth et al. 1996). The epothilone analog Ixabepilone (Ixempra™) was approved by the US Food and Drug Administration in 2007 for the treatment of advanced breast cancer.

Melithiazols and *Cystothiazoles*

Using a classic antibiotic screening approach, antifungal activity was observed in the cultures of two *Melittangium lichenicola*

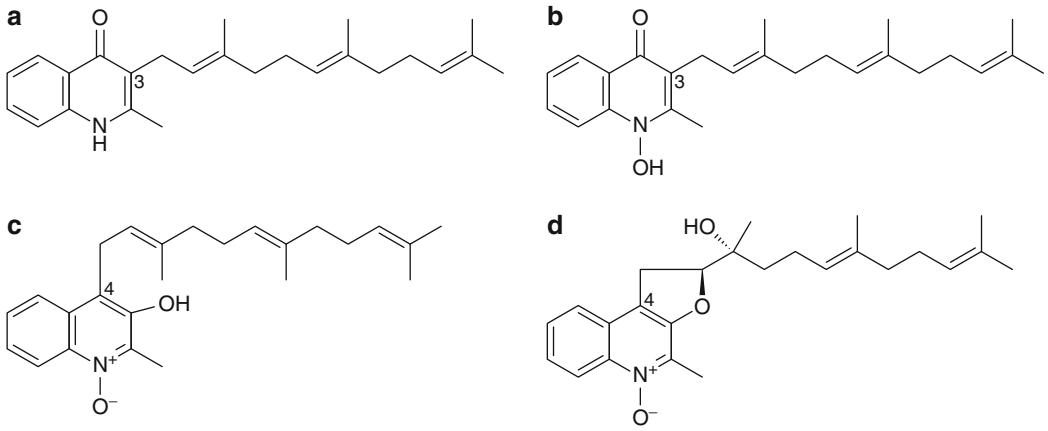


Fig. 2.4
Chemical structures of the four major aurachins from *Stigmatella aurantiaca*. A) Aurachin D (C-type); B) Aurachin C (C-type); C) Aurachin B (A-type); and D) Aurachin A (A-type)

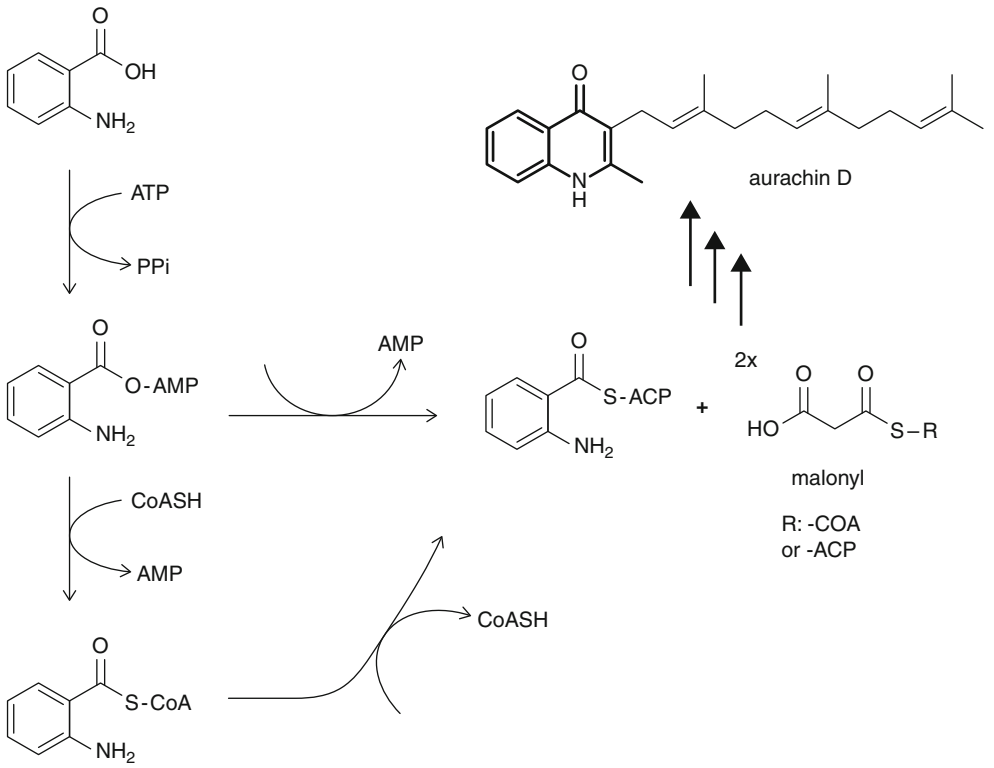


Fig. 2.5
General pathway of aurachin biosynthesis

strains (Me l26 and Me l46). The new compounds, named melithiazol A, B, and C, were also found in other myxobacteria. They are related to the previously characterized myxothiazol but are less toxic. However, antibacterial activity was not observed (Sasse et al. 1999). Cystothiazoles were identified as antibiotics in 1998 in a screen for bioactive compounds in myxobacteria. They were purified from a *Cystobacter fuscus* culture and characterized as new bithiazole-type antibiotics (Ojika et al. 1998).

Although most secondary metabolites of *Cystobacteraceae* members are antibiotics, some also show cytotoxic activity. However, further studies are needed to determine any potential

applications in cancer treatment. These molecules are extremely complex and diverse, motivating both the chemical manipulation of these molecules to construct semisynthetic analogs and the search for new secondary metabolites from these organisms. ❯ *Table 2.4* shows the structures and biosynthetic clusters of the main antibiotic compounds described above.

Concluding Remarks and Perspectives

Myxobacteria are a singular group of microorganisms that are characterized by their environmental adaptability, which is related to their multicellular behavior and complex social traits. The morphological changes that take place during their life cycle include cellular aggregation and fruiting body formation. The study of myxobacteria can contribute to the understanding of bacterial communication, social behavior, and adaptation to stressful conditions. In addition, these microorganisms have proved to be efficient producers of bioactive molecules.

Since the 1980s, many studies have described the characterization and classification of myxobacteria. In 2002, the first anaerobic myxobacterium was identified in this group, which was previously thought to consist of strict aerobes, suggesting an as yet undiscovered diversity in these bacteria. Two new members of the *Cystobacteraceae* family, *Anaeromyxobacter dehalogenans* and *Hyalangium minutum*, were recently described and characterized.

Cultivation and isolation of these slow-growing bacteria are challenging. Therefore, cultivation-independent methods are important tools to screen for metabolic pathway gene clusters that regulate morphogenetic changes during stressful conditions (e.g., starvation) and other physiological and adaptive events, such as secondary metabolite production. Determining the genetic mechanisms that regulate formation of the characteristic fruiting bodies and myxospores is of great importance to microbial ecology studies. The extremely large genomes of myxobacteria may account for some of their unique features; therefore, whole genome sequencing may be important to identify the genetic basis for such traits.

In addition to the screening and isolation of new active compounds, manipulating myxobacterial secondary metabolite core structures may produce semisynthetic molecules with a broader spectrum of activity, less toxicity, and higher efficiency. This is a promising alternative for antibiotic development, as commercially available antibiotics are becoming obsolete, primarily because of multidrug-resistant pathogens. This process could also contribute to anticancer drug development, as molecules with cytotoxic actions are being isolated and characterized. In particular, the secondary metabolites of *Stigmatella aurantiaca* should be noted for the diversity of their molecular structures, which increases the arsenal of active core structures that may be useful in the pharmaceutical and biotechnology industries.

Finally, research focusing on the lifestyle of these microorganisms could provide insights into the molecular pathways underlying the unique features of myxobacteria.

Supplement 1

WAT agar and WCX agar

$CaCl_2 \cdot 2H_2O$ 0.1 % (w/v)
Agar 1.5 % (w/v)
HEPES 20 mM

Prepare medium with distilled water, adjust pH to 7.2, and autoclave. After cooling, add cycloheximide (final concentration 25 mg/ml) from a filter-sterilized stock solution to produce the WCX agar. Because the pH is difficult to adjust in this unbuffered medium, adding 20 μM *N*-2-(hydroxy-ethyl)piperazine-*N*′-2-ethane-sulfonic acid (HEPES) is advisable.

The cultures should be incubated at 30 °C for 8–21 days, with daily inspection for swarms and/or fruiting bodies. *Cystobacter* species are fast spreaders and may reach the streaks within 1–2 days after inoculation. *Cystobacter* and *Archangium* swarms usually develop tough slime sheets, with branched and radiating veins (Shimkets et al. 2006).

VY/2 agar

Bakers' yeast (commercial yeast cake) 0.5 % (w/v)
$CaCl_2 \cdot 2H_2O$ 0.1 % (w/v)
Cyanocobalamin 0.5 mg/μl
Agar 1.5 % (w/v)

Prepare medium in distilled water, adjust pH to 7.2, and autoclave. The yeast may be stored as an autoclaved stock suspension for several weeks. To obtain a uniform suspension of yeast cells in the agar, add the yeast to the molten medium.

CY agar

Casitone (Difco) 0.3 % (w/v)
Yeast extract (Difco) 0.1 % (w/v)
$CaCl_2 \cdot 2H_2O$ 0.1 % (w/v)
Agar 1.5 % (w/v)

Prepare medium in distilled water, adjust the pH to 7.2, and autoclave.

Buffered Yeast Agar (Modified Version of VY/2 Agar)

Add the following to 1 l distilled water: 5-g baker's yeast, 1-g $CaCl_2 \cdot 2H_2O$, 5-mM HEPES, 10-g Bacto Agar. Adjust pH to 7.0 with KOH, and autoclave. After the medium cools, add 0.5-μg/ml vitamin B_{12} (filter-sterilized).

Table 2.4

Structure and general characteristics of some myxobacterial antibiotic compounds

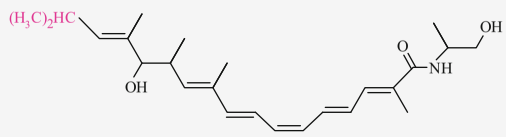
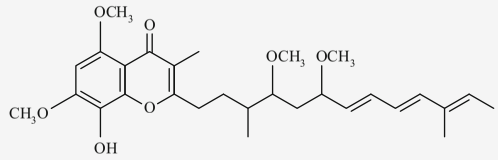
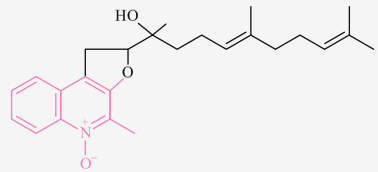
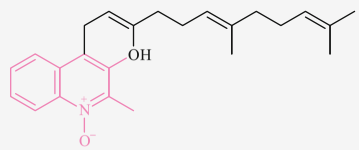
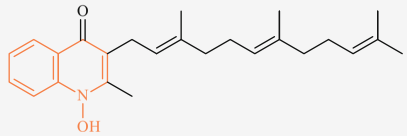
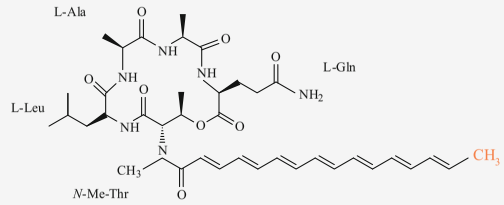
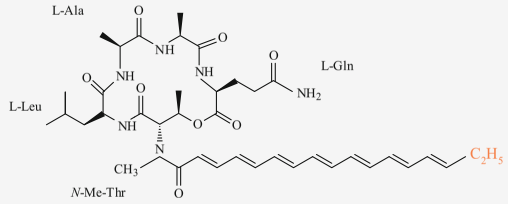
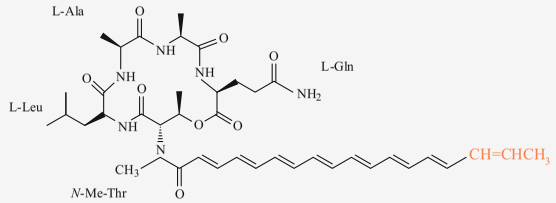
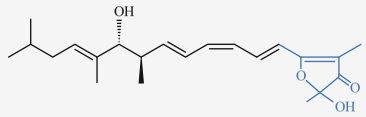
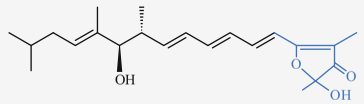
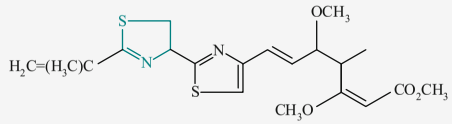
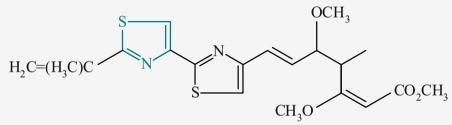
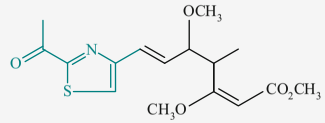
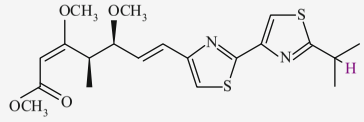
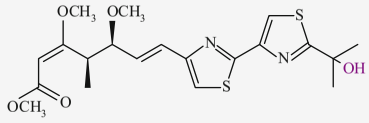
Compound	Strain	Structure	Genetic cluster	Observation	References
Myxalamid B	*Stigmatella aurantiaca* Sg a15		PKS/ NRPS	The substituted alkyl radical (pink structure) is the only one that differs among myxalamid compounds	Gerth et al. (1983), Silakowski et al. (2001)
Stigmatellin	*Stigmatella aurantiaca* Sg a15		PKS	This compound has no natural derivatives so far	Gaitatzis et al. (2002), Kunze et al. (1984)
Aurachin A	*Stigmatella aurantiaca* Sg a15		Non-PKS or NRPS	Structures of A and B are similar (pink). Aurachin C has slightly different rings (orange)	Kunze et al. (1987), Pistorius et al. (2011b)
Aurachin B					
Aurachin C					

Table 2.4 (continued)

Compound	Strain	Structure	Genetic cluster	Observation	References
Myxochromide S1	*Stigmatella aurantiaca* DW4/3-1		PKS/ NRPS	Compounds differ in the alkyl substitutions (shown in orange)	Wenzel et al. (2005)
Myxochromide S2					
Myxochromide S3					
Aurafuron B (2a)	*Stigmatella aurantiaca* DW4/3-1		PKS	Substitution and chiral differences are seen between the two aurafuron B molecules (identical structure in blue)	Frank et al. (2007), Kunze et al. (2005)
Aurafuron B (2b)					

Melithiazol A	*Melittangium lichenicola* Me l26 and Me l46		PKS/ NRPS	The three structures have similar rings (shown in green). A and B differ in one double bond	Sasse et al. (1999), Weinig et al. (2003)
Melithiazol B					
Melithiazol C					
Cystothiazole A	*Cystobacter fuscus*		PKS/ NRPS	Different radicals are seen in the core structure (shown in purple)	Feng et al. (2005), Ojika et al. (1998), Suzuki et al. (2003)
Cystothiazole B					

Abbreviations: NRPS nonribosomal peptide synthetase, *PKS* polyketide synthase

Supplement 2

Anaerobic Medium

Add the following (per liter): NaCl, 1.0 g; $MgCl_2 \cdot 6H_2O$, 0.5 g; KH_2PO_4, 0.2 g; NH_4Cl, 0.3 g; KCl, 0.3 g; $CaCl_2 \cdot 2H_2O$, 0.015 g; resazurin, 1 mg; trace element solution A, 1 ml; Trace element solution B, 1 ml; $Na_2S \cdot 9H_2O$, 0.048 g; L-cysteine, 0.035 g; $NaHCO_3$, 2.52 g; vitamin solution, 10 ml.

Trace Element Solution A

Add the following (per liter): HCl (25 % [wt/wt] solution), 10 ml; $FeCl_2 \cdot 4H_2O$, 1.5 g; $CoCl_2 \cdot 6H_2O$, 0.19 g; $MnCl_2 \cdot 4H_2O$, 0.1 g; $ZnCl_2$, 70 mg; H_3BO_3, 6 mg; $Na_2MoO_4 \cdot 2H_2O$, 36 mg; $NiCl_2 \cdot 6H_2O$, 24 mg; and $CuCl_2 \cdot 2H_2O$, 2 mg.

Trace Element Solution B

Add the following (per liter): Na_2SeO_3, 6 mg; $Na_2WO_4 \cdot 2H_2O$, 8 mg; NaOH, 0.5 g.

Vitamin Solution (Wolin et al. 1963)

After autoclaving, add the following to the medium (mg/l distilled water): biotin, 2; folic acid, 2; pyridoxine hydrochloride, 10; riboflavin, 5; thiamine, 5; nicotinic acid, 5; pantothenic acid, 5; vitamin B_{12} 0.1; p-aminobenzoic acid, 5; thioctic acid, 5. Add reductants to the medium after it has been boiled and cooled to room temperature. Flux the headspace with oxygen-free N_2:CO_2 (80:20) and adjust pH to 7.2–7.3 by varying the flow of CO_2 (Wolin et al. 1963).

Mineral Salts Medium (Sanford and Tiedje 1996/1997)

Add the following (per liter): $CaC1_2 \cdot 2H_20$, 0.015 g; $MgC1_2 \cdot 6H_20$, 0.02 g; $FeSO_4 \cdot 7H_20$, 0.007 g; and Na_2SO_4, 0.005 g. Add 2-mM potassium phosphate buffer (pH 7.2–7.5).

Trace Metals Solution

Add the following to give the final concentration per liter: $MnCl_2 \cdot 4H_20$, 5 mg; H_3BO_3, 0.5 mg; $ZnC1_2$, 0.5 mg; $COC1_2 \cdot 6H_20$, 0.5 mg; $NiSO_4 \cdot 6H_20$, 0.5 mg; $CuC1_2 \cdot 2H_20$, 0.3 mg; $NaMoO_4 \cdot 2H_20$, 0.1 mg. Add NH_4C1 (final concentration 8 mM). Add 10-mM $NaHCO_3$ to buffer the headspace containing N_2:CO_2 (95:5).

Vitamin Solution (Wolin et al. 1963)

After autoclaving the medium, add the following (mg/l distilled water): biotin, 2; folic acid, 2; pyridoxine hydrochloride, 10; riboflavin, 5; thiamine, 5; nicotinic acid, 5; pantothenic acid, 5; vitamin B_{12} 0.1; p-aminobenzoic acid, 5; thioctic acid, 5 (Wolin et al. 1963).

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3 The Family *Desulfarculaceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract
Desulfarculaceae is the only family within the order *Desulfarculales* (Kuever et al. 2005a) that embraces a single genus *Desulfarculus*. Besides their 16S rRNA gene sequence phylogeny, the only member of the family is defined by a wide range of morphological and chemotaxonomic properties for the delineation to other genera and species. Strictly anaerobic, having a respiratory type of metabolism. Fermentative metabolism was not observed. Members are mesophilic sulfate-reducing bacteria. Members of the family are found in various habitats as indicated by clone sequences. The only described species is chemoorganoheterotroph and chemolithoheterotroph. The only member oxidizes organic substrates completely to carbon dioxide with sulfate as electron acceptor.

This contribution is a modified and updated version of a previous family description (Kuever et al. 2005b).

Taxonomy, Historical and Current

Short Description of the Family

De.sul.far.cu.la'ce.ae. N.L. masc. n. *Desulfarculus*, type genus of the family; suff. -*aceae*, ending to denote family; N.L. fem. pl. n. *Desulfarculaceae*, the Desulfarculus family. The description is an emended version of the description given in Bergey´s Manual, 2nd edition (Kuever et al. 2005b, c).

The family belongs to the order *Desulfarculales* within the *delta-Proteobacteria*. The family *Desulfarculaceae* contains a single genus *Desulfarculus* (Kuever et al. 2006) which is the type genus of the family (Kuever et al. 2006). Gram-staining negative. Morphological forms are always vibrio-shaped cells. Spore formation is absent. The only member is motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory type of metabolism. Fermentative metabolism was not observed. Formate and short- and long-chain fatty acids are used as electron donors. The only described species oxidizes organic substrates completely to carbon dioxide. The only member is mesophilic. The only described species is chemoorganoheterotroph and chemolithoheterotroph. Sulfate, sulfite, and thiosulfate are used as electron acceptor and reduced to sulfide. The only member has been isolated from a freshwater habitat. Similar organism might occur in brackish water and marine habitats.

Phylogenetic Structure of the Family

The phylogenetic structure of the family and its neighboring families is shown on ❯ *Fig. 3.1*. The borders of the family are primarily based on the phylogenetic tree as framework and their unique properties (Physiology, chemotaxonomic markers) which are present in all members (see ❯ *Table 3.1*).

Molecular Analyses

DNA-DNA Hybridization Studies

Are absent.

Bioprinting and Ribotyping

Are absent.

MALDI-TOF

Are absent.

E. Rosenberg et al. (eds.), *The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria*, DOI 10.1007/978-3-642-39044-9_270,

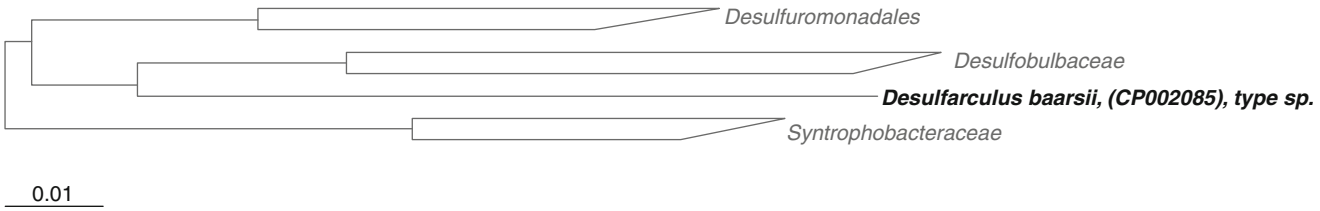

Fig. 3.1
Phylogenetic reconstruction of the family *Desulfarculaceae* based on the neighbor-joining algorithm with the Jukes-Cantor correction. Sequence dataset and alignments according to the All-Species Living Tree Project release LTPs108 (Yarza et al. 2010). The tree topology was stabilized with the use of a representative set of around 750 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 from the alignment. Scale bar indicates estimated sequence divergence 2005

Table 3.1
Selected characteristics of the only member of the genus *Desulfarculus*

Characteristic	*Desulfarculus baarsii*
Type strain	Strain 2st14, ATCC 33931, DSM 2075, VKM B-1802
Accession number of the 16S rRNA gene sequence of the type strain	CP002085
Morphology	Vibrio
Cell size (μm)	0.5–0.7 × 1.5–4
Motility	+
Mol% G + C content	65.7 (genome data)
Major menaquinone	MK-7(H_2)
Optimal pH	7.3
Optimal temperature (°C)	35–39
Optimal NaCl concentration (g/l)	7–20
Growth factor requirement	–
Oxidation of substrate	Complete
H_2/CO_2	–
Formate	+
Acetate	+
Fatty acids	C_3-C_{18}
Ethanol	–
Other n-alcohols	–
Lactate	–
Pyruvate	Nr
Fumarate	–
Succinate	–
Malate	–
Other	Isobutyrate, 2-methybutyrate, isovalerate
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Sulfate	+
Sulfite	+
Thiosulfate	+
Sulfur	–
Other	–
Literature	Widdel 1980; Collins and Widdel 1986; Kuever et al. 2006, gen. nov. comb. nov.; Kuever et al. 2005

Genome Comparison

The complete genome sequence of the only described species of the genus *Desulfarculus* which is also the only member of this family which has been analyzed (Sun et al 2010). The genome of the type strain of *Desulfarculus baarsii* 2st14 is 3,655,731 bp long and contains 3,355 genes with 3,303 coding for proteins and 52 coding for RNA genes. The mol% G + C of the DNA is 65.7. In the genome single copies of the dissimilatory, adenylylsulfate reductase (AprBA) and the dissimilatory sulfite reduction (DsrAB) were found. The aprBA gene is similar to gram-positive aprBA genes and the Qmo complex is incomplete and consists only out of QmoAB. The QmoC is lacking which is also similar to gram-positive sulfate-reducing (e.g., *Desulfotomaculum* and *Desulfosporosinus*).

Phages

Phages might be present, but are not reported so far.

Phenotypic Analyses

The main features of the only member of the family *Desulfarculaceae* is listed in *Table 3.1*.

All members are gram-negative vibrio. The only species is motile. All are strictly anaerobic, having a respiratory or fermentative metabolism.

The cellular fatty acid pattern of *D. baarsii* strain 2st14^T is dominated by saturated straight chain fatty acids (43.0 % C 14:0,

9.9 % C 16:0, and 2.3 % C 18:0), followed by saturated iso- and anteiso-branched fatty acids (21.3 % i-C 14:0, 12.3 % ai-C 15:0, and 2.8 % i-C 15:0). A comparison of the fatty acid profiles of *D. baarsii* and various gram-negative sulfate reducers by cluster analysis indicated a separate position of *D. baarsii* (Vainshtein et al. 1992) [31], corroborating the distinct phylogenetic position of the species as shown based on the 16S rRNA sequence analysis (*Fig. 3.1*).

The dominant respiratory quinone is MK-7(H_2) (Collins and Widdel 1986). Organic substrate oxidation is always complete. Typical electron donors for sulfate reduction are formate, short-chain and long-chain fatty acid, and short-chain branched fatty acids. Fermentation was never observed. Formate allows chemolithoautotrophic growth without acetate. Typical electron acceptors for growth are sulfate, sulfite, and thiosulfate.

Desulfoviridin is not present. The optimal growth is between 35 °C and 39 °C; NaCl is not required for growth.

Strains belonging to this genus might be isolated from various anoxic sediments.

Desulfarculus Kuever et al. 2006, 1^{VP} (Effective Publication Kuever et al. 2005a, 1004)

De.sul.far'cu.lus. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. masc. n. *arculus*, a small bow; N.L. masc. n. *Desulfarculus*, a bow-shaped sulfate-reducer.

The genus *Desulfarculus* contains a single validly described species. Its chemotaxonomic and physiological properties are listed in *Table 3.1*.

In *D. baarsii*, a high activity of carbon monoxide dehydrogenase is observed, indicating the operation of the anaerobic C_1-pathway (Wood-Ljungdahl pathway) for formate assimilation and CO_2 fixation or complete oxidation of acetyl-CoA (Schauder et al. 1986). This is also reflected from genome data (Sun et al. 2010).

Taxonomic comment: The phylogenetic tree (*Fig. 3.1*) clearly indicates that *Desulfarculus baarsii* represents an isolated and deeply branching lineage. Therefore, the classification as a separate order and family is justified. This is also reflected by phylogenetic trees based on the dsrAB and aprBA genes (Zverlov et al. 2005; Meyer and Kuever 2007).

Biolog

Biolog date are not available for sulfate-reducing bacteria.

Isolation, Enrichment, and Maintenance Procedures

The only described member of the family *Desulfarculaceae* requires anoxic media for growth like all other sulfate-reducing bacteria. The media are prepared under specific conditions, and the addition of a reductant is required, in general sulfide to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992). A detailed description is provided in the genus description (Kuever et al. 2005c).

For enrichment the used electron donor should be highly selective and would have a strong influence on what kind of sulfate-reducing bacteria will grow in the medium. The most selective substrates for the isolation of *Desulfarculus* spp. might be long-chain fatty acids and branched short-chain fatty acids. One might succeed also with formate in the absence of acetate. As usual electron acceptor sulfate is used. All these substrates might enrich also for other sulfate-reducing bacteria belonging to other genera because there are several which have quite similar nutritional requirements.

Most sulfate-reducing bacteria of this family have been enriched using batch cultures; other options are serial dilution techniques of natural samples. For isolation in general, roll-tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992; Kuever et al. 2005d).

For short-term preservation, stock cultures can be stored at 2–6 °C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. For long-term storage, cultures can be kept freeze dried, at −80 °C, or in liquid nitrogen.

Ecology

Habitat

Members of this family might be isolated from various habitats including freshwater, brackish, and marine systems. There are several clone sequences based on the 16S rRNA gene described which would fell into this family. So members of this family might be widespread, although isolates are lacking. The type strain of the genus *Desulfarculus*, strain 2st14, was isolated with stearate as only electron donor and sulfate as electron acceptor from anoxic sediment of a freshwater ditch close to the University of Konstanz, Germany.

Pathogenicity, Clinical Relevance

There is no pathogenic relevance known.

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4 The Family *Desulfobacteraceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract

Desulfobacteraceae, a family within the order *Desulfobacterales*, embraces 21 genera *Desulfobacter*, *Desulfatibacillum*, *Desulfatiferula*, *Desulfatirhabdium*, *Desulfatitalea*, *Desulfobacterium*, *Desulfobacula*, *Desulfobotulus*, *Desulfocella*, *Desulfococcus*, *Desulfoconvexum*, *Desulfofaba*, *Desulfofrigus*, *Desulfoluna*, *Desulfonatronobacter*, *Desulfonema*, *Desulforegula*, *Desulfosalsimonas*, *Desulfosarcina*, *Desulfospira*, *and Desulfotignum*. The family is primarily defined by their 16S rRNA gene sequence phylogeny. Morphological and chemotaxonomic properties are highly variable for the delineation of genera and species. Strictly anaerobic, having a respiratory type of metabolism. Fermentative metabolism might occur. Members are either mesophilic or psychrophilic sulfate-reducing bacteria. Members of the family are mainly found in freshwater, brackish water, marine, and haloalkaline habitats. Most described species are chemoorganoheterotroph; some are chemolithoheterotroph or chemolithoautotroph. Most members oxidize organic substrates completely to carbon dioxide, whereas some perform an incomplete oxidation of organic substrates to acetate.

This contribution is a modified and an updated version of a previous family description (Kuever et al. 2005a).

Taxonomy, Historical and Current

Sort Description of the Family

De.sul.fo.bac.ter.a′ce.ae. M.L. masc. n. *Desulfobacter* type genus of the family; -aceae, ending to denote a family; N.L. fem. pl. n. *Desulfobacteraceae* the Desulfobacter family (Modified from *Bergey's Manual*). The description is an emended version of the description given in *Bergey's Manual*, 2nd edition (Kuever et al. 2005a).

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The family represents the type family of the order *Desulfobacterales* within the *delta-Proteobacteria*. The family *Desulfobacteraceae* contains the genera *Desulfobacter* (Widdel 1981) which is the type genus of the family (Kuever et al. 2006) *Desulfatibacillum* (Cravo-Laureau et al. 2004a), *Desulfatiferula* (Cravo-Laureau et al. 2007), *Desulfatirhabdium* (Balk et al. 2008), *Desulfatitalea* (Higashioka et al. 2012), *Desulfobacterium* (Bak und Widdel et al. 1988), *Desulfobacula* (Rabus et al. 2000; Kuever et al. 2001), *Desulfobotulus* (Kuever et al. 2009), *Desulfocella* (Brandt et al. 1999), *Desulfococcus* (Widdel 1981), *Desulfoconvexum* (Könneke et al. 2012), *Desulfofaba* (Knoblauch et al. 1999), *Desulfofrigus* (Knoblauch et al. 1999), *Desulfoluna* (Suzuki et al. 2008), *Desulfonatronobacter* (Sorokin et al. 2012), *Desulfonema* (Widdel 1981), *Desulforegula* (Rees and Patel 2001), *Desulfosalsimonas* (Kjeldsen et al. 2010), *Desulfosarcina* (Widdel 1981), Desulfospira (Finster et al. 1997), and *Desulfotignum* (Kuever et al. 2001). Gram-staining negative. Morphological forms vary from coccoid to vibrio- and rod-shaped cells of varying length to multicellular filaments or aggregate-forming cells. Spore formation is absent. Most members are motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory type of metabolism. Some species can grow by fermentation. Simple organic molecules are used as electron donors; some species can use also H_2. Most members are mesophilic; a few one are psychrophilic. Substrate oxidation is either complete to carbon dioxide or incomplete to acetate. All species showing a complete oxidation use the anaerobic C_1 pathway (carbon monoxide pathway, Wood pathway) for the oxidation of acetyl-CoA except members of the genus *Desulfobacter* which use a modified TCA-cycle. Autotrophic growth is achieved by reverse action of both pathways.

Most described species are chemoorganoheterotroph; some are chemolithoheterotroph or chemolithoautotroph. Sulfate and thiosulfate are used as electron acceptor and reduced to sulfide; some species can also use sulfite. A few species can grow by disproportionation of reduced sulfur compounds with or without acetate as carbon source.

Members have been isolated from various sources, like freshwater, brackish, marine, and halosaline environments and haloalkaline habitats.

Phylogenetic Structure of the Family and Its Genera

The phylogenetic structure of the family and its neighboring families within the order *Desulfobacterales* is shown on *Fig. 4.1*. The borders of the family are primarily based on the phylogenetic tree as framework; other unique properties (Physiology, chemotaxonomic markers) which are present in all members are not found (see *Table 4.1*).

Molecular Analyses

DNA-DNA Hybridization Studies

Are absent.

Bioprinting and Ribotyping

Are absent.

MALDI-TOF

Are absent.

Genome Comparison

The complete genome sequences of three validated and two not validated members of this family have been analyzed.

The genome of the type strain of *Desulfobacter postgatei*, strain 2 ac9, is 3,972,458 bp long and contains 3,772 genes with 3,440 coding for proteins and 94 coding for RNA genes. The mol% G+C of the DNA is 47.2 (a publication is not available).

The genome of the type strain of *Desulfobacterium autotrophicum*, strain HRM2, is 5,589,073 bp long (one chromosome and a 68,709 bp plasmid) and contains 4,941 genes with 4,867 coding for proteins and 69 coding for RNA genes. The mol % G+C of the DNA is 48.8 (Strittmatter et al. 2009).

The genome of the type strain of *Desulfobacula toluolica*, strain Tol2, is 5197905 bp long and contains 4,435 genes with 4,375 coding for proteins. The mol% G+C of the DNA is 41.4 (publication absent).

Strain AK-01, not the type species of *Desulfatibacillum alkenivorans*, contains a genome which is 6,517,073 bp long and contains 5,359 genes with 5,252 coding for proteins and 76 coding for RNA genes. The mol% G+C of the DNA is 54.5 (Callaghan et al. 2012).

The genome of another alkane-oxidizing sulfate-reducing bacterium, strain Hxd3, is 3,944,167 bp long, contains 3,323 genes with 3,265 coding for proteins and 53 for RNA genes. The mol% G+C of the DNA is 56.2 (a publication is not available). This strain was named *Desulfococcus oleovorans*. This classification is definitely wrong. From its 16S rRNA gene, this strain would clearly resemble a new genus and has nothing to do with the genus *Desulfococcus* (data not shown).

All published genomes contain complete functional sulfate-reduction pathways. The aprBA genes are present as single copies and linked to a complete QmoABC complex, and all required genes for the dissimilatory sulfite reduction (dsrAB and dsrMKJOP) are present and found as a single copy.

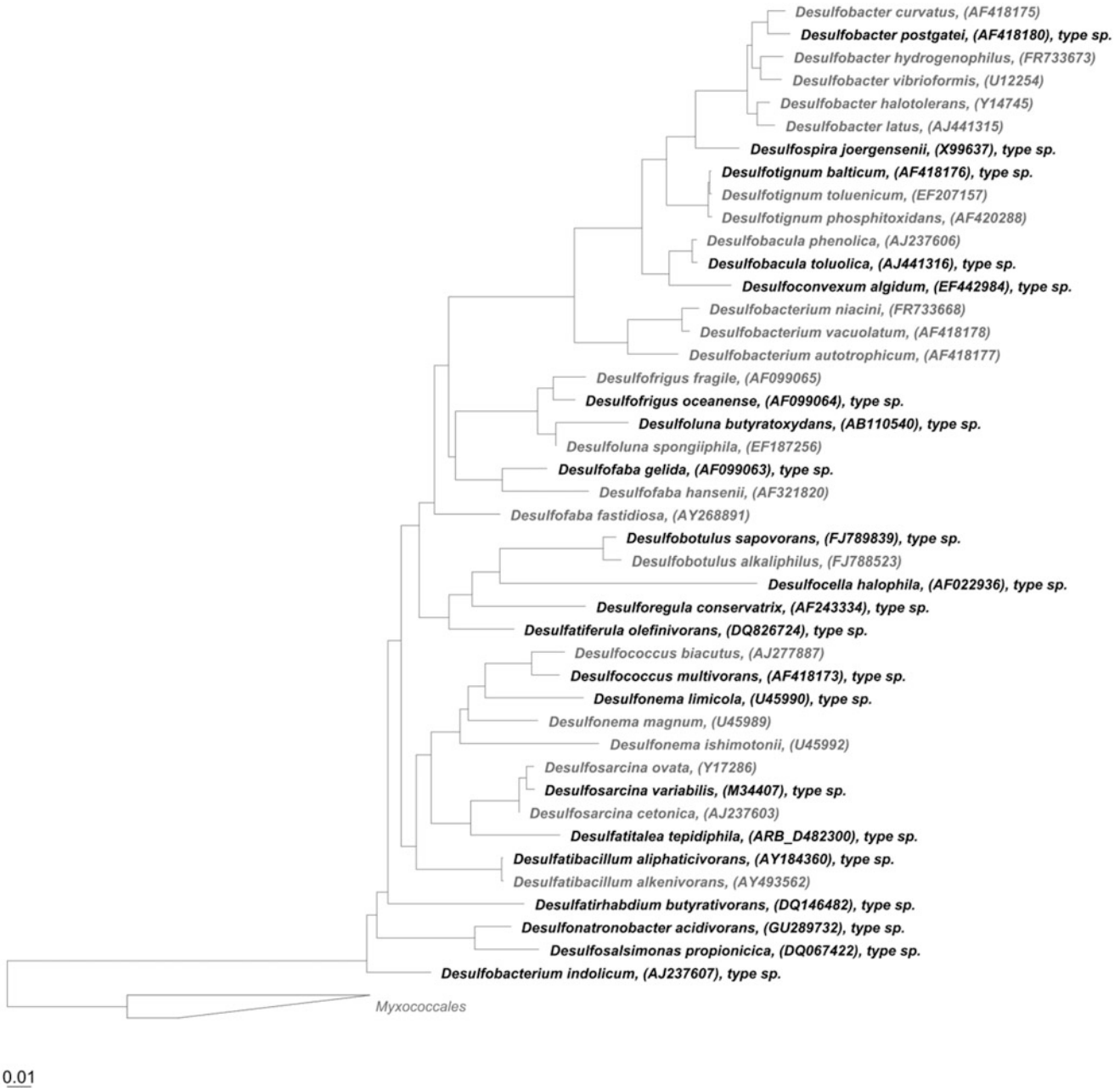

Fig. 4.1

Phylogenetic reconstruction of the family *Desulfobacteraceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Samatakis 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

There are several ongoing genome sequencing projects of members of the *Desulfobacteraceae* which includes the type strain of the following species: *Desulfobacter curvatus*, *Desulfonema magnum*, *Desulfonema limicola*, *Desulfosarcina variabilis, and Desulfospira joergensenii*. In addition, the genomes of strain BuS5 (a butane oxidizing sulfate-reducing bacterium falling into a new cluster close to the *Desulfococcus/Desulfosarcina* cluster) (Kniemeyer et al. 2007) and a multicellular organism tentatively named *Candidatus Magnetoglobus multicellularis* (Abreu et al. 2007) will be sequenced.

Table 4.1
Morphological and chemotaxonomic characteristics of genera of *Desulfobacteraceae*

	Desulfobacter	*Desulfatibacillum*	*Desulfatiferula*	*Desulfatirhabdium*	*Desulfatitalea*	*Desulfobacterium*	*Desulfobacula*
Morphology	Vibrio- or rod-shaped	Slightly curved rods	Slightly curved rods	Oval- to rod- shaped	Rod-shaped	Rod-shaped	Oval- to rod-shaped
Gram-stain	–	–	–	–	–	–	Negative
Motility	+/–	Nr	+	Nr	+	+/–	+/–
Metabolism	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Major fatty acids	$C_{14:0,\ iso}C_{15:0}$, $C_{16:0}$, 10Me-$C_{16:0}$, $C_{16:1\ w7c}$, $C_{17.0\ cyclo}$, $C_{18:1\ w7c}$, $C_{18:1\ w9c}$, $C_{:18:0}$[a]	Nr	Nr	Iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$	Anteiso-$C_{15:0}$, $C_{16:0}$, anteiso-$C_{17:0}$,	$C_{16:1\ c7}$, $C_{16:1\ c9}$, $C_{16:0}$, $C_{17:1\ w6}$, $C_{17:1\ c11}$[a]	$C_{16:1\ c9}$, $C_{16:0}$, 10-Me$C_{16:0}$,
Menaquinone	MK-7 or MK-7(H_2)	Nr	Nr	Nr	Nr	MK-7 or MK-7(H_2)	MK-7(H_2)
G+C content (mol%)	44.0–49.0	41.4–57.8	45.5	55.1	56.6	45.0–48.8	41.0–41.4
Substrate oxidation	Complete	Complete	Incomplete	Complete	Complete	Complete	Complete
CODH activity	Absent[b]	Nr	Nr	Nr	Nr	Present	Present
Typical electron donors	Acetate, (some species H_2, ethanol, pyruvate)	H_2, short- and long-chain fatty acids, alkenes	Butyrate, long-chain fatty acids, alkenes	H_2, formate, long- and short-chain fatty acids, dicarboxylic acids, aromatic compounds	H_2, formate, acetate, propionate, butyrate, succinate, fumarate, lactate, pyruvate	Pyruvate, propionate (no other typical electron donor, because of highly variable substrates)	Lactate, pyruvate, fumarate, butyrate, benzoate, p-cresol, toluene
Chemolithoautotrophic growth	One species	All species	No species	All species	All species	Some species	No species
Fermentative growth	No species	No species	No species	All species	Nr	Some species	Nr
Growth by disproportionation of reduced sulfur compounds	Nr	–	Nr	–	Nr	Nr	Nr
Typical electron acceptors	Sulfate, sulfite, thiosulfate	Sulfate, sulfite, thiosulfate	Sulfate	Sulfate, thiosulfate	Sulfate, thiosulfate	Sulfate	Sulfate, sulfite
Optimal growth temperature (°C)	28–34	28–30	30–36	28–30	34–42	20–30	28
Habitat	Anoxic brackish or marine sediment	Hydrocarbon-polluted marine sediment	Oil-polluted marine sediment	UASB reactor wastewater treatment	Tidal marine sediment	Anoxic brackish or marine sediment	Anoxic marine sediment

	Desulfobotulus	*Desulfocella*	*Desulfococcus*	*Desulfoconvexum*	*Desulfofaba*	*Desulfofrigus*	*Desulfoluna*
Morphology	Vibrio	Vibrio	Spherical or lemon-shaped	Curved, vibroid rod	Curved rod	Rod	Slightly curved rod
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+	+	–	+	+/–	+/–	+
Metabolism	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Major fatty acids	$C_{16:0}$, $C_{16.1\ w7c}$, $C_{18:1\ w7c}$, $C_{18:1\ w9c}$	$C_{16:0}$, $C_{18:1\ c9}$, $C_{18:1\ c11}$,	anteiso$C_{15:0}$, $C_{16:0}$, anteiso$C_{17:1\ w7}$,	$C_{14:0}$, $C_{16:1\ c9}$, $C_{16:0}$	$C_{15:0}$, $C_{15:1\ c9}$	$C_{16:0}$, $C_{16:1\ c9}$, $C_{18:1\ c11}$	$C_{14:0}$, $C_{16:0}$, $C_{16:1\ w7}$, $C_{18:1\ w7}$
Menaquinone	MK-7	Nr	MK-7	Nr	MK-8	MK-9	MK-8(H_4)
G+C content (mol%)	51.3–53.0	35	56.0–57.0	46.0	48.8–53.4	52.1–52.8	58.5–62.0
Substrate oxidation	Incomplete	Incomplete	Complete	Complete	Incomplete	Incomplete or complete	Incomplete
CODH activity	Nr	Nr	Present	Present	Nr	Nr	Nr
Typical electron donors	Pyruvate, short- and long-chain fatty acids	Pyruvate, short- and long-chain fatty acids, 2-methylbutyrate	Formate, short- and long-chain fatty acids, pyruvate, acetone	H_2, formate, short-chain fatty, dicarboxylic acids, amino acids, alcohols, benzoate	Pyruvate, propionate, alcohols	Formate, butyrate, lactate, pyruvate, malate, alcohols, serine	Lactate, pyruvate, formate, short-chain fatty acids
Chemolithoautotrophic growth	No species	No species	One species	All species	No species	No species	No species
Fermentative growth	All species	No species	One species	All species	Some species	All species	Some species
Growth by disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	Nr	No species	Nr
Typical electron acceptors	Sulfate, sulfite	Sulfate	Sulfate, sulfite	Sulfate, thiosulfate, sulfur	Sulfate	Sulfate, FeIII-citrate	Sulfate, thiosulfate, sulfite
Optimal growth temperature (°C)	32–34	34	28–35	14–16	Depending on the species, reaching from 7to 20 to 28	10–18	28–30
Habitat	Freshwater sediment, soda lakes; might occur also in marine sediment	Hypersaline lake	Freshwater, brackish, and marine sediment	Permanently cold, marine sediment	Marine sediment	Permanently cold, marine sediment	Marine estuarine sediment, marine sponge

Table 4.1 (continued)

	Desulfonatronobacter	*Desulfonema*	*Desulforegula*	*Desulfosalsimonas*	*Desulfosarcina*	*Desulfospira*	*Desulfotignum*
Morphology	Rod	Multicellular filaments	Rod	Rod (sometimes filaments)	Oval- to rod-shaped, sometimes forming packages	Vibrio	Slightly curved rod
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+	gliding	+	Nr	+/–	+	+/–
Metabolism	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Major fatty acids	$C_{16:0}$, $C_{18:1\ w7}$	Nr	Nr	Nr	Anteiso-$C_{15:0}$, $C_{16:1\ w7c}$, $C_{16:0}$,	$C_{16:1\ c9}$, $C_{16\ :\ 0}$	$C_{16:0,}$ 10Me $C_{16:0}$, $C_{16:1\ w7c}$, $C_{17.0\ cyc}$
Menaquinone	MK-8	MK-7 or MK-9	Nr	Nr	MK-7	MK-7 and MK-7(H2)	Nr
G+C content (mol%)	54.4	35.0–55.0	Nr	54.1	51.0–59.0	49.9	52.0–53.9
Substrate oxidation	Complete	Complete	Incomplete	Complete	Complete	Complete	Complete
CODH activity	Nr	Present	Nr	Nr	Present	Nr	Present
Typical electron donors	Short-chain fatty acids	Short- and long-chain fatty acids	Long-chain fatty acids	H_2, short-chain fatty acids, alcohols, lactate, pyruvate	Lactate, pyruvate, formate, acetate, and other short- and long-chain fatty acids, benzoate	H_2, lactate, pyruvate, formate, long- and short-chain fatty acids, dicarboxylic acids, betaine, proline, yeast extract	H_2, pyruvate,
Chemolithoautotrophic growth	No species	Some species	No species	No species	Some species	All species	All species
Fermentative growth	No species	No species	No species	No species	Some species	No species	Some species
Growth by disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Typical electron acceptors	Sulfate, thiosulfate	Sulfate	Sulfate	Sulfate, sulfite, thiosulfate	Sulfate	Sulfate, sulfite, thiosulfate, sulfur	Sulfate, sulfite
Optimal growth temperature (°C)	35	28–32	25–30	40	30–33	26–30	28–34
Habitat	Hypersaline soda lakes	Anoxic freshwater, brackish water, or marine sediment	Freshwater sediment	Hypersaline lake	Anoxic freshwater, brackish water, or marine sediment	Anoxic marine sediment	Anoxic brackish or marine sediment

[a]Depending on the species large variations; data from not validated strains not included (Kohring et al. 1994)
[b]Acetyl-CoA oxidation and CO_2 fixation with H_2 as electron donor is accomplished via a modified TCA-cycle

Phages

Phages might be present, but are not reported so far.

Phenotypic Analyses

The main features of all members of the family *Desulfobacteriaceae* are listed in ❯ *Table 4.1* and the tables featuring the genera (❯ *Table 4.2*–❯ *4.22*).

Desulfobacter Widdel 1981, 382[VP] (Effective Publication Widdel 1980, 376) Emend. Widdel 1987, 289

De.sul.fo.bac´ter. L. pref. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); N.L. masc. n. *bacter*, rod or staff; N.L. masc. n. *Desulfobacter*, a rod-shaped sulfate-reducing bacterium.

The genus *Desulfobacter* contains six described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table. 4.2*. Besides the validated species listed in ❯ *Table 4.2*, there are several strains which might represent new species (Widdel 1987). Another species was described by Tarpgaad and coworkers (2006) as *Desulfobacter psychrotolerans* but was never validated.

Desulfatibacillum Cravo-Laureau et al. 2004a, 81[VP]

De.sul.fa.ti.ba.cil'lum. L. pref. *de-*, from; N.L. masc. n. *sulfas*, sulfate; L. neut. n. *bacillum*, a small staff, a wand, a rod; N.L. neut. n. *Desulfatibacillum*, a sulfate-reducing rod.

The genus *Desulfatibacillum* contains two described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 4.3*.

Desulfatiferula Cravo-Laureau et al. 2007, 2701[VP]

De.sul.fa.ti.fe´ru.la. L. pref. de, from; N.L. n. *sulfas* -atis, sulfate; L. fem. n. *ferula*, a staff, a small rod; N.L. fem. n. *Desulfatiferula*, a rod-shaped sulfate reducer.

The genus *Desulfatiferula* contains a single described species. Its chemotaxonomic and physiological properties are listed in ❯ *Table 4.4*.

Desulfatirhabdium Balk et al. 2008, 113[VP]

De.sul.fa.rhab´di.um. L. pref. de-, from; N.L. n. *sulfas -atis*, sulfate; Gr. neut. n. *rhabdium*, a little rod; N.L. neut. n. *Desulfatirhabdium*, a sulfate-reducing small rod.

The genus *Desulfatirhabdium* contains a single described species. Its chemotaxonomic and physiological properties are listed in ❯ *Table 4.5*.

Desulfatitalea Higashioka et al. 2012, 764[VP]

De.sul.fa.ti.ta´le.a. L. pref. de-, from; N.L. n. *sulfas -atis*, sulfate; L. fem. n. *talea*, a slender staff, rod, stick; N.L. fem. n. *Desulfatitalea*, a rod-shaped sulfate reducer.

The genus *Desulfatitalea* contains a single described species. Its chemotaxonomic and physiological properties are listed in ❯ *Table 4.6*.

Desulfobacterium Bak and Widdel 1988, 136[VP] (Effective Publication Bak and Widdel 1986, 175)

De.sul.fo.bac.te´ri.um. L. pref. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. neut. n. bacterium, a rod; N.L. neut. n. Desulfobacterium, a rod-shaped sulfate reducer.

The type species of the genus *Desulfobacterium* is *Desulfobacterium indolicum* and not *Desulfobacterium autotrophicum* as indicated previously (Kuever et al. 2005a; see also J.P. Euzeby on http://www.bacterio.cict.fr/d/desulfobacterium.html). Both are listed as type species for the genus, but the older validation has priority. Therefore, the correct type species is *Desulfobacterium indolicum* (Bak and Widdel 1988).

The genus *Desulfobacterium* contains six validly described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 4.7*.

Taxonomic comment: As can be seen in ❯ *Fig. 4.2*, *Desulfobacterium catecholicum* is clearly a member of the family *Desulfobulbaceae* and not of the family *Desulfobacteraceae*. This is also reflected by the phylogeny based on the aprBA gene (Meyer and Kuever 2007). *Desulfobacterium anilini* forms an isolated deep-branching lineage and should be regarded as a separate order and family (see ❯ *Fig. 4.2*). Its isolated position is also reflected by phylogenetic trees based on the dsrAB gene (Klein et al. 2001). A very strong indication for a new order and family for *Desulfobacterium anilini* and close relatives (like strain Naph2) is the presence of an aprBA gene which is more similar to gram-positive SRB (Meyer and Kuever 2007). *Desulfobacterium indolicum*, *Desulfobacterium autotrophicum*, *Desulfobacterium niacini*, and *Desulfobacterium vacuolatum* are very obviously member of the family *Desulfobacteraceae* (see ❯ *Fig. 4.1*) but cannot be included in a single genus. *Desulfobacterium indolicum* forms a very isolated branch and is not at all linked to the other species in the tree. Therefore, *Desulfobacterium indolicum* would represent the genus *Desulfobacterium* as only species, and *Desulfobacterium autotrophicum*, together with *Desulfobacterium niacini* and *Desulfobacterium vacuolatum*, should be regarded as a new genus which has to be established.

Table 4.2

Comparison of selected characteristics of members of the genus *Desulfobacter*

Characteristic	*Desulfobacter postgatei*	*Desulfobacter curvatus*	*Desulfobacter halotolerans*	*Desulfobacter hydrogenophilus*	*Desulfobacter latus*	*Desulfobacter vibrioformis*
Type strain	Strain "Dangast" 2ac9, ATCC 33911, DSM 2034	Strain AcRM3, ATCC 43919, DSM 3379	Strain GSL-Ac1, DSM 11383	Strain AcRS1, ATCC 43915, DSM 3380	Strain AcRS2, ATCC 43918, DSM 3381	Strain B54, DSM 8776
Accession number of the 16S rRNA gene sequence of the type strain	AF418180	AF418175	Y14745	FR733673	AJ441315	U12254
Morphology	Oval-shaped	Vibrio-shaped	Rod-shaped	Rod-shaped	Oval-shaped	Vibrio-shaped
Cell size (μm)	1.0–1.5 × 1.7–2.5	0.5–1 × 1.7–3.5	0.8–1.2 × 3.0–5.0	1.0–1.3 × 2.0–5.0	1.6–2.4 × 5.0–7.0	1.9–2.3 × 4.5–8.0
Motility	+/–	+	+	–	+/–	+
Mol% G+C content	47.2	46.0	49.0	45.0	44.0 (48.0)	47.0
Major menaquinone	MK-7	MK-7(H_2)	Nr	MK-7(H_2)	MK-7	Nr
Optimal pH	7.3	6.8–7.2	6.2–7.4	6.6–7.0	7.0–7.3	6.8–7.0
Optimal temperature (°C)	28–32	28–31	32–34	29–32	30	33
Optimal NaCl concentration (g/l)	7	20	10–20	10–20	20	10–50
Growth factor requirement	Vitamins	Vitamins	Vitamins	Vitamins	Vitamins	–
Oxidation of substrate	Complete	Complete	Complete	Complete	Complete	Complete
Compounds used as electron donors and carbon sources						
H_2/CO_2	–	+[a]	–	+	–	–
Formate	–	–	Nr	–	–	–
Acetate	+	+	+	+	+	+
Fatty acids	–	–	–	–	–	–-
Ethanol	–	+	+	+	–	–
Other *n*-alcohols	–	Nr	–	–	–	–
Lactate	–	–	–	–	–	–
Pyruvate	–	+	+[a]	+	–	–
Fumarate	–	–	–	–	–	–
Succinate	–	Nr	–	Nr	Nr	–
Malate	–	–	–	–	–	–
Others	–	–	–	–	–	–
Fermentative growth	–	–	–	–	–	–
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	Nr	Nr
Electron acceptors used						
Sulfate	+	+	+	+	+	+
Sulfite	+	+	+	+	–	+
Thiosulfate	+	+	+	+	–	+
Sulfur[b]	–	–	–	–	–	Nr

Table 4.2 (continued)

Characteristic	*Desulfobacter postgatei*	*Desulfobacter curvatus*	*Desulfobacter halotolerans*	*Desulfobacter hydrogenophilus*	*Desulfobacter latus*	*Desulfobacter vibrioformis*
Other	–	Nr	–	Nr	Nr	Nr
Literature	Widdel 1981, gen. nov., sp. nov.; Widdel 1980; Widdel and Pfennig 1981	Widdel 1988, sp. nov.; Widdel 1987	Brandt and Ingvorsen 1998, sp. nov.; Brandt and Ingverson 1997	Widdel 1988, sp. nov.; Widdel 1987	Widdel 1988, sp. nov.; Widdel 1987	Lien and Beeder 1997, sp. nov.

[a]Poor growth

Table 4.3
Comparison of selected characteristics of members of the genus *Desulfatibacillum*

Characteristic	*Desulfatibacillum aliphaticivorans*	*Desulfatibacillum alkenivorans*
Type strain	Strain CV2803, ATCC BAA-743, DSM 15576	Strain PF2803, ATCC BAA-924, DSM 16219
Accession number of the 16S rRNA gene sequence of the type strain	AY184360	AY493562
Morphology	Slightly curved rods	Slightly curved rods
Cell size (μm)	0.6 × 2.2–5.5	0.7 × 1.2–4.5
Motility	Nr	Nr
Mol% G+C content	41.4	57.8
Major menaquinone	Nr	Nr
Optimal pH	7.5	6.8
Optimal temperature (°C)	28–35	28–30
Optimal NaCl concentration (g/l)	24	10
Growth factor requirement	–	–
Oxidation of substrate	Complete	Complete
Compounds used as electron donors and carbon sources		
H_2/CO_2	+	+
Formate	+	–
Acetate	+	.
Fatty acids	C_3 to C_{18}	C_4 to C_{18}
Ethanol	–	–
Other *n*-alcohols	*n*-butanol	Glycerol, *n*-butanol
Lactate	–	–
Pyruvate	+	+
Fumarate	+	–
Succinate	+	–
Malate	+	+

Table 4.3 (continued)

Characteristic	*Desulfatibacillum aliphaticivorans*	*Desulfatibacillum alkenivorans*
Others	Alkanes (C_{13} to C_{18}), alkenes (C_7 to C_{23}), crotonate, isobutyrate	Alkenes (C_8 to C_{23}), crotonate, isobutyrate,
Fermentative growth	–	–
Disproportionation of reduced sulfur compounds	–	–
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	+
Sulfur	–	–
Other	–	–
Literature	Cravo-Laureau et al. 2004a, gen. nov., sp. nov.	Cravo-Laureau et al. 2004b, sp. nov.

Strain Hxd3 which was named "*Desulfobacterium oleovorans*" but was not validly described, represents a new genus within the family *Desulfobacteraceae*. Another not validated member of the genus *Desulfobacterium* is strain N106A1-4.93, tentatively named *Desulfobacterium zeppellini* which is closely related to *Desulfobacterium vacuolatum* (98 % sequence identity of the 16S rRNA gene) but might resemble a new species within the genus (Meyer and Kuever 2007).

Desulfobacula Rabus et al. 2000, 1415[VP] (Effective Publication Rabus et al. 1993, 1450) Emend. Kuever et al. 2001, 176

De.sul.fo.ba´cu.la. L. pref. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. dim. n. *bacula*, a small berry; N.L. fem. n. *Desulfobacula*, sulfate-reducing small berry.

Table 4.4
Selected characteristics of the only member of the genus *Desulfatiferula*

Characteristic	*Desulfatiferula olefinivorans*
Type strain	Strain LM2801, DSM 18843, JCM 14469
Accession number of the 16S rRNA gene sequence of the type strain	DQ8236724
Morphology	Slightly curved or vibroid rod
Cell size (μm)	0.45 × 0.8–5
Motility	+
Mol% G+C content	45.5
Major menaquinone	Nr
Optimal pH	7.5
Optimal temperature (°C)	30–36
Optimal NaCl concentration (g/l)	6–10
Growth factor requirement	Vitamins
Oxidation of substrate	Incomplete
Electron acceptors used	
H_2/CO_2	–
Formate	–
Acetate	–
Fatty acids	C_4, C_{14}–C_{24}
Ethanol	–
Other *n*-alcohols	–
Lactate	–
Pyruvate	–
Fumarate	–
Succinate	–
Malate	–
Others	Alkenes (C_{14}–C_{23})
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	–
Thiosulfate	–
Sulfur	–
Other	–
Literature	Cravo-Laureau et al. 2007, gen. nov., sp. nov.

Table 4.5
Selected characteristics of the only member of the genus *Desulfatirhabdium*

Characteristic	*Desulfatirhabdium butyrativorans*
Type strain	Strain HB1, DSM 18734, JCM 14470
Accession number of the 16S rRNA gene sequence of the type strain	DQ146482
Morphology	Oval- to rod-shaped
Cell size (μm)	1–1.3 × 2.6–3.5
Motility	Nr
Mol% G+C content	55.1
Major menaquinone	Nr
Optimal pH	7.0
Optimal temperature (°C)	28–30
Optimal NaCl concentration (g/l)	5
Growth factor requirement	Nr
Oxidation of substrate	Complete
Electron acceptors used	
H_2/CO_2	+[a]
Formate	+
Acetate	–[b]
Fatty acids	C_3, C_4, C_{16}, C_{18}
Ethanol	+
Other *n*-alcohols	*n*-propanol, *n*-butanol, 2,3-butandiol
Lactate	–
Pyruvate	+
Fumarate	+
Succinate	+
Malate	–
Others	Crotonate, catechol, phenol, benzoate, 4-hydroxybenzoate
Fermentative growth	+
Disproportionation of reduced sulfur compounds	–
Electron acceptors used	
Sulfate	+
Sulfite	–
Thiosulfate	+
Sulfur	–
Other	–
Literature	Balk et al. 2008, gen. nov., sp. nov.

[a]Only in the presence of acetate
[b]Partially oxidized

Table 4.6
Selected characteristics of the only member of the genus *Desulfatitalea*

Characteristic	*Desulfatitalea tepidiphila*
Type strain	Strain S28bF, NBRC 107166, DSM 23472
Accession number of the 16S rRNA gene sequence of the type strain	AB614135
Morphology	Rod
Cell size (μm)	0.5–0.6 × 1.7–3.8
Motility	+
Mol% G+C content	56.6
Major menaquinone	Nr
Optimal pH	6.8–7.2
Optimal temperature (°C)	34–42
Optimal NaCl concentration (g/l)	Nr
Growth factor requirement	Nr
Oxidation of substrate	Complete
Electron acceptors used	
H_2/CO_2	+
Formate	+
Acetate	+
Fatty acids	C_3, C_4
Ethanol	–
Other *n*-alcohols	Nr
Lactate	+
Pyruvate	+
Fumarate	+
Succinate	+
Malate	Nr
Others	Yeast extract
Fermentative growth	Nr
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	Nr
Thiosulfate	+
Sulfur	Nr
Other	–
Literature	Higashioka et al. 2012, gen. nov., sp. nov.

The genus *Desulfobacula* contains two described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 4.8*.

Desulfobotulus Kuever et al. 2009, 923[VP] (Effective Publication Kuever et al. 2005d, 970)

De.sul.fo.bo´tu.lus. L. pref. de-, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. masc. n. *botulus*, a sausage; N.L. masc. n. *Desulfobotulus*, a sausage-shaped sulfate reducer.

The genus *Desulfobotulus* contains two described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 4.9*.

Desulfocella Brandt et al. 1999, 198[VP]

De.sul.fo.cel´la. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. *cella*, a store-room, a chamber, and in biology a cell; N.L. fem. n. *Desulfocella*, sulfate-reducing cell.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 4.10*.

Desulfococcus Widdel 1981, 382[VP] (Effective Publication Widdel 1980, 382)

De.sul.fo.coc´cus. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos*, grain, seed), berry, coccus; N.L. masc. n. *Desulfococcus*, a berry shaped (spherical) sulfate reducer.

The genus *Desulfococcus* contains two described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 4.11*.

Desulfoconvexum algidum et al. 2013, 963[VP]

De.sul.fo.con´ve.xum. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. n. n. *convexum*, bow, curve; N.L. n. n. *Desulfoconvexum*, sulfate reducer shaped like a curve.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 4.12*.

Desulfofaba Knoblauch et al. 1999, 1641[VP]

De.sul.fo.fa´ba. L. prefix *de*, off; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. *faba*, a bean; N.L. fem. n. *Desulfofaba*, a sulfate-reducing bean.

Table 4.7

Comparison of selected characteristics of members of the genus *Desulfobacterium*

Characteristic	*Desulfobacterium indolicum*	*Desulfobacterium autotrophicum*	*Desulfobacterium niacini*	*Desulfobacterium vacuolatum*	*Desulfobacterium anilini*[a]	*Desulfobacterium catecholicum*[a]
Type strain	Strain In04, ATCC 43938, DSM 3383	Strain HRM2, ATCC 43914, DSM 3382, VKM B-1955	Strain NAV-1, DSM 2650, JCM 12294	Strain IbRM, DSM 3385, JCM 12295	Strain Ani, ATCC 49792, DSM 4660	Strain NZva20, Nelson, NZ, ATCC 43955, DSM 3882
Accession number of the 16S rRNA gene sequence of the type strain	AJ237607	AF418177	FR733668	AF418178	AJ237601	AJ237602
Morphology	Oval- to rod-shaped	Oval-shaped	Oval- to irregular-shaped	Oval-shaped or spherical (chain forming)	Rod-shaped	Oval- to lemon-shaped
Cell size (μm)	0.7–1.5 × 2–2.5	0.9–1.3 × 1.5–3.0	1.5–3.0	1.5–2.0 × 2.0–2.5	1.25 × 1.5–3.0	1.3–1.8 × 2.2–2.8
Motility	+	+	+	–	–	–
Mol% G+C content	47.4	48.8	46	45	59.1	52.4
Major menaquinone	MK-7(H_2)	MK-7(H_2)	MK-7	MK-7(H_2)	Nr	Nr.
Optimal pH	Nr	6.7			6.9–7.5	6.9–7.1
Optimal temperature (°C)	28	25–28	29	25–30	35	28
Optimal NaCl concentration (g/l)	21	21	13.5	21	14	7
Growth factor requirement	Vitamins	Vitamins	Vitamins	–	Vitamins	Vitamins
Oxidation of substrate	Complete	Complete	Complete	Complete	Complete	Complete
Compounds used as electron donors and carbon sources						
H_2/CO_2	–	+	+	+	+[b]	+
Formate	+[c]	+	+	+	+	+
Acetate	+[c]	+[c]	+[c]	+[c]	+	+[c]
Fatty acids	C_3	C_3–C_{16}	C_3–C_{16}	C_3–C_{16}	C_3–C_{18}	C_3-C_{20}
Ethanol	+	+	+[c]	+[c]	–	+[c]

Other *n*-alcohols	*n*-propanol, *n*-butanol	*n*-propanol, *n*-butanol	*n*-propanol, *n*-butanol	Nr	–	Methanol, *n*-propanol, *n*-butanol
Lactate	–	+	–	+	–	+
Pyruvate	+	+	+	+	+	+
Fumarate	+	+	+	+	–	+
Succinate	+	+	+	+	–	Nr
Malate	+	+	+	+	–	+
Others	Maleinate, indole, quinoline, 2-aminobenzoate	Isobutyrate, 2-methylbutyrate, Glutamate	Glutamate, glutarate, nicotinate, pimelate	Isobutyrate, alanine, glutamate, glutamine, glutarate, Glycine, isoleucine, leucine, proline, serine, valine	Methanol, 2-methybutyrate, 3-methylbutyrate, 3-methyvalerate, phenol, p-cresol, benzoate, phenylpropionate, aniline, other aromatic compounds, cyclohexanol, cyclohexanone, cyclohexane carboxylate	Glutarate, glutamate, pimelate, cyclohexanecarboxylate, benzoate, 4-hydroxybenzoate, protocatechuate, catechol, resorcinol, hydroquinone, 2-aminobenzaote, phloroglucinol, pyrogallol
Fermentative growth	Nr	+	–	Nr	Nr	+
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	+	Nr
Electron acceptors used						
Sulfate	+	+	+	+	+	+
Sulfite	Nr	–	+	Nr	+	+
Thiosulfate	+	+	+	Nr	+	+
Sulfur	Nr	–	Nr	Nr	–	–
Other	Nr	Fumarate	–	Nr	–	+
Literature	Bak and Widdel 1988, gen. nov., sp. nov.; Bak and Widdel 1986	Brysch et al. 1988, sp. nov.; Brysch et al. 1987	Kuever et al. 2006, sp. nov.; Kuever et al. 2005; Imhoff-Stückle and Pfennig 1983	Kuever et al. 2006, sp. nov.; Kuever et al. 2005;	Schnell et al. 1990, sp. nov.; Schnell et al. 1989	Szewzyk and Pfennig 1988, sp. nov.; Szewzyk and Pfennig 1987

[a]Data not included in *Table 4.1*
[c]Poor growth

Table 4.8

Comparison of selected characteristics of members of the genus *Desulfobacula*

Characteristic	*Desulfobacula toluolica*	*Desulfobacula phenolica*
Type strain	strain Tol2 = DSM 7467.	strain Ph01 = ATCC 43956 = DSM 3384
Accession number of the 16S rRNA gene sequence of the type strain	AJ441316	AJ237606
Morphology	Oval	Oval- to rod-shaped
Cell size (μm)	1.2–1.4 × 1.2–2.0	1.0–1.5 × 2.0–3.0
Motility	+/–	+
Mol% G+C content	41.4	41.0
Major menaquinone	Nr	MK-7(H_2)
Optimal pH	7.0–7.1	7.0
Optimal temperature (°C)	28	28
Oxidation of substrate	Complete	Complete
Optimal NaCl concentration (g/l)	20	20
Growth factor requirement	vitamins	–
H_2/CO_2	–	+[a]
Formate	–	+[a]
Acetate	–	+[a]
Fatty acids	C_4	C_4
Ethanol	+	+[a]
Other *n*-alcohols	*n*-propanol, *n*-butanol	*n*-propanol, *n*-butanol
Lactate	–	–
Pyruvate	+	–
Fumarate	+	+[a]
Succinate	+	+[a]
Malate	+	+[a]
Others	Benzoate, p-cresol, toluene	Benzoate, p-cresol, toluene, phenol, 2-aminobenzoate
Fermentative growth	Nr	Nr
Disproportionation of reduced sulfur compounds	Nr	Nr
Electron acceptors used		
Sulfate	+	+
Sulfite		–
Thiosulfate	Nr	+
Sulfur	Nr	–
Other	Nr	–
Literature	Rabus et al. 2000, gen. nov., sp. nov. Rabus et al. 1993	Bak and Widdel 1987; Kuever et al. 2001, comb. nov.

[a]Poor growth

Table 4.9

Comparison of selected characteristics of members of the genus *Desulfobotulus*

Characteristic	*Desulfobotulus sapovorans*	*Desulfobotulus alkaliphilus*
Type strain	Strain 1pa3 Lindhorst, ATCC 33892, DSM 2055	Strain ASO4-4, DSM 22078, UNIQEM U759
Accession number of the 16S rRNA gene sequence of the type strain	FR733666	FJ788523
Morphology	Vibrio	Vibrio
Cell size (μm)	1.5 × 3.0–5.5	1.0 × 3.0–6.0
Motility	+	+
Mol% G+C content	53.0	51.3
Major menaquinone	MK-7	Nr
Optimal pH	7.7	9.9–10.1
Optimal temperature (°C)	34	32
Oxidation of substrate	Incomplete	Incomplete
Optimal NaCl concentration (g/l)	1.0–7.0	35
Growth factor requirement	–	Nr
H_2/CO_2	–	–
Formate	–	–
Acetate	–	–
Fatty acids	C_4 to C_{16}	C_4, C_6, C_8, C_9
Ethanol	–	–
Other *n*-alcohols	–	–
Lactate	+	–
Pyruvate	+	+
Fumarate	–	–
Succinate	–	–
Malate	–	–
Others	–	–
Fermentative growth	+	+

Table 4.9 (continued)

Characteristic	*Desulfobotulus sapovorans*	*Desulfobotulus alkaliphilus*
Disproportionation of reduced sulfur compounds	Nr	Nr
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	–	+
Sulfur	–	Nr
Other	–	–
Literature	Kuever et al. 2009, comb. nov.; Kuever et al. 2005d; Widdel 1981	Sorokin et al. 2010, sp. nov.; Sorokin et al. 2010

Table 4.10 (continued)

Characteristic	*Desulfocella halophila*
Malate	–
Others	Alanine, 2-methybutyrate
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	–
Thiosulfate	–
Sulfur	–
Other	–
Literature	Brandt et al. 1999, gen. nov., sp. nov.

Table 4.10
Selected characteristics of the only member of the genus *Desulfocella* (a table is used for easier comparison with other genera)

Characteristic	*Desulfocella halophila*
Type strain	Strain GSL-But2, ATCC 700426, CIP 106097, DSM 11763
Accession number of the 16S rRNA gene sequence of the type strain	AF022936
Morphology	Vibrio
Cell size (μm)	0.5–0.7 × 2.0–4.0
Motility	+
Mol% G+C content	35.0
Major menaquinone	Nr
Optimal pH	7.0
Optimal temperature (°C)	34
Optimal NaCl concentration (g/l)	40–50
Growth factor requirement	–
Oxidation of substrate	Incomplete
Compounds used as electron donors and carbon sources	
H_2/CO_2	–
Formate	–
Acetate	–
Fatty acids	C_4–C_6, C_8, C_{12}, C_{16}
Ethanol	–
Other *n*-alcohols	–
Lactate	–
Pyruvate	+
Fumarate	–
Succinate	–

Table 4.11
Comparison of selected characteristics of members of the genus *Desulfobotulus*

Characteristic	*Desulfococcus multivorans*	*Desulfococcus biacutus*
Type strain	Strain "Göttingen"1be1, ATCC 33890, DSM 2059	Strain KMRActs, DSM 5651
Accession number of the 16S rRNA gene sequence of the type strain	AF418173	AJ277887
Morphology	Spherical	Lemon-shaped
Cell size (μm)	1.5–2.2	1.4 × 2.3
Motility	–	–
Mol% G+C content	57.0	56.5
Major menaquinone	MK-7	Nr
Optimal pH	7.3	7.3
Optimal temperature (°C)	35	28–30
Oxidation of substrate	Complete	Complete
Optimal NaCl concentration (g/l)	5–10	0–1
Growth factor requirement	Vitamins	Nr
H_2/CO_2	–	Nr
Formate	+	Nr
Acetate	+[a]	+[a]
Fatty acids	C_3 to C_{16}	C_3 to C_7
Ethanol	+	+

Table 4.11 (continued)

Characteristic	*Desulfococcus multivorans*	*Desulfococcus biacutus*
Other *n*-alcohols	*n*-propanol, *n*-butanol	*N*-propanol, isopropanol, *n*-butanol, iso-butanol
Lactate	+	–
Pyruvate	+	+
Fumarate	–	–
Succinate	–	–
Malate	–	–
Others	2-Methybutyrate, 3-methylbutyrate, benzoate, 2-hydroxybenzoate, phenylacetate, cyclohexanecarboxylate, (some strains acetone)	Acetone, butanone, crotonate
Fermentative growth	+	–
Disproportionation of reduced sulfur compounds	Nr	Nr
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	Nr
Sulfur[b]	–	Nr
Other	–	Nr
Literature	Widdel 1981; Widdel 1980, gen. nov., sp. nov.	Platen et al. 1991, sp. nov.; Platen et al. 1990

[a]Poor growth

The genus *Desulfofaba* contains three described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 4.13*.

Taxonomic comment: As indicated in ❯ *Fig. 4.1*, members of the genus *Desulfofaba* form not a distinct cluster. The sequence identity of the 16S rRNA gene between *Desulfofaba gelida* and *Desulfofaba hansenii* is 93.6 % and to *Desulfofaba fastidiosa* is 91.9 %, whereas the identity between *Desulfofaba hansenii* and *Desulfofaba fastidiosa* lays at 91.0 %. Therefore, it would be justified to establish for the two last mentioned species a new separate genus for each of them. Here, obviously the ability to use propionate as substrate and to show an incomplete carbon oxidation was sufficient enough to combine them in one genus which is not reflected by their 16S rRNA gene sequences although they are similar to each other.

Table 4.12
Selected characteristics of the only member of the genus *Desulfoconvexum* (a table is used for easier comparison with other genera)

Characteristic	*Desulfoconvexum algidum*
Type strain	Strain JHA1, DSM 19738, JCM 16085
Accession number of the 16S rRNA gene sequence of the type strain	EF442914
Morphology	Curved, vibroid rod
Cell size (μm)	1.5 × 2.0–3.5
Motility	+
Mol% G+C content	46.0
Major menaquinone	Nr
Optimal pH	7.2–7.4
Optimal temperature (°C)	14–16
Optimal NaCl concentration (g/l)	20–30
Growth factor requirement	Nr
Oxidation of substrate	Complete
Compounds used as electron donors and carbon sources	
H_2/CO_2	+
Formate	+
Acetate	–
Fatty acids	C_3 to C_7
Ethanol	+
Other *n*-alcohols	Methanol, *n*-Propanol, *n*-butanol, glycerol
Lactate	+
Pyruvate	–
Fumarate	+
Succinate	–
Malate	+
Others	Glycine, alanine, serine, betaine, choline chloride, proline, sorbitol, mannitol, benzoate
Fermentative growth	+
Disproportionation of reduced sulfur compounds	
Electron acceptors used	
Sulfate	+
Sulfite	–
Thiosulfate	+
Sulfur[a]	+
Other	–
Literature	Könneke et al. 2013, gen. nov., sp. nov.

[a]Only in the presence of acetate

Table 4.13

Comparison of selected characteristics of members of the genus *Desulfobotulus*

Characteristic	*Desulfofaba gelida*	*Desulfofaba fastidiosa*	*Desulfofaba hansenii*
Type strain	Strain PSv29, DSM 12344	Strain P2, ATCC BAA-815, DSM 15249	Strain P1, ATCC 700811, DSM 12642
Accession number of the 16S rRNA gene sequence of the type strain	AF099063	AY268891	AF321820
Morphology	Rod	Curved rod	Curved rod
Cell size (μm)	3.1 × 5–6.2	0.8–1.0 × 3.0–3.8	2.0–3.0 × 3.0–6.0
Motility	+ (might occur in older cultures)	+	+
Mol% G+C content	52.5	48.8	48.8
Major menaquinone	MK-8	Nr	Nr
Optimal pH	7.1–7.6	6.8–7.1	7.2–7.4
Optimal temperature (°C)	7	28	20
Oxidation of substrate	Incomplete	Incomplete	Incomplete
Optimal NaCl concentration (g/l)	14–25	10–16	13
Growth factor requirement	Nr	Nr	Nr
Compounds used as electron donors and carbon sources			
H_2/CO_2	–	–	+[a]
Formate	+[a]	Nr	Nr
Acetate	–	–	–
Fatty acids	C_3 to C_4	C_3	C_3
Ethanol	+	–	+
Other *n*-alcohols	*n*-propanol, *n*-butanol, glycerol	*n*-propanol	*n*-butanol
Lactate	+	+	–
Pyruvate	+	–	+
Fumarate	+	–	+
Succinate	+	–	+
Malate	+	Nr	Nr
Others	Alanine, glycine	–	Alanine
Fermentative growth	+	+	–
Disproportionation of reduced sulfur compounds		Nr	Nr
Electron acceptors used			
Sulfate	+	+	+
Sulfite	+	+	+
Thiosulfate	+	+	–
Sulfur[b]	–	–	+
Other	–	–	
Literature	Knoblauch et al. 1999, gen. nov., sp. nov.	Abildgaard et al. 2004, sp. nov.	Abildgaard et al. 2004, comb. nov.; Finster et al. 2001.

[a]Only in the presence of acetate

Table 4.14
Comparison of selected characteristics of members of the genus *Desulfofrigus*

Characteristic	*Desulfofrigus oceanense*	*Desulfofrigus fragile*
Type strain	Strain ASv26, DSM 12341	Strain LSv21, DSM 12345
Accession number of the 16S rRNA gene sequence of the type strain	AF099064	AF099065
Morphology	Rod	Rod
Cell size (μm)	2.1 × 4.2–6.1	0.8 × 3.2–4.2
Motility	+/–	+/–
Mol% G+C content	52.8	52.1
Major menaquinone	MK-9	MK-9
Optimal pH	7.0–7.5	7.0–7.4
Optimal temperature (°C)	10	18
Oxidation of substrate	Complete	Incomplete
Optimal NaCl concentration (g/l)	25–30	25–30
Growth factor requirement	–	–
H_2/CO_2	+[a]	-
Formate	+[a]	+[a]
Acetate	+	–
Fatty acids	C_4, C_5	C_4, C_6, C_7, C_{16}
Ethanol	+	+
Other *n*-alcohols	*n*-propanol, *n*-butanol, glycerol	*n*-propanol, *n*-butanol, glycerol
Lactate	+	+
Pyruvate	+	+
Fumarate	–	+
Succinate	–	–
Malate	+	+
Others	Glycine, serine	Alanine, serine
Fermentative growth	+	+
Disproportionation of reduced sulfur compounds	–	–
Electron acceptors used		
Sulfate	+	+
Sulfite	+	–
Thiosulfate	+	–
Sulfur[b]	–	–
Other	FeIII-citrate	FeIII-citrate
Literature	Knoblauch et al. 1999, gen. nov., sp. nov.	Knoblauch et al. 1999, sp. nov.

[a]Only in the presence of acetate

Table 4.15
Comparison of selected characteristics of the members of the genus *Desulfoluna*

Characteristic	*Desulfoluna butyratoxydans*	*Desulfoluna spongiiphila*
Type strain	Strain MSL71, DSM 19427, JCM 14721	Strain AA1, ATCC BAA-1256, DSM 17682
Accession number of the 16S rRNA gene sequence of the type strain	AB110540	EF187256
Morphology	Slightly curved rod	Slightly curved rod
Cell size (μm)	08–09 × 1.6–3.4	1 × 2.0–4.0
Motility	+	–
Mol% G+C content	62.0	58.5
Major menaquinone	MK-8(H_4)	Nr
Optimal pH	6.3	7–8
Optimal temperature (°C)	30	28
Optimal NaCl concentration (g/l)	20	25
Growth factor requirement	Nr	Nr
Oxidation of substrate	Incomplete	Incomplete
Compounds used as electron donors and carbon sources		
H_2/CO_2	+[a]	Nr
Formate	+[a]	+[a]
Acetate	–	–
Fatty acids	C_4	C_3
Ethanol	+	Nr
Other *n*-alcohols	Glycerol, *n*-propanol, *n*-butanol	Nr
Lactate	+	+
Pyruvate	+	+
Fumarate	–	Nr
Succinate	–	+
Malate	+	+
Others	–	Citrate, glucose, benzoate
Fermentative growth	+	–
Disproportionation of reduced sulfur compounds	Nr	Nr
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+

Table 4.15 (continued)

Characteristic	*Desulfoluna butyratoxydans*	*Desulfoluna spongiiphila*
Thiosulfate	+	+
Sulfur	Nr	Nr
Other	–	2,6-dibromophenol, other bromo- and iodophenols
Literature	Suzuki et al. 2008, gen. nov., sp. nov.	Ahn et al. 2009, sp. nov.

[a]Only in the presence of acetate

Desulfofrigus Knoblauch et al. 1999, 1640[VP]

De.sul.fo.fri´gus. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. neut. n. *frigus*, cold; N.L. neut. n. *Desulfofrigus*, sulfate reducer living in the cold.

The genus *Desulfofrigus* contains two described species. Their chemotaxonomic and physiological properties are listed in Table 4.14.

Desulfoluna Suzuki et al. 2008, 831[VP]

De.sul.fo.lu´na. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. *cella*, a store-room, a chamber, and in biology a cell; N.L. fem. n. *Desulfocella*, sulfate-reducing cell.

The genus *Desulfoluna* contains two describes species. The chemotaxonomic and physiological properties are summarized in Table 4.15.

Desulfonatronobacter Sorokin et al. 2012, 2111[VP]

De.sul.fo.na.tro.no.bac´ter. L. prep. de, from; N.L. pref. sulfo-, prefix used for N.L. masc. n. sulfas -atis, sulfate; N.Gr. n. natron, arbitrarily derived from the Arabic n. natrun or natron, soda; N.L. masc. n. bacter, a rod; N.L. masc. n. Desulfonatronobacter, sulfate-reducing natronophilic rod.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in Table 4.16.

Table 4.16
Selected characteristics of the only member of the genus *Desulfonatronobacter* (a table is used for easier comparison with other genera)

Characteristic	*Desulfonatronobacter acidivorans*
Type strain	Strain APT2, DSM 24257, UNIQEM U853
Accession number of the 16S rRNA gene sequence of the type strain	GU289732
Morphology	Rod
Cell size (μm)	05–0.6 × 0.8–1.5
Motility	+
Mol% G+C content	54.4
Major menaquinone	MK-8
Optimal pH	10.0
Optimal temperature (°C)	35
Optimal NaCl concentration (g/l)	35
Growth factor requirement	Nr
Oxidation of substrate	Complete
Compounds used as electron donors and carbon sources	
H_2/CO_2	–
Formate	–
Acetate	–
Fatty acids	C_3 to C_8
Ethanol	–
Other *n*-alcohols	–
Lactate	–
Pyruvate	–
Fumarate	–
Succinate	Nr
Malate	–
Others	–
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	–
Thiosulfate	+
Sulfur	–
Other	–
Literature	Sorokin et al. 2012, gen. nov., sp. nov.

Desulfonema Widdel et al. 1981, 382[VP] (Effective Publication Widdel 1980, 378)

De.sul.fo.ne´ma. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); Gr. neut. n. *nema*, thread; N.L. neut. n. *Desulfonema*, thread-forming sulfate reducer.

The genus *Desulfonema* contains two describes species. The chemotaxonomic and physiological properties are summarized in Table 4.17.

Taxonomic comment: As indicated in Fig. 4.1, the genus *Desulfonema* is not forming a distinct cluster. To combine

Table 4.17

Comparison of selected characteristics of the members of the genus *Desulfonema*

Characteristic	*Desulfonema limicola*	*Desulfonema ishimotonii*	*Desulfonema magnum*
Type strain	Strain "Jadebusen" 5 ac10, ATCC 33961, DSM 2076	Strain Tokyo 01, DSM 9680	Strain "Montpellier" 4be13, ATCC 35288, DSM 2077
Accession number of the 16S rRNA gene sequence of the type strain	U45990	U45992	U45989
Morphology	Multicellular filaments, 10–400 cells	Multicellular filaments cells	Multicellular filaments, 10–200 cells
Cell size (μm)	2.5–3.0 × 2.5–3.0	2.5–3.0 × 3.0–6.0	6.0–8.0 × 9.0–13.0
Motility	Gliding	Gliding	Gliding
Mol% G+C content	35.0	55.0	42.0
Major menaquinone	MK-7	Nr	MK-9
Optimal pH	7.6	7.0	7.0
Optimal temperature (°C)	30	30	32
Optimal NaCl concentration (g/l)	15	20	20
Growth factor requirement	Vitamins	Nr	Vitamins
Oxidation of substrate	Complete	Complete	Complete
Compounds used as electron donors and carbon sources			
H_2/CO_2	+	+	–
Formate	+	+	+[a]
Acetate	+[b]	+	+[b]
Fatty acids	C_3 to C_{14}	C_3 to C_{14}	C_3 to C_{10}
Ethanol	–	+	–
Other *n*-alcohols	Nr	*n*-propanol, *n*-butanol	Nr
Lactate	+	+	–
Pyruvate	+	+	–
Fumarate	+	+	+
Succinate	+	+	+
Malate	–	–	+[b]
Others	Isobutyrate, 2-methylbutyrate, 3-methylbutyrate	Isobutyrate, 2-methylbutyrate, 3-methylbutyrate	Isobutyrate, 2-methylbutyrate, 3-methylbutyrate, benzoate, 4-hydroxybenzoate, hippurate, phenylacetate, 3-phenylpropionate
Fermentative growth	–	–	–
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr
Electron acceptors used			
Sulfate	+	+	+
Sulfite	+	Nr	–
Thiosulfate	+	Nr	–
Sulfur	–	Nr	–
Other	–	Nr	–
Literature	Widdel 1981, gen. nov., sp. nov. Widdel 1980	Fukui et al. 2000, sp. nov.; Fukui et al. 1999	Widdel 1981, sp. nov.; Widdel 1980

[a]Only in the presence of acetate
[b]Poor growth

Table 4.18

Selected characteristics of the only member of the genus *Desulforegula* (a table is used for easier comparison with other genera)

Characteristic	*Desulforegula conservatrix*
Type strain	Strain Mb1Pa, DSM 13527, ATCC BAA-134
Accession number of the 16S rRNA gene sequence of the type strain	AF243334
Morphology	Rod-shaped
Cell size (μm)	1–1.3 × 2.6–3.0
Motility	+
Mol% G+C content	Nr
Major menaquinone	Nr
Optimal pH	Nr
Optimal temperature (°C)	25–30
Optimal NaCl concentration (g/l)	1
Growth factor requirement	Vitamins or yeast extract
Oxidation of substrate	Incomplete
Compounds used as electron donors and carbon sources	
H_2/CO_2	–
Formate	–
Acetate	–
Fatty acids	C_4 to C_{17}
Ethanol	–
Other *n*-alcohols	
Lactate	–
Pyruvate	–
Fumarate	–
Succinate	–
Malate	–
Others	–
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	–
Thiosulfate	–
Sulfur	–
Other	Nr
Literature	Rees and Patel 2001, gen. nov., sp. nov.

Table 4.19

Selected characteristics of the only member of the genus *Desulfosalsimonas* (a table is used for easier comparison with other genera)

Characteristic	*Desulfosalsimonas propionicica*
Type strain	Strain PropA = DSM 17721 = VKM B-2385
Accession number of the 16S rRNA gene sequence of the type strain	DQ067422
Morphology	Rods, sometimes filaments
Cell size (μm)	0.8–1.0 × 2.0–4.0
Motility	Nr
Mol% G+C content	54.1
Major menaquinone	Nr
Optimal pH	7.0
Optimal temperature (°C)	40
Optimal NaCl concentration (g/l)	60
Growth factor requirement	Yeast extract
Oxidation of substrate	Complete (alcohols incomplete)
Compounds used as electron donors and carbon sources	
H_2/CO_2	+[a]
Formate	–
Acetate	–
Fatty acids	C_3 to C_4
Ethanol	+
Other *n*-alcohols	*n*-propanol, *n*-butanol
Lactate	+
Pyruvate	+
Fumarate	+
Succinate	–
Malate	–
Others	Isobutyrate, yeast extract
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	+
Sulfur[b]	–
Other	–
Literature	Kjeldsen et al. 2010, gen. nov., sp. nov.

[a]Only in the presence of acetate

[b]In most cases sulfur might be reduced to sulfide but is not coupled to growth

Table 4.20

Comparison of selected characteristics of members of the genus *Desulfosarcina*

Characteristic	*Desulfosarcina variabilis*	*Desulfosarcina cetonica*	*Desulfosarcina ovata*
Type strain	Strain "Montpellier" 3be13, ATCC 33932, DSM 2060, VKM B-1627	Strain 480, DSM 7267, JCM 12296, VKM B-1975	Strain oXyS1, DSM 13228, JCM 12297
Accession number of the 16S rRNA gene sequence of the type strain	M34407	AJ237603	Y17286
Morphology	Oval rod, packages	Oval	Rod
Cell size (μm)	1.0–1.5 × 1.5–2.5	0.8–1.2 × 1.8–2.7	0.8–1.0 × 2.5–4.0
Motility	+/-	–	Nr
Mol% G+C content	51.0	59.0	51.0
Major menaquinone	MK-7	Nr	Nr
Optimal pH	7.4		
Optimal temperature (°C)	33	30	32
Oxidation of substrate	Complete	Complete	Complete
Optimal NaCl concentration (g/l)	13.5	10	21
Growth factor requirement	–	–	Nr
H_2/CO_2	+	+	+
Formate	+	+	+
Acetate	+[a]	+	+
Fatty acids	C_3–C_{14}	C_3–C_{16}	C3 (long-chain fatty acids not tested)
Ethanol	+	+	+
Other *n*-alcohols	*n*-Propanol, *n*-butanol	*n*-Propanol, *n*-butanol	Nr
Lactate	+	+	+
Pyruvate	+	+	+
Fumarate	+	–	Nr
Succinate	+	–	+
Malate	+	–	+
Others	2-Methylbutyrate, 3-methylbutyrate, cyclohexanecarboxylate, hippurate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, phenylacetate, 3-phenylpropionate	Acetone, benzoate, *m*-cresol	Benzoate, toluene, *o*-xylene
Fermentative growth	+	Nr	Nr
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr
Electron acceptors used			
Sulfate	+	+	+
Sulfite	+	Nr	Nr
Thiosulfate	+	+	Nr
Sulfur	–	+	Nr
Other	–	–	Nr
Literature	Widdel 1981, gen. nov., sp. nov. Widdel 1980	Kuever et al. 2006, sp. nov.; Galushko and Rozanova 1994; Kuever et al. 2005e, comb. nov.	Kuever et al. 2006, sp. nov.; Kuever et al. 2005e; Harms et al. 1999

[a]Poor growth

Table 4.21
Selected characteristics of the only member of the genus *Desulfospira* (a table is used for easier comparison with other genera)

Characteristic	*Desulfospira joergensenii*
Type strain	Strain B331, ATCC 700409, DSM 10085
Accession number of the 16S rRNA gene sequence of the type strain	X99637
Morphology	Vibrio
Cell size (μm)	07–0.8 × 1.0–2.0
Motility	–
Mol% G+C content	49.9
Major menaquinone	MK-7 and MK-7(H_2)
Optimal pH	7.0–7.4
Optimal temperature (°C)	26–30
Optimal NaCl concentration (g/l)	12–20
Growth factor requirement	Vitamins
Oxidation of substrate	Complete
Compounds used as electron donors and carbon sources	
H_2/CO_2	+
Formate	+
Acetate	–
Fatty acids	C4, C8, C10, C12
Ethanol	Nr
Other *n*-alcohols	Glycerol
Lactate	+
Pyruvate	+
Fumarate	+
Succinate	+
Malate	–
Others	Crotonate, glutarate, malate, oxaloacetate, choline chloride, betaine, DMSP, proline, yeast extract
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	+
Sulfur	+
Other	–
Literature	Finster et al. 1997 gen. nov., sp. nov.; Finster et al. 1997

these three species into one genus is primarily based on their morphology. They are the only multicellular sulfate-reducing bacteria showing a gliding motility. In principle the described species might form separate genera based on the distance of their 16S rRNA gene sequences to each other (all below 95 % sequence identity) and the rest of the genera belonging into the family *Desulfobacteraceae*.

Desulforegula Rees and Patel 2001, 1915[VP]

De.sul.fo.re´gu.la. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. n. fem *regula*, a straight piece of wood or ruler; N.L. fem. n. *Desulforegula*, a sulfate-reducing bacterium shaped like a ruler.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in Table 4.18.

Desulfosalsimonas Kjeldsen et al. 2010, 1063[VP]

De.sul.fo.sal.si.mo´nas. L. pref. *de*, from; L. n. *sulfur*, sulfur; L. adj. *salsus*, salty, saline; L. fem. n. *monas*, unit, monad; N.L. fem. n. *Desulfosalsimonas*, a sulfate-reducing monad that thrives in (hyper)saline environments.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in Table 4.19.

Desulfosarcina Widdel 1981, 382[VP] (Effective Publication Widdel 1980, 382)

De.sul.fo.sar.´ci.na. L. pref. de-, from; L. n. sulfur, sulfur; N.L. pref. desulfo-, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. sarcina, a package, bundle, and also a generic name (Sarcina); N.L. fem. n. Desulfosarcina, sarcina-shaped sulfate reducer.

The genus *Desulfosarcina* contains three described species. Their chemotaxonomic and physiological properties are listed in Table 4.20.

Desulfospira Finster et al. 1997, 1274[VP] (Effective Publication Finster et al. 1997, 207)

De.sul.fo.spi´ra. L. pref. *de-*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. *spira*, a coil, spire; N.L. fem. n. *Desulfospira*, a sulfate-reducing coil.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in Table 4.21.

Table 4.22

Comparison of selected characteristics of members of the genus *Desulfotignum*

Characteristic	*Desulfotignum balticum*	*Desulfotignum phosphitoxidans*	*Desulfotignum toluenicum*
Type strain	Strain Sax, ATCC BAA-19, DSM 7044	Strain FiPS-3, DSM 13687, OCM 818	Strain H3, ATCC BAA-1460, DSM 18732
Accession number of the 16S rRNA gene sequence of the type strain	AF418176	AF420288	EF207157
Morphology	Rod-shaped	Rod-shaped	Slightly curved rod
Cell size (μm)	0.5–0.7 × 1.5–3.0	0.6–0.8 × 2.0–4.0	0.6–1.0 × 1.4–2.5
Motility	+	–	–
Mol% G+C content	52.7[a]	53.9	52.0
Major menaquinone	Nr	Nr	Nr
Optimal pH	7.3	6.9–7.2	7.2
Optimal temperature (°C)	28–32	30	34
Oxidation of substrate	Complete	Complete	Complete
Optimal NaCl concentration (g/l)	20	10–20	15
Growth factor requirement	Vitamins	Nr	Vitamins
H_2/CO_2	+[b]	+	+
Formate	+[b]	+	+
Acetate	+[b]	–	+
Fatty acids	C_4, C_{10}, C_{12}, C_{16}	–	C_4, C_6, C_7, C_9, C_{10}, C_{14}, C_{16}
Ethanol	–	Nr	–
Other *n*-alcohols	–	Nr	Nr
Lactate	+	–	–
Pyruvate	+	+	+
Fumarate	+	+	Nr
Succinate	+[b]	+[b]	+
Malate	+	+	–
Others	Malate, glutamate, glycine, betaine, proline, benzoate, 4-hydroxybenzoate, phenol, phenylacetate	Phosphite, malate, glutamate, glycine, betaine, proline, glucose, arabinose, xylose	Crude oil, toluene, benzoate, 4-hydroxybenzoate
Fermentative growth	+	Nr	+
Disproportionation of reduced sulfur compounds	Nr	–	Nr
Electron acceptors used			
Sulfate	+	+	+
Sulfite	+	+	+
Thiosulfate	+	+	–
Sulfur[b]	Nr	–	–
Other	–	–	Nr
Literature	Kuever et al. 2001, gen. nov., sp. nov.; Schink et al. 2002	Schink et al. 2002, sp. nov.; Schink et al. 2002	Ommedal and Torsvik 2007, sp. nov.

[a]Value of the original description was wrong, this value was provided by Stefan Spring (DSMZ)

[b]Poor growth

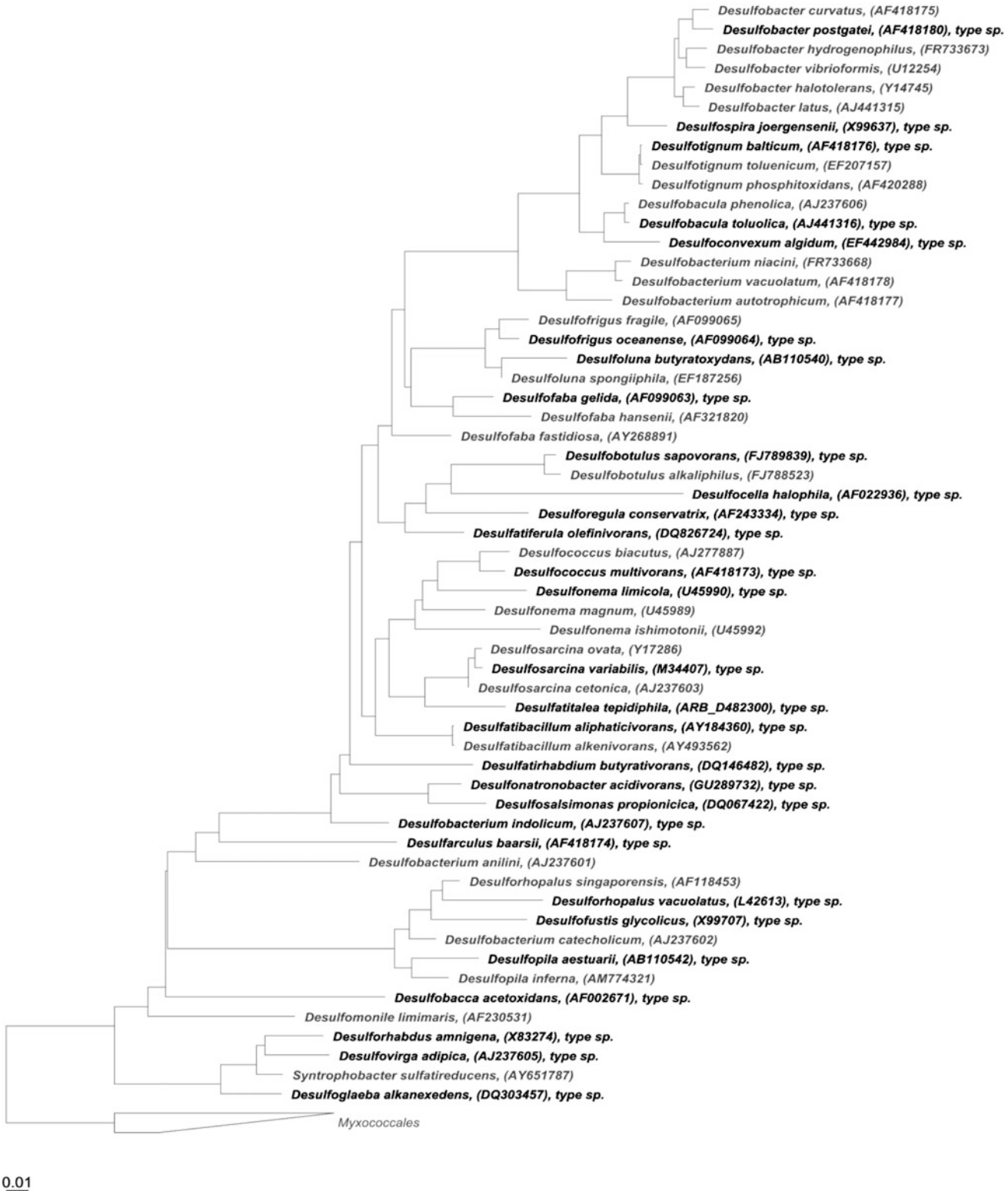

Fig. 4.2

Phylogenetic reconstruction of the family *Desulfobacteraceae* and neighboring lineages based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Samatakis 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

Desulfotignum Kuever et al. 2009, 923^{VP} (Effective Publication Kuever et al. 2005, 970)

De.sul.fo.tig´num. L. pref. *de-*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. neut. n. *tignum*, a piece or stick of timber; N.L. neut. n. *Desulfotignum*, sulfate-reducing stick.

The genus *Desulfotignum* contains three described species. Their chemotaxonomic and physiological properties are listed in *Table 4.22*.

Biolog

Biolog data are not available for sulfate-reducing bacteria.

Isolation, Enrichment, and Maintenance Procedures

All members of the family *Desulfobacteraceae* require anoxic media for growth like all other sulfate-reducing bacteria. The media are prepared under specific conditions, and the addition of a reductant is required in general sulfide to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992) and might be modified by other authors as listed in the original descriptions of various taxa. Typical modifications are simplifications, higher salt concentrations, or media for cultivation of haloalkaline organisms (e.g., Sorokin et al. 2012). For the isolation of organisms from brackish and marine sediment an elevated concentration of Mg^{2+} and sometimes Ca^{2+} ions is required.

For enrichment the used electron donor might be more or less selective and will have a strong influence on what kind of sulfate-reducing bacteria will grow in the medium. As usual electron acceptor sulfate is used. Most sulfate-reducing bacteria of this family have been enriched using batch cultures; other options are serial dilution techniques of natural samples. For isolation in general, roll-tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992; Kuever et al. 2005b).

For short-term preservation, stock cultures can be stored at 2–6 °C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. In general, multicellular filaments have to be kept as living culture and would not survive freezing. For long-term storage, cultures can be kept freeze dried, at −80 °C or in liquid nitrogen.

Ecology

Habitat

Members of this family were isolated from various habitats including freshwater, brackish water, and marine sediments.

All strains of the genus *Desulfobacter* were isolated with acetate as only electron donor and carbon source. The type strain of *Desulfobacter postgatei*, strain "Dangast" 2 ac9, was isolated from anoxic sediment of a brackish water ditch at the Jadebusen area, Northern Germany (Widdel 1980; Widdel and Pfennig 1981). The type strains of *Desulfobacter hydrogenophilus* and *Desulfobacter latus*, strain AcRS1 and strain AcRS2, were isolated from anoxic marine sediment from Rio di Giacomo, Venice, Italy (Widdel 1987). Strain AcRM3, the type strain of *Desulfobacter curvatus*, was isolated from anoxic marine sediment obtained from Rio Marin, Venice, Italy (Widdel 1987). Anoxic sediment form the Great Salt Lake (Utah, USA) was used for the isolation of the type strain of *Desulfobacter halotolerans* (Brandt and Ingverson 1997). Strain B54, the type strain of *Desulfobacter vibrioformis*, was isolated from a water-oil separation system of the Statfjord B platform in the Norwegian sector of the North Sea (Lien and Beeder 1997).

The type strain of *Desulfatibacillum aliphativorans*, strain CV2803, was isolated with alkanes as substrates from hydrocarbon-polluted marine sediment (Gulf of Fos, France) (Cravo-Laureau et al. 2004a). From the same location, the type strain of *Desulfatibacillum alkenivorans*, strain PF2803, was isolated using 1-tetradecene as electron donor and carbon source (Cravo-Laureau et al. 2004b).

Using 1-eicosene as electron donor and carbon source, strain LM2801, the type strain of *Desulfatiferula olefinivorans*, was isolated from an enrichment culture inoculated with brackish sediment of a wastewater decantation facility of an oil refinery (Berre lagoon, France) (Cravo-Laureau et al. 2007).

From a UASB reactor treating paper-mill wastewater, strain HB1, the type strain of *Desulfatirhabdium butyrativorans*, was isolated using butyrate as electron donor and carbon source.

The type strain of *Desulfatitalea tepidiphila*, strain S28bF, was isolated from an enrichment culture growing on toluene which was inoculated with anoxic marine sediment from Sanbanze (Tokyo Bay, Japan) using fumarate as electron donor and carbon source (Higashioka et al. 2012).

With indole as electron donor and carbon source, strain In04, the type strain of Desulfobacterium indolicum, was isolated from anoxic marine sediment obtained from Rio Marin (Venice, Italy) (Bak and Widdel 1986). The type strain of *Desulfobacterium autotrophicum*, strain HRM2, was isolated with hydrogen as electron donor and carbon dioxide as carbon source from anoxic marine mud, Venice, Italy (Brysch et al. 1988). Strain NAV-1, the type strain of *Desulfobacterium niacini*, was isolated from anoxic marine mud from Rio Marin, Venice, Italy, using nicontinate as electron donor and carbon source (Imhoff-Stuckle and Pfennig 1983). Isobutyrate was used as electron donor and carbon source for the isolation of strain IbRM, the type strain of *Desulfobacterium vacuolatum* from anoxic marine mud, Venice, Italy (Rees et al. 1998). The type strain of *Desulfobacterium anilini*, strain Ani 1, was isolated with aniline as electron donor and carbon source from anoxic marine sediment obtained from the North Sea coast close to Norddeich, Germany (Schnell et al. 1989). From a vanillate degrading mixed culture inoculated with anoxic sediment from Delaware bay

(Nelson, New Zealand), the type strain of *Desulfobacterium catecholicum*, strain NZva20, was isolated using catechol as substrate (Szewzyk and Pfennig 1987).

Strain Tol2, the type strain of *Desulfobacula toluolica*, was isolated from anoxic, sulfide-rich marine sediment from Eel Pond, a seawater pond in Woods Hole, MA, USA, using toluene substrate (Rabus et al. 1993). The type strain of *Desulfobacula phenolica*, strain Ph01, was isolated with phenol as electron donor and carbon source from marine mud (Venice, Italy) (Bak and Widdel 1988).

From freshwater sediment of a ditch close to Lindhorst (Hannover, Germany), strain 1pa3, the type strain of *Desulfobotulus sapovorans*, was isolated using palmitate as only electron donor and carbon source (Widdel 1980). The type strain of *Desulfobotulus alkaliphilus*, strain ASO4-4, was isolated with butyrate as electron donor and carbon source from sediment of the hypersaline soda lakeBitter-1 in Kulunda Steppe, Altai, Russia (Sorokin et al. 2010). *Desulfobotulus* spp. might get isolated also from brackish and marine habitats using short- and long-chain fatty acids as substrates.

Butyrate was used as electron donor and carbon source for the isolation of strain GSL-But2, the type strain of *Desulfocella halophila*, from hypersaline, thalassohaline sediment of the Great Salt Lake (UT, USA) (Brandt et al. 1999).

The type strain of *Desulfococcus multivorans*, strain 1be1, was isolated with benzoate from sewage sludge obtained in Göttingen, Germany (Widdel 1980). Using acetone as only electron donor and carbon source, strain KMRActS, the type strain of *Desulfococcus biacutus*, was isolated from anoxic sludge of a wastewater treatment plant (Konstanz, Germany) (Platen et al. 1990).

The type strain of *Desulfoconvexum algidum*, strain JHA1, was isolated from permanently cold, marine sediment of an Arctic fjord at the northwest coast of Svalbard (Smeerenburgfjorden; 79°42′815 N, 11°05′189E) using hydrogen as electron donor and carbon dioxide as carbon source (Könneke et al. 2012).

Strain PSv29, the type strain of *Desulfofaba gelida*, was isolated with propionate as electron donor and carbon source from an anoxic marine sediment sample obtained from Hornsund sediment (76′58.2′ N, 15′34.5′ E), Spitzbergen, Norway (Knoblauch et al. 1999). The type strain of *Desulfofaba fastidiosa*, strain P2, was isolated with propionate as only electron donor and carbon source from the sulfate-methane transition zone of a marine sediment located at the east coast of Jutland, Denmark (59° 09′ 20″ N 10° 19′ 24″ E) (Abildgaard et al. 2004). From the roots of the eelgrass *Zostera marina* (location unknown), strain P1, the type strain of *Desulfofaba hansenii*, was isolated using propionate as only electron donor and carbon source (Finster et al. 2001).

The inoculum for the isolation of strain ASv26, the type strain of *Desulfofrigus oceanense*, originated from Hornsund sediment (76′58.2′ N, 15′34.5′ E), Spitzbergen, Norway, and the substrate used was acetate (Knoblauch et al. 1999). From the same location, the type strain of *Desulfofrigus fragile*, strain LSv21, was isolated using lactate as only electron donor and carbon source (Knoblauch et al. 1999).

The type strain of *Desulfoluna butyratoxydans*, strain MSL7, was isolated from estuarine sediment at the Niida River estuary in Sakata Harbour, Japan, using lactate as substrate (Suzuki et al. 2008). A sponge (*Aplysina aerophoba*) from the Mediterranean Sea (Banyuls sur Mer, France) was used as inoculum for the isolation of strain AA1, the type strain of *Desulfoluna spongiiphila*, with lactate as electron donor and carbon source (Ahn et al. 2009).

The type strain of *Desulfonatronobacter acidivorans*, strain APT2, was isolated with propionate as electron donor and carbon source and thiosulfate as electron acceptor from anoxic sediment of lake Bitter 1, a soda lake in southwestern Siberia (Altai region, Russia)(Sorokin et al. 2012).

Acetate was used as electron donor and carbon source for the isolation of strain 5ac10, the type strain of *Desulfonema limicola*, from anoxic mud obtained from the Jadebusen area, Northern Germany (Widdel 1980). Using benzoate as electron donor and carbon source, strain "Montpellier" 4be13, the type strain of *Desulfonema magnum*, was isolated from anoxic black, mud obtained from a lagoon close to Montpellier, France (Widdel 1980). The type strain of *Desulfonema ishimontonii*, strain Tokyo 01, was isolated from anoxic marine sediment obtained from Tokyo Bay, Japan, using a mixture of acetate (7 mM) and isobutyrate (1.5 mM) as electron donors and carbon sources (Fukui et al. 1999).

The type strain of *Desulforegula conservatrix*, strain MB1Pa, was isolated with palmitate as electron donor and carbon source using sediment of a wetland lake of the floodplain of the River Murray, Albury, Australia (Rees and Patel 2001).

Strain PropA, the type strain of *Desulfosalsimonas propionica*, was isolated with propionate as electron donor and carbon source from the northern arm of the Great Salt Lake, Utah, USA (Kjeldsen et al. 2010).

The type strain of *Desulfosarcina variabilis*, strain 3be13, was isolated with benzoate from anoxic mud obtained from a lagoon close to Montpellier, France (Widdel 1980). Strain 480, the type strain of *Desulfosarcina cetonica*, was isolated with butyrate as donor (electron donor and carbon source) from a flooded oil deposit located at the Absheron peninsula (Baku, Aserbaidschan) (Galuskko and Rozanova 1990).

With o-xylene as only electron donor and carbon source, the type strain of *Desulfosarcina ovata*, strain oXYS1, was isolated from a North Sea oil tank located in Wilhelmshaven, Northern Germany (Harms et al. 1999).

The type strain of *Desulfospira joergensenii*, strain B331, was isolated with butyrate as electron donor and carbon source from oxidized iron-rich surface sediment from a *Zostera noltii* overgrown mud flat (Arcachon, France) (Finster et al. 1997).

The type strain of *Desulfotignum balticum*, strain Sax, was isolated with benzoate as electron donor and carbon source from anoxic marine mud (Saxild, Denmark) (Drzyzga et al. 1993). With phosphate as electron donor, strain FiPS-3, the type strain of *Desulfotignum phosphitoxidans*, was isolated from anoxic sediment of the Canal Grande (Venice, Italy) (Schink et al. 2002). Strain H3, the type strain of *Desulfotignum*

toluenicum, was isolated with crude oil as electron donor and carbon source from an oil-reservoir model column at the University of Bergen, Norway (Ommedal and Torsvik 2007).

Pathogenicity, Clinical Relevance

There is no clinical relevance known.

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5 The Family *Desulfobulbaceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract
Desulfobulbaceae, a family within the order *Desulfobacterales*, embraces the genera *Desulfobulbus*, *Desulfocapsa*, *Desulfofustis*, *Desulfopila*, *Desulforhophalus*, *Desulfotalea*, and *Desulfurivibrio*. Besides their 16S rRNA gene sequence phylogeny, all members of the family are defined by a wide range of morphological and chemotaxonomic properties for the delineation of genera and species. Strictly anaerobic, having a respiratory type of metabolism. Fermentative metabolism might occur. Most members are mesophilic sulfate-reducing bacteria; some are psychrophilic. A few members are obligate alkaliphilic. Some members cannot use sulfate as terminal electron acceptor but grow by disproportionation of reduced sulfur compounds or use thiosulfate or sulfur/polysulfide as electron acceptors. Members of the family are found in marine, brackish, or freshwater habitats. Most described species are chemoorganoheterotroph; some are chemolithoheterotroph or chemolithoautotroph. Most members oxidize organic substrates incompletely to acetate.

This contribution is a modified and updated version of a previous family description (Kuever et al. 2005a).

Taxonomy, Historical and Current

Short Description of the Family

De.sul.fo.bul.ba′ce.ae. M.L. masc. n. *Desulfobulbus* type genus of the family; -aceae, ending to denote a family; N.L. fem. pl. n. *Desulfobulbaceae* the Desulfobulbus family (Modified from *Bergey's Manual*). The description is an emended version of the description given in *Bergey's Manual*, 2nd edition (Kuever et al. 2005a).

The family *Desulfobulbaceae* contains the genera *Desulfobulbus* (Widdel et al. 1981) which is the type genus of the family (Kuever et al. 2006), *Desulfocapsa* (Janssen et al. 1997), *Desulfofustis* (Friedrich et al. 1996), *Desulfopila* (Suzuki et al. 2007b), *Desulforhopalus* (Isaksen and Teske 1999), *Desulfotalea* (Knoblauch et al. 1999), and *Desulfurivibrio* (Sorokin et al. 2008). Gram-staining is negative. Morphological forms are in general oval- or rod-shaped cells; only the genus *Desulfurivibrio* contains vibrio-shaped cells. Spore formation is absent. Most members are motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory type of metabolism. Some species can grow by fermentation. Simple organic molecules are used as electron donors; some species can use also H_2. Most members oxidize organic substrates incompletely to acetate.

Most described species are chemoorganoheterotroph; some are chemolithoheterotroph or chemolithoautotroph. Sulfate and thiosulfate are used as electron acceptor and reduced to sulfide; some species can also use sulfite. A few species can grow by disproportionation of reduced sulfur compounds with or without acetate as carbon source. Some members cannot use sulfate as electron acceptor and use thiosulfate or sulfur/polysulfide instead. A few members of this family can use nitrate as terminal electron acceptor which is reduced to ammonia.

Members have been isolated from various sources, like freshwater, brackish water, marine, and haloalkaline habitats. A few members are obligate alkaliphilic. Most members are mesophilic except members belonging to the genus *Desulfotalea* which are obligate psychrophilic.

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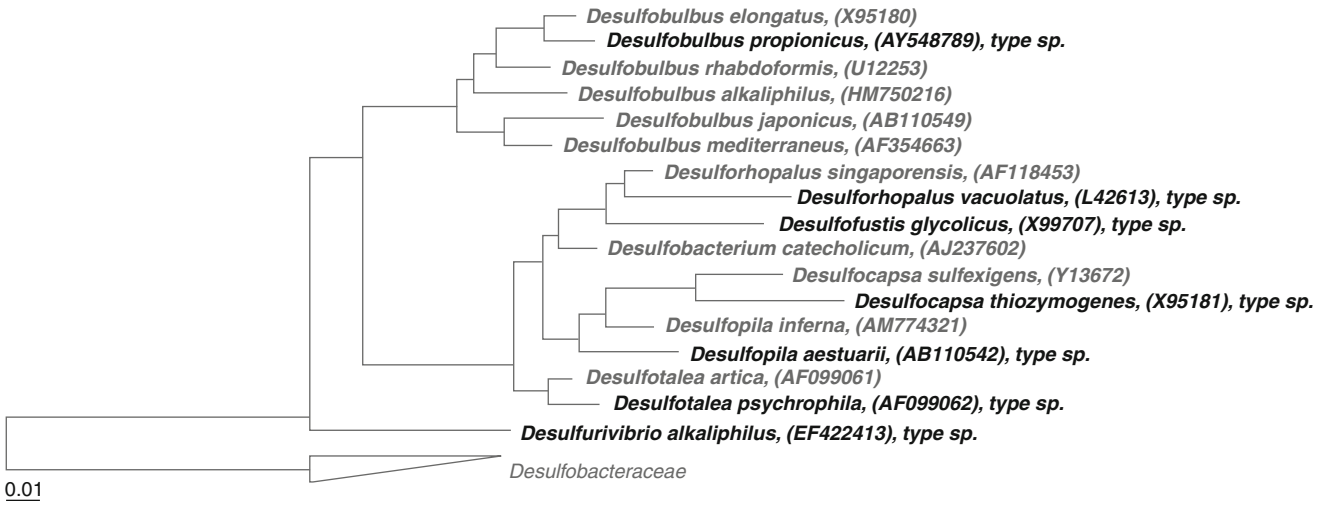

Fig. 5.1

Phylogenetic reconstruction of the family *Desulfobulbaceae* 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 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. The sequence of *Desulfobulbus marinus* was not included in the tree because length and quality were not sufficient, but it is clearly a member of the genus

One single species, *Desulforhopalus vacuolatus*, contains gas vacuoles (Isaksen and Teske 1999).

Phylogenetic Structure of the Family and Its Genera

The phylogenetic structure of the family and its neighboring family *Desulfobacteraceae* within the order *Desulfobacterales* is shown on *Fig. 5.1*. The borders of the family are primarily based on the phylogenetic tree as framework; other unique properties (physiology, chemotaxonomic markers) which are present in all members are not found (see *Table 5.1*).

Taxonomic Comment: As can be seen on *Fig. 5.1*, *Desulfobacterium catecholicum* (Szewzyk and Pfennig 1987) is clearly not a member of the genus *Desulfobacterium* but also a member of the *Desulfobulbaceae* family and should be regarded as a new genus. Another not validated and described member not shown in this tree is *Desulfobacterium corrodens*, a highly corrosive iron-oxidizing sulfate-reducing bacterium (Dinh et al. 2004) which cluster with *Desulfopila inferna*; both should be considered as a new separate genus (Gittel et al. 2010). Another strain named MLMS-1 (DSM 17814) isolated from Mono Lake, California, USA, was used for genome sequencing and is also clearly a new genus within this family (Hoeft et al. 2004).

Molecular Analyses

DNA-DNA Hybridization Studies

Are absent.

Bioprinting and Ribotyping

Are absent.

MALDI-TOF

Are absent.

Genome Comparison

The complete genome sequences of four members of this family have been analyzed. The genome of the type strain of *Desulfotalea psychrophila* DSM 12343 is 3,659,632 bp long (one chromosome of 3,523,383 bp, a 121,586 bp plasmid, and a 14,663 bp plasmid) and contains 3,322 genes with 3,234 coding for proteins and 98 RNA genes (Rabus et al. 2004). The mol% G+C of the DNA is 46.6. In the genome single copies of the dissimilatory adenylylsulfate reductase (AprBA) linked to the membrane-bound Qmo complex (QmoABC) and the dissimilatory sulfite reduction (DsrAB) linked to the DsrMKJOP complex were found.

For the type strain of *Desulfobulbus propionicus* DSM 2032, the genome is 3,881,859 bp long and contains 3,408 genes with 3,351 coding for protein and 57 RNA genes (Paganini et al. 2011). The mol% G+C of the DNA is 58.93. Again both aprBA linked to a complete QmoABC complex and the dsrAB are present as a single copy.

Table 5.1

Morphological and chemotaxonomic characteristics of genera of *Desulfobacteraceae*

	Desulfobulbus	*Desulfocapsa*	*Desulfofustis*	*Desulfopila*	*Desulforhopalus*	*Desulfotalea*	*Desulfurivibrio*
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Vibrio
Gram stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+ or −	+	+	+	+	+ or −	−
Metabolism	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Major fatty acids	$C_{14:0}$, $C_{15:0}$, $C_{16:1w7}$, $C_{16:1w5}$, $C_{17:1w6}$, $C_{18:1w7}$ (in *D. alkaliphilus* a high amount of $C_{17:0}$ cyclo is present)	Nr	Nr	$C_{16:0}$, $C_{16:1w5}$, $C_{16:1w7}$, $C_{17:0}$ cyclo (only in D. inferna), C18:0	$C_{15:1w9}$, $C_{17:1w11}$	$C_{16:1w9}$, $C_{16:1w11}$,	$C_{16:0}$, $C_{16:1w5}$, $C_{18:w7}$
Menaquinone	Mk-5(H_2)	Nr	Mk-5(H_2)	Mk-8(H_4)	MK-6(H_2) or MK-6(H_4)	Mk-6 or Mk-(H_2)	Nr
G+C content (mol%)	47.3–59.9	47.2–50.7	56.2	50.3–54.4	40.6–48.4	41.8–46.8	60.3[d]
Substrate oxidation	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Nr
CODH activity	Nr	Present[a]	Present	Present[a]	Nr	Nr	Nr
Typical electron donors	H_2 (most species), propionate, lactate, pyruvate, alcohols	Reduced sulfur compounds used for disproportionation, alcohols (one species)	H_2, lactate, pyruvate, alcohols, fumarate, succinate, malate, glyoxylate, glycolate	Lactate, pyruvate, alcohols, fumarate	Propionate, alcohols	H_2, formate, propionate, lactate, pyruvate, alcohols	H_2, formate, butyrate, isobutyrate, lactate, pyruvate, ethanol, fumarate, succinate, malate, yeast extract
Chemolithoautotrophic growth	No species	One species	No species	One species	No species	No species	All species
Fermentative growth	All species	Growth by disproportionation of reduced sulfur compounds not by fermentation of organic compounds	Nr	All species	All species	All species	Growth by disproportionation of reduced sulfur compounds[b]
Growth by disproportionation of reduced sulfur compounds	+ or −	+	Nr	Nr	+ or −	−	+[b]
Typical electron acceptors	Sulfate, thiosulfate, sulfite	Sulfate, thiosulfate, sulfite	Sulfate, sulfite	Sulfate, sulfite	Sulfate, sulfite, thiosulfate	Sulfate	Thiosulfate, sulfur/ polysulfide
Optimal growth temperature (°C)	25–39	30	28	28–35	18–31	10–18	35[c]
Habitat	Freshwater, brackish or marine sediment, alkaline lake	Freshwater, brackish or marine sediment	Brackish or marine sediment	Marine sediment	Marine sediment	Marine sediment	Haloalkaline lake

[a]In *Desulfocapsa sulfexigens* and *Desulfopila inferna* CODH activity was present

[b]Personal Communication K. Finster

[c]Personal Communication D.Y. Sorokin

[d]Data taken from the genome sequencing project. In the original description the values was 40.5 mol% for the G+C content

The genome analysis of the type strain of *Desulfurivibrio alkaliphilus* DSM 19089 is finished, but no publication is available. The size of the genome is 3,097,763 bp. It contains 3,913 genes and 3,663 coding for proteins. Interestingly typical enzymes for sulfate reduction are found in the genome although this organism cannot use sulfate as electron acceptor for growth. The aprBA linked to a complete QmoABC complex and the dsrAB genes are present and found as a single copy.

From strain MLMS-1 the genome was also sequenced, but a publication is also absent. The genome is 6,058,912 bp long and contains 5,487 genes with 5,370 coding for proteins and 108 RNA genes. This strain is also described as non-sulfate-reducing bacterium. Surprisingly, it owns single copies of the aprBA linked to a complete QmoABC complex and the dsrAB genes.

In principle, *Desulfurivibrio alkaliphilus* and strain MLMS-1 might be able to use sulfate as terminal electron acceptor. Why they cannot use sulfate remains unclear. A lacking transport system could be an explanation.

For the type strain of both *Desulfocapsa* species, a genome sequencing project has already started, but so far no data are published.

Phages

Phages might be present but are not reported so far.

Phenotypic Analyses

The main features of all members of the family *Desulfobulbaceae* are listed in ❯ *Table 5.1* and the tables featuring the genera (❯ *Tables 5.2–5.8*).

Desulfobulbus Widdel 1981, 382[VP] (Effective Publication Widdel 1980, 372)

De.sul.fo.bul´bus. L. prep. de, from; L. n. sulfur, sulfur; N.L. pref. desulfo-, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. masc. n. *bulbus*, a bulb, an onion; N.L. masc. n. *Desulfobulbus*, onion-shaped sulfate reducer.

The genus *Desulfobulbus* contains seven described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 5.2*.

Taxonomic Comment: Because of the poor quality and length of the sequence, *Desulfobulbus marinus* is not included in the phylogenetic tree (❯ *Fig. 5.1*). Using the shorter sequences for tree construction, it is clearly indicated that *Desulfobulbus marinus* is a member of this genus. This is also reflected by phylogenetic analyses based on dsrAB (Klein et al. 2001) and arpBA genes (Meyer and Kuever 2007).

Desulfocapsa Janssen et al. 1997, 601[VP] (Effective Publication Janssen et al. 1996, 190)

De.sul.fo.cap´sa. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. *capsa*, box; N.L. fem. n. *Desulfocapsa*, a sulfate-reducing box.

The genus *Desulfocapsa* contains two described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 5.3*.

Taxonomic Comment: The phylogenetic tree (❯ *Fig. 5.1*) and the low identity of 92.2 % of the 16S rRNA gene sequence to the type strain *Desulfocapsa thiozymogenes* indicates that *Desulfocapsa sulfexigens* should not be regarded as a member of this genus. This has to be confirmed by additional varying physiological properties and phylogenetic trees based on dsrAB and aprAB which are absent so far. Compared to other genera, leaving these two species in the same genus would be highly inconsistent.

Desulfofustis Friedrich et al. 1996, 1067 [VP]

De.sul.fo.fus´tis. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. masc. n. *fustis*, club; N.L. masc. n. *Desulfofustis*, a club-shaped sulfate reducer.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 5.4*.

Desulfopila Suzuki et al. 2007a, 524[VP]

De.sul.fo.pi ´la. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. pila, pillar; N.L. fem. n. Desulfopila, a sulfate-reducing pillar.

The genus *Desulfopila* contains two described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 5.5*.

Taxonomic Comment: The phylogenetic tree (❯ *Fig. 5.1*) and the low identity of 94.3 % of the 16S rRNA gene sequence to the type strain *Desulfopila aestuarii* clearly indicates that *Desulfopila inferna* should not be regarded as a member of this genus. This is also reflected by varying physiological properties and phylogenetic trees based on dsrAB and aprAB (Gittel et al. 2010). Compared to other genera leaving these two species in the same genus would be highly inconsistent.

Desulforhopalus Isaksen and Teske 1999, 935[VP] (Effective Publication Isaksen and teske 1996, 167)

De.sul.fo.rho´pa.lus. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize

Table 5.2

Comparison of selected characteristics of members of the genus *Desulfobulbus*

Characteristic	*Desulfobulbus propionicus*	*Desulfobulbus alkaliphilus*	*Desulfobulbus elongatus*	*Desulfobulbus japonicus*	*Desulfobulbus marinus*	*Desulfobulbus mediterraneus*	*Desulfobulbus rhabdoformis*
Type strain	Strain "Lindhorst" 1pr3, ATCC 33891, DSM 2032, VKM B-1956	Strain APS1, DSM 24258, UNIQEM U900	Strain FP, ATCC 43118, DSM 2908	Strain Pro1, DSM 18378, JCM 14043	Strain 3pr10, DSM 2058, JCM 13489	Strain 86FS1, DSM 13871, JCM 15784	Strain M16, ATCC 700652, DSM 8777
Accession number of the 16S rRNA gene sequence of the type strain	CP001734, AY548789	HM750216	X95180	AB110549	M34411	AF354663	U12253
Morphology	Oval- or lemon-shaped rod	Oval- or lemon-shaped rod	Rod	Rod	Oval-shaped rod	Oval-shaped rod	Rod
Cell size (μm)	1.0–1.3 x 1.5–2.0	1.0–1.5 x 2.0–2.5	0.6–1.0 x 1.5–2.5	0.8–1.6 x 1.4–2.9	1.0–1.3 x 1.8–2.0	1.2–1.7 x 1.4–3.2	
Motility	–	+	+	+		+	–
Mol% G+C content	58.9[e]	52.8	59.0	46–48.6	47.3	58.6	50.6
Major menaquinone	Mk-5(H_2)	Mk-5(H_2)	Mk-5(H_2)	Mk-5(H_2)	Mk-5(H_2)	Nr	Mk-5(H_2)
Optimal pH	7.2	9.4	7.0	6.7	7.1–7.4	7.1–7.4	6.8–7.2
Optimal temperature (°C)	39	33	31	35	29	25	31
Optimal NaCl concentration (g/l)	0–1	11.7	0–1	30	21	21	15–20
Growth factor requirement	Vitamins	Nr	Vitamins	Nr	Vitamins	–	–
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	–[f]	+[a]	+[a]	+[a]	–	+[a]
Formate	–	–[f]	–	+[a]	+[a]	–	–
Acetate	–	–[f]	–	–	–	–	–
Fatty acids	C_3	C_3 [f]	C_3	C_3	C_3	C_3	C_3
Ethanol	+	–[f]	+	+	+	+	+
Other *n*-alcohols	*n*-propanol	–[f]	*n*-propanol	*n*-propanol, *n*-butanol, glycerol	*n*-propanol	*n*-propanol, *n*-butanol	*n*-propanol
Lactate	+	–[f]	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	–	–[f]	–	+	–	+	+
Succinate	–	–[f]	–	–	–	+	+

Table 5.2 (continued)

Characteristic	*Desulfobulbus propionicus*	*Desulfobulbus alkaliphilus*	*Desulfobulbus elongatus*	*Desulfobulbus japonicus*	*Desulfobulbus marinus*	*Desulfobulbus mediterraneus*	*Desulfobulbus rhabdoformis*
Malate	–	–[f]	–	+	–	+	+
Other	–	Nr	–	Alanine, fructose, glucose	–	Glucose, galactose, lactose, cellobiose, alanine. Poor growth on other mono- and disaccharides	–
Fermentative growth	+	–[f]	+	+	+	+	+
Disproportionation of reduced sulfur compounds	+[b]	–[f]	Nr	Nr	Nr	Nr	Nr
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	+	+	–	+	+	+
Thiosulfate	+	–[d]	+	+	+	+	+
Sulfur	–[b]	–[d]	–	Nr	Nr	Nr	Nr
Other	Nitrate	Nr	–	–	Nr	–[c]	–
Literature	Widdel (1981), sp. nov.; Widdel (1980)	Sorokin et al. (2012), sp. nov.	Samain et al. (1985), sp. nov.; Samain et al. (1984)	Suzuki et al. (2007a), sp. nov.	Kuever et al. (2013), sp. nov.; Kuever et al. (2005b)	Sass et al. (2002), sp. nov.; Sass et al. (2002)	Lien et al. (1998), sp. nov.

[a]Only in the presence of acetate
[b]Sulfur can be disportionated in the presence of FeIII
[c]Oxygen is used as electron acceptor but allows no growth
[d]Both are reduced under nongrowing conditions to sulfide
[e]Data taken from the genome published (Paganini et al. 2011)
[f]D.Y. Sorokin personal communications

Table 5.3

Comparison of selected characteristics of members of the genus *Desulfocapsa*

Characteristic	*Desulfocapsa thiozymogenes*	*Desulfocapsa sulfexigens*
Type strain	Strain Bra2, DSM 7269	Strain SB164P1, ATCC 700424, DSM 10523
Accession number of the 16S rRNA gene sequence of the type strain	X95181	Y13672
Morphology	Rod-shaped rod	Rod-shaped rod
Cell size (μm)	0.8–0.9 x 2.0–3.5	0.5 x 2.0–4.0
Motility	+	+
Mol% G+C content	50.7	47.2
Major menaquinone	Nr	Nr
Optimal pH	7.3–7.5	6.7–7.3
Optimal temperature (°C)	30	30
Optimal NaCl concentration (g/l)	0–1	15
Growth factor requirement	–	–
Oxidation of substrate	Incomplete	Unknown
Compounds used as electron donors and carbon sources		
H_2/CO_2	–	+[a]
Formate	–	+[a]
Acetate	–	–
Fatty acids	–	–
Ethanol	+	–
Other *n*-alcohols	*n*-propanol, *n*-butanol	Nr
Lactate	–	–
Pyruvate	–	–
Fumarate	–	–
Succinate	–	–
Malate	–	–
Others	–	Nr
Fermentative growth	–	–
Disproportionation of reduced sulfur compounds	+b	+[b]
Electron acceptors used		
Sulfate	+	–[a]
Sulfite	+	+
Thiosulfate	+	+
Sulfur	+[c]	+[c]
Other	–	–
Literature	Janssen et al. (1997), sp. nov., Janssen et al. (1996)	Finster et al. (2000), sp. nov.; Finster et al. (1998)

[a]Utilized without growth
[b]Chemolithoautotrophic growth
[c]Only in the presence of ferrihydrite as sulfide scavenger

a dissimilatory sulfate-reducing procaryote); L. masc. n. *rhopalus*, cudgel; N.L. masc. n. *Desulforhopalus*, cudgel-formed sulfate reducer.

The genus *Desulforhopalus* contains two described species. Their chemotaxonomic and physiological properties are listed in *Table 5.6*.

Taxonomic Comment: The phylogenetic tree (*Fig. 5.1*) and the low identity of 94.2 % of the 16S rRNA gene sequence to the type strain *Desulforhopalus vacuolatus* clearly indicates that *Desulforhopalus singaporensis* should not be regarded as a member of this genus. This is also reflected by varying physiological properties and a phylogenetic tree based on dsrAB

Table 5.4

Selected characteristics of the only member of the genus *Desulfofustis* (a table is used for easier comparison with other genera)

Characteristic	*Desulfofustis glycolicus*
Type strain	Strain PerGlyS, ATCC 700454, DSM 9705
Accession number of the 16S rRNA gene sequence of the type strain	X99707
Morphology	Straight or slightly curved rod
Cell size (μm)	0.55 × 2.0–4.5
Motility	+
Mol% G+C content	56.2
Major menaquinone	Mk-5(H_2)
Optimal pH	7.3
Optimal temperature (°C)	28
Optimal NaCl concentration (g/l)	5.8
Growth factor requirement	Vitamins
Oxidation of substrate	Incomplete
Compounds used as electron donors and carbon sources	
H_2/CO_2	+[a]
Formate	–
Acetate	–
Fatty acids	–
Ethanol	–
Other *n*-alcohols	–
Lactate	+
Pyruvate	Nr
Fumarate	+
Succinate	+
Malate	+
Others	Glycolate[b], glyoxylate[b], yeast extract
Fermentative growth	Nr
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	–
Sulfur[c]	+
Other	–
Literature	Friedrich et al. (1996), sp. nov., Friedrich and Schink, (1995)

[a]Only in the presence of acetate
[b]These compounds are completely oxidized to carbon dioxide
[c]In most cases sulfur might be reduced to sulfide but is not coupled to growth

Table 5.5

Comparison of selected characteristics of members of the genus *Desulfopila*

Characteristic	*Desulfopila aestuarii*	*Desulfopila inferna*
Type strain	Strain MSL86 = DSM 18488 = JCM 14042.	Strain JS_SRB250Lac = DSM 19738 = NBRC 103921.
Accession number of the 16S rRNA gene sequence of the type strain	AB110542	AM774321
Morphology	Rod	Rod
Cell size (μm)	0.7–1.2 × 1.9–3.8	0.3–0.5 × 1.0–2.0
Motility	+	–
Mol% G+C content	54.4	50.3
Major menaquinone	Mk-8(H_4)	Nr
Optimal pH	7.5–7.6	7.0–7.2
Optimal temperature (°C)	35	28
Optimal NaCl concentration (g/l)	10	20–30
Growth factor requirement	Vitamins, yeast extract	Vitamins
Oxidation of substrate	Incomplete	Incomplete
H_2/CO_2	–	+
Formate	+[a]	+
Acetate	–	–
Fatty acids	–	C_4, C_5, C_6
Ethanol	+	+
Other *n*-alcohols	*n*-propanol, *n*-butanol, glycerol	*n*-propanol, *n*-butanol, glycerol
Lactate	+	+
Pyruvate	+	+
Fumarate	+	+
Succinate	–	+
Malate	–	–
Others	–	Proline
Fermentative growth	+	+
Disproportionation of reduced sulfur compounds	Nr	Nr
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	–
Sulfur	–	–
Other	Fumarate	–
Literature	Suzuki et al. (2007a), sp. nov.	Gittel et al. (2010), sp. nov.

[a]Only in the presence of acetate

Table 5.6
Comparison of selected characteristics of members of the genus *Desulforhopalus*

Characteristic	*Desulforhopalus vacuolatus*	*Desulforhopalus singaporensis*
Type strain	Strain ltk 10, ATCC 700480, DSM 9700	Strain Singapore T1, DSM 12130
Accession number of the 16S rRNA gene sequence of the type strain	L42613	AF118453
Morphology	Rod	Rod
Cell size (μm)	1.5–1.8 × 3.9–5.0	0.9–1.2 × 1.7–2.3
Motility	–	–
Mol% G+C content	48.4	40.6
Major menaquinone	Nr	Mk-5(H_2)
Optimal pH	7.0	7.8
Optimal temperature (°C)	18–19	31
Optimal NaCl concentration (g/l)	20	20
Growth factor requirement	Vitamins	–
Oxidation of substrate	Incomplete	Incomplete
Compounds used as electron donors and carbon sources		
H_2/CO_2	+[a]	Nr
Formate	–	+[a]
Acetate	–	–
Fatty acids	C_3	C_3, C_4
Ethanol	+	+
Other *n*-alcohols	*n*-propanol, *n*-butanol	*n*-propanol, *n*-butanol
Lactate	+	–
Pyruvate	+	+
Fumarate	–	+
Succinate	Nr	+
Malate	Nr	+
Others	–	Alanine, casamino acids, fermentation of taurine
Fermentative growth	+	+
Disproportionation of reduced sulfur compounds	–	+
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	+
Sulfur	–	Nr
Other	–	nitrate
Literature	Isaksen and Teske (1997), sp. nov. Isaksen and Teske, (1996)	Lie et al. (2000), sp. nov.; Lie et al. (1999)

[a]Only in the presence of acetate

(Gittel et al. 2010). Compared to other genera, leaving these two species in the same genus would be highly inconsistent.

Desulfotalea Knoblauch et al. 1999, 1641[VP]

De.sul.fo.ta´le.a. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. talea, a slender staff, a rod; N.L. fem. n. *Desulfotalea*, a sulfate-reducing rod.

The genus *Desulfotalea* contains two described species. Their chemotaxonomic and physiological properties are listed in Table 5.7.

Desulfurivibrio Sorokin et al. 2008, 1993 (Effective Publication Sorokin et al. 2008, 438)

De.sul.fu.ri.vi´bri.o. L. pref. *de-*, from, off, away; L. n. *sulfur*, sulfur; N.L. masc. n. *vibrio*, that which vibrates, a vibrio; N.L. masc. n. *Desulfurivibrio*, vibrio that reduces sulfur compounds

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in Table 5.8.

Biolog

Biolog data are not available for sulfate-reducing bacteria.

Isolation, Enrichment, and Maintenance Procedures

All members of the family *Desulfobulbus* require anoxic media for growth like all other sulfate-reducing bacteria. The media are prepared under specific conditions and the addition of a reductant is required, in general sulfide to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992) and might be modified by other authors as listed in the original descriptions of various taxa.

For enrichment the used electron donor might be highly selective and will have a strong influence on what kind of sulfate-reducing bacteria will grow in the medium. As usual electron acceptor sulfate is used. In some cases thiosulfate or sulfite might enrich for organisms capable to grow by disproportionation of these compounds. Most sulfate-reducing bacteria of this family have been enriched using batch cultures; other options are serial dilution techniques of natural samples. For isolation in general, roll tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992; Kuever et al. 2005c).

For short-term preservation stock cultures can be stored at 2–6 °C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. For long-term storage cultures can be kept freeze dried, at −80 °C or in liquid nitrogen.

Table 5.7

Comparison of selected characteristics of members of the genus *Desulfotalea*

Characteristic	*Desulfotalea psychrophila*	*Desulfotalea arctica*
Type strain	Strain LSv54 = DSM 12343	Strain LSv514 = DSM 12342
Accession number of the 16S rRNA gene sequence of the type strain	AF099062	AF099061
Morphology	Rod	Rod
Cell size (μm)	0.6 × 4.5–7.4	0.7 × 1.6–2.7
Motility	−/+[a]	−
Mol% G+C content	46.8	41.8
Major menaquinone	Mk-6(H_2)	Mk-6
Optimal pH	7.1–7.3	7.1–7.3
Optimal temperature (°C)	10	18
Oxidation of substrate	Incomplete	Incomplete
Optimal NaCl concentration (g/l)	20	20
Growth factor requirement	−	−
Compounds used as electron donors and carbon sources		
H_2/CO_2	+[b]	+[b]
Formate	+[b]	+[b]
Acetate	−	−
Fatty acids	−	−
Ethanol	+	+
Other *n*-alcohols	*n*-propanol, *n*-butanol	Glycerol
Lactate	+	+
Pyruvate	+	+
Fumarate	+	−
Succinate	−	−
Malate	+	−
Others	Alanine, serine	Serine
Fermentative growth	+	+
Disproportionation of reduced sulfur compounds	−	−
Electron acceptors used		
Sulfate	+	+
Sulfite	+	−
Thiosulfate	+	−
Sulfur	−	+[c]
Other	−	−
Literature	Knoblauch et al. (1999), sp. nov.	Knoblauch et al. (1999), sp. nov.

[a]Motile cells may not be observed in cultures at the beginning of growth but might occur later
[b]Only in the presence of acetate
[c]Sulfur is reduced to sulfide but is not coupled to growth

Table 5.8

Selected characteristics of the only member of the genus *Desulfurivibrio* (a table is used for easier comparison with other genera)

Characteristic	*Desulfurivibrio alkaliphilus*
Type strain	Strain AHT2, DSM 19089, UNIQEM U267
Accession number of the 16S rRNA gene sequence of the type strain	EF422413
Morphology	Vibrio
Cell size (μm)	0.4–0.5 × 1.5–2.0
Motility	−
Mol% G+C content	60.3[d]
Major menaquinone	Nr
Optimal pH	9.5
Optimal temperature (°C)	35
Optimal NaCl concentration (g/l)	29.2–58.4
Growth factor requirement	Nr
Oxidation of substrate	Nr
Compounds used as electron donors and carbon sources	
H_2/CO_2	+[a]
Formate	+[a]
Acetate	+
Fatty acids	C_4
Ethanol	+
Other *n*-alcohols	Nr
Lactate	+
Pyruvate	+
Fumarate	Nr
Succinate	+
Malate	+
Others	Isobutyrate
Fermentative growth	−[c]
Disproportionation of reduced sulfur compounds	+[e]
Electron acceptors used	
Sulfate	−
Sulfite	−
Thiosulfate	+
Sulfur	+[b]
Other	Nitrate
Literature	Sorokin et al. (2008), sp. nov.; Sorkin et al. (2008)

[a]Only in the presence of acetate
[b]Here sulfur is provided as (polysulfide mixture)
[c]Personal Communication D.Y. Sorokin
[d]Data taken from the genome sequencing project. In the original description the value was 40.5 mol% for the G+C content
[e]Personal Communication K. Finster

Ecology

Habitat

All members of this family were isolated from sediments or other typical anoxic habitats. The type strain of the genus *Desulfobulbus* strain 1pr3 was isolated using propionate as electron donor from anoxic mud of a freshwater ditch located at Lindhorst close to Hannover, Northern Germany (Widdel 1980). Strain APS1 (type strain of *Desulfobulbus alkaliphilus*) was isolated also with propionate from sediment of a soda lake located in the Altai region, Russia (Sorokin et al. 2012). The source for *Desulfobulbus elongatus* was a mesophilic anaerobic digester fed with vegetable canning waste water (Samain et al. 1984). Again propionate was used for enrichment and isolation. The type strain of *Desulfobulbus japonicus* was obtained also using propionate from anoxic sediment from the Niida river estuary in Sakata harbor located at the coast of the Sea of Japan (38° 54.5' N 139° 50.6' E) (Suzuki et al. 2007). Strain 3pr10, the type strain of *Desulfobulbus marinus*, was isolated from an anoxic mud flat of the Jadebusen area, Lower Saxony, Northern Germany, using propionate (Widdel 1980). With lactate as electron donor, the type strain of *Desulfobulbus mediterraneus*, strain 86FS1, was isolated from sediment obtained from the continental slope off the coast of NE Spain (41° 12.36′ N 2° 50.02′ E) (Sass et al. 2002). The water–oil separation system on the deck of Statfjord A platform in the Norwegian sector of the North Sea was used for isolation of strain M16, the type strain of *Desulfobulbus rhabdoformis*, with propionate as electron donor (Lien et al. 1998).

The type strain of *Desulfocapsa thiozymogenes*, strain Bra2, was isolated with thiosulfate as electron donor and acetate as carbon source from a freshwater lake in Arhus, Denmark (Janssen et al. 1996). In contrast, strain SB164P1, type strain of *Desulfocapsa sulfexigens*, was obtained with elemental sulfur in the presence of ferrihydrite using oxidized surface sediment from an eelgrass-covered mud flat located in the basin of Arcachon, France (Finster et al. 1998).

Strain PerGlyS, type strain of *Desulfofustis glycolicus*, was isolated with glycolate from anoxic marine mud of Rio della Pergola, Venice, Italy (Friedrich and Schink 1995).

With lactate the type strain of *Desulfopila aestuarii*, strain MSL86, was isolated from the same location as *Desulfobulbus japonicus* (sediment from the Niida river estuary in Sakata harbor located at the coast of the Sea of Japan (38° 54.5' N 139° 50.6' E) (Suzuki et al. 2007a). Strain JS_SRB250Lac, type strain of *Desulfopila inferna*, was obtained from sediment of 2.5 m depth from a tidal sand flat in the German Wadden Sea (Janssand; 53° 44177' N 007° 41.970' E) using lactate as electron donor and carbon source (Gittel et al. 2010).

The type strain of *Desulforhopalus vacuolatus*, strain ltk10, was isolated with lactate as electron donor and thiosulfate as electron acceptor from an enrichment inoculated with anoxic mud from Kysing Fjord, Denmark (Isaksen and Teske 1996). Strain S´pore T1, the type strain of *Desulforhophalus vacuolatus*, was isolated with malate and taurine from anoxic mud from a saltwater marsh in the Republic of Singapore (Lie et al. 1999).

Strain Lsv54, type strain of *Desulfotalea psychrophila*, and strain Lsv514, type strain of *Desulfotalea arctica*, were both isolated from permanently cold Arctic sediment from Storfjord (77° 33.0' N 19° 0.50' E), Svalbard, Norway, using lactate as substrate (Knoblauch et al. 1999).

The type strain of *Desulfurivibrio alkaliphilus*, strain AHT 2, was isolated from anoxic sediment of the haloalkaline Wadi Natrun Lake, Egypt, using hydrogen as electron donor and polysulfide as electron acceptor (Sorokin et al. 2008).

Pathogenicity, Clinical Relevance

There is no clinical relevance known.

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6 The Family *Desulfohalobiaceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract
Desulfohalobiaceae, a family within the order *Desulfovibrionales*, embraces the genera *Desulfohalobium*, *Desulfonatronospira*, *Desulfonatronovibrio*, *Desulfothermus*, *Desulfonauticus*, *Desulfovermiculus*, and *Desulfohalophilus*. Besides their 16S rRNA gene sequence phylogeny, all members of the family are defined by a wide range of morphological and chemotaxonomic properties for the delineation of genera and species. Strictly anaerobic, having a respiratory type of metabolism. Fermentative metabolism might occur. Members are either mesophilic or moderately thermophilic sulfate-reducing bacteria. Members of the family are mainly found in marine, highly saline, and haloalkaline habitats. Most described species are chemoorganoheterotroph; some are chemolithoheterotroph or chemolithoautotroph. Most members oxidize organic substrates incompletely to acetate, whereas some perform a complete oxidation of organic substrates.

This contribution is a modified and updated version of a previous family description (Kuever et al. 2005a).

Taxonomy, Historical and Current

Short Description of the Family

De.sul.fo.ha.lo.bi.a′ce.ae. N.L. neut. n. *Desulfohalobium* type genus of the family; -aceae, ending to denote a family; N.L. fem. pl. n. *Desulfohalobiaceae* the Desulfohalobium family (modified from *Bergey's Manual*). The description is an emended version of the description given in *Bergey's Manual*, 2nd edition (Kuever et al. 2005a).

The family belongs to the order *Desulfovibrionales* within the *Deltaproteobacteria*. The family *Desulfohalobiaceae* contains the genera *Desulfohalobium* (Ollivier et al. 1991) which is the type genus of the family (Kuever et al. 2006), *Desulfonatronospira* (Sorokin et al. 2008a), *Desulfonatronovibrio* (Zhilina et al. 1997), *Desulfonauticus* (Audiffrin et al. 2008), *Desulfothermus* (Kuever et al. 2006), and *Desulfovermiculus* (Belyakova et al. 2007). Gram-staining is negative. Morphological forms vary from vibrio- and rod-shaped cells of varying length to spirillum-like forms. Spore formation is absent. Most members are motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory type of metabolism. Some species can grow by fermentation. Simple organic molecules are used as electron donors; some species can use also H_2. Most mesophilic members oxidize organic substrates incompletely to acetate, whereas all members of the thermophilic genus *Desulfothermus* and the mesophilic genus *Desulfovermiculus* perform a complete oxidation of organic substrates.

Most described species are chemoorganoheterotroph; some are chemolithoheterotroph or chemolithoautotroph. Sulfate and thiosulfate are used as electron acceptor and reduced to sulfide; some species can also use sulfite. A few species can grow by disproportionation of reduced sulfur compounds with or without acetate as carbon source.

Members have been isolated from various sources, like marine and halosaline environments and haloalkaline habitats. The origin of members growing at elevated temperature are marine hydrothermally vents or hydrothermally influenced sediments.

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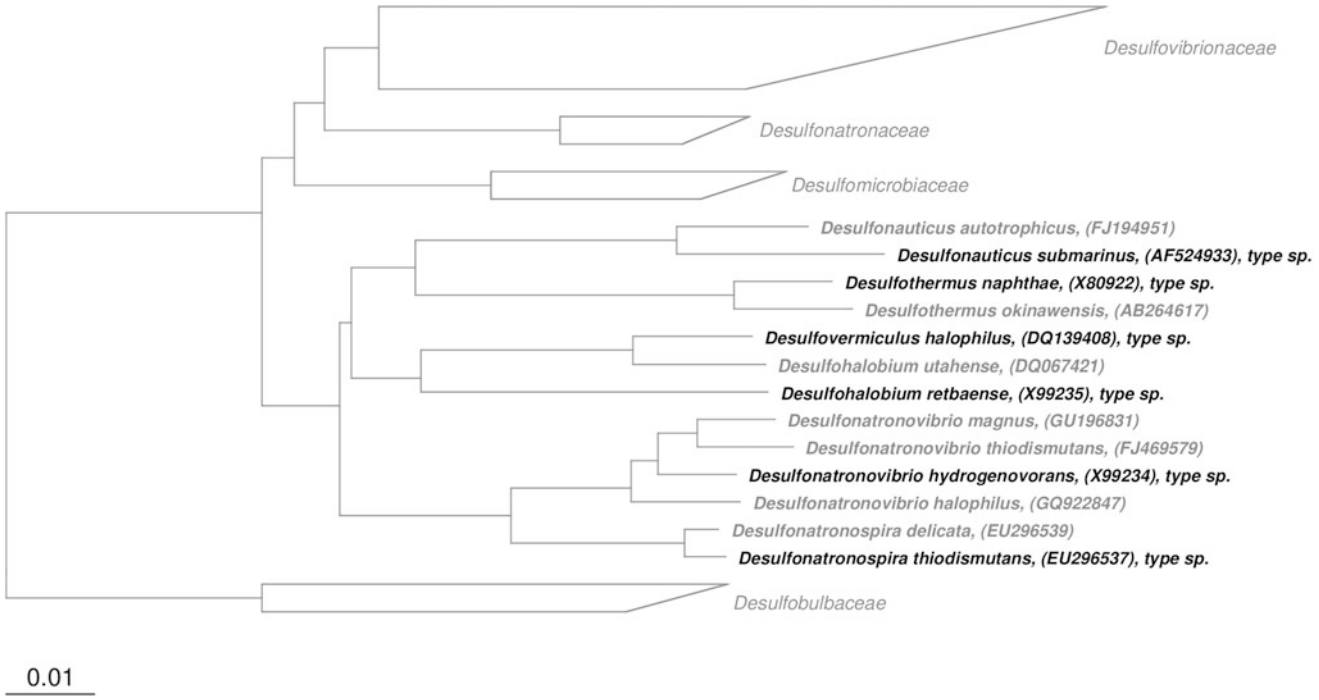

Fig. 6.1
Phylogenetic reconstruction of the family *Desulfohalobiaceae* based on the neighbor-joining algorithm with the Jukes-Cantor correction. Sequence dataset and alignments according to the All-Species Living Tree Project release LTPs108 (Yarza et al. 2010). A 40 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence

Phylogenetic Structure of the Family and Its Genera

The phylogenetic structure of the family and its neighboring families within the order *Desulfovibrionales* is shown on *Fig 6.1*. The borders of the family are primarily based on the phylogenetic tree as framework; other unique properties (Physiology, chemotaxonomic markers) which are present in all members are not found (see *Table 6.1*).

Molecular Analyses

DNA-DNA Hybridization Studies

Are absent.

Bioprinting and Ribotyping

Are absent.

MALDI-TOF

Are absent.

Genome Comparison

The complete genome sequences of two members of this family have been analyzed (Spring et al. 2010). The genome of the type strain of *Desulfohalobium retbaense* DSM 5692 is 2,909,567 bp long (one chromosome and a 45,263 bp plasmid) and contains 2,609 genes with 2,552 coding for proteins and 57 coding for RNA genes. The mol% G+C of the DNA is 57.3. In the genome single copies of the dissimilatory adenylylsulfate reductase (AprBA) linked to the membrane-bound Qmo complex (QmoABC) and the dissimilatory sulfite reduction (DsrAB) linked to the DsrMKJOP complex were found.

The genome analysis of the type strain of *Desulfonatronospira thiodismutans* DSM 19093 remains unfinished, and no publication is available. The size of the genome is 4,11 Mb. It contains 3,913 genes and 3,663 coding for proteins. Again, aprBA is linked to a complete QmoABC complex, and all required genes for the dissimilatory sulfite reduction (dsrAB and dsrMKJOP) are present and found as a single copy.

Phages

Phages might be present, but are not reported so far.

Table 6.1

Morphological and chemotaxonomic characteristics of genera of *Desulfohalobiaceae*

	Desulfohalobium	*Desulfonatronospira*	*Desulfonatronovibrio*	*Desulfonauticus*	*Desulfothermus*	*Desulfovermiculus*
Morphology	Straight or slightly curved rods	Vibrio	Vibrio	Straight or curved rods	Straight or slightly curved rods	Vibrio or spirilla shaped
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+ or −	+	+	+	+	+
Metabolism	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Major fatty acids	$C_{15:0\ iso}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$	$C_{15:0\ iso}$, $C_{16:0}$, $10Me_{16:0}$, $C_{17:1\ iso}$, $C_{17:0\ iso}$	$C_{15:0\ iso}$, $C_{15:0\ ante}$, $C_{16:0\ iso}$, $C_{16:0}$, $C_{17:1\ w8}$, $C_{17:0}$, $C_{18:1\ w7c}$, $C_{18:0}$	$C_{15:0\ iso}$, $C_{15:0\ ante}$, $C_{16:0}$, $C_{17:0\ iso}$, $C_{17:0\ ante}$, $C_{18:0}$	$C_{16:0}$, $12Me_{16:0}$ (only *D. okinawensis*)	nr
Menaquinone	Nr	Nr	Nr	MK-6(H_2) or MK-6(H_4)	nr	MK-7
G + C content (mol%)	51.4–57.3	49.8–50.4	41.8–48.6	34.4–41.3	34.9–37.4	55.2
Substrate oxidation	Incomplete	Incomplete	Incomplete	No organic substrate used except formate	Complete	Complete
CODH activity	Nr	Present	Nr	nr	Present	Present
Typical electron donors	Lactate, pyruvate, ethanol, H_2, formate; one species propionate, butyrate, fumarate, succinate, malate	H_2, formate, lactate, pyruvate, ethanol	H_2, formate, pyruvate	H_2, formate	One species alkanes and long-chain fatty acids; one species formate, short-chain fatty acids, pyruvate, citrate, fumarate, glucose	Lactate, pyruvate, ethanol, H_2, formate, propionate, butyrate, fumarate, succinate, malate
Chemolithoautotrophic growth	No species	All species	Some species	Some species	No species[a]	All species
Fermentative growth	Some species	All species	Some species	No species	No species	All species
Growth by disproportionation of reduced sulfur compounds	Nr	+	+ or −	−	−	nr
Typical electron acceptors	Sulfate, thiosulfate; some species sulfite, sulfur	Sulfate, thiosulfate, sulfite	Sulfate, thiosulfate, sulfite	Sulfate, thiosulfate, sulfite, sulfur	Sulfate, thiosulfate	Sulfate, thiosulfate, sulfite, sulfur
Optimal growth temperature (°C)	37–40	30	30–37	45–58	50–65	37
Habitat	Hypersaline lake	Highly alkaline and saline soda lakes	Highly alkaline and saline soda lakes	Hydrothermal vent or oil field brine	hydrothermal vent	Oil field

[a]One species can grow autotrophically on formate without acetate

Phenotypic Analyses

The main features of all members of the family *Desulfohalobiaceae* are listed in Table 6.1 and the tables featuring the genera (Table 6.2).

Desulfohalobium Ollivier et al. 1991, 78[VP]

De.sul.fo.ha.lo´bi.um. L. prep. de, from; L. n. sulfur, sulfur; N.L. pref. desulfo-, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); Gr. n. hals halos, salt; Gr. n. bios, life; N.L. adj. halobius -a -um, living on salt; N.L. neut. n. *Desulfohalobium*, a sulfate-reducing, salt-requiring, rod-shaped bacterium.

The genus *Desulfohalobium* contains two described species. Their chemotaxonomic and physiological properties are listed in Table 6.2.

Taxonomic comment: The phylogenetic tree (Fig. 6.1) clearly indicates that *Desulfohalobium utahense* should not be regarded as a member of this genus but of the genus *Desulfovermiculus*. This has already been indicated by Spring and coworkers (2010). The problem might have been publication of both descriptions to the same time. Confirmation of the misclassification might be possible by phylogenetic analyses based on dsrAB and aprBA genes, but so far no data for *Desulfovermiculus halophilus* are available.

Desulfonatronospira Sorokin et al. 2008, 1511[VP] (Effective Publication: Sorokin et al. 2008, 1451)

De.sul.fo.na.tro.no.spi´ra. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); N.L. n. *natron* (arbitrarily derived from the Arabic n. *natrun* or *natron*) soda, sodium carbonate; N.L. pref. *natrono-*, pertaining to soda; L. fem. n. *spira*, a spire; N.L. fem. n. *Desulfonatronospira*, desulfurizing soda-loving spirillum.

The genus *Desulfonatronospira* contains two described species. Their chemotaxonomic and physiological properties are listed in Table 6.3.

Desulfonatronovibrio Zhilina et al. 1997, 149[VP]

De.sul.fo.na.tro.no.vi´bri.o. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); N.L. n. *natron* (arbitrarily derived from the Arabic n. *natrun* or *natron*) soda, sodium carbonate; N.L. pref. *natrono-*, pertaining to soda; L. v. *vibro*, 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. *Desulfonatronovibrio*, sulfate-reducing vibrio from soda environment.

Table 6.2
Comparison of selected characteristics of members of the genus *Desulfohalobium*

Characteristic	*Desulfohalobium retbaense*	*Desulfohalobium utahense*
Type strain	Strain HR100, ATCC 49708, DSM 5692, JCM 16813	Strain EtOH3, DSM 17720, VKM B-2384
Accession number of the 16S rRNA gene sequence of the type strain	CP001734	DQ067421
Morphology	Curved rod	Oval-shaped rod
Cell size (μm)	0.7–0.9 × 1.0–3.0	1.0–1.2 × 2.5–3.0
Motility	+	–
Mol% G + C content	57.3	51.4
Major menaquinone	Nr	Nr
Optimal pH	6.5–7.0	6.8
Optimal temperature (°C)	37–40	37
Optimal NaCl concentration (g/l)	100	80–100
Growth factor requirement	Yeast extract	–
Oxidation of substrate	Incomplete	Incomplete
Compounds used as electron donors and carbon sources		
H_2/CO_2	+[a]	+[a]
Formate	+[a]	+[a]
Acetate	–	–
Fatty acids	–	C_3–C_4
Ethanol	+	
Other *n*-alcohols	Nr	C_1, C_3–C_5
Lactate	+	+
Pyruvate	+	+
Fumarate	–	+
Succinate	–	+
Malate	–	+
Other	–	Casamino acids, yeast extract
Fermentative growth	+	–
Disproportionation of reduced sulfur compounds	Nr	Nr
Electron acceptors used		
Sulfate	+	+
Sulfite	+	–
Thiosulfate	+	+
Sulfur[b]	+	–
Other	nr	nr
Literature	Ollivier et al. (1991), gen. nov; sp. nov.	Fredlund Jacobsen et al. (2006),sp. nov.

[a]Only in the presence of acetate

[b]In most cases sulfur might be reduced to sulfide but is not coupled to growth

Table 6.3
Comparison of selected characteristics of members of the genus *Desulfonatronospira*

Characteristic	*Desulfonatronospira thiodismutans*	*Desulfonatronospira delicata*
Type strain	Strain ASO3-1, DSM 19093, UNIQEM U234	Strain AHT 6, DSM19491, UNIQEM U275
Accession number of the 16S rRNA gene sequence of the type strain	EU296537	EU296539
Morphology	Vibrio or spirillum	Oval-shaped rod
Cell size (μm)	0.6–0.8 × 2.0–30.0	1.0–1.2 × 2.5–3.0
Motility	+	–
Mol% G + C content	49.8–50.4	51.4
Major menaquinone	nr	nr
Optimal pH	10.0	6.8
Optimal temperature (°C)	30	30
Optimal NaCl concentration (g/l)	117–146	58
Growth factor requirement	Vitamins	Vitamins
Oxidation of substrate	Incomplete	Incomplete
Compounds used as electron donors and carbon sources		
H_2/CO_2	+[a]	+[a]
Formate	+[a]	+[a]
Acetate	–	–
Fatty acids	nr	nr
Ethanol	+	+
Other *n*-alcohols	Butanol	nr
Lactate	+	+
Pyruvate	+	+
Fumarate	nr	nr
Succinate	nr	nr
Malate	nr	nr
Others	nr	nr
Fermentative growth	+	+
Disproportionation of reduced sulfur compounds	+	+
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	+
Sulfur[b]	+	–
Other	nr	nr
Literature	Sorokin et al. 2008a gen. nov., sp. nov.; Sorokin et al. 2008b	Sorokin et al. 2008a gen. nov., sp. nov.; Sorokin et al. 2008b

[a]Only in the presence of acetate
[b]In most cases sulfur might be reduced to sulfide but is not coupled to growth

The genus *Desulfonatronovibrio* contains four described species. Their chemotaxonomic and physiological properties are listed in *Table 6.4*.

Desulfonauticus Audiffrin et al. 2003, 1589[VP]

De.sul.fo.na.tro.nau´ti.cus. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. adj. *nauticus*, nautical; N.L. masc. n. *Desulfonauticus*, a marine sulfate reducer.

The genus *Desulfonauticus* contains two described species. Their chemotaxonomic and physiological properties are listed in *Table 6.5*.

Desulfothermus Kuever et al. 2006, 2[VP] (Effective Publication: Kuever et al. 2005b, 955)

De.sul.fo.ther´mus. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); Gr. adj. *thermos*, hot; N.L. masc. n. *Desulfothermus*, sulfate reducer living in hot places.

The genus *Desulfothermus* contains two described species. Their chemotaxonomic and physiological properties are listed in *Table 6.6*.

Desulfovermiculus Belyakova et al. 2007, 1371 (Effective Publication: Belyakova et al. 2006, 169)

De.sul.fo.ver.mi´cu.lus. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. masc. n. *vermiculus*, a little worm; N.L. masc. n. *Desulfovermiculus*, vermiform sulfate-reducing bacterium.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in *Table 6.7*.

Biolog

Biolog data are not available for sulfate-reducing bacteria.

Isolation, Enrichment, and Maintenance Procedures

All members of the family *Desulfohalobiaceae* require anoxic media for growth like all other sulfate-reducing bacteria. The media are prepared under specific conditions and the addition of a reductant is required, in general sulfide, to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992) and might be modified by other authors as listed in the original descriptions of various taxa. Typical modifications are simplifications, higher salt concentrations (e.g., *Desulfohalobium retbaense* medium, Ollivier et al. 1991),

Table 6.4

Comparison of selected characteristics of members of the genus *Desulfonatronovibrio*

Characteristic	*Desulfonatronovibrio hydrogenovorans*	*Desulfonatronovibrio halophilus*	*Desulfonatronovibrio magnus*	*Desulfonatronovibrio thiodismutans*
Type strain	Strain Z-7935, DSM 9292.	Strain HTR1, DSM 24312, UNIQEM U802	Strain AHT22, DSM 24400, UNIQEM U844	Strain AHT9, DSM 21540, UNIQEM U754
Accession number of the 16S rRNA gene sequence of the type strain	X99234	GQ922847	GU196831	FJ469579
Morphology	Vibrio	Vibrio	Vibrio	Vibrio
Cell size (μm)	0.5 × 1.5–2.0	0.4 × 1.0–2.0	0.8–1.0 × 2.0–3.0	0.3–0.4 × 1.0–3.0
Motility	+	+	+	+
Mol% G + C content	48.6	45.7	43.0	41.8–42.9
Major menaquinone	Nr	Nr	Nr	Nr
Optimal pH	9.6	8.0	10	9.5–10
Optimal temperature (°C)	37	37	30	30
Optimal NaCl concentration (g/l)	30	29	23	23–35
Growth factor requirement	vitamins	–	Vitamins	Vitamins
Oxidation of substrate	No organic substrate used except formate	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources				
H_2/CO_2	+[a]	+[a]	+[a]	+
Formate	+[a]	+[a]	+[a]	+
Acetate	–	Nr	Nr	Nr
Fatty acids	–	Nr	Nr	Nr
Ethanol	–	Nr	Nr	Nr
Other *n*-alcohols	–	Nr	Nr	Nr
Lactate	–	Nr	Nr	Nr
Pyruvate	–	+	+	+
Fumarate	–	Nr	Nr	Nr
Succinate	–	Nr	Nr	Nr
Malate	–	Nr	Nr	Nr
Others	–	Nr	Nr	Nr
Fermentative growth	–	+	+	+
Disproportionation of reduced sulfur compounds	Nr	–	+	+
Electron acceptors used				
Sulfate	+	+		+
Sulfite	+	+		+
Thiosulfate	+	+		+
Sulfur[b]	–	–		–
Other	Nr	Nr		Nr
Literature	Zhilina et al. (1997), gen. nov; sp. nov.	Sorokin et al. (2012a), sp. nov.; Sorokin et al. (2012b)	Sorokin et al. (2011a), sp. nov.; Sorokin et al. (2011b)	Sorokin et al. (2011a), sp. nov.; Sorokin et al. (2011b)

[a]Only in the presence of acetate

[b]In most cases sulfur might be reduced to sulfide but is not coupled to growth

or media for cultivation of haloalkaline organisms (e.g., see Zhilina et al. 1997; Sorokin et al. 2008).

For enrichment the used electron donor might be highly selective and will have a strong influence on what kind of sulfate-reducing bacteria will grow in the medium. As usual electron acceptor sulfate is used. In some cases thiosulfate or sulfite might enrich for organisms capable to grow by disproportionation of these compounds. Most sulfate-reducing

Table 6.5

Comparison of selected characteristics of members of the genus *Desulfonauticus*

Characteristic	*Desulfonauticus submarinus*	*Desulfonauticus autotrophicus*
Type strain	Strain 6 N, CIP 107713, DSM 15269	Strain TeSt, DSM 4206, JCM 13028
Accession number of the 16S rRNA gene sequence of the type strain	AF524933	FJ194951
Morphology	Curved rod	Straight or slightly curved rod
Cell size (μm)	0.35–0.5 × 5.0–6.0	0.6 × 1.2–4.0
Motility	+	–
Mol% G + C content	34.4	41.3
Major menaquinone	Mk-6(H2)	Mk-6(H2) and MK-6(H4)
Optimal pH	7.0	7.8
Optimal temperature (°C)	45	58
Optimal NaCl concentration (g/l)	20[c]	25
Growth factor requirement	Yeast extract	–
Oxidation of substrate	No organic substrate used	No organic substrate used
Compounds used as electron donors and carbon sources		
H_2/CO_2	+[a]	+
Formate	+[a]	+
Acetate	–	–
Fatty acids	–	–
Ethanol	–	
Other *n*-alcohols	–	–
Lactate	–	–
Pyruvate	Nr	–
Fumarate	–	–
Succinate	–	–
Malate	–	–
Others	–	–
Fermentative growth	–	–
Disproportionation of reduced sulfur compounds	Nr	–
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	+
Sulfur[b]	+	+
Other	nr	–
Literature	Audiffrin et al. (2003), gen. nov., sp. nov.; Mayilraj et al. (2009), emend. desc.	Mayilraj et al. (2009), sp. nov.

[a]Only in the presence of acetate

[b]In most cases sulfur might be reduced to sulfide but is not coupled to growth

[c]According to the original description the strain can also grow without NaCl

Table 6.6

Comparison of selected characteristics of members of the genus *Desulfothermus*

Characteristic	*Desulfothermus naphthae*	*Desulfothermus okinawensis*
Type strain	Strain TD3, DSM 13418, JCM 12298	Strain TFISO9, DSM 17375, JCM 13304
Accession number of the 16S rRNA gene sequence of the type strain	X80922	AB264217
Morphology	Curved rod	Rod
Cell size (μm)	0.8–1.0 × 2.5–3.5	0.6–0.9–1.2 × 2.5–5.0
Motility	(+)	+
Mol% G + C content	37.4	34.9
Major menaquinone	nr	nr
Optimal pH	6.5–6.8	5.9–6.4
Optimal temperature (°C)	60–65	50
Oxidation of substrate	Complete	Complete
Optimal NaCl concentration (g/l)	21	25
Growth factor requirement	–	–
Compounds used as electron donors and carbon sources		
H_2/CO_2	–	–
Formate	–	+
Acetate	–	+
Fatty acids	C_6–C_{18}	C_3, C_5
Ethanol	–	+
Other *n*-alcohols	nr	Glycerol
Lactate	–	+
Pyruvate	–	+
Fumarate	–	+
Succinate	–	+
Malate	–	+
Others	Alkanes (C_6–C_{11})	Isobutyrate, isovalerate, Citrate, tartrate, glucose, yeast extract
Fermentative growth	+	–
Disproportionation of reduced sulfur compounds	nr	nr
Electron acceptors used		
Sulfate	+	+
Sulfite	–	–
Thiosulfate	+	+
Sulfur[a]	–	–

Table 6.6 (continued)

Characteristic	*Desulfothermus naphthae*	*Desulfothermus okinawensis*
Other	nr	nr
Literature	Kuever et al. (2006), gen. nov., sp. nov.; Kuever et al. (2005b)	Nunoura et al. (2007), sp. nov.

[a]In most cases sulfur might be reduced to sulfide but is not coupled to growth

bacteria of this family have been enriched using batch cultures; other options are serial dilution techniques of natural samples. For isolation in general, roll-tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992; Kuever et al. 2005c).

For short-term preservation, stock cultures can be stored at 2–6 °C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. For long-term storage, cultures can be kept freeze-dried, at −80 °C or in liquid nitrogen.

Ecology

Habitat

All members of this family were isolated from hypersaline and haloalkaline lakes or marine systems influenced by hydrothermal activity. The type strain of the genus *Desulfohalobium* strain HR_{100} was isolated from sediment of the Lake Retba (Senegal) (Ollivier et al. 1991). Together with the misclassified *Desulfohalobium utahense* (should be reclassified as a member of the genus *Desulfovermiculus*, see *Fig 6.1*) which was isolated from sediment of the Great Salt Lake (Utah, USA), they are the most halophilic sulfate-reducing bacteria described so far (Fredlund Jacobsen et al. 2006). Very close to both with respect to salinity and its phylogenetic position in the family tree (see *Fig 6.1*) is *Desulfovermiculus halophilus* which was isolated from brine formation water of an oil field (location not described) (Belyakova et al. 2006). Typical habitats for *Desulfonatronospira* seem to be haloalkaline lakes. *Desulfonatronospira thiodismutans* was isolated from sediment of Kulunda Steppe Lake (Altai, Russia), whereas the source for the *Desulfonatronospira delicata* was sediment of lakes of the Wadi El Natrun (Egypt) (Sorokin et al. 2008). Soda Lakes are the typical environment for members of the genus *Desulfonatronovibrio*. The type species *Desulfonatronovibrio hydrogenovorans* was isolated from sediment of Lake Magadi (East Africa Rift, Kenya) (Zhilina et al. 1997). The type strains of *Desulfonatronovibrio thiodismutans* and *Desulfonatronovibrio magnus* were isolated from the lake Tanatar-5 in the Kulunda Steppe (Altai, Russia). Other strain of *Desulfonatronovibrio thiodismutans* originated from other lakes of the same area and Owens Lake (California, USA) (Sorokin et al. 2012). Less alkaline high saline lakes of the Kulunda Steppe Area (Altai, Russia) were used for the isolation of *Desulfonatronovibrio halophilus* (Sorokin et al. 2012).

Table 6.7
Selected characteristics of the only member of the genus *Desulfovermiculus* (a table is used for easier comparison with other genera)

Characteristic	*Desulfovermiculus halophilus*
Type strain	Strain 11–6, DSM 18834, VKM B-2364
Accession number of the 16S rRNA gene sequence of the type strain	DQ130408
Morphology	Vibrio or spirilla
Cell size (μm)	0.5–0.6 × 1.0–20.0
Motility	+
Mol% G + C content	55.2
Major menaquinone	MK-7
Optimal pH	7.2
Optimal temperature (°C)	37
Optimal NaCl concentration (g/l)	80–100
Growth factor requirement	–
Oxidation of substrate	Complete
Compounds used as electron donors and carbon sources	
H_2/CO_2	+
Formate	+
Acetate	–
Fatty acids	C_3–C_4
Ethanol	+
Other *n*-alcohols	–
Lactate	+
Pyruvate	+
Fumarate	+
Succinate	+
Malate	+
Others	Alanine, crotonate
Fermentative growth	+
Disproportionation of reduced sulfur compounds	nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	+
Sulfur[a]	+
Other	nr
Literature	Belyakova et al. (2007), gen. nov., sp. nov.; Belyakova et al. (2006)

[a]In most cases sulfur might be reduced to sulfide but is not coupled to growth

The matrix of the tubeworms *Riftia* and *Alvinella* found at a deep-sea hydrothermal vent (13°N East Pacific Rise, 2,900 m depth) was the source for the isolation of *Desulfonauticus submarinus* (Audiffrin et al. 2003). The other member of this genus, *Desulfonauticus autotrophicus*, was isolated from brine taken from an oil–water separator of an oil field located in Northern Germany (close to Hamburg) (Cord-Ruwisch et al. 1987; Mayilraj et al. 2009). Elevated temperature habitats are also typical for the genus *Desulfothermus*. The type species, *Desulfothermus naphthae*, was isolated from sediment of a natural oil seepage in the Guaymas Basin (Gulf of Mexico) (Rueter et al. 1994). The other species, *Desulfothermus okinawensis*, was isolated from a black smoker chimney at the Yonaguni Knoll IV hydrothermal vent field in the South Okinawa Trough (24° 50.938′ N, 122° 42.020′ E) (Nunoura et al. 2007).

Pathogenicity, Clinical Relevance

There is no clinical relevance known.

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7 The Family *Desulfomicrobiaceae*

Jan Kuever[1] · *Alexander Galushko*[2,3]
[1]Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany
[2]Department of Plant Physiology and Biotechnology, Tomsk State University, Tomsk, Russia
[3]Department of Microbial Ecology, Vienna University, Vienna, Austria

Abstract
Desulfomicrobiaceae, a family within the order *Desulfovibrionales*, embraces a single genus *Desulfomicrobium*. Besides their 16S rRNA gene sequence phylogeny, all members of the family are defined by a wide range of morphological and chemotaxonomic properties for the delineation of genera and species. Strictly anaerobic, having a respiratory type or fermentative type of metabolism. Members are either mesophilic or moderately thermophilic sulfate-reducing bacteria. Members of the family are found in various, predominantly marine habitats. All described species are chemoorganoheterotroph and chemolithoheterotroph with the exception of *Desulfomicrobium macestii* and *Desulfomicrobium thermophilum* which are described to be chemolithoautotroph. All members oxidize organic substrates incompletely to acetate with sulfate as electron acceptor, except some substrates which are exclusively fermented.

This contribution is a modified and updated version of a previous family description (Kuever et al. 2005a).

Taxonomy, Historical and Current

Short Description of the Family

De.sul.fo.mi.cro.bi.a'ce.ae. N.L. neut. n. *Desulfomicrobium* type genus of the family; -aceae, ending to denote a family; N.L. fem. pl. n. *Desulfomicrobiaceae* the *Desulfomicrobium* family (Modified from *Bergey's Manual*). The description is an emended version of the description given in *Bergey´s Manual*, 2nd edition (Kuever et al. 2005a).

The family belongs to the order *Desulfovibrionales* within the *delta-Proteobacteria*. The family *Desulfomicrobiaceae* contains a single genus *Desulfomicrobium* (Rozanova et al. 1994) which is the type genus of the family (Kuever et al. 2006). Gram-staining is negative. Morphological forms are always rod-shaped cells. Spore formation is absent. Most members are motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory or fermentative type of metabolism. Simple organic molecules and H_2 are used as electron donors. All members oxidize organic substrates incompletely to acetate. Most members are mesophilic; one member is moderately thermophilic. All described species are chemoorganoheterotroph and nearly all are chemolithoheterotroph with the exception of *Desulfomicrobium macestii* and *Desulfomicrobium thermophilum* that are described to grow as autotrophs on H_2 and CO_2 (Hippe et al. 2003; Thevenieau et al. 2007). Sulfate and thiosulfate are used as electron acceptor and reduced to sulfide; some species can also use sulfite. All species can grow well by fermentation of fumarate or malate. Growth on these substrates is not coupled to sulfate reduction. Members have been isolated from various sources, like freshwater, brackish water, and marine habitats. All species have very high similarity value for the 16S rRNA and DsrAB sequences (Dias et al. 2008).

Phylogenetic Structure of the Family

The phylogenetic structure of the family and its neighboring families within the order *Desulfovibrionales* is shown on ❯ *Fig. 7.1*. The borders of the family are primarily based on the phylogenetic tree as framework and their unique properties (physiology, chemotaxonomic markers) which are present in all members (see ❯ *Table 7.1*).

E. Rosenberg et al. (eds.), *The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria*, DOI 10.1007/978-3-642-39044-9_310,

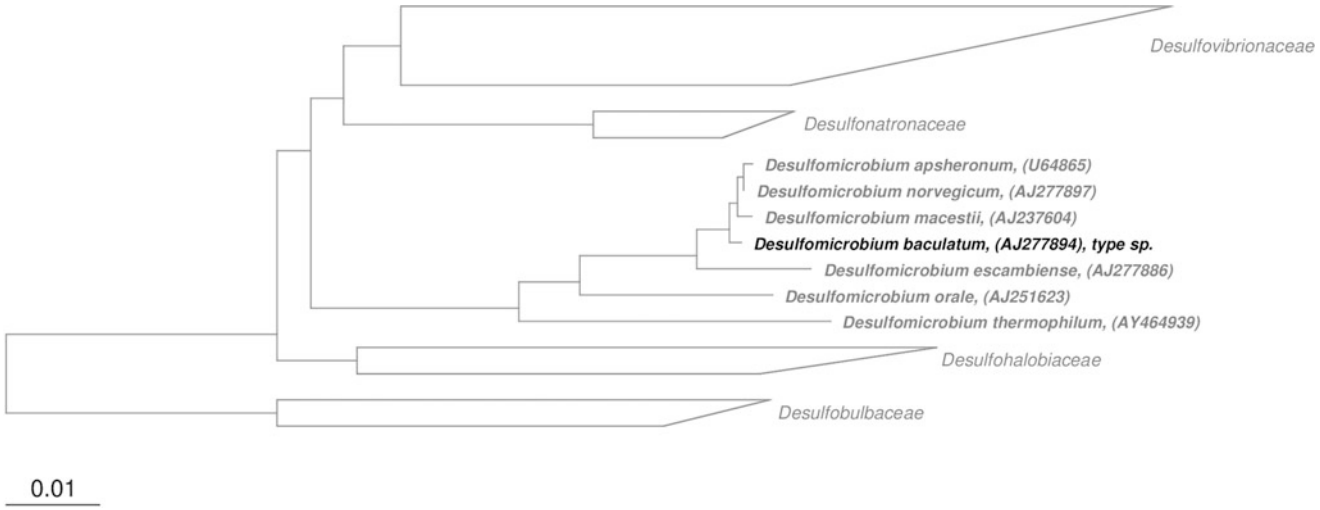

Fig. 7.1

Phylogenetic reconstruction of the family *Desulfomicrobiaceae* 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). A 40 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence

Molecular Analyses

DNA-DNA Hybridization Studies

DNA-DNA hybridization study was done with four strains described till 1997 (Sharak Genthner et al. 1997). All of them had very low level of DNA-DNA similarity (11–27 %) and even lower value to *Desulfovibrio desulfuricans* proving that they represented separate species and are very distantly related to *Desulfovibrio*. At least one member of a family, *Desulfomicrobium macestii*, showed much DNA similarity to *Desulfomicrobium norvegicum*, very close to the recommended threshold for species differentiation (Hippe et al. 2003).

Bioprinting and Ribotyping

Are absent.

MALDI-TOF

Are absent.

Genome Comparison

The complete genome sequence of one member of this family has been analyzed (Copeland et al. 2009). The genome of the type strain of *Desulfomicrobium baculatum* strain X (DSM 4028) is 3,942,657 bp long and contains 3,565 genes with 3,494 coding for proteins and 72 coding for RNA genes. The mol% G + C of the DNA is 58.7. In the genome, single copies of the dissimilatory adenylylsulfate reductase (aprBA) linked to the membrane-bound Qmo complex (QmoABC) and the dissimilatory sulfite reduction (DsrAB) linked to the DsrMKJOP complex were found. Two 16S rRNA gene sequences in the genome were almost identical with the difference of 1 bp.

Phages

Phages might be present, but are not reported so far.

Phenotypic Analyses

The main features of all members of the family *Desulfomicrobiaceae* are listed in *Table 7.1*.

All members are gram-negative rods. Nearly all are motile. All are strictly anaerobic, having a respiratory or fermentative metabolism.

The cellular fatty acid pattern of members of the genus *Desulfomicrobium* was found to be dominated by anteiso- (ai) and isomethyl-branched unsaturated and saturated fatty acids and varies depending on the species.

Typical dominant fatty acids found are $C_{15:0\ iso}$, $C_{16:0\ iso}$, $C_{17:0}$, $C_{17:1\ iso}$, C17:1 $\omega 10_c$, and $C_{18:0}$ (Vainshtein et al. 1992; Tourova et al. 1998; Copeland et al. 2009).

The dominant respiratory quinone seems to be MK-6, but data for most members are lacking (Collins and Widdel 1986; Hippe et al. 2003). Organic substrate oxidation is always incomplete. Typical electron donors for sulfate reduction are H_2, formate, lactate, and ethanol. Fumarate, malate, and pyruvate are fermented to succinate and acetate as end products without sulfate reduction. Although some descriptions claim

Table 7.1

Comparison of selected characteristics of members of the genus *Desulfomicrobium*

Characteristic	*D. baculatum*	*D. apsheronum*	*D. escambiense*	*D. macestii*	*D. norvegicum*	*D. orale*	*D. thermophilum*
Type strain	Strain X, DSM 4028, VKM B-1378	Strain 1105, DSM 5918, VKM B-1804	Strain ESC 1, ATCC 51164, DSM 10707	Strain M-9, DSM 4194, VKM B-1598	Strain Norway 4, DSM 1741, NCIMB 8310	Strain NY678, DSM 12838, JCM 17150	Strain P6-2, DSM 16697, CCUG 49732
Accession number of the 16S rRNA gene sequence of the type strain	CP001629	U64865	AJ277886	AJ237604	AJ277897	AJ251623	AY464939
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cell size (μm)	0.5–0.7 × 0.9–1.9	0.7–0.9 × 1.4–2.9	0.5 × 1.7–2.2	0.7 × 1.9–2.7	0.5–1.0 × 3.0–5.0	0.6–0.8 × 1.8–3.0	0.7 × 2.0–3.0
Motility	+	+	Nr	+	+	+/−	+
Mol% G + C content	56.8	52.5	63.6[d]	58.0	56.8	59.7	58.7
Major menaquinone	Nr	Nr	Nr	MK-6	MK-6	Nr	Nr
Optimal pH	7.2	7.2	Nr	7.2	Nr	Nr	6.6
Optimal temperature (°C)	28–37	25–30	28[d]	35	25–30	37	55
Optimal NaCl concentration (g/l)	10	10	5[d]	13	23 [f]	Nr	5
Growth factor requirement	−	−	−	−	−	Nr	−
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	+[a]	+[a]	+[e]	+[a]	+[a]	+[e]
Formate	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]	Nr
Acetate	−	−	−	−	−	−	−
Fatty acids	−	−	Nr	−	−	−	−
Ethanol	−	+	+	+	+	+	+
Other *n*-alcohols	−	−	−	Propanol, butanol, 1,2-pronediol	−	Nr	Propanol
Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	+[c]	+[c]	−	+	+[c]	Nr	+[c]
Succinate	−	−	−	Nr	−	Nr	−
Malate	+[c]	+	−	+	+[c]	Nr	Nr
Other	−	−	−	−	−	Nr	−
Fermentative growth	+	+	+	+	+	+	+
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	+	Nr	+	+	Nr	+

Table 7.1 (continued)

Characteristic	*D. baculatum*	*D. apsheronum*	*D. escambiense*	*D. macestii*	*D. norvegicum*	*D. orale*	*D. thermophilum*
Thiosulfate	+	+	+	+	+	Nr	+
Sulfur[b]	–	–	Nr	Nr	+	Nr	–
Other	–	–	–	Nr	–	Nr	–
Literature	Rozanova and Nazina (1976); Rozanova et al. (1988), comb. nov.; gen. nov., sp. nov. Sharak Genthner et al. (1997)	Rozanova et al. (1998), sp. nov.; Galouchko and Rozanova (1996); Sharak Genthner et al. (1997)	Sharak Genthner et al. (1994, 1997), sp. nov.	Gogotova and Vainshtein (1989); Hippe et al. (2003), comb. sp. nov.,	Sharak Genthner et al. (1997), comb. nov.; Collins and Widdel (1986); Biebl and Pfennig 1977; Miller and Saleh, 1964	Langendijk et al. (2001), sp. nov.	Thevenieau et al. (2007a, b), sp. nov.

[a]Only in the presence of acetate or yeast extract
[b]In some cases sulfur might be reduced to sulfide but is not coupled to growth
[c]Only by fermentation, not coupled to sulfate reduction
[d]Data from Dias et al. 2008; the mol% G + C in the original description was 59.9
[e]In the original description, autotrophic growth is listed. This remains questionable because the medium contains 0.1 % yeast extract. It is very likely that these strains are also chemolithoheterotroph
[f]Data from Miller Saleh (1962)

autotrophic growth (Hippe et al. 2003; Thevenieau et al. 2007), a chemolithoheterotrophic mode might be common for all species. The addition of substantial amounts of yeast extract in the media used for *Desulfomicrobium macestii* and *Desulfomicrobium thermophilum* might imply heterotrophic growth. A real proof for autotrophic growth of these organisms is lacking. Growth by disproportionation of reduced sulfur compounds was not reported for the type strains. Typical electron acceptors for growth are sulfate, sulfite, and thiosulfate.

Biochemical studies of activity and UV/visible absorption of sulfite-reducing enzymes showed the presence of desulforubidin-type protein in two species of the genus (*Desulfomicrobium baculatum* and *Desulfomicrobium norvegicum*) (Lee et al. 1973; Moura et al. 1988; DerVartanian 1994). The biochemical type of sulfite reductases of other species of the family is not known; however, it was generalized that desulfoviridin was absent in all known species (Thevenieau et al. 2007; Dias et al. 2008). In comparison to desulfoviridin with two catalytically active sirohemes and two inactive sirohydrochlorins, desulforubidin has four sirohemes, but only two of them are active (Oliveira et al. 2011).

For most members, the optimal growth temperature is between 30 °C and 37 °C; the only exception is *D. thermophilum* which grows at 55 °C.

Strains belonging to this genus have been isolated from various sources including moistened manganese ore dumps, oil-bearing strata water, harbor and other marine sediments, subsurface waters, terrestrial sulfur springs, and the oral cavity of human beings.

Desulfomicrobium Rozanova et al. 1994, 370[VP] (Effective Publication Rozanova et al. 1988, 518)

De.sul.fo.mi.cro´bi.um. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing prokaryote); N.L. neut. n. *microbium* (from Gr. adj. *mikros*, small and Gr. n. *bios*, life), a microbe; N.L. neut. n. *Desulfomicrobium*, sulfate-reducing, small life.

The genus *Desulfomicrobium* contains seven validly described species. Their chemotaxonomic and physiological properties are listed in *Table 7.1*.

Taxonomic Comment: The phylogenetic tree (*Fig. 7.1*) clearly indicates that *Desulfomicrobium thermophilum* should not be regarded as a member of this genus but of a new genus belonging in the same family. The 16S rRNA gene sequence identity is much lower to the other members of this genus. Similar to the situation in other families and genera (e.g., *Desulfovibrio*), the rules for classification or definition of a new genus are highly inconsistent and variable. Here the sequence identity lays between 92 % and 94 % to the other *Desulfomicrobium* spp. This together with its much higher growth temperature would justify the status of a new genus for this species. The status of a new genus is also supported by the aprBA phylogeny for a highly similar organism called strain Hobo which would be member of the same species (Meyer and Kuever 2007). The similar fatty acid profile and substrate pattern should not be used as argument for the same genus because there are many examples where this is identical, and still separate genera are described.

Desulfomicrobium orale which has a sequence identity of 94–95 % for the 16S rRNA gene to all members of this genus except *D. thermophilum* should be included in this genus although it represents an isolated branch and might also be a separate genus (Fig. 7.1). This is also supported by DsrAB phylogeny (Dias et al. 2008).

In addition to the validly published species shown in Table 7.1, there are three other species described. These are *Desulfomicrobium hypogeium* (Krumholz et al. 1998), *Desulfomicrobium aestuarii* (Dias et al. 2008), and *Desulfomicrobium salsuginis* (Dias et al. 2008). For *Desulfomicrobium baculatum* several different strains from Biebl and Pfennig (1977) are deposited at the DSMZ (Braunschweig, Germany). It was suggested to reclassify strain New Jersey of *Desulfovibrio desulfuricans* subspecies *desulfuricans* (NCIMB 8313) as a strain of *Desulfomicrobium baculatum* (Pereira et al. 1996). All of them are not validated and therefore not included in Table 7.1.

Biolog

Biolog data are not available for sulfate-reducing bacteria.

Isolation, Enrichment, and Maintenance Procedures

All members of the family *Desulfomicrobiaceae* require anoxic media for growth like all other sulfate-reducing bacteria. The media are prepared under specific conditions, and the addition of a reductant is required, in general sulfide to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992) and might be modified by other authors as listed in the original descriptions of various taxa.

For enrichment, the used electron donor should be highly selective and would have a strong influence on what kind of sulfate-reducing bacteria will grow in the medium. There are no very selective substrates for *Desulfomicrobium* species known. One might succeed using such electron donors as lactate, ethanol, formate, and H_2; the last two compounds should be used in combination with small amount of acetate (1–2 mM). As usual, electron acceptor sulfate is used. Fumarate or malate might be used for specific enrichment without sulfate. However in all cases, the environmental sample should already contain significant amount of cells of *Desulfomicrobium* spp. but not of *Desulfovibrio* spp. that have quite similar nutritional requirements.

Most sulfate-reducing bacteria of this family have been enriched using batch cultures; other options are serial dilution techniques of natural samples. For isolation in general, roll tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992; Kuever et al. 2005b).

For short-term preservation, stock cultures can be stored at 2–6 °C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. For long-term storage, cultures can be kept freeze-dried, at −80 °C, or in liquid nitrogen.

Ecology

Habitat

Members of this family were isolated from various habitats including freshwater, brackish, and marine systems. The type strain of the genus *Desulfomicrobium* strain X was isolated from water-saturated manganese carbonate ore and described as *Desulfovibrio baculatum* (Rozanova and Nazina 1976). Strain 1105, the type strain of *Desulfomicrobium apsheronum*, was isolated from syntrophic sulfidogenic acetate-oxidizing coculture enriched from strata water of an oil-bearing deposit located at the Apsheronum Peninsula (Azerbaijan) (Galouchko and Rozanova 1996). The type strain of *D. esambiense* was isolated from freshwater sediment of the Escambia River, Florida, USA, whereas the type strain of *D. norvegicum* which was first also classified as a *Desulfovibrio* sp. originates from marine water of the Oslo harbor (Norway). *Desulfomicrobium macestii* was first described as a member of the genus *Desulfobacterium*. The type strain was isolated from a sulfide spring located at Matsesta, Russia (Gogotova and Vainstein 1989). From a very similar habitat, the type strain of *Desulfomicrobium thermophilum* originates. Here also a terrestrial sulfur spring located in Paipa, Colombia, was used for isolation (Thevenieau et al. 2007). For the type strain of *Desulfomicrobium orale*, the most unusual habitat is listed which was isolated from the subgingival plaque of a patient with periodontitis (Langendijk et al. 2001).

Cells of *Desulfomicrobium* spp. were assumed to be important H_2 utilizers in anoxic environments (Krumholz et al. 1999) and also in oxic-anoxic transition zones (Ito et al. 2002). The presence of several hydrogenases with different cellualr localization was shown in several strain of Desulfomicrobium species. However, till present mostly NiFeSe hydrogenase of strain 9974 (DSM 1743; Biebl and Pfennig, 1977) of *Desulfomicrobium baculatum* was investigated in detail (Texeira et al. 1987). It was very active for H_2 production, but not for consumption, and it was not very sensitive to oxygen. H_2-uptake hydrogenases of *Desulfomicrobium* spp. are not investigated, yet.

Pathogenicity, Clinical Relevance

There seems to be evidence that *Desulfomicrobium orale* is associated with periodontitis (Langendijk et al. 2001; Riggio et al. 2011).

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8 The Family *Desulfonatronaceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract
Desulfonatronaceae, a family within the order *Desulfovibrionales*, embraces a single genus *Desulfonatronum*. Besides their 16S rRNA gene sequence phylogeny, all members of the family are defined by a wide range of morphological and chemotaxonomic properties for the delineation of genera and species. Strictly anaerobic, having a respiratory type or fermentative type of metabolism. Members are mesophilic and alkaliphilic sulfate-reducing bacteria. Members of the family are found in alkaline habitats. All described species are chemoorganoheterotroph; some species are either chemolithoheterotroph or chemolithoautotroph (*Desulfonatronum thiodismutans* and *Desulfonatronum thioautotrophicum*). All members oxidize organic substrates incompletely to acetate with sulfate as electron acceptor.

This contribution is a modified and updated version of a previous family description (Kuever et al. 2005).

The family belongs to the order *Desulfovibrionales* within the *Deltaproteobacteria* and contains a single genus *Desulfonatronum* (Pikuta et al. 1998) which is the type genus of the family (Kuever et al. 2006). Gram-staining negative. Morphological forms are always rod-shaped cells, but may be spirilloid under suboptimal conditions. Spore formation is absent. All members are motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory type of metabolism, some species are capable to grow by fermentation. Simple organic molecules and H_2 are used as electron donors. All members oxidize organic substrates incompletely to acetate. All members are mesophilic. All described species are chemoorganoheterotrophs and some are chemolithoheterotrophs with the exception of *Desulfonatronum thiodismutans* and *Desulfonatronum thioautotrophicum* that are described to grow as autotrophs on H_2 and CO_2 (Pikuta et al. 2003; Sorokin et al. 2011a). Sulfate, sulfite, and thiosulfate are used as electron acceptor and reduced to sulfide. Most species can grow by fermentation of organic substrates and by disproportionation of reduced sulfur compounds. Sodium and carbonate are required for growth. Members have been isolated exclusively from haloalkaline habitats.

Phylogenetic Structure of the Family

The phylogenetic structure of the family *Desulfonatronaceae* and its neighboring families within the order *Desulfovibrionales* are shown on ❯ *Fig. 8.1*. The borders of the family are primarily based on the phylogenetic tree as framework and their unique properties (physiology, chemotaxonomic markers) which are present in all members (see ❯ *Table 8.1*).

Molecular Analyses

DNA-DNA Hybridization Studies

DNA-DNA hybridization study was done between the type strains of *Desulfonatronum lacustre*, strain Z-7951, and *Desulfonatronum thiodismutans*, strain MLF1, and exhibited 51 % homology (Pikuta et al. 2003).

Bioprinting and Ribotyping

Are absent.

MALDI-TOF

Are absent.

E. Rosenberg et al. (eds.), *The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria*, DOI 10.1007/978-3-642-39044-9_273,

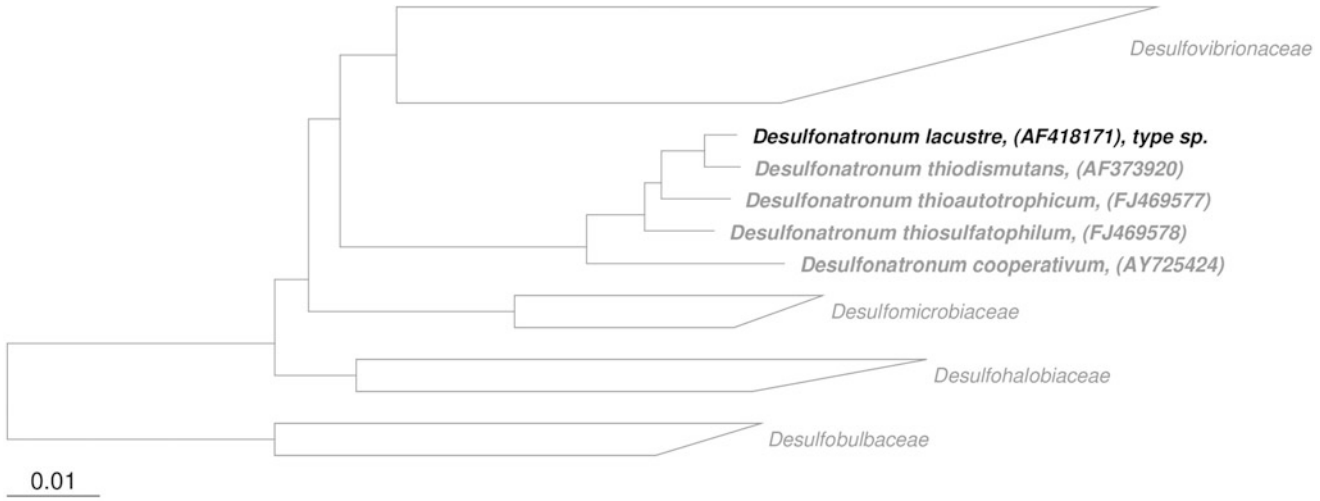

Fig. 8.1
Phylogenetic reconstruction of the family *Desulfonatronaceae* based on the neighbor-joining algorithm with the Jukes-Cantor correction. Sequence dataset and alignments according to the All-Species Living Tree Project release LTPs108 (Yarza et al. 2010). A 40 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence

Genome Comparison

The complete genome sequence of the type strain of *Desulfonatronum lacustre* should be available soon. So far this project remains unfinished.

Phages

Phages might be present but are not reported so far.

Phenotypic Analyses

The main features of all members of the family *Desulfonatronaceae* are listed in Table 8.1.

All members are gram-negative vibrio. All are motile. All are strictly anaerobic, having a respiratory or fermentative metabolism. All members are obligate alkaliphilic.

The cellular fatty acid pattern of members of the genus *Desulfonatronum* was found to be dominated by iso and normal unsaturated and saturated fatty acids and varies depending on the species.

Typical dominant fatty acids found are $C_{14:0}$, $isoC_{15:0}$, $isoC_{17:1\ \omega8}$, $C_{16:1\ \omega7t}$ $C_{16:1\ \omega7c}$, $C_{18:1\ \omega11}$, $C_{18:1\ \omega7c}$, $C_{18:1\ \omega11t}$, and $C_{18:0}$ (Pikuta et al. 1998, 2003; Sorokin et al. 2011a).

Data for the dominant respiratory quinone are lacking. Organic substrate oxidation is always incomplete. Typical electron donors for sulfate reduction are H_2, formate, lactate, and ethanol. Autotrophic growth was demonstrated for *Desulfonatronum thiodismutans* and *Desulfonatronum thioautotrophicum*. Growth by disproportionation of sulfite and thiosulfate seems to be a common feature of this genus. Typical electron acceptors for growth are sulfate, sulfite, and thiosulfate.

For most members the optimal growth temperature is between 30 °C and 37 °C. Strains belonging to this genus have been only isolated from haloalkaline and hypersaline habitats.

Desulfonatronum Pikuta et al. 1998, 631^{VP} (Effective Publication Pikuta et al. 1998, 112)

De.sul.fo.na.tro´um. L. prep. de, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); N.L. neut. n. *natron* (arbitrarily derived from the Arabic n. natrun or natron) soda; N.L. neut. n. *Desulfonatronum*, a sulfate reducer inhabiting soda lakes.

The genus *Desulfonatronum* contains five validly described species. Their chemotaxonomic and physiological properties are listed in Table 8.1.

Biolog

Biolog data are not available for sulfate-reducing bacteria.

Isolation, Enrichment, and Maintenance Procedures

All members of the family *Desulfonatronaceae* require anoxic media for growth like all other sulfate-reducing bacteria. The media are prepared under specific conditions and the addition of a reductant is required, in general sulfide to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992). Modifications required for the isolation of these obligate alkaliphilic organisms are listed in the descriptions for

Table 8.1

Comparison of selected characteristics of members of the genus *Desulfonatronum*

Characteristic	*D. lacustre*	*D. cooperativum*	*D. thioautotrophicum*	*D. thiodismutans*	*D. thiosulfatophilum*[a]
Type strain	Strain Z-7951, DSM 10312	Strain Z-7999, DSM 16749, VKM B-2329	Strain ASO4-1, DSM 21337, UNIQEM U756	Strain MLF1, ATCC BAA-395, DSM 14708	Strain ASO4-2, DSM 21338, UNIQEM U757
Accession number of the 16S rRNA gene sequence of the type strain	AF418171	AY725424	FJ469577	AF373920	FJ469578
Morphology	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio
Cell size (μm)	0.7–0.9 × 2.5–3.0	0.4–0.5 × 1.0–2.5	0.5–0.6 × 2.0–4.0	0.6–0.7 × 1.2–2.7	0.4–0.5 × 1.5–4.0
Motility	+	+	+	+	+
Mol% G + C content	57.3	56.5	57.6	63.1	57.0
Major menaquinone	Nr	Nr	Nr	Nr	Nr
Optimal pH	9.3–9.5	8.0–9.0	9.3	9.5	9.5
Optimal temperature (°C)	40	35–38	30–41	37	30–40
Optimal NaCl concentration (g/l)	0	5–15	23.4–35	30	17.5
Growth factor requirement	–	–	–	Vitamins	–
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
H_2/CO_2	+[b]	+[b]	+	+	+[b]
Formate	+[b]	+[b]	+	+	+[b]
Acetate	–	–	–	–	–
Fatty acids	–	–	–	–	–
Ethanol	+	–	+	+	+
Other n-alcohols	–	Nr	–	–	–
Lactate	–[c]	+	+	–	+
Pyruvate	–	–[d]	+	–	+
Fumarate	–	–	–	Nr	–
Succinate	–	–	–	Nr	–
Malate	–	–	–	Nr	–
Other	–	–	–	–	–
Fermentative growth	–	–	+	–	+
Disproportionation of reduced sulfur compounds	+	Nr	+	+	+
Sulfate	+	+	+	+	+
Sulfite	+	+	+	+	+
Thiosulfate	+	+	+	+	+
Sulfur	–	–		–	–
Other	–	–	–	–	–
Literature	Pikuta et al. (1998), gen. nov. sp. nov.; Zhilina et al. (2005); Sorokin et al. (2011a)	Zhilina et al. (2005), sp. nov.	Sorokin et al. (2011a, b), sp. nov.	Pikuta et al. (2003);	Sorokin et al. (2011a, b), sp. nov.

[a]Correct spelling would be *D. thiosulfatiphilum* according to J.P. Euzeby

[b]Only in the presence of acetate or yeast extract

[c]Some strains but not the type strain can use lactate (Zhilina et al. 2005)

[d]Unusual, because lactate is metabolized

the different species (Pikuta et al. 1998, 2003; Sorokin et al. 2011). Essential are sodium and carbonate for growth (Zhilina 2005).

For enrichment the used electron donor and the alkaline medium should be highly selective and would have a strong influence on what kind of sulfate-reducing bacteria will grow in the medium. For isolation of members of the genus *Desulfonatronum*, formate, ethanol, lactate, and pyruvate might be selective electron donors in combination with sulfate as electron acceptor. Use of formate together with thiosulfate might enrich for *Desulfonatronovibrio*; the same might be true for H_2 as electron donor.

Most sulfate-reducing bacteria of this family have been enriched using batch cultures; other options are serial dilution techniques of natural samples. For isolation in general, roll tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992). For certain members of this family, the plating technique was the only successful method to obtain pure cultures (Sorokin et al. 2011a). In contrast to *Desulfonatronovibrio* which form usual black colonies, members of the genus *Desulfonatronum* form disc-like (up to 3 mm in diameter) colonies with orange-pink color inside the alkaline agar medium. Therefore, it might be that they form some kind of pigment and are more oxygen tolerant than members of the genus *Desulfonatronovibrio* (D.Y. Sorokin pers. com.).

For short-term preservation stock cultures can be stored at 2–6 °C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. For long-term storage cultures can be kept freeze dried, at −80 °C or in liquid nitrogen.

Ecology

Habitat

Members of this family were isolated exclusively from haloalkaline habitats.

Strain Z-7951, the type strain of *Desulfonatronum lacustre*, was isolated using H_2 or formate as electron donors in combination with acetate as carbon source and sulfate as electron acceptor from an enrichment which was inoculated with anoxic mud from lakes of the Ulug-Khem valley in Tuva, Russia (Pikuta et al. 1998).

The type strain of *Desulfonatronum cooperativum*, strain Z-7999, was isolated from an acetate-utilizing syntrophic co-culture which was inoculated with anoxic deposits and water from soda lake Khadin, located near Kysil-city, Tuva, Russia (Zhilina et al. 2005).

The type strain of *Desulfonatronum thioautotrophicum*, strain ASO4-1, was isolated with formate as electron donor and sulfate as electron acceptor with acetate as carbon source from the soda lake Tanatar-1 in the Kulunda Steppe (Altai, Russia) (Sorokin et al. 2011a).

In contrast, the type strain of *Desulfonatronum thiodismutans*, strain MLF1, was isolated from a different continent. Here anoxic mud from the south shore of Mono Lake, California, USA, was used as inoculum for an enrichment culture with formate as electron donor and sulfate as electron acceptor (Pikuta et al. 2003).

Strain ASO4-2, the type strain of *Desulfonatronum thiosulfatiphilum*, was isolated from sediment of soda lake Picturesque in the Kulunda Steppe (Altai, Russia) using pyruvate as electron donor and sulfate as electron acceptor (Sorokin et al. 2011a).

Pathogenicity, Clinical Relevance

No clinical relevance known.

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9 The Family *Desulfovibrionaceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract
Desulfovibrionaceae, a family within the order *Desulfovibrionales*, embraces the genera *Desulfovibrio*, *Desulfobaculum*, *Desulfocurvus*, *Bilophila*, and *Lawsonia*. Besides their 16S rRNA gene sequence phylogeny, all members of the family are defined by a wide range of morphological and chemotaxonomic properties for the delineation of genera and species. Strictly anaerobic, having a respiratory type or fermentative type of metabolism. Members are all mesophilic or moderately psychrophilic. Members of the family are found in various habitats, including freshwater, brackish water and marine sediments, biofilms, soil, sewage sludge, and animals. Most described species are chemoorganoheterotroph, and some are chemolithoheterotroph. All members oxidize organic substrates incompletely to acetate.

This contribution is a modified and updated version of a previous family description (Kuever et al. 2005a, b).

Taxonomy, Historical, and Current

Short Description of the Family

De.sul.fo.vi.bri.o.na′ce.ae. N.L. masc. n. *Desulfovibrio*, type genus of the family; suff. -*aceae*, ending to denote family; N.L. fem. pl. n. *Desulfovibrionaceae*, the *Desulfovibrio* family (Modified from *Bergey's Manual*). The description is an emended version of the description given in *Bergey's Manual*, 2nd edition (Kuever et al. 2005b).

The family belongs to the order *Desulfovibrionales* within the *delta-Proteobacteria* (Kuever et al. 2005a). The family *Desulfovibrionaceae* contains the genera *Desulfovibrio* (Kluyver and van Niel 1936; Skerman et al. 1980) which is the type genus of the family (Kuever et al. 2005b), *Desulfobaculum* (Zhao et al. 2012), *Desulfocurvus* (Klouche et al. 2009), *Bilophila* (Baron et al. 1990, 1989), and *Lawsonia* (McOrist et al. 1995). Gram-staining negative. Morphological forms vary from vibrio- and rod-shaped cells of varying length to spirillum-like forms. Spore formation is absent. Most members are motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory or fermentative type of metabolism. In general, simple organic molecules are used as electron donors; some species can use also H_2. Members of the genus *Bilophila* might be dependent on more complex media (Baron et al. 1989), whereas members of the genus *Lawsonia* are obligate intracellular parasite of intestinal cells and can only be cultivated in cell cultures (McOrist et al. 1995). Nearly all members are mesophilic; some are slightly psychrophilic. All members oxidize organic substrates incompletely to acetate.

All described species are chemoorganoheterotroph, most are also chemolithoheterotroph. No species is chemolithoautotroph (although this is sometimes listed in the description). Members of the genera *Desulfovibrio*, *Desulfobaculum*, and *Desulfocurvus* use all sulfate as electron acceptor which is reduced to sulfide (these are regarded to be sulfate-reducing bacteria), most species can also use sulfite and thiosulfate. A few species of the genus *Desulfovibrio* can grow by disproportionation of reduced sulfur compounds with acetate as carbon source. In contrast to most other sulfate-reducing bacteria (see other families), members of the genus *Desulfovibrio* (and probably *Desulfobaculum* and *Desulfocurvus*) are regarded to be relative robust against oxygen stress and are much easier to isolate and to cultivate than most sulfate-reducing bacteria of the other families.

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Members of the genus *Bilophila* cannot use sulfate as electron acceptor, but use sulfite, thiosulfate, taurine, or sulfur-containing amino acid derivatives instead. Members of the genus *Lawsonia* are obligate intracellular parasites. Their metabolism remains unclear and use of oxidized sulfur compounds as electron acceptor was not documented so far.

Members have been isolated from various sources, like freshwater, brackish water and marine sediments, biofilms, soil, sewage sludge, aquifers, hydrothermal vent systems, hindgut of insects, feces of mammals and humans, and infected areas of humans and animals.

Phylogenetic Structure of the Family and Its Genera

The phylogenetic structure of the family and its neighboring families within the order *Desulfovibrionales* is shown in *Fig. 9.1*. The borders of the family are primarily based on the phylogenetic tree as framework; other unique properties are the ability to use sulfate as electron acceptor and simple organic compounds like lactate as electron donor and carbon source. The use of cellular fatty acids and menaquinones as typical chemotaxonomic markers remains highly vague and might only work for certain clusters within the tree.

Taxonomic comments: The entire situation within this family is highly unsatisfactory and unclear compared to all the other families. The only exception has been the establishment of new genera for *Bilophila* and *Lawsonia* which was more based on their unique physiological properties (like inability to use sulfate as electron acceptor). Looking on the tree shown in *Fig. 9.1*, they can also be regarded as a *Desulfovibrio* spp. which would not be useful from a taxonomic point of view.

The genus *Desulfovibrio* consists of species which are related to each other at levels far below 95 % or even lower at 90 % based on their 16S rRNA gene sequence identity. The only exceptions are the new genera *Desulfobaculum* and *Desulfocurvus*. From some perspective these are simply members of the genus *Desulfovibrio*, and their physiological properties and also chemotaxonomic markers are identical to members of this genus. Favoring the 16S rRNA gene identity values which are the base for the tree shown in *Fig. 9.1*, it is justified to establish these new genera. The main task here is to use the same rules as for the other taxa for the sake of consistency. Therefore, the entire genus *Desulfovibrio* has to be split in several genera, most likely as the clusters shown in *Fig. 9.1*. This would form a solid substructure and might show some physiological properties which are similar in certain clusters (e.g., like marine *Desulfovibrios* or similar habitats).

To demonstrate the unsatisfactory situation, here are a few examples. To justify the new genus *Desulfobaculum*, the presence of MK-7 and certain cellular fatty acids was used to be unique in contrast to other *Desulfovibrio* spp. This is not the case. For most *Desulfovibrio* spp., data on cellular fatty acids and menaquinones are lacking other and cannot be considered at all, but MK-7 is also found in *Desulfovibrio* sp. (see *Table 9.2*), and for cellular fatty acids, the situation is similar. For *Desulfocurvus* only the low 16S rRNA gene sequence identity was used as argument for the formation of a new genus. But what about the closest relatives *Desulfovibrio bizertensis* and *Desulfovibrio senezii*? Are they members of the same genus, or new genera?

Another example is the use of DNA-DNA hybridization (DDH). There are certain values used more or less as a general rule (Stackebrandt et al. 2000; Wayne et al. 1987). A value of 70 % DDH was proposed by Wayne et al. (1987) as a recommended standard for delineating species. Although this recommendation is not a strict standard, it is more or less accepted. Within the genus *Desulfovibrio* these rules are ignored. Some wrong classifications on the subspecies level (primarily based on physiological properties) have already been corrected (e.g., status of *Desulfovibrio oxamicus*); others are still there (see taxonomic comment genus *Desulfovibrio*). Using the value of 70 % the two subspecies of *Desulfovibrio africanus* would resemble a single species. On the other hand the value of 40 % between the two subspecies of *Desulfovibrio oceani* would clearly justify a new separate species and not at all a subspecies.

Molecular Analyses

DNA-DNA Hybridization Studies

DDH-data are limited and have not been done on a systematic base. Here are some of them summarized. Between *Desulfovibrio africanus* ssp. *africanus* and *Desulfovibrio africanus* ssp. *uniflagellum*, the level of DDH is 72 % (Castaneda-Carrion et al. 2010). The DDH identity between *Desulfovibrio vietnamensis* and *Desulfovibrio alaskensis* is 10.2 % (Feio et al. 2004). DDH value between *Desulfovibrio ferrireducens* and *Desulfovibrio frigidus* is 14.5 % (Vandieken et al. 2006). *Desulfovibrio intestinalis* has 44.6 % DNA-DNA homology to *Desulfovibrio desulfuricans* ssp. *desulfuricans* (strain Essex 6) (Fröhlich et al. 1999). The DDH between *Desulfovibrio marinus* to *Desulfovibrio indonensis* is 15.7 % and to *Desulfovibrio gabonensis* 26.0 % (Ben Dhia Thabet et al. 2007). The DNA-DNA homology between *Desulfovibrio marinisediminis* and *Desulfovibrio acrylicus* is 10.3 % (Takii et al. 2008). The DDH identity for *Desulfovibrio oceani* ssp. *galateae* to *Desulfovibrio oceani* ssp. *oceani* is 40 % (Finster and Kjedsen 2010). The DDH homology between *Desulfovibrio oxamicus* and *Desulfovibrio termiditis* is 58 % (Lopez-Cortes et al. 2006). *Desulfovibrio paquesii* has a DDH homology of 56 % to *Desulfovibrio gigas* (van Houten et al. 2009).

Bioprinting and Ribotyping

Are absent.

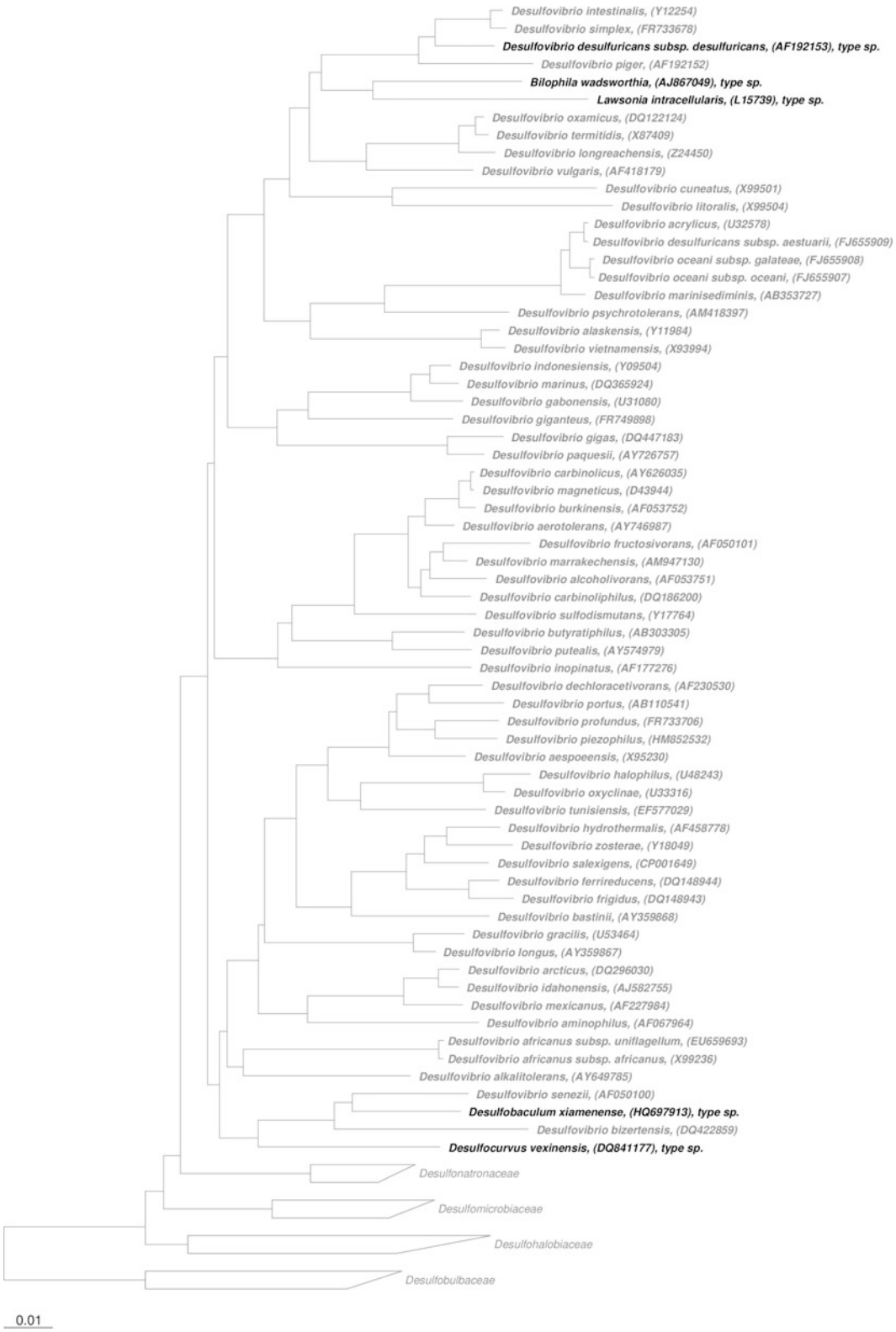

Fig. 9.1

Phylogenetic reconstruction of the family *Desulfovibrionaceae* 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). 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

MALDI TOF

Are absent.

Genome Comparison

The complete genome sequences of seven type strains and nine other strains of members of this family have been analyzed.

The genome of the type strain of *Desulfovibrio aespoeensis* is 3,629,109 bp long and contains 3,406 genes with 3,304 coding for proteins and 63 for RNA genes. The mol% G+C content of the DNA is 62.6. A publication is absent.

The genome *Desulfovibrio alaskensis* strain G20 is 3,730,232 bp long and contains 3,364 genes with 3,258 coding for proteins and 81 rNA genes. The mol% G+C content is 57.8 (Hauser et al. 2011).

The genome of the type strain of *Desulfovibrio piger* is 2,826,240 bp long and contains 3,185 genes with 3,114 coding for protein and 71 coding for RNA genes. The mol% G+C is 63.0. A publication is absent.

The genome of the type strain of *Desulfovibrio fructosivorans* is 4,673,595 bp long and contains 4,210 genes with 4,159 coding for proteins and 51 coding for RNA genes. The mol% G+C content of the DNA is 63.9. A publication is absent.

The genome of the type strain of *Desulfovibrio magneticus* has been analyzed and contain one chromosome (5,248,049 bp) and two plasmids (58,704 bp and 8,867 bp). The genome contains 4,760 genes coding for 4,700 proteins and 60 RNA genes. The mol% G+C content of the DNA is 62.7 (Nakazawa et al. 2009).

The genome of the type strain of *Desulfovibrio salexigens* is 4,289,847 bp long and contains 3,941 genes with 3,807 coding for proteins and 108 coding for RNA. The mol% G+C content of the DNA is 47.1. A publication is absent.

The genome of the type strain of *Desulfovibrio vulgaris* has been analyzed and contains one chromosome (3,570,858 bp) and one plasmid (202,301 bp). The genome contains 3,639 genes coding for 3,536 proteins and 86 RNA genes. The mol% G+C content of the DNA is 63.2 (Heidelberg et al. 2004).

The genome of *Desulfovibrio vulgaris* strain RCH1 has been analyzed and contains one chromosome (3,532,052 bp) and one plasmid (198,504 bp). The genome contains 3,190 genes coding for 3,091 proteins and 87 RNA genes. The mol% G+C content of the DNA is 63.2 (Walker et al. 2009)

The genome of the *Desulfovibrio vulgaris* strain DP4 has been analyzed and contains one chromosome 3,462,887 bp) and one plasmid (202,305 bp). The genome contains 3,338 genes coding for 3,221 proteins and 87 RNA genes. The mol% G+C content of the DNA is 63.2 (Walker et al. 2009).

The genome of strain "Myazaki F" which is wrongly classified as *Desulfovibrio vulgaris* is 4,040,304 bp long and contains 3,286 genes with 3,180 coding for proteins. The mol% G+C content of the DNA is 67.1 (Walker et al. 2009).

The genome of strain ATCC 27774, wrongly classified as *Desulfovibrio desulfuricans*, is 2,873,437 bp long and contains 2,450 genes with 2,356 coding for proteins and 68 coding for RNA genes. The mol% G+C of the DNA is 58.1. There is no publication available.

Another genome of strain ND132, also wrongly classified as a *Desulfovibrio desulfuricans*, is 3,858,580 bp long, contains 3,533 genes with 3,454 coding for proteins and 64 coding for RNA genes (Brown et al. 2011a).

The genome of strain Walvis Bay, wrongly classified as *Desulfovibrio africanus*, is 4,200,534 bp long, contains 3,884 genes with 3,725 coding for proteins and 69 coding for RNA gens. The mol% G+C content of the DNA is 61.4 (Brown et al. 2011b).

All genomes of members of the genus *Desulfovibrio* contain an aprBA gene linked to a complete QmoABC complex and all required genes for the dissimilatory sulfite reduction (dsrAB and dsrMKJOP).

From *Bilophila wadsworthia*, two genomes have been analyzed, both not resembling the type strain. Strain 3_1_6 isolated from human feces is 4.37 Mb long and contains 3,730 genes with 3,651 coding for proteins and 62 for RNA genes. The mol% G+C content was not published. Another strain 4_1_30 was also isolated from human feces is 447 Mb long and contains 4,172 genes coding for 4,114 proteins and 58 RNA genes. Also the mol% G+C content was not calculated. For both *Bilophila* genomes, a publication is absent.

The genome of Lawsonia intracellularis PHE/MN1-00 (not the type strain) has been analyzed and contains one chromosome (1,457,619 bp) and three plasmids (27,048 bp, 39,794 bp, and 194,553 bp). The genome contains 1,391 genes coding for 1,340 proteins and 49 RNA genes. The mol% G+C content of the DNA is 33.1. A publication is absent.

Phages

Phages might be present but are not reported so far.

Phenotypic Analyses

The main features of all members of the family *Desulfovibrionaceae* are listed in ❯ *Table 9.1* and the tables featuring the genera (❯ *Tables 9.2*–❯ *9.6*).

Desulfovibrio Kluyver and van Niel 1936, 397[VP]

De.sul.fo.vi´bri.o. L. pref. *de*, from; L. n. *sulfur*, sulfur; L. v. *vibro*, 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. *Desulfovibrio*, a vibrio that reduces sulfur compounds.

Table 9.1
Morphological and chemotaxonomic characteristics of genera of *Desulfovibrionaceae*

	Desulfovibrio	*Desulfobaculum*	*Desulfocurvus*	*Bilophila*	*Lawsonia*
Morphology	Vibrio or rod shaped	Rod shaped	Vibrio shaped	Rod shaped	Rod shaped
Gram-stain	Negative	Negative	Negative	Negative	Negative
Motility	+/– (most species motile)	+	+	–	+(intracellular)
Metabolism	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Major fatty acids	$C_{14:0}$, iso $C_{15:0}$, anteiso $C_{15:0}$, $C_{16:1\ w7c}$, $C_{16:0}$, iso $C_{17:1\ w7}$, iso $C_{17:0}$, anteiso $C_{17:0}$, $C_{18:0}$, $C_{18.1\ w9c}$, $C_{18:1\ w7c}$[a]	Iso $C_{15:0}$, iso $C_{16:0}$, iso $C_{17:1\ w9c}$	Nr	Iso $C_{15:0}$, $C_{16:0}$, cyclo $C_{17:0\ w7c}$, cyclo $C_{19:0\ w9c}$	Nr
Menaquinone	MK-6 or MK-7	MK-7	Nr	Nr	Nr
G+C content (mol%)	36.7–69.0	64.5	67.2	59.2 (not the type strain)	33.1
Substrate oxidation	Incomplete	Incomplete	Incomplete	Incomplete	Nr
CODH activity	Absent	Nr	Nr	Nr	Nr
Typical electron donors	Lactate, pyruvate, most species: H_2, formate, primary alcohols	Lactate, pyruvate, succinate, malate, citrate, oxalate, cysteine, casamino acids, yeast extract, cellobiose, galactose, acetate[b]	Lactate, pyruvate, formate	Pyruvate, formate	Only complex media
Chemolithoautotrophic growth	No species	Nr	Nr	Nr	Nr
Fermentative growth	Most species	All species	All species	No species	Nr
Growth by disproportionation of reduced sulfur compounds	Some species	Nr	Nr	Nr	Nr
Typical electron acceptors	Sulfate, most species also sulfite and thiosulfate	Sulfate, sulfite, thiosulfate	Sulfate, sulfite, thiosulfate	Sulfite, thiosulfate, taurine, DMSO, nitrate, cysteate	Nr
Optimal growth temperature (°C)	20–43	35–40	37	35–37 (other not tested)	35–37
Habitat	Various sources, including freshwater, brackish water, marine sediments, sewage sludge, intestinals of insects	Anoxic mangrove sediment	Deep saline aquifer	Clinical isolates from gastrointestinal, genital, and oral (periodontal) cavities of humans and other mammals; anoxic freshwater sediment and sewage sludge	Clinical isolates, obligate intracellular parasite of intestinal cells of pigs

[a]The fatty acids are highly variable between all species of the genus *Desulfovibrio*
[b]Utilization of acetate remains highly questionable

The genus *Desulfovibrio* contains 62 described species or subspecies. Their chemotaxonomic and physiological properties are listed in *Table 9.2*. There are several members of the genus *Desulfovibrio* described which have not been validated, so far. They are not listed here. Some of them are listed in the genome section of the family, although many of these are lacking several information required for a formal description (Kuever et al. 2005c).

Taxonomic comment: The phylogenetic tree (*Fig. 9.1*) clearly indicates that *Desulfovibrio desulfuricans ssp. aestuarii* is not related to *Desulfovibrio desulfuricans ssp. desulfuricans* but might resemble *Desulfovibrio acrylicus* or a subspecies of this. This has already been indicated by Stefan Spring (pers. com.). See also the taxonomic comments for the family.

Table 9.2
Comparison of selected characteristics of members of the genus *Desulfovibrio*

Characteristic	*Desulfovibrio desulfuricans* ssp. *desulfuricans*	*Desulfovibrio acrylicus*	*Desulfovibrio aerotolerance*	*Desulfovibrio aespoeensis*	*Desulfovibrio africanus* ssp. *africanus*	*Desulfovibrio africanus* ssp. *uniflagellum*	*Desulfovibrio alaskensis*
Type strain	Strain Essex 6, ATCC 29577, CCUG 34226, CIP 107039, DSM 642, LMG 7529, NCIB (now NCIMB) 8307, VKM B-1799	Strain W218, DSM 10141	Strain DvO5, DSM 16695, JCM 12613	Strain Aspo-2, ATCC 700646, DSM 10631	Strain ATCC 19996, DSM 2603, NCIMB 8401, VKM B-1757	Strain SR-1, JCM 15510, KCTC 5649	Strain Al1, DSM 16109, NCIMB 13491
Accession number of the 16S rRNA gene sequence of the type strain	AF192153, M34113	U32578	AY746987	X95230, CP002431 (genome)	X99236	EU659693	Y11984
Morphology	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio
Cell size (μm)	0.5–0.8 × 1.5–4.0	0.8 × 2.0–4.0	1.0 × 2.0–5.0	0.5 × 1.7–2.5	0.5–0.6 × 2.0–3.0	0.5 × 3.5	0.5–1.2 × 1.0–5.0
Motility	+	+	+	+	+	+	+
Mol% G+C content	59.0	45.1	57.2	61.0	63.1	62.4	64.1
Major menaquinone	Mk-6	Nr	Nr	Nr	Mk-6(H_2)	Nr	Nr
Optimal pH	7.2–7.8	7.4	6.9	7.5	7.0	7.0	7.0
Optimal temperature (°C)	30–36	30–37	29	25–30	34–37	37	37
Optimal NaCl concentration (g/l)	0–1	18	0.5	7	0–1	0–1	25
Growth factor requirement	–	Yeast extract	–	Nr	–	Nr	–
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]	Nr
Formate	+[a]	+[a]	+[a]	–	+[a]	+[a]	Nr
Acetate	–	–	–	–	–	–	–
Fatty acids	–	–	–	–	–	–	–
Ethanol	+	+	+	–	+	+	+[b]
Other *n*-alcohols	*N*-propanol, *n*-butanol	Glycerol, *n*-propanol	Glycerol, *n*-propanol, *n*-butanol, *n*-pentanol	Nr	Simple primary alcohols	Nr	–
Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	+	+	+	–	–	Nr	Nr

Succinate	–	+	–	Nr	–	Nr	+
Malate	+	+	+[b]	–	+	–	Nr
Other	Choline	Glycine, serine, alanine, cysteine	–	–	–	–	–
Fermentative growth	+	Nr	+	+	–	+	Nr
Disproportionation of reduced sulfur compounds	Nr	Nr	+	Nr	Nr	Nr	Nr
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	–	+	Nr	+	+	+
Thiosulfate	+	+	+	+	+	+	+
Sulfur[c]	–	–	+	+	+	+	Nr
Other	Fumarate, nitrate, FeIII[d], UVI[d]	Acrylate	oxygen[d]	–	–	–	–
Literature	Postgate and Campbell 1966, subspecies; Postgate 1951	van der Maarel et al. 1997, sp. nov.; van der Maarel et al. 1996	Mogensen et al. 2009, sp. nov.; Mogensen et al. 2005	Motamedi and Pedersen 1998, sp. nov.	Campbell et al. 1966; Postgate and Campbell 1966; Castaneda-Carrion et al. 2010, subsp. nov.	Castaneda-Carrion et al. 2010, subsp. nov.	Feio et al. 2004, sp. nov.

Characteristic	*Desulfovibrio alcoholivorans*	*Desulfovibrio alkalitolerans*	*Desulfovibrio aminophilus*	*Desulfovibrio arcticus*	*Desulfovibrio bastinii*	*Desulfovibrio bizertensis*	*Desulfovibrio burkinensis*
Type strain	Strain SPSN, ATCC 49738, DSM 5433, VKM B-1761	Strain RT2, DSM 16529, JCM 12612	Strain ALA-3, DSM 12254	Strain B15, DSM 21064, VKM B-2367	Strain SRL4225, ATCC BAA-903, DSM 16055	Strain MB3, DSM 18034, NCIMB 14199	Strain HDv, ATCC 700846, DSM 6830
Accession number of the 16S rRNA gene sequence of the type strain	AF053751	AY649785	AF067964	DQ296030	AY359868	DQ422859	AF053752
Morphology	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio	Curved rod
Cell size (μm)	0.2 × 3.0–4.0	0.5–0.8 × 1.4–1.9	0.2 × 3.0–4.0	0.4–0.5 × 3.0–4.0	0.5 × 2.0–3.0	0.5 × 2.0–3.0	0.8–1.2 × 2.2–3.1
Motility	+	+	+	+	+	+	+
Mol% G+C content	66.0/64.5	64.7	66.0	55.2	44.6	51.0	67.0
Major menaquinone	MK-6	Nr	Nr	MK-6(H_2)	Nr	Nr	Nr
Optimal pH	7.3	9.0–9.4	7.5	6.7–7.0	5.8–6.2	7.0	6.8
Optimal temperature (°C)	35–37	43	35	24	35–40	40	37
Optimal NaCl concentration (g/l)	7.5	1.3	0–7.5	2	40	20	0

Table 9.2 (continued)

Characteristic	*Desulfovibrio alcoholivorans*	*Desulfovibrio alkalitolerans*	*Desulfovibrio aminophilus*	*Desulfovibrio arcticus*	*Desulfovibrio bastinii*	*Desulfovibrio bizertensis*	*Desulfovibrio burkinensis*
Growth factor requirement	–	Yeast extract	Yeast extract (depends on the substrate)	–	Vitamins	–	Vitamins, yeast extract
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]
Formate	+[a]	+[a]	+[a]	+[a]	–	Nr	–
Acetate	–	–	–	–	–	–	–
Fatty acids	–	–	–	–	–	–	–
Ethanol	+	–	+	+	+	+	+
Other *n*-alcohols	*n*-propanol, *n*-butanol, *n*-pentanol, glycerol, 1,2-propanediol, 1,3-propanediol, 1,4-butandiol	–	–	–	*n*-butanol, glycerol	*n*-propanol, *n*-butanol	*n*-propanol, *n*-butanol, glycerol, 1,2-propanediol, 1,3-propanediol
Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	+[c]	–	–	–	–	+	+
Succinate	Nr	–	Nr	–	–	+	+
Malate	+[c]	–	–	–	–	+	+
Other	Tyrosol	–	Alanine, cysteine, glycine, threonine, casamino acids	Choline	–	–	Dihydroxyacetone
Fermentative growth	+	+	+	–	+	+	+
Disproportionation of reduced sulfur compounds	Nr	Nr	+	Nr	Nr	Nr	Nr
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+
Sulfur[d]	–	–	–	+	+	+	+
Other	Fumarate, nitrate	–	–	$FeIII^{d}$, DMSO[c]	–	Fumarate	Fumarate
Literature	Qatibi et al. 1995, sp. nov.; Qatibi et al. 1991; Ouattara et al. 1999; Chamk et al. 2009	Abilgaards et al. 2006, sp. nov.	Baena et al. 1999, sp. nov.; Baena et al. 1998	Pecheritsyna et al. 2012, sp. nov.	Magot et al. 2004, sp. nov.	Haoari et al. 2006, sp. nov.	Ouattara et al. 1999, sp. nov.

Characteristic	*Desulfovibrio butyratiphilus*	*Desulfovibrio carbinolicus*	*Desulfovibrio carbinoliphilus*	*Desulfovibrio cuneatus*	*Desulfovibrio dechloracetivorans*	*Desulfovibrio desulfuricans* ssp. *aestuarii*	*Desulfovibrio ferrireducens*
Type strain	Strain BSY, DSM 21556, JCM 15519	Strain EDK82, DSM 3852, VKM B-1758	Strain D41, ATCC BAA-1241, DSM 17524	Strain STL1, DSM 11391	Strain SF3, ATCC 700912	Strain Sylt 3, ATCC 29578, NCIB (now NCIMB) 9335	Strain 61, DSM 16995, JCM 12925
Accession number of the 16S rRNA gene sequence of the type strain	AB303305	AY626035	DQ186200	X99501	AF230530	FJ655909	DQ148944
Morphology	Curved rod	Rod	Curved rod	Curved rod	Curved rod	Vibrio	Vibrio
Cell size (μm)	0.8–0.9 × 2.4–5.6	0.6–1.1 × 1.5–5.0	0.6–0.7 × 2.4–3.0	0.4–0.6 × 1.6–3.0	0.4–0.6 × 1.0–4.0	0.5–1.0 × 3.0–5.0	0.7 × 2.5–5.5
Motility	+	–	+	+	+	+	+
Mol% G+C content	63.3	65.0	63.0	52.7	Nr	55–59	42.0
Major menaquinone	MK-6(H_2)	Nr	Nr	Nr	Nr	Nr	Nr
Optimal pH	7.1	7–7.3	7.0–8.0	Nr	Nr	7.4–7.6	7.1–7.5
Optimal temperature (°C)	35	37-38	30	28	30	30-36	23
Optimal NaCl concentration (g/l)	5	0	Nr	0.25	1.6–25	25	10–25
Growth factor requirement	–	Yeast extract	–	–	Nr	–	–
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[e]	+[a]	+[a]	+[a]	–	+[a]	+[a]
Formate	–	+[a]	+[a]	+[a]	–	+[a]	+[a]
Acetate	–	–	–	–	+[f]	–	–
Fatty acids	C_4–C_5	–	–	Nr	C_3[f]	–	–
Ethanol	+	+	+	–	+[f]	+	+
Other n-alcohols	*n*-propanol, *n*-butanol	Methanol, *n*-propanol, *n*-butanol, *n*-pentanol, glycerol, 1,3-propanediol	Methanol, *n*-propanol, iso-propanol, *n*-butanol, iso-butanol, 3-methylbutanaol, ethylene gylcol, 1,2-propanediol, 1,3-propanediol, 1,4-butandiol, benzyl alcohol, phenylethanol	–	Nr	*n*-propanol, *n*-butanol	*n*-propanol

Table 9.2 (continued)

Characteristic	*Desulfovibrio butyratiphilus*	*Desulfovibrio carbinolicus*	*Desulfovibrio carbinoliphilus*	*Desulfovibrio cuneatus*	*Desulfovibrio dechloracetivorans*	*Desulfovibrio desulfuricans* ssp. *aestuarii*	*Desulfovibrio ferrireducens*
Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	–	+	+	+	+[f]	+	+
Succinate	–	+	–	–	–	–	+
Malate	–	+	+	+	Nr	+	+[b]
Other	–	Oxaloacetate	Maleate	–	Alanine[f]	Glucose	–
Fermentative growth	+	+	+	+	Nr	+	+
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr	+	Nr	Nr	Nr
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	–	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+
Sulfur[c]	Nr	+	–	+	Nr	+	–
Other	–	Fumarate	–	Fumarate, O_2[d]	Nitrate, halo-aromatic compounds	–	FeIII-citrate[d]
Literature	Suzuki et al. 2010, sp. nov.	Nanninga and Gottschal 1995, sp. nov. ; Nanninga and Gottschal 1987	Allen et al. 2008, sp. nov.	Sass et al. 1998, sp. nov. ; Sass et al. 1998	Sun et al. 2001, sp. nov. ; Sun et al. 2000	Postgate and Campbell 1966, subspecies. ; Stüven 1960	Vandieken et al. 2006, sp. nov.

Characteristic	*Desulfovibrio frigidus*	*Desulfovibrio fructosivorans*	*Desulfovibrio furfuralis*	*Desulfovibrio gabonensis*	*Desulfovibrio giganteus*	*Desulfovibrio gigas*	*Desulfovibrio gracilis*
Type strain	Strain 18, DSM 17176, JCM 12924	Strain JJ, ATCC 49200, DSM 3604, VKM B-1801	Strain F1 (not deposited in any culture collection)	Strain SEBR 2840, ATCC 700201, DSM 10636	Strain 8601, DSM 4123	Strain ATCC 19364, DSM 1382, NCIB (now NCIMB) 9332, VKM B-1759	Strain SRL6146, ATCC BAA-904, DSM 16080
Accession number of the 16S rRNA gene sequence of the type strain	DQ148943	AF050101	No accession number available	U31080	FR749898	DQ447183, AY726757	U53464
Morphology	Vibrio	Vibrio	Vibrio	Rod	Rod	Vibrio	Vibrio
Cell size (μm)	0.7 × 2.0–5.0	0.5–0.7 × 2.0–4.0	0.3–1.2 × 0.8–3.0	0.4 × 2.0–4.0	1.0 × 5.0–10.0	0.8–1.0 × 6.0–11.0	0.3 × 4.5–9.0
Motility	+	+	+	+	+	+	+

Mol% G+C content	43.3	64.1	61.0	59.5	56.0	65.0	59.0
Major menaquinone	Nr	MK-6	Nr	Nr	Nr	MK-6	Nr
Optimal pH	7.1	6.5–7.0	6.8–6.9	6.9–7.3	7.5	7.2–7.4	6.8–7.2
Optimal temperature (°C)	20–23	35	38	30	35	30–36	37–40
Optimal NaCl concentration (g/l)	20–30	0	Nr	5–6	20–30	0–2	50–60
Growth factor requirement	–	–	Nr	Vitamins	Vitamins	Vitamins	Vitamins
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	+[a]	Nr	+[a]	+[a]	+[a]	+[a]
Formate	+[a]	+[a]	Nr	+[a]	–	+[a]	+[a]
Acetate	–	–	–	–	–	–	–
Fatty acids	–	–	–	–	–	–	–
Ethanol	+	+	+	+	+	+	–
Other *n*-alcohols	*n*-propanol	Glycerol	1,4-butanediol	*n*-butanol	*n*-propanol, isopropanol, *n*-butanol, isobutanol, *n*-pentanol, glycerol	Nr	–
Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	+	+	+	+	–	+[b]	–
Succinate	–	–	–	+	–	+	–
Malate	+[d]	+	Nr	+	-	+[d]	-
Other	Alanine	Fructose, other sugars fermented	Furfural, furfuryl alcohol, 2-furoic acid, 4-hydroxybutyrate	Fructose	Cysteine	–	–
Fermentative growth	+	+	Nr	+	+	+	–
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	+	+	+	+	+	+
Thiosulfate	–	+	Nr	+	+	+	+
Sulfur[c]	+	+	Nr	+	–	+	+

Table 9.2 (continued)

Characteristic	*Desulfovibrio frigidus*	*Desulfovibrio fructosivorans*	*Desulfovibrio furfuralis*	*Desulfovibrio gabonensis*	*Desulfovibrio giganteus*	*Desulfovibrio gigas*	*Desulfovibrio gracilis*
Other	FeIII[d]	Fumarate	Nitrate	Fumarate	–	Fumarate, haloaromatic compounds	Fumarate
Literature	Vandieken et al. 2006, sp. nov.	Ollivier et al. 1990, sp. nov. ; Ollivier et al. 1988	Folkerts et al. 1989, sp. nov.; Folkerts et al. 1989	Tardy-Jacquenod et al. 1996, sp. nov. ; Ben Dhia Thabet et al. 2007	Esnault et al. 1988, sp. nov. ; Esnault et al. 1988; Caumette et al. 1991	Le Gall 1963, species. ; Boyle et al. 1999	Magot et al. 2004, sp. nov.
Characteristic	***Desulfovibrio halophilus***	***Desulfovibrio hydrothermalis***	***Desulfovibrio idahonensis***	***Desulfovibrio indonesiensis***	***Desulfovibrio inopinatus***	***Desulfovibrio intestinalis***	***Desulfovibrio litoralis***
Type strain	Strain SL 8903, ATCC 51179, DSM 5663	Strain AM13, CIP 107303, DSM 14728	Strain CY1, DSM 15450, JCM 14124	Strain Ind 1, DSM 15121, NCIMB 13468	Strain HHQ 20, DSM 10711	Strain KMS2, DSM 11275	Strain STL6, DSM 11393
Accession number of the 16S rRNA gene sequence of the type strain	U48243	AF458778	AJ582755	Y09504	AF177276	Y12254	X99504
Morphology	Vibrio	Vibrio	Curved rod	Rod	Vibrio	Vibrio	Curved rod
Cell size (μm)	0.6 × 2.5–5.0	0.5–1.0 × 1.0–2.0	0.6 × 1.3–2.5	0.5–1.2 × 3.0–5.0	1.0–1.5 × 4.0–12.0	0.4–0.5 × 1.0–1.4	0.4–0.6 × 1.8–2.7
Motility	+	+	+	+	+	+	+
Mol% G+C content	60.7	47.0	63.5	58.1	55.0	55.0	36.7
Major menaquinone	Nr	Nr	MK-6(H_2)	Nr	Nr	Nr	Nr
Optimal pH	6.5	7.8	6.5	6.8–7.2	6.9–7.1	Nr	7.2–7.4
Optimal temperature (°C)	35	35	28	10–37 (range)	30	37	28
Optimal NaCl concentration (g/l)	60–70	25	0–1	5–6	10–25	0.1	0.25
Growth factor requirement	–	Nr	–	Nr	–	–	–
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	+[a]	+[a]	Nr	+[a]	+[a]	+[a]
Formate	+[a]	+[a]	+[a]	Nr	+[a]	+[a]	+[a]
Acetate	–	–	–	–	–	–	–
Fatty acids	–	–	–	–	–	–	Nr
Ethanol	+	+	–	–	+	+	+
Other *n*-alcohols	*n*-propanol	Glycerol	–	–	–	–	Nr

Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	–	+	+	–	+	+	+
Succinate	–	–	+	Nr	–	+	–
Malate	–	+	+	–	+	+	–
Other	Serine	Choline	–	–	Hydroxyhydroquinone, fructose, ribose	Alanine	Nr
Fermentative growth	–	+	+	Nr	–	+	+
Disproportionation of reduced sulfur compounds	+[g]	Nr	Nr	Nr	Nr	Nr	–
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	+	+	Nr	+	+	+
Thiosulfate	+	+	+	Nr	+	+	+
Sulfur[c]	+	–	+	–	–	–	+
Other	–	–	FeIII, AQHS, Fumarate, DMSO, MnIV	–	–	–	Fumarate, O_2[d]
Literature	Caumette et al. 1991, sp. nov. ; Caumette et al. 1991	Alazard et al. 2003, sp. nov.	Sass et al. 2009, sp. nov.	Feio et al. 2000, sp. nov. , Feio et al. 1998; Ben Dhia Thabet et al. 2007	Reichenbecher and Schink 1999, sp. nov. ; Reichenbecher and Schink 1997.	Fröhlich et al. 1999, sp. nov. ; Fröhlich et al. 1999	Sass et al. 1998, sp. nov. ; Sass et al. 1998
Characteristic	***Desulfovibrio longreachensis***	***Desulfovibrio longus***	***Desulfovibrio magneticus***	***Desulfovibrio marinus***	***Desulfovibrio marinisediminis***	***Desulfovibrio marrakechensis***	***Desulfovibrio mexicanus***
Type strain	Strain AB16910a, ACM 3958	Strain SEBR 2582, ATCC 51456, DSM 6739	Strain RS-1, ATCC 700980, DSM 13731	Strain E-2, DSM 18311, JCM 14040	Strain C/L2, DSM 17456, JCM 14577, NBRC 101113	Strain EMSSDQ4, ATCC BAA-1562, DSM 19337	Strain Lup1, DSM 13116, JCM 15822
Accession number of the 16S rRNA gene sequence of the type strain	Z24450	AY359867	D43944	DQ365924	AB353727	AM947130	AF227984
Morphology	Vibrio	Rod	Vibrio	Vibrio	Vibrio	Rod	Curved rod
Cell size (μm)	0.5 × 2.0–4.0	0.4–0.5 × 5.0–10.0	1.0 × 3.0–5.0	0.5 × 1.5–2.5	0.7–1.0 × 1.0–3.5	1.3–1.6 × 3.0–4.0	0.5 × 1.7–2.5
Motility	+	+	+	+	+	–	–
Mol% G+C content	69.0	62.3	66.0	60.4	46.2	65.1	66.0
Major menaquinone	Nr	Nr	MK-7(H_2)	Nr	MK-6(H_2)	Nr	Nr
Optimal pH	7.0–7.4	7.4	7.0	7.0	7.5	7.0	7.2
Optimal temperature (°C)	37	35	30	37	37	37	37

Table 9.2 (continued)

Characteristic	*Desulfovibrio longreachensis*	*Desulfovibrio longus*	*Desulfovibrio magneticus*	*Desulfovibrio marinus*	*Desulfovibrio marinisediminis*	*Desulfovibrio marrakechensis*	*Desulfovibrio mexicanus*
Optimal NaCl concentration (g/l)	Nr	10–20	0–1	5	20–25	0	0
Growth factor requirement	Nr	Vitamins	Nr	Nr	–	Nr	-
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	+[a]	–	+[a]	+[a]	+[a]	+[a]
Formate	Nr	+[a]	–	+[a]	+[a]	+[a]	+[a]
Acetate	–	–	–	–	–	–	–
Fatty acids	–	–	–	–	–	–	–
Ethanol	Nr	–	+	+	+	+	+
Other *n*-alcohols	Nr	–	Glycerol	–	*n*-propanol, glycerol	*n*-propanol, *n*-butanol, isopropanol, 1,2-propanediol, 1,3-propnaediol, 2-methoxyethanol, 1,4-butandiol, glycerol	
Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	+	–	–	+	+	+	–
Succinate	Nr	–	Nr	+	+	–	–
Malate	–	–	+	+	+	+	–
Other	–	–	Oxaloacetate	Fructose	Alanine, glycine, serine, aspartate, casein, peptone, yeast extract	1,4 tyrosol	Serine, cysteine, choline, alanine, yeast extract, casamino acids
Fermentative growth	+	–	+	+	+	+	+
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	–	Nr	–
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	+	–	+	+	+	+
Thiosulfate	+	+	+	+	+	+	+

Sulfur[c]	Nr	+	–	+	–	+	+
Other	Fumarate	Fumarate	Fumarate	–	–	Fumarate	Fumarate, DMSO, FeIII
Literature	Redburn and Patel 1995, sp. nov. ; Redburn and Patel 1994	Magot et al. 1992, sp. nov.	Sakaguchi et al. 2002, sp. nov.	Ben Dhia Thabet et al. 2007, sp. nov.	Takii et al. 2008, sp. nov.	Chamk et al. 2009, sp. nov.	Hernandez-Eugenio et al. 2001, sp. nov. ; Hernandez-Eugenio et al. 2000; Sass et al. 2009; Pecheritsyna et al. 2012
Characteristic	*Desulfovibrio oceani ssp. galateae*	*Desulfovibrio oceani ssp. oceani*	*Desulfovibrio oxamicus*	*Desulfovibrio oxyclinae*	*Desulfovibrio paquesii*	*Desulfovibrio piezophilus*	*Desulfovibrio piger*
Type strain	Strain I.9.1, I9, DSM 21391, JCM 15971	Strain I.8.1, DSM 21390, JCM 15970	Strain Monticello 2, ATCC 33405, DSM 1925, NCIMB 9442	Strain P1B, DSM 11498	Strain SB1, DSM 16681, JCM 14635	Strain C1TLV30, DSM 21447, JCM 15486	Strain ATCC 29098, DSM 749
Accession number of the 16S rRNA gene sequence of the type strain	FJ655908	FJ655907	DQ122124	U33316	AY726757, DQ447183	HM852532	AF192152
Morphology	Vibrio	Vibrio	Vibrio	Curved rod	Vibrio	Vibrio	Rod
Cell size (μm)	0.3 × 1.0–1.5	0.3 × 1.0–1.5	0.4 × 2.0	0.5 × 2.0–3.0	1.0 × 5.0–8.0	0.5 × 2.0–4.0	0.8–1.3 × 1.2–5.0
Motility	+	+	+	+	+	+	–
Mol% G+C content	45.1	45.9	65.2	Nr	62.2	49.6	64.0
Major menaquinone	MK-6	Nr	Nr	Nr	Nr	Nr	MK-6
Optimal pH	7.0–8.0	7.0–8.0	7.2–7.4	6.8–7.0	6.5–8.5	7.3	7.0–7.2
Optimal temperature (°C)	20	20	37	30–37	10–45 (range)	30	37
Optimal NaCl concentration (g/l)	25–35	25–35	0–10	50–100	Nr	25	0–2
Growth factor requirement	Nr	Nr	Nr	–	Nr	Nr	Vitamins
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]
Formate	+[a]	+[a]	+[a]	+[a]	+[a]	+[a]	–
Acetate	–	–	–	–	–	–	–
Fatty acids	Nr	Nr	–	–	–	–	–
Ethanol	–	–	+	+	+	+	+

Table 9.2 (continued)

Characteristic	*Desulfovibrio oceani ssp. galateae*	*Desulfovibrio oceani ssp. oceani*	*Desulfovibrio oxamicus*	*Desulfovibrio oxyclinae*	*Desulfovibrio paquesii*	*Desulfovibrio piezophilus*	*Desulfovibrio piger*
Other *n*-alcohols	–	–	*n*-butanol	*n*-propanol, *n*-butanol	Glycerol	Nr	–
Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	+	+	–	–	+	+	–
Succinate	–	–	Nr	–	+	–	Nr
Malate	+	+	Nr	+	+	+	–
Other	Peptone, casamino acids, yeast extract, glycine	Peptone, casamino acids, yeast extract	Citrate, choline, oxamate, oxalate	–	–	–	–
Fermentative growth	+	+	+	+	+	+	–
Disproportionation of reduced sulfur compounds	–	–	Nr	+	Nr	Nr	Nr
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	+	Nr	+	+	+	Nr
Thiosulfate	+	+	Nr	+	+	+	Nr
Sulfur[c]	–	–	Nr	+	Nr	–	–
Other	Taurine, fumarate	Taurine, fumarate	Nitrate	– (O_2[d])	–	–	Nr
Literature	Finster and Kjeldsen 2012, sp. nov.; Finster and Kjeldsen 2012	Finster and Kjeldsen 2012, sp. nov.; Finster and Kjeldsen 2012	Postgate and Campbell 1966; Lopez-Cortes et al. 2006, comb. nov.; Postgate 1963	Krekeler et al. 2000, sp. nov.; Krekeler et al. 1997	van Houten et al. 2009, sp. nov.	Khelaifia et al. 2011, sp. nov.	Moore et al. 1976; Loubinoux et al. 2002, comb. nov.

Characteristic	*Desulfovibrio portus*	*Desulfovibrio profundus*	*Desulfovibrio psychrotolerans*	*Desulfovibrio putealis*	*Desulfovibrio salexigens*	*Desulfovibrio senezii*	*Desulfovibrio simplex*
Type strain	Strain MSL79, DSM 19338, JCM 14722	Strain 500-1, DSM 11384	Strain JS1, DSM 19430, JCM 14597, KCTC 5573	Strain B7-43, ATCC BAA-905, DSM 16056.	Strain ATCC 14822, DSM 2638, VKM B-1763	Strain CVL, DSM 8436, JCM 16814	Strain XVI, DSM 4141, JCM 16812
Accession number of the 16S rRNA gene sequence of the type strain	AB110541	FR733706	AM418397	AY574979	CP001649 (genome)	AF050100	FR733678
Morphology	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio
Cell size (μm)	0.7–1.0 × 1.8–2.3	0.5–1.0 × 1.0–2.0	0.3–0.8 × 0.5–2.0	0.7 × 2.3–4.1	0.5–0.8 × 1.3–2.5	0.3 × 1.0–1.3	0.5–1.0 × 1.5–3.0

Motility	+	+	+	+	+	+	+
Mol% G+C content	62.1	54.0	49.3	57.8	47.1	62.0	48.0
Major menaquinone	MK-6(H_2)	Nr	Nr	Nr	MK-6(H_2)	Nr	Nr
Optimal pH	6.5	7.0	8.5–9.0	7.0	7.8	7.6	7.0
Optimal temperature (°C)	35	25	28-30	30	30–36	37	37
Optimal NaCl concentration (g/l)	20	6–80	0–20	0–1	20–40	25	6–8
Growth factor requirement	Yeast extract	–	Vitamins	Nr	–	–	Nr
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources							
H_2/CO_2	+[a]	+[a]	–	+[a]	+[a]	+[a]	+[a]
Formate	+[a]	–	+[a]	–	+[a]	–	+[a]
Acetate	–	–	–	–	–	–	–
Fatty acids	–	–	–	–	–	–	–
Ethanol	+	–	+	+	+	–	+
Other *n*-alcohols	*n*-propanol, n-butanol	Nr	*n*-butanol	*n*-butanol	*n*-propanol, n-butanol	–	*n*-propanol, *n*-butanol
Lactate	+	+	+	+	+	+	+
Pyruvate	+	+	+	+	+	+	+
Fumarate	+	–	–	+	+	–	+
Succinate	+	Nr	+	–	+	–	–
Malate	+	–	+	+	+	–	+
Other	–	Toluene[h]	–	Glycine	–	–	Aromatic aldehydes[i]
Fermentative growth	+	+	Nr	+	–	–	Nr
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Electron acceptors used							
Sulfate	+	+	+	+	+	+	+
Sulfite	+	+	–	+	+	+	–
Thiosulfate	+	+	–	+	+	+	+
Sulfur[c]	Nr	–	–	Nr	–	–	Nr

Table 9.2 (continued)

Characteristic	*Desulfovibrio portus*	*Desulfovibrio profundus*	*Desulfovibrio psychrotolerans*	*Desulfovibrio putealis*	*Desulfovibrio salexigens*	*Desulfovibrio senezii*	*Desulfovibrio simplex*
Other	–	Nitrate, FeIII, DMSO, lignosulfonate, fumarate	Fumarate	–	Fumarate	Fumarate	Nitrate
Literature	Suzuki et al. 2010, sp. nov.; Suzuki et al. 2009	Bale et al. 1997, sp. nov.	Sasi Jyothsna et al. 2008, sp. nov.	Basso et al. 2005, sp. nov.	Postgate and Campbell 1966, species; Zellner et al. 1990	Tsu et al. 1999, sp. nov.; Tsu et al. 1998	Zellner et al. 1990, sp. nov.; Zellner et al. 1989

Characteristic	*Desulfovibrio sulfodismutans*	*Desulfovibrio termitidis*	*Desulfovibrio tunisiensis*	*Desulfovibrio vietnamensis*	*Desulfovibrio vulgaris*	*Desulfovibrio zosterae*
Type strain	Strain ThAc01, ATCC 43913, DSM 3696, VKM B-1764	Strain HI 1, ATCC 49858, DSM 5308	Strain RB22, DSM 19275, JCM 15076, NCIMB 14400	Strain G3 100, DSM 10520, JCM 15785	Strain ATCC 29579, CCUG 34227, CIP 107040, DSM 644, LMG 7563, NCAIM B.01489, VKM B-1760	Strain lac, DSM 11974
Accession number of the 16S rRNA gene sequence of the type strain	Y17764	X87409	EF577029	X93994	AF418179	Y18049
Morphology	Vibrio	Vibrio	Vibrio	Vibrio	Vibrio	Curved rod
Cell size (μm)	0.5–1.0 × 1.5–3.0	0.4 × 3.0	0.5 × 1.0–1.5	0.8–1.0 × 2.5–3.0	0.5–0.8 × 1.5–4.0	0.5 × 3.0
Motility	+	+	+	+	+	+
Mol% G+C content	48.0	67.5	59.6	60.6	65	42.7
Major menaquinone	Nr	Nr	Nr	Nr	MK-6	Nr
Optimal pH	7.3	6.6	7.0	7.5	7.0–7.4	6.8–7.3
Optimal temperature (°C)	30–35	37	37	37	30–36	32.5–34.5
Optimal NaCl concentration (g/l)	0–1	0–1	40	50	0.1	11.7
Growth factor requirement	Vitamins	Nr	Nr	Yeast extract	–	–
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources						
H_2/CO_2	+[a]	–	+[a]	Nr	+[a]	+[a]
Formate	–	+[a]	+[a]	+[a]	+[a]	+[a]
Acetate	–	–	–	–	–	–
Fatty acids	–	–	–	Nr	–	–
Ethanol	+	Nr	–	+	+	+

Other *n*-alcohols	*n*-propanol, n-butanol	Nr	Methanol, glycerol	Glycerol	*n*-propanol, *n*-butanol	–
Lactate	+	+	+	+	+	+
Pyruvate	–	+	+	+	+	+
Fumarate	–	Nr	+	+	+	+
Succinate	–	–	+	–	+/–	–
Malate	–	–	–	+	+	+
Other	–	Fructose, rhamnose, mannose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, shikimic acid	–	–	–	Choline, fructose, alanine
Fermentative growth	–	+	+	–	+	+
Disproportionation of reduced sulfur compounds	+	Nr	Nr	Nr	Nr	Nr
Electron acceptors used						
Sulfate	+	+	+	+	+	+
Sulfite	+	+	+	+	+	+
Thiosulfate	+	+	+	+	+	+
Sulfur[c]	–	+	+	Nr	–	–
Other	–	Nitrate	–	Fumarate	FeIII[d], CrVI[d]	Fumarate
Literature	Bak and Pfennig 1988, sp. nov.; Bak and Pfennih 1987	Trinkerl et al. 1991, sp. nov.; Trinkerl et al. 1990	Ben Ali Gam et al. 2009, sp. nov.	Dang et al. 2002, sp. nov.; Dang et al. 1996	Postgate and Campbell 1966, subspecies.	Nielsen et al. 1999, sp. nov.

[a]Only in the presence of acetate or yeast extract
[b]Only fermented, not coupled to sulfate reduction
[c]In most cases sulfur might be reduced to sulfide but is not coupled to growth
[d]Reduced but not coupled to growth
[e]Lithoautotrophic growth claimed in original description, this might not be the case
[f]Oxidation coupled to the reduction of 2-chlorophenol
[g]Not mentioned in the description, but positive test procedures indicate thiosulfate dismutation
[h]Highly questionable, if this substrate is really used
[i]Are oxidized to the corresponding acid, probably widespread among *Desulfovibrio* spp.

Table 9.3
Selected characteristics of the only member of the genus *Desulfobaculum* (a table is used for easier comparison with other genera)

Characteristic	*Desulfobaculum xiamenense*
Type strain	Strain P1, CGMCC 1.5166, DSM 24233
Accession number of the 16S rRNA gene sequence of the type strain	HQ697913
Morphology	Rod shaped
Cell size (μm)	0.6 × 1.5–2.2
Motility	+
Mol% G+C content	64.5
Major menaquinone	MK-7
Optimal pH	7.0
Optimal temperature (°C)	35–40
Optimal NaCl concentration (g/l)	5
Growth factor requirement	Nr
Oxidation of substrate	Incomplete[a]
Compounds used as electron donors and carbon sources	
H_2/CO_2	+[b]
Formate	–
Acetate	+[a]
Fatty acids	–
Ethanol	–
Other *n*-alcohols	–
Lactate	+
Pyruvate	+
Fumarate	+
Succinate	+
Malate	+
Others	Cellobiose, galactose, citrate, oxalate, cysteine, casamino acids, yeast extract
Fermentative growth	+
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	–
Sulfur[b]	–
Other	–
Literature	Zhao et al. 2012, gen. nov., sp. nov.

[a]Description claims oxidation of acetate which should be regarded as highly questionable
[b]Should only be possible in the presence of acetate or yeast extract

Table 9.4
Selected characteristics of the only member of the genus *Desulfocurvus* (a table is used for easier comparison with other genera)

Characteristic	*Desulfocurvus vexinensis*
Type strain	Strain VNs36, AS36, DSM 17965, JCM 14038
Accession number of the 16S rRNA gene sequence of the type strain	DQ841177
Morphology	Vibrio
Cell size (μm)	0.5 × 3.0–5.0
Motility	+
Mol% G+C content	67.2
Major menaquinone	Nr
Optimal pH	6.9
Optimal temperature (°C)	37
Optimal NaCl concentration (g/l)	0–20 (range)
Growth factor requirement	–
Oxidation of substrate	Incomplete
Compounds used as electron donors and carbon sources	
H_2/CO_2	–
Formate	+[a]
Acetate	–
Fatty acids	–
Ethanol	–
Other *n*-alcohols	–
Lactate	+
Pyruvate	+
Fumarate	–
Succinate	–
Malate	–
Others	–
Fermentative growth	+
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	+
Sulfur	–
Other	–
Literature	Klouche et al. 2009, gen. nov., sp. nov.

[a]Only in the presence of acetate

Table 9.5
Selected characteristics of the only member of the genus *Bilophila* (a table is used for easier comparison with other genera)

Characteristic	*Bilophila wadsworthia*
Type strain	Strain WAL 7959, ATCC 49260, CCUG 32349
Accession number of the 16S rRNA gene sequence of the type strain	AJ867049
Morphology	Rod shaped
Cell size (μm)	0.7–1.0 × 1.0–2.0
Motility	–
Mol% G+C content	Nr (other strains 59.2)
Major menaquinone	Nr
Optimal pH	Nr
Optimal temperature (°C)	35–37 (others not tested)
Optimal NaCl concentration (g/l)	Nr
Growth factor requirement	Requires complex media
Oxidation of substrate	Incomplete
Compounds used as electron donors and carbon sources	
H_2/CO_2	–
Formate	+[a]
Acetate	–
Fatty acids	Nr
Ethanol	Nr
Other n-alcohols	Nr
Lactate	–
Pyruvate	+
Fumarate	Nr
Succinate	Nr
Malate	Nr
Others	Nr
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	–
Sulfite	+[b]
Thiosulfate	+[b]
Sulfur	–
Other	Taurine, cysteate[b], nitrate[b]
Literature	Baron et al. 1990, gen. nov.,sp. nov.; Baron et al. 1989; Laue et al 1997

[a]Only in the presence of acetate or complex media
[b]Some strains

Table 9.6
Selected characteristics of the only member of the genus *Lawsonia* (a table is used for easier comparison with other genera). Electron donors, carbon sources, and electron acceptors are not known so far, because *Lawsonia intracellularis* seems to be an obligately intracellular parasite and cannot be grown as pure culture on defined media

Characteristic	*Lawsonia intracellularis*
Type strain	Strain 1482/89, NCTC 12656
Accession number of the 16S rRNA gene sequence of the type strain	L15739
Morphology	Vibrio or rod shaped
Cell size (μm)	0.3–0.4 × 1.5–2.0
Motility	+
Mol% G+C content	33.1
Major menaquinone	Nr
Optimal pH	Nr
Optimal temperature (°C)	35–37
Optimal NaCl concentration (g/l)	Nr
Growth factor requirement	Only intracellular growth documented
Oxidation of substrate	Nr
Literature	McOrist et al. 1995 gen. nov., sp. nov.

Desulfobaculum Zhao et al. 2012, 1574[VP]

De.sul.fo.ba´cu.lum. L. pref. *de*, from; L. n. *sulfur*, sulfur; L. neut. n. *baculum*, a stick or rod; N.L. neut. n. *Desulfobaculum*, a rod sulfate-reducing bacterium.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 9.3*.

Desulfocurvus Klouche et al. 2009, 3102[VP]

De.sul.fo.cur´vus. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating, used to characterize a dissimilatory sulfate-reducing prokaryote; L. adj. *curvus*, curved; N.L. masc. n. *Desulfocurvus*, a curved sulfate-reducing bacterium.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 9.4*.

Bilophila Baron et al. 1990, 320 (Effective Publication Baron et al. 1989, 3410)

Bi.lo'phi.la. L. n. *bilis*, bile; N.L. adj. *philus -a -um* (from Gr. adj. philos -ê -on), friend, loving; N.L. fem. n. *Bilophila*, bile-loving organism.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 9.5*.

Lawsonia McOrist et al. 1995, 824

Law.so'ni.a. N.L. fem. n. *Lawsonia*, named after Gordon H.K. Lawson, the Scottish veterinarian who first recognized the organism causing porcine proliferative enteropathy.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 9.6*.

Biolog

Biolog data are not available for sulfate-reducing bacteria.

Isolation, Enrichment, and Maintenance Procedures

All members of the family *Desulfovibrionaceae* require anoxic media for growth like all other sulfate-reducing bacteria. The media are prepared under specific conditions, and the addition of a reductant is required, in general sulfide, to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992) and might be modified by other authors as listed in the original descriptions of various taxa. The only known members of the genus *Bilophila* and *Lawsonia* are not able to use sulfate as electron acceptor and require specific media. *Lawsonia* seems to be an obligately intracellular and cell-dependent bacterium growing in intestinal cells of pigs (and other mammals) (McOrist et al. 1995; McOrist and Gebhart 2005). *Bilophila* can be isolated using complex media from human feces, saliva, and vaginal discharge and in periodontal pockets of dogs (Baron 2005).

For enrichment of members of the genera *Desulfovibrio*, *Desulfobaculum*, and *Desulfocurvus*, the used electron donor might be highly selective and will have a strong influence on what kind of sulfate-reducing bacteria will grow in the medium. H_2, formate, lactate, primary alcohols, and some dicarboxylic acids or amino acids might be useful for selective enrichment. As usual electron acceptor sulfate is used. In some cases, thiosulfate or sulfite might enrich for organisms capable to grow by disproportionation of these compounds. Most sulfate-reducing bacteria of this family have been enriched using batch cultures, other options are serial dilution techniques of natural samples. For isolation in general, roll-tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992; Kuever et al. 2005d).

For short-term preservation, stock cultures can be stored at 2–6 °C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. For long-term storage, cultures can be kept freeze dried, at –80 °C or in liquid nitrogen.

Ecology

Habitat

Members of this family were isolated from various sources including freshwater, brackish water, marine water, sewage sludge, hindgut of insects, animals, and humans.

Strain Essex 6, the type strain of Desulfovibrio desulfuricans ssp. desulfuricans, was isolated with lactate from a tar and sand mix around a corroded gas main, Essex, United Kingdom (Postgate and Campbell 1966). Strain W218, the type strain of *Desulfovibrio acrylicus*, was isolated with DMSP as electron donor and yeast extract as carbon source from anoxic intertidal sediment from the Wadden Sea near Westernieland, The Netherlands (van der Maarel et al. 1996). The type strain of *Desulfovibrio aerotolerans*, strain DvO5, was isolated with lactate as electron donor and carbon source from activated sludge from a wastewater treatment plant in Odder, Denmark (Mogensen et al. 2005). Strain Aspo-2, the type strain of *Desulfovibrio aerotolerans*, was isolated with lactate from deep subterranean granitic groundwater obtained from borehole KAS03 at a depth of 600 m located in South-Eastern Sweden (Motamedi and Pedersen 1998). The type strain of *Desulfovibrio africanus* ssp. *africanus*, strain Benghazi, was isolated with lactate from well water from Benghazi, Libya (Campbell et al. 1966). Strain SR-1, the type strain of *Desulfovibrio africanus* ssp. *uniflagellum*, was isolated with lactate from a uranium-contaminated shallow, subsurface aquifer in Shiprock, New Mexico, USA (Castaneda-Carrion et al. 2010). The type strain of *Desulfovibrio alaskensis*, strain Al1, was isolated with lactate as electron donor and carbon source from production water of a soured oilfield Pardu Bay, Alaska, USA (Feio et al. 2004).

Strain SPSN, the type strain of *Desulfovibrio alcoholivorans*, was isolated with lactate from a pilot fermenter containing alcohol distillery waste water, INRA, Norbonne, France (Qatibi et al. 1991). Strain RT2 was isolated from a biofilm attached to a steel coupon from a reactor connected to the return line of the Skanderborg district heating plant (Jutland, Denmark) using a substrate mix (glucose, pyruvate, lactate, propionate and acetate)(Abildgaard et al. 2008). Strain ALA-3, the type strain of *Desulfovibrio aminophilus*, was isolated with alanine as electron donor and carbon source and thiosulfate as electron acceptor from anoxic sludge of a dairy wastewater treatment plant (Santa Fe de Bogota, Columbia) (Baena et al. 1998). Lactate was used for the isolation of strain B15, the type strain of *Desulfovibrio arcticus*, from a cryopeg within permafrost on the Varandey Peninsula, on the southern coast of the Barent Sea, Russia (Pecheritsyna et al. 2012). Strain SRL4225, the type strain of *Desulfovibrio bastinii*, was isolated with lactate from a water sample collected in the oil field pipeline linking offshore production platforms of the Emeraude offshore oilfield (Congo) to onshore treatment facilities (Magot et al. 2004). The type strain of *Desulfovibrio bizertensis*, strain MB3, was isolated from enrichment cultures obtained with H_2/CO_2, yeast extract, or peptone in combination with acetate which were inoculated with anoxic marine sediment recovered

near Bizerte, Tunesia (Haoari et al. 2006). Strain HDv, the type strain of *Desulfovibrio burkinensis*, was isolated with lactate from an anoxic layer (10–25 cm) of ricefield soil of the Kou Valley in Burkina Faso (Ouattara et al. 1999).

Butyrate was used as substrate for the isolation of strain BSY, the type strain of *Desulfovibrio butyratiphilus*, from an anaerobic municipal sewage sludge digester, Yokohama, Japan (Suzuki et al. 2010). Strain EDK82, the type strain of *Desulfovibrio carbinolicus*, was isolated using a substrate mix of lactate, butyrate, propionate, and acetate from a purification plant, De Krim, Netherlands (Nanninga and Gottschal. 1987). The type strain of *Desulfovibrio carbinophilus*, strain D41, was isolated from a syntrophic methanogenic culture growing on benzyl alcohol which was inoculated from a gas condensate contaminated aquifer near Fort Lupton, CO, USA (Allen et al. 2008). The type strain of *Desulfovibrio cuneatus*, strain STL1, was isolated with lactate from the uppermost 30 mm of the sandy littoral sediment of Lake Stechlin, Germany (Sass et al. 1998). With 2-chlorophenol as electron acceptor and acetate as electron donor and carbon source strain SF3, the type strain of *Desulfovibrio dechloracetivorans*, was isolated from enrichment cultures obtained from Palo Alto Baylands Preserve on the west coast of San Francisco Bay, CA, USA (Sun et al. 2000). Strain Sylt 3, the type strain of *Desulfovibrio desulfuricans* ssp. *aestuarii*, was isolated with lactate from anoxic sediment obtained from the coast of the island of Sylt, Germany (Stüven 1960). The type strain of *Desulfovibrio ferrireducens*, strain 61, was isolated from an enrichment culture obtained from permanently cold surface sediment from Tempelfjorden, station CD (78° 25.267′ N 17° 8.277′ E), Svalbard, Norway using lactate as electron donor and carbon source and poorly crystalline iron oxide as electron acceptor (Vandieken et al. 2006).

The type strain of *Desulfovibrio frigidus*, strain 18, from an enrichment culture obtained from permanently cold surface sediment from Tempelfjorden, station CC (78° 26.039′ N 17° 19.772′ E), Svalbard, Norway using a mixture of lactate and formate as electron donor and carbon source and sulfate as electron acceptor (Vandieken et al. 2006). Strain JJ, the type strain, was isolated from an methanogenic consortium growing on sucrose obtained from estuarine sediment (location unknown) probably using lactate (Jones et al. 1984). The type strain of *Desulfovibrio fructosivorans*, strain F1, was isolated from a continuous fermented culture which converted the organic constituents of sulfite evaporator condensate to methane and carbon dioxide (Brune et al. 1983). The type strain of *Desulfovibrio gabonensis*, strain SEBR 2840, was isolated from a water sample taken from an oil pipeline linking offshore production platform to onshore treatment facilities in Gabon using lactate as substrate (Tardy-Jacquenod et al. 1996). Strain 8601, the type strain of *Desulfovibrio giganteus*, was isolated with lactate from anoxic sediment of Berre Lagoon ("Etang de Berre") near Marseile, France (Esnault et al. 1988). The type strain of *Desulfovibrio gigas*, strain ATCC 19364, was isolated from a water sample obtained from the same location using lactate as substrate (Le Gall 1963). The type strain of *Desulfovibrio gracilis*, strain SRL6146, was isolated with lactate from oilfield production water collected at the E153 wellhead of Emeraude oil field (Congo) (Magot et al. 2004).

The type strain of *Desulfovibrio halophilus*, strain SL 8903, was isolated with lactate from the upper photosynthetic microbial mat that developed at the sediment surface of Solar Lake, Sinai, Egypt (Caumette et al. 1991). Strain AM13, the type strain of *Desulfovibrio hydrothermalis*, was isolated from a hydrothermal chimney sample taken from the Grandbonum vent site at latitude 13° N along the East Pacific Rise at a depth of 2,600 m (Alazard et al. 2003). From a heavy metal contaminated sediment of Lake Coeur d'Alene, Idaho, USA, strain CY1, the type strain of *Desulfovibrio idahonensis*, was isolated using lactate as electron donor and carbon source (Sass et al. 2009). Strain Ind 1, the type strain of *Desulfovibrio indonesiensis*, was recovered from the biofilm of a heavily corroded hull of an oil storage vessel moored of the Indonesian coast using lactate as substrate (Feio et al. 1998.). Hydroxyhydroquinone was used as electron donor and carbon source for the isolation of strain HHQ20, the type strain of *Desulfovibrio inopinatus*, from anoxic marine sediment from Venice, Italy (Reichenbecher and Schink 1997). Strain KMS2, the type strain of *Desulfovibrio intestinalis*, was isolated with lactate from the hindgut of the lower termite *Mastotermes darwiniensis* Frogatt (Fröhlich et al. 1999). The type strain of *Desulfovibrio litoralis*, strain STL6, was isolated with lactate from the uppermost 30 mm of the sandy littoral sediment of Lake Stechlin, Germany (Sass et al. 1998).

Strain AB16910a, the type strain of *Desulfovibrio longreachii*, was isolated with lactate from a water sample obtained from a bore hole at a depth of 400 m from the deep aquifer Great Artesian Basin (Longreach district, Queensland, Australia) (Redburn and Patel 1994). A mixture of acetate, lactate, and yeast extract was used for the isolation of strain SEBR 2582, the type strain of *Desulfovibrio longus*, from an oil-producing well in the Paris Basin, France (Magot et al. 1992). Strain RS-1, the type strain of *Desulfovibrio magneticus*, was isolated from an iron-reducing enrichment obtained from freshwater sediment of Kameno River waterway (Wakayama, Japan) Sakaguchi et al. 2002). The type strain of *Desulfovibrio oxyclinae*, strain P1B, was isolated using ethanol as electron donor and carbon source from a cyanobacterial mat from the surface sediment of the Solar Lake, Sinai, Egypt) (Krekeler et al. 1997). Strain E-2, the type strain of *Desulfovibrio marinus*, was isolated from marine sediment contaminated by phosphogypsum disposal near Sfax, Tunesia, the substrate used was not mentioned (Ben Dhia Thabet et al. 2007). Casamino acids were used as electron donor and carbon source from the isolation of strain C/l2, the type strain of *Desulfovibrio marinisediminis*, from coastal marine sediment of the Tokyo Bay, Japan (Takii et al. 2008). The type strain of *Desulfovibrio marrakechensis*, strain EMSSDQ4, was isolated with lactate from olive mill wastewater obtained in Marrakech, Morocco (Chamk et al. 2009). Strain Lup1, the type strain of *Desulfovibrio mexicanus*, was isolated with lactate from an UASB reactor treating cheese factory water near Queretaro, Mexico (Hernandez-Eugenio et al. 2000).

Strain I.9.1, the type strain of *Desulfovibrio oceani* ssp. *galateae*, and strain 1.8.1, the type strain of *Desulfovibrio oceani* ssp. *oceani*, were isolated with lactate from the oxygen minimum zone off the coast of Peru at a depth of 400–500 m (13.52.310 S; 76.46.232 W) (Finster and Kjeldsen 2010). Oxamate was used as substrate for the isolation of strain Monticello 2, the type strain of *Desulfovibrio oxamicus*, from sediment of a stream close to Monticello, Illinois, USA (Postgate 1963). Using H_2 as electron donor and acetate as carbon source strain SB1, the type strain of *Desulfovibrio paquesii*, was isolated from the sulfidogenic sludge of a full-scale THIOPAQ gas lift reactor, Budel-Dorplein, Netherlands (van Houten et al. 2009). Strain C1TLV30, the type strain of *Desulfovibrio piezophilus*, was isolated with lactate from wood falls at a depth of 1693 m in the Mediterranean Sea, Nile Deep Sea Fan (32° 31' 97" N 30° 21'18" E) (Khelaifia et al. 2011). Strain ATCC 29098, the type strain of *Desulfovibrio piger*, was isolated with lactate from human feces Moore et al. 1976).

The type strain of *Desulfovibrio portus*, strain MSL79, was isolated with lactate as substrate from anoxic sediment collected at water depth of 2 m in the Niida river estuary in Sakata Harbor, Japan (Suzuki et al. 2009). Strain 500-1, the type strain *of Desulfovibrio profundus*, was isolated from deep marine sediment at a depth of 500 m obtained from the Japan Sea (ocean Drilling Program Leg 128, site 798B) using lactate as substrate (Bale et al. 1997). The type strain of *Desulfovibrio psychrotolerans*, strain JS1, was isolated with lactate as substrate from lake Pangong, Himalaya Leh District, and Jammu and Kashmir, India (Sasi Jyothsna et al. 2008). Strain B7-43, the type strain of *Desulfovibrio putealis*, was isolated with lactate as substrate from a well-collecting water from a deep aquifer at a depth of 430 m, Paris Basin, France (Basso et al. 2005). The type strain of *Desulfovibrio salexigens*, strain British Guiana, was isolated with lactate from sling mud of the coast of British Guyana (today Guyana, South America) (Postgate and Campbell 1966). Strain CLV, the type strain of *Desulfovibrio senzii*, was isolated with lactate as electron donor and carbon source from sediment obtained from the Western Salt Company solar saltern, Chula Vista, California, USA (Tsu et al. 1998). From an anaerobic sour whey digester (Germany) strain XVI, the type strain of *Desulfovibrio simplex*, was isolated with lactate as substrate (Zellner et al. 1989).

The type strain of *Desulfovibrio sulfodismutans*, strain ThAc01, was isolated with thiosulfate as electron donor and acceptor and acetate as carbon source from anoxic mud of the litoral zone Lake Constanze, Germany (Bak and Pfennih 1987). With a mixture of substrates including several sugars strain HI1, the type strain of *Desulfovibrio termiditis*, was isolated from the hindgut of the termite *Heterotermes indicola* (Wasmann) (Trinkerl et al. 1990). Strain RB22, the type strain of *Desulfovibrio tunisiensis*, was isolated from exhaust water of a Tunisian oil refinery, Bizerte, Tunisia using lactate as substrate (Ben Ali Gam et al. 2009). Strain G3 100, the type strain of *Desulfovibrio vietnamensis*, was isolated with lactate from production water near the offshore platform of the coastal town of Vung Tau in Southern Vietnam (Dang et al. 1996). Strain Hildenborough, the type strain of *Desulfovibrio vulgaris*, was isolated with lactate from soil obtained in Kent, United Kingdom (Postgate 1951). The type strain *of Desulfovibrio zosterae*, strain lac, was isolated with lactate from the roots of *Zostera marina*, Loegstoer Broad, Denmark (Nielsen et al. 1999).

The type strain of *Desulfobaculum xiamenensis*, strain P1, was isolated with a medium containing yeast extract, tryptone, casein, and beef extract from a mangrove sediment sample collected from Qinglan Port area, Hainan, China (Zhao et al. 2012).

The type strain of *Desulfocurvus vexinensis*, strain VNs36, was isolated with lactate from a well that collected water from a deep saline aquifer used for gas storage at a depth of 830 m in Paris Basin, France (Klouche et al. 2009).

The type strain of *Bilophila wadsworthia*, strain WAL 7959, was isolated from gangrenous, perforated, inflamed, and non-inflamed appendices, and also from normal human feces on Brucella blood agar (Baron et al. 1989).

The type strain of *Lawsonia intracellularis*, strain 1482/89, was obtained from a pig with proliferative enteropathy and is cocultured in a rat enterocyte cell line that was infected with a suspension of bacteria obtained from the infected pig (McOrist et al. 1995).

Pathogenicity, Clinical Relevance

There are several isolates of *Desulfovibrio* which have been isolated from humans or animals. For members of the genera *Bilophila* and *Lawsonia*, the clinical relevance is well documented (McOrist and Gebhart 2005; Baron 2005). *Lawsonia intracellularis* is known to cause proliferative enteropathy in pigs and a few other mammals (McOrist and Gebhart 2005).

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10 The Family *Desulfurellaceae*

Anthony C. Greene
School of Biomolecular and Physical Sciences, Griffith University, Brisbane, QLD, Australia

Abstract
The family *Desulfurellaceae* is part of the class *Deltaproteobacteria* in the phylum Proteobacteria and contains the genera *Desulfurella* and *Hippea*. There are seven valid species in the family which are all strictly anaerobic and moderately thermophilic bacteria. Members of the family are mostly obligately sulfur respiring, though *Desulfurella propionica* can use thiosulfate as an electron acceptor besides elemental sulfur. During growth, organic substrates are completely oxidized with CO_2 and H_2S as products. Lithotrophic growth with hydrogen as an electron donor is possible by all species except *Desulfurella acetivorans*. The genomes of *D. acetivorans, Hippea maritima, Hippea alviniae*, and *Hippea* sp. strain KM1 have been sequenced and are all slightly smaller than 2 mega base pairs in length. Members of the family are found mostly in warm-hot sulfur-containing anoxic environments, such as hydrothermal springs and sediments, and often in association with microbial mats. The DNA base composition (mol% G+C) is typically low for family members and is a distinguishing characteristic between the two genera, *Desulfurella* (31–33 mol%) and *Hippea* (35–40 mol%). While officially classified as part of *Deltaproteobacteria*, the family is more closely related phylogenetically to members of the class *Epsilonproteobacteria*.

Introduction

The class *Deltaproteobacteria*, a phenotypically distinct mixture of aerobic and anaerobic bacteria, contains eight valid orders and three suborders. The anaerobes within *Deltaproteobacteria* have a vital role in cycling sulfur, metals, and organic compounds in many anoxic environments. The ability to reduce elemental sulfur is a trait widespread across the prokaryotic domains: however, many use sulfur as an electron sink in fermentation reactions and not all are capable of dissimilatory sulfur reduction (Rabus et al. 2006). Dissimilatory sulfur reduction is an anaerobic energy yielding process where elemental sulfur is converted to hydrogen sulfide coupled to the oxidation of a suitable electron donor. Many significant dissimilatory sulfur-reducing bacteria belong to *Deltaproteobacteria*, particularly those that completely oxidize organic substrates such as all members of *Desulfurellales* and some *Desulfuromonadaceae* and *Geobacteraceae*.

Elemental sulfur is common in volcanically heated environments including solfataric fields, hot springs, and hydrothermal vents. As such, many thermophilic and hyperthermophilic sulfur-reducing bacteria and archaea can be found in these environments. Sulfur reduction is a common trait among deeply branching hyperthermophilic bacteria such as *Aquificales* and archaea from the *Euryarchaeota* and *Crenarchaeota* phyla, often linked to the use of hydrogen as an electron donor (Bonch-Osmolovskaya 2005). *Desulfurellales* is a bacterial order belonging to *Deltaproteobacteria*, in which all members are moderately thermophilic sulfur-respiring bacteria, which is unique in the domain *Bacteria*. The order contains the single family of *Desulfurellaceae*. This chapter describes current knowledge of the taxonomy, characteristics, ecology, and applications of the family *Desulfurellaceae*.

Taxonomy, Historical and Current

Short Description of the Family

De.sul.fu.rel.la'ce.ae. N. L. fem. n. *Desulfurella* type genus of the family; *-aceae* ending to denote family; N. L. fem. pl. n. *Desulfurellaceae* the *Desulfurella* family. The description is a

E. Rosenberg et al. (eds.), *The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria*, DOI 10.1007/978-3-642-39044-9_312,

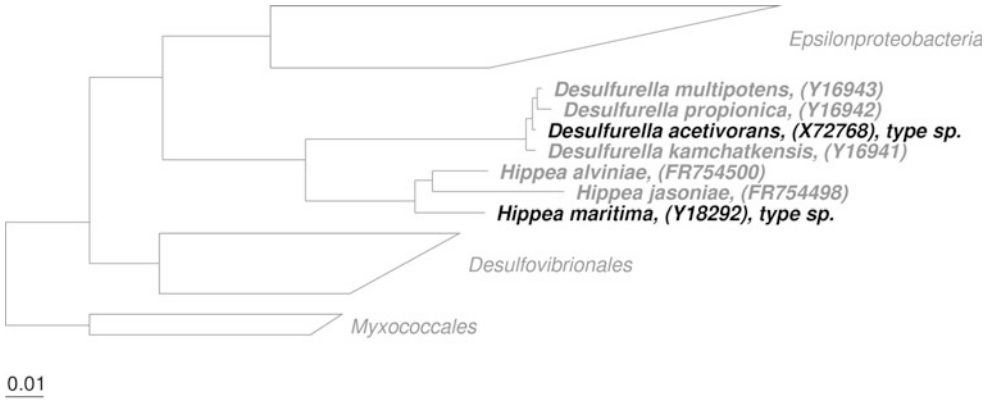

Fig. 10.1

Neighbor-joining phylogenetic tree of the family *Desulfurellaceae*. Sequences for all members were from the LTP database (Munoz et al. 2011). Trees have been filtered for hypervariable positions below 40 % conservation threshold. Bar indicates 0.1 substitution per nucleotide position. The list of type strains used for tree construction: *Desulfurella acetivorans* A63^T, *Desulfurella multipotens* RH-8^T, *Desulfurella propionica* U-8^T, *Desulfurella kamchatkensis* K-119^T, *Hippea maritima* MH$_2^T$, *Hippea jasoniae* Mar08-272r^T, *Hippea alviniae* EP5-r^T

modified version of that given in *Bergey's Manual of Systematic Bacteriology*, 2nd edition (Kuever et al. 2005a).

Phylogenetically, the family is a member of the order *Desulfurellales* (Kuever et al. 2005b), class *Deltaproteobacteria*, and phylum Proteobacteria. The family contains the type genus *Desulfurella* (Bonch-Osmolovskaya et al. 1990) and *Hippea* (Miroshnichenko et al. 1999). There are seven valid species across the two genera in the family.

Cells stain Gram negative and are strictly anaerobic. All species are moderate thermophiles. They are mostly motile and short rod or ovoid in shape. Motility occurs by means of a single polar flagellum. Growth is mainly respiratory with sulfur used as an electron acceptor by all members. Hydrogen and fatty acids are the main electron donors. Most species are neutrophiles though some are moderately acidophilic. The G+C content of DNA is relatively low ranging from 31 to 41 mol%. Species are typically found in sulfur-rich warm sediments or hydrothermal vents and often associated with thermophilic microbial communities.

Phylogenetic Structure of the Family

Current taxonomic status has the family *Desulfurellaceae* forming a distinct deep branch of the class *Deltaproteobacteria* and the sole member of the order *Desulfurellales* (Kuever et al. 2006). However, the phylogenetic tree reveals that the family is more closely related to the members of the class *Epsilonproteobacteria* (Fig. 10.1), in particular families *Nautiliaceae, Helicobacteraceae, and Campylobacteraceae*, rather than Deltaproteobacteria. The most related families of *Deltaproteobacteria* are *Desulfovibrionaceae, Desulfonatronaceae, Desulfomicrobiaceae*, and *Desulfohalobiaceae*. The four *Desulfurella* species are closely related (>99 % similarity) and are quite distinct from *Hippea* (85–88 % similarity). In fact, the species of *Hippea* vary considerably in pairwise similarity to each other at 95–96 % (Flores et al. 2012).

The origin of the family was with the isolation of *Desulfurella acetivorans* (Bonch-Osmolovskaya et al. 1990). Phenotypically, the genus *Desulfurella* was similar to *Desulfuromonas* with the new genus proposed primarily on the basis of differences in G+C content of DNA and absence of cytochromes. Subsequent 16S rDNA analysis revealed that *Desulfurella* formed a distinct branch as part of the epsilon class of *Proteobacteria* (Rainey et al. 1993) and was considerably different phylogenetically to *Desulfuromonas*. The isolation of the other three species in the genus, *D. multipotens, D. kamchatkensis*, and *D. propionica*, revealed a cohesive closely related grouping separated on the basis of DNA-DNA hybridization (Miroshnichenko et al. 1994, 1998). The genus *Hippea* was proposed with the isolation of *Hippea maritima* (Miroshnichenko et al. 1999). *Hippea* was the most related genus to *Desulfurella* but was still substantially different phylogenetically (88 % similarity). Subsequent isolation of *H. jasoniae* and *H. alviniae* strains again revealed substantial differences to *Desulfurella* species (85–88 % similarity) but a closer relationship to *H. maritima* (94 and 96 %, respectively).

Molecular Analyses

The description of the family *Desulfurellaceae* has been based largely on physiological and 16S rRNA sequence comparisons. Relatively few molecular studies have been done on the isolates. Whole genome sequencing has been completed on *Hippea maritima, Hippea alviniae*, and *Desulfurella acetivorans*. An additional strain, *Hippea medeae*, has been sequenced as well. However, *H. medeae*, which is most related to *H. maritima* (98.5 %), has yet to be validated as a species of *Hippea*. All genomes sequenced so far are relatively small of less than 2 mega base pairs in size and have low G+C content.

The genome of *Hippea maritima* MH_2^T was the first in the family to be fully sequenced (GOLD ID Gc01705). The genome, completed by US Department of Energy Joint Genome Institute and DSMZ, is 1,694,430 base pairs in length with 1,723 protein coding and 57 RNA genes in one circular chromosome (Huntemann et al. 2011). The G+C content of DNA is 37.5 %. Over 76 % of the genes have a predicted function. According to clusters of orthologous group (COG) categories, the highest numbers of genes are associated with amino acid transport and metabolism at 9.07 % (150) followed by translation, ribosomal structure, and biogenesis at 8.16 % (135). A significant proportion of genes were for essential functions, which is consistent with the relatively small genome size.

The full genome of *Hippea alviniae* EP5-r^T has been sequenced in permanent draft form (GOLD ID Gi14057). The genome was completed by the US Department of Energy Joint Genome Institute and is 1,749,386 base pairs in length with a G+C ratio of 37.0 %. There are 1,814 open reading frames with 1,757 protein coding genes and 57 RNA genes. Over 73 % of the genes have a predicted function. Similar to *H. maritima*, the largest numbers of genes are associated with amino acid transport and metabolism at 8.89 % (148) followed by genes for translation, ribosomal structure, and biogenesis at 8.17 % (136), according to the highest clusters of orthologous group (COG) categories. In addition, the importance of electron transport is clearly exhibited with an abundance of genes involved.

The genome of *Hippea medeae* strain KM1 also has been sequenced in permanent draft form (GOLD ID Gi14058). The genome was completed by the US Department of Energy Joint Genome Institute and is 1,742,311 base pairs in length with a DNA base composition slightly higher than other species in the genus (42.9 mol% G+C). There are 1,776 open reading frames with 1,719 protein coding genes and 57 RNA genes. Over 86 % of the genes have a predicted function. Like *H. maritima*, almost the same numbers of genes are associated with amino acid transport and metabolism at 8.97 % (150) and translation, ribosomal structure, and biogenesis at 8.13 % (136), which are the highest clusters of orthologous group (COG) categories. Again, the importance of electron transport is clearly exhibited with an abundance of genes involved.

Genomic sizes of the type stains of *Desulfurella acetivorans* (1,939 kilobase pairs) and *D. multipotens* (1,894 kilobase pairs) were determined through macrorestriction fragment analysis using pulsed field gel electrophoresis (Pradella et al. 1998). Further analysis revealed a high degree of genetic similarity between the two. Later, whole genome sequencing of *D. acetivorans* $A63^T$ revealed a slightly smaller genome size of 1,819,992 based pairs with 1,819 protein coding and 56 RNA genes (GOLD ID Gi03019). The genome was completed by the US Department of Energy Joint Genome Institute and has a DNA base composition of 32.1 mol% G+C. Over 85 % of the genes have a predicted function. According to clusters of orthologous group (COG) categories, the highest numbers of genes are associated with energy production and conversion at 9.98 % (177). The next most abundant genes were similar in the number of genes to *H. maritima*; next most abundant were amino acid transport and metabolism at 7.95 % (141) then translation, ribosomal structure, and biogenesis genes at 7.50 % (133).

DNA-DNA hybridization studies were done to compare a number of *Desulfurella* isolates (Miroshnichenko et al. 1994, 1998). The highest degree of relatedness was between *D. acetivorans* and *D. multipotans* (69 %), followed by *D. acetivorans* and *D. propionica* (55 %), *D. acetivorans* and *D. kamchatkensis* (40 %), and *D. kamchatkensis* and *D. propionica* (32 %). *D. multipotans* was not compared with *D. kamchatkensis* or *D. propionica*. Likewise, none of the *Hippea* species have been compared.

Phenotypic Analyses

Desulfurella Bonch-Osmolovskaya, Sokolova, Kostrikina, and Zavarzin 1993, 624^{VP}, (Effective Publication: Bonch-Osmolovskaya et al. 1990, 155), Emend. Miroshnichenko, Rainey, Hippe, Chernyh, Kostrikina, and Bonch-Osmolovskaya 1998, 478

De.sul.fur.el'la. L. pref. *de* from L. n. *sulfur* sulfur; N. L. dim. ending *-ella*; N. L. fem. n. *Desulfurella* sulfur-reducing bacterium.

There are four valid species in the genus. The type species is *Desulfurella acetivorans*, type strain $A63^T$, and was isolated from a thermophilic cyanobacterial mat found in the Uzon caldera in Kamchatka, Russia. Cells are Gram negative, with an outer S-layer. Strictly anaerobic short rods are variable in length and mostly motile by means of a single polar flagellum (◉ *Table 10.1*). *D. propionica* is the only nonmotile species. Moderate thermophiles with a temperature range between 45 °C and 80 °C and an optimum of 55–60 °C. Growth is mainly respiratory with sulfur used as an electron acceptor by all members. Hydrogen, acetate, pyruvate, palmitate, and stearate are used as electron donors by all four species. Lactate, fumarate, and malate are used by *D. kamchatkensis* and *D. propionica*. Propionate is used by only *D. propionica* and butyrate by *D. multipotans*. Organic substrates are completely oxidized with H_2S and CO_2 as products. *D. propionica* can also ferment pyruvate and use thiosulfate as an electron acceptor. Yeast extract is required for the growth of *D. acetivorans* but not for the other three species. Cytochromes are absent but menaquinones are present. Species are neutrophiles, though *D. acetivorans* can grow at moderately acidic conditions.

Hippea Miroshnichenko, Rainey, Rhode, and Bonch-Osmolovskaya 1999, 1037^{VP}

Hip'pea. L. fem. n. named after Hans Hippe, a German microbiologist, in recognition of his significant contribution to the characterization of new, obligately anaerobic prokaryotes and the understanding of their physiology.

Table 10.1
A comparison of the major characteristics of species in the genus *Desulfurella*

Characteristic	*D. acetivorans*[a]	*D. multipotens*[b]	*D.kamchatkensis*[c]	*D. propionica*[c]
Motility	+	+	+	–
Width (μm)	0.5–0.7	0.5–0.7	0.5–0.8	1.0
Length (μm)	1–2	1.5–1.8	1.5–2.0	1.5–2.0
Temperature optimum (°C)	52–57	58–60	54	55
Temperature range (°C)	44–70	42–77	40–70	33–63
pH optimum	6.8–7.0	6.4–6.8	6.9–7.2	6.9–7.2
pH range	4.2–7.6	6.0–7.2	ND	ND
DNA composition (mol% G+C)	31.4	33.5	31.6	32.2
Electron donors				
H_2	–	+	+	+
Acetate	+	+	+	+
Lactate	–	–	+	+
Propionate	–	–	–	+
Butyrate	–	+	–	–
Fumarate	–	–	+	+
Malate	–	–	+	+
Pyruvate	+	+	+	+
Palmitate	+	+	+	+
Stearate	+	+	+	+
Electron acceptors				
Sulfur	+	+	+	+
Thiosulfate	–	–	–	+
Sulfate	–	–	–	–
Sulfite	–	–	–	–
Nitrate	–	–	–	–
Fe(III)	–	ND	–	–
Mn(IV)	–	ND	ND	ND

ND not determined
[a]Bonch-Osmolovskaya et al. (1990)
[b]Miroshnichenko et al. (1994)
[c]Miroshnichenko et al. (1998)

There are three valid species in the genus. The type species is *Hippea maritima*, type strain MH_2^T, and was isolated from hot vents in the tidal zone of Matupi Harbour, Papua New Guinea, and shallow-water hot vents of the Bay of Plenty, New Zealand. Cells are rod-shaped to ovoid typically ranging 0.3–1.0 μm wide and 0.7–3.5 μm long (*Table 10.2*). Gram negative, strictly anaerobic, and moderate thermophilic with growth between 40 °C and 75 °C. Neutrophiles to obligate acidophiles. *H. maritima* optimum is pH 5.8–6.2, while *H. jasoniae* and *H. alviniae* is pH 4.5–5.0. Growth is respiratory with sulfur used as the sole electron acceptor. Other potential electron acceptors that were tested but did not support growth were sulfate, sulfite, thiosulfate, nitrate, and Fe(III). Lithotrophic growth occurs with hydrogen as the electron donor. Other electron donors used by all three species were acetate, fumarate, and yeast extract. In addition, *H. maritima* uses stearate, palmitate, and ethanol, while *H. jasoniae* and *H. alviniae* use succinate, propionate, and casamino acids. Organic substrates are oxidized with H_2S and CO_2 as products. Substrates not used by any of the species include pyruvate, lactate, formate, butyrate, glucose, starch, methanol, and peptone. All species require NaCl (1–5 % w/v) for growth. Yeast extract (0.02 % w/v) or vitamins are required for growth.

Isolation, Enrichment, and Maintenance Procedures

Members of the family *Desulfurellaceae* have been routinely enriched and isolated in anaerobically prepared media (Ljungdahl and Wiegel 1986). A suitable CO_2-bicarbonate buffered mineral salts medium supplemented with elemental sulfur

Table 10.2

A comparison of the major characteristics of species in the genus *Hippea*

Characteristic	*H. maritima*[a]	*H. jasoniae*[b]	*H. alvinia*[b]
Motility	+	+	+
Width (μm)	0.4–0.8	0.5–0.7	0.3–0.6
Length (μm)	1.0–3.0	2.0–3.5	0.7–1.5
Temperature optimum (°C)	52–54	60–65	60
Temperature range (°C)	40–65	40–72	45–75
pH optimum	6.0	4.5–5.0	4.5–5.0
pH range	5.4–6.5	3.5–5.0	3.5–5.5
NaCl requirement (%)	2.5–3.0	1–6	1–5
DNA composition (mol% G+C)	40.4	35.6	37.1
Substrates			
H_2	+	+	+
Yeast extract	+	+	+
Casamino acids	–	+	+
Acetate	+	+	+
Fumarate	+	+	+
Succinate	–	+	+
Propionate	–	+	+
Ethanol	+	–	–
Palmitate	+	ND	ND
Stearate	+	ND	ND

ND not determined

[a]Miroshnichenko et al. (1999)

[b]Flores et al. (2012)

as electron acceptor and a suitable electron donor such as acetate or hydrogen is used. *Hippea* species are marine isolates so NaCl addition is a requirement in their enrichment. As all members of the family are moderate thermophiles, incubation temperature is 55–60 °C. Any special conditions existing in the habitat should be factored in to the medium or incubation conditions.

A suitable basal salts medium that can be used for enrichment of *Desulfurellaceae* isolates was defined by Miroshnichenko and colleagues (1998, 1999). The components are as follows (g/l of distilled water): NH_4Cl, 0.33; KCl, 0.33; KH_2PO_4, 0.33; $CaCl_2.2H_2O$, 0.33; $MgCl_2.6H_2O$, 0.33; yeast extract, 0.1; $Na_2S.9H_2O$, 0.5; $NaHCO_3$, 1.5; and resazurin, 0.0002. Elemental sulfur can be added as flowers of the sulfur aqueous suspension at a final concentration of 10 g/l. Organic substrates such as acetate are added at 5 g/l. For *Hippea* enrichments, NaCl is added at a final concentration of 25 g/l. The pH is adjusted to pH 6.5 with 2.5 M H_2SO_4. However, pH may be altered depending on the sample conditions, for example, *H. jasoniae* and *H. alviniae* were isolated from deep-sea hydrothermal sulfur deposits with thermoacidophilic microorganisms at pH 4.5 targeted. The medium is dispensed under N_2-CO_2 (80:20) gas mixture into either serum bottles with butyl rubber stoppers or hungate tubes with a 30–50 % headspace gas to medium ratio. When molecular hydrogen is used as substrate, the headspace is filled with H_2-CO_2 gas mixture (80:20) in place of N_2-CO_2.

After autoclaving, sterile solutions of trace elements (1 ml/l) and vitamins (1 ml/l) are added. Trace elements such as those devised by Widdel and Pfennig (1981) can be used and are composed of (per liter) HCl (25 %), 10 ml; $FeCl_2.4H_2O$, 1.5 g; $CoCl_2.6H_2O$, 190 mg; $MnCl_2.4H_2O$, 100 mg; $ZnCl_2$, 70 mg; H_3BO_3, 62 mg; $Na_2MoO_4.2H_2O$, 36 mg; $NiCl_2.6H_2O$, 24 mg; and $CuCl_2.2H_2O$, 17 mg. The vitamin solution (Wolin et al. 1963) is composed of (milligrams per liter) biotin, 2; folic acid, 2; pyridoxine hydrochloride, 10; riboflavin, 5; thiamine, 5; nicotinic acid, 5; pantothenic acid, 5; vitamin B_{12}, 0.1; p-aminobenzoic acid, 5; and thioctic acid, 5. Suspensions and soluble substrates also are added from sterile anoxic stock solutions.

Enrichments are initiated by adding samples directly to fresh media. Microbial mat samples need to be mixed thoroughly or homogenized to break up the mat structure. The time taken for positive enrichments is generally 3–5 days. Positive enrichments are shown by the appearance of turbidity and confirmed by microscopy and also in many cases by accumulation of hydrogen sulfide. Pure cultures are isolated by serial dilutions and from roll tube solidified with agar or Gelrite using a fine suspension of elemental sulfur or polysulfide. Colonies are typically white-greyish, translucent, round or irregular shaped, and

0.5–2 mm in diameter. Cultures can be maintained at room temperature or in the refrigerator and should be transferred at least monthly in the short term. Longer-term storage is in liquid nitrogen or lyophilized.

Ecology

Members of *Desulfurellaceae* inhabit anaerobic freshwater or marine environments, in particular warm-hot sulfurous springs, sediments, and hydrothermal vents. All members have an important role ecologically in being able to mineralize organic compounds, reduce sulfur as part of the sulfur cycle, and often are associated with microbial communities in moderately thermophilic environments.

D. multipotans was isolated from hot sediments and cyanobacterial mats from shallow-water hot vents of Green Lake, Raoul Island, Kermadec archipelago, New Zealand (Miroshnichenko et al. 1994). Prior to that, *D. acetivorans* also had been isolated from a thermophilic cyanobacterial mat found in the Uzon caldera in Kamchatka peninsula in Russia (Bonch-Osmolovskaya et al. 1990). Large numbers of *D. acetivorans* cells indicated an active role in the community possibly in the degradation of cyanobacterial mass (Bonch-Osmolovskaya 2005). Subsequently, *D. kamchatkensis* and *D. propionica* were also isolated from the Kamchatka thermal areas. *D. kamchatkensis* was found associated with *Thermothrix thiopara* from Pauzhetka hot spring and *D. propionica* from cyanobacterial mat from sulfide-rich hot pond in the Uzon caldera (Miroshnichenko et al. 1998). When microbial communities of the thermoacidophilic (pH 4 and 50 °C) groundwaters of the East Thermal Field of Uzon Caldera were examined, 70 % were Archaea and *Desulfurella* represented only 0.7 % of all bacterial sequences (Mardanov et al. 2011). This suggests that, while present, *Desulfurella* require specialized niches such as microbial mats or may be in lower percentages due to the acidic conditions.

The latter is unlikely as many natural sulfurous environments can be acidic as well as thermophilic, and members of the *Desulfurellaceae* have been regularly detected in these environments. Members of *Desulfurellaceae* range from mildly acid tolerant, e.g., *D. multipotens*, to acidophilic, e.g., *H. jasoniae* and *H. alvinia*. Uncultured *Desulfurella* species were detected as dominant members of yellow and brown microbial mat communities in a thermal (58–62 °C) spring in Norris Basin, Yellowstone National Park (Jackson et al. 2001). These mats were arsenite oxidizing and highly acidic (pH 3.1), and the authors suggested that the phylotypes were likely to represent new acidophilic species. Likewise, Hall and colleagues (2008) found a phylotype branching close to *D. kamchatkensis* in a bacterial community at Coffee Pots Hot Spring, also at Yellowstone National Park, which had a temperature range of 39.3 to 74.1 °C and pH range of 5.75 to 6.91. Bacteria from the *Desulfurella* group (rRNA sequence similarity *D. kamchatkensis* 98.2 %, *D. multipotens* 98.1 %, *D. acetivorans* 97.8 %, *D. propionica* 97.8 %) were also detected in moderate temperature (48 °C) acidic pools in the geothermal springs on the Caribbean island of Montserrat (Burton and Norris 2000). *Desulfurella* were found to be present in dam sediment cores with pH 3.9–5.7 from the highly acidic Tinto River in Spain (Sánchez-Andrea et al. 2011). Heat from the exothermic oxidation of pyrite and depth gave the mild thermophilic conditions suitable for *Desulfurellaceae*. The *Desulfurella* phylotypes were present only in the deeper sediments where the pH was higher.

Members of the family have also been detected in marine environments, in particular *Hippea* species. *H. maritima* was first isolated from hot vents in the tidal zone of Matupi Harbour, Papua New Guinea, and shallow-water hot vents of the Bay of Plenty, New Zealand (Miroshnichenko et al. 1999). More recently, *H. jasoniae* and *H. alviniae* were isolated from thermal sulfide deposits in a deep-sea hydrothermal vent field along the mid-Atlantic ridge in Atlantic Ocean (Flores et al. 2012). *H. maritima* and *D. kamchatkensis* and related uncultured *Desulfurellaceae* have been found as part of a bacterial community in CO_2-hydrate bearing deep-sea sediments at the Yonaguni Knoll IV hydrothermal field at the southern Okinawa Trough, Japan (Inagaki et al. 2006). *Desulfurella* also were detected as a major part of the dominant Deltaproteobacteria phylotypes in sediment samples from the Amsterdam mud volcano, East Mediterranean Sea (Pachiadaki et al. 2011). Similarly, community analysis of a deep-sea hydrothermal vent at Brothers volcano off the north coast of New Zealand revealed that Deltaproteobacteria were a substantial proportion of the bacterial population and that the *Desulfurella-Hippea* grouping was a significant part and the only sulfur-reducing phylotypes detected (Stott et al. 2008). Thermophilic anaerobic oxidation of methane by marine microbial consortia enriched from a hydrothermal vent site in the Guaymas Basin, in the Gulf of California off Mexico, was dominated by a lineage most closely related to *Desulfurella* spp. and *H. maritima* (Holler et al. 2011).

Petroleum reservoirs are known to be a rich source of thermophilic anaerobic respirers (Orphan et al. 2000; Greene et al 1997). However, no members of *Desulfurellaceae* had been detected until Liu and colleagues (2009) found bacteria related to *H. maritima* in the production water from Changqing Oil Field in Northwest China.

Pathogenicity, Clinical Relevance

No members of the family *Desulfurellaceae* are pathogenic. All are bacteria isolated from natural anoxic environments typically of volcanic origin at moderately high temperatures which are generally not conducive for pathogen growth or survival. All *Desulfurellaceae* bacteria are risk group 1 bacteria.

Applications

Currently there are no direct applications of *Desulfurellaceae*. However, a wide variety of anaerobic respiring bacteria have been investigated for potential applications in bioremediation.

The ability to degrade organic contaminants and reduce toxic metals is seen as traits that have possible applications in anoxic environments such as contaminated groundwaters and sediments. Anaerobic respirers such as *Geobacter metallireducens, G. sulfurreducens, and Shewanella oneidensis* have been the subject of great interest due to their ability to degrade aromatic hydrocarbons and reduce metals such as uranium, cobalt, and chromium. Other anaerobes such as *Dehalococcoides ethanogenes* are capable of reductive chlorination of chlorinated solvents. The ability of *Desulfurellaceae* members to degrade organic contaminants is yet to be tested, as is the reduction of toxic metals which can serve as alternative electron acceptors to sulfur in numerous anaerobic respirers. *Desulfurella* have been detected as part of communities in lactate-fed acidic mineral processing wastewater treatment reactors (Kaksonen et al. 2004). *Desulfurella* were found also as major microbial players of a multistage bioremediation system utilizing composted agricultural waste products for the purification of acidic and iron- and sulfate-rich wastewater enriched from a pretreatment potato cell (Dann et al. 2009). If *Desulfurellaceae* are to have applications in bioremediation, a particular advantage would be their growth under acidic and moderately thermophilic conditions.

Microbial fuel cells use microorganisms for the generation of electrical energy through the flow of electrons from the oxidation of organic compounds to a cathode in an electric circuit. Numerous anaerobic respirers such as *Geobacter, Shewanella*, and *Desulfuromonas* species have been studied for use in microbial fuel cells. A number of recent studies have detected populations related to *Desulfurella* as part of mixed communities in microbial fuel cells suggesting that they may have an important role under certain conditions. The use ofmicrobial fuel cells for the removal of sulfur pollutants in wastewater was investigated recently (Liang et al. 2013). The authors recognized the importance of *Desulfurella* in the treatment of sulfur-containing wastewater under weak acid conditions. Populations closely related to *D. acetivorans* were detected in microbial fuel cells enriched with glucose and glutamate (Choo et al. 2006). Li and colleagues (2011) enhanced the performance of microbial fuel cells by coating polymers on to anodes which enriched for electrochemically active bacteria. A major group of the electrochemically active bacteria were members of *Desulfurellaceae*, most closely related to *H. maritima*. As the *Desulfurellaceae* group is studied further and new species are identified, it is likely that there will be future applications for the family.

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11 The Family *Desulfuromonadaceae*

Anthony C. Greene
School of Biomolecular and Physical Sciences, Griffith University, Brisbane, QLD, Australia

Abstract
The family *Desulfuromonadaceae* is part of the Deltaproteobacteria class in the phylum Proteobacteria and contains the genera *Desulfuromonas*, *Desulfuromusa*, *Pelobacter*, *Malonomonas*, and *Geoalkalibacter*. The genera *Geothermobacter* and *Geopsychrobacter*, while currently part of the closely related family *Geobacteraceae*, have phylogenetic and phenotypic characteristics more closely aligned with *Desulfuromonadaceae* and are therefore discussed in this chapter. Members of the family are strictly anaerobic, although there is some tolerance to oxygen, and found widely in anoxic environments, particularly freshwater and marine sediments. They play important roles in the degradation of organic matter and are involved in syntrophic associations especially with methanogens and phototrophic green sulfur bacteria. Most members of *Desulfuromonadaceae* have a respiratory metabolism; however, *Pelobacter* and *Malonomonas* are almost exclusively fermentative with the exception of *Pelobacter seleniigenes* which is primarily a respirer. The majority of species are mesophiles, but there are several psychrophilic, alkaliphilic/alkalitolerant, and one thermophilic species. A wide range of substrates are metabolized by family members, ranging from organic acids to aromatic and chlorinated compounds. Sugars are used only by one out of the twenty-three species, *Geothermobacter ehrlichii*. The family requires some rearrangement with several species, needing reclassification to new genera. In particular, the genus *Pelobacter* has significant phylogenetic disparity.

Introduction

The class *Deltaproteobacteria* contains a phenotypically distinct mixture of aerobic and anaerobic bacteria. The aerobic bacteria are either gliding bacteria with a complex life cycle or predatory bacteria that prey on other Gram-negative bacteria. The anaerobic group is made up of primarily anaerobic respirers that utilize sulfur, sulfate, and/or metals, particularly iron and manganese, as terminal electron acceptors. There are a small number of fermenters belonging to this group as well. The anaerobes within Deltaproteobacteria have a vital role in cycling sulfur, metals, and organic compounds in many anoxic environments including marine and freshwater sediments and subsurface environments. *Desulfuromonadales* is a bacterial order belonging to Deltaproteobacteria, in which all members are strictly anaerobic. *Desulfuromonadales* contains some of the most important dissimilatory sulfur and metal reducing bacteria. Of particular significance is the wide variety of substrates and electron acceptors used by members of the order.

Currently, *Desulfuromonadales* contains the two closely related families of *Desulfuromonadaceae* and *Geobacteraceae*. The type genus/species of these two families, *Desulfuromonas acetoxidans*, and *Geobacter metallireducens* were the first dissimilatory sulfur-reducing and iron-reducing bacteria, respectively, discovered. Since their discovery, sulfur and iron reduction have been found to be widespread traits across the prokaryotic domains. Anaerobic respirers within the order are able to completely oxidize organic compounds using sulfur and/or metals. Recently some of these bacteria have been of particular interest for their potential in the bioremediation of aromatic, chlorinated, and

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metal contaminants, which can be coupled to reductive processes or used directly as electron acceptors. This chapter describes current knowledge of the taxonomy, characteristics, ecology, and applications of the family *Desulfuromonadaceae*.

Taxonomy: Historical and Current

Short Description of the Family

De.sul.fu.ro.mo.na.da'ce.ae. N. L. fem. n. *Desulfuromonasadis* type genus of the family; -*aceae* ending to denote family; N. L. fem. pl. n. *Desulfuromonadaceae* the *Desulfuromonas* family. The description is a modified version of that given in *Bergey's Manual of Systematic Bacteriology*, 2nd edition (Kuever et al. 2005a; emended by Greene et al. 2009).

Phylogenetically, the family is a member of the order *Desulfuromonadales* (Kuever et al. 2005b), class *Deltaproteobacteria*, and phylum Proteobacteria. The family contains the type genus *Desulfuromonas* (Pfennig and Biebl 1976), *Desulfuromusa* (Liesack and Finster 1994), *Malonomonas* (Dehning and Schink 1989), *Pelobacter* (Schink and Pfennig 1982), and *Geoalkalibacter* (Zavarzina et al. 2006, emended by Greene et al. 2009). *Geothermobacter* (Kashefi et al. 2003) and *Geopsychrobacter* (Holmes et al. 2004b) have been included in this family based on phylogenetic and phenotypic characteristics even though current taxonomic status still has them placed in *Geobacteraceae* (Euzeby 2013; Kuever et al. 2006). The EzTaxon-e database (Kim et al. 2012) and the Living Tree Project (Munoz et al. 2011) also have these genera in *Desulfuromonadaceae*. Currently, if we include *Geothermobacter* and *Geopsychrobacter*, there are 23 species across the 7 genera in the family.

Cells stain Gram negative and are mainly strictly anaerobic although some are aerotolerant. Cells are mostly motile and rod-shaped of variable length, some slightly curved and others ovoid. Motility occurs by means of single polar or subpolar flagella, or peritrichous flagella; some species are only motile in young cultures and some others are nonmotile. Growth is by either anaerobic respiration or fermentation. Fatty acids and alcohols are the main electron donors, while sulfur and Fe(III) compounds are major electron acceptors. Species are mainly mesophilic though some are psychrophilic and one is thermophilic. Cells contain a variety of cytochromes.

Phylogenetic Structure of the Family

The family is closely related to the family *Geobacteraceae*. The two families make up the order *Desulfuromonadales* and share many similarities. Moderately related families from other orders include *Desulfobacteraceae*, *Desulfobulbaceae*, and *Bacteriovoraceae*. The order *Desulfuromonadales* originated with the isolation of *Desulfuromonas acetoxidans* (Pfennig and Biebl 1976). This was followed by the description of *Pelobacter* with the isolation of *Pelobacter acidigallici* (Schink and Pfennig 1982). The genus *Malonomonas* was next described after *Malonomonas rubra* (Dehning and Schink 1989) was isolated. *M. rubra*, still the sole species in the genus, branches close to several *Pelobacter* species. These two genera have been grouped in reference to a third possible family in the order "*Pelobacteraceae*" (Sayers et al. 2009; Wagner et al. 2012), but currently this is an invalid family. However, while *Pelobacter* and *Malonomonas* share some physiological traits, they differ in other distinguishing characteristics such as the presence of c-type cytochromes and fumarate and malate fermentation. The genus *Desulfuromusa* was described later and formed a separate cluster in the family (Liesack and Finster 1994). Subsequently, three further genera have been described and all have distinctive phylogenetic and phenotypic characteristics. *Geothermobacter* (Kashefi et al. 2003) is thermophilic growing best at 55 °C, *Geopsychrobacter* (Holmes et al. 2004b) is psychrotolerant while *Geoalkalibacter* (Zavarzina et al. 2006) is alkalitolerant/alkaliphilic. *Geothermobacter* and *Geopsychrobacter* each have just a single species and *Geoalkalibacter* has two.

The placement of members of the family *Desulfuromonadaceae* has changed considerably in recent times, which has shown particularly the closeness and common ancestry of the family with *Geobacteraceae*. The type genus, *Desulfuromonas*, was originally classified in a broad group of "dissimilatory sulfate- or sulfur-reducing bacteria" (Krieg and Holt 1984). Then based on phylogenetic data and the ability to reduce Fe(III) and/or sulfur, *Desulfuromonas*, *Geobacter*, *Desulfuromusa*, and *Pelobacter* were proposed under the single monophyletic family of *Geobacteraceae* (Lonergan et al. 1996). The family contained two subgroups based on signature sequences and lines of descent which were *Desulfuromonas* and *Geobacter* clusters. It was not until later that the family *Geobacteraceae* was first formally described by Holmes et al. (2004a) and validated. The authors suggested that the family was composed of three distinct clusters, *Geobacter*, *Desulfuromonas*, and *Desulfuromusa*, based on phylogenetic analysis of 16S rRNA, *fusA*, *gyrB*, *rpoB*, and *recA* genes. Subsequently, the clusters were separated into the two current families of *Desulfuromonadaceae* and *Geobacteraceae* (Kuever et al. 2006).

Phylogenetically, there is generally a clear distinction between members of *Desulfuromonadaceae* and *Geobacteraceae* (*Fig. 11.1*). In *Desulfuromonadaceae*, species of *Pelobacter* are interspersed throughout the family. Three species, *P. acetylenicus*, *P. venetianus*, and *P. carbinolicus*, group as part of the *Desulfuromonas* clade. However, *D. palmitatis* was the one species of *Desulfuromonas* not part of this clade, branching with *Geoalkalibacter*. *Malomonas rubra* branches close to several more *Pelobacter* species, *P. seleniigenes*, *P. acidigallici*, and *P. massiliensis* which are distinct from the final member of the genus, *P. propionicus*. In fact, *P. propionicus* is an anomaly as phylogenetically it is placed in the middle of *Geobacteraceae* but shares the distinctive physiological characteristics of most *Pelobacter*. In contrast, *P. seleniigenes* does not share the distinctive characteristics of the other *Pelobacter* species and is actually more related phylogenetically to *Malomonas rubra* and several *Desulfuromusa* species (*D. succinoxidans* and *D. bakii*). According to phylogenetic properties, logically *Pelobacter* species should be broken up into various other genera; however, *Pelobacter* species share some unique characteristics which differ

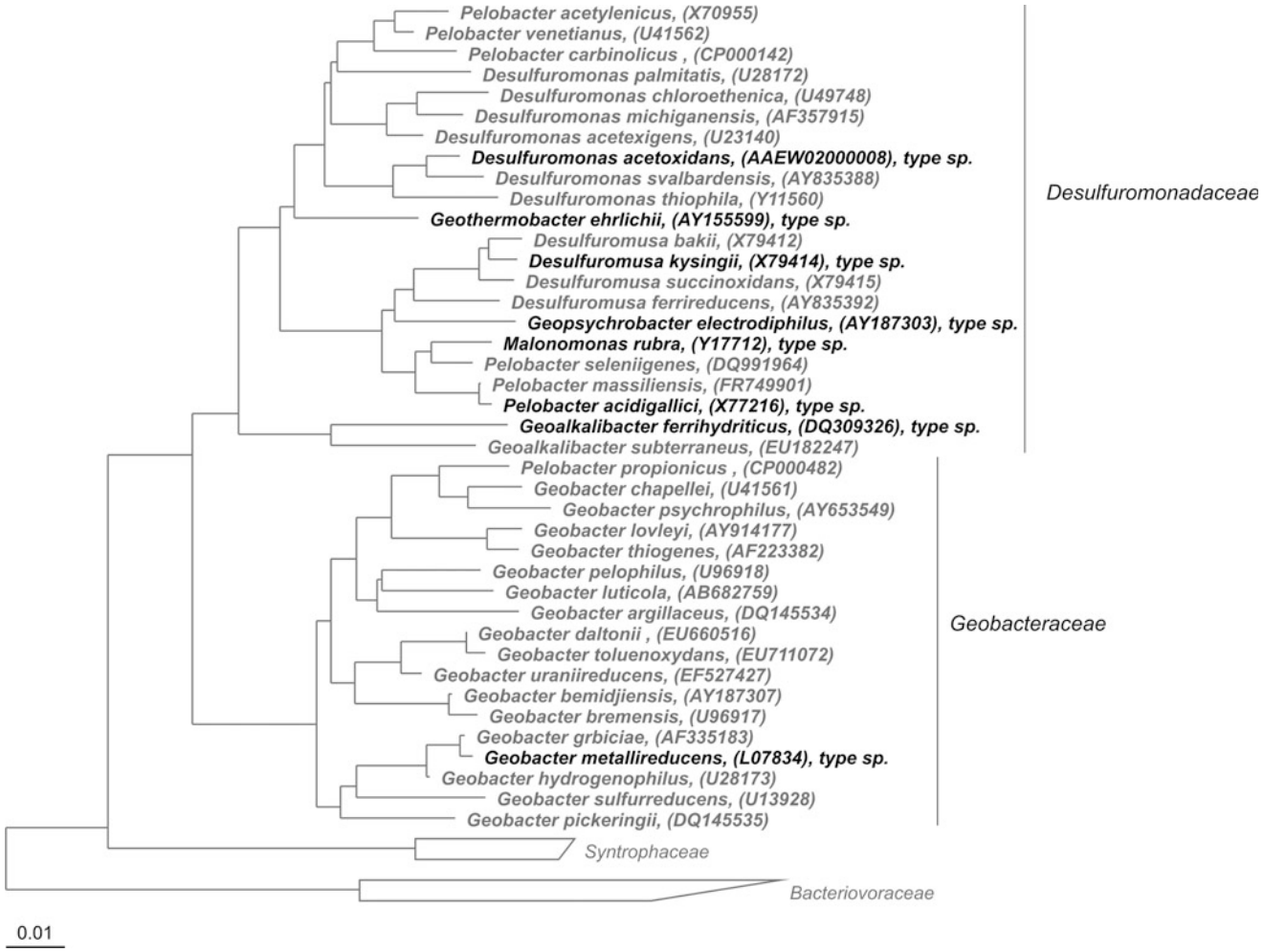

Fig. 11.1

Neighbor Joining phylogenetic tree of the families *Desulfuromonadaceae* and *Geobacteraceae*. Sequences for all members were from the LTP database (Munoz et al. 2011). Trees have been filtered for hypervariable positions below 40 % conservation threshold. Bar indicates 0.1 substitution per nucleotide position. The list of type strains used for tree construction: *Desulfuromusa bakii*, Gyprop^T, *Desulfuromusa kysingii* Kysw2^T, *Desulfuromusa succinoxidans* Gylac^T, *Desulfuromusa ferrireducens* 102^T, *Geopsychrobacter electrodiphilus* A1^T, *Malonomonas rubra* GraMal1^T, *Pelobacter seleniigenes* KM^T, *Pelobacter acidigallici* MaGal12^T, *Pelobacter massiliensis* HHQ7^T, *Geothermobacter ehrlichii* SS015^T, *Geoalkalibacter ferrihydriticus* Z-0531^T, *Geoalkalibacter subterraneus* Red1^T, *Desulfuromonas palmitatis* SDBY1^T, *Desulfuromonas acetexigens* 2873^T, *Desulfuromonas chloroethenica* TT4B^T, *Desulfuromonas michiganensis* BB1^T, *Desulfuromonas acetoxidans* 11070^T, *Desulfuromonas svalbardensis* 112^T, *Desulfuromonas thiophila* NZ27^T, *Pelobacter acetylenicus* WoAcy1^T, *Pelobacter venetianus* GraPEG1^T, *Pelobacter carbinolicus* GraBD1^T, *Pelobacter propionicus* OttBD1^T, *Geobacter bemidjiensis* Bem^T, *Geobacter bremensis* Dfr1^T, *Geobacter sulfurreducens* PCA^T, *Geobacter daltonii* FRC-32^T, *Geobacter uraniireducens* Rf4^T, *Geobacter chapellei* 172^T, *Geobacter psychrophilus* P35^T, *Geobacter thiogenes* K1^T, *Geobacter pelophilus* Dfr2^T, *Geobacter argillaceus* G12^T, *Geobacter hydrogenophilus* H-2^T, *Geobacter metallireducens* GS-15^T, *Geobacter grbiciae* TACP-2^T, *Geobacter pickeringii* G13^T, *Geobacter lovleyi* SZ^T, *Geobacter luticola* OSK6^T, *Geobacter toluenoxydans* TMJ1^T

with other genera in the order. In particular, most *Pelobacter* do not completely oxidize organic substrates with S^o or Fe(III) as electron acceptors, generally lack c-type cytochromes and have a fermentative metabolism. Future studies may reveal that the *Pelobacter* species may need to be divided into a number of genera, *P. propionicus* on its own into *Geobacteraceae*, *P. seleniigenes* with *Desulfuromusa*, *P. acidigallici*, and *P. massiliensis* together with *Malomonas rubra*, and finally *P. acetylenicus*, *P. venetianus* with *P. carbinolicus*. Of the remaining genera, *Geopsychrobacter* branches as part of the *Desulfuromusa* cluster, while *Geothermobacter* is quite distinct from other members of the family.

Molecular Analyses

The description of the family *Desulfuromonadaceae* has been based largely on physiological and 16S rRNA

sequence comparisons. Relatively few molecular studies have been done on the isolates other than whole genome sequencing of four isolates, *Desulfuromonas acetoxidans*, *Pelobacter carbinolicus*, *Pelobacter propionicus*, and *Geopsychrobacter electrodiphilus*. However a number of molecular techniques have been used recently to detect family members in a range of environments including retrieving and amplifying functional genes (Zapata-Penasco et al. 2012), DGGE (Ketep et al. 2013), T-RFLP (Luna et al. 2009) and real time-PCR (Lourdes et al. 2010).

DNA-DNA hybridization studies were done to compare a number of *Desulfuromusa* isolates (Liesack and Finster 1994). With a level of DNA relatedness of less than 30 %, the isolates were classified into three *Desulfuromusa* species. Finster et al. (1997) performed DNA-DNA hybridization studies on four *Desulfuromonas* isolates. Of these, the highest degree of relatedness was between *D. acetexigens* and *D. palmitatis* (37 %), followed by *D. palmitatis* and *D. thiophila* (31 %), *D. acetoxidans* and *D. thiophila* (31 %) and the remaining combinations were 20–22 %.

Holmes et al. (2004a) analyzed the sequences of five conserved genes, *nifD*, *recA*, *gyrB*, *rpoB*, and *fusA* in the 30 valid *Desulfuromonadaceae*/*Geobacteraceae* isolates at that time. The study revealed similar phylogeny to the 16S rRNA. However with *Geopsychrobacter electrodiphilus*, *nifD*, *recA*, *gyrB* sequences were most similar to *Desulfuromonas palmitatis*, while *rpoB* and *fusA* sequences were most like *Desulfuromusa succinoxidans*. The *nifD* gene was present in all isolates tested, suggesting that nitrogen fixation is highly conserved trait in *Desulfuromonadaceae* and *Geobacteraceae*. Subsequently, the gene has been found in all strains isolated and characterized since then. The G+C content (mol%) of genomic DNA has been tested in most of the isolates in the family and are given in the Phenotypic Analyses section.

The full genome of *Desulfuromonas acetoxidans* DSM 684^T has been sequenced in permanent draft form (GOLD ID Gi00197). The genome was completed by US Department of Energy Joint Genome Institute and is 3,828,328 base pairs in length with a G+C ratio of 51.8 %. There are 3,289 open reading frames with 3,234 protein-coding genes and 55 RNA genes. Over 71 % of the genes have a predicted function. According to clusters of orthologous group (COG) categories, the highest numbers of genes are associated with signal transduction mechanisms (334) followed by energy production and conversion (197). A significant amount of genes were for chemotactic functions which would be expected due to complex regulatory requirements. The importance of electron transport is clearly exhibited with an abundance of genes involved.

Similar to several related *Geobacter* species, the *D. acetoxidans* genome contains a very large number of genes coding for c-type cytochromes that contain multiple heme cofactors, in fact over a 100 genes that have heme-binding motif characteristics of c-type cytochromes (Butler et al. 2009). Alves et al. (2011) reported coding for 47 putative multiheme cytochromes, and expression of these proteins varies depending on growth conditions. The large percentage of genes coding for c-type cytochromes confirms the importance of electron transport.

The genome of the *Pelobacter carbinolicus* DSM 2380^T (GOLD ID Gc00306) was completed by US Department of Energy Joint Genome Institute and is 3,665,893 base pairs in length and contains 3,234 genes. There are 3,148 protein-coding genes along with 86 RNA genes and 29 non-functional pseudogenes. The G+C ratio of the genome is 55.11 %. Genome annotation revealed more metabolic and physiological versatility than expected (Aklujkar et al. 2012). Catabolic genes were identified for numerous substrates, including 2,3-butanediol, acetoin, glycerol, 1,2-ethanediol, ethanolamine, choline, and ethanol. Pathways for the production of hydrogen and formate are present, which reflects the syntrophic growth with methanogens. The genome contains 14 genes encoding c-type cytochromes (Haveman et al. 2006), which is far fewer than *Desulfuromonas* and *Geobacter* species. Consequently, *P. carbinolicus* lacks essential components for electron transfer to Fe(III). Therefore, Fe(III) is reduced only indirectly by sulfide, the product of elemental sulfur reduction (Haveman et al. 2008). Pathways for electron transfer to elemental sulfur have been identified. In contrast to *Desulfuromonas* and *Geobacter* species, the *Pelobacter* species can be seen as primarily fermentative and syntrophic species (Sun et al. 2010).

The complete genome of *Pelobacter propionicus* DSM 2379^T (GOLD ID Gc00467) was completed by US Department of Energy Joint Genome Institute and has 4,241,119 base pairs. The genome is made up of a single chromosome of 4,008,000 base pairs and two separate circular plasmids, pPRO1 and pPRO2, which have 202,397 and 30,722 base pairs, respectively. The G+C ratio of the chromosomal DNA is 59.02 % and contains 3,685 genes coding for 3,576 proteins, 67 structural RNAs, 42 pseudogenes, and 6 unresolved genes. The plasmids pPRO1 has a G+C ratio of 48.10 % with 195 genes and pPRO2 has 56.32 % G+C content with 33 genes. Like *P. carbinolicus*, the highest numbers of genes are associated with energy production and conversion according to COG categories.

Geopsychrobacter electrodiphilus DSM 16401^T (GOLD ID Gc11526) is the only other strain in *Desulfuromonadaceae* to have its complete genome sequenced. The genome was completed by US Department of Energy Joint Genome Institute in permanent draft form and is 4,241,730 base pairs long. There are 4,021 open reading frames including 3,954 protein-coding genes, 67 RNA genes, and 96 pseudogenes. The G+C ratio is 53.21 %.

Phenotypic Analyses

Desulfuromonas Pfennig and Biebl 1977, 306^{AL} (Effective Publication: Pfennig and Biebl **1976**, 11)

De.sul.fu.ro.mo'nas. L. pref. *de* from; L. n. *sulfur* sulfur; Gr. n. *monas* a unit, monad; N.L. fem. n. *Desulfuromonas*, a sulfur-reducing monad.

Table 11.1
Differentiating characteristics of *Desulfuromonas* species

Characteristic	*D. acetoxidans*[a]	*D. acetexigens*[b]	*D. palmitatis*[c]	*D. thiophila*[d]	*D. chloroethenica*[e]	*D. michiganensis*[f]	*D. svalbardensis*[g]
Temperature optimum (°C)	30	30	40	30	21–31	25	14
G+C content (mol%)	53.6	62.3	54.7	61.6	NT	NT	50.1
Motility	+	+	–	+	+	–[h]	+
Electron donors							
Propionate	–	–	–	–	–	–	+
Ethanol	+	–	–	–	–	–	+
Malate	+	–	–	–	–	+	–
Succinate	–	–	+	+	–	+	–
Electron acceptors							
Mn(IV)	+	+	+	NT	–	NT	+
Malate	+	+	–	–	–	+	–
Fumarate	+	+	+	–	+	+	+
Other	–	–	–	–	PCE, TCE	PCE, TCE	–

NT not tested, *PCE* tetrachloroethylene, *TCE* trichloroethylene
[a]Pfennig and Biebl (1976)
[b]Finster et al. (1994)
[c]Coates et al. (1995)
[d]Finster et al. (1997)
[e]Krumholz (1997)
[f]Sung et al. (2003)
[g]Vandieken et al. (2006)
[h]Small percentage (<5 %) motile

Cells are rod-shaped to ovoid typically ranging 0.4–0.9 μm wide and 0.8–4.0 μm long. Gram negative, strictly anaerobic chemoorganotrophs with mainly a respiratory metabolism although malate or fumarate can be fermented. The type species is *Desulfuromonas acetoxidans*, type strain 11070^T, and was isolated from anaerobic sulfide-containing marine sediments of the South Orkney Islands in the Southern Ocean. Elemental sulfur and Fe(III) are used as electron acceptors and acetate as electron donor. The use of other electron acceptors varied between species, *D. thiophila* was the only one not to use fumarate, while Mn(IV) was used by a number of species (*Table 11.1*). Two isolates, *D. chloroethenica* and *D. michiganensis*, were capable of using tetrachloroethylene and trichloroethylene as electron acceptors. Biotin was required as a growth factor by some of the isolates. Marine strains, *D. acetoxidans*, *D. palmitatis*, *D. svalbardensis*, require a salinity of at least 20 g/L. All members contain c-type cytochromes and are capable of syntrophic growth. For instance, *D. acetoxidans* grows syntrophically with the green sulfur bacterium *Chlorobium vibrioforme* in H_2-producing cocultures. *D. acetoxidans* reduced the sulfur to sulfide with acetate as electron donor, while *C. vibrioforme* oxidized sulfide to sulfur (Warthmann et al. 1992). Fatty acids analyzed in two species, *D. acetoxidans* (Kohring et al. 1994) and *D. svalbardensis* (Vandieken et al. 2006), revealed that $C_{16:0}$ and $C_{16:1}\omega7c$ predominated.

Pelobacter Schink and Pfennig 1983, 89^{VP} (Effective Publication: Schink and Pfennig **1982**, 200)

Pe.lo.bac'ter. Gr. masc. n. *pelos* mud; N.L. masc. n. *bacter* equivalent of Gr. neut. n. *bakterion* a rod; N.L. masc. n. *Pelobacter* a mud-inhabiting rod.

Fermentative rods that are strictly anaerobic, chemoorganotrophic and have a Gram-negative cell structure, with sizes ranging 0.5–1.0 μm wide and 1.5–6.0 μm long. There are seven valid species in the genus. The type species is *Pelobacter acidigallici*, type strain Ma Gal2^T, which was isolated from anaerobic mud of Rio Marin channel, Venice Italy. As indicated previously, species are dispersed across *Desulfuromonadaceae* and *Geobacteraceae* in three distinct phylogenetic clusters. However, the species do share common phenotypic characteristics. Members of the genus have a fermentative metabolism and are only able to use a narrow range of substrates with none fermenting sugars (*Table 11.2*). While some species of *Pelobacter* are capable of Fe(III) reduction and sulfur reduction, organic substrates are only incompletely oxidized. *P. seleniigenes* also has a respiratory metabolism, reducing selenate, Fe(III), nitrate, sulfur, fumarate, chlorate, and anthraquinone disulfonate when acetate is the electron donor. *Pelobacter* generally lack c-type cytochromes; however, no data is available for *P. seleniigenes*.

Table 11.2
Differentiating characteristics of *Pelobacter* species

Characteristic	*P. acidigallici*[a]	*P. acetylenicus*[b]	*P. carbinolicus*[c]	*P. propionicus*[c]	*P. venetianus*[d]	*P. massiliensis*[e]	*P. seleniigenes*[f]
Temperature optimum (°C)	32	25–30	32	14	33	30	NT
Temperature range (°C)	4–35	8–32	4–35	−2 to 20	10–40	20–40	NT
pH optimum	6.5–7.9	6.7–7.4	6.5–7.9	7.0–7.3	7.0–7.5	7.2	NT
pH range	5.8–8.25	6.2–7.8	5.8–8.25	6.5–7.9	5.5–8.0	6.5–7.8	NT
G+C content (mol%)	51.8	57.1	52.3	57.4	52.2	59.0	54.1
Substrates fermented	Gallic acid	Acetylene	Acetoin	Acetoin	Acetoin	Gallic acid	Pyruvate
	PGCA	Acetoin	2,3-Butanediol	2,3-Butanediol	2,3-Butanediol	Phloroglucinol	Citrate
	Phloroglucinol	Ethanolamine	Ethylene glycol	Ethanol	PEG	Pyrogallol	Lactate
		Choline	Ethanol[g]	Butanol[h]	1,2-propanediol	Hydroxyhydroquinone	
		1,2-propanediol	Butanol[g]	Propanol[h]	Ethanol[g]		
		Ethanol[g]	Propanol[g]	Lactate	Butanol[g]		
		Butanol[g]		Pyruvate	Propanol[g]		
		Propanol[g]			Glycerol[h]		
		Glycerol[h]					

NT not tested, *PGCA* phloroglucinol carboxylate, *PEG* polyethylene glycols
[a]Schink and Pfennig (1982)
[b]Schink (1985)
[c]Schink (1984)
[d]Schink and Stieb (1983)
[e]Schnell et al. (1991)
[f]Narasingarao and Häggblom (2007)
[g]Growth was possible in mixed culture with *Acetobacterium woodii* or *Methanospirillum hungatei* (Schink 1984; Schink 2006)
[h]Growth was possible only in the presence of 10 mM acetate (Schink 1984; Schink 2006)

Fatty acid content has been only reported for *P. seleniigenes* and is composed of predominantly the straight chain fatty acids of $C_{15:0}$, $C_{16:0}$ and $C_{17:0}$. *Pelobacter* are able to grow syntrophically in anoxic environments. *P. acetylenicus*, *P. carbinolicus*, and *P. venetianus* will only ferment the primary aliphatic alcohols, ethanol, propanol, and butanol when H_2-consuming methanogens are present (Schink 2006). Rotaru et al. (2012) demonstrated that *P. carbinolicus* exchanged electrons with *Geobacter sulfurreducens* via interspecies hydrogen transfer.

Malonomonas Dehning and Schink 1990, 320^{VP} (Effective Publication: Dehning and Schink **1989**, 431)

Ma.lo.no.mo'nas. N.L. n. *acidum malonicus* malonic acid; Gr. n. *monas* a unit, monad; N.L. fem. n. *Malonomonas* malonic acid utilizing monad.

The genus contains a single species, *Malonomonas rubra*. The type strain GraMal1^T was isolated from marine anoxic sediments of Canal Grande in Venice, Italy. Cells are microaerotolerant anaerobic, chemoorganotrophic, Gram negative, straight to slightly curved rods with rounded ends, 0.4-µm wide and 3.1–4.0-µm long, and motile in younger cultures by means of 1–2 polar flagella. Colonies are red and disk-shaped. At least 150 mM NaCl is required for growth. Growth occurs at 22–45 °C (optimum 28–30 °C) and pH 6.0–8.5 (optimum pH 7.1–7.3). Growth is fermentative, with malonate, fumarate, and malate being the only substrates fermented. Malonate is decarboxylated to acetate, and fumarate or malate is fermented to succinate and CO_2. A high concentration of a mainly periplasmic c-type tetraheme cytochrome was identified with maximum absorption bands at 420, 522, and 551 nm. The cytochrome was up to 12 % of the total cell protein and not involved in energy metabolism (Kolb et al. 1998). DNA has a G+C content of 48.3 mol %.

Desulfuromusa Liesack and Finster **1994**, 756^{VP}

De.sul.fu.ro.mu'sa. L. pref. *de* from; L. n. *sulfur* sulfur; N.L. fem. n. *musa* banana; N.L. fem. n. *Desulfuromusa* a banana-shaped bacterium that reduces sulfur.

Cells are Gram negative, chemoorganotrophic, and slightly curved or rod-shaped. All four described species are motile by polar flagella. Strictly anaerobic metabolism, though *D. kysingii* tolerates 2 % oxygen but no growth occurs in its presence. There are four species in the genus. All species use elemental sulfur, Fe (III), and fumarate as electron acceptors. *D. kysingii*, *D. bakii*, and *D. succinoxidans* reduce Fe(III)-nitrilotriacetic acid, and *D. kysingii* and *D. ferrireducens* Fe(III)-citrate. *D. ferrireducens* also reduces poorly crystalline Fe(III) oxide (Vandieken et al. 2006). In addition, all species grow by disproportionation of fumarate, and use acetate, lactate, succinate, pyruvate as electron donors. The use of several other substrates varied between species (*Table 11.3*). No sugars are used by any of the species. *D. kysingii*, *D. bakii*, and *D. succinoxidans* share many similar characteristics which is reflected by their cohesive phylogenetic clustering. *D. ferrireducens*, however, differed more from the other species particularly with significantly lower temperature optimum and higher G+C content in DNA.

Geothermobacter Kashefi Holmes Baross and Lovley 2005, 547^{VP} (Effective Publication Kashefi et al. **2003**, 2992)

Ge.o.ther.mo.bac'ter. Gr. n. *ge* earth; Gr. n. *thermos* heat; N.L. masc. n. *bacter* rod; N.L. masc. n. *Geothermobacter* a rod from hot earth.

The genus contains a single species, *Geothermobacter ehrlichii*. The type strain SS015^T was isolated from the Bag City hydrothermal vent at the Axial Seamount (46°N, 130°W) at the intersection of the Juan de Fuca Ridge and the Cobb Seamount chain in the northeastern Pacific Ocean. Cells are strictly anaerobic, chemoorganotrophic, Gram-negative rods, 0.5-µm wide and 1.2–1.5-µm long, and highly motile by means of a single subpolar flagellum. Growth occurs at 35–65 °C (optimum of 55 °C), pH 5.0–7.75 (optimum pH 6.0), and NaCl concentrations ranging from 0.5 % to 5.0 % (optimum 1.9 %).

The oxidation of acetate, pyruvate, malate, formate, propionate, butyrate, glutamate, isoleucine, arginine, asparagine, glutamine, histidine, serine, ethanol, methanol, isopropanol, maltose, fructose, starch, peptone, tryptone, and casamino acids is coupled to the reduction of Fe(III) and nitrate. Dimethyl sulfoxide is also used as an electron acceptor. Fe(III) is used in the form of poorly crystalline Fe(III) oxide and not as Fe(III) citrate, Fe(III) pyrophosphate, goethite, or hematite. Growth is inhibited by ampicillin, chloramphenicol, erythromycin, kanamycin, novobiocin, penicillin G, phosphomycin, rifampin, streptomycin, and vancomycin HCl. Cells contain an abundance of c_3-cytochrome, with maximum absorption bands at 420, 523, and 552 nm.

Geopsychrobacter Holmes Nicoll Bond and Lovley 2005, 547^{VP} (Effective Publication: Holmes et al. **2004b**, 6029)

Ge.o.psych.ro.bac'ter. Gr. n. *ge* earth; Gr. adj. *psukhros* cold; N.L. masc. n. *bacter* rod; N.L. masc. n. *Geopsychrobacter* a rod from cold earth.

The genus contains a single species, *Geopsychrobacter electrodiphilus*. The type strain A1^T was isolated from a laboratory marine sediment fuel cell. Sediments were from Boston Harbour, USA, at a water depth of 5 m. Cells are nonmotile, strictly anaerobic, chemoorganotrophic, Gram-negative curved rods, 0.22-µm wide and 2.5-µm long. Growth occurs at 4–30 °C with an optimum of 22 °C. The oxidation of acetate, malate, succinate, citrate, fumarate, pyruvate, benzoate,

Table 11.3
Differentiating characteristics of *Desulfuromusa* species

Characteristic	*D. kysingii*[a]	*D. bakii*[a]	*D.succinoxidans*[a]	*D. ferrireducens*[b]
Morphology	Curved rods	Curved rods	Curved rods	Thin rods
Width (μm)	0.5–0.6	0.5–0.6	0.8–1.0	0.7–1.0
Length (μm)	2–5	2–4	4–6	3–5
Temperature optimum (°C)	32	25–30	32	14
Temperature range (°C)	4–35	8–32	4–35	−2–20
pH optimum	6.5–7.9	6.7–7.4	6.5–7.9	7.0–7.3
pH range	5.8–8.25	6.2–7.8	5.8–8.25	6.5–7.9
G+C content (mol%)	47	46	46	52.3
Electron donors				
Formate	−	−	−	+
Propionate	+	+	+	−
Malate	+	+	+	−
Citrate	+	+	−	−
Ethanol	−	−	−	+
Propanol	−	−	−	+
Butanol	−	−	−	+
Alanine	+	+	+	−
H_2	−	−	−	+
Electron acceptors				
Nitrate	+	−	−	−
Malate	+	−	−	−
Disproportionation				
Malate	+	+	+	−
Maleate	+	−	−	NT
Aspartate	+	−	+	NT

NT not tested
[a]Liesack and Finster (1994)
[b]Vandieken et al. (2006)

stearate, hydrogen, ethanol, acetoin, aspartic acid, glutamic acid, glycine, alanine, methionine, peptone, tryptone, casamino acids, and yeast extract is coupled to the reduction of Fe(III). Colloidal S^0, Mn(IV), and anthraquinone 2,6,-disulfonate are also used as electron acceptors. Cells contain an abundance of c-type cytochromes, with maximum absorption bands at 420, 522, and 552 nm.

Geoalkalibacter Zavarzina Kolganova Boulygina Kostrikina Tourova and Zavarzin 2007, 893[VP] (Effective Publication: Zavarzina et al. 2006, 680), Emend Greene Patel and Yacob 2009, 784

Ge.o.al.ka.li.bac'ter. Gr. n. *ge* earth; Arabian adj. *al qaliy* ashes of saltwort; N.L. n. *alkali*, alkali; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Geoalkalibacter*, a rod from alkaline earth.

Short rods that are non-fermentative, strictly anaerobic, and have a Gram-negative cell structure. Size is typically 0.3–0.6-μm wide and 1–5-μm long though could vary depending on the substrate. The type species is *Geoalkalibacter ferrihydriticus*, type strain Z-0531[T], and was isolated from anoxic bottom sediments of Lake Khadyn, Tuva, Russian Federation. *G. ferrihydriticus* and the other type strain *Geoalkalibacter subterraneus* Red1[T] isolated from Redwash oilfield, USA, share 95.8 % 16S rRNA gene sequence similarity. Both species are motile by either lateral (*G. ferrihydriticus*) or polar (*G. subterraneus*) flagella. Fe(III) in the form of amorphous Fe(III) hydroxide and ferrihydrite, Mn(IV,) and S° are used as electron acceptors, with *G. ferrihydriticus* also using anthraquinone-2,6-disulfonate and *G. subterraneus* using nitrate and trimethylamine N-oxide additionally. The oxidation of acetate, pyruvate, ethanol, propionate, lactate, tryptone, and yeast extract is coupled to the reduction of Fe(III). Growth of *G. ferrihydriticus* is inhibited by streptomycin, penicillin, and rifampin but not vancomycin. *G. subterraneus* is not inhibited by any of these antibiotics. Differentiating characteristics are shown in *Table 11.4*.

Table 11.4
Characteristics that differentiate *Geoalkalibacter ferrihydriticus*, and *Geoalkalibacter subterraneus*

Characteristic	*G. ferrihydriticus*[a]	*G. subterraneus*[b]
Temperature optimum (°C)	35	40
Temperature range (°C)	18–39	30–50
pH optimum	8.5	7
pH range	7.8–10	6–9
Salinity optimum (%)	0	2
Salinity range (%)	0–5	0–10
Flagella	polar	lateral
G+C content (mol%)	55.3	52.5
Electron acceptors		
Fe(III) citrate	–	+
Nitrate	–	+
Electron donors		
Yeast extract	–	+
Peptone	–	+
Hydrogen	–	+
Formate	–	+
Succinate	–	+
Pyruvate	+	+
Butyrate	–	+
Tartrate	+	–
Malonate	–	+
Methanol	–	+
Glycerol	–	+

[a]Zavarzina et al. (2006)
[b]Greene et al. (2009)

Isolation, Enrichment, and Maintenance Procedures

Members of the family *Desulfuromonadaceae* have been routinely enriched and isolated in anaerobically prepared media (Ljungdahl and Wiegel 1986). The components and incubation conditions are varied depending on the habitat and characteristics of isolates. Most isolates are capable of respiratory growth with sulfur or Fe(III) coupled to oxidation of acetate either under freshwater or marine conditions. As the enrichment medium for most *Desulfuromonadaceae* isolates differs, typically a bicarbonate-buffered, sulfide-reduced (except if enriched on Fe(III) compounds) basal salts medium can be used. The conditions existing in the habitat need to be factored in to the medium or incubation conditions.

A suitable basal salts medium that can be used for freshwater or marine enrichment of a broad range of *Desulfuromonadaceae* isolates was defined by Schink and Pfennig (1982). The components are as follows (g/L of distilled water): KH_2PO_4, 0.2; NH_4Cl, 0.25; $CaCl_2{\cdot}2H_2O$, 0.15. For the freshwater medium $MgCl_2{\cdot}2H_2O$, 0.4 and NaCl, 1.0 were added as well, while for marine medium $MgCl_2{\cdot}2H_2O$, 3.0 and NaCl, 20.0 were used. After autoclaving, sterile solutions of $NaHCO_3$, 2.5 g/L; Na_2S, 0.12 g/L; trace elements SL7, 1 mL/L, and vitamins, 1 mL/L are added. Trace elements (Widdel and Pfennig 1981) composed of (per liter): HCl (25 %), 10 mL; $FeCl_2{\cdot}4H_2O$, 1.5 g; $CoCl_2{\cdot}6H_2O$, 190 mg; $MnCl_2{\cdot}4H_2O$, 100 mg; $ZnCl_2$, 70 mg; H_3BO_3, 62 mg; $Na_2MoO_4{\cdot}2H_2O$, 36 mg; $NiCl_2{\cdot}6H_2O$, 24 mg; $CuCl_2{\cdot}2H_2O$, 17 mg. The vitamin solution (modified Pfennig 1978) was composed of (per liter): biotin, 10 mg; calcium pantothenate, 25 µg; thiamine, 50 µg; *p*-aminobenzoic acid, 50 µg; nicotinic acid, 100 µg; pyridoxamine, 250 µg. The addition of biotin was particularly important as some strains, e.g., *D. acetoxidans* and *D. svalbardensis*, are unable to grow without it. The pH is adjusted to 7.2–7.3. The medium is dispensed under N_2–CO_2 (80:20) gas mixture into either serum bottles with butyl rubber stoppers or hungate tubes with a 50–70 % headspace gas to medium ratio. Suspensions and soluble substrates are added from sterile anoxic stock solutions. Solid substrates need to be added prior to gassing and autoclaving.

Enrichment preparation, substrates, and incubation conditions will vary depending on the source of the organisms. Many *Desulfuromonadaceae* strains can be enriched from freshwater or marine sediments by the addition of sodium acetate (5–20 mM) and an excess of elemental sulfur or ferric iron (30–100 mM) to basal salts medium. Alternate electron acceptors such as tetrachloroethylene or trichloroethylene can be used for the enrichment of chloroethylene-reducing strains, e.g., *D. chloroethenica* and *D. michiganensis*. Black anoxic sediment is added to the medium at approximately 5–10 % of the total volume. Anaerobic conditions can be maintained by using an anaerobic chamber or flushing enrichment cultures with sterile N_2–CO_2 gas. Enrichment cultures are incubated at 25–30 °C, and the time taken for positive enrichments was generally 1–2 weeks but could take up to 10 weeks. Positive enrichments were shown by the appearance of turbidity and confirmed by microscopy and also in many cases by accumulation of reduced electron acceptors, Fe(II), sulfide, and dehalogenated organic compounds. Similarly, acetate and sulfur or Fe(III) can be used for the enrichment of *Desulfuromusa* strains, though succinate, propionate, or lactate can be used as electron donors in enrichment cultures.

Isolates from the genera *Geoalkalibacter*, *Geopsychrobacter*, and *Geothermobacter* are enriched by similar techniques with important variations in media or incubation conditions. To enrich for *Geoalkalibacter*, media should around pH 8.5–9 and the source of bacteria is soda lake sediments or petroleum reservoir fluids. *Geopsychrobacter* and *Geothermobacter* should be enriched at 15 °C and 55 °C, respectively. In addition, *D. svalbardensis* and *Desulfuromusa ferrireducens* are psychrophilic and isolation should be at 15 °C as well. *Malonomonas* can be enriched from marine sediments in basal salts medium with the addition of 20 mM disodium malonate. As growth is fermentative, no added electron acceptor is necessary. Likewise, *Pelobacter* can also grow fermentatively and is enriched from marine or freshwater sediments. Substrates for enrichment are

variable and include 5 mM gallic acid or 5 mM phloroglucinol (*P. acidigallici*), 10 mM 2,3-butanediol (*P. carbinolicus* and *P. propionicus*), 0.2 bar partial pressure acetylene (*P. acetylenicus*), 1 mM hydroxyhydroquinone (*P. massiliensis*), 20 mM pyruvate and 10 mM selenate (*P. seleniigenes*), 0.1 % polyethylene glycol (*P. venetianus*). Acetate is a fermentation product of most species rather than a substrate for growth, although *P. seleniigenes* is able to use acetate as an electron donor in anaerobic respiration.

Enrichment cultures should be transferred up to five times into fresh medium. As most *Desulfuromonadaceae* isolates are strict anaerobes, subsequent isolation is typically by either dilution-to-extinction and/or by picking and subculturing colonies from roll tube or deep-agar dilutions. In some cases, agar plates were used, provided manipulations were done in an anaerobic chamber and plates were incubated anaerobically. Colonies were generally picked from the highest positive dilutions. Colonies either were yellow, pink-red, or white.

Cultures can be maintained in the refrigerator and should be transferred every 2–3 months in the short term. In some instances, cells have remained viable for much longer periods under these conditions. Longer term storage would be at −20 °C in 20 % glycerol and even longer in liquid nitrogen or lyophilized.

Ecology

Members of *Desulfuromonadaceae* inhabit anaerobic environments, in particular freshwater or marine sediments and muds. These habitats can be pristine or contaminated, surface or deep subsurface environments. All members of the family have important roles in sulfur and metal cycles, and in the degradation of organic matter. In natural habitats, members of *Desulfuromonadaceae* can have a variety of syntrophic associations with other anaerobic respirers, fermentative bacteria, methanogens, and phototrophic green sulfur bacteria. Due to their capacity for sulfur and metal reduction, many have been isolated from sulfur or metal-containing environments. The first described member of the family, *D. acetoxidans*, was isolated from both marine or freshwater sulfide-containing water and mud, syntrophic green sulfur bacteria culture. Subsequently, *D. acetexigens* was isolated from freshwater sulfide-containing mud and *D. thiophila* from an anoxic mud of a freshwater sulfur spring.

All of the strains from the family *Pelobacter* are from either freshwater or marine sediments. However, *P. propionicus* along with *D. acetexigens* could be isolated from sewage sludge digesters as well as sediments. *Malonomonas rubra*, which clusters with several *Pelobacter* isolates, was also from anoxic marine sediments. *Pelobacter* occupy an important niche in anoxic environments, particularly sediments. They play an integral role providing fermentation products for respiratory organisms and in syntrophic relationships, particularly with H_2-consuming methanogens (Schink 1997; Butler et al. 2009). Depending on the species, these bacteria are able to ferment a number of substrates such as butanediol, acetoin, ethanol, lactate, and pyruvate, and produce acetate, H_2, and CO_2 as end products.

Geopsychrobacter originated from marine sediment material but was subsequently isolated from an experimental fuel cell. The low temperature operation (15 °C) of this fuel cell enabled the isolation of the psychrotolerant strains. *Desulfuromonas* species have also been detected in biofilms on anodes in fuel cells specifically inoculated with wastewater effluent from a kraft pulp mill (Ketep et al. 2013).

Psychrophilic isolates are typically from low temperature environments, *D. svalbardensis* and *Desulfuromusa ferrireducens* from permanently cold (2–3 °C) Arctic fjord sediments. The other *Desulfuromusa* isolates were isolated from a Danish fjord and deep-sea sediments (2,000 m depth) but were not psychrophilic though they had minimum growth temperatures of 4–8 °C. Not all deep-sea samples have yielded bacteria that grow at low temperature. Several members of *Desulfuromonadaceae* have been isolated from deep-sea hydrothermal vent fluids and sediments. The sole thermophilic member of the family, *Geothermobacter ehrlichii*, was isolated from hydrothermal vent fluids. Community analysis revealed the presence of *D. Palmitatis* in hydrothermal vent sediments though enrichment temperatures were at 37 °C (Guerrero-Barajas et al. 2011). Other extreme environments have revealed the presence of *Desulfuromonadaceae*. *Geoalkalibacter ferrihydriticus* was isolated from an alkaline soda lake, and the most abundant clone in hypersaline soils was most related to *D. palmitatis* (Caton and Schneegurt 2012).

Contaminated sediments and aquifers are a major source of *Desulfuromonas* isolates. *D. palmitatis* was isolated from marine hydrocarbon-contaminated sediments (Coates et al. 1995), and copper-contaminated marine sediments with tolerance to copper exceeding 1,000 ppm (Besaury et al. 2013). *D. chloroethenica* was found in freshwater sediments contaminated with industrial solvents particularly chlorinated ethylenes and toluene (Krumholz et al. 1996; Krumholz 1997). Likewise, *D. michiganensis* was first isolated from a chloroethene-contaminated aquifer (Sung et al. 2003) and later consistently detected in trichloroethene-contaminated aquifer cores (Lourdes et al. 2010). *D. michiganensis* was also major component of a 1,1,1,-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) dechlorinating community enriched from paddy soils (Chen et al. 2013) and found in pristine river sediments as well (Sung et al. 2003). *Desulfuromonadaceae* dominated dechlorinating communities enriched from tidal flat sediments and were most similar to *D. michiganensis* in particular, as well as *D. thiophila* and *Pelobacter acidigallici* (Lee et al. 2011). Ribotypes detected in groundwaters were found to belong to the genera *Desulfuromonas*, *Pelobacter*, and *Desulfuromusa* (Luna et al. 2009). *Pelobacter* was detected as part of a community structure of a pesticide-contaminated (Paul et al. 2006).

Petroleum reservoirs have been the source of a wide variety of anaerobic bacteria, including sulfur and metal reducing bacteria. *Geoalkalibacter subterraneus* was isolated from production waters fluids (Greene et al. 2009). Clones related to

Desulfuromonas, *Desulfuromusa*, and *Pelobacter* species were detected as significant proportions of microbial populations in saline produced waters (Zapata-Penasco et al. 2012; Zhang et al. 2012). The presence of these bacteria in reservoirs may represent a problem for oil recovery and quality. The production of sulfide can cause oil souring and corrosion, and degradation of oil components can be linked to respiratory processes. However, as these reservoirs had been water-flooded, it is likely that these bacteria originated in surrounding aquifers or sediments and may not be indigenous to reservoirs. Furthermore, work in our laboratory has failed to detect members of *Desulfuromonadaceae* in high temperature and non-water-flooded reservoirs.

Pathogenicity, Clinical Relevance

No members of the family *Desulfuromonadaceae* are pathogenic. All are bacteria isolated from natural anoxic environments, typically freshwater and marine sediments. All are risk group 1 bacteria.

Applications

Bioremediation

The capacity of a numbers of isolates to dechlorinate industrial pollutants offers potential in bioremediation. Tetrachloroethene (PCE) and trichloroethene (TCE) have widespread use as industrial solvents and, as a result, have left aquifers and soils contaminated. *D. michiganensis* and *D. chloroethenica* are able to dechlorinate PCE and TCE to *cis*-1,2-dichloroenthane (cDCE) (Sung et al. 2003). However, the limitation in bioremediation is that they are unable to further dechlorinate cDCE to vinyl chloride and ethylene. Though Sung et al. (2003) suggest other advantages offset this, such as they operate at typical aquifer temperatures and high PCE concentrations, alternative electron acceptors do not inhibit dechlorination, and acetate or lactate can be used as electron acceptors. *D. michiganensis* and *D. chloroethenica* have been developed for use in bioaugmentation processes for bioremediation of chlorinated solvents (Stroo et al. 2010). Likewise, *Desulfuromonas michiganensis* was also major component of a 1,1,1,-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) dechlorinating community enriched from paddy soils (Chen et al. 2013).

In a field study of diesel contaminated groundwater, *Desulfuromonas* and *Desulfuromusa* species were detected as part of a benzene, toluene, ethylbenzene, and xylene (BTEX) degrading community (Ramos et al. 2012). However, whether these bacteria degraded the diesel hydrocarbons or were part of a syntrophic community was not established. Similarly in a separate study, uncultured *Pelobacter* species previously detected in biodegraded oil reservoirs were dominant members (up to 70 %) of several BTEX anaerobic degradation communities (Berlendis et al. 2010). The ability of *P. venetianus* to degrade polyethylene glycol and detection of enzymes involved offers potential for use in remediation in industrial wastes (Frings et al. 1992). Other *Pelobacter* species are able to degrade trihydroxybenzenes which can also be environmental contaminants.

Many members of the family *Desulfuromonadaceae* are Fe(III) reducers; however, their ability to reduce more toxic metals has not been widely investigated. Given that related *Geobacter* species are capable of reducing a number of toxic metals and radionuclides, some *Desulfuromonadaceae* may also have that capability. *Desulfuromonas* were the major organisms detected in microbial communities when acetate was added to stimulate the reduction of soluble U(VI) to insoluble U(IV) in sediments from uranium-contaminated sites in New Mexico, USA (Holmes et al. 2002). *D. palmitatis* was used in a combined chemical treatment scheme for the removal of arsenic and other metals by 90 % in contaminated soils (Vaxevanidou et al. 2008). The same authors looked at *D. palmitatis* for the reduction of hexavalent chromium and removal of Fe oxides from bauxite ore (Papassiopi et al. 2008, 2010).

Microbial Fuel Cells

The flow of electrons from the oxidation of organic compounds to a cathode in an electric circuit will generate electricity. The idea of microbial fuel cells and the use of microorganisms to oxidize the organic matter has been around for many years. However, the involvement and better understanding of the physiology of anaerobic respirers like some *Desulfuromonadaceae* isolates has been investigated more recently in a number of studies. Holmes et al. (2004c) found that *Desulfuromonadaceae* and *Geobacteraceae* isolates dominated microbial communities in fuel cells. *Desulfuromonas* species predominate in natural marine sediment fuel cells as they are highly efficient at transferring electrons to an anode when oxidizing organic matter (Lovley 2006). In such cases, *D. acetoxidans* was found to be the most abundant organism (Bond et al. 2002; Logan and Regan 2006).

In situ and large-scale operations are being investigated as a useful source of power for operational equipment or electronic devices. Optimizing the system components such as design, site, microbial balance, substrate, electrode composition, and temperature will lead to increases in the power generated (Ozkaya et al. 2012; Ketep et al. 2013; Holmes et al. 2004c). The variable environmental conditions under which *Desulfuromonadaceae* isolates are able to grow offers the potential to apply microbial fuels cells to varying environments (Badalamenti et al. 2013). In fact, the psychrotolerant *Geopsychrobacter electrodiphilus* was originally isolated from a marine sediment fuel cell. This offers the potential of constructing fuel cells using psychrotrophs to power monitoring equipment in deeper marine environments where low temperatures prevail. The use of *Geoalkalibacter* species has produced high current densities in microbial electrochemical cells under alkaline and saline conditions (Badalamenti et al. 2013).

The combined approach of using wastes for the generation of electrical current has great potential (Ketep et al. 2013). *D. acetoxidans* was investigated for combined use of current production in sediment batteries and bioremediation of organic contaminants in the marine sediments use part of a community (Bond et al. 2002). Furthermore, it may be able to be used to complement anaerobic digestion in wastewater treatment processes (Pham et al. 2006). *Desulfuromonas* species were detected as significant members of fuel cell communities when anaerobic sludge from an anaerobic digester at a wastewater treatment plant was used as inoculum (Kim et al. 2007).

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12 The Family *Geobacteraceae*

Wilfred F. M. Röling
Faculty of Earth and Life Sciences, Molecular Cell Physiology, VU University Amsterdam, Amsterdam, The Netherlands

Abstract
Geobacteraceae, a family within the order *Desulfuromonadales*, is in this chapter described as consisting of the genus *Geobacter* and the sole species *Pelobacter propionicus*. The genus *Geobacter* comprises anaerobic, non-fermenting chemoorganotrophic mesophiles. Their hallmark feature is the ability to reduce insoluble Fe(III) and Mn(IV) for which they can employ several mechanisms, of which the most notably is extracellular electron transfer via electric conductive nanowires. *Geobacter* species are physiological versatile. They all oxidize acetate and mainly use small organic acids and alcohols. Several species also oxidize monoaromatic hydrocarbons, such as toluene and benzene. Next to Fe(III), a range of other electron acceptors can be used, and *Geobacter* species can transfer electron directly to other microbial species or graphite electrodes. Sulfur is often respired, and some species respire organohalides. *P. propionicus* is phylogenetically located within the *Geobacter* clade, but several of its physiological characteristics are distinct from *Geobacter* properties: it can ferment but does not utilize acetate as an electron donor nor oxidizes organic compounds completely. Furthermore, it does not contain the c-type cytochromes that are involved in electron transfer to Fe(III) in *Geobacter* species. Members of the genus *Geobacter* often dominate in iron-reducing settings, in particular in environments that have been subject to anthropogenic influences. They represent a rare case of environmental dominant species that are relatively easy to enrich and isolate. Genome-scale metabolic models have strongly contributed to understanding their physiology and ecology. The physiological characteristics of *Geobacter* species are employed in environmental biotechnology, such as the natural attenuation of organic matter, bioremediation of aromatic hydrocarbons, heavy metals and organohalides, and generating bioenergy in microbial fuel cells and microbial electrolysis cells.

Taxonomy, Historical and Current

Ge.o.bac.ter.a' ce.ae. M.L. masc. n. *Geobacter* type genus of the family; *-aceae* ending to denote a family; M.L. fem. Pl. n. *Geobacteraceae* the *Geobacter* family. The description is an emended version of the validated description in *Bergey's Manual* (Garrity et al. 2005; Validation List 107 2006).

The family *Geobacteraceae* is located in the order *Desulfuromonadales* in the δ-subclass of the Proteobacteria. The order branches phylogenetically between the orders *Desulfovibrionales* and *Desulfobacterales*. The family *Geobacteraceae* is currently not consistently described, which has led to erroneous assignments of genera to this family. In this chapter an emended description of the *Geobacteraceae* by Garrity et al. (2005) will be presented, consisting of one genus, *Geobacter*, and the species *Pelobacter propionicus*. The family branches between the family *Desulfuromonadaceae* (Kuever et al. 2005) and *Myxococcales* (*Fig. 12.1*).

The current inconsistent assignment of genera and species to the family *Geobacteraceae* appears to relate to the nearly parallel description of the novel family *Geobacteraceae* by Holmes et al. (2004a), and by Garrity et al. (2005), with differences in the genera assigned by these authors to the family. Holmes et al. (2004a) described the family *Geobacteraceae* as consisting of six genera divided over three subclades on basis of phylogenetic

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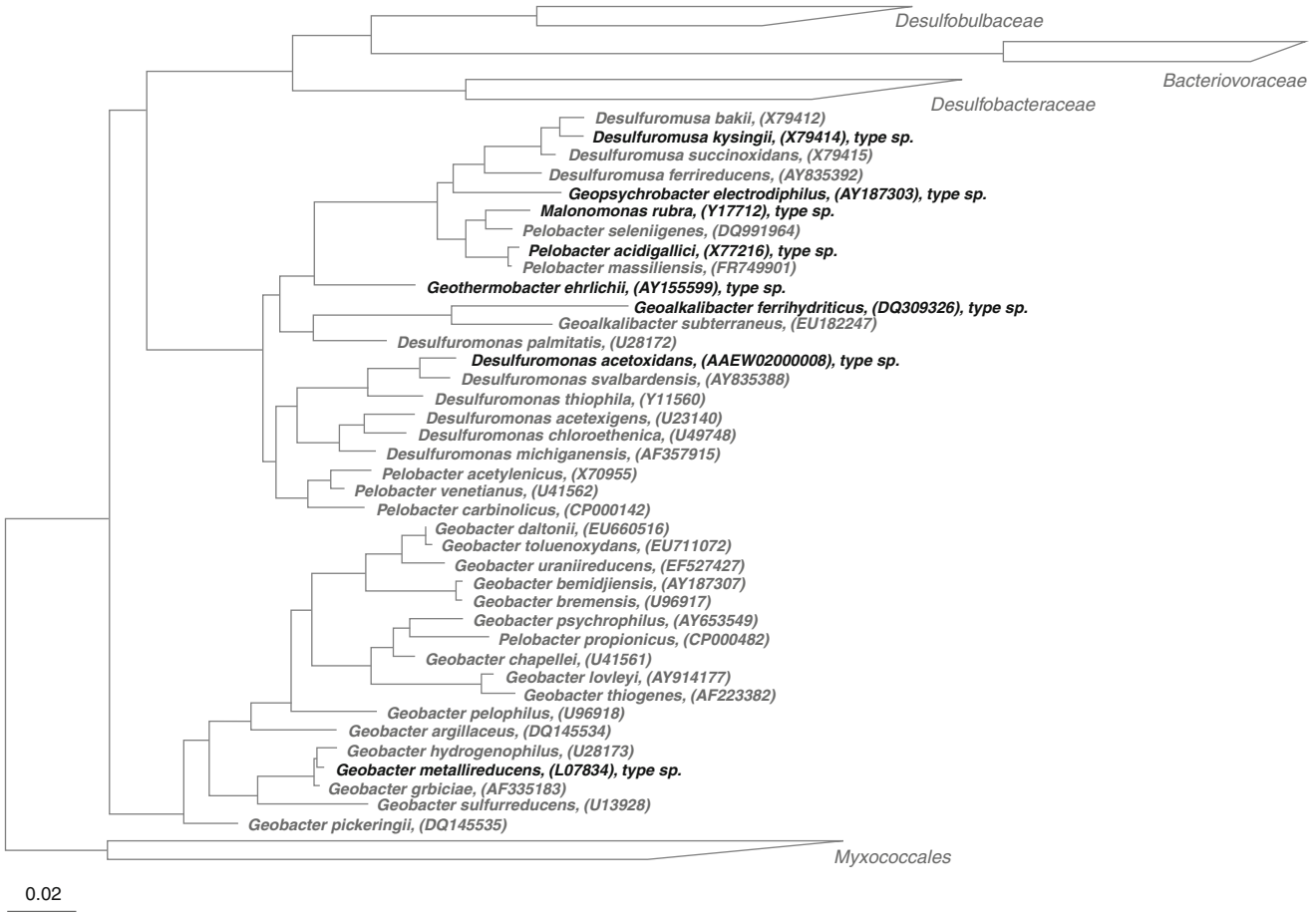

Figure 12.1

Maximum likelihood genealogy reconstruction of the 16S rRNA gene sequences of all validly described species (March 2013) in the family *Geobacteraceae*. Also members of the *Desulfuromonadaceae* are shown, to indicate that the genera *Geothermobacter*, *Geoalkalibacter*, and *Geopsychrobacter* fall within the *Desulfuromonadaceae*. The bar indicates 2 % sequence divergence. Type strains for genera are indicated in bold

analysis of six genes: the genera *Geobacter* and *Trichlorobacter* in the *Geobacter* subclade; the genera *Desulfuromonas*, *Pelobacter*, and *Malonomonas* in the *Desulfuromonas* subclade; and the genera *Desulfuromusa* in the *Desulfuromusa* subclade. 16S-rRNA gene-based evolutionary distances between the *Geobacter* and *Desulfuromonas* subclades were observed to be large and generally confirmed by the additional analysis of five other marker genes (Holmes et al. 2004a). Garrity et al. (2005) and Kuever et al. (2005) assigned in *Bergey's Manual* the same six genera to two different families, the *Geobacteraceae* and the *Desulfuromonadaceae*, with *Geobacteraceae* (Garrity et al. 2005) comprising members of the genera *Geobacter* and *Trichlorobacter*, while the other genera were included in the *Desulfuromonadaceae* (Kuever et al. 2005). Garrity et al. (2005) described the family *Geobacteraceae* in also on basis of the phylogenetic analysis of 16S rRNA gene sequences. There appear to be no unique physiological characteristics that distinguish *Geobacteraceae* from *Desulfuromonadaceae*, although members of the family *Geobacteraceae* have only been isolated from anoxic fresh water-related settings, while the *Desulfuromonadaceae* are more, but not exclusively, associated with marine settings (Garrity et al. 2005; Kuever et al. 2005).

The *Geobacteraceae* description in *Bergey's Manual* (Garrity et al. 2005) is currently (March 2013) most adhered to (e.g., http://www.bacterio.cict.fr/classifgenera-families.html#Geobacteraceae; http://www.ncbi.nlm.nih.gov/taxonomy; http://www.arb-silva.de/projects/living-tree/) and largely followed here. The sole species assigned to the genus *Trichlorobacter* (De Wever et al. 2000) was later renamed to *Geobacter thiogenes* (Nevin et al. 2007), on basis of its phylogenetic and physiological similarities to *Geobacter* species. A few new genera, *Geothermobacter*, *Geoalkalibacter*, and *Geopsychrobacter*, are currently assigned to the *Geobacteraceae* (e.g., see abovementioned webpages); however, based on their 16S rRNA gene phylogeny (*Fig. 12.1*) and phylogeny on basis of other genes (Holmes et al. 2004a), these genera clearly belong to the family *Desulfuromonadaceae*. Greene et al. (2009) have proposed to amend the description of *Desulfuromonadaceae* to include also the genus *Geoalkalibacter*. In this chapter therefore the description of the *Geobacteraceae* comprises one genus,

Geobacter, and the species *Pelobacter propionicus*. This description is currently also followed by http://eztaxon-e.ezbiocloud.net/. While *Pelobacter propionicus* belongs to the family *Geobacteraceae* on basis of its phylogeny of six genes (Holmes et al. 2004a), all other isolates currently described as belonging to the *Pelobacter* genus are in the *Desulfuromonadaceae* (Kuever et al. 2005), including the type strain for the *Pelobacter* genus, *Pelobacter acidigallici* (Fig. 12.1). Therefore, *Pelobacter propionicus* requires taxonomic reassignment.

All members of the *Geobacteraceae* are Gram-negative, nonspore-forming rods with rounded ends. They are anaerobic chemoorganotrophic mesophiles able to reduce Fe(III). They mainly use small organic acids and alcohols. They contain genes for nitrogen fixation, which are functional in the strains (*G. metallireducens, G. sulfurreducens*) that have been tested for nitrogen fixation (Bazylinski et al. 2000; Holmes et al. 2004b). Several characteristics of *P. propionicus* are distinct from *Geobacter* properties: it cannot utilize acetate as an electron donor (Schink 1984) or completely oxidize organic acids. Furthermore, although it can reduce Fe(III) (Lonergan et al. 1996), it does not contain the c-type cytochromes that are involved in electron transfer to Fe(III) in *Geobacter* species (Leang et al. 2003). It has been suggested that the fermentative members in the *Geobacteraceae* and *Desulfuromonadaceae* have developed their fermentative metabolism as a secondary evolutionary event (Stackebrandt et al. 1989). *P. propionicus* clusters among *Geobacter* species (Fig. 12.1).

Molecular Analyses

DNA-DNA Relatedness Studies

Conventional DNA-DNA hybridization has been infrequently used in the taxonomy of *Geobacteraceae*, even though species delineation based on 16S rRNA gene sequences is often difficult, as species with clearly different physiological characteristics can have more than 97 % identity in their 16S rRNA genes. Hybridization of *G. bemidjiensis* and *G. bremensis* yielded a DNA-DNA relatedness value of 63.5 %, below the species threshold of 70 %, while their 16S rRNA genes were 99 % identical (Nevin et al. 2005). Genomic DNAs of *G. grbiciae* and *G. hydrogenophilus* were, respectively, 30 and 54 % homologous to *G. metallireducens*, while they were more than 99 % similar at the 16S rRNA gene level (Coates et al. 2001). *G. daltonii* revealed 21 % relatedness to its closest relative *G. uraniireducens*, 16S rRNA gene sequence similarity was 98.1 %, but *rpoB* similarity was 81.1 % and below the suggested species threshold of 97.7 % (Prakash et al. 2010). Prakash et al. (2010) concluded that DNA-DNA hybridization data are redundant for *Geobacter* species delineation when molecular data other than rRNA gene sequences indicate low genome similarity.

Genome sequencing can replace DNA-DNA hybridization studies and allow for an estimate for DNA relatedness using regression analysis, which indicated a relatedness of below 20 % between *G. daltonii* and *G. uraniireducens* (Prakash et al. 2010). The average nucleotide identity (ANI) was 73.4 %, and the percentage of DNA conserved (>90 % nucleotide identity) only 0.27 % (Prakash et al. 2010). G+C content is standard reported, ranging from 50.2 to 63.8 %, P. *propionicus* in the middle at 57.8 %.

PCR-Based Analysis of Phylogenetic Markers

Phylogeny of the currently recognized 17 species is mainly based on 16S rRNA gene sequences. However, delineation of *Geobacter* species solely on basis of 16S rRNA sequences and using the 97 % identity threshold generally employed to delineate novel species (Stackebrandt and Goebel 1994) are not definitive, as several distinct species share greater than 97 % sequence identity (Coates et al. 2001; Nevin et al. 2005; Prakash et al. 2010). Phylogeny of type strains of species of the *Geobacteraceae* and *Desulfuromonadaceae* has been studied in large detail by Holmes et al. (2004a) on the basis of six conserved genes: next to 16S rRNA, also *nifD*, *recA*, *gyrB*, *rpoB*, and *fusA* gene sequences were obtained. This study included eight *Geobacter* species and *Pelobacter propionicus*. The phylogenies derived from the later five genes also clustered *Geobacter* species together, with *P. propionicus* falling in the *Geobacter* cluster. Also *gltA*, encoding an eukaryote-like citrate synthase, has been used as a marker, since this gene is phylogenetic distinct from those of other prokaryotes and a key enzyme in the central metabolism of *Geobacteraceae* and *Desulfuromonadaceae* (Prakash et al. 2010).

Due to their high abundances in iron-reducing environments, members of the *Geobacteraceae* are already easily encountered by culturing-independent approaches using general, bacteria-specific 16S rRNA gene-directed primers. In order to specifically target and quantify *Geobacter* species, a number of specific 16S rRNA gene primers have been developed (Cummings et al. 2003; Holmes et al. 2002; Snoeyenbos-West et al. 2000). This work dates from before the family *Desulfuromonadaceae* was separated from the *Geobacteraceae*, thus the primers also target the *Desulfuromonadaceae*. All reported primer sets have their drawbacks, by either also amplifying 16S rRNA genes from other Deltaproteobacteria, such as *Syntrophus* species (Cummings et al. 2003; Holmes et al. 2002; Snoeyenbos-West et al. 2000), or being too specific and missing dominant members of the *Geobacteraceae*/*Desulfuromonadaceae* (Lin et al. 2005). Functional genes are therefore more preferred. Of these, in particular *gltA* has been applied to samples from metal-reducing environments (Holmes et al. 2005). Published primers do not distinguish fermentative *Pelobacter propionicus* from iron-reducing *Geobacter* species. Quantification of functional genes unique to *Geobacter* species, such as the gene encoding the multicopper protein OmpB which is required for Fe(III) oxide reduction, may allow for specific targeting of iron-reducing *Geobacter* species (Holmes et al. 2008).

Rep-PCR has been reported for *G. lovleyi* and *G. thiogenes* (Sung et al. 2006).

Table 12.1
Genomic features of sequenced, validly described members of the *Geobacteraceae*

	G. bemidjiensis	*G. daltonii*	*G. lovleyi*	*G. metallireducens*	*G. sulfurreducens PCA*	*G. sulfurreducens KN400*	*P. propionicus*
NCBI ID	NC_011146	CP001390	NC_010814	NC_007517	NC_002939	NC_017454	NC_008609
Type strain	Yes	Yes	Yes	Yes	Yes	No	Yes
Length (nt)	4615150	4304501	3917761	3997420	3814128	3714272	4000800
GC content (%)	60	53.5	54	59	60.9	61.3	58.5
Protein coding	4023	3798	3606	3519	3446	3328	3831
rRNA operons	4	1	2	2	2	2	4
Cytochrome	73	68	61	76	89	ND	0
Hemes/ cytochrome	7.6	9.6	4.8	7.3	7.5	ND	–
Plasmids	None	ND	77 kb	14 kb	None	None	31,202 kb
Publication[a]	a, b	a, c	a, d	a, e	a, f, g	g	h

ND not determined
[a]Publications in a, Butler et al. (2010); b, Aklujkar et al. (2010); c, Prakash et al. (2010); d, Wagner et al. (2012); e, Aklujkar et al. (2009); f, Methe et al. (2003); g, Butler et al. (2012); h, Aklujkar et al. (2012)

Genome Comparison

The complete genome sequences of five validly described *Geobacter* species and *Pelobacter propionicus* have been reported. Key characteristics are summarized in *Table 12.1*. Genome length ranges from 3.8 to 4.6 Mb. The number of 16S rRNA genes varies from one to four but is generally two. Between 3,434 and 4,023, coding genes have been deduced. A phylogeny of the family was constructed using 697 protein families that had a single ortholog in all sequenced genomes. The resulting phylogeny was in line with the 16S rRNA gene phylogeny (Butler et al. 2010).

The genes for acetate transport and its oxidation via the TCA cycle and for proton transport and energy generation across the inner membrane are well conserved in *Geobacter* species (Butler et al. 2010). Predicted pathways for use of other electron donors can vary between species, as for instance for propionate (Aklujkar et al. 2010). Orthologs to the four subunits of the uptake hydrogenase were only found in those species capable of hydrogen oxidation (Butler et al. 2010). Unexpected capacity for the use of carbon sources has been revealed: transporters for glucose were found in the genome of *G. bemidjiensis*, and its ability to grow on glucose was subsequently confirmed experimentally (Aklujkar et al. 2010). The central metabolism of *G. metallireducens* contains several energy-inefficient reactions that are not present in *G. sulfurreducens* and may aid it to improve flux for rapid energy generation when growing with complex substrates such as benzoate (Sun et al. 2009).

Although the reduction of insoluble Fe(III) is an ubiquitous feature of *Geobacter* species and occurs on the outer membrane, intriguingly, most (86 %) of the cytochromes for Fe(III) reduction are not conserved, including many of the outer-membrane cytochromes (Butler et al. 2010). While poorly conserved, cytochromes are very abundant in all *Geobacter* genomes, comprising about 2 % of all genes. Also their diversity is large, with 472 different predicted cytochromes encountered so far. Cytochrome duplication and divergence appear to play a role in these genotypes, while lateral gene transfer has a minor role at most as only 19 of the 472 predicted cytochromes have been identified as lateral gene transfer candidates. This abundance and diversity of weakly conserved cytochromes is postulated to indicate that they are important in their heme-bearing capacity, as sinks for electrons between the inner-membrane transport chain and the extracellular acceptor, and they may not be specific terminal reductases (Butler et al. 2010). Deletion studies confirmed that the outer c-type cytochromes involved in Fe(III) oxide reduction are different between *G. sulfurreducens* and *G. metallireducens* (Smith et al. 2013).

The genome of *G. sulfurreducens* provides evidence for aerobic metabolism (Methe et al. 2003), in line with its reported capability to withstand and even grow with oxygen (Lin et al. 2004). Several genomic features indicate that also *G. bemidjiensis* has enhanced abilities to respire, detoxify, and avoid oxygen (Aklujkar et al. 2010). Key genes related to oxygen tolerance and reactive oxygen species detoxification are absent from *G. lovleyi*, which does not tolerate oxygen (Wagner et al. 2012).

Geobacter species encode enzymes for the acetyl-coA pathway, which is employed to assimilate carbon dioxide (Methe et al. 2003). *G. bemidjiensis* is possibly capable of carbon dioxide fixation via citrate lyase (Aklujkar et al. 2010). Whereas most *Geobacter* species are expected to obtain reducing equivalents for biosynthesis from electron transfer pathways via a ferredoxin oxidoreductase, *G. metallireducens* derives them from the oxidative pentose phosphate pathway (Aklujkar et al. 2009). Genome-scale models combined with experiments revealed that *Geobacteraceae* have an uncommon isoleucine biosynthesis route, via citramalate as intermediate and with acetyl-coA and pyruvate are precursors (Tang et al. 2007).

Geobacter species encode a remarkable large number of genes for two-component signaling proteins (Aklujkar et al. 2009, 2010; Methe et al. 2003), suggesting a large adaptive potential (Lovley et al. 2011). They also contain multiple chemotaxis systems or homologs of the chemotaxis system and an unusually large number of chemoreceptor genes (Tran et al. 2008). One chemotaxis (−like) system is predicted to be involved in flagellar motility and appears to be unique for *Geobacter* species. The large numbers of methyl-accepting chemotaxis proteins homologs may enable the sensing of a great variety of environmental signals.

Comparative genomic analysis of *P. propionicus*, *G. metallireducens*, and *G. sulfurreducens* revealed several common features. All tricarboxylic acid cycle reactions are present, even though *P. propionicus* does not completely oxidize organic electron acceptors to carbon dioxide while *Geobacter* species can (Sun et al. 2010). Unique reactions for *P. propionicus* are methylmalonyl-coA-dependent propionate formation, corresponding to its propionate formation, and a citrate lyase reaction.

Several features that are unique for individual *Geobacter* species have been obtained by lateral acquisition and are located on genomic islands. *G. metallireducens* contains a 300 kb island predicted to encode enzymes for the degradation of many aromatic compounds except toluene; the toluene degradation genes are encoded by a separate island (Butler et al. 2007). *G. lovleyi* is the only *Geobacter* species known to respire the organohalide perchloroethene (PCE). Based on GC content and the codon adaptation index, its duplicated reductive dehalogenase (RDase) genes are on a genomic island that has recently been obtained from an ancestral gene cluster (Wagner et al. 2012). Gram-positive *Desulfitobacterium* also appear to have obtained its RDase genes from this cluster. A second genomic island in the *G. lovleyi* genome contains a predicted F-factor conjugative pilus tra-gen cluster, which does not have homologs in other sequenced *Geobacter* genomes. Cobalamin is required for organohalide respiration. Surprisingly, 15 of the 24 genes needed for de novo cobalamin biosynthesis are located on a 77 kb plasmid. However, newly isolated PCE-respiring strains did not carry a similar plasmid, indicating that this plasmid is not a requirement for PCE respiration in this species (Wagner et al. 2012).

The genomes of the *G. sulfurreducens* strains PCA and KN400 have been compared (Butler et al. 2012). The faster reduction of insoluble Fe(III) and higher current production by strain KN400 does not appear to be due to novel gene acquisition but to changes in the common metabolic network. There is significant enrichment for single-nucleotide polymorphisms in noncoding or synonymous amino acid sites, suggesting selective pressure since the divergence of the two strains. Substantial sequences differences occur especially in cytochromes and integral membrane proteins (Butler et al. 2012).

Phenotypic Analyses

The main features of members of the *Geobacteraceae* are shown in ❯ *Tables 12.2* and ❯ *12.3*.

Table 12.2
Contrasting physiological characteristics of the genus *Geobacter* and *Pelobacter propionicus*

	Geobacter	*Pelobacter propionicus*
Fermentation	−	+
Growth on acetate	+	−
Complete mineralisation	+	−
Reduction of chelated Fe(III)	+	+
Reduction of soluble Fe(III)	+	ND
Temperature tolerance (°C)	4–40	4–45

ND not determined

Geobacter (Lovley et al. 1993)

The type strain of *Geobacter* (Ge.o.bac′ter; Gr n. ge the earth; masc. bacter equivalent of Gr. Nl. Bacterion a small rod; M.L masc. n. Geobacter a rod from the earth) is *Geobacter metallireducens*. Sixteen species have so far been validly described. *Geobacter* cells are rod shaped with rounded ends and grow as single cells, in pairs or in chains. They are catalase and carotenoid negative. Cells contain menaquinone, mainly the MK8 type, and c cytochromes. Cytochromes cause pink-colored colonies on plate. They are diverse in their use of electron acceptors and donors (❯ *Table 12.3*). *Geobacter* species are mesophiles, although *G. psychrophilus* can grow at 4 °C, and prefer circumneutral pH and low salt concentrations. Physiology is influenced by culturing conditions, with for instance the type of Fe(III) used affecting substrate utilization and motility (Childers et al. 2002; Straub et al. 1998). A key phenotypic characteristic of *Geobacter* species is the conservation of energy from the oxidation of organic compounds to carbon dioxide with Fe(III), Mn(IV), and graphite electrodes as the electron acceptor. All Geobacter species contain the *nifH* gene (Holmes et al. 2004a), and nitrogen fixation has been reported for *G. metallireducens* (Bazylinski et al. 2000) and *G. sulfurreducens* (Holmes et al. 2004b).

Metal Reduction and Other Electron Acceptors

A hallmark physiological feature of *Geobacter* species is their capability to directly reduce insoluble Fe(III) forms, with the accessibility relating to the surface area (Yan et al. 2008). Poorly crystalline Fe(III) hydroxides and structural Fe(III) of phyllosilicates are well reducible, while crystalline Fe(III) are poorly accessible (Shelobolina et al. 2004). *Geobacter* species produce electric, metallic-like conductive pili (Malvankar et al. 2011; Reguera et al. 2005) that extracellular transfer electrons to insoluble Fe(III) forms. C-cytochromes are likely important in the transfer of electrons from the outer membrane to the pili and from the pili to Fe(III) but not for electron transport along the pili (Leang et al. 2010; Malvankar et al. 2011; Shi et al. 2007). *G. metallireducens* can access insoluble Fe(III) and Mn(IV) by

Table 12.3
Comparison of selected physiological and distinguishing characteristics of validly published *Geobacter* species

	G. argillaceus	*G. bemidjiensis*	*G. bremensis*	*G. chapellei*	*G. daltonii*	*G. grbiciae*	*G. hydrogenophilus*	*G. lovleyi*	*G. metallireducens*	*G. pelophilus*	*G. pickeringii*	*G. psychrophilus*	*G. sulfurreducens*	*G. thiogenes*	*G. toluenoxydans*	*G. uraniireducens*
Publications[a]	a	b	c, d	e	f, g	e	e	h, i	J, k, l	c	c	b	m-p	h, q, r	s	t
Cell size (um)	1–2 × 0.6	2.5–4 × 0.8	1.8 × 0.6	1–2 × 0.6	1–1.5 × 0.3–0.5	1–2 × 0.6	1–2 × 0.6	1–14 × 0.4	2–4 × 0.5	1.5 × 0.6	1–2 × 0.6	2.5–3 × 0.8	2–3 × 0.5	na	2.1–3.8 × 0.4	1.2–2 × 0.5–0.6
Temp. range	10–36	15–37	10–35	na	10–40	na	na	10–40	na	10–35	10–36	4–<37	na	na	16–30	10–34
Temp. optimum	30	30	30–32	25	30	35	35	30	30–35	30–32	30	17–30	30–35	30	30	32
pH	5.8–7.4	na	5.0–7.5	na	6–8	na	na	na	na	5.7–7.5	5.8–8.0	6.0–9.0	na	na	6.5–7.2.	6.0–7.7
pH optimum	6.2–6.8	7	5.5–6.7	na	6.7–7.3	na	6.5	6.5–7.2	na	6.7–7	6.6–7.2	na	na	na	6.6–7.0	6.5–7.0
salt tolerance (%)	na	na	0–1	na	0–.07	na	0–1	na	na	0–0.5	na	0–3	0–1.5	na	na	na
e-donor use																
e-accepter used in test[b]	Sol	Ch	Fum	Ch	Fum	Sol	Ch	Ch, PCE	Sol	Fum	Sol	Sol	Ch	TCA	Ch	Sol
H_2	–	+	+	–	–	+	+	+	–	+	–	–	+	–	na	–
Acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Formate	na	–	+	+	+	+	+	–	–	+	na	+	–	–	+	–
Propionate	na	–	+	–	–	+	+	–	na	+	na	–	na	–	+	–
Butyrate	+	+	+	–	+	+	+	–	+	–	+	–	–	–	+	–
Valerate	+	+	–	na	na	na	na	na	+	–	+	–	na	na	na	–
Lactate	+	+	+	+	na	–	–	–	na	–	+	+	+	–	–	+
Pyruvate	na	+	+	–	na	+	+	+	+	+	na	+	–	na	+	+
Fumarate	na	na	+	na	na	–	na	na	na	+	–	na	–	na	–	na
Malate	na	+	+	na	na	na	na	na	na	+	–	+	na	na	–	na
Succinate	–	+	+	–	–	–	+	–	na	+	–	+	–	na	–	–
Glycerol	+	–	–	na	na	na	na	na	–	–	+	–	na	–	na	na
Methanol	–	–	–	–	na	–	–	–	–	–	+	na	–	na	–	na
Ethanol	+	+	+	+	na	+	+	–	+	+	+	+	–	–	+	+

Propanol	na	na	+	na	na	na	na	na	+	+	na	na	–	na	+	na
Butanol	+	+	+	na	+	na	na	na	+	–	+	–	–	na	+	–
Acetoin	na	–	–	na	na	na	na	na	na	–	na	–	na	+	na	na
Glucose	na	+	–	–	na	–	–	–	–	–	na	na	–	na	–	na
Toluene	na	–	–	–	+	v	na	–	+	–	na	na	na	na	+	na
Benzene	na	na	na	–	+	–	–	–	+	na	na	na	na	na	–	na
Phenol	na	–	na	–	na	–	–	na	+	na	na	na	–	na	+	na
m-Cresol	na	na	na	na	na	na	na	na	–	na	na	na	na	na	+	na
p-Cresol	na	na	na	–	na	na	na	na	+	na	na	na	na	na	+	na
Benzoate	na	+	+	–	+	v	+	–	+	–	na	–	–	na	+	na
3-Hydroxybenzoate	na	–	na	na	na	na	na	na	–	na	na	na	na	na	+	na
e-acceptor use																
Fe(III) (amorphous)	+	+	+	+	+	+	+	+	+	+	+	+	+	na	+	+
Fe(III) pyrophosphate	+	+	na	+	na	+	+	na	na	na	+	+	+	na	na	+
Fe(III) citrate	+	+	+	–	+	v	+	+	+	na	+	v	+	na	+	–
Fe(III) NTA	+	+	na	+	na	+	+	na	na	na	+	+	na	+	na	+
Mn(IV)	+	+	+	+	na	na	–	+	+	+	+	v	–	na	–	+
U(VI)	+	na	na	+	+	na	+	+	+	na	+	na	+	na	na	+
Graphite electrode	na	–	+	na	na	na	na	+	+	na	na	+	+	na	na	na
AQDS	–	+	na	+	na	+	+	na	+	na	+	v	na	na	na	+
Sulfur	+	–	+	+	+	na	+	+	–	+	+	–	+	+	–	–
Nitrate	+	–	–	–	–	na	–	+	+	–	–	–	–	na	–	–
Fumarate	–	+	+	+	+	na	+	+	–	+	+	+	+	+	+	+
Malate	–	+	+	–	+	na	–	+	na	+	+	+	+	na	na	+
TCA	na	na	na	na	na	na	na	–	na	na	na	na	na	+	na	na
TCE	na	na	na	na	na	na	na	+	na	na	na	na	na	–	na	na
Oxygen (air)	–	na	–	–	na	na	na	na	–	–	–	na	+	na	–	–

v variable among strains of the same species, *na* not available

[a]Characteristics were published in: a, Shelobolina et al. (2007); b, Nevin et al. (2005); c, Straub and Buchholz-Cleven (2001), Straub et al. (1998); d, Nercessian et al. (2012); e, Coates et al. (1996, 2001); f, Prakash et al. (2010); g, Kostka (personal communication); h, Sung et al. (2006); i, Strycharz et al. (2008); j, Lovley et al. (1989, 1993); Lovley and Lonergan (1990); k, Zhang et al. (2012); l, Tremblay et al. (2012); m, Caccavo et al. (1994); n, Bond and Lovley (2003); o, Lin et al. (2004); p, Ueki and Lovley (2010); q, De Wever et al. (2000); r, Nevin et al. (2007); s, Kunapuli et al. (2010); t, Shelobolina et al. (2008)

[b]Indicated is the electron acceptor used in assays to determine electron donor usage, with *Sol* solid Fe(III), *Ch* chelated Fe(III), *Fum* fumarate, *PCE* perchloroethene, and *TCA* trichloroacetic acid

chemotaxis towards their reduced products (Childers et al. 2002), for which it uses flagella and pili.

Most *Geobacter* species also reduce quinone moieties in natural and artificial humic acids (AQDS), which function as extracellular electron shuttles and transfer abiotically their electrons to insoluble Fe(III) compounds, including crystalline Fe (III) oxides (Bauer and Kappler 2009; Scott et al. 1998). The small size of humic acids enables the reduction of Fe(III) that is not physically accessible by the microbes. Cysteine can also be utilized as electron shuttle (Doong and Schink 2002). *Geobacter* species grow faster with dissolved, chelated Fe(III), or the availability of electron shuttles; however, they have not been found to produce metabolic energy-expensive chelators or electron shuttles themselves, in contrast to other iron reducers (Nevin and Lovley 2000). Extracellular magnetite can be produced during iron reduction, as well as other Fe(II)-containing minerals.

Geobacter species are versatile in the use of electron acceptors (Table 12.3). Most species can also reduce fumarate, malate, sulfur, and heavy metals, such as U(VI), and are electricigens, able to transfer electrons to electrodes. Nitrate, when used, is reduced to ammonia. Nitrite, sulfate, sulfite, thiosulfite, selenate, or selenite reduction has never been observed. Organohalide respiration has been observed for two species: *G. thiogenes* reduces trichloroacetate (TCA) while *G. lovleyi* respires per- and trichloroethene (PCE, TCE). Dechlorination can occur in the presence of alternative electron acceptors (Sung et al. 2006). De Wever et al. (2000) postulated a sulfur-sulfide cycle to be involved in TCA reduction, but this mechanism was not observed for PCE reduction by *G. lovleyi* (Sung et al. 2006).

While *Geobacter* species were considered to be strict anaerobes, at least *G. sulfurreducens* is capable of growth in the presence of 10 % O_2, and it tolerates atmospheric oxygen for at least 24 h (Lin et al. 2004). The aerobic metabolism is in line with genomic information (Methe et al. 2003).

Organic and Inorganic Electron Donors

Acetate is the only carbon source found to be used by all *Geobacter* species, but a large number of carbon sources can be mineralized to carbon dioxide. Differentiating substrate utilization characteristics are shown in Table 12.3. C_1-C_5 organic acids and C_2-C_4 alcohols are frequently utilized, with some species using nearly all of these substrates (e.g., *G. bremensis*) while others consume only a few (e.g., *G. sulfurreducens*, *G. thiogenes*). Species that used iso-propionate or/and iso-butyrate also use propionate or/and butyrate, respectively.

Also aromatic compounds are used by several species, with benzoate utilization most frequently observed. Toluene degradation has been observed for four species, two of these species are also able to degrade benzene: *G. metallireducens* (Zhang et al. 2012) and *G. daltonii* (Kostka, personal communication). Other environmental pollutants that are oxidized by some *Geobacter* species are phenol, p-cresol, and m-cresol; no isolates are known to degrade ethylbenzene or xylene. Species that use toluene also degrade benzoate, benzaldehyde, and benzyl alcohol and have been found to use 4-hydroxy benzaldehyde, 4-hydroxy benzyl alcohol, and 4-hydroxybenzoate.

G. *bemidjiensis* is the sole *Geobacter* species known to degrade the carbohydrate glucose; a property predicted from genome analysis and then experimentally confirmed (Aklujkar et al. 2010). *Geobacter* species do not ferment but can disproportionate fumarate (Aklujkar et al. 2010).

Geobacter species have not been found to use as sole source of carbon: amino acids, polyaromatic hydrocarbons, alkanes, palmitate, citrate, caproate, caprylate, and intermediates of aerobic aromate degradation such as ferulate, syringate and salicylic acid, and the sugars fructose and mannitol.

Geobacter species can also utilize inorganic electron donors. Many species grow with hydrogen, in the presence of organic carbon (Table 12.3). *G. metallireducens* can also oxidize Fe (II) and U(IV) or reduced humic acids with nitrate or fumarate as an electron acceptor, although it is unclear if energy conservation to support growth occurs with these electron donors (Finneran et al. 2002; Lovley and Blunt-Harris 1999; Weber et al. 2006). Sulfur oxidation has not been observed.

Chemotaxonomic Properties

G. metallireducens, *G. sulfurreducens*, and G. *bemidjiensis* have been investigated in a single study for their polar lipid fatty acids (PFLAs), lipopolysaccharide (LPS) hydroxyl fatty acids, and respiratory quinones. Lipid profiles varied with strains and less with electron acceptor (Hedrick et al. 2009). Variation in culturing conditions influences PFLA profiles, complicating comparisons between different studies for the same species (Hedrick et al. 2009; Lovley et al. 1993; Shelobolina et al. 2008). The most abundant PFLAs are 14:0, i15:0, 16:1ϖ7c, 16:1ϖ5c, and 16:0. LPS hydroxyl fatty acids were more variable between species and electron acceptors than PFLAs. They were dominated by 3oh14:0, unusual high levels of 3oh16:0, and the uncommon fatty acids 9oh16:0 and 10oh16:0; the latter may be used as biomarkers for *Geobacter*. *G. metallireducens* and *G. sulfurreducens* contain a range of hopanoid lipids, in particular squalene, diploptene, and bishomohopan-32-ol acetate. Their quantities were highest in *G. metallireducens*. These biomarkers were long used as indicators for ancient oxic environments (Hartner et al. 2005).

Menaquinone-8 is the most abundant respiratory quinone, constituting about 85 % of the total. Ubiquinones have not been detected (Hedrick et al. 2009). Reduced cytochrome c spectra show absorption maxima at 419–424, 521–524 and 551–555 nm.

Pelobacter propionicus (Schink 1984)

Three Gram-negative, nonspore-forming, rod-shaped *Pelobacter propionicus* strains were isolated by Schink (1984). Several features are contrasting those of *Geobacter* species (Table 12.2).

P. propionicus is capable of fermentation of 2,3-butanediol, acetoin, and acetate and variable in the fermentation of diacetyl, pyruvate, ethanol, propanol, and butanol. Propanol and butanol are fermented to the corresponding fatty acid with concomitant reduction of acetate and carbonate to propionate (Schink 1984). In contrast to *Geobacter* species and despite the possession of the tricarboxylic acid cycle (Sun et al. 2010), it produces acetate and propionate as fermentation products. Its growth requires carbonate and a reductant. It does not express cytochrome c (Schink 1984). Besides fermentation, it can use chelated Fe(III) and sulfur as electron acceptor with lactate as electron donor (Lonergan et al. 1996) but not nitrate. Electron acceptors that are also not used by *Geobacter* are also not used by *P. propionicus*. The temperature range (4–45 °C) over which *P. propionicus* can grow is wider than reported for any *Geobacter* species.

Enrichment, Isolation and Maintenance Procedures

Culturing *Geobacteraceae*

Geobacter species are relatively easy to enrich and isolate, although it should be noted that some in situ dominant *Geobacter* phylotypes have resisted isolation (Botton et al. 2007; Lin et al. 2007b; Rooney-Varga et al. 1999). Most studies describing the enrichment and/or isolation of *Geobacter* species were not specifically aiming for *Geobacteraceae* but for iron-reducing microorganisms in general. For a more specific enrichment and isolation of *Geobacter* species, an anaerobic, liquid mineral medium containing acetate as sole source of carbon and amorphous Fe(III) as electron acceptor is recommended. Acetate is a substrate used by all *Geobacter* members, and only a few other iron reducers (e.g., *Rhodoferax metallireducens*) (Finneran et al. 2003) apart from members of the *Desulfuromonadaceae*. It should be noted, however, that *P. propionicus* does not grow with acetate. The use of amorphous or poorly crystalline Fe(III) as electron acceptor is advised, even though the preparation of amorphous Fe (III) is more laborious and faster growth is observed when chelated Fe (III) is used. The use of iron chelators, such as citrate or nitrilotriacetic acid (NTA), bears the risk that microorganisms that ferment the chelator may be enriched and use Fe(III) non-dissimilatory (Caccavo et al. 1994). If chelators are to be used, Fe(III)NTA is preferred. A dilution-to-extinction approach (comparable to a most probable number approach) is recommended to enrich for the most dominant iron reducers. Sample dilution and to a lesser extent the type of Fe(III) affects the type of iron-reducing consortia that will grow (Lin et al. 2007a, b).

Although iron reducers grow in liquid cultures to cell densities that are too low to be seen by eye, iron reduction itself can easily be observed in enrichments. Upon reduction of insoluble Fe(III), its brown color will start to disappear. A black color, due to magnetite formation, may appear over time. A color change from orange/brownish to colorless will be observed in media with soluble, chelated Fe(III). Frequently, the formation of Fe(II)-containing precipitates will occur. This formation depends on the type of medium, especially on the buffering agent employed. For instance, white precipitates ($FeCO_3$) may occur in carbonate buffered media. The color change depends also on the amount of Fe(III) and acetate employed. Complete oxidation of one molecule of acetate requires eight molecules of Fe(III). An excess of acetate in the medium will enable complete iron reduction but also bears the risk of growth of acetoclastic methanogens on the remaining acetate in enrichments.

Soon after the observation of iron reduction, positive enrichments must be transferred a couple of times to fresh medium at 1–10 % (v/v), before strain isolation is attempted. The transfers are needed to remove compounds (e.g., sediment particles, organics) associated with the original inoculum. For strain isolation, the enrichments need to be tenfold serially diluted into a (semi)solid medium; after incubation, single colonies can be picked with sterile glass Pasteur pipettes in an anaerobic glove box and subcultured on solid or liquid medium. Insoluble Fe(III) can easily be employed in liquid media but is more difficult to use in (semi)solid media where chelated Fe(III) is preferred over the use of other soluble electron acceptors, such as nitrate, which are not used by all *Geobacteraceae*.

Strict anaerobic conditions must be employed in the preparation of media and handling of *Geobacteraceae*. The addition of oxygen scavengers aids growth. Commonly used oxygen scavengers for anaerobic culturing, such as sulfide, can react abiotically with Fe (III). Fe(II), added to a final concentration of 0.5 mM $FeCl_2$, is a more appropriate oxygen scavenger. Anaerobic conditions are more easily maintained in liquid cultures than in solid media. Incubations should be performed in the dark. Vitamins are generally not required for *Geobacter* growth but enhance growth (Coates and Lovley 2005). Genome analysis and subsequent experimental validation revealed that *Geobacter bemidjiensis* is deficient in 4-aminobenzoate synthesis (Aklujkar et al. 2010).

A frequently employed medium to enrich, isolate, and study *Geobacter* species is a freshwater Fe(III) oxide basal medium (Lovley and Phillips 1988), containing per liter:

- 30–100 mM amorphous Fe(III)oxide or 5–10 mM Fe(III) NTA
- 1.36 g Na-acetate (10 mM)
- 1.5 g NH_4Cl
- 2.5 g $NaHCO_3$
- 0.6 g $NaH_2PO_4.H_2O$
- 0.1 g KCl
- 0.1 g $CaCl_2.2H_2O$
- 10 mL vitamin solution according to DSMZ medium 141
- 10 mL trace element solution, prepared according to DSMZ medium 141

The medium is dispensed in serum bottles or tubes and sparged with 80–90 % N_2 and 20–10 % CO_2, capped with butyl rubber stoppers (Viton stoppers in case of PCE or monoaromatic hydrocarbons as carbon source), and sterilized. 1.5–2.0 % bacteriological agar is added for solid medium. Fe(III) is generally added after autoclaving and cooling down of the medium.

Amorphous, poorly crystalline Fe(III) is prepared by dissolving $FeCl_3{\cdot}6H_2O$ in 400 mL water to a concentration

of 0.4 M. Subsequently, the pH must be carefully adjusted to pH 7.0 by slowly and dropwise adding a 10 M NaOH solution to the stirred Fe(III) solution. A (temporarily) too high pH may result in the formation of less bioavailable Fe(III) forms. Once pH 7.0 is reached, the solution must be stirred for an additional 30 min and pH further adjusted, when needed. The suspension is then centrifuged for 15 min at 5,000 rpm, and the supernatant is discarded. The Fe(III)oxide is resuspended in water, and the procedure of centrifugation and resuspension is repeated six times, to obtain chloride-free Fe(III) oxide. In the final step, the Fe(III)oxide is dissolved in 100 mL, to a concentration of ~1 mol per liter. It is advisable to determine the Fe(III) content, using ferrozine (Viollier et al. 2000). Autoclaving this solution is not recommended, and filter sterilization is not possible: an alternative is to prepare an acidic 6-line ferrihydrite solution (Schwertmann and Cornell 1991) which can be filter sterilized at concentrations of 25–75 mM.

A 100 mM stock solution of Fe(III)NTA is prepared by dissolving 1.64 g $NaHCO_3$ in 80 mL water, followed by adding 2.56 g sodium nitrilotriacetic acid and then 2.7 g $FeCl_3.6H_2O$. After bringing the volume to 100 mL, the solution must be sparged with an 80–90 % N_2 and 20–10 % CO_2 gas mixture and filter sterilized into an anaerobic, sterile serum bottle.

The addition of tungsten (0.00025 g/L $NaWO_4 \cdot 2H_2O$) to the medium is recommended to aid the enrichment and isolation of aromatic compound degrading *Geobacter* species: obligate anaerobic microorganisms employ a tungsten-containing class of benzoyl-coenzyme A reductases to open the ring of benzoyl-coA, a central intermediate in aromatic degradation (Kung et al. 2009).

A second medium for culturing iron reducers is based on the medium used for the cultivation of sulfate-reducing bacteria (Widdel and Pfennig 1982), with Fe(III) replacing $NaSO_4$. The medium of Widdel and Pfennig is also used to enrich for fermentative *Pelobacter*, in which case the $NaSO_4$ is omitted.

Maintenance

Geobacter cultures can be maintained as frozen stocks at −70 °C, after growth to mid-exponential phase. The stocks can be prepared in by transferring aliquots (1–5 mL) to small anaerobic serum vials (10 mL), to which an anaerobic solution of glycerol is added to final concentrations of 10 %. After mixing, the vial is immediately frozen at −70 °C. The frozen stocks should be checked regularly for viability. The cultures can also be stored at 4 °C but then need to be transferred every 4 weeks (Coates and Lovley 2005).

Ecology

A large number of culturing-based studies, and in particular molecular analyses, which avoid cultivation bias, have revealed that *Geobacteraceae* are of large global biogeochemical significance (reviewed in Lovley et al. 2011). They frequently dominate in soils and sediments in which Fe(III) reduction is an important process, such as aquatic sediments, wetlands, rice paddies, and the subsurface. *Geobacter* species are especially present in relative high numbers in iron-reducing environments subjected to anthropogenic increases in organic matter but are certainly not only limited to these environments (reviewed by Lovley et al. 2011). In wetland sediments, *Geobacter* species may contribute significantly to anaerobic redox cycling of iron by performing both dissimilatory Fe(III) reduction and oxidation of Fe(II) with nitrate (Weber et al. 2006), although other microorganisms might be more important in the Fe(II) oxidation (Coby et al. 2011). A culturing-based study indicated that *Geobacteraceae* might also be important in the reduction of electron-shuttling humic acids (Coates et al. 1998). *Geobacter* species have mainly been observed in environments with circumneutral pH but also have been encountered in acidic springs, peat or sediments (Adams et al. 2007; Kusel et al. 2008; Percent et al. 2008). *Geobacter* species are also frequently abundant on the surface of electrodes harvesting electricity from organic compounds in water or sediments (reviewed in Lovley et al. 2011).

Geobacter species have a number of properties that contribute to their high abundances in iron-reducing settings: their ability to converse energy from the oxidation of the key organic intermediate in anaerobic fermentation (acetate) coupled to Fe(III) or Mn(IV), chemotaxis towards iron (Childers et al. 2002), the use of nanowires to access iron via extracellular electron transfer (Reguera et al. 2005), capacity to fix nitrogen (Bazylinski et al. 2000), and a very low-maintenance energy (Lin et al. 2009). Furthermore, the high number of genes for the two-component signal proteins suggest a very large adaptive potential (Lovley et al. 2011). The relative importance of these characteristics varies with the type of environment in which they thrive. *Geobacter* species may become dominant in acetate-amended, nitrogen-limited environments due to their ability to fix nitrogen (Zhuang et al. 2011) but are also dominant in ammonium-rich landfill leachate plumes (Röling et al. 2001), where more likely the low-maintenance energy is of importance (Lin et al. 2009).

Geobacter species can have substantial geochemical impacts in anaerobic soils and sediments. These include the release of soluble Fe(II) from insoluble Fe(III) into groundwater, accompanied by trace metals, metalloids, and phosphates, which may have secondary effects such as stimulation of microbial growth and generation of groundwater that is unsuitable as drinking water. Also changes in soil porosity can occur, and iron reduction may lead to the precipitation of Fe(II)-containing minerals.

Besides their importance in metal reduction, *Geobacteraceae* may also be important as fermenting microorganisms in interspecies electron transfer under nonmetal-reducing conditions. Syntrophic interactions of *Geobacter* species with other microbial species via extracellular transfer of electrons to them via metabolites or with humic acid, cysteine, or sulfide as electron shuttles have been shown in laboratory cocultures with acetate

or toluene as the source of carbon (Cord-Ruwisch et al. 1998; Kaden et al. 2002; Meckenstock 1999). Surprisingly, while syntrophic interactions would be expected for the fermenting *P. propionicus*, when this species was cocultured with organisms that could potentially scavenge metabolites excreted by *P. propionicus*, its biomass concentration did not increase (Schink 1984). Recently, *Geobacter* species were found to be also capable to transfer electrons directly to syntrophic partners (Summers et al. 2010). Geobacter species can also receive electrons directly from fermenting partners (Summers et al. 2010), although this ability also depends on the partner microorganism (Rotaru et al. 2012). The environmental significance of direct interspecies electron transfer still has to be shown. Molecular analyses revealed that *Geobacter* and closely related species can constitute 20 % or more of cell numbers in methanogenic aggregates in anaerobic digesters that treat organic brewery waste water (Morita et al. 2011). This suggests that direct interspecies electron transfer can be important in such methanogenic settings.

The availability of genome sequences has enabled an iterative genome-scale modeling and experimental approach that resulted in an in-depth understanding of the central metabolism and ecology of *Geobacter* species (Mahadevan et al. 2011).

Pathogenicity, Clinical Relevance

While pathogenic *Geobacteraceae* have not been described, the activity of *Geobacteraceae* may still have major impacts on human health. Upon reduction of subsurface Fe(III) oxides by *Geobacter* species, also toxic heavy metals can be released into groundwater, which is in many countries a major source of drinking water. A major groundwater pollutant is arsenic, constituting a major toxic hazard to millions of people worldwide, especially East Asia. Microbial communities in microcosms containing As-mobilizing sediments from West Bengal were dominated by *Geobacter* species. Arsenic release occurred after iron reduction and was limited by the availability of electron donor (Islam et al. 2004).

Growth of *G. sulfurreducens* is inhibited by chloramphenicol (10 mg/mL), nalidixic acid (10 mg/mL), tetracycline (10 mg/mL), kanamycin (200 mg/mL), spectinomycin (50 mg/mL), streptomycin (400 mg/mL), and ampicillin (400 mg/mL) (Coppi et al. 2001).

Application

Besides being of global ecological significance, members of the *Geobacteraceae* have found utility in a large variety of environmental biotechnological applications. They play major roles in bioremediation and in recent years are also employed to harvest electricity from organic wastes and aquatic sediments. *Geobacter* species are generally not purposely applied in environmental biotechnology but become dominant in microbial communities due to the combination of the applied treatment and the physiological characteristics of *Geobacteraceae*, in particular their ability to use acetate and to make electrical contact with extracellular electron acceptors.

Natural Attenuation of Hydrocarbons and Organic Compounds

Iron is the fourth element on Earth and an important component of subsurface sediments. When subsurface becomes polluted with organic compounds and hydrocarbons, e.g., due to petroleum spills, highway runoff of acetate-containing deicing compounds or after disposal on unlined landfills, these pollutants are often disappearing under iron-reducing conditions, without requiring human intervention (e.g., van Breukelen et al. 2003). *Geobacter* species are frequently dominant in these settings and present at relative higher abundances than in control samples (Holmes et al. 2007; Lin et al. 2005; Röling et al. 2001; Rooney-Varga et al. 1999; Winderl et al. 2008).

Important groundwater pollutants are the monoaromates benzene, toluene, ethylbenzene, and xylene (BTEX), as they dissolve relatively well and are carcinogenic. BTEX were long considered to be degradable under iron-reducing conditions solely by *Geobacter* species, based on enrichments (e.g., Botton et al. 2007), and properties of isolated species (◉ *Table 12.3*). In recent years, several species outside the *Geobacteraceae* family have also been found to be capable of degradation of toluene and benzene coupled to iron reduction (Kunapuli et al. 2007, 2010; Weelink et al. 2009). Analysis of marker genes of anaerobic BTEX degradation has suggested that these non-*Geobacter* species may play specific, important roles in anaerobic monoaromatic degradation in some iron-reducing settings, next to *Geobacter* species (Pilloni et al. 2011; Staats et al. 2011).

Active Bioremediation of Hydrocarbons

Several strategies aiming to stimulate aromatic hydrocarbon degradation by *Geobacter* species have been designed and successfully tested. These approaches are especially directed at enhancing the availability of Fe(III) present in solid Fe(III) forms for reduction, by the addition of chelators (Lovley et al. 1994, 1996b), or introduction of humic acids as electron shuttles (Lovley et al. 1996a). Nanoparticles of iron oxides are better reducible than their larger counter partners. Their small size may allow their injection into polluted aquifers, to stimulate iron reduction in aquifers with low amounts of bioavailable iron (Bosch et al. 2010). Inserting electrodes as in situ electron acceptor provides an interesting alternative (Zhang et al. 2010). Lovley (2011) proposed the concept of "subsurface snorkels" with graphite rods functioning in contaminated anaerobic sediments, extending into aerobic soil or water. The part of the rod in the anaerobic soil functions as the anode, while the portion of the rod in the aerobic environments above comprises the cathode.

Active Bioremediation of Heavy Metals

Bioremediation has been used to reductively precipitate heavy metals in the subsurface, with a main focus on U(VI). Removal of soluble U(VI) in groundwater can be achieved by acetate amendment. Injection with acetate often leads to the preferential growth of *Geobacter* species and concomitant precipitation of U(IV) (Holmes et al. 2002). As an attractive alternative, the insertion of electrodes to provide *Geobacter* cells in situ with electrons has been tested (Gregory and Lovley 2005). This approach would allow for a continuous, simple supply of electrons to *Geobacter* growing on the electrodes for the reduction of the soluble U(VI), with the produced U(IV) precipitating on the electrode. By periodically removing and cleaning the electrode, U(VI) can be irreversibly removed.

Laboratory studies have revealed that *Geobacter* species also can reduce other heavy metals to less mobile forms: Ag(I) (Law et al. 2008), Co(III) (Caccavo et al. 1994), V(V) (Ortiz-Bernad et al. 2004), Tc(VII), Np(V) (Lloyd et al. 2000), Pu(IV) (Boukhalfa et al. 2007), and Hg(II) (Wiatrowski et al. 2006). In situ removal of V(V) and Tc(VII) has been revealed, however may also be due to the abiotic reduction of these heavy metals by biological produced Fe(II) (Lloyd et al. 2000; Ortiz-Bernad et al. 2004). Although *Geobacter* species may contribute to arsenic mobilization (Islam et al. 2004), it was observed that *G. sulfurreducens* does not reduce As(V) to more soluble As(III) and that the formation of solid Fe(II)-bearing phases during Fe(III) reduction aids to capture arsenic species (Islam et al. 2005).

Bioremediation of Chlorinated Contaminants

PCE and nitrate are frequently co-contaminants at uranium-contaminated sites. *G. lovleyi* is so far the only *Geobacter* species known to be capable of PCE respiration but dechlorinates PCE incompletely, with cis-dichloroethene as a more toxic end product than the parent molecule (Sung et al. 2006). *G. lovleyi* is, however, frequently found in enrichments with *Dehalococcoides* species that completely degrade PCE to ethane (Yan et al. 2012). It has been postulated that incomplete organohalide-respiring microorganisms enhance overall organohalide detoxification. Also, *G. lovleyi* provides *Dehalococcoides* species with specific cobamides for their growth (Yan et al. 2012). Based on energetic consideration, it was expected that PCE respiration precedes nitrate and U(IV) reduction; however, *G. lovleyi* simultaneously reduces these compounds (Sung et al. 2006). This may provide a growth advantage over competitors in poor substrate environments with time-variant substrates. *G. lovleyi* is also only organohalide-respiring strain known to use hydrogen and acetate. As for heavy metals, electrode-based removal strategies for PCE have been suggested (Strycharz et al. 2008).

The formation of reactive Fe(II) minerals or reduced humic acids by *Geobacter* species may aid the abiotic removal of carbon tetrachloride (Cervantes et al. 2004; McCormick et al. 2002).

Bioenergy

Microbial fuel cells and microbial electrolysis cells to decompose organic wastewater are intensively investigated. Microbial fuel cells generate an electric current, while microbial electrolysis cells yield hydrogen or methane from organic matter by applying a current. *Geobacter* species are consistently enriched on anodes in microbial fuel cells (Lovley et al. 2011), while molecular analysis has frequently revealed sequences closest related to *P. propionicus* in hydrogen-producing microbial electrolysis cells (Chae et al. 2008; Selembo et al. 2010), although *P. propionicus* dominated also anodes in acetate-fed microbial fuel cells (Kiely et al. 2011). While *Geobacter* species are already efficient in the transfer of electrons to electrodes, there is scope for further optimization of *Geobacter* strains for improved current output by metabolic engineering (Izallalen et al. 2008).

Recent observations may lead to further application of *Geobacteraceae* in energy production as *Geobacter* species are abundant syntrophs in conductive aggregates in methanogenic digesters (Morita et al. 2011), possibly enabling the rapid conversion of organic matter to methane. In an enrichment converting coal to methane, *Geobacter* was one of the most abundant microorganisms (Jones et al. 2010). Cocultures of *Clostridium beijerinckii* and *G. metallireducens* on xylose in the presence of a humic acid analogue as electron shuttle enhanced biohydrogen production by *C. beijerinckii* by up to 52 %. Hydrogen production was enhanced due to removal of *Clostridium*-produced, inhibitory acetate by *G. metallireducens* and the cycling of humic acids between the two species (Zhang et al. 2013).

Ecological Systems Biology and Environmental Biotechnology

Understanding in situ physiological status is key to bioremediation and other environmental biotechnological applications, in order to monitor and rationally engineer the activities of *Geobacter* species. Methods to quantify key gene transcripts and proteins, and relating their abundances to growth rates and metabolic rates, oxidative stress, acetate availability, and limitations in iron, ammonium, and phosphate, have been developed (Chin et al. 2004; Elifantz et al. 2010; Holmes et al. 2004b, 2008; Mouser et al. 2009; N'Guessan et al. 2010; O'Neil et al. 2008). The development of these indicators of in situ physiological status took optimally advantage of the availability of pure *Geobacter* isolates that are closely related to those in metal-reducing environments and the ability to grow these *Geobacter* species in chemostats under environmental relevant conditions.

The availability of sequenced genomes has contributed to constrained-based, genome-scale models of several *Geobacter* species and *P. propionicus* (Mahadevan et al. 2011). Genome-scale metabolic models integrated into reactive transport models allowed for in silico evaluation of various strategies to stimulate in

situ uranium bioremediation (Scheibe et al. 2009). Flux balance analysis based on genome-scale models also contributed to a better understanding on when and why *Geobacter* species become dominant iron reducers or lose to other iron-reducing microorganisms (Zhuang et al. 2011) or microorganisms that employ other electron accepting processes (Barlett et al. 2012; Zhuang et al. 2012). These models have contributed to directed rational engineering to accelerate rates of respiration and power output, by creating ATP consuming futile cycles (Izallalen et al. 2008).

Genetic systems for the manipulation of *G. sulfurreducens* (Coppi et al. 2001) and *G. metallireducens* (Tremblay et al. 2012) by gene deletion and replacement have been developed and allow for metabolic engineering. Adaptive evolution by applying selective pressure for rapid Fe(III) oxide reduction resulted in the isolation of *G. sulfurreducens* strains with tenfold higher rates than those of the parent strain. Genome resequencing revealed mutations in a c-type cytochrome and transcriptional regulator and when these were introduced into the parent strains they were found to be responsible for the higher rates (Tremblay et al. 2011).

Future Applications

Inspired by the unique properties of *Geobacter* species to interact bidirectionally with electrodes, Lovley et al. (2011) presented a number of exciting potential applications. Microbial electrosynthesis is a process of synthesizing organic compounds from carbon dioxide, with the input of electrons from electrodes. Electronically functional material consisting of living *Geobacter* cells would allow for cheap, self-renewable applications in bioelectronics.

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13 The Family *Haliangiaceae*

Ronald Garcia · Rolf Müller
Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland – Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology, Saarland University, Saarbrücken, Germany

Abstract
Haliangiaceae represent a unique myxobacterial taxon occupying a novel and distinct phylogenetic cluster in the suborder *Nannocystineae* with *Kofleriaceae* emerging as their most closely related family. To date, there is no valid standing nomenclature to classify the monotypic genus *Haliangium* and member species (*H. ochraceum*, *H. tepidum*) into a family rank level. So far, all members of this taxon were isolated from marine environment. *Haliangiaceae* represent bacteriolytic- and non-cellulolytic-type obligate halophilic myxobacteria, and currently the only known application is the production of novel biologically active compounds.

Taxonomy, Historical and Current

Proposed Short Description of the Family

Ha.li.an'gi.a'ce.ae. M.L. fem. n. *Haliangium* type genus of the family; *-aceae* ending to denote a family; M.L. pl. fem. n. *Haliangiaceae* the *Haliangium* family.

Based on 16S rRNA gene sequences, *Haliangiaceae* form a phylogenetic distinct clade in suborder *Nannocystineae*, order *Myxococcales* (Garcia et al. 2010), class *Deltaproteobacteria*, and phylum Proteobacteria.

The family is composed of the monotypic type genus *Haliangium* (Fudou et al. 2002). Vegetative rod cells stain Gram-negative and are non-flexuous, slender, and fat with more or less blunted ends. Cells move by swarming or gliding. Fruiting or fruiting-like bodies are mound shape to sporangiole type containing rounded spore or spore-like cells. Swarm colonies are thin and filmlike on lean medium. Colony edge may appear flame-like to ridges on surface, or as bands and pseudoplasmodia in agar. Radial vein architecture, agar depressions, and breaks may also be produced by the swarming colony. The members of this family are regarded aerobic, mesophilic, and chemoorganotrophic. They tolerate sodium chloride and resist wide spectra of antibiotics. No hydroxy-type fatty acid is found in this family (Fudou et al. 2002; Garcia et al. 2011; Stadler et al. 2010). Mol percent G+C ranges from 67 to 69.

Historical and Comments on *Haliangiaceae*

Haliangium is the first isolated and described truly marine myxobacterial genus (Fudou et al. 2002) validated about 10 years ago (Validation list No. 87). The assignment of the genus *Haliangium* to a family rank appears not clear. *Haliangiaceae* family is currently not existing in the List of Prokaryotic Names with Standing Nomenclature (LPSN) (http://www.bacterio.net/). In an editorial note in *Bergey's Manual of Systematic Bacteriology*, 2nd edition, it was stated that the genus *Haliangium* and member species (*H. ochraceum*, *H. tepidum*) were proposed by Fudou and co-workers (2002) to be included in the *Kofleriaceae* family (Reichenbach 2005). In addition, *Kofleriaceae* only include the monotypic genus *Kofleria* comprising of the monotypic species *Kofleria flava* (Reichenbach et al. 2005). However, *Haliangium* was already enlisted in a newly erected *Haliangiaceae* family in the Taxonomic Outline of the Prokaryotes, Release 5.0 of the *Bergey's Manual of Systematic Bacteriology* (Garrity et al. 2004; http://www.taxonomicoutline.org/index.php/toba), as well as in a more recently updated version of the Taxonomic Outline of Bacteria and Archaea, TOBA Release 7.7 (Garrity et al. 2007). Albeit the *Haliangiaceae* family was enlisted in these outlines, it is indeed not validly described in the taxonomic nomenclature. Since *Haliangium* has neither been

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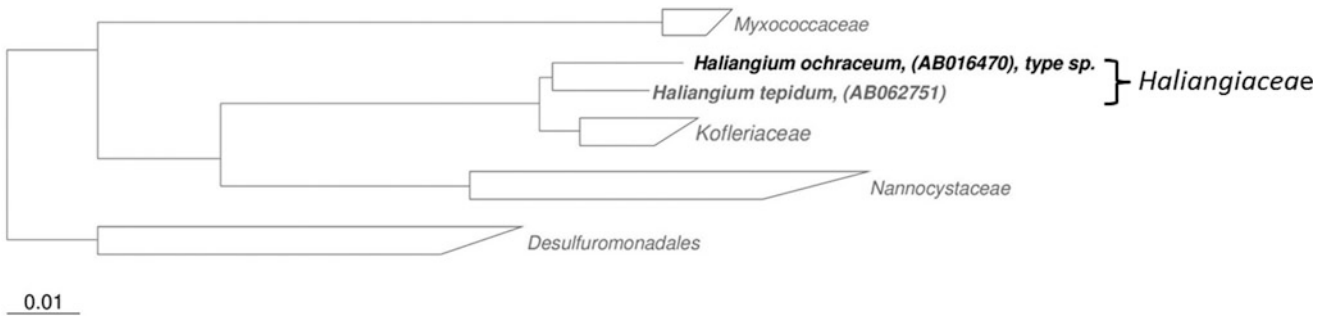

Fig. 13.1

Phylogenetic reconstruction of the family *Haliangiaceae* based on 16S rRNA gene sequences created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence dataset and alignment used were 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

directly connected to the *Kofleriaceae* nor with the *Haliangiaceae*, the family rank remains to be determined. In light of the facts presented in this chapter, the creation of a family *Haliangiaceae* seems justified; hence such proposal is made in description of the family.

The proposal to include the two species of *Haliangium* in *Kofleriaceae* (a reclassification of *Polyangium vitellinum*) was made on the basis of a 16S rRNA gene phylogenetic tree and in accordance with morphological similarities to *P. vitellinum* (Fudou et al. 2002). However, recent studies have shown that phylogenetic sequence difference greater than 4–5 % (equivalent to 96–94 % similarity) could already mark a delineation of myxobacteria into subgroups or clades (Garcia et al. 2010; Jiang et al. 2007). For *Phaselicystis flava*, the highest 16S rRNA gene similarity (96 %) to *Sorangium cellulosum* and its novel cluster occupied in the phylogenetic tree determined major points for the proposal to a novel family in *Sorangiineae* (Garcia et al. 2009). A high sequence divergence (>5 %) and differences in chemo-physiological (including fatty acid profile) and morphological characteristics between *Haliangium* and *Kofleria* again suggest that they belong to two distinct separate families.

Phylogenetic Structure of the Family and Related Taxa

The phylogenetic position of *Haliangium* based on 16S rRNA gene was first unveiled more than a decade ago after its successful isolation and proposal to a novel genus (Iizuka et al. 1998; Fudou et al. 2002). *Haliangium ochraceum* strain SMP-2^T (*H. ochraceum*) represents the first isolate in this genus and was shown to be affiliated with *Nannocystis* (Iizuka et al. 1998). Later studies revealed the phylogenetic positions of the two *Haliangium* species (*H. tepidum*, *H. ochraceum*) clustering with *Polyangium vitellinum* Pl vt1^T (Fudou et al. 2002; Iizuka et al. 2003b). The unique positioning of *Haliangium* in the *Nannocystineae* suborder was further supported in the extensive phylogenetic analysis covering most myxobacterial-type strains (Garcia et al. 2010; Ivanova et al. 2010). Based on phylogenetic distance, topology, and physiological differences, *Haliangium* was already discussed to occupy a family rank (Garcia et al. 2010). Using the neighbor-joining tree algorithm in the All-Species Living Tree Project (LTP) (Yarza et al. 2010; ❯ *Fig. 13.1*), *Haliangiaceae* was affirmed to occupy a unique cluster of its own bifurcating from *Kofleriaceae*. Both families are phylogenetically divergent within the *Enhygromyxa-Plesiocystis* and *Nannocystis* clades in *Nannocystaceae* (Garcia et al. 2010; ❯ Chap. 16).

Molecular and Genome Analysis

Haliangium ochraceum strain SMP-2^T is the only known member of this family with genome sequence available from Sanger and 454 platforms. The genome size is 9,446,314 bp and is found in a circular chromosome (Ivanova et al. 2010). There are about 6,898 protein-coding genes, 53 RNAs, and 2 copies of an identical 16S rRNA gene, and the genome contains 69.5 % GC (see also ❯ Chap. 14). About 62.1 % of these protein-coding genes were assigned to a putative function, while the rest were annotated as hypothetical proteins (Ivanova et al. 2010).

The genome contains some predominant functional clusters of orthologous group (COG) in the following categories: (1) 549 genes involved in signal transduction mechanisms; (2) 488 genes for transcription factors; (3) 439 genes for replication, recombination, and repair; (4) 332 genes associated to yet unknown function; and (5) 308 genes involved in amino acid transport and metabolism. Since myxobacteria are known for their diverse

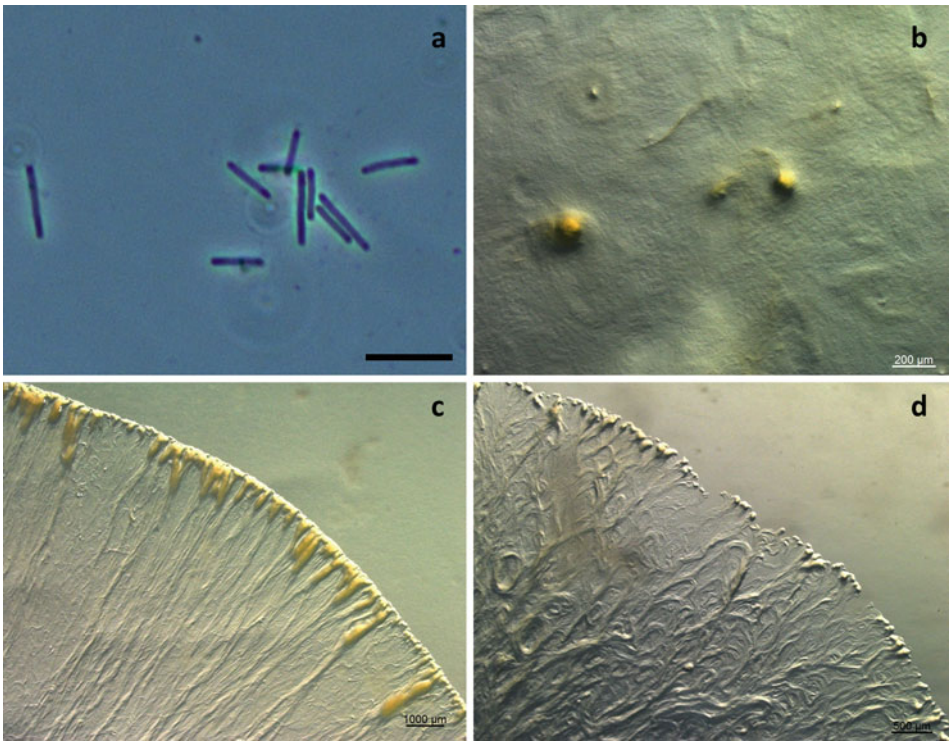

Fig. 13.2
***Haliangium ochraceum* DSM 14365^T growth stages. Phase-contrast photomicrograph of vegetative cells (a). Fruiting-like body aggregates (b). Swarming colony on VY/2SWS medium showing the concentration of *yellow* cells at the edge (c) and tortuous veins with flare-like ends (d). Stereophotomicrographs (b–c). Bar, 10 µm (a)**

natural products, it is worth mentioning that there are 174 genes assigned to COG categories associated with secondary metabolite biosynthesis, transport, and catabolism (Ivanova et al. 2010). The polyketide synthases (PKS) multidomain proteins are found in COG 3221. The most striking feature of the SMP-2^T genome is the unusually high number of genes (16) in the cytoskeleton COG category encoding proteins of the actin family (COG 5277). In most bacteria this feature is absent, and out of these 16 genes, 15 belong to COG5184 which is associated to α-tubulin suppressor function and related to the eukaryotic cell cycle regulator RCC1 domain-containing proteins (Ivanova et al. 2010). However, the function of these hypothetical proteins remains to be determined in myxobacteria. No plasmids and phages are known to date in this family.

Phenotypic Analysis

The family is characterized phenotypically by phase-dark, fat, cylindrical, and stout vegetative cells with blunted ends. Swarm colony is thin and filmlike on lean medium and can penetrate, depress, corrode, etch, cut, or split the agar. Myxospores or myxospore-like cells are short rods to spherical shape. Fruiting bodies are either sporangiole-type (*H. tepidum*) or aggregate-like structure (*H. ochraceum*) (Fudou et al. 2002; Iizuka et al. 1998). *Figures 13.2* and *13.3* show the growth characteristic pattern of *Haliangium ochraceum* and *Haliangium tepidum*, respectively.

Haliangium Fudou, Jojima, Iizuka, Yamanaka 2002

Ha.li.an'gi.um. Gr. adj. *halios*, belonging to the sea or marine; Gr. neut. n. *angion* vessel; M.L. neut. n. *Haliangium* vessel found in the sea (Fudou et al. 2002).

Fruiting bodies are composed of sessile aggregate-like structure or sporangioles containing more or less spherical myxospores. Swarm shows vein with flare-like edges and composed of slender rod vegetative cells with blunted ends. Partially degrades agar, lyses bacteria, and does not decompose cellulose. Congo-red stain is not adsorbed. Exhibits strict aerobic and moderately halophilic characteristics. Major menaquinone is MK-8. Major fatty cellular acids are *iso*-$C_{16:0}$, $C_{16:0}$, and *iso*-$C_{17:0}$. Anteiso fatty acids are also present. The DNA mol% G+C is 67–69 %. The type species is *Haliangium ochraceum*, and the type strain is SMP-2^T (=AJ 13395^T = DSM 14365^T = JCM 11303^T). Source was dry coastal *Laminariales* seaweed or sea grass collected from sandy beach (Aburatsubo-wan) of Miura Peninsula, Kanagawa, Japan, collected

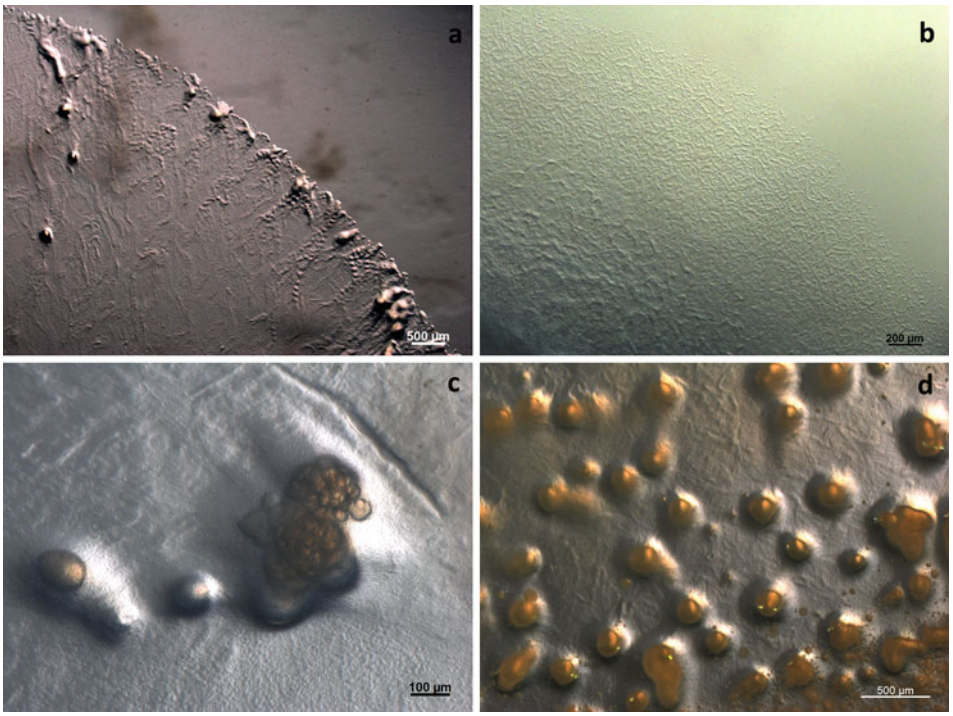

Fig. 13.3
Stereophotomicrographs of *Haliangium tepidum* DSM 14436^T growth stages. Swarming colony on VY/2SWS agar showing flame-like edges with ripplings (a) and fine flares (b). Cluster of yellowish and tiny sporangioles on agar (c). Huge cell mounds on peptone-based medium showing similarity to a *Myxococcus* or a developing stage of myxobacterial fruiting body (d)

in 1997 (Iizuka et al. 1998; Fudou et al. 2002). *Table 13.1* shows the diagnostic characteristics of the two *Haliangium* species.

Enrichment, Isolation, Growth Cultivations, and Preservations

Just like *Plesiocystis*, *Haliangium* could also be isolated in ASW-WCX agar medium (gram per liter, Bacto agar 15, $CaCl_2{\cdot}2H_2O$ 1.0, dissolved in 0.75× artificial seawater (ASW), pH adjusted to 7.5 with NaOH before autoclaving, supplemented with cyanocobalamin 0.5 mg, and cycloheximide 25 mg after autoclaving) cross-streaked with live *Escherichia coli* (Iizuka et al. 1998). Small amount of sample can be inoculated on several areas of the streaked bacterium. Incubation can be set from 25° C to 28 °C. Growth can be recognized by clearing of the bacterial bait produced by the swarming cells. Pure culture can be obtained by repeated transfers of the swarm colony to the same fresh medium containing bait.

Haliangium can be maintained in 1/5 CY/SWS agar (grams per liter, Bacto Casitone 0.6, Bacto yeast extract 0.2, NaCl 20, agar 15, seawater salt solution (SWS) 1 L, adjust pH to 7.4 with 1 M NaOH) (Iizuka et al. 2003a). *Haliangium ochraceum* can also grow in CY/SWS with 3 g/L Casitone (Difco). SWS contain (grams per liter distilled water) $MgSO_4{\cdot}7H_2O$ 8.0, $CaCl_2{\cdot}2H_2O$ 1.0, KCl 0.5, $NaHCO_3$ 0.16, H_3BO_3 0.02, KBr 0.08, $SrCl_2$ 0.03, glycerophosphate-2Na 0.01, and ferrous citrate 0.1; supplement with 1 mL trace element solution, and adjust pH to 7.5 with KOH (Iizuka et al. 1998). Alternatively, SWS was reformulated in a modified version where ferrous citrate was reduced to 10 mg/L, and pH was adjusted to 7.4 using NaOH (Iizuka et al. 2003a). Trace element solution contain (milligrams per liter distilled water or specified) $MnCl_2{\cdot}4H_2O$ 100, $CoCl_2$ 20, $CuSO_4$ 10, $Na_2MoO_4{\cdot}2H_2O$ 10, $ZnCl_2$ 20, LiCl 5, $SnCl_2{\cdot}2H_2O$ 5, H_3BO_3 10, KBr 20, KI 20, EDTA, and Na-Fe^{+3} salt trihydrate 8 g, and filter sterilize (Drews 1974).

Haliangium can be routinely cultivated in VY/2-ASW agar (grams per liter, Baker's yeast 5, Bacto agar 15, artificial seawater (ASW) 0.75×, pH adjusted to 7.5 with NaOH, supplemented with cyanocobalamin 0.5 mg/L after autoclaving) (Iizuka et al. 1998), Vy2/SWS agar (grams per liter, Baker's yeast paste prepared from dried yeast and washed 3× with deionized water 5, NaCl 20, Bacto agar 15, dissolved in modified version of SWS, but with pH readjusted lower to 7.3 with 1 M NaOH, supplemented with cyanocobalamin 0.5 mg/L after autoclaving) (Iizuka et al. 2003a), and casein/SWS (grams per liter, casein sodium 2, NaCl 20, dissolved in modified SWS, supplemented with 0.5 mg/mL cyanocobalamin after autoclaving) (Iizuka et al. 2003a). *Haliangium* can be incubated at 30 °C (*H. ochraceum*) to 37 °C (*H. tepidum*) and stored for around 2–4 weeks.

Table 13.1
Diagnostic characteristics of *Haliangium* species

	Haliangium ochraceum DSM 14365^T (=SMP-2^T)	*Haliangium tepidum* DSM 14436^T (=SMP-10^T)
Isolation source	Dry seaweed (*Laminariales*) (Iizuka et al. 1998)	Sea grass
Vegetative cell: shape	Long, slender, cylindrical rods with blunted ends	Long, slender, cylindrical rods with blunted ends
Type	*Non-flexuous*	*Non-flexuous*
Size L × W (μm) Fudou et al. 2002; Iizuka et al. 2003a	0.5–0.6 × 3.0–8.0/0.5–0.8 × 3.0–8.0	0.5–0.6 × 3.0–8.0/0.5–0.8 × 3.0–8.0
Swarm colony	*Colorless to yellow shades*	*Colorless to pale yellow*
Edge	*Flare- to flame-like*	*Flare- to flame-like*
Diffusable pigment (VY2/SWS agar)	*None*	*None*
Diffusable pigment (CY/SWS agar)	*Yellow to amber*	*None*
Fluorescent pigment	None	None
Myxospore shape	Oval to spherical	Oval to spherical
Size (μm)	0.5–0.7	0.5–0.7
Fruiting body	Fruiting-like aggregate (Iizuka et al. 1998)	Sporangiole
Arrangement	*Often solitary*	*Often as clusters*
Shape	Oval	Slight polyhedral
Color	Light yellow to yellowish brown	*Pale yellow*
Size (μm)	50–150	15–70
Congo red stain	Negative	Negative
Oxygen requirement	Aerobic	Aerobic
Temperature tolerance (°C)	20–40	27–45
Optimum (°C)	30–34	37–40
Growth at 15 °C	Negative	Negative
18 °C	Positive	Negative
37 °C	Positive	Positive
45 °C	Negative/weak (Iizuka et al. 2003a)	Positive
NaCl range (%) Fudou et al. (2002); Iizuka et al. (2003a)	0.5–6.0/0.2–6.5	0.5–6.0/0.2–6.5
Optimum (%)	1.0–3.0	1.0–3.0
Cation requirement (Iizuka et al. 2003a)	Ca^{2+}, Mg^{2+}	Ca^{2+}
Catalase	Positive	Negative
Oxidase	Weak reaction	Weak reaction
Cellulose	Negative	Negative
Agar	Positive	Positive
Chitin	Positive	Negative
Casein	Positive	Positive
DNA	Positive	Positive
Gelatin	Positive	Positive
Starch	Positive	Positive
Tween 80 (Iizuka et al. 2003a)	Positive	Positive
Bacterial lysis (*Escherichia coli*)	Positive	Positive
Yeast lysis (Baker's yeast)	Positive	Positive
Alkaline phosphatase	Weak reaction	Weak reaction

Table 13.1 (continued)

	Haliangium ochraceum DSM 14365^T (=SMP-2^T)	*Haliangium tepidum* DSM 14436^T (=SMP-10^T)
Acid phosphatase	Weak reaction	Weak reaction
C4 esterase	Negative	Negative
C8 esterase	Weak reaction	Weak reaction
C14 lipase	Weak reaction	Weak reaction
Cystine arylamidase	Negative	Negative
Leucine arylamidase	Weak reaction	Weak reaction
Valine arylamidase	Weak reaction	Weak reaction
Trypsin	Weak reaction	Weak reaction
Chymotrypsin	Weak reaction	Weak reaction
Naphthol-AS-BI-phosphohydrolase	Positive	Positive
α-Glucosidase	Negative	Positive
β-Glucosidase	Positive	Positive
α-Galactosidase	Negative	Negative
β-Galactosidase	Negative	Negative
β-Glucuronidase	Negative	Negative
α-Mannosidase	Negative	Negative
α-Fucosidase	Negative	Negative
N-acetyl-beta-glucosaminidase	Negative	Negative
Antibiotic resistance (50 μg/mL)		
Ampicillin	*No growth*	*No growth*
Bacitracin	*Growth*	*Growth*
Cephalosporin	*No growth*	*No growth*
Fusidic acid	*Growth*	*Growth* (*poor*)
Gentamicin	*Growth*	*No growth*
Hygromycin	*Growth*	*Growth*
Kanamycin	*No growth*	*No growth*
Kasugamycin	*No growth*	*Growth*
Neomycin	*No growth*	*No growth*
Oxytetracycline	*Growth* (*poor*)	*Growth*
Polymyxin	*Growth*	*Growth*
Spectinomycin	*No growth*	*No growth*
Thiostrepton	*Growth*	*No growth*
Trimethoprim	*Growth* (*poor*)	*Growth* (*poor*)
Major menaquinone	MK8	MK8
Major fatty acids (Fudou et al. 2002; Garcia et al. 2011)	*n*-$C_{16:0}$, *iso*-$C_{16:0}$/*iso*-$C_{16:1}$, C16:1ω7c	*n*-$C_{16:0}$, *iso*-$C_{16:0}$/*iso*-C16:1, *iso*-C17:0, *iso* C-$_{15:0}$ OAG
n-$C_{16:0}$	Greater	Lesser
iso-$C_{16:0}$	Lesser	Greater
BCFA:SCFA ratio (Garcia et al. 2011)	BCFA ≥ SCFA	BCFA > SCFA
Hydroxy FAs (Fudou et al. 2002; Garcia et al. 2011)	Not detected	Not detected
PUFAs (Garcia et al. 2011)	None	None
Mol% G+C content	67	69/70 (Iizuka et al. 2003a)

Note: Unless specified, most data were obtained from Fudou et al. (2002). Text in italics were determined by the authors, except for the taxonomic names
BCFA branched-chain fatty acids, *SCFA* straight-chain fatty acids, *FAs* fatty acids, *PUFAs* polyunsaturated fatty acids

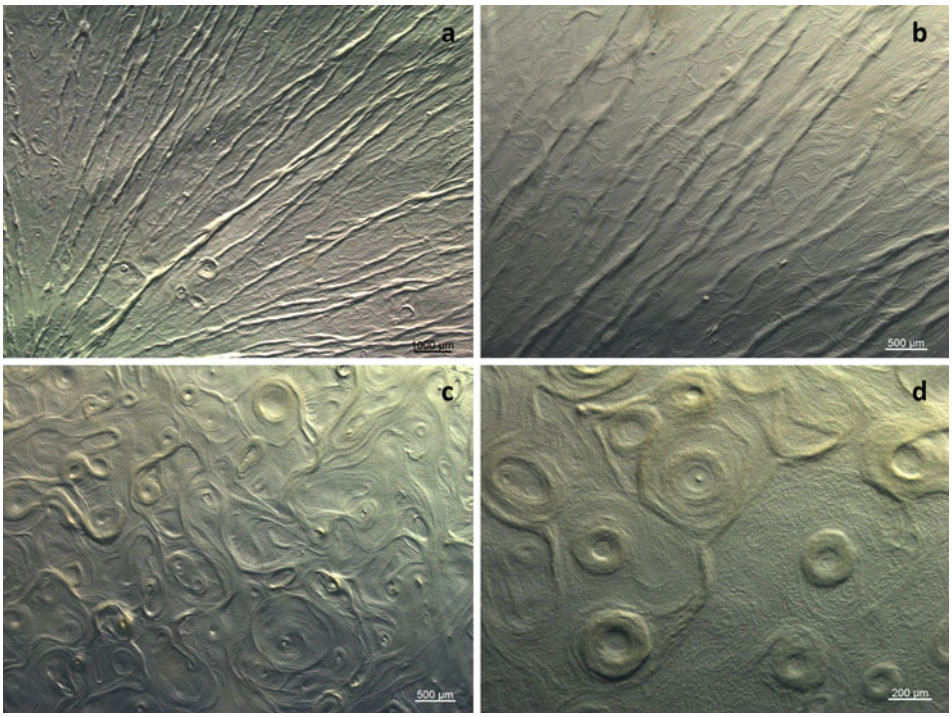

Fig. 13.4
Stereophotomicrographs of *Haliangium ochraceum* DSM 14365^T swarm colony on VY/2SWS agar. *Long* and *thick* (a) and mixed of both *thick* and fine radial veins (b). Spinning-like and tortuous vein swarming pattern (c–d)

Haliangiaceae can be preserved by slow freezing of the vegetative cells for at least 3 h in an isopropanol-freezing container (e.g., Nalgene® Mr. Frosty) before permanent storage at −80 °C. Cryopreservation could be achieved by using 20 % glycerol cryoprotectant (final concentration) mixed with suspending medium (e.g., CY/SWS) and by quick freezing using liquid nitrogen. The revival of cells is performed by thawing the sample and plating on growth-supporting media, for example, VY/2-SWS and 1/3 CY-SWS agar. In all cases, strains could be reactivated by plating or liquid inoculation.

Morphology and Unusual Bacterial Features

Halangium ochraceum exhibits slimy to radial vein swarming on agar, often in spinning pattern close to the center of colony (*Fig. 13.4*). This pattern is most famous to members of the *Cystobacteraceae* family and less common to *Nannocystineae* suborder. In Casitone-based medium (e.g., CY/SWS), huge yellow cell aggregates resembling fruiting bodies of some myxobacterial genera, and agar diffusing pigment (yellowish brown to pale amber color) can be produced (*Fig. 13.5*). In addition, deep agar excavations and cuts can also be produced by the swarming cells (*Fig. 13.6*).

Although *Haliangium ochraceum* and *Kofleria flava* (*Kofleriaceae*) share yellow vegetative cell color, fatty acid analysis revealed significant differences in the amount of straight- and branched-chain fatty acids and their respective major fatty acids (Garcia et al. 2011). *Haliangium ochraceum* contains a high amount of straight-chain $C_{16:0}$ and $C_{16:1\omega7c}$ fatty acids, and these were only found in small to trace amounts in *K. flava* (Garcia et al. 2011). In addition to the vegetative cell color, the globular sporangiole-like mass elevated by means of stalk-like slime on yeast-based medium (e.g., VY/2 agar) cannot be found in *H. ochraceum* on yeast-based/SWS agar.

Ecology, Physiology, and Metabolism

So far, all members of *Haliangiaceae* were isolated from marine environment, particularly from a sandy beach of Miura Peninsula, Kanagawa, Japan (Fudou et al. 2002). The fact that, out of 500 samples processed for isolation collected in 24 different coastal sites, it only yielded two isolates (Fudou et al. 2002) indicates the rarity of these organisms in marine environment. However, they were only isolated in one country, and hence it is currently impossible to comment on their overall geographical distribution. Despite their isolation from seaweed (*Laminariales*) or sea grass, it currently cannot be established whether these species are indeed living in association with such.

Both species described in *Haliangium* are regarded aerobic and chemoorganotroph. They are considered mesophilic with growth temperature range of 20–45 °C but with varying growth optimum depending on the species. Best growth was observed at 37–40 °C for *H. tepidum*, while 30–34 °C was found optimal for

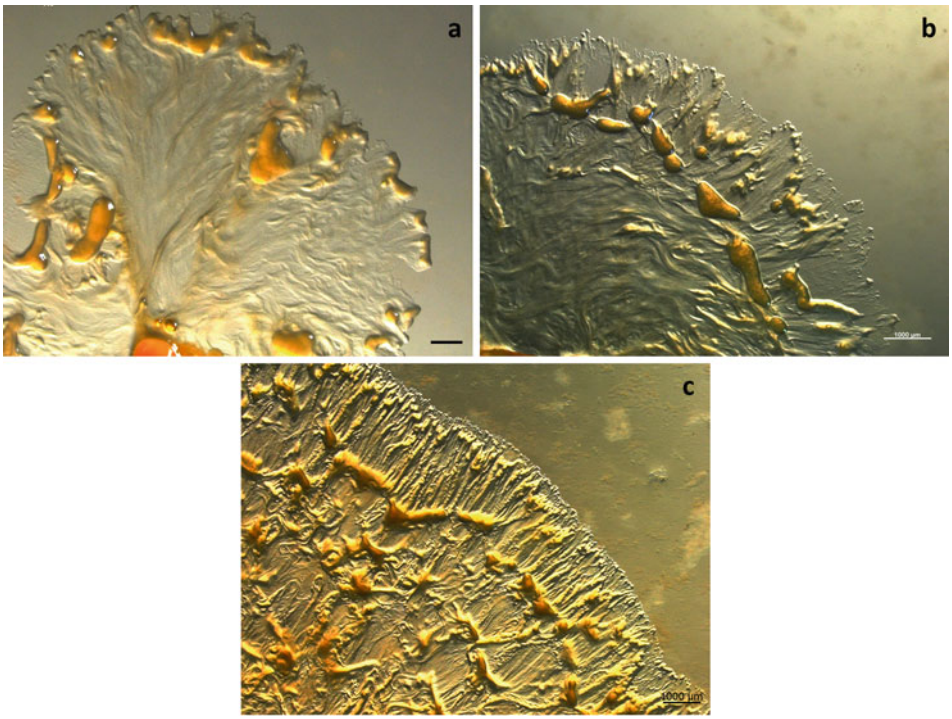

Fig. 13.5
Stereophotomicrographs of *Haliangium ochraceum* DSM 14365^T cell aggregates. *Large yellow* cell knobs, humps, and ridges on the surface of the agar (a–c). *Yellowish brown* agar diffusing pigment produced by the swarm colony in older culture cultivated in CY/SWS agar

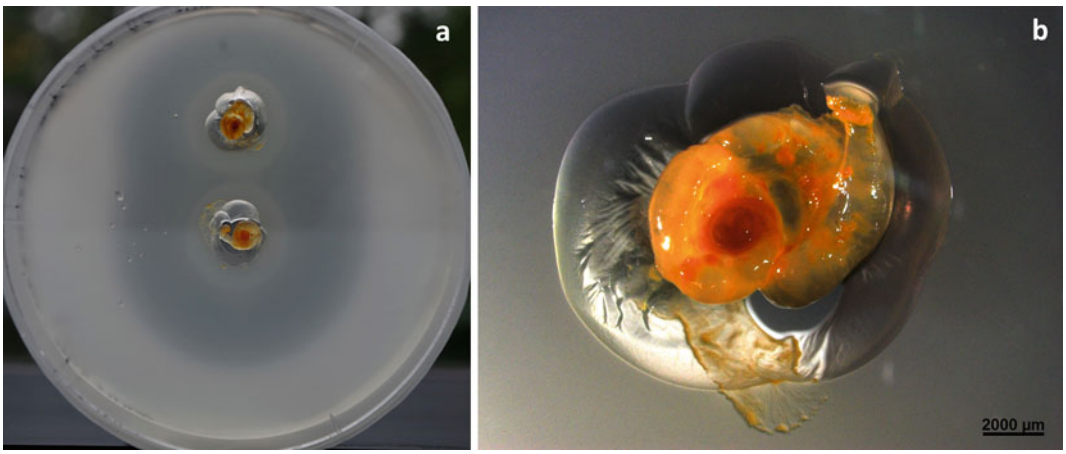

Fig. 13.6
***Haliangium ochraceum* DSM 14365^T agar degradation on VY/2SWS agar. Growing and swarming *yellow* colonies with small halo around and yeast clearing indicated by huge halo (a). Stereophotomicrograph of the top colony in figure a (b)**

H. ochraceum (Fudou et al. 2002). The pH required for growth is within the neutral range. *Haliangium* tolerates 0.5–6.0 % sodium chloride and shows optimum growth concentration at 1–3 %. Calcium cation (Ca^{2+}) appears required for growth. In *H. ochraceum*, both calcium (Ca^{2+}) and magnesium cations (Mg^{2+}) are required (Iizuka et al. 2003a). The members of this family are considered predators and decomposers capable of lysing microorganisms (bacteria and yeast) and degrading biomacromolecules including agar, gelatin, and casein, but not cellulose.

Symbiosis, Pathogenicity

No known microbial symbiosis and no implication to cause disease. *Haliangiaceae* are classified WHO Risk Group I organism having low or no risk to individual or community.

Application

So far, the first and only known secondary metabolite compound family from this group is haliangiacin (Fudou et al. 2001) including its derivatives (Kundim et al. 2003). The producer strain was previously identified as *Haliangium luteum* (Fudou et al. 2001), but was subsequently renamed to *H. ochraceum* after its valid description (Fudou et al. 2002). Haliangiacins are β-methoxyacrylate-type polyene antifungal antibiotics acting against phytopathogens such as *Phytophthora capsici* (Kundim et al. 2003). They are also considered cytotoxic compounds inhibiting the complex III respiration (Weissman and Müller 2010). To the best of our knowledge besides this antibiotic, no other application is currently known from this family. However, in search for type-1 polyketide synthase genes by PCR in marine *Nannocystineae* including *Haliangium tepidum*, novel and diverse PKS genes were discovered (Komaki et al. 2008) indicating further potential to find additional metabolites. In conclusion, the novel marine *Haliangiaceae* are likely to become an important source of novel secondary metabolites in the future.

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14 The Family *Kofleriaceae*

Elke Lang
Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany

Abstract
The family *Kofleriaceae* belongs to the order *Myxococcales*, widely known as myxobacteria. The phylogenetic neighboring genus is *Haliangium*. *Kofleria flava* is the only recognized species in the genus, which is the only genus of the family. Within *Kofleria* 11 cultured strains, all aerobic and isolated from soil, dung, or bark, are known. Based on the metagenome analysis of a freshwater lake sediment, it is concluded that members of *Kofleriaceae* are relatively abundant among the myxobacteria within this habitat. They may be distinguished from *Haliangiaceae* by being halophobic, from *Nannocystaceae* by the form of the slender cells, and the yellow swarms which do not etch the agar. No fruiting bodies with mature myxospores have been detected yet. The cells are well equipped to cleave macromolecules such as starch, casein, or chitin but not cellulose. *Kofleriaceae* grow well on dead or living food cells (bacteria or yeast), a feature being utilized for their isolation and maintenance.

Taxonomy, Historical and Current

The family *Kofleriaceae* belongs to the order *Myxococcales* within the Deltaproteobacteria. Its description is based on 11 strains which were originally assigned to the species *Polyangium vitellinum*, based on morphological characters (Reichenbach 2005a, b). On closer examination, Reichenbach perceived that these organisms do not exactly conform to the description of the genus *Polyangium*, for which *P. vitellinum* is the type species. Instead, these strains perfectly met Kofler's description of *Polyangium flavum*. Nonetheless, the analysis of the 16S rRNA gene sequence of strain Pl vt1^{T} placed the strain within the *Nannocystis* lineage but not next to *Polyangium* (Spröer et al. 1999). Because of this position and subtle morphological characters, the authors revived Kofler's *Polyangium flavum* in a new genus, *Kofleria*, and designated strain Pl vt1^{T} as the type strain of the species *Kofleria flava* (*ex* Kofler 1913), nom. rev., comb. nov. (Reichenbach 2005b). *Kofleria flava* is yet the sole recognized species in the genus *Kofleria*, which is still the sole genus of the family *Kofleriaceae* (http://www.bacterio.cict.fr/). The family was established because of the relatively large phylogenetic distance to its closest neighbor, *Nannocystis exedens*. Additionally, the appearance of the swarms and cells deviates from those of *Nannocystis* or *Polyangium* cultures (Reichenbach 2005c). Meanwhile more myxobacterial genera were described and the 16S rRNA gene of their members was sequenced. The separate phylogenetic position of a branch consisting of *K. flava* and two marine sister species, *Haliangium ochraceum* and *Haliangium tepidum*, has been consistently approved (*Fig. 14.1*; Mohr et al. 2012). Unfortunately, no 16S rRNA gene sequence for any *Polyangium* type strain is currently available which would give a more complete picture (LTP http://www.arb-silva.de/projects/living-tree/; Munoz et al. 2011; http://eztaxon-e.ezbiocloud.net/ezt_hierarchy; Kim et al. 2012), in particular about the relationship of the genera *Kofleria* and *Polyangium*.

The suggestion to establish a new family for the new genus *Kofleria* was printed in 2005, and the family name obtained its standing in nomenclature not earlier than 2007 (Validation list 115), much later than the effective description of the genus *Haliangium* was published (Fudou et al. 2002). In the meantime, several "taxonomic authorities" introduced the family *Haliangiaceae* to harbor this separate branch of descend (LTP, The Taxonomic Outline of Bacteria and Archaea, http://www.taxonomicoutline.org/index.php/toba/index). Although the family name *Haliangiaceae* has no standing in nomenclature until the date of writing, this family name is more frequently used in scientific data banks than the name *Kofleriaceae* (EzTaxon-e, IMG-JGI). The results of the molecular and phenotypic analyses, as described below, substantiate the perception that the two genera should be incorporated in one single family, a view which was expressed already by Reichenbach (2005a). He mentioned in a note added during the edition of *Bergey's Manual* that he regarded the two *Haliangium* species as members of the family *Kofleriaceae*. Fudou and coauthors (2002) also highlighted the overall resemblance of the members of the two organisms.

Until today, the description of the family and the species *K. flava* is based on the 11 strains isolated by Hans Reichenbach. The sequencing of the 16S rRNA genes (1,380 nt) of three of them revealed that they possibly represent more than one species

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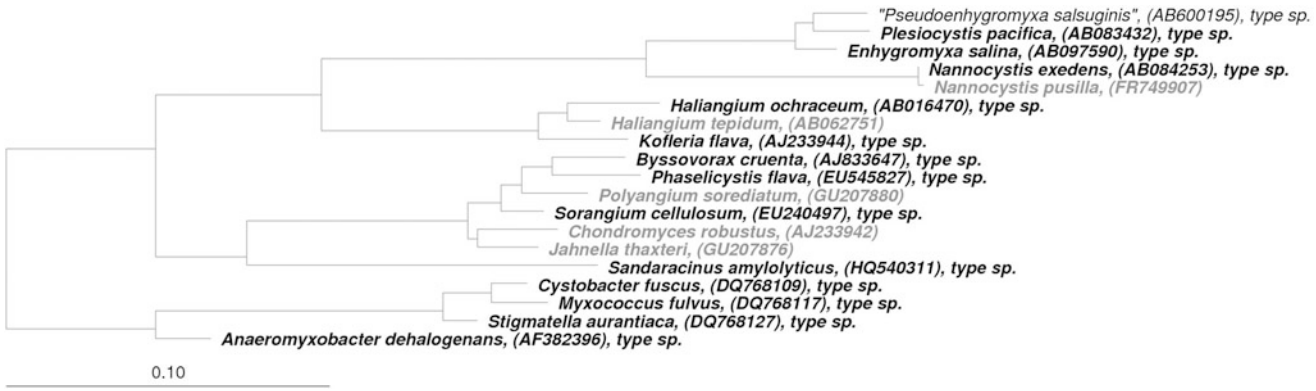

Fig. 14.1
Phylogenetic reconstruction of the family *Kofleriaceae* and related myxobacteria based on the maximum likelihood algorithm RAxML (Stamatakis 2006). Sequence dataset and alignments according to the All-Species Living Tree Project, release LTPs108 (Yarza et al. 2013). Representative sequences 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

since their sequences show similarities not higher than 98 % to the type strain Pl vt1^T (unpublished results). The members of the family share the common properties of the myxobacteria: They are Gram-negative large rods which are motile by gliding. The myxobacteria are known for the concerted action of thousands to millions of cells resulting in the formation of special morphological entities called fruiting bodies. These contain the myxospores which are highly resistant to desiccation. The metabolism of myxobacteria is strictly respiratory. The substrates predominantly used are macromolecules rather than low molecular compounds such as monosaccharides. The swarm colonies are able to predate on bacterial and yeast cells. Since peptone media generally do not support growth, VY/2 agar including heat-killed cells of baker's yeast is a favorite medium to cultivate, observe, and maintain myxobacterial strains. In the past, the taxonomic grouping and hierarchy of the myxobacteria were strongly based on the morphological features of the swarm colonies, the vegetative cells, the fruiting bodies, and the enclosed myxospores. The morphological resemblance of *Kofleria* strains to those of the genus *Polyangium*, especially to *P. vitellinum*, demolished a consistent circumscription of the cell morphology of the (now phylogenetically defined) suborder *Nannocystineae*, harboring the family *Kofleriaceae* (Reichenbach 2005c). Features which may help to differentiate members of *Kofleria* species from *Polyangium* species are as follows: The vegetative cells with rounded ends are more slender than *Polyangium* cells. The swarm colonies form globular knobs of different size all over and do not edge the agar surface. However, no completed sporangioles or myxospores have been detected up to now (Reichenbach 2005c).

The most closely related sister genus of *Kofleria* is the genus *Haliangium* with its two species *H. ochraceum* and *H. tepidum*. The two genera have many properties in common (*Table 14.1*). However, the most important difference consists of the salt requirements. While *Kofleria*, as most of the other recognized myxobacteria, are of terrestrial origin and grow optimally without or at low salt concentrations, *Haliangium* strains were isolated from marine materials, and their growth depends on the presence of NaCl (optimal concentration 1–3 % w/v) (Fudou et al. 2002). The irregular knobs and nodules of *Kofleria* resemble very much the fruiting bodies of *Haliangium* thus suggesting that the former may be incompletely developed versions of fruiting bodies.

Molecular Analyses

The 16S rRNA gene sequence of strain *K. flava* Pl vt1^T places it into one group with *H. ochraceum* and *H. tepidum* (*Fig. 14.1*). The sequences of the two *Haliangium* species showed less than 94 % similarity to strain Pl vt1^T (Ivanova et al. 2010). Regardless of the set of strains selected and of the algorithms applied for generating the phylogenetic trees, the two genera always appear as phylogenetic sister genera (Fudou et al. 2002; Garcia et al. 2010; Ivanova et al. 2010; Iizuka et al. 2013). This underpins the fact that both are descendants of a common ancestor, even though *Kofleria* strains are soilborne and *Haliangium* strains are derived from marine environments. The amount of base substitutions per site between the two *Haliangium* species and between *K. flava* and *H. tepidum* is 0.049 and 0.046, respectively, when calculated using the MCL model (MEGA5). These facts justify the question whether the three species should be unified in one and the same family.

Such considerations may be the reason why the authors of EzTaxon-e (Kim et al. 2012) list the 16S rRNA gene sequence of *Kofleria flava* with the family *Haliangiaceae* (see above). Since there are no other molecular data apart from the 16S rRNA gene available for *Kofleriaceae*, some data of the phylogenetic neighbor may give an idea about the genome of the *Kofleriaceae*: The genome of the *H. ochraceum* type strain comprises one main circular chromosome with a G + C content of 69.5 mol%. It is 9.4 Mbp long and contains 6,898 protein-coding genes, two rRNA operons, and a relatively high percentage of genes not assigned to COGs (42 %). Sixteen genes of the type strain

Table 14.1
Characters of strains of the genus *Kofleria* and the phylogenetic neighbor genus *Haliangium*

Reaction	*Kofleria*	*Haliangium*
Morphology		
Cells	Rounded ends 0.6–0.7 × 4–6 μm	Blunt ends 0.5–0.6 × 3–8 μm
Myxospores or similar structures	Spherical cell inclusions 0.5 μm in aging cultures	Oval to spherical 0.5–0.7 μm
Swarm	Tough slime sheets; may slightly sink into the agar; with or without veins, yellow rim	Tough slime sheets; may slightly sink into the agar; yellow, no veins
Fruiting bodies or similar structures	Questionable; yellow knobs in agar or on surface	Yellow-brown Fb consisting of one or several sporangioles (15–150 μm) packed in envelopes
NaCl requirements	Not required; no growth at 1 % or higher	Required, growth at 0.5–6.0 %; optimum at 1–3 %
Strains with optimum at 37 °C	+	+
Oxidase	+	w
Hydrolysis of starch	+[a]	+
DNA	–	+
Casein	+	+
Gelatin	nd	+
Esculin	++	nd
Cellulose	–[a]	–
Chitin	++[a]	var
Yeast cells	++	++
Enzymes, API ZYM		
Alkaline phosphatase	++	+
Esterase	+	–
Esterase lipase	+	+
Lipase	var	w
Leucine arylamidase	++	+
Valine arylamidase	var	+
Cystine arylamidase	–	–
Trypsin	var	+
α-chymotrypsin	var	w
Acid phosphatase	var	w
Naphthol-AS-BI-phosphohydrolase	w	+
α-galactosidase	–	–
ß-galactosidase	(–) 1/7	–
ß-glucuronidase	–	–
α-glucosidase	– or w	var
ß-glucosidase	(–) 1/7	+
N-Acetyl-ß-glucosaminidase	var	–
α-mannosidase	–	–
α-fucosidase	–	–
Enzymes, API Campy		
Urease	var	nd
Nitrate reduction	–	nd
Esterase	+	nd
Hippurate hydrolysis	(+) (6/7)	nd
γ-glutamyltransferase	var	nd
Triphenyl tetrazolium reduction	var	nd
Pyrrolidonyl arylamidase	var	nd
L-arginine arylamidase	+	nd
L-aspartate arylamidase	var	nd

w weak; *var* reaction varies among strains; 1/7: one out of seven strains positive; *nd* not determined
[a]Data from Reichenbach (2005b), data about *Haliangium* from Fudou et al. (2002)

SMP-2^T were putatively assigned to the cluster of orthologous group (COG) "cytoskeleton." Recognizing that other bacterial genomes may not comprise any genes assigned to this COG category, it is worth mentioning that all myxobacterial genomes studied so far include several copies in this category (Ivanova et al. 2010).

Phenotypic Analyses

Vegetative cells of the 11 strains assigned to the species *Kofleria flava* stain Gram-negative and form cylindrical rods with blunt ends, measuring 0.6–0.7 by 4–6 μm (*Fig. 14.2*). These cell form and size resemble those of *Haliangium* and *Polyangium* but deviate from the much shorter rods described for members of the *Nannocystaceae*, the phylogenetic co-family in the suborder *Nannocystineae* (Reichenbach 2005c). The colonies are spreading on the agar surface, forming film-like layers, and thus are called "swarms." The extending motion is propelled by gliding. The strains grow as evenly spreading swarms on VY/2 agar plates (*Fig. 14.3*) but do not grow on E-medium agar. The swarms are slightly yellow, the pigmentation being most distinctive at the thickened ridge following the very lateral rim of the swarm of some strains (*Fig. 14.3a*). The swarms consist of a more or less tough slime sheet with radial veins and spherical or irregular yellow knobs measuring 50–300 μm described in detail in Reichenbach (2005b). These globular masses are

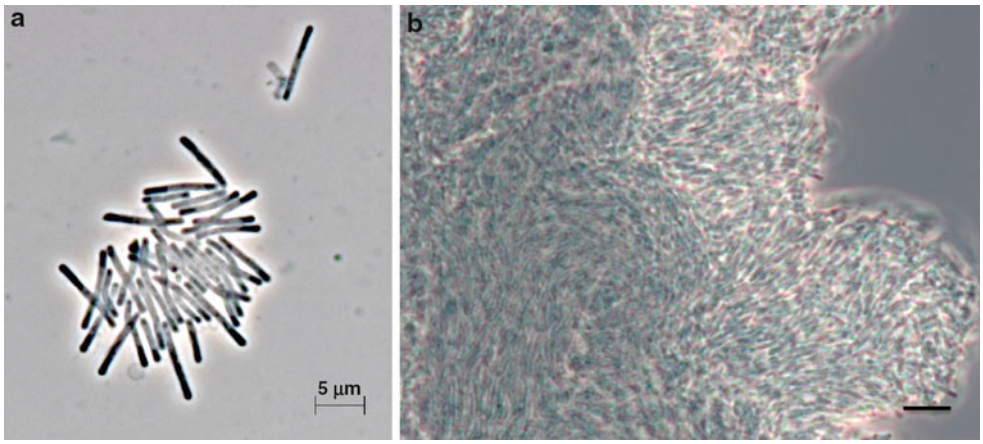

Fig. 14.2
Cell morphology of *Kofleria* strains. (a) Vegetative cells of the type strain of *Kofleria flava*, Pl vt1^T (DSM 14601^T) grown on VY/2 agar; bar = 5 µm. (b) Cells densely packed in an irregular knob on the agar surface, DSM 53745 (compare *Fig. 14.3d*) grown on VY/2 agar plus 0.5 % NaCl; bar = 10 µm

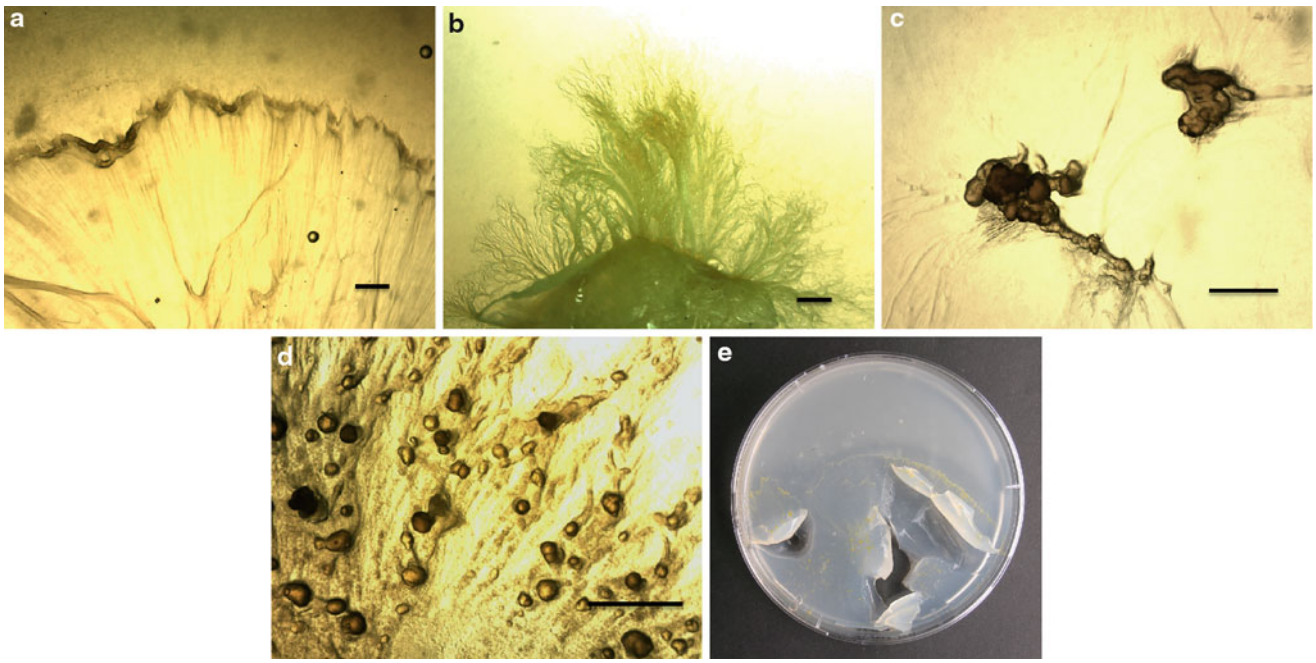

Fig. 14.3
Details of *Kofleria* swarms on agar plates. Strains grown on VY/2 medium if not given otherwise. All bars = 1 mm. (a) Edge of a swarm of DSM 14659. Note the fine first rim of the swarm, followed by a dense ridge. (b) Weak growth of DSM 14659 on PT agar with distinctive veins, starting from an inoculation cube at the bottom of the photo. (c) Fruiting body-like irregular knobs in older parts of the swarm of *Kofleria flava*, Pl vt1^T (DSM 14601^T). (d) Irregular knobs on a swarm of DSM 53743. Note the difference in size, compared to *Fig. 14.3c*. (e) Culture of DSM 14659 after 6 weeks. The swarm matrix is constricted thereby pulling off agar triangles from the petri dish

sometimes elevated over the agar surface by short stalks and may be interpreted as incompletely developed fruiting bodies. The agar surface is not edged, but the swarm matrix may contract when aging though pulling off the agar from the petri dish (*Fig. 14.3e*). Congo red is not adsorbed by the swarms (Reichenbach 2005a).

Kofleria strains are heterotrophic aerobes expressing oxidase activity. Being myxobacteria isolated under aerobic conditions, they are presumed to be strictly aerobic organisms. They are excellently equipped with enzymatic capabilities to decompose macromolecules such as starch, casein, xylan, or chitin. Cellulose or DNA, however, is not cleaved. The organisms

are specialized to decompose cells of other bacteria or yeasts; thus, the swarms on VY/2 agar are surrounded by a lysis halo.

The fatty acid profile of strain Pl vt^T is dominated by iso-branched components which make up 86 % of the total (Garcia et al. 2011). The main fatty acids are *iso*-$C_{16:0}$, *iso*-$C_{16:1}$, and *iso*-$C_{17:0}$. No hydroxylated fatty acids were detected, a feature shared with members of *Nannocystis, Polyangium*, and *Sorangium* and all marine genera such as *Haliangium, Plesiocystis*, and *Enhygromyxa* (Garcia et al. 2011). Fudou et al. (2002) stated that the presence of *anteiso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$ is a unique feature of the *Haliangium* species, not having measured strain Pl vt1^T. The presence of *anteiso*-$C_{17:0}$ (but not of *anteiso*-$C_{16:0}$) in *Kofleria* and in *Haliangium* was confirmed by Garcia et al. (2011), thus underscoring the relatedness of the two genera. However, the presence of *anteiso*-$C_{17:0}$ was also found by these authors in specific single species scattered in different families, for example, in *Melittangium lichenicola, Cystobacter armeniaca*, or, in minor amounts, in *Polyangium sorediatum*. The quinone composition of *Kofleria* was not determined yet. Twenty terrestrial species investigated so far and members of *Haliangium* contain menaquinone MK-8 (Fudou et al. 2002; Shimkets et al. 2006), but other menaquinones were found in *Plesiocystis* or *Enhygromyxa* (Iizuka et al. 2003).

Isolation, Enrichment, and Maintenance Procedures

All strains of *K. flava* grow well on VY/2 agar. Corresponding to the multicellular lifestyle, high inocula are generally needed for subcultivation. The most favorable method for transferring is by cutting small cubes from the edge of a swarm including some of the subjacent agar and placing them upside down onto a fresh agar plate. The methods of how to isolate, enrich, and maintain myxobacteria are described in detail in Shimkets et al. (2006). There are no specific instructions to be given for *Kofleria* strains. Single strains show non-spreading growth of an intensely orange-yellow biomass on CY agar, SP agar, PT agar, or HP14 agar. The establishment of liquid cultures is usually an elaborate task and can only be achieved by using high inocula, as presumed from experiences with other members of the *Nannocystineae* and *Sorangineae*. Shimkets et al. (2006) and Iizuka et al. (2013) give several hints how to achieve growth of myxobacteria in liquid media.

Short-term maintenance may be achieved by storage at 18–22 °C of VY/2 agar plates which had been inoculated at the periphery and incubated at growth temperature until growth has started. Since *Kofleria* strains do not produce myxospores under the conditions tested so far, they do not survive lyophilization. Long-term storage at −80 ° C or in liquid nitrogen is recommended. For this purpose, scrape off the swarms or, even better, cut small (3–5 mm edge length) agar cubes comprising the swarm rim from well-grown agar cultures, distribute 3–4 cubes into suitable cryovials containing 200 µl of a cryoprotecting solution (per L: 250 ml glycerol, 10.0 g casitone, and 1.0 g $MgSO_4 \times 7\ H_2O$; modified from Shimkets et al. 2006), and incubate for 10–20 min at room temperature. Subsequently, transfer the samples to the deep freezer or to the gas phase of a liquid nitrogen tank. After 1 h, the samples may be submersed into the liquid phase if appropriate. Preservation success of a series of vials should be tested immediately by using one of them. For the resuscitation, bring the frozen samples to room temperature as quickly as possible, for example, in a water bath, and transfer the agar cubes or swarm sheets to a VY/2 agar plate, thereby avoiding to transfer much of the protecting liquid. Nevertheless, be sure to transfer all biomass, as the swarm pieces may detach from the agar tubes during freezing and thawing.

Composition of the above mentioned media (Shimkets et al. 2006):

- VY/2 agar
  Per L: 5.0 g baker's yeast, 1.0 g $CaCl_2 \times 2\ H_2O$, 0.50 mg vitamin B12, 14.0 g agar (Difco). Sterilize a stock solution of vitamin B12 separately by filtration. Prepare and store yeast cells as an autoclaved stock suspension (5 g baker's yeast in 100 ml distilled water, adjust pH to 6.5 and autoclave). Adjust pH of medium to 7.2 with KOH before autoclaving and after autoclaving and cooling to 50 °C (use pH indicator paper).
- CY agar
  Per L: 3.0 g casitone, 1.0 g $CaCl_2 \times 2\ H_2O$; 1.0 g yeast extract; 14.0 g agar. pH 7.2.
- E-medium
  Per L: 4.0 g skim milk, 4.0 g defatted soy flour, 2.0 g yeast extract, 10 g starch, 50 mM HEPES, 7 mg Fe-EDTA, 5.0 ml glycerol, 14.0 g agar. pH 7.4.
- SP agar
  Per L: 1.0 g raffinose, 1.0 g sucrose, 1.0 g galactose, 5.0 g soluble starch, 2.5 g casitone, 0.5 g $MgSO_4 \times 7\ H_2O$, 0.25 g K_2HPO_4, 2.5 ml vitamin solution, 14.0 g agar. pH 7.4.
- PT agar
  Per L: 4.0 g casitone, 4.0 g yeast extract, 2.0 g $MgSO_4 \times 7\ H_2O$, 1.0 g $CaCl_2 \times 2\ H_2O$, 14.0 g agar. pH 7.2.
- Hp14 agar
  Per L: 2.0 g Na-glutamate, 1.0 g yeast extract, 1.0 g $MgSO_4 \times 7\ H_2O$, 14.0 g agar. pH 7.2.

Ecology

The hitherto cultured strains have been isolated from soil with decomposing plant material, goat dung, or bark of a *Ficus* tree. The samples were collected in the USA, Kazakhstan, India, or Germany (Reichenbach, 2005b). Accordingly, all strains were isolated and cultivated routinely on media without NaCl additions. They tolerate 0.5 % NaCl in VY/2 medium. In the presence of 1 % NaCl, only one out of six strains grows poorly and after a prolonged lag phase. This reaction classifies them as terrestrial organisms. The pH of growth for most strains ranges between pH 5 and 8 (VY/2 medium buffered with 10 mM MES, HEPES, or TAPS). The temperature range is as follows: Four out of seven strains grow at 37 °C but not at 8 °C. One of them had to be incubated at 37 °C and did not grow at 28 °C when

resuscitated from the cryopreserved state. Two strains were adapted to a lower temperature range; they multiplied at 8 °C but not at 37 °C. Lysis of yeast cells was observed at all temperatures tested.

The results of a BLAST search for similar 16S rRNA gene sequences using the "EMBL release" or "EMBL environmental" databases indicate that members of the *Kofleriaceae* are very rare in the environment since there are only two sequences of uncultured bacteria displayed which show identities higher than 94 % to the sequence derived from *K. flava* Pl vt1^{T}. One of these clones was found on concrete and one in soil from tall grass prairie. However, metagenome studies directed specifically to myxobacteria revealed that *Kofleria* relatives are detected in different environments: They constitute one of the most abundant myxobacterial branches in (freshwater) lake mud (Chenghai Lake, China) (Li et al. 2012), they are the next relatives of a methylotrophic clade in a saline, alkaline lake (Antony et al. 2010), and their 16S rDNA could be detected in samples of a glacier of the Himalaya (Liu et al. 2011) or of snow layers from a Japanese mountain (Tanaka et al. 2011). Jiang and coauthors (2010) depict 30 sequences from marine-uncultured clones, available at that time, which appear monophyletically, yet distantly related, with *Kofleria* and *Haliangium*. Most of them originate from marine sediments.

Pathogenicity, Clinical Relevance

Since the time when the definition of "myxobacteria" was coined, they were not reported as pathogenic agents to humans, animals, or plants to the best of the author's knowledge. Reports of myxobacteria being, for example, pathogenic to fish date back to a time when several gliding bacteria such as *Flavobacterium columnare* and *Flavobacterium psychrophilum* had been classified as myxobacteria.

Application

Myxobacteria are increasingly recognized due to their extraordinary potential to produce secondary metabolites. Many of these are novel chemical compounds and are affecting pro- or eukaryotic cells. Hence, these natural products are awaiting exploitation for applications in pharmacy or plant protection (Gerth et al. 2003; Kundim et al. 2003; Nett and König 2007; Weissmann and Müller 2010). Key enzymes in the biosynthesis of the metabolites are polyketide synthases and nonribosomal peptide synthetases, respectively (Silakowski et al. 2001; Gross et al. 2006). However, strains of *Kofleria* were not among the species which have been described to exhibit such capabilities. Since the metabolite spectrum is strain-specific, this fact might be due to the low number of strains isolated yet. It can be expected that together with the increasing knowledge about the specific biology of the different myxobacteria families, the possibility of finding more isolates and concordantly other producers of metabolites will rise as well.

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15 The Family *Myxococcaceae*

Ronald Garcia · Rolf Müller
Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland – Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology, Saarland University Campus, Saarbrücken, Germany

Abstract
Myxococcaceae belong to the suborder *Cystobacterineae* in order *Myxococcales* and comprise three genera validly described as *Myxococcus*, *Corallococcus*, and *Pyxidicoccus*. Members of this family are widely distributed in soil and also occur in freshwater and marine environment. *Myxococcus* and *Corallococcus* appear to be the most commonly isolated myxobacterial genera in almost every soil sample. *Myxococcaceae* family covers the best explored and studied myxobacteria with a wide scope of research. The family's pioneering and significant contributions to the order *Myxococcales* include (1) first genome to be sequenced, (2) discovery of first replicating plasmid, and (3) discovery of first bacteriophage. To date, several genomes are available belonging to *Myxococcus* and *Corallococcus*, and sequencing of *Pyxidicoccus* is ongoing. Albeit being common in the environment, metagenomics studies revealed that many members of this family still remain not cultivated. The important application of *Myxococacceae* family is reflected primarily on diverse secondary metabolites acting as antimicrobials, antiparasitics, antivirals, cytotoxins, and anti-blood coagulants. Bacteriocin-like activity and the potential application as biocontrol agent were also determined. Natural product discovery in this family appears promising as an important source of future drugs.

Taxonomy, Historical, and Current

Short Description of the Family

Myx.o.coc.ca'ce.ae. M.L. masc. n. *Myxococcus* type genus of the family; *-aceae* ending to denote a family; M.L. fem. pl. n. *Myxococcaceae* the *Myxococcus* family (Reichenbach 2005).

Myxococcaceae family occupies a unique clade in suborder *Cystobacterineae*, order *Myxococcales* (Garcia et al. 2010), class *Deltaproteobacteria*, and phylum Proteobacteria (Shimkets and Woese 1992; Kaiser 1993). Members of this family are morphologically and phylogenetically coherent allowing clear distinction among other myxobacterial families.

Myxococcaceae is composed of genus *Corallococcus* (Reichenbach 2007a), *Pyxidicoccus* (Reichenbach 2007b), and the type genus *Myxococcus* (Thaxter 1892). Members show close similarity in vegetative cells, swarm colony pattern, and myxospores (*Fig. 15.1*). Vegetative cells are flexuous, long, rod-shaped with more or less tapering ends, and stained Gram negative. Cells move by swarming or gliding on agar surfaces (Supplementary data *15.1*). Except for *Pyxidicoccus*, fruiting

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E. Rosenberg et al. (eds.), *The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria*, DOI 10.1007/978-3-642-39044-9_303,

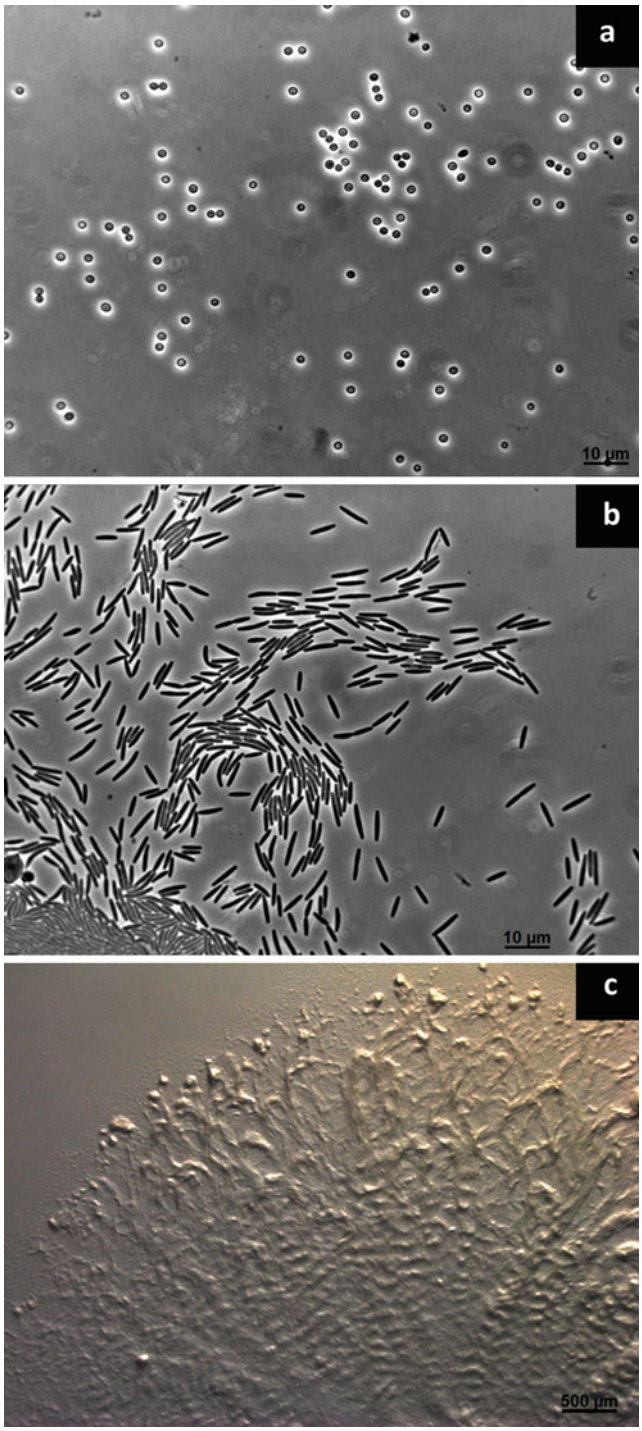
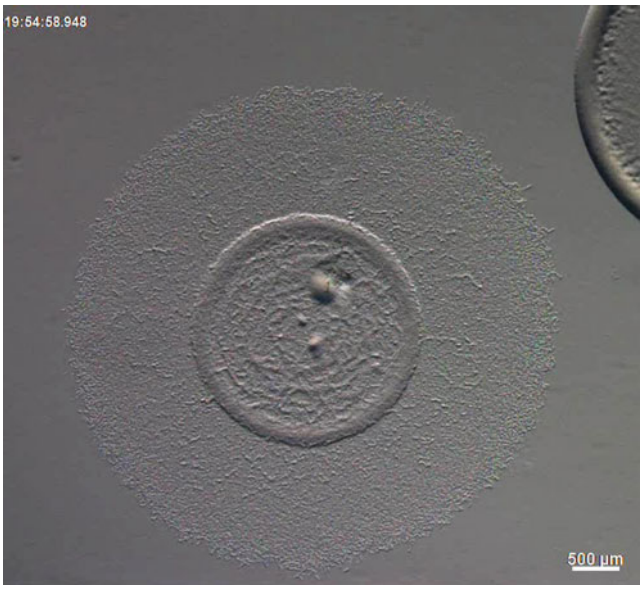


Fig. 15.1
Phenotypic characteristics of *Myxococcaceae*. Rounded and refractile myxospores of *Myxococcus stipitatus* DSM 14675^{PN} (a). Long slender flexuous rods with tapered ends in *Pyxidicoccus fallax* DSM 14698^{T} (b). Thin and filmlike colony on agar (VY/2) with flare-like ends and ripples in *Corallococcus exiguus* DSM 14697^{T} (c)

bodies are born naked without enclosing wall or sporangiole. Myxospores are rounded and often optically refractile. On lean medium, colony is thin and filmlike with flare- to flame-like edges. Swarm cells do not etch or corrode the agar



Supplementary data 15.1
Time-lapse movie of *Myxococcus stipitatus* SBMx178 swarming on lean agar

and are commonly produced on surface of the substrate. Colony may be slimy but not tough and stringy compared with members of the *Cystobacteraceae*. *Myxococcaceae* are classified aerobic, are mesophilic, and grow at a neutral to slightly alkaline pH. Unless determined halophilic, almost all members do not require sodium chloride for growth. Wide-spectrum antibiotic resistance is also known in this family. Mol percent DNA G+C of determined genera (*Myxococcus*, *Corallococcus*) ranges from 66 to 71 (Reichenbach 2005). Branched-chained cellular fatty acids are more predominant compared to the straight-chained type, with *iso*-$C_{15:0}$ as the major type fatty acid. The family is widespread in the environment and commonly isolated in terrestrial soil.

Historical and Comments on *Myxococcaceae*

The family *Myxococcaceae* is remarkable for quite a number of taxonomic changes in addition to some neotype strain proposals for replacement of those nonexisting type isolates. Modern analytics combined with molecular and phylogenetic studies contributed to the improvement of myxobacterial systematics. The reclassification of *Myxococcus flavescens* back to *Myxococcus virescens* appears supported by their similarities in the fatty acid profile, some physiological reactions, and DNA-DNA relatedness; thus, *M. flavescens* described by Yamanaka and colleagues (1987) was proposed to be a synonym to Thaxter's described type *M. virescens* (Lang et al. 2008).

In *Myxococcus cruentus* (Thaxter 1897), its designation to place in a new genus (*Byssovorax*) under a different family (*Polyangiaceae*) and suborder (*Sorangiineae*) has been supported by differences in morphological and physiological characteristics which is strongly confirmed by molecular phylogenetic data;

hence, *M. cruentus* was reclassified to *Byssovorax cruenta*, a novel genus and species (Reichenbach et al. 2006, see comments in Chap. 19). In a very similar case, Thaxter's described *Myxococcus disciformis* (Thaxter 1904) was also reclassified and proposed for a neotype strain bearing the name *Angiococcus disciformis* (Hook et al. 1980), which was later further reclassified to *Cystobacter disciformis* (Brockman and McCurdy 1989). The reclassification from genus *Myxococcus* to *Angiococcus* appears phenotypically justified since the proposed neotype was described to exhibit a fruiting body with enclosing sporangiole wall, a distinct and unique characteristic differentiating from *Myxococcus* (characterized by naked fruiting body). Furthermore, the subsequent transfer of *Angiococcus* to *Cystobacter* seems also justified based on their morphological similarities.

In the absence of *Myxococcus stipitatus* live type strain (Windsor M78^T) in the open culture collection (Lang and Stackebrandt 2009), Mx s8 (Helmholtz Zentrum für Infektion Forschung – HZI collection, formerly called Gesselschaft für Biotechnologie Forschung – GBF) = DSM 14675 has been introduced to replace the "type" strain (Reichenbach 2005), and the typification for its validity was formally requested in the publication (Lang and Stackebrandt 2009). In the List of Prokaryotic Names with Standing Nomenclature – LPSN (http://www.bacterio.net) – *Myxococcus coralloides* was included as validly described species belonging to *Myxococcus*; however, it should be noted that this species was recently reclassified to *Corallococcus coralloides* (Reichenbach 2007a). Many of the previously described species which to date do not occupy a valid standing nomenclature are perhaps similar to currently available *Myxococcus* type species. In the second edition of the *Bergey's Manual of Systematic Bacteriology*, these probable similar species in *Myxococcus* have been enumerated (Reichenbach 2005). In the absence of live type strain or genetic material, verification and identification seems to be impossible.

Members of the *Myxococcaceae* previously known as genus *Chondrococcus* were renamed to *Corallococcus* to accommodate myxobacterial isolates with horn- or coral-like, hard fruiting body, and bearing rounded myxospores (Reichenbach 2005). *Corallococcus macrosporus* is among those members with tortuous history. To simplify the story, the species bears the earlier name under the genus *Chondrococcus* (Jahn 1924; Krzemieniewska and Krzemieniewski 1926). Although the name was considered illegitimate as it was already used for an alga (Jeffers and Holt 1961), the name was still retained (Breed et al. 1957). Later, the classification became even more complicated as *Chondrococcus*, *Myxococcus*, and *Angiococcus* were merged together in the genus *Myxococcus* (Buchanan and Gibbons 1974). In the following years, *Myxcoccus macrosporus* became included in the Approved Lists of Bacterial Names, while *Chondrococcus* and *Angiococcus* were dropped (Skerman et al. 1980). The generic name *Corallococcus* was introduced in referral to *Chondrococcus* in the first edition of The Prokaryotes (Reichenbach and Dworkin 1981), while the species *Corallococcus macrosporus* became formally enlisted in the second edition of *Bergey's Manual of Systematic Bacteriology* (Reichenbach 2005). Molecular phylogenetic studies and further characterizations provide clear evidence that *Corallococcus macrosporus* should be reclassified back to the genus *Myxococcus* (Stackebrandt et al. 2005, 2007; Stackebrandt and Päuker 2005; Lang and Stackebrandt 2009). These findings are in agreement with recent phylogenetic (Garcia et al. 2010) and chemo-marker studies based on fatty acids (Garcia et al. 2011).

Pyxidicoccus is the most recently described genus in *Myxococcaceae* which formerly bear the name *Pyxicoccus* in the second edition of the *Bergey's Manual of Systematic Bacteriology* and in the third edition of this book (Reichenbach 2005; Shimkets et al. 2006). The corrected generic epithet appears in the Validation List number 115. *Pyxidicoccus* is a distinct genus in *Myxococcaceae* based on phenotypic (Reichenbach 2005) and phylogenetic studies (Garcia et al. 2010). Some strains which are named "*Angiococcus*" were shown phylogenetically closely related to *Myxococcus fulvus-Myxococcus stipitatus* clade (Spröer et al. 1999), as exemplified by a designated reference strain An d1 (Reichenbach 2005), while other strains (An d4, An d6) are not related at all but appear affiliated to other taxon (*Archangium*) (Spröer et al. 1999). *Pyxidicoccus* close affiliation to *Myxococcus fulvus-Myxococcus stipitatus* clade in genus *Myxococcus* has been affirmed in a previous phylogenetic study (Garcia et al. 2010).

Editorial note: A novel species *Agreggicoccus edonensis* belonging to a novel genus *Agreggicoccus* was published after completion of this chapter to be included in the Myxococcaceae family (Sood et al. 2014).

Phylogenetic Structure of the Family and Related Taxa

Myxococcaceae is one of those family under the *Cystobacterineae* suborder composed of three genera and eight species. *Figure 15.2* shows the 16S rRNA gene phylogenetic tree of the *Myxococcaceae* family constructed by the neighbor-joining algorithm using the sequence data set based on All-Species Living Tree Project – LTP database (http://www.arb-silva.de/projects/living-tree) (Yarza et al. 2010). *Myxococcaceae* family is monophyletic based on the 16S rRNA gene sequence with *Cystobacteraceae* appearing as the closest neighbor. The coherency among *Myxococcaceae* genera (*Myxococcus, Corallococcus*, and *Pyxidicoccus*) appears to be reflected in high-percentage 16S rRNA gene sequence similarity (Garcia et al. 2010). Although *Pyxidicoccus* shows 99.3 % similarity to closest type species *Myxococcus fulvus* in the 16S rRNA gene sequence (Garcia et al. 2010), it appears that this low-percentage difference (<1 %) could be considered for the proposal of a novel taxon, provided supported by unique and distinct characteristics. Moreover, *Pyxidicoccus* seems to be exceptional from the rest of myxobacteria since most taxa appear to be separated by at least 4 % phylogenetic distance (Garcia et al. 2010).

The branching of proposed neotype strain of *Mellitangium lichenicola* (ATCC 25946) in *Myxococcaceae* family appears not surprising after its position was determined in coherence with previous studies (Spröer et al. 1999; Garcia et al. 2010). Since this

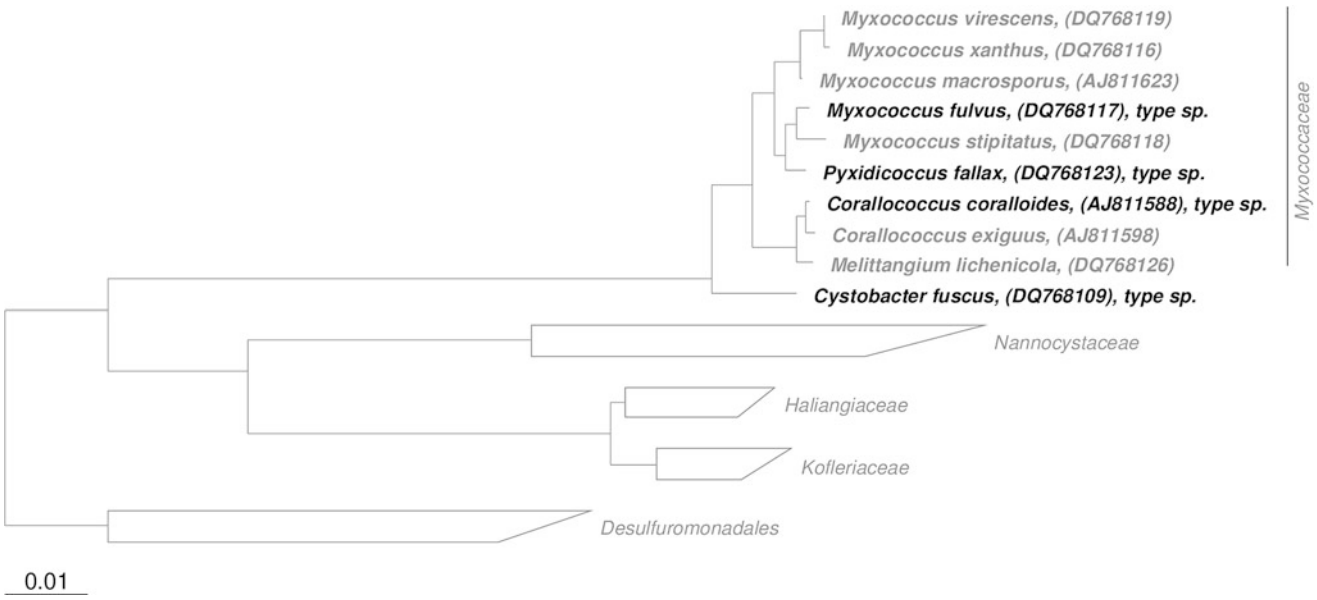


Fig. 15.2

Phylogenetic reconstruction of the family *Myxococcaceae* based on 16S rRNA gene sequence 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

appears to be a case of possible misclassification (Spröer et al. 1999; Garcia et al. 2010), the neotype strain was proposed for a replacement (Lang and Spröer 2008).

Molecular Analysis

To determine the degree of similarity among *Corallococcus* strains, matrix-assisted laser desorption ionization/time of flight (MALDI-TOF) was used in reference with two *Myxococcus* species (*M. xanthus* and *M. macrosporus* – previously known as "*Corallococcus macrosporus*") (Stackebrandt et al. 2005). In contrast to the housekeeping genes *gyrB* and 16S rRNA which show very high percentages of similarity among closely related strains and species, MALDI-TOF analysis of ribosomal proteins reveals that 20 % overall mass identity in *Corallococcus* may already indicate high relatedness among strains (Stackebrandt et al. 2005).

Besides 16S rRNA ((Spröer et al. 1999; Stackebrandt et al. 2005; Stackebrandt and Päuker 2005; Lang and Stackebrandt 2009; Garcia et al. 2010), *gyrB*, or both (Stackebrandt and Päuker 2005) several other housekeeping genes (fusA, lepA, rpoB) were also used to descriminate related strains of Corallococcus (Stackebrandt et al. 2007). Among them, the 16S rRNA gene appears to be the most conserved as judged by the similarities in intra- and inter-clustering, while *fusA* seems to be the least conserved. Based on the degree of gene conservatism as determined by similarities, the order is as follows: 16S rRNA > *rpoB* > *lepA* > *gyrB* > *fusA*. The disadvantages of some of these genes for such studies (*fusA*, *lepA*) are that they cannot be all amplified by universal primers, at least in *Corallococcus* strains studied previously, and none of these genes show the same tree topology (Stackebrandt et al. 2007). Analysis between 16S rRNA and *gyrB* genes, also in *Corallococcus*, has shown that strains with 100 % 16S rRNA gene identity may only show 96.4–99.7 % *gyrB* sequence similarity, while unrelated strains were found 10 % lower (Stackebrandt and Päuker 2005). In *Myxococcus*, in addition to the 16S rRNA gene, the 16S–23S rRNA gene internal transcribed spacer region was also used to delineate among member species and strains (Miyashita et al. 2008).

Although the riboprint pattern may seem to be important in assessing the diversity at the rrn operon level, analysis in *Corallococcus* strains suggests no significance, as it shows no direct correlation with the established gene sequence (Stackebrandt et al. 2007). The analysis of *Corallococcus* species as judged by high-percentage 16S rRNA gene similarity (>99 %) appears no direct DNA-DNA correlation (Stackebrandt et al. 2007). Thus, the decision to propose a strain to a novel species in myxobacteria based on DNA-DNA hybridization and ribotyping seems not very convincing which contradicts to the previous hypothetical expectations (Stackebrandt and Päuker 2005).

Genome Analysis

To date, six genomes are published in *Myxococcaceae* comprising three *Myxococcus xanthus* belonging to strain DK1622 (Goldman et al. 2006), DZF1 (Müller et al. 2013b), DZ2

(Müller et al. 2013a), one *Myxococcus stipitatus* strain DSM14675^{PN} = Mx s8^{PN} (Huntley et al. 2013), a halotolerant *Myxococcus fulvus* strain HW-1 (Li et al. 2011), and a terrestrial *Corallococcus coralloides* strain DSM 2259^{T} (Huntley et al. 2012). They contain a single circular chromosome with genome size ranging from 9 to 10 Mbp and exhibiting approximately 70 % GC content. In five sequenced *Myxococcus* strains, four have more or less 9 Mbp size except for *M. stipitatus* with 10.3 Mbp. In six *Myxococcaceae* genomes, the number of protein-coding sequences (CDS) ranges from 7,285 to 8,043 (*Table 15.1*). In *Myxococcus xanthus* DK1622 alone, more than 90 % of its genome consists of CDS which 48 % (= 3,542 CDS) belong to the families of paralogous genes (Goldman et al. 2006), while *Corallococcus coralloides* DSM2259^{T} contains a bit higher percentage of protein-coding genes which were predicted to account for 8,033 equivalent to 91.7 % of the genome sequence length (Huntley et al. 2013). The high number of CDS in DK16222 accounting for >15 % of the total genes was suggested to be a result of an extensive gene duplication which are found more or less equally distributed in the chromosome (Goldman et al. 2006). Some of these duplicated genes in DK1622 were predicted to be involved in cell-cell signaling, small molecule sensing, and control of transcription.

Comparative analyses of two *M. xanthus* genomes from strains DK1622 and DZ2 revealed high similarities except for few specific and remarkable genes. Strain DZ2 contains specific genes coding for hypothetical proteins similar to those found in *Myxococcus fulvus*, *Stigmatella aurantiaca*, and *Sorangium cellulosum* genomes. In addition, some of these unique genes may likely functionally be involved in regulation of transcription, translation, signaling transduction, protein transport, and fatty acid modifications (Müller et al. 2013a).

A striking feature of *M. xanthus* DK1622 is that it contains large gene clusters associated with secondary metabolism (8.6 % of the genome). Although these genes may be hypothesized to be acquired by horizontal gene transfer (HGT), this seems unlikely since these genes are clustered, functionally duplicated, and lack sequence discontinuities (Goldman et al. 2006). In addition, the genes responsible for secondary metabolism may likely play an important role in microbial predation. Genes for secondary metabolism are found superclustered within regions from 4.4–5.8 Mbp to 1.5–3.5 Mbp counterclockwise from the origin of replication. In comparison with *Streptomyces coelicolor* and *Streptomyces avermitilis*, DK1622 is predicted to have a higher capacity for synthesizing polyketides and mixed polyketides-polypeptides (Goldman et al. 2006).

To understand the genetic programs involved in the evolution of fruiting body morphogenesis and their basis for differences between myxobacteria, the proposed neotype strain (DSM 14675) of *Myxococcus stipitatus* was chosen to be sequenced (Huntley et al. 2013). *Table 15.1* shows some features of the genome in comparison with other sequenced *Myxococacceae*. *M. stipitatus* genome shows closest overall synteny similarity with *Myxococcus xanthus* (Huntley et al. 2013).

In our program to discover and identify novel natural products from myxobacteria, some strains belonging to the genus *Myxococcus*, *Corallococcus*, and *Pyxidicoccus* were selectively chosen for genome sequencing because of their intriguing natural products. Complete sequencing of the genome aims to identify and elucidate the biosynthetic pathways involved in secondary metabolism of the target compound and to explore other metabolomic potentials of the strain.

Table 15.1
Comparative genomes overview among members of the *Myxococcaceae* family

Strain	*M. xanthus* DK1622	*M. xanthus* DZF1	*M. xanthus* DZ2	*M. fulvus* HW-1	*M. stipitatus* DSM 14675	*C. coralloides* DSM 2259^{T}
Sequencing method	4.5-fold by Monsanto	454GS-FLX titanium platform	454GS-FLX titanium platform	454 + Illumina + Sanger	454 XLR titanium platform + Illumina + Sanger	454 XLR titanium flatform
Chromosome	Single circular			Single circular	Single	
Genome size	9,139,763 bp	ca. 9.28 Mb	9.287 Mb	9,003,593 bp	10,350,586 bp	10,080,619 bp
GC content				70.6 %	69.2 %	69.9 %
CDS	7,388	7,704	7,709	7,285	8,043	8,033
Genomic islands				9		
GenBank accession no.	CP000113	AOBT00000000	AKYI00000000	CP002830	CP004025	CP003389
Reference	Goldman et al. (2006)	Müller et al. (2013b)	Müller et al. (2013a)	Li et al. (2011)	Huntley et al. (2013)	Huntley et al. (2012)

Phages and Plasmids

Myxobacterial phages had been studied decades ago and not much follow-up was reported. Several phages that had been investigated in myxobacteria were mostly isolated from *Myxococcus xanthus* (Burchard and Dworkin 1966; Brown et al. 1976a, b; Martin et al. 1978; Rodrigues et al. 1980). These myxobacterial bacteriophages were grouped into four types, namely, MX-1, MX-4, MX-8, and MX-9, and all were identified as double-stranded DNA-type bacteriophages. The MX-1 group phage is characterized to have complex morphology with close resemblance to T4 coliphage (Brown et al. 1976a, b). MX-1 was among the first myxobacterial phages isolated from *Myxococcus xanthus* and morphologically characterized by the presence of icosahedral head and contractile sheath tail and classified as lytic with host restricted only to *M. xanthus* (Burchard and Dworkin 1966). This phage was determined to have large genome with size about 130–150 10^6 Da with 56 mol% GC content. The second group (MX-8) was characterized to be a generalized-type transducing phage containing linear double-stranded DNA (Stellwag et al. 1985; Orndorff et al. 1983). MX-4 group was classified as generalized transducing phage which shows similarity in morphology and genome size to λ coliphage (Campos et al. 1978). The MX-8 group is also a generalized-type but a small transducing phage which has similarity to T3 coliphage (Martin et al. 1978). The fourth group, MX-9, was the only member characterized to have a very short tail with characteristic antigen (Martin et al. 1978). Several additional new *Myxococcus xanthus* phages have also been isolated and characterized, and all were also determined within these groupings (Rodrigues et al. 1980).

To date, the only plasmid known to *Myxococcaceae* or myxobacteria in general is pMF1. The plasmid was isolated in *Myxococcus fulvus* strain 124B02 (Zhao et al. 2008). The detection of this plasmid from 150 myxobacterial strains composed mainly of *Myxococcus* and *Corallococcus* strains suggests that the plasmid is quite rare in myxobacteria. It was characterized to be circular with a size of 18,634 bp and with 68.7 mol% GC content (Zhao et al. 2008). Furthermore, it was determined as autonomously replicating after a gene encoding for an unknown protein was determined as transformable with high efficiencies in *Myxococcus xanthus* strains DZ1 and DK1622. Twenty-three open reading frames (ORF) were predicted in the complete sequence of the plasmid; nine of these show similarity to known sequences in *Stigmatella* (ORFs pMF1.8, pMF1.12), *Anaeromyxobacter* (ORF pMF1.16), *Myxococcus* (ORFs pMF1.19-20), and other organisms. The other predicted ORFs show unique sequences without significant homology in the GenBank database. ORFs pMF1.4, pMF1.11, and pMF1.14 produce very large products comprising of 487, 316, and 634 amino acids, respectively, which cannot be correlated yet to any known function. Remarkably, 86 % of the sequenced plasmid resembles 90 % in the protein-coding sequences of *Myxococcus* genome. Since the pMF1 replication region cannot be determined by bioinformatics, it is hypothesized to represent a new locus (Zhao et al. 2008).

Phenotypic Analysis

All members of this family exhibit phase-dark rod vegetative cells with tapering ends. Myxospores are rounded to ovoid, optically refractile, nonmotile, and with thick coat. Swarms are filmlike and thin on lean medium with flare- to flame-like edges and often show rippling pattern. Colonies do not corrode, etch, depress, or degrade the agar. Fruiting bodies are either enclosed in sporangiole or born naked.

Myxococcus Thaxter 1892, 403[AL]

Myx.o.coc'cus. Gr. fem. n. *myxa* mucus, slime; Gr. masc. n. *kokkos* berry; M.L. masc. n. *Myxococcus slime coccus.* (Reichenbach 2005).

Fruiting bodies contain no sporangiole, appearing as humpto knob shaped, and in some species stalk is present (Fig. 15.1). They consist of aggregates of optically rounded myxospores and often are arranged solitary on agar. Swarms are thin, filmlike, and almost transparent in lean medium, non-agar penetrating, and non-agar degrading. Swarms show flare- to-flamelike appearance at the colony edge and rippling pattern within the colony. Vegetative cells are long, flexuous, and phase-dark rods with slight tapering ends. They exhibit predatory bacteriolytic and noncellulolytic type of nutrition. Swarm stains positive in Congo red. They are characterized chemoorganotrophic, strict aerobic, and mesophilic. Catalase is positive and oxidase is negative. Major cellular fatty acids are *iso*-$C_{15:0}$, and $C_{16:1\omega5c}$. The DNA mol% G+C is 65–71 %. The type species is *Myxococcus fulvus*, and the type strain is ATCC25199^T (= M17^T = DSM 16525^T). Table 15.2 shows differentiating characteristics among other *Myxococcaceae* genera. Figure 15.4 shows the growth stages of *Myxococcus*.

Corallococcus (*Chondrococcus* Jahn 1924) Reichenbach 2007, 115[VL]

Co.ral.lo.coc'cus. Gr. neut. n. *korallion* coral; Gr. masc. n. *kokkos* berry; M.L. masc. n. *Corallococcus coral-shaped coccus* (referring to coral-shaped fruiting bodies) (Reichenbach 2005).

Fruiting bodies are naked and hard in structure without sporangiole and composed of optically refractile rounded myxospores. Vegetative cells are long, flexuous, and phase-dark rods with slightly tapering ends. Swarm colonies are thin, transparent, and form ripples on lean agar medium (Figs. 15.5 and 15.6). They can lyse bacteria and yeast cells, cannot degrade agar and cellulose. Congo red stains positive. They exhibit strict aerobic, chemoorganotrophic, and mesophilic growth characteristics. They also show catalase positive and weak positive oxidase. They hydrolyse aesculin and degrade starch and xylan. NaCl is not required for growth and inhibited at 1.5 % concentration. Major cellular fatty acids are branched-chained *iso*-$C_{15:0}$, *iso*-$C_{17:0\ 2\text{-}OH}$, *iso*-$C_{17:0}$, and *iso*-$C_{17:1\omega5c}$. The mol % G+C of DNA is 66–68. The type species is *Corallococcus*

Table 15.2
Differentiating characteristics among *Myxococcaceae* genera

Genus	*Myxococcus*				*Corallococcus*		*Pyxidicoccus*
Species	See legend below				*C. coralloides* DSM 2259^T, *C. exiguus* DSM 14696^T		*P. fallax* DSM 14698^T
Morphology (buffered VY/2 agar)							
Vegetative cell	Long, slight tapering rods				Long, sligth tapering rods		Long, tapering rods, close to spindle
Type	Flexuous				Flexuous		Flexuous
Size L × W (μm)	2.6–7.9 × 0.7–1.0				4.0–9.0 × 0.9–1.0		4–8.5 × 0.9–1.3
Swarm colony	Colorless to shade of orange and yellow				Colorless		Colorless
Edge	Flares, flames				Flares, flames		Flares, flames
Diffusable pigment	None				None		None
Myxospore	Rounded to oval				Rounded		Rounded
Size range (μm) (D)	1.2–2.5				1.2–2.2		1.6–2.0
Fruiting body	Naked (no sporangiole)				Naked (no sporangiole)		Sporangiole
Consistency	Soft, deliquescent				Hard		Hard
Arrangement	Often solitary				Often solitary, dense amount		Often clusters
Shape	Hump, knob, mound				Coral-, hornlike		Ovoid
Color	Varies in species (beige, yellow, orange)				Peach/pale orange		Yellow to rust orange
Size (μm)	50–200 (D), 50–300 (H)				20–1,000 (L)		30–80 (sporangiole)
Congo-red stain	Positive				Positive		Positive
Oxygen requirement	Aerobic				Aerobic		Aerobic
pH optimum	Neutral range				Neutral range		Neutral range
Temperature optimum	Mesophilic range				Mesophilic range		Mesophilic range
NaCl 1.5 %[a]	Positive, except *M. fulvus* DSM16525^T				Negative		ND
Catalase	Positive				Positive		Positive
Oxidase	Negative				Weak positive		Positive
Degradation: agar	Negative				Negative		Negative
Cellulose	Negative				Negative		Negative
Aesculin[a]	Negative				Weak reaction to positive		ND
Xylan[a]	Negative				Positive		ND
Starch[a]	Negative				Positive		ND
Bacterial lysis (*E. coli*)	Positive				Positive		Positive
Yeast lysis (Baker's yeast)	Positive				Positive		Positive
Antibiotic resistance (50μg/mL, buffered VY/2 agar)	DSM 16525^T	DSM 16526^T	DSM 2260^T	DSM 14697^T	DSM 2259^T	DSM 14696^T	DSM 14698^T
Ampicillin	G	G	NG	NG	G	G	G
Bacitracin	G	G	G	G	G	G	G
Fusidic acid	G	G	G	NG	NG	NG	G
Gentamycin	G	G	G	G	G	NG	G
Hygromycin	G	G	G	NG	NG	G	G
Kanamycin	NG	NG	NG	NG	G	NG	NG
Kasugamycin	G	G	G	NG	NG	NG	G
Neomycin	G	G	G	G	G	G	G
Oxytetracycline	NG	NG	NG	NG	NG	NG	NG
Polymyxin	G	G	G	G	G	G	G
Sodium Cephalothin	G	NG	NG	NG	NG	NG	G

Table 15.2 (continued)

Genus	*Myxococcus*				*Corallococcus*		*Pyxidicoccus*
Species	See legend below				*C. coralloides* DSM 2259^T, *C. exiguus* DSM 14696^T		*P. fallax* DSM 14698^T
Spectinomycin	G	G	G	G	NG	NG	G
Thiostrepton	NG	NG	NG	NG	NG	NG	NG
Trimetophrim	G	G	G	G	NG	G	G
Fatty acids (FAME method in comparison with type/neotype strains) 2							
Major fatty acids	*iso*-$C_{15:0}$ > $C_{16:1\omega5c}$				*iso*-$C_{15:0}$ > *iso*-$C_{17:0\ 2\text{-}OH}$, *iso*-$C_{17:0}$, *iso*-$C_{17:1\omega5c}$		$C_{16:1\omega5c}$ > *iso*-$C_{15:0}$, > *iso*-$C_{17:0\ 2\text{-}OH}$
BCFA: SCFA ratio	BCFA > SCFA				BCFA > SCFA		BCFA > SCFA (nearly equal)
$C_{17:1\ 2OH}$	None				None		None
PUFAs[b]	$C_{16:2}$				None		GLA
	LA, GLA (*M. fulvus* DSM 16525^T)						
Mol % G+C content[c]	68–71				66–68		ND

Myxococcus includes *M. fulvus* DSM 16525^T, *M. xanthus* DSM 16526^T, *M. stipitatus* DSM 14675^{PN}, *M. virescens* DSM 2260^T, and *M. macrosporus* DSM 14697^T. Fatty acids: DSM14698^T (unpublished)

FAs fatty acid, *FAME* fatty acid methyl ester, *GLA* gamma linolenic acid, *LA* linoleic acid, *G* growth, *NG* no growth, *ND* not determined, *D* diameter, *H* height, *L* length, *W* width

[a]Lang and Stackebrandt (2009),[b]Garcia et al. (2011),[c]Reichenbach (2005)

coralloides, and the type strain is ATCC 25202^T (= DSM 2259^T = NBRC 100086^T, M2^T) from Canadian soil. Diagnostic characteristics among *Myxococcaceae* genera are shown in Table 15.2.

Pyxidicoccus corrig. Reichenbach 2007, 155^{VL}

Py.xi.di.coc'cus. Gr. fem. n. *pyxis* box, case, container; Gr. masc. n. *kokkos* berry; M.L. masc. n. *Pyxidicoccus* boxed coccus.

Fruiting body is composed of brownish-orange rounded sporangioles commonly arrange as clusters or chains on agar. Myxospores are rounded to slightly oval, optically refractile with thick wall enclosed in sporangiole. Swarm colony is thin and almost colorless in lean agar to orange in casitone-based medium. The swarm edge appears flare- to flame-like protrusions on agar with rippling characteristic of the colony. Vegetative cells are long and phase-dark rods, mostly appearing as spindle shaped. Figures 15.7 and 15.8 show the phenotypic characteristics. Bacteria and yeasts are preyed and lysed. Colony cannot degrade agar and cellulose. Swarm stains Congo-red positive. They exhibit aerobic, chemoorganotrophic, and mesophilic characteristics and grow within the neutral pH range. Sodium chloride is not required for growth. They show positive reactions in oxidase and catalase. Major fatty acids are *iso*-$C_{15:0}$ and $C_{16:1\omega5c}$. The mol% DNA G+C of the type strain is not determined. The type species is *Pyxidicoccus fallax*, while the type strain is Py f1^T (=DSM 14698^T = JCM12639^T), isolated in 1992 from soil with decaying plant material collected in Crete island, Greece (Reichenbach 2005). Table 15.2 shows the diagnostic-characteristics among genera of the *Myxococcaceae*.

Collection, Processing, Treatment, and Storage of Samples

Myxococcaceae can be isolated in almost all types of samples. Topmost layer of soil is the most common source for this family and can be processed directly after collection. Leftover and unprocessed materials can be kept air-dried for weeks before permanent storage in vials at room temperature. In our experience, members of this family can be re-isolated over and over again from the same sample even after years of storage under dried condition. Dried sample has the advantage of preventing growth of unwanted contaminating microorganisms during the isolation process (see Chap. 19). Since myxobacteria are able to produce desiccated and heat-resistant myxospores, isolation can still be performed in the future.

Setups for Cultivation, Isolation, and Purification

The isolation of members of *Myxococcaceae* follows the standard setup of bacterial baiting on lean water agar medium (e.g., WAT,

WCX) (Shimkets et al. 2006). Although they are considered noncellullolytic type, they may also appear in mineral salt agar (Stan-21, see Chap. 19), a setup commonly used for the isolation of cellulolytic myxobacteria. The strains belonging in this family can be recognized by the formation of fruiting bodies and swarms after almost a week of incubation at 30 °C, or sometimes a bit longer. *Corallococcus* appears as corral- or hornlike structure (*Fig. 15.9*) on the surface of the agar, while *Myxococcus* are visible as colored hump-, knob-, or mound-shaped slimy fruiting bodies. A stalk can also be recognized in some species of *Myxococcus* (e.g., *M. stipitatus*) (*Fig. 15.3b*). *Pyxidicoccus* is different as it produces sporangioles which appear almost similar to *Cystobacter*. Fruiting bodies are typically huge and vary in different color shades from cream, brownish, and yellow to orange. All these three genera show common swarming pattern producing flare-like edges of the colony and with rippling pattern. In addition, the colony most often appears thin and filmlike on lean agar setup. Isolation of *Corallococcus* and *Pyxidicoccus* can be performed by lifting the fruiting bodies using fine needle and transferring into a new fresh agar medium, preferably also a lean medium (e.g., WAT agar baited with bacteria). Since *Myxococcus* fruiting body is soft and contains no sporangiole, myxospores could easily be taken simply by just only touching with tip of fine needle. Isolation from the swarm is carried out by cutting the farthest or the outermost part of the colony using also a fine needle. Although the farthest edge is presumed to be clean, this is not always the case because

Fig. 15.3
Fruiting body morphologies of *Myxococcus* (stereophotomicrographs). Fruiting bodies on soil sample from the enrichment setups (a–b). *Myxococcus stipitatus* phenotypically characterized by the presence of stalk (b). Morphologies of different *Myxococcus* spp. in pure culture showing color variations in the fruiting bodies (c–f)

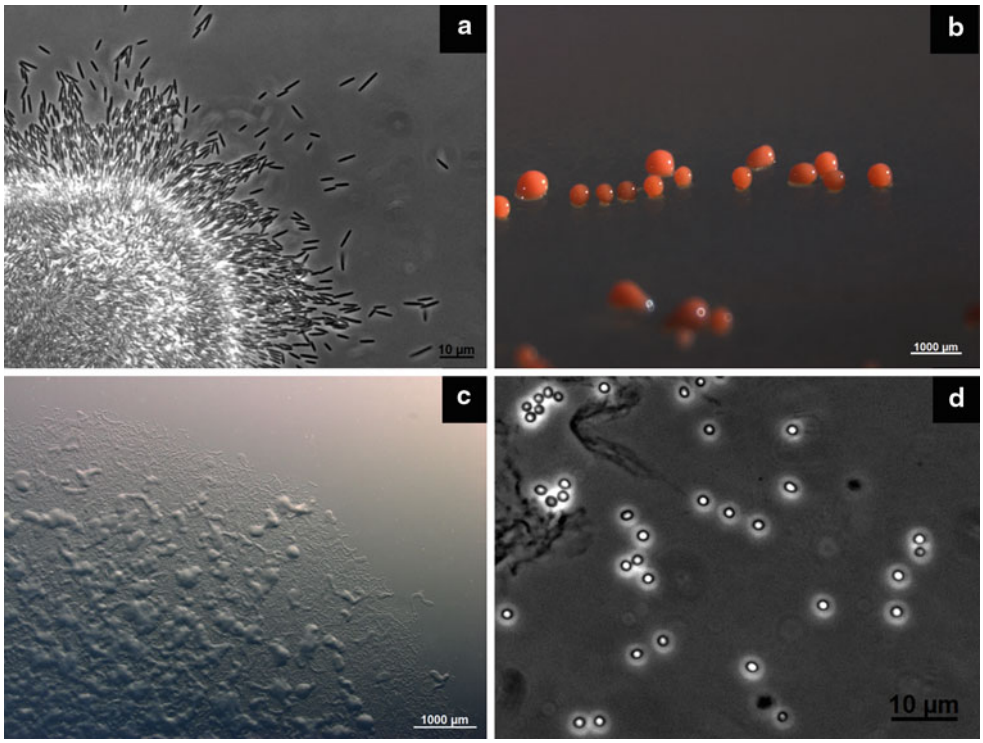


Fig. 15.4
Growth stages of *Myxococcus*. Phase-dark rod vegetative cells in *M. fulvus* DSM 16525^T (a). Knob-to-mound-shaped soft fruiting bodies in *Myxococcus* sp. SBMx178 (b). Flare-like swarming in *Myxococcus xanthus* DSM 16526^T (c). Optically refractile rounded myxospores in *Myxococcus stipitatus* DSM 14675^T (d). Phase-contrast photomicrographs (a, d). Stereophotomicrographs (b–c)

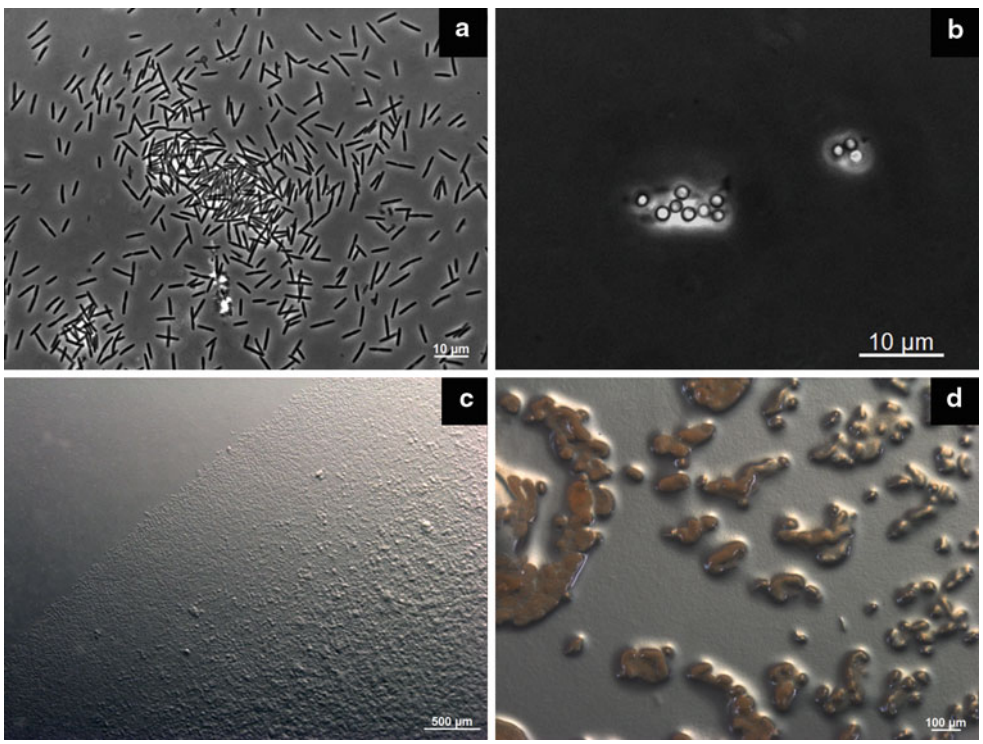


Fig. 15.5
Growth stages of *Corallococcus coralloides* DSM 2259^T. Phase-dark rod vegetative cells (a). Optically refractile and rounded myxospores (b). Thin and filmlike swarm with fine flare edges on agar (WAT) (c). Peach to orange fruiting bodies on buffered VY/2 agar (d). Phase-contrast photomicrographs (a–b). Stereophotomicrographs (c–d)

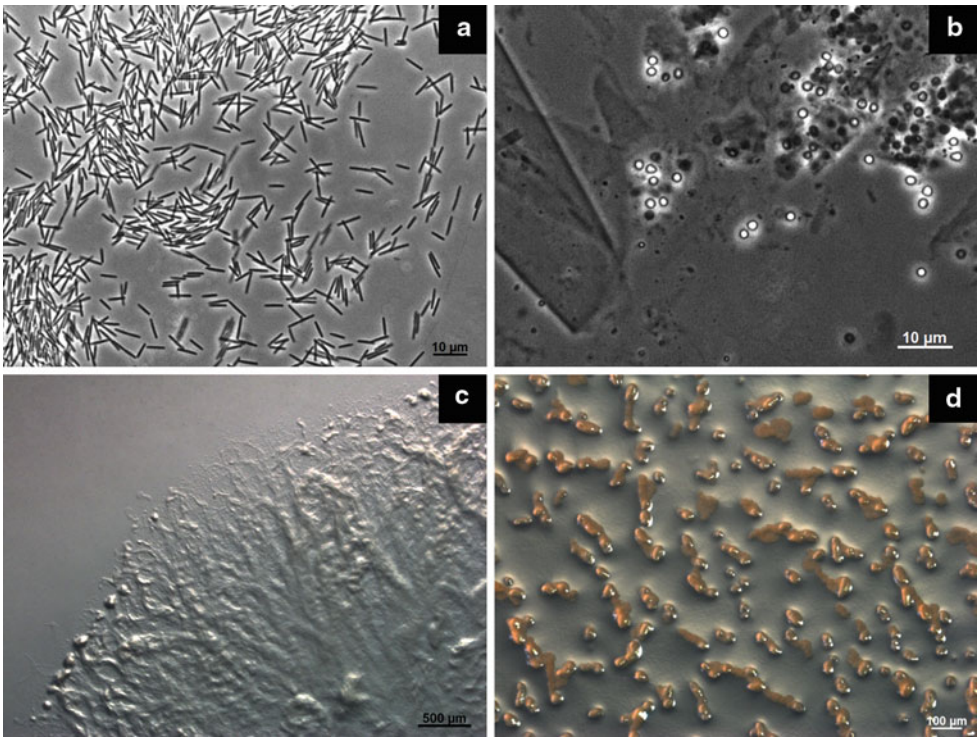


Fig. 15.6
Growth stages of *Corallococcus exiguus* DSM 14696^T. Phase-dark rod vegetative cells (a). Optically refractile and rounded myxospores (b). Swarm colony in buffered VY/2 agar (c). Tiny and dense fruiting bodies (d). Phase-contrast photomicrographs, slide mounts (a–b). Stereophotomicrographs from buffered VY/2 agar (c–d)

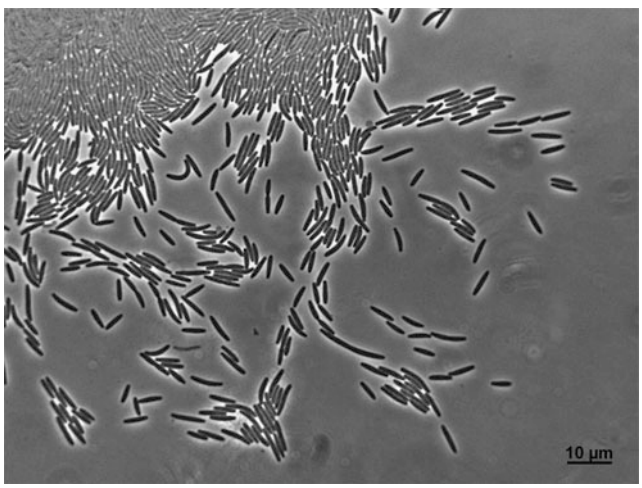


Fig. 15.7
Swarming pattern of *Pyxidicoccus fallax* DSM 14698^T on mounted slide

other bacterial contaminants may sometimes be able to survive and not be killed by myxobacteria. *Myxococcus* and *Corallococcus* are examples of myxobacterial genera where purification is not a serious problem (Reichenbach 1983). In our experience, all the three genera in this family could easily be isolated compared with other myxobacterial families. One to three subcultivations of the colony edge are usually enough to obtain a pure culture isolate. Water agar or yeast-based medium (VY/2) are the best choice for inoculation. In some cases where contaminants (e.g., bacteria) are hard to eliminate, antibiotics or cocktails can be used as the last option to purify. This could be accomplished by taking the contaminated fruiting bodies and suspending in 1 mL EBS broth with 0.1 mL antibiotic solution (e.g., AB-1) (Reichenbach 1983). Fruiting body purification could be applied with *Corallococcus* and *Pyxidicoccus* since their fruiting bodies are hard and not coalescing in liquid medium. The use of levamisole and cycloheximide may also be useful to inhibit the growth of amoeba and fungi, respectively in heavily contaminated samples (see Chap. 19). In addition to these chemical agents, previous studies have also pioneered the purification methods which include heating and freezing of fruiting body and use of ammonia vapors (Reichenbach 1983) which might also be applicable for the isolation of *Myxococcaceae*. Purity of the isolate can be evaluated in any clear liquid medium (e.g., MD1, CY, CTT, SP, etc.) which supports the growth of myxobacteria and as well of the contaminants for detection through microscopy and agar plating. Members of the *Myxococcaceae* can be identified by the

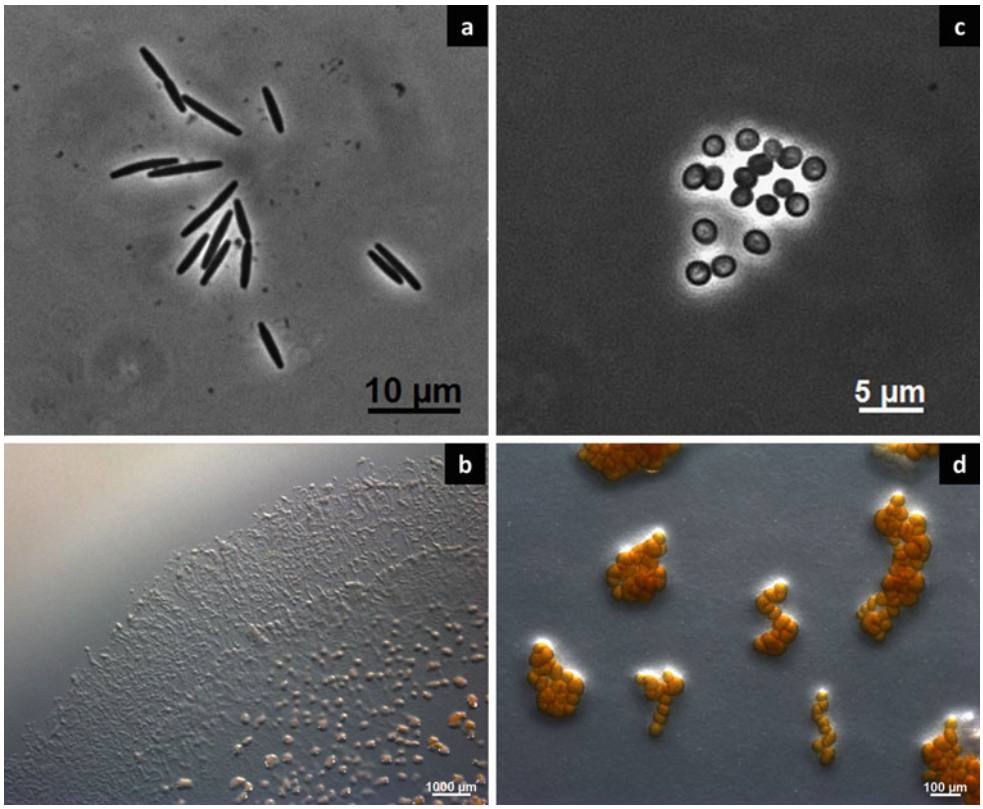


Fig. 15.8
Growth stages of *Pyxidicoccus fallax* DSM 14698^T. Phase-dark, vegetative rods with tapering ends (a). Swarm on buffered VY/2 agar showing flares on the edges (b). Optically refractile and rounded myxospores (c). Yellow-orange fruiting sporangioles arrange in clusters (d). Phase-contrast photomicrographs, slide mounts (a,c). Stereophotomicrographs from buffered VY/2 agar (b,d)

Table 15.3
Isolation sources of *Myxococcaceae*

Sources	*Myxococcus*	*Corallococcus*	*Pyxidicoccus*
Terrestrial			
Herbivore dung	+	+	–
Soil	+	+	+
Bark/rotting wood[a]	+	+	–
Plant leaf (phyllosphere)[b]	+	+	–
Lake	+	+	–
River bank	+	+	–
Marine			
Shore mud	+	+	–
Beach sand	+	+	–

+ encountered, – not encountered
[a]Reichenbach and Dworkin (1992),[b]Rückert (1981)

Fig. 15.9
Typical horn- to coral-like fruiting bodies of *Corallococcus coralloides* on agar

family's distinct vegetative cell morphology and by nonmotile behavior in liquid medium which can produce swarming colony when plated on solid agar (*Table 15.3*).

Growth Cultivation and Maintenance

All members of this family could be cultivated and maintained in yeast-based media either from fresh (VY/2, buffered VY/2) or dried yeast (YCA). Culture could stay viable at room

temperature for a month, or longer, especially if fruiting bodies or myxospores are produced. Other useful media for cultivations are based on casitone (e.g., Amb, CAS, CB, CT, CTT, CTT-YE, CY, CYE, CYG2, MD1, PT, SP). Defined medium (A1) based on amino acids has also been used for cultivation of *Myxococcus xanthus* (Bretscher and Kaiser 1978). For fruiting body development assay in *Myxococcus xanthus*, starvation TPM and CF media are recommended (Higgs and Merlie 2008). Some media (e.g., PT) were indicated for cultivation of some strains (*Myxococcus fulvus* strain Mx f2) but may also be useful for other members of the family.

List of Media and Solutions

A1-Defined Medium (Bretscher and Kaiser 1978).

Tris–HCl 10 mM (pH7.6), KH_2PO_4-$KHPO_4$ (pH7.6), $MgSO_4$ 8 mM, $(NH_4)_2SO_4$ 0.5 mg/L, L-Asp 100 μg mL^{-1}, L-Ile 100 μg mL^{-1}, L-Phe 100 μg mL^{-1}, L-Val 100 μg mL^{-1}, L-Leu 50 μg mL^{-1}, L-Met 10 μg mL^{-1}, sodium pyruvate (prepared from 25 % sodium pyruvate, pH 7.6 stock), potassium aspartate 0.5 % (prepared from 25 % aspartic acid, pH 7.6 adjust with KOH), $FeCl_3$ 10 μM, $CaCl_2$ 10 μM, vitamin B_{12} (filter sterilized, add after autoclaving), and agarose 0.8 % (for solid medium). Adjust pH to 7.6.

Antibiotic Solution AB-1 (Reichenbach 1983).

(mg/50 mL water) chloramphenicol 20, streptomycin sulfate 30, tetracycline HCl 25, and sodium cephalothin 20, filter sterilized

Amb Agar (Ringel et al. 1977).

(g/L) Difco Casitone 2.5, soluble starch 5, $MgSO_4 \cdot 7H_2O$ 0.5, K_2HPO_4 0.25, Bacto Agar 1.5. Adjust pH to 7.0 with KOH.

Buffered Yeast Agar/Buffered VY/2 Agar (Garcia et al. 2009).

(g/L) baker's yeast 5, $CaCl_2 \cdot 2H_2O$ 1, Bacto Agar 10, HEPES 5 mM, and vitamin B_{12} 0.5 μg/mL (filter sterilized, add after autoclaving). Adjust pH to 7.0 with KOH.

CAS (Shimkets et al. 2006).

(g/L) Difco Casitone 10 and $MgSO_4 \cdot 7H_2O$ 1. Adjust pH to 6.8 with KOH.

CB (Sarao et al. 1985).

(g/L), Difco Casitone 2.5, $MgSO_4 \cdot 7H_2O$ 0.5, and K_2HPO_4 0.025. Adjust pH to 7.0 with KOH.

CF Agar (Hagen et al. 1978).

(g/L) Difco Casitone 0.15, sodium pyruvate 1, sodium citrate 2, $(NH_4)_2SO_4$ 0.2, $MgSO_4 \cdot 7H_2O$ 8 mM, potassium phosphate buffer (pH 7.6) 1 mM, tris buffer (pH 7.6) 10 mM, and agar 15

CT Agar (Dworkin 1962).

(g/L) Difco Casitone 20, $MgSO_4 \cdot 7H_2O$ 8 mM, potassium phosphate buffer (pH 7.2) 0.01 M, and Bacto Agar 1.5

CTT Agar (Bretscher and Kaiser 1978).

(g/L) Difco Casitone 10, $MgSO_4 \cdot 7H_2O$ 8 mM, potassium phosphate buffer (pH 7.6) 1 mM, tris buffer (pH 7.6) 10 mM, and Bacto Agar 15

CTT-YE Agar (Bretscher and Kaiser 1978).

(g/L) CTT medium and yeast extract 5

CY Agar (Reichenbach and Dworkin 1992).

(g/L) Difco Casitone 3, Difco yeast extract 1, $CaCl_2 \cdot 2H_2O$ 1, and agar 15. Adjust pH to 7.2 with KOH.

CYE (Campos and Zusman 1975).

(g/L) Difco Casitone 10, Difco yeast extract 5, MOPS buffer (pH7.6) 10 mM, and $MgSO_4$ 4 mM

CYG2 Agar (Shimkets et al. 2006).

(g/L) Difco Casitone 3, Difco yeast extract 1, $CaCl_2 \cdot 2H_2O$ 1, $MgSO_4 \cdot 7H_2O$ 1, and Bacto Agar 15

EBS lm (Reichenbach 1983).

(g/L) Merck tryptically digested casein peptone 5, Difco proteose peptone 5, Merck meat peptone 1, and Difco yeast extract 1. Adjust pH to 7.0.

Hp6 Agar (Reichenbach 1983).

(g/L) sodium glutamate 10, Difco yeast extract 1, vitamin B_{12} 0.5 μg/ml, $MgSO_4 \cdot 7H_2O$ 1, glucose (separately autoclave) 5, and agar 15. Adjust pH to 7.2.

MD1 (Behrens et al. 1976).

(g/L) Difco Casitone 3, $CaCl_2 \cdot 2H_2O$ 0.7, $MgSO_4 \cdot 7H_2O$ 2, trace elements, and vitamin B_{12} 0.5 mg/L (filter sterilized). Adjust pH to 7.2 with KOH.

MYX Agar (Shimkets et al. 2006).

(g/L) sodium glutamate 5, Difco yeast extract 1, $MgSO_4 \cdot 7H_2O$ 1, glucose 2, and Bacto Agar 15. Adjust pH to 7.2 with KOH.

PT Agar (Shimkets et al. 2006).

(g/L) Difco Casitone 4, Difco yeast extract 4, $MgSO_4 \cdot 7H_2O$ 2, $CaCl_2 \cdot 2H_2O$ 1, and Bacto Agar 15

SP Agar (McDonald and Peterson 1962; McCurdy 1963).

(g/L) raffinose 1, sucrose 1, galactose 1, soluble starch 5, Difco Casitone 2.5, $MgSO_4 \cdot 7H_2O$ 0.5, K_2HPO_4 0.25, and agar 15. Vitamin solution 2.5 ml/L

TPM Agar (Higgs and Merlie 2008).

(g/L) Tris–HCl (pH 7.6) 10 mM, K_2HPO_4 1 mM, $MgSO_4$ 8 mM, and Difco Agar 15

Trace Element Solution (Drews 1974).

(mg/L distilled water) $MnCl_2 \cdot 4H_2O$ 100, $CoCl_2$ 20, $CuSO_4$ 10, $Na_2MoO_4 \cdot 2H_2O$ 10, $ZnCl_2$ 20, LiCl 5, $SnCl_2 \cdot 2H_2O$ 5, H_3BO_3 10, KBr 20, KI 20, and EDTA Na-Fe^{+3} salt trihydrate 8 g, filter sterilized

Vitamin Solution (McDonald and Peterson 1962).

(mg/L ethanol) thiamine 100, riboflavin 75, pyridoxine 75, calcium pantothenate 200, *p*-aminobenzoic acid, nicotinamide 75, choline HCl 200, folic acid 1, inositol 1,000, biotin 0.05, and vitamin B_{12} 0.05, filter sterilized

WAT Agar (Reichenbach and Dworkin 1992; Shimkets et al. 2006).

(g/L) Bacto Agar 15, $CaCl_2 \cdot 2H_2O$ 1.0, and HEPES 20 mM. Adjust pH to 7.2 with KOH.

WCX Agar (Reichenbach and Dworkin 1992; Shimkets et al. 2006).

WAT agar and cycloheximide 25 μg/mL, filter sterilized. Add after autoclaving.

YCA (Miyashita et al. 2008).

(g/L) dry yeast 10, $CaCl_2 \cdot 2H_2O$ 1, and agar 15. Adjust pH to 7.2.

Preservations and Reactivations of Cultures

Myxococcaceae can be cheaply preserved by desiccating the matured fruiting bodies for 2–3 weeks in desiccator. Fruiting bodies can be scraped or cut out with small agar pieces to place on small piece of rectangular or rounded sterile filter paper. Completely desiccated fruiting bodies are permanently kept in sterile vials which can be stored at room temperature. Reactivation is usually performed by taking the whole piece of paper containing the desiccated material and inoculated on agar. Normally, growth takes only a couple of days or sometimes can be extended for weeks. Desiccation is our most preferred method to regenerate back the fruiting bodies. Actively growing vegetative cells can be preserved in 5–20 % glycerol and DMSO as cryoprotectant. Cell pellet from 50 to 100 mL broth culture growing in logarithmic phase is suspended in 5–10 mL (10 %) fresh medium. Cells are homogenized and aliquoted to several cryo vials containing cryoprotectants. Each batch of preservation is carefully checked for viability and purity. *Myxococcaceae* vegetative cells can survive −80 °C freezing temperature even without the addition of cryoprotectant; however, it is recommended to slowly freeze in a cold isopropanol cryo chamber (e.g., Nalgene Mr. Frost®) prior to permanent storage. Alternative to vegetative cells, myxospores and fruiting bodies can also be used for cryo freezing. In *Myxococcus xanthus*, DMSO (0.5 M) chemically induced myxospores were determined more stable for cryo stock culture compared with cryo-preserved vegetative cells (Higgs and Merlie 2008). Reactivation of cryo-preserved cells containing cryoprotectant can be done by taking small cell aliquot to plate on agar (e.g., VY/2, CY) or to inoculate on small volume of liquid medium (e.g., Amb, MD1). Thawing the whole cryo culture and inoculating to suitable agar and broth medium can be performed for cryo-preserved strains without cryoprotectant.

Morphology and Growth Stages

Vegetative Cells: *Myxococcaceae* vegetative cells are phase-dark and flexuous long rods with slight variation in size and shape among members. Among the three genera in this family, *Pyxidicoccus* appears slightly different as it resembles spindle or fusiform shaped. Cells' average length size is about 3–6 μm for *Myxococcus* and 4–8 μm for *Corallococcus* and *Pyxidicoccus*. This type of cell is unable to burrow in agar and move by gliding or swarming on surface. This behavior is shared by *Cystobacteraceae*, the closest myxobacterial family in *Cystobacterineae* suborder. In shaken liquid medium, strains start to grow as clumps, flakes, aggregates, or as a "ring" around the flask attached to the glass surface. Homogeneous suspension could be observed as soon as the cells become adapted to the condition and nutrition.

Swarm: Members of the *Myxococcaceae* have generally thin and film swarming colony with color varies from orange to yellow tint on lean medium, and may get intense in may get intense in casitone- or peptone-based medium, as in the case of *Myxococcus xanthus*, *M. macrosporus*, and *M. virescens*. Swarm of *M. stipitatus* can be recognized by slimy surface which perhaps important for a stalk and fruiting body formation (*Fig. 15.10*). Swarm colony edge resembles flares or flames on agar culture. Waves or ripples are also commonly observed among members of this family and appears to have role in the predatory lifestyle and fruiting body formation, for example, in *Corallococcus coralloides* (Supplementary data *15.2*).

Fruiting Body: *Myxococcaceae* are often easily recognized in culture by fruiting body color which vary from white, peach, yellow, and orange depending on the species. Members can be differentiated by shape and consistency of fruiting body, and for presence or absence of sporangiole. As most *Myxococcus* species produced soft hump- to knob-shaped fruiting bodies, *M. stipitatus* differ for having a stalk which sometimes could

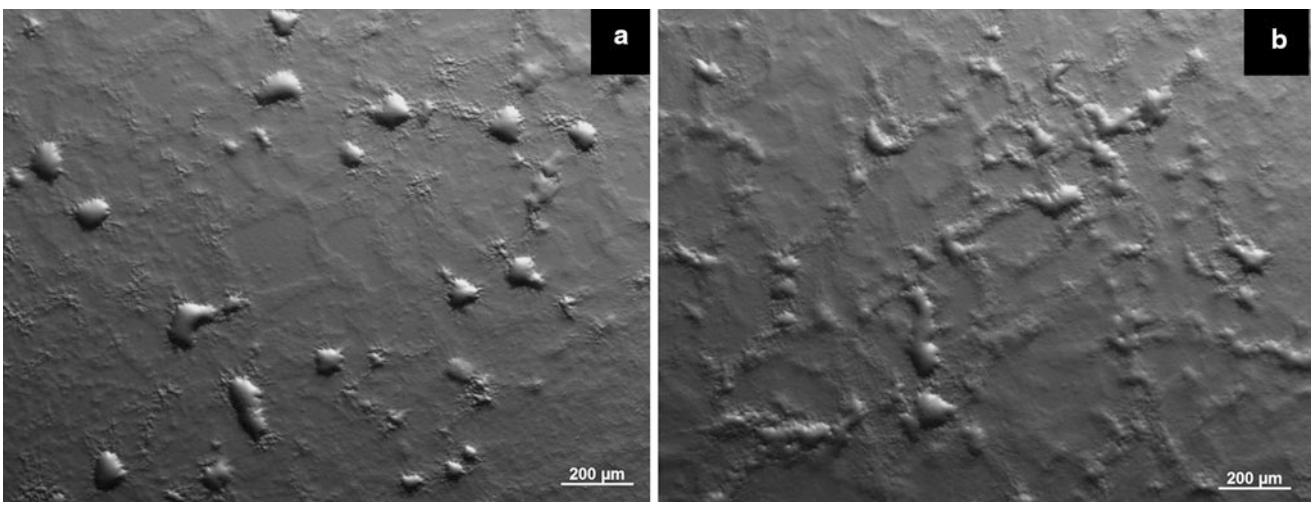
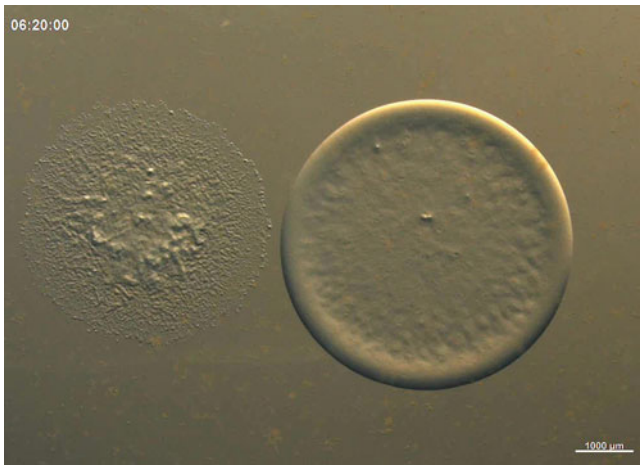


Fig. 15.10
***Myxococcus stipitatus* DSM 14675^T showing slimy swarm architecture on buffered VY/2 agar**



Supplementary data 15.2
Time-lapse movie of *Corallococcus coralloides* showing rippling swarming pattern, *Escherichia coli* predation, and fruiting body formation

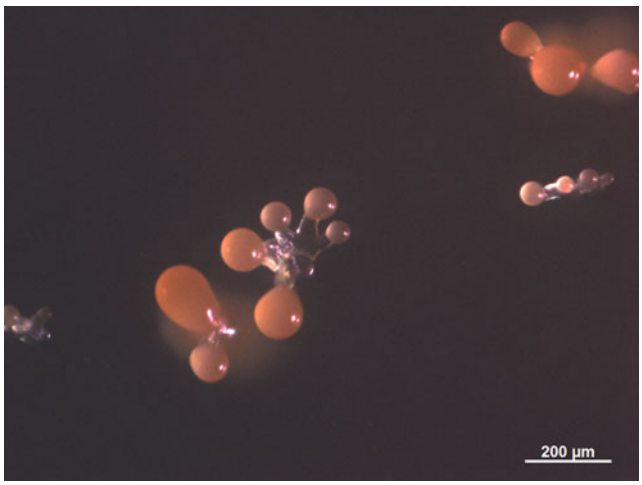


Fig. 15.11
***Myxococcus stipitatus* unusual branched/multi-stalk fruiting body on buffered VY/2 agar**

be branched (Fig. 15.11). *Corallococcus* differed from *Myxococcus* by its hard and horn- to coral-like fruiting bodies. *Myxococcus* fruiting bodies coalesce after some time while *Corallococcus* does not. This characteristic has also been found in *Myxococcus macrosporus* (a reclassification of *Corallococcus macorsporus*) in addition to some other differences in morphology (Fig. 15.12). However, the transfer of *C. macrosporus* to genus *Myxococcus* appears justified despite some phenotypic differences (see section of this chapter "Historical and Comment on *Myxococcaceae*"). *Pyxidicoccus* differed from *Corallococcus* and *Myxococcus* clearly by the presence of sporangiole.

Myxospores: *Myxococcaceae* myxospores are optically refractile containing thick coat which seems to play a protective function in desiccation and resistance to extreme temperature conditions. The characteristic spore shape in this family is rounded to slightly oval with variation in diameter size. Cells in the transitional stage between vegetative cells and myxospores differ since they are more oval in shape and phase dark. *Myxococcus* are morphologically delineated into species by the size of myxospores (Reichenbach 2005). *Myxococcus fulvus* and *Myxococcus stipitatus* contain myxospores measuring 1.2–1.6 µm, whereas *M. stipitatus* and *M. virescens* produced much larger spore measuring 1.8–2.1 µm and 2.0–2.5 µm, respectively. Myxospores of *Corallococcus* and *Myxococcus* are packed in fruiting body without enclosing wall which differed from sporangiole-encased spores in *Pyxidicoccus*.

Pigments and Unusual Bacterial Features

Members of the *Myxococcaceae* are generally colored with usually varying shades of orange and yellow. The color can be seen in swarm colony, vegetative cell pellet after centrifugation from liquid culture, in fruiting body or in concentrated spores. In some uncommon *Myxococcus* strains, fruiting bodies can be deep purple (Fig. 15.3c). Pigments sometimes could be seen in culture supernatant but are usually associated with secondary metabolites as in the case of myxochromide and myxovirescins in *Myxococcus*. In addition, the yellow color in *Myxococcus xanthus* may also indicate the presence of myxalamid compound family (Gerth et al. 1983). The fluorescing colony of some *Myxococcus* strains under UV light has been found correlated with the compound phenylamide/stipiamide (Lampky and Brockmann 1977). So far, the most typical and characterized pigment in myxobacteria belongs to the carotenoid family. The monocyclic glycoside ester (e.g., myxobacton) is the most common carotenoid type found in *M. xanthus*, for which cyclization is catalyzed by lycopene cyclase type enzymes (Iniesta et al. 2008). Carotenoids in myxobacteria appear to play a photo-protective function (Burchard and Dworkin 1966), and carotenoid synthesis has been shown to be light induced and controlled by different carotenoid biosynthetic genes (Burchard and Dworkin 1966; Burchard and Hendricks 1969; Reichenbach and Kleinig 1971; Hodgson and Murillo 1993; Hodgson and Berry 1998).

Ecology

Members of the *Myxococcaceae* are widespread in the environment. The most common habitat for them is soil sample with almost all members of the family can be found. *Myxococcus* and *Corallococcus* predominate in most samples and usually appear in every enrichment setup for isolation. Among *Myxococcus* species, *M. stipitatus* is often appearing on soil and decaying plant material. In case of *M. fulvus*, barks of tree and rotting wood appear to be the habitat as it is commonly found there (Reichenbach and Dworkin 1992). *Myxococcus* and *Corallococcus* are the two common genera found on dung of herbivores (e.g., rabbit, goat, sheep, deer, hare, and moose), and

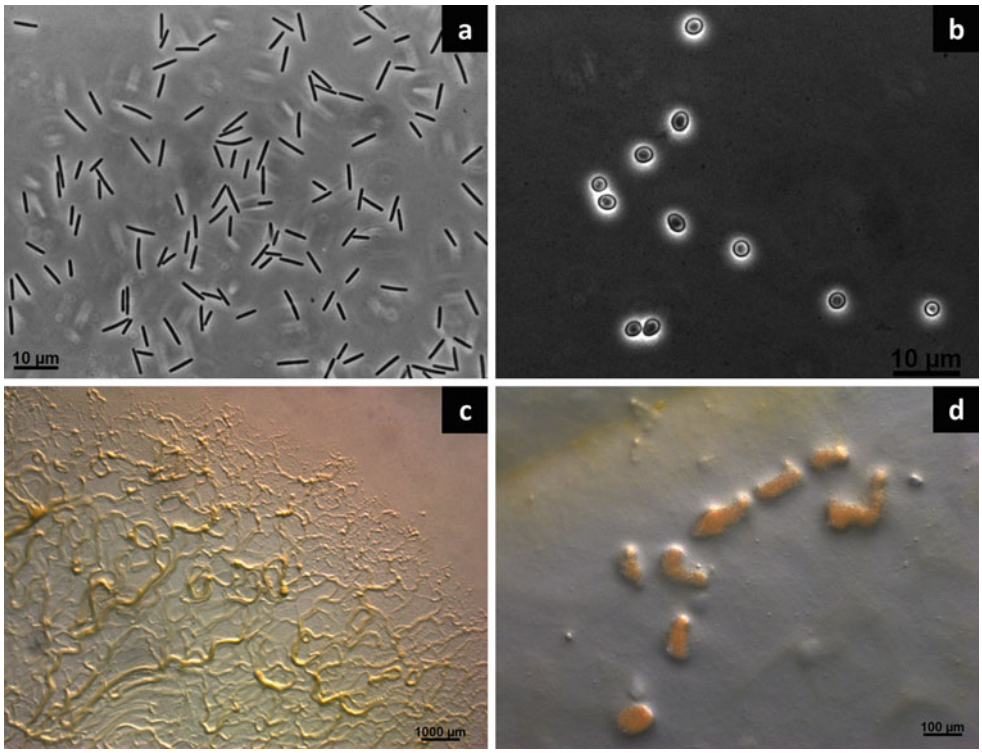


Fig. 15.12
Growth stages of *Myxococcus* (*Corallococcus*) *macrosporus* DSM 14697^{PN}. Phase-dark, vegetative long-rod cells (a). Optically refractile, large, and rounded myxospores (b). Tortuous and veiny swarm (c). Mound- to ridge-shape fruiting bodies (d). Phase-contrast photomicrographs (a–b). Stereophotomicrographs (c–d)

colonization appears associated with the presence of prey microorganisms and plant nutrients to support their growth.

Some *Myxococcus* are distinguished among other *Myxococcaceae* as they can tolerate salt; thus, it is not surprising to find *Myxococcus* in muddy or sandy shore sediments (Li et al. 2002; Zhang et al. 2005; Brinkhoff et al. 2011) and in saline alkaline soils (Zhang et al. 2013). However, no obligate halophile has been discovered yet in this family to counterpart some members of the *Nannocystaceae* and *Haliangiaceae* (Iizuka et al. 2003a, b; Fudou et al. 2002). Occurrence of *Myxococcaceae* has also been documented in cave sediments yielding *Corallococcus* and *Myxococcus* strains (Menne 1999). In acidic environment such as peat bogs, occurrence of *Myxococcus* and *Corallococcus* has also been documented (Dawid 1984). Despite the wide distribution of *Myxococcus* in soil, samples obtained from Antarctic did not yield isolate from this genus (Dawid et al. 1988), indicating that temperature is a factor for their survival.

Based on the number of isolated strains and those observed during the enrichment setups, *Corallococcus* appears more common than *Myxococcus* or even with other myxobacterial genera. This supports the finding that *C. coralloides* shows highest global incidence in soil – accounting for up to 61.1 % (Dawid 2000). The fast growth and dense amount of fruiting bodies produced by *Corallococcus* are perhaps associated to its highest worldwide incidence rate. Since *Corallococcus* and *Myxococcus* are among of the few myxobacterial genera that can be found in restrictive environment of Sahara (Reichenbach 1999), this suggest that they have very high resistance to drastic conditions which could also be accounted to contribute in their high global incidence.

In general, myxobacteria tend to be dominant in the tropics and show a pattern that the diversity of species increases from polar to tropical zones (Dawid 2000). Several *Myxococcus* species, including *M. coralloides* (a reclassified *Corallococcus coralloides*), were reportedly found on phylosphere (plant leaves) (Rückert 1981). All three genera of *Myxococcaceae* can also be found in lake sediments but with much higher abundance recorded on the "uncultured" members of this family, and they appear to be part of the so-called exclusive limnetic myxobacteria (Li et al. 2012).

Physiology and Metabolism

Myxococcaceae are a strictly aerobic family. As judged by their early appearance in the isolation setups, they may be considered as one of the fast-growing myxobacteria. In *Myxococcus xanthus* strain DK101, generation time was determined between 22 and 30 h at 29 °C in defined medium (Bretscher and Kaiser 1978). Members of this family can grow as clumps or flakes at the start but could easily adjust into suspension after several transfers or subcultivations in casitone-containing liquid medium.

Growth is optimum at mesophilic temperature and within the neutral to slightly alkaline pH range. They are also characterized by their ability to prey on microorganisms (e.g., bacteria). Complex bio-macromolecules such as cellulose, gelatin, agar, agarose, and chitin cannot be degraded.

Utilization of sugar has been tested in some strains of *Corallococcus* and the effect is generally correlated to the increase in cell density (ca. double) but not in the growth rate (Irschik and Reichenbach 1985a). Polysaccharides such as starch, maltotriose, amylose, amylopectin, and pullulan were determined to enhance growth of *C. coralloides* whereas mono- and disaccharides appear not to be utilized (Irschik and Reichenbach 1985a). Same finding was reported in *Myxococcus xanthus*, in which mono- and disaccharides cannot serve as carbon-energy sources (Bretscher and Kaiser 1978). The utilization of starch, in addition to xylan, was also described in *Corallococcus exiguus* and *Corallococcus coralloides* (Lang and Stackebrandt 2009). These two sugars appear not to be utilized in *Myxococcus*, thus could be used as physiological markers to differentiate these two closely related genera.

The effect of amino acids in *Myxococcus xanthus* was determined outstanding for carbon and energy sources, while pyruvate appears to be the best source in comparison with acetate, aspartate, glutamate, and majority of the tricarboxylic acid cycle intermediates (Bretscher and Kaiser 1978). Leucine, isoleucine, and valine were among the required amino acids for vegetative cell growth, while phenyalanine appears not essential in *M. xanthus* (Bretscher and Kaiser 1978). Ammonium sulfate appears not to be an essential inorganic nitrogen source. However, it was determined stimulatory for *M. xanthus* growth just as well vitamin B_{12} but not found in other vitamins (thiamine, calcium pantothenate, riboflavin, nicotinic acid, choline chloride, pyridoxamine, *p*-aminobenzoic acid, folic acid, biotin, inositol) (Bretscher and Kaiser 1978).

Sodium chloride seems to be not required and not essential for growth by members of the *Myxococcaceae.* In some *Myxococcus* isolates, salt can be tolerated by some marine-derived strains (Brinkhoff et al. 2011). Study has shown that 1.5 % sodium chloride concentration can still support growth of several *Myxococcus* strains which include all types and proposed neotype strains (except for *M. fulvus*) but not in *Corallococcus* (Lang and Stackebrandt 2009). The effect of commercial agar (e.g., Bacto) for cultivation of myxobacteria has shown advantageous compared with agarose due to the contaminating protein present in the agar (Bretscher and Kaiser 1978).

Symbiosis, Pathogenicity, and Clinical Relevance

To the best of our knowledge, there is no prokaryotic or eukaryotic symbiosis in *Myxococcaceae.* There is also no indication that it can cause any disease. *Myxococcaceae* could be classified as WHO Risk Group I for having low or no risk to individuals or community.

Application

Myxococcaceae is one of the outstanding producers of novel bioactive compounds in myxobacteria, which follow the rank of *Polyangiaceae* and *Cystobacteraceae.* Twenty-one basic scaffolds with no intergeneric overlapping were identified and elucidated in *Myxococcaceae*, many of which acting as antimicrobials, cytotoxins, and rarely as antivirals (*Table 15.4*). Antibacterial and cytotoxic compounds account for the same percentage (33 % = 7/21), followed by antifungals (24 % = 5/21). Some of these compounds are acting as broad spectra antibiotics for microorganisms or eukaryotic cell lines. Although not that often reported the antiparasitic (e.g., *Plasmodium*) and antiviral (e.g., anti-HIV) activities, this may be due to the fact that compounds from myxobacteria are not routinely tested for these properties.

DKxanthenes are an example of a polyene-type compound acting like pheromone by showing activity on myxospores' morphogenesis (Meiser et al. 2006). So far, and together with stigmolone (Plaga et al. 1998), this is the only known myxobacterial compound exhibiting specific activity during bacterial development.

Corallopyronin is one of the few examples of promising myxobacterial compounds isolated from *Corallococcus coralloides* which was determined as a specific inhibitor of the bacterial RNA polymerase. Interestingly, the compound was also found active in vivo against filaria with activity in the range of doxycycline (Schiefer et al. 2012). Its derivative corallopyronin A is currently on its early stage of preclinical evaluation (Schmitz et al. 2013). Some related strains of *Myxococcus* were also discovered producing a structurally related compound, named myxopyronin. Together, this compound family appears to have high potential to become a broad-spectrum antibacterial drug due to their RNAP inhibition which specifically targeting the "switch region" of the protein (Mukhopadhyay et al. 2008; Sucipto et al. 2013).

Application from this family is not limited to antibiotics, cytotoxic, and antiviral compounds. A glycopeptide with MW of 6,300 was discovered acting as blood anticoagulant (myxaline) at 10 IU mg^{-1} in *Myxococcus xanthus* strain CM011 (El Akoum et al. 1987). Low molecular weight bacteriocin-like activity has been discovered in *Myxococcus fulvus* strain Mx f16 (Hirsch 1977). The potential of some *Myxococcus* spp. to become an agricultural biocontrol agent has been demonstrated with soil-born plant pathogenic fungi (Bull et al. 2002).

Concluding Remarks and Perspectives

Although *Myxococcaceae* appears to be the most explored family in myxobacteria, most of these studies were mainly done in the genus *Myxococcus* and specifically focused on a single species (*M. xanthus*). To further explore and discover mechanisms involved in the growth development from unicellular to

Table 15.4

Secondary metabolites in *Myxococcaceae* and their biological activities

Genus	Compound	Structural class	Biological activity	Mode of action	No.	References
Corallococcus	Corallopyronin	α-Pyrone	Antibacterial	RNAP inhibitor		Irschik et al. (1985), Mukhopadhyay et al. (2008), Erol et al. (2010)
	Corallorazines	Peptide	None reported	–		Schmitz et al. (2013)
	Pyrrolnitrin	Phenylpyrrole	Antibacterial, antifungal	Respiratory inhibitor (complex I)		Triphati and Gottlieb (1969), Gerth et al. (1982b)
	Myxothiazol	ß-Methoxyacrylate	Antifungal, cytotoxic	Respiratory inhibitor (complex III)		Gerth et al. (1980), Thierbach and Reichenbach (1981), Steinmetz et al. (2000)
	Myxovalargin	Peptide	Antibacterial, anti-plasmodia, weak cytotoxic	Protein synthesis inhibitor, acts on cell membrane	5	Irschik et al. (1983b), Irschik and Reichenbach (1985b)
Myxococcus	Althiomycin	Cyclic peptide	Antibacterial	Peptidyltransferase inhibitor		Kunze et al. (1982), Fujimoto et al. (1970)
	Cittilin	Bicyclic tetrapeptide		Moderate inhibitor of pancreatic elastase		Reichenbach and Höfle (1999)
	DKxanthene	Polyene	Pheromone-like activity	Affecting myxospore morphogenesis		Meiser et al. (2006)
	Harman					Reichenbach and Höfle (1999)
	Melithiazols	ß-Methoxyacrylate	Antifungal, cytotoxic	Inhibits synthesis of DNA, RNA, protein		Sasse et al. (1999), Böhlendorf et al. (1999)
	Myxalamid	Polyene	Antifungal	Respiratory inhibitor (complex I)		Gerth et al. (1983)
	Myxochelin	Catechol	Iron chelator	–		Kunze et al. (1989), Ambrosi et al. (1998)
	Myxochromide	Lipopeptide	Weak cytotoxic	–		Ohlendorf et al. (2008)
	Myxopyronin	α-Pyrone	Antibacterial	RNAP inhibitor		Irschik et al. (1983a), Mukhopadhyay et al. (2008), Sucipto et al. (2013)
	Myxoprincomide	Peptide	None reported	–		Cortina et al. (2012)
	Myxothiazol	Bithiazole	Antifungal, cytotoxic	Respiratory inhibitor (complex-III)		Gerth et al. (1980), Thierbach and Reichenbach (1981), Steinmetz et al. (2000)
	Myxotyroside	Rhamnoside	Weak cytotoxic, weak antiplasmodium	–		Ohlendorf et al. (2009)
	Myxovalargin	Peptide	Antibacterial, anti-plasmodia, weak cytotoxic	Protein synthesis inhibitor, act on cell membrane		Irschik et al. (1983b), Irschik and Reichenbach (1985b)
	Myxovirescin/ antibiotic TA	Macrolide	Antibacterial	Inhibits type II signal peptidase		Gerth et al. (1982a), Zafriri et al. (1981)
	Phenalamide/ stipiamide	Polyene	Anti-HIV (HIV-1)	Reverses multidrug resistance		Trowitzsch-Kienast et al. (1992), Kim et al. (1991)
	Phenoxan	γ-Pyrone	Antifungal	Respiratory inhibitor (complex I)		Kunze et al. (1992)

Table 15.4 (continued)

Genus	Compound	Structural class	Biological activity	Mode of action	No.	References
	Pyrrolnitrin	Phenylpyrrole	Antibacterial, antifungal	Respiratory inhibitor (complex I)		Triphati and Gottlieb (1969), Gerth et al. (1982b)
	Rhizophodin	Macrodiolide	Antifungal, weak cytotoxic	Actin polymerization inhibitor		Sasse et al. (1993), Jansen et al. (2008), Hageleuken et al. (2009)
	Saframycin Mx1	Isoquinoline	Antibacterial, cytotoxic	Binds to DNA	19	Irschik et al. (1988)
Pyxidicoccus[a]	–	–	–	–	0	
Grand total					24	
Total (no overlapping)					21	

Note: Overlapping compounds within genera are written in boldface
[a]Nothing published yet

multicellular level, five *Myxococcus* and a *Corallococcus* strain were sequenced. Several more representatives of the *Myxococcaceae* family are currently being sequenced to primarily explore and discover the secondary metabolome.

Secondary metabolites isolated and elucidated in *Myxococcaceae* are not far away from the most endowed family, *Polyangiaceae*. Although majority of the activities produced was antimicrobial and cytotoxic, rare anti-HIV and antiparasitic compounds have also been found. It is also worth mentioning that compounds like the corallopyronins are already advancing to the stage of clinical trials, hoping to become a future antibiotic. Further exploration among members of this family from diverse and unexplored environment may likely succeed in finding additionally new bioactive compounds; although metagenomic approach has shown evidence for possible expansion of the family to accommodate taxa which are only known to date as sequences derived from clones of "uncultured" bacteria, we believe that they could be cultivated in the future and could be tapped for novel drug applications.

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16 The Family *Nannocystaceae*

Ronald Garcia · Rolf Müller
Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland – Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology, Saarland University, Saarbrücken, Germany

Abstract
Nannocystaceae belong to the suborder *Nannocystineae* in order *Myxococcales* and comprise two marine-derived genera *Plesiocystis* and *Enhygromyxa*, an estuarine *Pseudenhygromyxa*, and a terrestrial genus *Nannocystis*. To date, only five species have been validly described in this family. Members of the *Nannocystaceae* appear more ecologically diverse than other families of myxobacteria as they are widely distributed in the environment. Most members of this family are regarded halotolerant and halophilic organisms capable of degrading complex macromolecules and lysing microorganisms. One of the most remarkable characteristic of this family, shared with *Haliangiaceae* and *Kofleriacea*e, is the absence of hydroxy-type fatty acids, also a distinguishing landmark of the suborder *Nannocystineae*. This family is recognized for the production of long-chained polyunsaturated fatty acids and unusual steroids, although a large number of *Nannocystaceae* were isolated to date, mostly comprising *Nannocystis*, and very little is known about their secondary metabolites. However, the marine-derived members of this family turn out to be interesting producers of some novel biologically active compounds. Several of these strains are sequenced in ongoing genome projects to unfold genes responsible for their biological lifestyle and to allow studies aiming at the discovery of complex enzymes. Metagenomics data from both terrestrial and marine environment suggest that currently most of the members of this family remain not cultivated. Therefore, unraveling and exploiting these sources in the future hold promise for drug discovery programs.

Taxonomy, Historical, and Current

Short Description of the Family

Nan.no.cys.ta'ce.ae. M.L. fem. n. *Nannocystis*-type genus of the family; *-aceae* ending to denote a family; M.L. pl. fem. n. *Nannocystaceae* the *Nannocystis* family (Reichenbach 2005).

E. Rosenberg et al. (eds.), *The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria*, DOI 10.1007/978-3-642-39044-9_305,

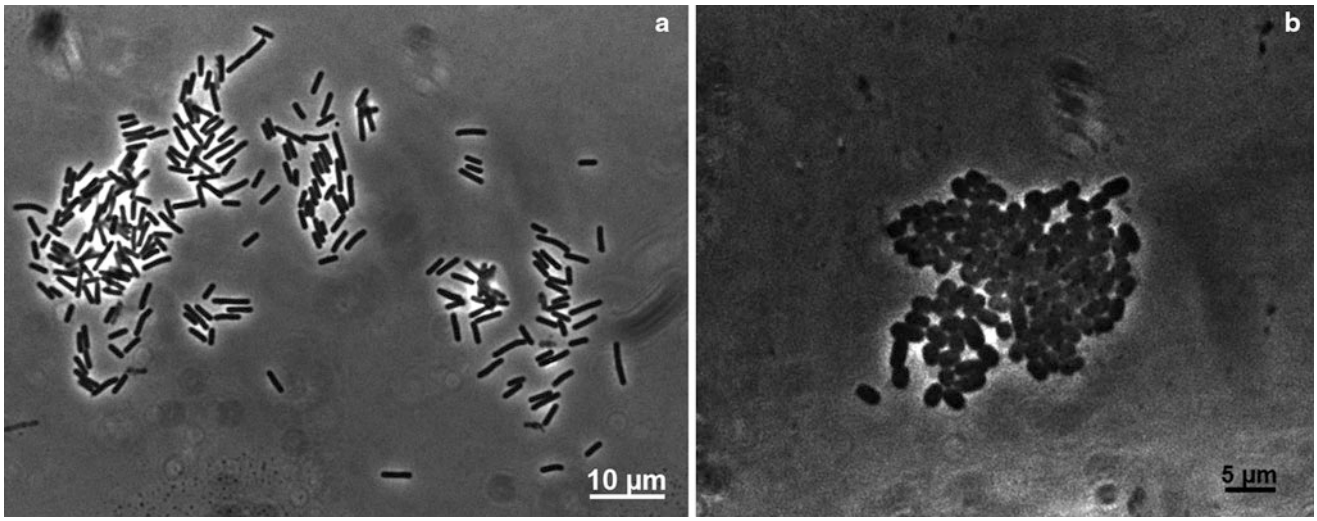

Fig. 16.1
Phase contrast of *Nannocystaceae* vegetative cell types. *Pseudenhygromyxa salina* long, slender, and non-flexuous-type cell (a). *Nannocystis exedens* short and fat rods (b)

The 16S rRNA gene sequence revealed that *Nannocystaceae* form a phylogenetic distinct clade in suborder *Nannocystineae*, order *Myxococcales* (Garcia et al. 2010), class *Deltaproteobacteria*, and phylum Proteobacteria (Shimkets and Woese 1992; Kaiser 1993).

The family is composed of genera *Enhygromyxa* (Iizuka et al. 2003b), *Pseudenhygromyxa* (Iizuka et al. 2013), *Plesiocystis* (Iizuka et al. 2003a), and *Nannocystis* (Reichenbach 1970), the type genus (Reichenbach 2005); each is represented by monotypic species except for *Nannocystis*. Members of this family share nearly common growth-stage characteristics. Vegetative rod cells stain Gram-negative and are non-flexuous, slender, and fat with more or less blunted ends (*Fig. 16.1*). Movement is by gliding on solid surface. Fruiting bodies are either sporangiole-type or naked cell aggregates containing rounded spore or spore-like cells. Swarm colonies are thin and almost filmlike on lean medium. Colony edge may appear flame-like to ridges on surface or bands and pseudoplasmodium in agar. Radial vein architecture, agar depressions, and cuts may also be produced by the swarming colony. Members of this family not only are considered aerobic and mesophilic but mostly tolerate higher sodium chloride than terrestrial myxobacteria. Some members are regarded obligate halophile, while others are classified halotolerant. They resist a wide spectrum of antibiotics. Mol percent G+C ranges from 65 to 72. To date, all members of this family were found to lack hydroxy-type fatty acids (Garcia et al. 2011). They are distributed in terrestrial and also well represented in estuarine and marine environments.

Historical and Comments on *Nannocystaceae*

Terrestrial myxobacteria from work in the last three decades are well-explored groups representing the bulk of the described species known to date. Novel taxa derived from soil are still the most prominent source for the continuously increasing number of novel isolates. Saline environment which yielded three isolated genera in *Nannocystaceae* is gaining attention for peculiar differences with the terrestrial counterpart and lately also for interesting natural products (Felder et al. 2013a, b). Attempts to isolate myxobacteria from the ocean has been documented in the past (Starr and Ordal 1953; Brockman 1967; Rückert 1975) including the isolation of truly marine obligates and a halotolerant strain belonging to *Nannocystineae* (Iizuka et al. 1998, 2003a, b, 2013; Fudou et al. 2002). Although metagenomics unfolds the diversity of marine myxobacterial groups, unfortunately no single representative of the so-called marine myxobacterial cluster (MMC) has been cultivated under laboratory conditions to date (Brinkhoff et al. 2011), likewise with the exclusively limnetic myxobacterial communities (Li et al. 2012). Based on metagenomics, *Nannocystaceae* or *Nannocystineae* suborder shows vast distribution in the environment for future discoveries. To date, this family is the largest in the suborder *Nannocystineae*, both in number of strains and species described, respectively.

Since not many strains have been described earlier to clearly delineate taxa, the classification of the genus *Nannocystis* to the suborder *Sorangiineae* was not surprising (Reichenbach 1970; Spröer et al. 1999). *Nannocystis* was even included in *Polyangiaceae* in the 9th edition of the *Bergey's Manual of Determinative Bacteriology* (Brockman 1993). The reclassification to *Nannocystaceae* as lone genus appeared in the latest edition of the *Bergey's Manual of Systematic Bacteriology* (Reichenbach 2005). Similar reclassification was applied to *Plesiocystis* and *Enhygromyxa* as they were also classified previously within the *Sorangiineae* suborder (Iizuka et al. 2003a, b). The position of these three genera in *Nannocystaceae* was clarified in an extensive myxobacterial phylogenetic study covering type, neotype, and

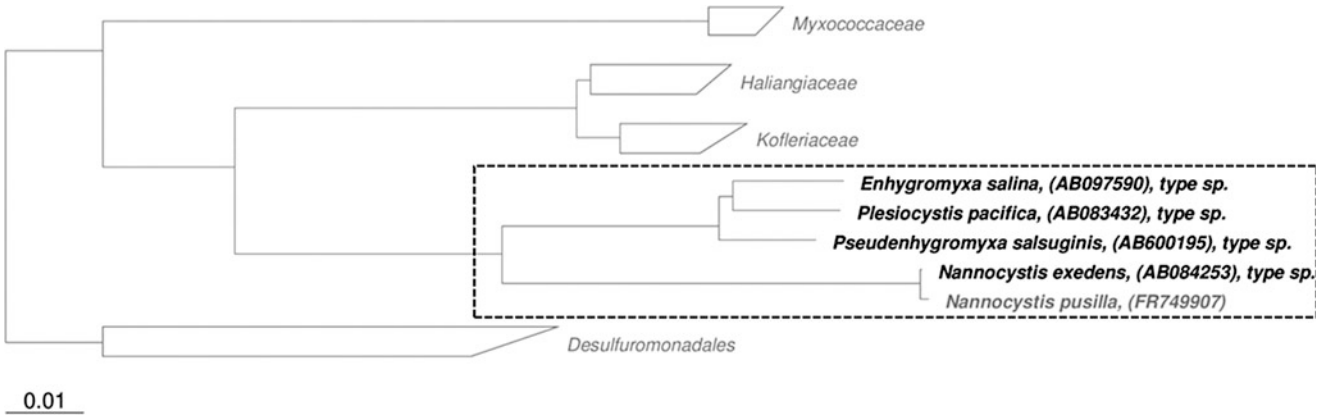

Fig. 16.2

Phylogenetic reconstruction of the family *Nannocystaceae* (*box*) based on 16S rRNA gene sequences 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

novel strains (Garcia et al. 2010). Except for *Haliangium* all validly described marine myxobacteria are currently confined to *Nannocystaceae* family in *Nannocystineae* suborder.

Phylogenetic Structure of the Family and Related Taxa

Earlier phylogenetic studies which include *Nannocystis* clearly show its unique position in the myxobacterial tree (Ludwig et al. 1983; Shimkets and Woese 1992; Spröer et al. 1999). The phylogenetic analysis based on 16S rRNA gene sequences shows *Nannocystaceae* cluster coherently in the *Nannocystineae* suborder, with *Kofleriaceae* and "*Haliangiaceae*" as closest family neighbors (Garcia et al. 2010). This analysis was confirmed by neighbor-joining tree algorithm in the All-Species Living Tree Project – LTP (Yarza et al. 2010) (*Fig. 16.2*). In the 16S rRNA gene-based phylogenetic tree, halotolerant (*Pseudenhygromyxa*) and halophile genera (*Enhygromyxa*, *Plesiocystis*) clustered together, while the terrestrial genus *Nannocystis* diverged to a different clade. Based on topology, the delineation is compelling between these two myxobacterial clades of different ecological sources. This pattern of clustering also conforms to a yet not validly described taxon, *Paraliomyxa* (Iizuka et al. 2006b). This novel slightly halophilic myxobacterium was isolated from a nearshore soil sample and was phylogenetically determined earlier to be closely located within the *Enhygromyxa-Plesiocystis* clade (Iizuka et al. 2006b; Garcia et al. 2010).

Molecular Analysis

Within the four described genera of *Nannocystaceae*, no DNA-DNA hybridization, multi-locus sequencing, riboprinting-ribotyping, or genome-based taxonomy studies were conducted. In the proposed subspecies of *Nannocystis exedens*, it may be worth performing the DNA-DNA hybridization to determine whether morphological variations support their delineation into different species.

Based on 16S rDNA sequence, strains of *Enhygromyxa salina* were determined 99.9–100 % similar, suggesting that all described isolates belong to the same species (Iizuka et al. 2003b). Similar findings were reported for the two strains (SIR-1^T, SHI-1) of *Plesiocystis pacifica* which show 99.5 % similarity in the 16S rDNA sequence (Iizuka et al. 2003a).

Genome Analysis

So far, only for *Plesiocystis pacifica* SIR-1^T, a genome sequence has been published, although there is a report of ongoing sequencing of *Enhygromyxa salina* (Schäberle et al. 2010). *P. pacifica* SIR-1^T genome is 10.6Mbp in size (http://mistdb.com/bacterial_genomes/summary/3004) and thus is significantly smaller than *Sorangium cellulosum* So ce56 (13, 033,779 bp) (Schneiker et al. 2007) but almost of the same size as *Myxococcus xanthus* DK1622 (9,139,763 bp) (Goldman et al. 2006). However, its genome is only about half the size of the facultative anaerobic myxobacterium *Anaeromyxobacter dehalogenans* 2CP-C (5,013,479 bp) (http://genome.jgi-psf.org/anade/anade.home.html). SIR-1^T genome as currently published comprises 237scaffolds in which 8,526 genes are identified, and exhibiting 70.7 mol% G+C (http://mistdb.com/bacterial_genomes/summary/3004).

Phages and Plasmids

No plasmids and phages reported in this family.

Phenotypic Analysis

The family is characterized phenotypically by phase-dark, fat, cylindrical, and stout vegetative cells with blunted ends. Swarm colony can penetrate, depress, corrode, etch, cut, or split the agar. Myxospores and myxospore-like cells are ovoid to spherical in shape. Fruiting and fruiting-like bodies are either sporangiole-type or aggregate-like structure.

Enhygromyxa (Iizuka, Jojima, Fudou, Tokura, Hiraishi, Yamanaka 2003)

En.hygro.my'xa. Gr. adj. *enhygro*, growing or living in wet or moist habitats; Gr. fem. n. *myxa*, slime; N. L. fem. n. *Enhygromyxa*, slime of wet or moist habitat, which means aquatic myxobacteria (Iizuka et al. 2003b).

Fruiting-like bodies are composed of large (60–80 μm W, 80–150 μm L) solitary and orange-brown aggregates which contain non-refractile, small, and spherical myxospore-like structures (0.5–0.7 μm). Swarm concentrates at the edge of the colony appearing as yellow bands or short pseudoplasmodium in agar. Swarm may also appear delicate slimy veins with flare-like edges on surface of the substrate. Vegetative cells are slender rods with blunted ends. Agar is cleaved and partially degraded and shows bacteriolytic but non-cellulolytic type of nutrition. Stains negative in Congo red. Exhibits chemoorganotrophic, strict aerobic, and moderately halophilic growth. Catalase and oxidase positive. Contains major menaquinone MK-7. Major cellular fatty acids are *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, and *iso*-$C_{17:0}$. Anteiso $C_{16:0}$, anteiso $C_{17:0}$, and polyunsaturated $C_{20:4}$ fatty acids are also present. The DNA mol% G+C is 65–67 %. The type species is *Enhygromyxa salina*, and the type strain is SHK-1^T (= DSM 15217^T = JCM 11769^T). The source was wet black mud collected in a shore facing Saroma-ko Lagoon in Japan in 1999 (Iizuka et al. 2003b). *Table 16.1* shows differentiating characteristics among other *Nannocystaceae* genera. Growth stages are illustrated in *Figs. 16.3* and *16.4*.

Plesiocystis (Iizuka, Jojima, Fudou, Hiraishi, Ahn, Yamanaka 2003)

Ples.i.o.cys'tis. Gr. masc. n. *plesion*, neighbor; Gr. fem. n. *cystis*, bladder; N. L. fem. n. *Plesiocystis*, neighbor bladder (to imply the genus is phylogenetically clustered next to the genus *Nannocystis* on the dendrogram) (Iizuka et al. 2003a).

Fruiting-like bodies are pinkish to brownish orange, composed of aggregate of cells without distinct enclosing wall. They are arranged solitary but may also appear as cluster on surface of the substrate (*Fig. 16.5*). Vegetative cells are phase-dark and delicate rod-shaped cells with blunted ends. Swarm colony may appear thin, transparent, and pseudoplasmodium-like producing cell aggregates (*Fig. 16.6*). Myxospore-like cells are non-refractile, small (0.5–0.7 μm), and spherical in shape. Can lyse bacterial cells and degrade agar but not cellulose. Congo red negative stained. Exhibits strict aerobic, mesophilic, chemoorganotrophic, and moderately halophilic growth characteristics. Shows oxidase-positive and catalase weak positive reaction. Contains major menaquinone MK-8(H_2). Major cellular fatty acids are *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, and *iso*-$C_{17:0}$. Anteiso fatty acids $C_{16:0}$ and $C_{17:0}$ and long-chained polyunsaturated $C_{20:4}$ are present. The mol% G+C of DNA is 70. The type species is *Plesiocystis pacifica*, and the type strain is SIR-1^T (= DSM 14875^T = $AJ13960^T$), isolated from a semi-dried sea grass sample (*Zostera* sp.) collected in 1999 from sandy beach of Iriomote-jima Island, Japan (Iizuka et al. 2003a). Diagnostic characteristics among *Nannocystaceae* genera are shown in *Table 16.1*.

Pseudenhygromyxa (Iizuka, Jojima, Hayakawa, Fujii, Yamanaka, Fudou 2013)

Pseud.en.hyg.ro.myx'a. Gr. adj. *pseudes*, false, N. L. n. *Enhygromyxa*, a genus of marine myxobacteria; N. L. fem. n. *Pseudenhygromyxa*, a false *Enhygromyxa* (Iizuka et al. 2013)

Fruiting-like body and cell aggregates show orange-brown to rusty color on agar (*Fig. 16.7a* and *b*). Myxospore-like cells are small and spherical to ellipsoidal. Swarm colony is colorless to pale peach, agar depressing and shows flare-like appearance on the agar edge (*Fig. 16.7c* and *d*). Vegetative cells are slender rods with blunted ends. Lyse and prey on bacteria; degrades agar but not cellulose. Congo red stains negative. Shows strict aerobic, chemoorganotrophic, neutrophilic, and mesophilic growth between 20 °C and 40 °C. Grows in absence of sodium chloride or tolerates concentration lower than found in seawater (halotolerant). Shows oxidase-positive and weak catalase reactions. Contains major menaquinone MK-7. Major fatty acids are *iso*-$C_{15:0}$, *iso*-$C_{17:0}$, and *iso*-$C_{16:0}$, and contain long-chained $C_{20:4}$ polyunsaturated fatty acids. The mol% DNA G+C of the type strain is 69.7. The type species is *Pseudenhygromyxa salsuginis*. The type strain is SYR-2^T (=DSM 21377^T = $NBRC104351^T$), isolated from muddy marsh sediment of Yoshino River (Yoshinogawa), Tokushima Prefecture, Shikoku, Japan, collected in August 1998 (Iizuka et al. 2013).

Nannocystis (Reichenbach 1970) (Type Genus of the Family *Nannocystaceae* and Suborder *Nannocystineae*, Reichenbach 2005)

Nan.no.cys'tis. Gr. masc. n. *nannos* dwarf; Gr. fem. n. *kystis* bladder; M.L. fem. n. *Nannocystis* tiny bag (Reichenbach 2005).

Fruiting bodies are small spherical, oval to short sausage-shaped sporangiole, usually embedded in the agar. Myxospores are small, rounded, and refractile packed in translucent sporangiole and commonly arranged in solitary. Swarm edge shows pattern of trails or fine wave, while center is commonly depressed and excavated and sometimes deep tunnels are formed. Produce almost colorless colony growth with agar degradation in lean medium. Vegetative cells are short slender

Table 16.1

Differentiating characteristics among *Nannocystaceae* genera as represented by the type species

Characteristics	*Nannocystis exedens* DSM 71^{T} [a]	*Plesiocystis pacifica* DSM 14875^{T} [b]	*Enhygromyxa salina* DSM 15217^{T} [c]	*Pseudenhygromyxa salsuginis* DSM 21377^{T} [d]
Vegetative cell: shape	Short fat rods to nearly cuboidal	Long slender fat rods	Long slender fat rods	Long slender fat rods
Type	*Non-flexuous*	*Non-flexuous*	*Non-flexuous*	*Non-flexuous*
Size L × W (μm)	1.75–4.8 × 1.1–2.0	1.5–7.0 × 0.5–0.8	1.5–7.0 × 0.5–0.7	2.0–5.0 × 0.5–0.8
Swarm colony (yeast/any Peptone based)	Colorless/light pink	Colorless/*peach orange*	Colorless/light orange to red	Colorless/light orange, light pink, light red
Edge	*Wave, trail, pseudoplasmodium, never as flare*	*Flare to pseudoplasmodium*	*Flare to pseudoplasmodium*	*Flare to pseudoplasmodium*
Diffusible pigment (Baker's yeast-based agar)	*None*	*None*	*None*	*None*
Myxospore/myxospore-like: shape	Rounded to oval	Rounded/spherical	Rounded/spherical	Spherical to ellipsoidal
Size (μm)	0.75–1.5	0.5–0.7	0.5–1.0	1.5–3.0 × 0.5–0.8
Fruiting/fruiting-like body	Sporangiole	Fruiting-like aggregate (FLA) without walls[b, e]	FLA	FLA
Arrangement	*Often solitary*	*Often solitary*	*Often solitary*	*Often solitary*
Shape	Rounded to ovoid	*Rounded, hump*, globular to polyhedral	*Rounded, hump*, globular	*Rounded, hump*, globular
Color	Colorless, yellowish brown to reddish	Pinkish to brownish orange	Orange to brownish orange	Brownish orange to red
Size (μm)	6 × 3.5–110 × 40	100–500	50–300	50–800
Congo red stain	Negative	Negative	Negative	Negative
Oxygen requirement	Aerobic	Aerobic	Aerobic	Aerobic
pH range	5.5–10.0	5.5–9.0	5.5–9.0	5.5–8.3
Optimum	7.0–9.5	7.0–8.5	7.0–8.5	7.0–7.5
Temperature tolerance (°C)	23–36	15–32	5–34	18–40
Optimum (°C)	30	28–30	28–30	30–35
NaCl range (%)	*0–0.2*[b, c]	1.0–4.0	0.1–4.0	0–2.5
Optimum (%)	*0–0.2*[b, c]	2.0–3.0	1.0–2.0	0.2–1.0
Cation requirement	ND	Ca^{2+}, Mg^{2+}, K^{+}	Ca^{2+} or Mg^{2+}	Ca^{2+} and Mg^{2+}
Catalase	Positive	Weak positive to negative	Positive	Negative
Oxidase	Positive	Positive	Positive	Positive
Degradation: agar	Very strong	Strong	Strong	Strong
Agarose	ND	ND	ND	Positive
Alginate	ND	ND	Negative	ND
Cellulose	Negative	Negative	Negative	Negative
Chitin	Negative[b, c]	Negative	Negative	Negative
Hydrolysis:				
Casein	ND	Positive	Positive	Positive
DNA	Negative[b, c]	Weak positive	Negative	Negative
Gelatin	Positive	ND	Positive	ND
Skim milk	ND	ND	Negative	ND
Starch	Negative[b, c]	Negative	Negative	Weak positive to negative
Tween 80	Weak positive[b, c]	Weak positive to negative	Negative	Negative
Bacterial lysis (*E. coli*)	Positive	ND	Positive	ND
Yeast lysis (Baker's yeast)	Negative	Negative	Negative	Negative

Table 16.1 (continued)

Characteristics	*Nannocystis exedens* DSM 71^{T} [a]	*Plesiocystis pacifica* DSM 14875^{T} [b]	*Enhygromyxa salina* DSM 15217^{T} [c]	*Pseudenhygromyxa salsuginis* DSM 21377^{T} [d]
Reaction to APIZYM				
α-glucosidase	Negative[b, c]	Negative	Negative	ND
β-glucosidase	Negative[b, c]	Negative	Negative	ND
C4 esterase	Weak positive[b, c]	Negative	Negative	ND
C8 esterase	Weak positive[b, c]	Negative	Weak positive to negative	ND
Antibiotic resistance (50 μg/mL)				
Ampicillin	*ND*	*No growth*	*No growth*	*Growth*
Bacitracin	*No growth*	*Growth*	*No growth*	*No growth*
Cephalosporin	*ND*	*No growth*	*No growth*	*No growth*
Fusidic acid	*Growth*	*Growth*	*Growth*	*Growth*
Gentamicin	*No growth*	*Growth*	*No growth*	*No growth*
Hygromycin	*No growth*	*Growth*	*Growth*	*Growth (poor)*
Kanamycin	*No growth*	*Growth*	*No growth*	*Growth (poor)*
Kasugamycin	*Growth*	*Growth*	*Growth*	*Growth (poor)*
Neomycin	*No growth*	*No growth*	*No growth*	*Growth (poor)*
Oxytetracycline	*No growth*	*No growth*	*No growth*	*No growth*
Polymyxin	*No growth*	*Growth*	*No growth*	*Growth (poor)*
Spectinomycin	*ND*	*No growth*	*No growth*	*No growth*
Thiostrepton	*No growth*	*No growth*	*No growth*	*No growth*
Trimethoprim	*Growth*	*Growth*	*Growth*	*Growth*
Major menaquinone	MK8[b, c]	MK8 (H_2)	MK7	MK7
Major fatty acids	*$C_{16:1w5c}$, $C_{14:0}$, iso-$C_{17:0}$, $C_{16:0}$, iso-$C_{15:0}$[f]	iso-$C_{15:0}$, iso-$C_{16:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$	iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{16:0}$
		($C_{16:0}$, $C_{16:1\omega7c}$, $C_{16:1\omega9c}$, $C_{18:1\omega9c}$[f])	($C_{16:0}$, $C_{16:1\omega7c}$, $C_{18:1\omega9c}$[f])	
Anteiso FAs	Not detected[f]	$C_{16:0}$[b, g], $C_{17:0}$ (ta)	$C_{16:0}$[c, *], $C_{17:0}$ (ta)	$C_{15:0}$, $C_{17:0}$ (ta)
Hydroxy FAs	Not detected[f]	Not detected[b, f]	Not detected[c, f]	Not detected
PUFAs	None[f]	AA[b, d, f]	AA[c, d, f], EPA[f]	AA
Mol% G+C content	70–72	69.3	65–67	69.7

Note: Data were obtained from each respective publication. Text in italics were determined by the authors, except for the taxonomic names
References: [a]Reichenbach 1970, [b]Iizuka et al. 2003a, [c]Iizuka et al. 2003b, [d]Iizuka et al. 2013, [e]Iizuka et al. 1998, [f]Garcia et al. 2011, [g]not detected (Iizuka et al. 2013)
Legend: *AA* arachidonic acids, *EPA* eicosapentaenoic acids, *FLA* fruiting-like aggregate, *ND* not determined, *PUFAs* polyunsaturated fatty acids, *ta* trace amount

fat rods with more or less rounded ends. *Figure 16.8* shows the growth stages. Exhibit bacteriolytic- and non-cellulolytic- type nutrition. Congo red stain is negative. Strict aerobic, mesophilic, and chemoorganotrophic. Oxidase and catalase positive. Contains major menaquinone MK-8. Major cellular fatty acids are *iso*-$C_{15:0}$, *iso*-$C_{17:0}$, $C_{16:1\omega5c}$, and $C_{14:0}$. Straight-chained fatty acids (SCFAs) predominate over branch-chained fatty acids (BCFAs). The mol% G+C of DNA ranges 70–72. The type species is *Nannocystis exedens*, isolated in 1968, and the type strain is Na e1^{T} (Microbial Culture Collection Department, Helmholtz Center for Infection Research, Braunschweig, Germany) (=DSM 71^{T} = ATCC25963^{T}). The strain was isolated from red sandy desert soil collected from Navajo reservation, north of Flagstaff, Arizona, USA (Reichenbach 1970). Differentiating characteristics among *Nannocystaceae* genera are shown in *Table 16.1*.

Isolation, Enrichment, and Maintenance Procedures

Collection, Processing, Treatment, and Storage of Samples

Samples collected from the environment can be used directly for enrichment or processed later after air-drying at room temperature. Growth and survival of the myxobacterium after drying or desiccation conditions seem associated to the

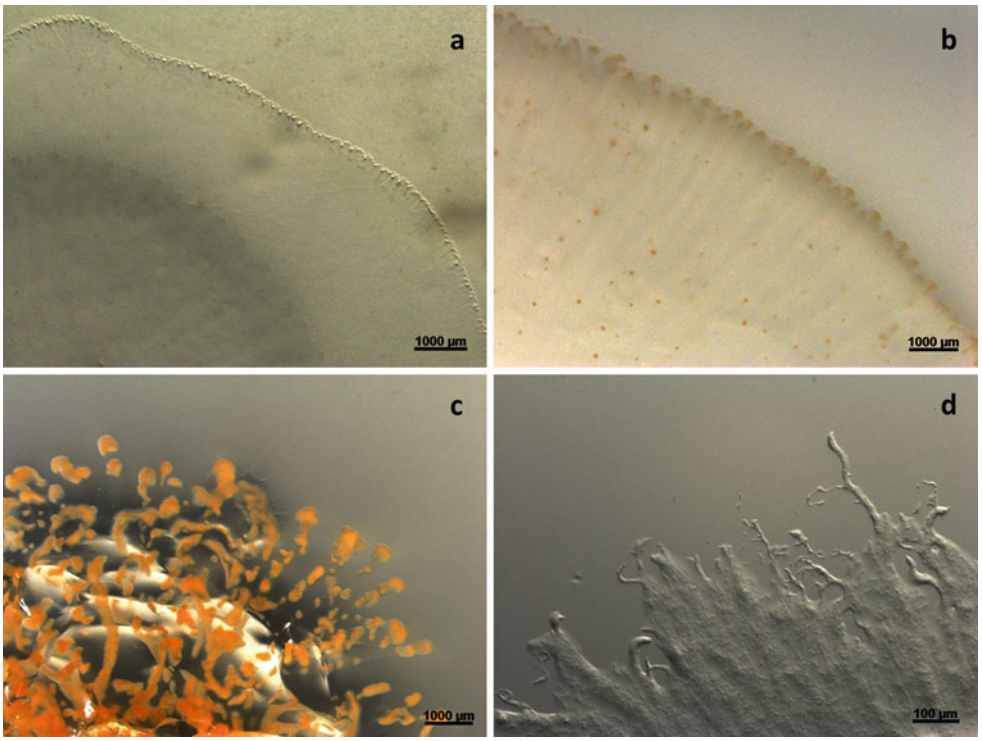

Fig. 16.3
Stereophotomicrographs of *Enhygromyxa salina* DSM 15217^T swarming patterns. Film and transparent colony with slight agar depressions (a). Migrating pseudoplasmodial-like cell bands in agar (b). *Yellow-orange thick* pseudoplasmodial swarm with agar degradation (c). Flare- to flame-like colony edges on surface of the agar (d). Growth in yeast-based SWS agar (a–b) and in Casitone-based SWS agar (c–d)

Fig. 16.4
***Enhygromyxa salina* DSM 15217^T growth stages. Phase-contrast photomicrograph of the vegetative cells (a). Stereophotomicrograph of solitary fruiting bodies held in transparent glistening slime (b) and sporangiole-like aggregate on VY/2-SWS agar (c). Bar, 10 µm (a)**

development of fruiting bodies or possible conversion of vegetative cells directly into myxospores. Dried soil materials including marine samples can be placed in clean vials and stored at room temperature. Completely dried samples prevent growth of microorganisms including unwanted molds, nematodes, helminths, and amoeba that contribute to further decomposition of the samples. The growth of these contaminants can be suppressed using antibiotics during enrichment.

Setups for Cultivation, Isolation, and Purification

Samples can be processed for *Nannocystaceae* enrichment by simulating the conditions of the sample source. ❯ *Table 16.2* shows the identified sources for members of this family. Among the most important factor to consider in the enrichment setups for marine sample are salinity, pH, and temperature. It is worth to consider that myxobacteria living in this type of environment

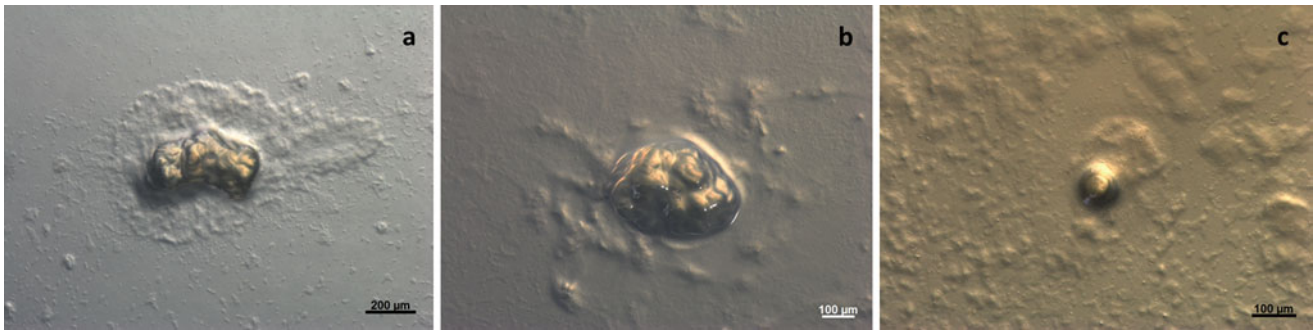

Fig. 16.5
Plesiocystis pacifica **DSM 14875^T fruiting-like bodies on yeast-based SWS agar medium. Large sporangiole-like aggregates (a–b). Solitary mound fruiting-like body (c)**

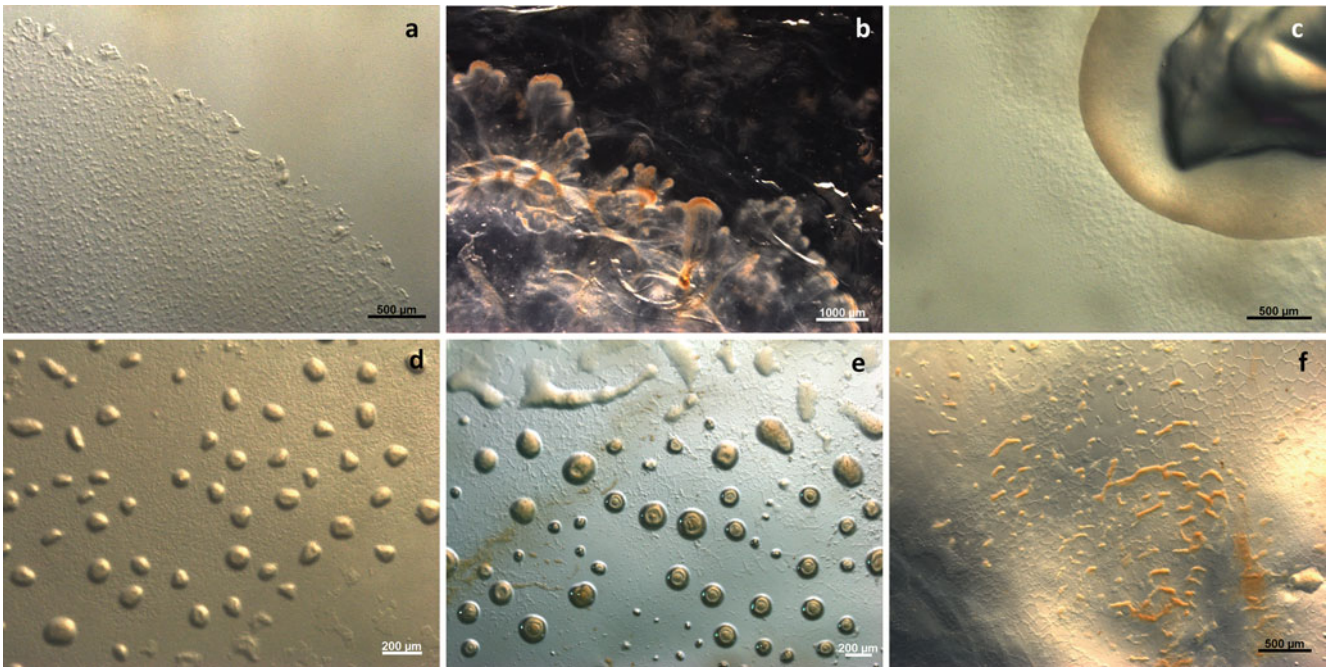

Fig. 16.6
Plesiocystis pacifica **DSM 14875^T swarm and cell aggregation stages. Thin and filmlike colony swarm on deionized yeast-based SWS agar showing migrating cells with tiny flares at the edges (a). *Orange* pseudoplasmodial swarms in 1/3 CY/SWS agar (b). Coherent agar penetrating colony (arc-shaped swarm on piece of inoculated agar) and filmlike surface swarming on natrose-based SWS agar (c). Scattered cell mounds on deionized yeast-based SWS (d) and 1/3 CY/SWS agar (e). Ridge- and roll-shaped *orange* cell aggregates in 1/3CY/SWS agar (f)**

are potentially halotolerant or halophilic, and sodium chloride needs to be adjusted accordingly. The use of either seawater or artificial commercial sea salts with pH adjustment according to the sample condition plays key roles in the enrichment and isolation of marine-derived myxobacteria belonging to genera *Plesiocystis*, *Enhygromyxa*, and a brackish-water isolate *Pseudenhygromyxa*. The latter genus appears ideally isolated provided that the estuarine conditions are met, for example, lower seawater and sodium chloride concentration. Since all members of these genera are bacteriolytic type, baiting with live bacterium (e.g., *Escherichia coli*) either streaked or spotted on agar stimulates their appearance in the setup.

Terrestrial *Nannocystaceae* can be processed for enrichment using the standard setup of bacterial baiting on lean water agar medium (gram per liter, Bacto Agar 15, $CaCl_2 \cdot 2H_2O$ 1.0, HEPES 20 mM, pH adjusted to 7.2 with KOH before autoclaving) (Shimkets et al. 2006). Purification follows the standard protocol for myxobacteria by cutting the farthest swarm edge and by repeated transferring into a new growth-supporting medium.

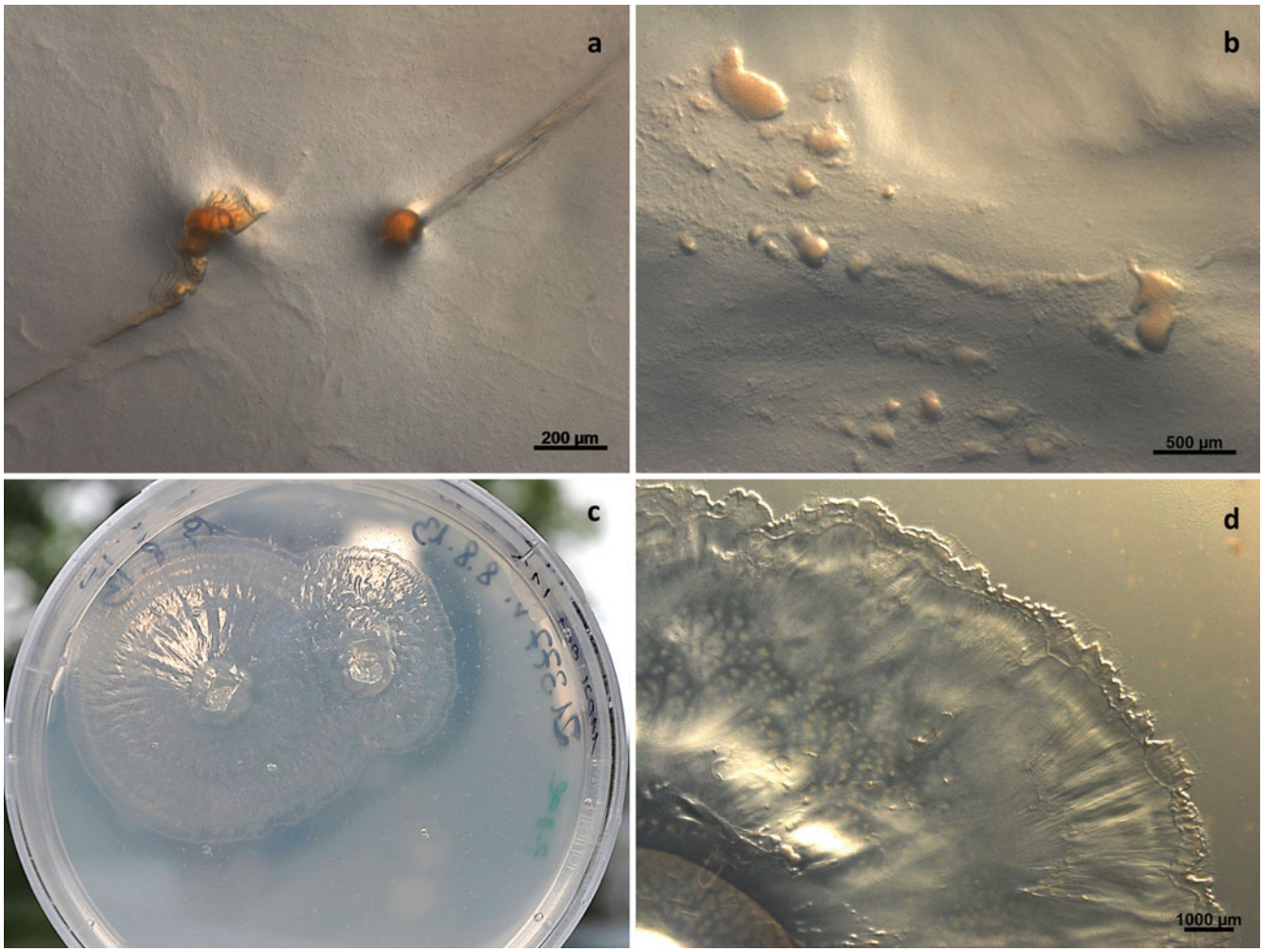

Fig. 16.7

Growth stages of *Pseudenhygromyxa salsuginis* DSM 21377^T. *Reddish-orange* fruiting body-like held in slime sheath in yeast-based SWS agar (a). Initial swarming aggregates on agar (b). Agar-degrading colonies radiating from two agar inocula (*center* of each colony) in natrose-based medium (c). Stereophotomicrograph of the colony edge showing deep agar depression (d)

Since *Nannocystaceae* family shares many features with *Polyangiaceae* in *Sorangiineae* suborder, careful attention is needed. These two families show very close resemblance in the vegetative cell, except for *Nannocystis* appearing almost cuboidal rods. Knowledge regarding the taxonomic designation during at the start of the isolation is advantageous for determining the growth, nutritional, and physicochemical requirements required for further steps in the purification process.

In general the use of ASW-WCX agar medium (gram per liter: Bacto Agar 15, $CaCl_2 \cdot 2H_2O$ 1.0, dissolved in 0.75 x artificial seawater (ASW), pH adjusted to 7.5 with NaOH before autoclaving, supplemented with cyanocobalamin 0.5 mg and cycloheximide 25 mg after autoclaving, Iizuka et al. 1998) appears ideal for the enrichment of *Plesiocystis* and *Enhygromyxa*. This medium seems to be a reformulation of WCX agar (gram per liter: Agar 15, $CaCl_2 \cdot 2H_2O$ 1.0, pH adjusted to 7.2, cycloheximide 25 µg/mL added after autoclaving, Reichenbach and Dworkin 1992) but was modified to adapt to marine conditions. Enrichment for *Pseudenhygromyxa* can be performed in Ec-$S_{20,10}$ agar (gram per liter: NaCl 10, Bacto Agar 15; dissolve in 20 % v/v seawater salt solution (SWS), adjust the pH to 7.2 before autoclaving with NaOH, supplement after autoclaving with cyanocobalamin 0.5 mg/L and cycloheximide 25 mg/L, and bait with parallel streaks of live *Escherichia coli* on agar surface) (Iizuka et al. 2013). Seawater salt solution (SWS) contains (grams per liter) $FeC_6H_5O_7 \cdot nH_2O$ 0.1, $MgSO_4 \cdot 7H_2O$ 8, $CaCl_2 \cdot 2H_2O$ 1, KCl 0.5, $NaHCO_3$ 0.16, H_3BO_3 0.02, KBr 0.08, $SrCl_2$ 0.03, and ß-glycerophosphate · 2Na 0.01 and 1 ml trace element solution (Iizuka et al. 2013). Trace element solution contains (milligrams per liter distilled water) $MnCl_2 \cdot 4H_2O$ 100, $CoCl_2$ 20, $CuSO_4$ 10, $Na_2MoO_4 \cdot 2H_2O$ 10, $ZnCl_2$ 20, LiCl 5, $SnCl_2 \cdot 2H_2O$ 5, H_3BO_3 10, KBr 20, KI 20, EDTA, Na-Fe^{+3} salt trihydrate 8 g, and filter sterilize (Drews 1974).

Nannocystaceae are recognized by clearing of the microbial bait and appearance of swarming cells and later fruiting or

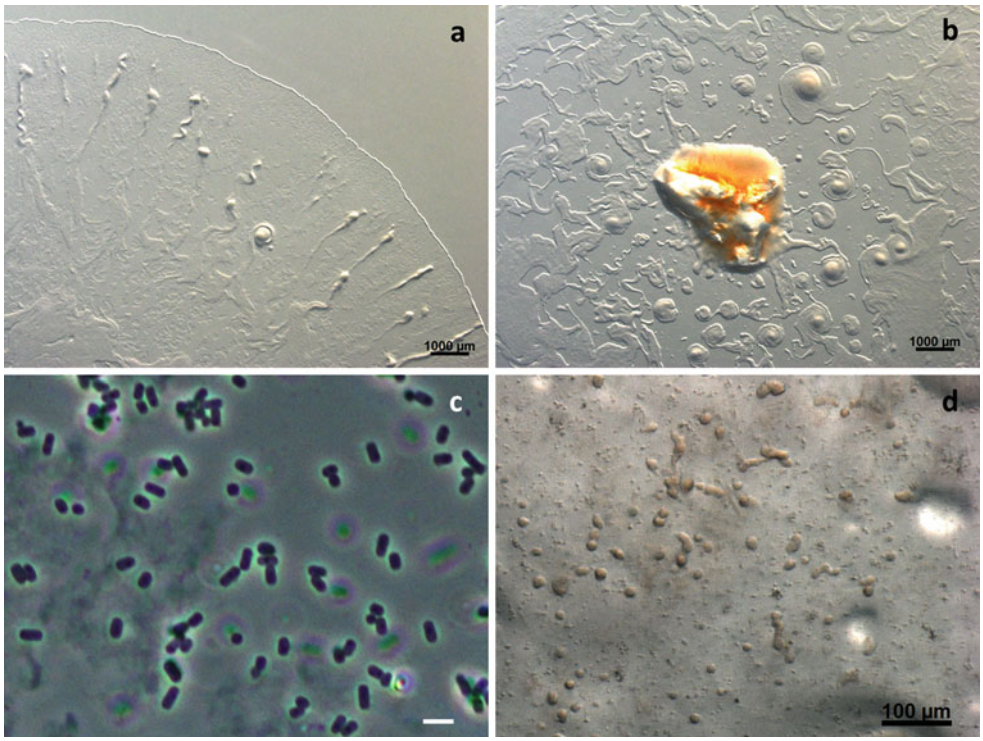

Fig. 16.8
Growth stages of *Nannocystis exedens* DSM 71^T. Colony edge showing some spiral plications (a). Middle of the colony showing the agar inoculum and spinning-like swarm trails (b). Phase-dark, short, fat to almost cuboidal vegetative rod cells obtained from VY/2 agar (background) (c). Tiny and agar submerged fruiting bodies in VY/2 (d). Bar, 5 µm (b)

Table 16.2
Isolation sources of *Nannocystaceae*

Sources	*Nannocystis*	*Plesiocystis*[a]	*Enhygromyxa*[b]	*Pseudenhygromyxa*[c]
Terrestrial:				
Herbivore dung	+[d]	–	–	–
Soil	+[e]	–	–	–
Lake (mud)	+[f]	–	–	–
Marine:				
Alga	–	–	+	–
Mud	–	–	+	+ (estuarine marsh)
Sand	+[g](intertidal region)	+	+	–
Sea grass	–	+	–	–

References: [a]Iizuka et al. 2003a, [b]Iizuka et al. 2003b, [c]Iizuka et al. 2013, [d]Reichenbach and Dworkin 1992, [e]Reichenbach 1970, [f]Li et al. 2012, [g]Ohlendorf et al. 2008

fruiting-like bodies on agar. *Nannocystis* is peculiar for swarm trails that are easily recognized on agar (*Fig. 16.9*). Isolation is performed by lifting the fruiting bodies or by cutting the swarm on the agar. Like all other myxobacteria, purification is performed by repeated subculturing of the swarm colony edge onto fresh medium baited with bacterium. Pure isolates are then kept in suitable maintenance media.

Growth Cultivation and Maintenance

Nannocystis is maintained in VY/2 and CY agar (grams per liter, Difco Casitone 3, Difco Yeast Extract 1, $CaCl_2 \cdot 2H_2O$ 1, Agar 15, pH 7.2 adjusted with KOH, Reichenbach and Dworkin 1992). Culture can be kept at 30 °C for 2–3 weeks in VY/2 agar (Reichenbach 1970) and around 2 weeks for CY agar.

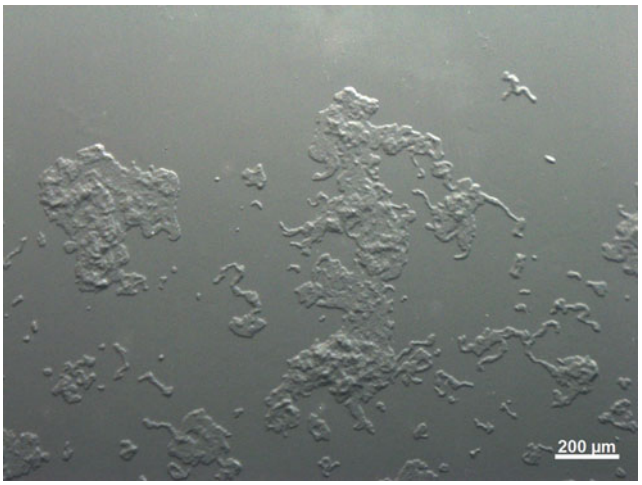

Fig. 16.9
Migrating swarm trails of *Nannocystis* produce at the edge of the agar colony

Nannocystis also grows in PL medium (grams per liter, single cell protein Hoechst Probion LS600 10, $MgSO_4 \cdot 7H_2O$ 1, $CaCl_2 \cdot 2H_2O$ 1, pH 7.0) and MD1 (grams per liter, Difco Casitone 3, $CaCl_2 \cdot 2H_2O$ 0.5, $MgSO_4 \cdot 7H_2O$ 2, cyanocobalamin 0.5 mg/L, trace elements, pH 7.2) (Kohl et al. 1983).

Pseudenhygromyxa is maintained in $N_{1.0}$-$S_{75,15}$ agar (grams per liter, natrose/casein natrium 1, NaCl 15, Bacto Agar 15; dissolve in 75 % v/v SWS, adjust the pH before autoclaving to 7.2 with NaOH, supplement with cyanocobalamin 0.5 mg/L after autoclaving), $N_{2.0}$-$S_{75,15}$ (same as $N_{1.0}$-$S_{75,15}$ agar, except that it contains 2 g/L natrose), and VY/2-$S_{75,15}$ agar (grams per liter, Baker's yeast paste 5, Bacto Yeast Extract 0.1, NaCl 15, Bacto agar 15, dissolved in 75 % v/v SWS, pH adjusted to 7.2 before autoclaving with NaOH, cyanocobalamin 0.5 mg/L added after autoclaving) (Iizuka et al. 2013). Seawater salt solution contained (grams per liter) $FeC_6H_5O_7 \cdot nH_2O$ 0.1, $MgSO_4 \cdot 7H_2O$ 8, $CaCl_2 \cdot 2H_2O$ 1, KCl 0.5, $NaHCO_3$ 0.16, H_3BO_3 0.02, KBr 0.08, $SrCl_2$ 0.03, and ß-glycerophosphate · 2Na 0.01 and 1 ml trace element solution (Iizuka et al. 2013).

Plesiocystis and *Enhygromyxa* can be maintained in 1/3 CY/SWS agar (grams per liter, Bacto Casitone 0.3, Bacto Yeast Extract 1, NaCl 20, Agar 15, seawater salt solution (SWS) 1 L; adjust pH to 7.4 with 1 M NaOH) (Iizuka et al. 2003a). SWS contain (grams per liter distilled water) $MgSO_4 \cdot 7H_2O$ 8.0, $CaCl_2 \cdot 2H_2O$ 1.0, KCl 0.5, $NaHCO_3$ 0.16, H_3BO_3 0.02, KBr 0.08, $SrCl_2$ 0.03, glycerophosphate-2Na 0.01, and iron (III) citrate 10 mg/L; supplement with 1 mL trace element solution, and adjust pH to 7.4 with 1 M NaOH (Iizuka et al. 2003a). They could also be routinely cultivated in Vy2/SWS agar (grams per liter), Baker's yeast paste prepared from dried yeast and washed 3x with deionized water 5, NaCl 20, Bacto Agar 15, dissolved in SWS, pH adjusted to 7.3 with 1 M NaOH, after autoclaving supplement with cyanocobalamin 0.5 mg/L (Iizuka et al. 2003a).

Additionally, *Plesiocystis* can also grow in VY/2-ASW agar (grams per liter, Baker's yeast 5, Bacto Agar 15, artificial seawater (ASW) 0.75x, pH adjusted to 7.5 with NaOH, supplemented with cyanocobalamin 0.5 mg/L after autoclaving) (Iizuka et al. 1998) and Casein/SWS (grams per liter), casein sodium 2, NaCl 20, dissolve in SWS (see composition in 1/3 CY/SWS), pH adjusted to 7.4 before autoclaving with 1 M NaOH, supplemented with 0.5 mg/mL cyanocobalamin after autoclaving (Iizuka et al. 2003a). Members of the *Nannocystaceae* can be kept in agar for around 2–4 weeks or before the culture reaches the end of the Petri dish. These myxobacterial cultures rarely reverse back to the medium and eventually face death at the edge of the plate. In other myxobacteria (e.g., *Chondromyces*, *Polyangium*, *Cystobacter*, etc.), swarms that reach the ends of the plate often develop into fruiting bodies.

It is our observation that *Nannocystaceae* and all other myxobacteria are best maintained and backed up in a lean medium (Peptone-/Casitone-free), for example, in Baker's yeast-based medium to keep as much as possible the morphology stages intact. Fruiting bodies and myxospores are the most vulnerable stages in myxobacteria which are often lost after series of successive cultivations, especially in Peptone-based medium.

Preservations and Reactivations of Cultures

Nannocystaceae can be preserved directly by storing in a −80 °C freezer or by suspending vegetative cells, swarm colony edge, fruiting bodies, and myxospores in glycerol (e.g., 20 %) or DMSO (e.g., 5 %). Concentration of the cryoprotectant must be tested for every strain to ensure the highest viability. For cryopreservation, *Plesiocystis* can be prepared by cultivating in VY/2-ASW agar (Iizuka et al. 2003a). Reactivation is performed simply by plating on several suitable maintenance media. *Plesiocystis* and *Enhygromyxa*, for example, can be plated onto VY/2-SWS and 1/3 CY/SWS agar. *Pseudenhygromyxa* can be reactivated in $N_{1.0}$-$S_{75,15}$ agar, $N_{2.0}$-$S_{75,15}$, and VY/2-$S_{75,15}$ agar media. For *Nannocystis*, frozen cell aliquots can be plated in VY/2 and CY agar. *Nannocystis* can additionally be kept as dried desiccated fruiting body on filter paper and stored at room temperature. No data are available regarding lyophilization and culture desiccation in *Plesiocystis*, *Enhygromyxa*, and *Pseudenhygromyxa*; however, dried fruiting bodies of *Nannocystis* on filter can be reactivated by simply placing them on agar medium (e.g., VY/2). Myxospores packed in sporangioles will eventually germinate and develop into a swarm within several days to few weeks.

Morphology and Developmental Stages

Morphological characterization has been regarded as an important criterion for myxobacterial identification and classification (Garcia et al. 2009a). *Nannocystaceae* share common phenotypic characteristics distinct from other myxobacterial families, although fruiting stage and development of myxospores are not clearly described for some members. Fruiting bodies in marine-derived isolates appear not well defined under

laboratory condition, only somehow reflecting ecological environments. Whether fruiting body is indeed developed and required in constantly fluctuating aquatic conditions (e.g., salinity, pH, temperature, pressure, nutrition, etc.) remains unknown. The terrestrial genus *Nannocystis* appears as evolved similarly with other land-isolated myxobacteria-possessing fruiting bodies enclosing myxospores.

Vegetative Cells

Members of the *Nannocystaceae* possess a vegetative cell type similar to *Polyangiaceae*, *Phaselicystidaceae*, *Kofleriaceae*, and *Haliangiaceae* showing slender fat rods with blunted ends (Reichenbach 2005; Garcia et al. 2009b; Fudou et al. 2002). *Nannocystis* was an exception among the *Nannocystaceae* as it exhibits shorter rods, many of which are almost cuboidal in shape. Vegetative cells of the *Nannocystaceae* commonly burrow in agar or appear floating carried by current in liquid medium. In shaken culture, vegetative cells grow in the form of flakes, clumps, or aggregates. In general, *Nannocystaceae* vegetative cells (ave. 1.5–7.0 μm L) appear a bit shorter than *Polyangiaceae* (ave. 3–10 μm L), *Kofleriaceae* (ave. 4–6 μm L, Reichenbach 2005), and *Haliangiaceae* (ave. 3–8 μm L). However, cell size may vary to some extent depending on the growth conditions (Reichenbach 1999a), for example, they may appear longer if cultivated in Peptone-based medium.

Swarm

The swarm colony produced by the *Nannocystaceae* is typically depressed into the agar medium, with counterparts in *Polyangiaceae, Phaselicystidaceae Kofleriaceae*, and *Haliangiaceae*. In yeast-based medium, the colony appears pale and almost transparent. Colony color is usually evident in Peptone- and Casitone-based media from peach to pale orange shades. Swarm architecture varies also in the medium. Semisoft to soft medium favors pseudoplasmodial garland swarming with agar depressions, while hard agar content supports film- to veinlike pattern on surface of the medium. Swarm edge may show scattered trails (e.g., *Nannocystis*) or flare to flame ends (e.g., *Enhygromyxa*). In the presence of microbial bait (e.g., *Escherichia coli*), colony edge may appear as ridge or roll as a response to bait preying.

Fruiting Body

In general, myxobacteria swarm and aggregate to culminate into fruiting structure bearing myxospores. In *Nannocystaceae* fruit or fruit-like bodies of *Plesiocystis, Enhygromyxa, Pseudenhygromyxa* post controversy on the function of bearing desiccated and heat resistant myxospores similar to their terrestrial counterparts. Among the four genera in this family, true sporangiole enclosing myxospores is most clearly defined in *Nannocystis* (Reichenbach 1970), whereas from *Plesiocystis* naked fruiting body bearing spores are described (Iizuka et al. 2003a). These fruiting or fruiting-like bodies are stereomicroscopically visible by their color (e.g., reddish, pale orange, etc.) appearing either on surface or within the agar substrate. *Nannocystis* is one of the myxobacterial genera with extremely tiny sporangiole only recognized at higher microscopic magnification.

Myxospores

Myxospores are a unique cell stage and a terminology exclusively attached to myxobacteria. Like most other bacterial spores, they ensure survival of the next cell generation. In myxobacteria, germination starts from a population of cells in a fruiting body. To date, *Nannocystaceae* are distinguished in myxobacteria for their ovoid or very short rod to rounded myxospore shape developed from fat rod, blunted-end-type vegetative cells. The rounded myxospore characteristic is shared with *Haliangiaceae* and with counterparts to *Myxococcaceae* and some members of the *Cystobacteraceae* in *Cystobacterineae* suborder. Although desiccation resistance characterizes the myxobacterial myxospores (Reichenbach 1984), this function appears not to be clear in marine genera connected to the *Nannocystaceae* family. In *Nannocystis*, myxospore resistance appears as an important role in harsh environmental conditions such as during summer drought or winter freezing season. The various conditions existing in marine ecosystems may lead to myxospores performing different adaptive functions in response to environmental stimuli.

Pigments and Unusual Bacterial Features

In *Nannocystis* the major pigments appear to be aromatic-type carotenoids lacking glycosidic attachments (Shimkets et al. 2006). These differ from *Cystobacterineae* monocyclic carotenoid glycosides containing a keto group and from *Sorangiineae* carotenoid glycosides bearing a hydroxyl group instead of the keto group (Kleinig et al. 1971). Other than carotenoids, brown to black melanoid pigments have also been speculated to be produced by *Nannocystis* (Reichenbach 1970). For *Plesiocystis*, *Enhygromyxa*, and *Pseudenhygromyxa*, nothing is known about their pigments, but most likely they contain carotenoid-type pigments as visually judged because of their yellow-orange color.

Members of *Nannocystis* are remarkable for their unusual swarming colony patterns producing long tunnels and holes, described as bubble-/sponge-/cheese-like and convolutions on agar (*Fig. 16.10*). They can also appear as spherical mounds, brick-like, and long tracks of excavations on agar (*Fig. 16.11*) which has counterpart with *Polyangium* in *Sorangiineae* suborder (see Chap. 19).

Actively growing *Plesiocystis* culture is unusual for transparent and hump shaped structures on agar medium (*Fig. 16.12*). These are not fruiting bodies and appear to be just a mound of slime similar to *Polyangium* (Chap. 19).

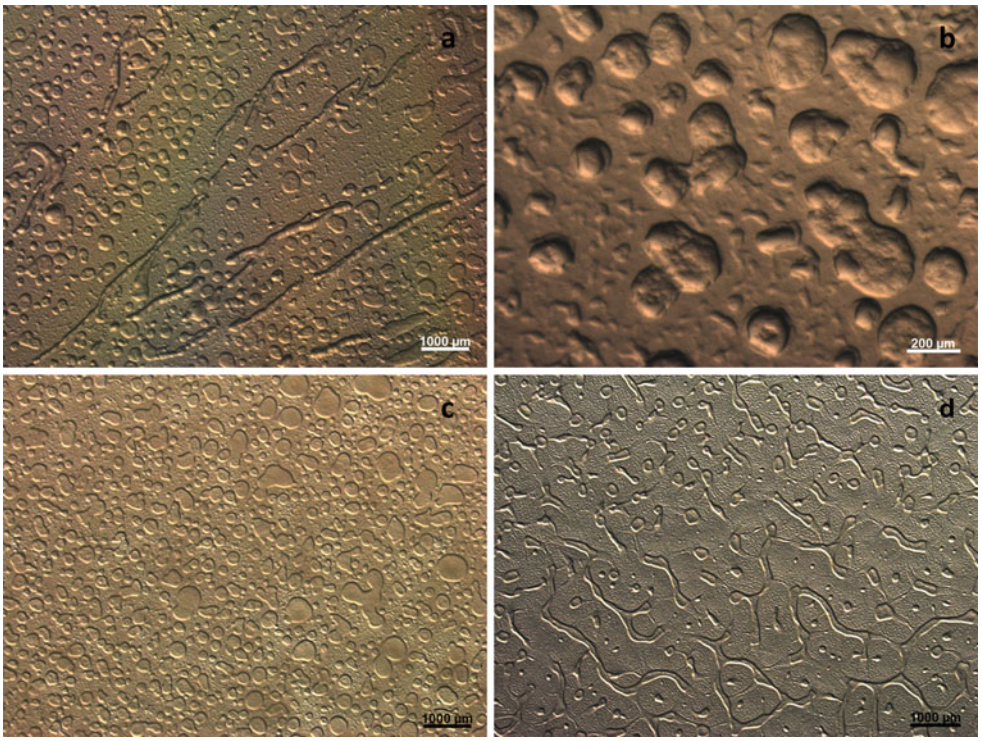

Fig. 16.10
Unusual swarming pattern of *Nannocystis exedens* DSM 71^T on VY/2 agar. Long tunnels of agar degradations (a). Deep agar holes (b). Bubble-/sponge-/cheese-like pattern (c). Convoluted shape appearance (d)

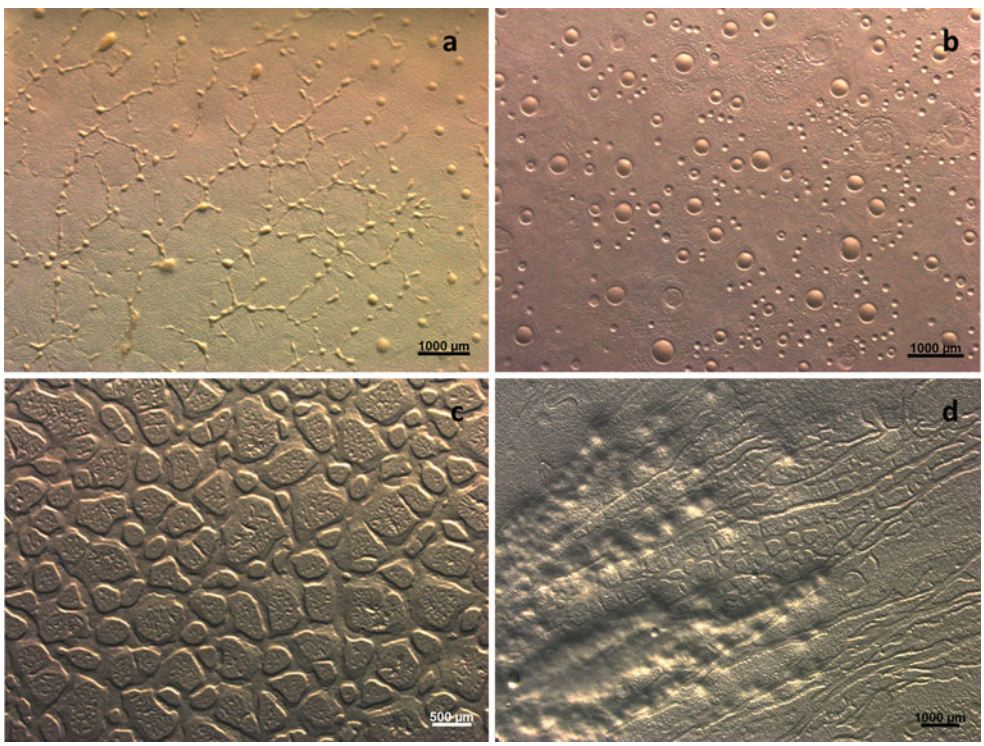

Fig. 16.11
Stereophotomicrographs of *Nannocystis exedens* DSM 71^T colony patterns on VY/2 agar. Netlike appearance (a). Mounds of spherical cell aggregates partially sunken in agar and crater-shaped holes scattered on agar (b). Brick-/puzzle-shaped pattern (c). Long tracks of agar excavations (d)

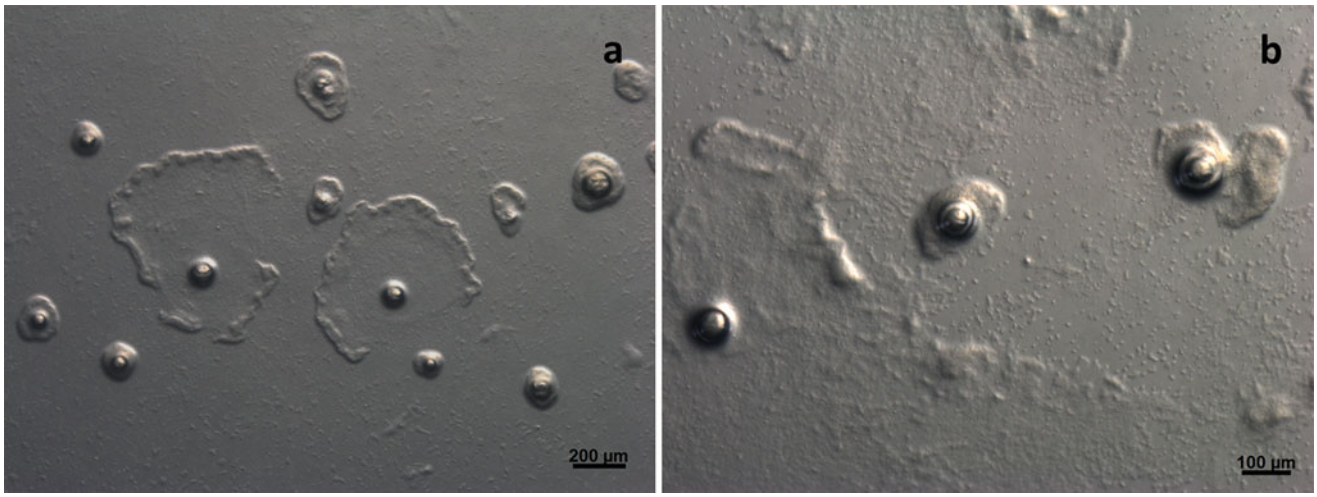

Fig. 16.12
***Plesiocystis pacifica* DSM 14875^T unusual structure on deionized yeast-based SWS agar. Solitary, transparent, shiny, globular-/knob-like structures surrounded by swarming vegetative cells (a–b). The structure appears to be acellular and seems to be composed of slime material**

Ecology

Nannocystaceae represents a family with members widely distributed in the environment. In this family, terrestrial members isolated from soils containing decaying plant material are all found in *Nannocystis*. These isolates are represented in various geographical locations and land habitats and were recorded to have 33.5 % global incidence in soil (Dawid 2000). *Nannocystis* are also ubiquitous in grasslands, dry pastures, sagebrush steppes, deserts, and different forest types (Reichenbach 1970). They are one of the very few myxobacterial genera successfully surviving in Saharan desert, high-altitude environments (Reichenbach 1999b), and cave deep sediments (Menne and Rückert 1988). A previous study has also shown that *Nannocystis*-type cells can be found in Antarctic soil (Dawid et al. 1988). *Nannocystis exedens* is one of the most common myxobacterial species found in warm temperate subtropical zones (Dawid 2000). The occurrence of *Nannocystis* does not seem limited to the terrestrial habitat: They were also isolated from a sandy beach intertidal region (Ohlendorf et al. 2008). *Nannocystis* rare finding in mud Chenghai Lake, China, was also documented (Li et al. 2012). In our collection, *Nannocystaceae* account for approximately 19 % of the overall isolates, equivalent to rank 3 position after *Myxococcaceae* and *Polyangiaceae* (Chap. 19).

Plesiocystis and *Enhygromyxa* appear distributed in marine sand samples. *Enhygromyxa* is additionally inhabiting the muddy shore samples and was the only marine myxobacteria reported isolated from brown (*Laminariales*) and green (*Ulvales*) seaweeds (Iizuka et al. 2003b). Occurrence of marine myxobacteria in sea grass *Zostera* sp. has also been described for *Plesiocystis* (Iizuka et al. 2003a). In the muddy estuarine environment where sodium chloride fluctuates up to 2 %, *Pseudenhygromyxa* was isolated as the first classified brackish-water myxobacterium (Iizuka et al. 2013).

Physiology and Metabolism

The four genera in this family are considered aerobic to microaerophilic. To date, no anaerobic *Nannocystaceae* member has been discovered to counterpart with *Anaeromyxobacter* in *Cystobacterineae* (Sanford et al. 2002; Thomas et al. 2009), although some uncultured strains exist in hot spring environments (Iizuka et al. 2006b), which may potentially be anaerobic. Their growth rate is slow and may be comparable with *Polyangiaceae*.

Nannocystaceae are considered neutrophilic and mesophilic to slightly thermophilic (e.g., *Pseudenhygromyxa*) with broader temperature tolerance ranging from 5 °C to 40 °C. In most strains, optimal growth is usually observed between 28 °C and 30 °C. The pH required for growth among members ranges from 5.5 to 9.0 and is considered within the range of slightly acidophilic to moderately alkalinophilic. However, the optimal pH is at the neutrophilic value. So far, no cultivated extremophiles regarding pH and temperature adaptation are reported in this family. Salt tolerance varies from sample source of the isolated strain. *Nannocystis* isolated from terrestrial samples does not require sodium chloride, and growth is inhibited with increasing sodium chloride concentration. However, some *Nannocystis* strains isolated from sandy beach of the intertidal region can grow even in the absence of sodium chloride and other sea salts (Ohlendorf et al. 2008), indicating their probable flushing out from the soil. In estuary where salt concentration fluctuates, myxobacterial inhabitants may be expected to tolerate lower salt concentration, such as in the case of *Pseudenhygromyxa*.

Table 16.3

***Nannocystaceae* secondary metabolites and their biological activities**

Genus	Compound	Structural class	Biological activity	Mode of action	No.
Enhygromyxa	Enhygrolide[a]	Aromatic lactone	Antibacterial	–	
	Salimabromide[b]	Tricyclic lactone	Antibacterial	–	
	Salimyxins[a]	Degraded sterol	Antibacterial	–	3
Paraliomyxa[c]	Miuraenamide[c]	Depsipeptide	Antifungal	Inhibits complex III respiration	1
Plesiocystis	–	–	–	–	0
Pseudenhygromyxa	–	–	–	–	0
Nannocystis	Geosmin[d]	Sesquiterpene			
	Germacran[e]	Sesquiterpene			
	Nannochelin[f]	Hydroxamate	Antibacterial, antifungal, siderophore		
	Phenylnannolone[g]	Polyene	Weak antiviral (influenza A)	Reverses daunorubicin resistance	4
Total					8

References: [a]Felder et al. 2013a, [b]Felder et al. 2013b, [c]Not a validly described genus (Iizuka et al. 2006b; Ojika et al. 2008), [d]Trowitzsch et al. 1981. [e]Reichenbach and Höfle 1999, [f]Kunze et al. 1992, [g]Ohlendorf et al. 2008

Plesiocystis and *Enhygromyxa* represent the only *Nannocystaceae* genera with sodium chloride tolerance equivalent to the seawater concentration (Iizuka et al. 2003a, b). These genera appear dependent on sodium chloride as absence inhibits their growth; hence, they are regarded as obligate halophilic. Bacterial lysis and degradation of biomacromolecules including gelatin, agar, agarose, and casein but not cellulose are also known in *Nannocystaceae* (*Table 16.1*).

Symbiosis, Pathogenicity, and Clinical Relevance

Unlike some species in *Polyangiaceae* (Jacobi et al. 1996, 1997), no prokaryotic symbiosis is described for *Nannocystaceae*. To date, isolated members of this family have not been implicated to cause any disease, neither to plants nor to animals. Just like all other validly described myxobacteria, members of the *Nannocystaceae* are also classified as WHO Risk Group I organisms having low- or no risk implication to individuals or the community.

Application

Nannocystaceae family according to our current knowledge is not equally diverse in natural products compared with some other families of myxobacteria belonging to the *Polyangiaceae* and *Myxococcaceae*. From over 100 basic core structures described in myxobacteria (Weissman and Müller 2009, 2010), so far only few compounds have been elucidated from *Nannocystaceae*, and these were mostly derived from the genus *Enhygromyxa* and *Nannocystis* (*Table 16.3*). The number of compounds elucidated in *Nannocystis* is considered very small in comparison to *Sorangium* and shows inverse proportionality to the number of strains.

Although marine genera belonging to this family face challenges making isolates amenable to natural product screening, compounds from these taxa are worth analyzing simply because they turn out to be novel and biologically active. *Enhygromyxa* are getting attention recently after three novel antibiotics were found (Felder et al. 2013a, b).

Just like some members of the *Polyangiaceae*, *Nannocystaceae* have also been found to produce polyunsaturated fatty acids (PUFAs), particularly omega-3 eicosapentaenoic acid (EPA) and omega-6 arachidonic acid (AA) (Garcia et al. 2011; Stadler et al. 2010; Iizuka et al. 2013). These important fatty acids are used as supplements in many pharmaceutical products and are also been implicated to cure several diseases in humans. Interestingly, *Nannocystaceae* exemplified by *Nannocystis exedens* (e.g., DSM 71^T) was discovered to produce squalene and unusual steroids (cholest-8(9)-en-3β-ol) (Kohl et al. 1983).

Concluding Remarks and Perspectives

Nannocystaceae appear to be an interesting and promising family for novel strain discovery. The expectation that this family will continue to grow is high as more environmental types are explored. Thus, they can also be seen as emerging sources for new biologically active and unique natural products. The discovery of marine *Nannocystaceae* and related obligate halophilic myxobacteria in the past decades came as a surprise and opens up opportunities to explore the marine environment and other ecology systems more. As metagenomics of marine and terrestrial ecosystems indicate that *Nannocystaceae* represent a significant proportion of the myxobacteria present in various types of samples, great number of this family have yet to be brought to culture (Jiang et al. 2007, 2010). The latter remains a serious challenge for these so-called "uncultured" members, but at the same time such efforts raise hope for the identification of a novel source of secondary metabolites and other interesting applications.

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17 The Family *Nitrospinaceae*

Sebastian Lücker · Holger Daims
Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, University of Vienna, Vienna, Austria

Abstract
The only genus within the family *Nitrospinaceae* Garrity et al. 2006 is *Nitrospina*, encompassing two isolated type strain species, *N. gracilis* Watson and Waterbury 1971 and *N. watsonii* Spieck et al. 2014. Historically, the family has been thought to belong to the *Deltaproteobacteria*, but recently was tentatively reclassified into the candidate phylum Nitrospinae phyl. nov., with emended classification as *Nitrospinia* class. nov., *Nitrospinales* ord. nov., and with *N. gracilis* Watson and Waterbury 1971 as the type species. *Nitrospina* species are obligate marine aerobic chemolithoautotrophic nitrite-oxidizing bacteria that grow with nitrite and carbon dioxide as sole energy and carbon source, respectively. Genomic analyses of *N. gracilis* strain 3/211 have shown that *Nitrospina* use the reverse tricarboxylic acid cycle for carbon fixation and lack classical defense mechanisms against oxidative stress, thus indicating a microaerophilic evolutionary origin and an adaptation to elevated partial oxygen pressures by yet unidentified mechanisms. This observation is in accordance with their environmental distribution, as *Nitrospina*-like organisms are frequently detected by molecular methods in oxic waters but still also in suboxic environments like marine oxygen minimum zones and apparently anoxic sediments. The isolated, closely related *Nitrospina* species represent only a small subgroup of the genus, as phylogenetic analyses of environmental 16S rRNA sequences reveal a huge undiscovered diversity forming at least four additional sequence clusters that contain no cultured representatives.

Taxonomy, Historical and Current

Ni.tro.spi.na'ce.ae. N.L. fem. n. *Nitrospina*, type genus of the family; suff. *–aceae*, ending to denote a family; N.L. fem. pl. n. *Nitrospinaceae*, the *Nitrospina* family

The family contains one genus, *Nitrospina*. All isolated members of this genus meet their energy and carbon demands by chemolithotrophic nitrite oxidation and autotrophic carbon fixation, respectively. Thus, all members of the genus *Nitrospina* belong to the functional guild of nitrite-oxidizing bacteria (NOB), characterized by the ability to perform the second step of nitrification, oxidation of nitrite to nitrate. The preceding reaction within this important part of the biogeochemical nitrogen cycle, the oxidation of ammonia to nitrite, is catalyzed by ammonia-oxidizing bacteria (AOB) and, as discovered more recently, archaea (Treusch et al. 2005; Hallam et al. 2006). Historically, all nitrifying bacteria were viewed as one coherent group, the *Nitrobacteriaceae* Buchanan 1917 (Watson 1971), and cell shape and morphological structures like the highly characteristic intracytoplasmic membrane (ICM) systems were used to differentiate genera. Based on this classification system, *Nitrospina* was already recognized as independent genus, as its type strain *N. gracilis* formed long slender rods and lacked an extensive ICM system, unlike the other NOB known at that time (Watson and Mandel 1971). Comparative 16S rRNA analyses refined this classification and clearly separated AOB and NOB in different phylogenetic groups (Teske et al. 1994). Among the NOB, for the genera *Nitrobacter* and *Nitrococcus*, these analyses indicated a clear affiliation to the *Alpha-* and *Gammaproteobacteria*, respectively, whereas for the genera *Nitrospina* and *Nitrospira*, a peripheral relationship to the *Deltaproteobacteria* was proposed. While the genus *Nitrospira* was moved into a distinct novel phylum (the Nitrospirae) shortly after (Ehrich et al. 1995), the phylogenetic position of *Nitrospina* remained controversial but unrefined (Lipski et al. 2001; Schramm 2003). The family *Nitrospinaceae* was circumscribed based on phylogenetic analysis of 16S rRNA genes (Garrity et al. 2005), but was still considered to be affiliated with the *Deltaproteobacteria*.

Only recently, the phylogenetic placement of *Nitrospina* could be resolved by phylogenetic analyses based on a concatenated dataset of established phylogenetic marker proteins, which were extracted from the draft genome sequence of *N. gracilis* strain 3/211 (Lücker et al. 2013). These analyses clearly separated the genus *Nitrospina* from the *Proteobacteria* and supported its reclassification in a novel phylum within the *Bacteria*.

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For this phylum the tentative name Nitrospinae phyl. nov. was proposed, with reclassification of the family *Nitrospinaceae* Garrity et al. 2006 in the *Nitrospinia* class. nov., *Nitrospinales* ord. nov., and with *N. gracilis* Watson and Waterbury 1971 as the type species (Lücker et al. 2013). The validity of employing concatenated protein datasets for resolving higher-order phylogenetic relationships has been demonstrated in multiple studies (e.g., Strous et al. 2006; Rinke et al. 2013), and the application of this approach allowed the phylogenetic affiliation of *Nitrospina* to be assessed with an unprecedented high degree of confidence. The validity of the proposed phylum gained additional support from an independent study, which used a 16S rRNA dataset containing the described *Nitrospina*, a novel isolate, and a large number of related environmentally derived sequences for phylogenetic analyses (Spieck et al. 2014). Furthermore, the overall 16S rRNA sequence identities of this novel *Nitrospina* isolate to any bacterial type strain outside the *Nitrospinaceae* range from 70.0 % to 84.8 % (Spieck et al. 2014). These low values are in accordance with higher taxon boundaries found by comprehensive analyses within the All-Species Living Tree Project (Yarza et al. 2008), thus supporting the phylum status of the Nitrospinae.

Members of the genus *Nitrospina* are marine, aerobic, obligate chemolithoautotrophic bacteria which oxidize nitrite to nitrate and fix carbon dioxide to fulfil their energy and carbon needs, respectively. Glycogen is formed as storage compound. Cells are Gram negative, form straight slender rods with a width of 0.3–0.4 µm and a length of 1–6.5 µm, occur as single cells, and divide by equal and unequal binary fission. Motility is not observed. Major fatty acids are 14:0 and 16:1 *cis*9. Growth medium is seawater enriched with nitrite and inorganic salts, and organotrophy is not observed. Optimal growth occurs in 70–100 % seawater at 25–30 °C and pH 7.5–8.0 (modified from Watson and Waterbury 1971; Spieck et al. 2014).

Molecular Analyses

DNA and 16S rRNA Characterization

The genus *Nitrospina* contains two type strains, *N. gracilis* Watson and Waterbury 1971 (strain Nb-211; L35504) and *N. watsonii* Spieck et al. 2014 (strain 347; JX645704). The two species have DNA G + C values of 57.7 and 55.6 mol%, respectively (Watson and Waterbury 1971; Spieck et al. 2014). *N. gracilis* Nb-211 was isolated from Atlantic Ocean surface waters by Watson and Waterbury (1971), but the culture appears not to be available any more. However, the closely related *Nitrospina* strain 3/211 (FR865038; 99.0 % 16S rRNA similarity to strain Nb-211) can be obtained from the culture collection of the University of Hamburg. This strain most likely is derived from strain Nb-3 (L35503; 99.6 % 16S rRNA similarity), which was isolated from Pacific Ocean surface waters in parallel to strain Nb-211, but has never been published (Lipski et al. 2001). The DNA G + C value of *N. gracilis* strain 3/211 is 57.3 mol% (Spieck et al. 2014). A DNA/DNA hybridization study was performed for *N. watsonii* and *N. gracilis* 3/211 and revealed a similarity of 30 % (Spieck et al. 2014). This low value and the 16S rRNA identity of 97.9 %, which is below the proposed minimal 16S rRNA identity for members of the same bacterial species (Stackebrandt and Ebers 2006), justify the classification of the two strains as two different species (Spieck et al. 2014).

Genomic Properties

To date only one genome of a member of the *Nitrospinaceae* has been analyzed. The draft genome of *Nitrospina gracilis* 3/211 (GOLD ID Gi39306; Lücker et al. 2013) consists of 109 contigs, which are arranged on four scaffolds. The largest scaffold encompasses 106 contigs, whereas the other three consist of single contigs whose location relative to the other contigs could not be determined unambiguously. The obtained sequence has a size of 3,067,213 bp and contains 3,147 predicted coding sequences (CDS), 1 *rrn* operon, and 45 tRNA genes. The average G + C content is 56.2 mol%, which is slightly lower but in good agreement with the HPLC-derived value of 57.3 mol% (Spieck et al. 2014). In total 2,301 CDS were automatically assigned to clusters of orthologous groups (COG) functional categories, with the largest number (637 CDS) belonging to the poorly characterized classes R and S, followed by amino acid transport and metabolism (class E, 216 CDS), cell wall/membrane/envelope biogenesis (class M, 214 CDS), and energy production and conversion (class C, 190 CDS). Although motility has not been observed for any *Nitrospina* isolate, the genome of *N. gracilis* 3/211 encodes a complete flagellar apparatus.

In accordance with the experimentally observed autotrophic growth, but surprisingly for an aerobic organism, the *N. gracilis* 3/211 genome encodes the reverse tricarboxylic acid (rTCA) cycle for inorganic carbon fixation. As some enzymes of the rTCA cycle interact with ferredoxin and are highly oxygen sensitive, this pathway is usually found in anaerobic and microaerophilic organisms (Berg 2011). In *Nitrospina*, however, these enzymes are of types that have been shown to tolerate oxic conditions (Yoon et al. 1996, 1997). Ferredoxin is required as low-potential electron donor for pyruvate and 2-oxoglutarate biosynthesis, but intriguingly none of the canonical ferredoxin-reducing mechanisms could be identified in the genome. Instead, *Nitrospina* encodes an alternative NADH-quinone oxidoreductase, which might make use of the proton motive force (*pmf*) for the reduction of ferredoxin with electrons from the quinone pool (Lücker et al. 2013).

Chemolithotrophic energy conservation from nitrite oxidation is catalyzed by the nitrite oxidoreductase (NXR), a molybdopterin-binding enzyme of the complex iron–sulfur molybdoenzyme (CISM) family (Rothery et al. 2008). The *N. gracilis* 3/211 genome contains two highly similar copies of NXR, and the complex has been reported to be anchored to the cytoplasmic membrane with the catalytic subunit facing the periplasm (Bartosch et al. 1999). Accordingly, the presence of signal peptides for transport across the cytoplasmic membrane was confirmed by genome analysis (Lücker et al. 2013).

Interestingly, the NXR gamma subunit lacks any predicted transmembrane helices, implying that an additional subunit may be responsible for anchoring NXR to the membrane. The genome encodes several proteins with low similarities to the canonical NXR gamma subunit that are predicted to contain transmembrane helices, thus making them probable candidates for a fourth NXR subunit. In accordance with this hypothesis, isolation of the membrane proteins of *N. gracilis* by ultracentrifugation revealed the presence of four major proteins. These proteins with apparent molecular weights of 116, 48, and 32 kDa probably are the NXR alpha, beta, and gamma subunits, respectively, while the fourth protein of 66 kDa most likely corresponds to an additional subunit as mentioned above (Spieck et al. 2014). The electrons derived from nitrite oxidation are shuttled to a terminal cytochrome *c* oxidase of the cbb_3 type. Here, *pmf* across the membrane is built up, which drives ATP formation by a canonical F_1F_0 ATP synthase and also the reverse electron transport (through respiratory complexes I to III) required for anabolic processes (Lücker et al. 2013).

Phenotypic Analyses

Genus *Nitrospina* Watson and Waterbury 1971

Ni.tro.spi'na. N.L. n. *nitrum*, nitrate; N.L. pref. *nitro-*, pertaining to nitrate; L. fem. n. *spina*, spine; N.L. fem. n. *Nitrospina*, nitrate spine

Nitrospina cells appear as long, slender rods with a diameter of 0.3–0.4 µm and stain Gram negative. In young cultures, the cell length ranges between 1 and 3 µm and increases to max. 6.5 µm when the cultures are stirred. Spherical forms with a size of 0.7 × 1 µm can be found in senescent batches. In these spheres the outer membrane becomes partly detached from a vibrio-shaped cytoplasm, leading to a coccoid-like appearance (Watson and Waterbury 1971; Spieck et al. 2014). Cells divide by binary fission and sometimes unequal cell division is observed, resulting in short rods or even coccoid cells. Motility or formation of microcolonies has not been observed. In old cultures, however, cells tend to adhere to the culture vessel walls. Carboxysomes and intracytoplasmic membrane systems as found in many other nitrifying bacteria are absent, but bleb-like invaginations of the cytoplasmic membrane occur occasionally. When stained for glycogen, *Nitrospina* cells reveal dark particulate inclusions 20–40 nm in diameter, which are assumed to represent glycogen deposits. Fatty acid profiles of all *Nitrospina* strains investigated are highly similar, with the major lipids being tetradecanoic (14:0) and *cis*-9-hexadecenoic acid (16:1 *cis*9). In addition, significant amounts of *cis*-9-tetradecenoic (14:1 *cis*9), hexadecanoic (16:0), 3-hydroxy-hexadecanoic (16:0 3OH), and the cyclopropane fatty acid 17:0 *cyclo* are present (Lipski et al. 2001). The only cytochromes detectable are of the *c*-type, and *a*-type cytochromes are missing, as indicated by the characteristic absorption peaks at 430, 524, and 555 nm (corresponding to the alpha, beta, and gamma peaks of cytochrome *c*) measured in cell suspensions (Spieck et al. 2014).

All isolated *Nitrospina* strains are aerobic obligate chemolithoautotrophic nitrite oxidizers. Their sole energy and carbon sources are nitrite and carbon dioxide, respectively, and oxygen serves as the terminal electron acceptor. Organic growth factors are not required. Doubling times in autotrophic mineral salt media amended with low concentrations of nitrite are between 24 and 28 h. Interestingly, growth and nitrite oxidation rates of *N. watsonii* are slightly stimulated in mixotrophic media containing low concentrations of yeast extract, peptone, and pyruvate (Spieck et al. 2014). In contrast, *N. gracilis* does not benefit from or is even inhibited by the addition of organic compounds (Watson and Waterbury 1971). Organoheterotrophic growth was not observed for any *Nitrospina* strain.

Isolation, Enrichment, and Maintenance Procedures

Cultivation

Nitrospina are grown in liquid mineral salts medium (Watson and Waterbury 1971) amended with low concentrations of nitrite at 25–30 °C, pH 7.5–8.0. No growth is observed on solid media, below 14 °C or above 40 °C and below pH 6.0 or above pH 8.5. Optimal growth occurs in medium containing 70–100 % seawater, which cannot be substituted with distilled water amended with NaCl. Optimal substrate concentrations are in the range of 0.5–3 mM nitrite. Concentrations higher than 20 mM inhibit growth, but *N. watsonii* tolerates up to 30 mM (Spieck et al. 2014). The marine mineral salts medium is most conveniently prepared by diluting stock solutions. Nitrite is added last at the final concentration required by the respective strain. Growth rates and biomass yields of some *Nitrospina* strains might be enhanced by the addition of simple organic carbon and nitrogen sources, resulting in a "mixotrophic" medium (Spieck et al. 2014). However, while some *Nitrospina* isolates might be stimulated, other strains will be inhibited by the presence of organics (Watson and Waterbury 1971), and the utilization of any substrates other than nitrite and CO_2/bicarbonate should be tested with care. Furthermore, for initial enrichment cultures, the addition of organics is not recommended, as it would stimulate the faster growth of heterotrophic bacteria. To test for the presence of contaminating heterotrophic organisms, the use of a marine heterotrophic medium without nitrite is recommended.

Nitrospina grow well in Erlenmeyer flasks of different volumes. The large liquid surface area in these culturing vessels ensures sufficient aeration. Cultivation should be carried out in the dark and without agitation, as light and high partial oxygen pressures can be inhibitory to NOB (Spieck and Bock 2005a). Large culture batches (5–10 l) should be stirred after nitrite has been completely consumed for the first time, and the stirring speed can be increased with increasing cell density. Incubation temperatures of new isolates might differ depending on the in situ conditions of the isolation source, but the strains isolated so far grow well at 28 °C. Cell growth will result in slight culture turbidity. Due to the low

substrate concentrations, nitrite must be replaced every 7–30 days depending on the culture density and nitrite-oxidizing activity. Nitrite consumption (and nitrate production) can be monitored semiquantitatively by using test strips or by the modified Griess–Ilosvay spot test (Schmidt and Belser 1994). For the latter assay, a small culture aliquot is transferred and mixed with a small drop of reagent, which can be provided on filter paper or in small-volume reaction tubes. If no pink color appears, nitrite must be replenished aseptically. Excessive nitrate accumulation should be avoided as product inhibition has been reported for some nitrite-oxidizing bacteria. Therefore, the transfer of 1 to 10 % (v/v) inoculum to fresh medium is recommended every 3–6 months. Seed cultures should be kept at 17 °C as a backup in case of contaminations of the main cultures or other problems. For harvesting cells, the cultures are centrifuged at 15,000 x g for 30 min at 4 °C. A brownish color of the pellet indicates that the cells are rich in iron-containing proteins (mainly NXR and cytochromes) and thus have a high nitrite-oxidizing activity (Spieck and Lipski 2011).

Marine mineral salts media composition (Spieck and Lipski 2011)*:*

- *Tenfold concentrated stock solution:* 0.05 g $CaCl_2 \cdot 2\ H_2O$, 1 g $MgSO_4 \cdot 7\ H_2O$, 0.01 g $FeSO_4 \cdot 7\ H_2O$, 0.017 g KH_2PO_4, 700 ml filter-purified seawater, 300 ml distilled water.
- *Marine trace elements:* 1,000 ml distilled water, 6 mg $CuSO_4 \cdot 5\ H_2O$, 25 mg $Na_2MoO_4 \cdot 2\ H_2O$, 50 mg $MnCl_2 \cdot 4\ H_2O$, 0.5 mg $CoCl_2 \cdot 6\ H_2O$, 25 mg $ZnSO_4 \cdot 7\ H_2O$.
  - *Final medium:* 630 ml seawater, 270 ml distilled water, 100 ml stock solution, 1 ml marine trace elements, 0.069 gl^{-1} $NaNO_2$ (= 1 mM; 0.5–3 mM nitrite is optimal for the cultivation of *Nitrospina*). The pH is initially adjusted to 6.5–7.0 and changes to pH 7.4–7.6 within 2 days after autoclaving. If no natural seawater is available, artificial seawater can be tested, but growth might fail.
  - *Heterotrophic medium:* 1,000 ml marine mineral salt medium (without addition of nitrite) amended with 0.15 g yeast extract, 0.15 g peptone, 0.055 g Na-pyruvate, pH 6.5. The pH changes to 7.4–7.6 after autoclaving.

Reagent for the Griess–Ilosvay spot test (Schmidt and Belser 1994):

- *Solution 1 (diazotization reagent):* 50 ml distilled water, 4 g sulfanilamide, 10 ml orthophosphoric acid.
- *Solution 2 (coupling reagent):* 40 ml distilled water, 0.2 g *N*-(1-Naphthyl)-ethylenediamine dihydrochloride.

  Combine both solutions and store in the dark at 4 °C.

Enrichment and Isolation

Enrichment cultures of *Nitrospina* can be started in 300 ml Erlenmeyer flasks by using 1 ml seawater (or 1 g of saline sediments) as inoculum in 150 ml marine mineral salt medium. Samples with a low cell density can also be inoculated into smaller volumes of medium, such as 50 ml medium in 100 ml flasks (Spieck and Lipski 2011). Alternatively, seawater samples can directly be amended with 1 mM $NaNO_2$ (or $(NH_4)_2SO_4$) and 50 µM K_2HPO_4 for the pre-enrichment of NOB (or NOB and also ammonia oxidizers) (Watson and Waterbury 1971; Watson et al. 1986). Since the enrichment efficiency is low, incubation of multiple samples in parallel is recommended. As mentioned above, the cultures should be kept in the dark at ambient temperatures. Nitrite must be measured and replenished regularly, and nitrite consumption accompanied by stoichiometric nitrate formation indicates growth of NOB. Once active nitrite oxidation occurs and more than half of the nitrite has been used, purification of NOB can be achieved by serial dilution (10^{-1} to 10^{-9}) in mineral salt medium. The diluted cultures must be incubated for one to several months and periodically tested for nitrite consumption. Aliquots from the highest dilution, which is tested positive for nitrite oxidation, should be used for subcultivation in fresh medium. Microscopic techniques can be used to observe the purity status of the culture. In parallel, the culture should be tested regularly for growth of heterotrophic contaminants in marine heterotrophic medium, or also in other standard complex media (like half-strength Luria-Bertani) prepared with natural seawater. The absence of turbidity after several days of incubation indicates the absence of heterotrophs from the culture.

An alternative method for the purification of *Nitrospina* and for the separation of different NOB co-enriched in the same culture is the use of Percoll density gradient centrifugation. Here, cells from up to 5 l of an enrichment culture are harvested by centrifugation (15,000 × g, 30 min, 4 °C) and resuspended in 1 ml 2 % NaCl. The cell suspension is mixed with 28 ml Percoll and 12 ml 1.5 M NaCl solutions, transferred into 40 ml centrifugation tubes, and centrifuged in a fixed-angle rotor (2 h, 12,000 × g, 4 °C). A distinct brownish band should form in the middle part of the gradient. This band is sampled with a sterile Pasteur pipette and resuspended in a small volume of 2 % NaCl. Light microscopy should reveal the presence of rod-shaped cells. Subsequently, the cell suspension is serially diluted (up to 10^{-12}) in marine mineral salt medium supplemented with 0.5–3 mM nitrite and incubated for one to several months. The highest dilution, which is tested positive for nitrite oxidation, is used as inoculum for further incubations and tested for heterotrophic contaminants as described above. These steps must be repeated until a pure culture is obtained, which can take up to several years (Spieck and Lipski 2011; Spieck et al. 2014).

Maintenance

Storage of liquid cultures is recommended at 17 °C in the dark. While many NOB can survive starvation for several years, it is recommended to transfer *Nitrospina* cultures to fresh media every 3–6 months. To revive starved cultures, large inoculum volumes (10 % v/v) and low initial nitrite concentrations

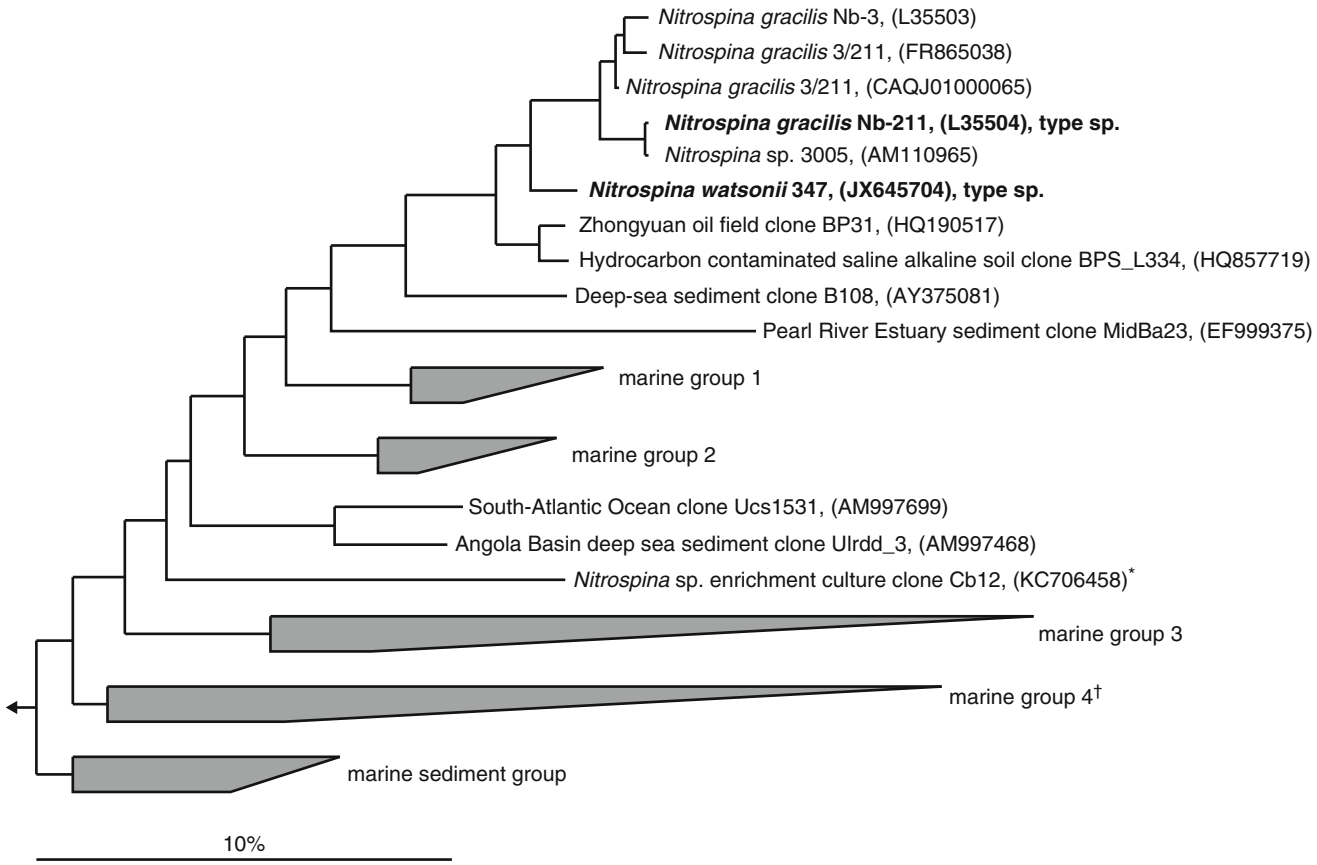

Fig. 17.1

Phylogenetic reconstruction of the family *Nitrospinaceae*. The 16S rRNA-based Bayesian interference tree was calculated using MrBayes 3.2.2 (run for 5,000,000 generations, s.d. = 0.014629). The overall tree topology was confirmed by other treeing methods (maximum likelihood, maximum parsimony). A 50 % conservation filter based on all high-quality sequences within the family was used to remove hypervariable regions, leaving 1,453 alignment positions for the tree calculations. The tree contains a representative subset of all *Nitrospina*-like sequences included in the SILVA nonredundant dataset (release 115; www.arb-silva.de) and additional sequences retrieved from NCBI. Only sequences >1,250 nucleotides and with a pintail chimera check quality score >90 were included. Type strain species are indicated in *boldface*. The *asterisk* marks a sequence derived from a nitrifying enrichment culture, and the *dagger* indicates a sequence group containing single-cell *Nitrospina* genomes (refer to the main text for details). The scale bar corresponds to 10 % estimated sequence divergence

(0.15 mM) should be used (Spieck and Lipski 2011). Long-time storage is also possible under liquid nitrogen, either without cryoprotectant (Watson and Waterbury 1971) or after resuspension in a cryoprotecting buffer that contains sucrose and histidine (Spieck and Bock 2005b). A recent study proposed the use of mineral salts medium supplemented with 10 % DMSO, optionally with tenfold diluted trypticase soy broth, as preservation medium for the storage of all NOB (Vekeman et al. 2013). For marine NOB, the use of marine growth medium is required, although this results in lower revival rates due to increased salt stress during freezing. For *Nitrospina* the survival rates were only 34 %, and higher survival rates were achieved by storage in marine mineral salt medium with 1 % sucrose and 1 % DMSO, but these results were inconsistent as not all cultures could be revived after storage in liquid nitrogen.

Ecology

Nitrospina appear to be confined to marine habitats. The isolates described to date were derived from Atlantic (strain Nb-211; L35504) or Pacific Ocean (strain Nb-3; L35503) surface waters (Watson and Waterbury 1971) and from the suboxic zone in the Black Sea (strain 347; JX645704) (Spieck et al. 2014). While strain Nb-211 and Nb-3 both belong to *N. gracilis*, strain 347 was classified as novel species, *N. watsonii*. In phylogenetic analyses these strains form a small monophyletic cluster that encompasses only one further sequence (*Nitrospina* sp. 3005; AM110965) closely related to *N. gracilis* Nb-211, which apparently was derived from deep-sea sediment by a cultivation-based approach (*Fig. 17.1*). Interestingly, even studies based on high-throughput sequencing approaches did not reveal any environmental 16S rRNA sequences from this cluster, indicating that the

cultivable *Nitrospina* species might be of minor importance in the environment. Accordingly, the current release of the SILVA 16S rRNA sequence database (SSU r117; www.arb-silva.de) classifies no less than 1,504 16S rRNA sequences from uncultured organisms as belonging to the genus *Nitrospina*, but only three of them have identities >95.5 % to any of the type strains, with the remaining ones ranging from 89.4 % to 94.8 %. Due to these large 16S rRNA sequence dissimilarities to the characterized isolates, one cannot infer a function of these uncultured *Nitrospina*-like bacteria. There are, however, two indications that these sequences indeed belong to nitrite-oxidizing organisms. First, a nitrifying enrichment inoculated with Dutch coastal North Sea water initially contained a novel *Nitrospina*-like bacterium (KC706458) (Haaijer et al. 2013). In phylogenetic analyses, the *Nitrospina*-like enrichment clone is only distantly affiliated with the *Nitrospina* type strains and falls between the marine groups 2 and 3 (*Fig. 17.1*). However, since this enrichment was dominated by *Nitrospira*-like NOB and *Nitrosomonas*-like AOB, a nitrite-oxidizing lifestyle of the *Nitrospina*-like bacterium is highly probable but was not demonstrated. Second, marine group 4 (*Fig. 17.1*) contains two *Nitrospina*-like 16S rRNA sequences derived from single-cell genomes (*Nitrospina* sp. SCGC AAA288-L16 and AB-629-B18). One of these draft genomic sequences (AB-629-B18, AQVV00000000) contains NXR alpha (WP_018047900) and beta (WP_018047899) subunits with very high similarities to the proteins in the *N. gracilis* 3/211 genome (90 % and 97 % amino acid identity, respectively). These two findings strongly indicate that many or even all *Nitrospina*-like sequences in the marine groups 1– 4, as well as sequences falling between these groups, belong to organisms capable of nitrite oxidation. Consequently, bacteria from these phylogenetic clusters are tentatively considered as members of the *Nitrospinaceae*, whereas no functional inference can be made yet for more distantly related organisms as exemplified by the marine sediment group in *Fig. 17.1*.

Molecular data indicate that *Nitrospina* usually are the most abundant NOB in marine systems. Consistent with the properties of cultured *Nitrospina* (Watson and Waterbury 1971; Spieck et al. 2014), *Nitrospina*-like sequences are frequently obtained from oxygenated ocean surface and deep-sea waters (Fuchs et al. 2005; DeLong et al. 2006; Mincer et al. 2007; Fuchsman et al. 2011; Crespo et al. 2013; Kim et al. 2013; Georges et al. 2014) but also from marine sediments (Davis et al. 2009; Jorgensen et al. 2012) and hydrothermal mat systems (Davis and Moyer 2008). Furthermore, the distribution profiles of *Nitrospina* and ammonia-oxidizing archaea (AOA) were found to correlate in some coastal and open ocean habitats (Mincer et al. 2007; Beman et al. 2010; Santoro et al. 2010), and prolonged coexistence with AOA closely related to "*Candidatus* Nitrosopumilus maritimus" was observed in an enrichment culture (Park et al. 2010). These results indicate a functional coupling of AOA and *Nitrospina*-like NOB in marine ecosystems. Most intriguingly, high abundances of *Nitrospina*-like bacteria have also been detected in marine oxygen minimum zones (Fuchs et al. 2005; Labrenz et al. 2007; Zaikova et al. 2010; Fuchsman et al. 2011; Beman et al. 2013; Ganesh et al. 2014), and active nitrite oxidation was measured in these suboxic environments (Casciotti and Buchwald 2012; Füssel et al. 2012). As nitrite oxidation is an aerobic process, this finding was highly unexpected. However, since the additional oxygen in nitrate stems from water, O_2 is not directly required as co-substrate for nitrite oxidation. As terminal electron acceptor for energy conservation, O_2 may theoretically be replaced by other thermodynamically favorable acceptors such as iodate, Fe(III), and Mn(IV) (Casciotti and Buchwald 2012). Still, the use of alternative electron acceptors to sustain nitrite oxidation remains to be experimentally proven. The observation that nitrite accumulates in the truly anoxic core of oxygen minimum zones (Thamdrup et al. 2012) rather indicates that *Nitrospina* have a high affinity to oxygen and thus remain active under suboxic conditions, whereas their activity ceases in the complete absence of O_2.

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18 The Family *Phaselicystidaceae*

Ronald Garcia · Rolf Müller
Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland – Helmholtz, Centre for Infection Research and Pharmaceutical Biotechnology, Saarland University, Saarbrücken, Germany

Abstract
Phaselicystidaceae belong to the suborder *Sorangiineae* in the order *Myxococcales* and are comprised of the monotypic genus *Phaselicystis* and the monotypic species *P. flava*. So far, all strains in this family have been isolated from soil samples and decomposing plant materials. The family is morphologically and chemo-physiologically distinct within *Sorangiineae*, with *Polyangiaceae* and *Sandaracinaceae* being closely related families based on 16S rRNA gene phylogenetic analysis. *Phaselicystidaceae* have a high GC content, typical for myxobacteria. Members of this family are bacteriolytic and non-cellulolytic. The family's interesting and compelling application includes the production of the polyunsaturated fatty acid omega-6 arachidonic acid.

Taxonomy, Historical, and Current

Short Description of the Family

Phaselicystidaceae (Pha.se.li.cys.ti.dá ce.ae. N. L. fem. n. *Phaselicystis* type genus of the family; L. suff. -aceae ending to denote a family; N. L. pl. fem. n. *Phaselicystidaceae* the *Phaselicystis* family, Garcia et al. 2009b).

Based on phylogeny, *Phaselicystidaceae* formed a distinct clade in *Myxococcales*, class *Deltaproteobacteria*, phylum Proteobacteria (Garcia et al. 2010). The family is composed only of the type genus *Phaselicystis* (Garcia et al. 2009b) and is unique for its phenotypic characteristics. Vegetative cells appear to be non-flexuous, long slender rods with round blunted ends, phase-dark, and stained Gram-negative. Cells move by gliding on substrate, producing radial and net-like veins with characteristic agar colony depression. Fruiting bodies are composed of sessile sporangioles, appearing solitary either in chains or in clusters. Myxospores are partially refractile slender rods with blunted ends. Swarm colony exhibits thin and long fine radial veins and does not absorb Congo red stain. *Phaselicystidaceae* exhibits aerobic, mesophilic growth and is resistant to a wide range of antibiotics. It has bacteriolytic type of nutrition and cannot degrade cellulose and chitin. Cellular straight-chained fatty acids are much more abundant than branched-chain type, with arachidonic acid, *iso*-$C_{15:0}$ and $C_{17:1}$ 2-OH, as the major components. The mol G+C content is 69 % (Garcia et al. 2009b). They are widely distributed in terrestrial soil samples containing decaying plant materials.

Comments on *Phaselicystidaceae*

Phaselicystidaceae represents a novel and unexplored myxobacterial family in *Sorangiineae*. There are only few studies on this group of bacteria even with the earliest strain (*Phaselicystis flava* NOSO-1) discovered in 1988. To date, the family comprises only of one species and two strains; however, these numbers may increase in the future as more isolates have been discovered belonging to this group.

Phylogenetic Structure of the Family and Related Taxa

Phaselicystidaceae occupies an isolated cluster in *Sorangiineae* based on recent and comprehensive myxobacterial phylogenetic analysis (Garcia et al. 2010) and RaxML 16S rRNA gene tree construction of the Living Tree Project – LTPs (Yarza et al. 2010) (*Fig. 18.1*). The family's sister clades are the *Polyangiaceae* (Garcia et al. 2009b) and *Sandaracinaceae* (Mohr et al. 2012). The phylogenetic position of the reference strain

E. Rosenberg et al. (eds.), *The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria*, DOI 10.1007/978-3-642-39044-9_307,

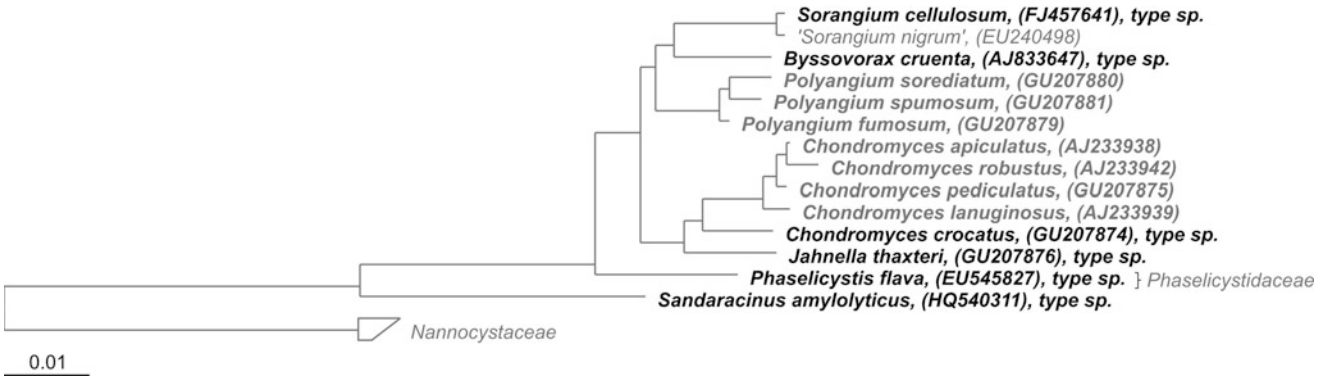

Fig. 18.1

Phylogenetic reconstruction of the family *Phaselicystidaceae* 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 50 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence

P. flava NOSO-1 (unidentified *Sorangiineae* isolate-1) has been shown in a previous study of a myxobacterial tree (Spröer et al. 1999). The family is composed of two validly published strains, both classified in *Phaselicystis flava*.

The affiliation of *Phaselicystidaceae* to *Sorangiineae* has also been correlated in the 16S rRNA gene and cellular fatty acids, where the family shows a high overall content of straight-chained fatty acids (Garcia et al. 2011). In addition, its clustering to *Sorangiineae* has also been associated with the occurrence of $C_{17:1}$ 2-OH fatty acid. The presence of hydroxy fatty acids suggests that *Phaselicystidaceae* clearly do not belong to *Nannocystineae* suborder, as the latter suborder is characterized by the complete absence of these fatty acids.

Molecular and Genome Analysis

DNA-DNA hybridization, multi-locus sequencing, riboprinting-ribotyping, genome-based taxonomy, genomic signatures, and mass spectrometric MALDI-TOF analyses have not been performed in *Phaselicystidaceae*. Phages and plasmids were also never investigated for their presence. Lastly, no representative strain of this family has been sequenced for a complete genome study. As this group of organism represents a novel family, future genome sequencing should be considered.

Phenotypic Analysis

The family is characterized by long cylindrical rod vegetative cells and short myxospores, both with blunted ends. In *Sorangiineae*, the colony morphology appears unusual, exhibiting long radial vein swarms on agar surfaces. The veins are tough and slimy and depress the agar. These phenotypic combinations of vegetative cells and swarm are unique in myxobacteria. Typical radial vein swarm patterns in myxobacteria are previously known from *Cystobacteraceae* (*Cystobacterineae* suborder), but with needle-shaped vegetative cells. Except for *Nannocystis*, with short rod vegetative cell morphology, all other members of the *Nannocystineae* suborder share nearly the same shape and size and have nonradial veins (Fudou et al. 2002).

Phaselicystis Garcia, Reichenbach, and Müller 2009

Pha.se.li.cys'tis. L. masc. n. phaselos an edible bean, kidney bean; Gr. fem. n. cystis-bladder; N.L. fem. n. *Phaselicystis* bean-shaped bladder, pertaining to the shape of the sporangiole.

The fruiting bodies are of the sporangiole type, appearing bean, sausage, or ovoid shaped with glistening sporangioles, and are arranged in clusters, chains, or solitary flat mats (*Figs. 18.2* and *18.3*). Vegetative cells are long and cylindrical rods with blunted ends similar to *Polyangiaceae*. Myxospores are shorter rods with blunted ends and are partially refractile. Swarm spreads as tough, slimy net-like veins on substrate with partial agar depressions barely recognized on agar (*Fig. 18.4*). The edge of the colony appears flame- or flare-like, with ridges of cell aggregates. *Figure 18.5* shows the morphological growth characteristics of the strain.

Swarm colonies appear to be bacteriolytic (*Fig. 18.6*) and stain Congo red-negative. Yeast is not degraded. It contains straight-chain $C_{20:4}$ fatty acid (arachidonic acid). The phylogeny based on 16S rRNA gene sequence analysis indicates independent branching from the *Polyangiaceae* in the suborder *Sorangiineae*.

The mol G+C content of DNA is 69.2 %. The type species is *Phaselicystis flava*, and the type strain is SBKo001^T = DSM 21295^T = NCCB 100230^T. It was isolated from a forest soil sample containing decaying plant collected in the Mt. Makiling Forest Reserve, Laguna Province, Philippines (Garcia et al. 2009b). *Table 18.1* summarizes the characteristics of *Phaselicystis flava*.

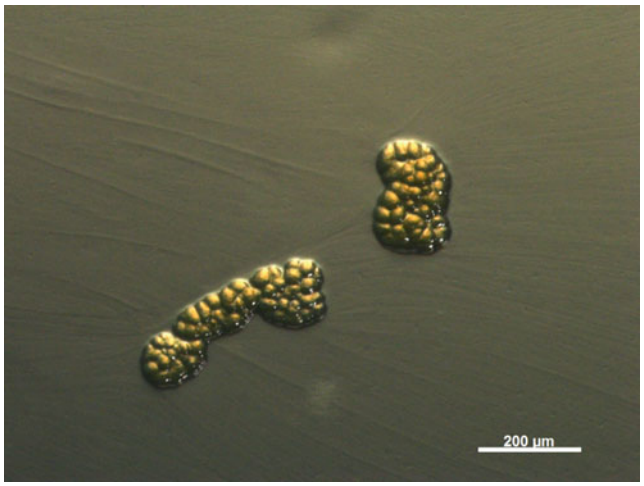

Fig. 18.2
Phaselicystis flava **bunch of sporangioles on agar resembling sorus fruiting body type of** ***Sorangium***

Isolation, Enrichment, and Maintenance Procedures

Collection, Processing, Treatment, and Storage of Samples

Soil samples and decaying plant materials can be processed directly for isolation soon after collection. Alternatively, they can be air-dried for weeks to prevent growth of other contaminating organisms (see Chap. 19). Completely dried sample stored for years could still yield isolates. Even after 6 years of storage at room temperature, *P. flava* could still be isolated from the original soil sample used to obtain for the isolation of the type strain. The ability of the bacterium to be re-isolated from the same sample source indicates that fruiting bodies containing resistant myxospores were developed before drying.

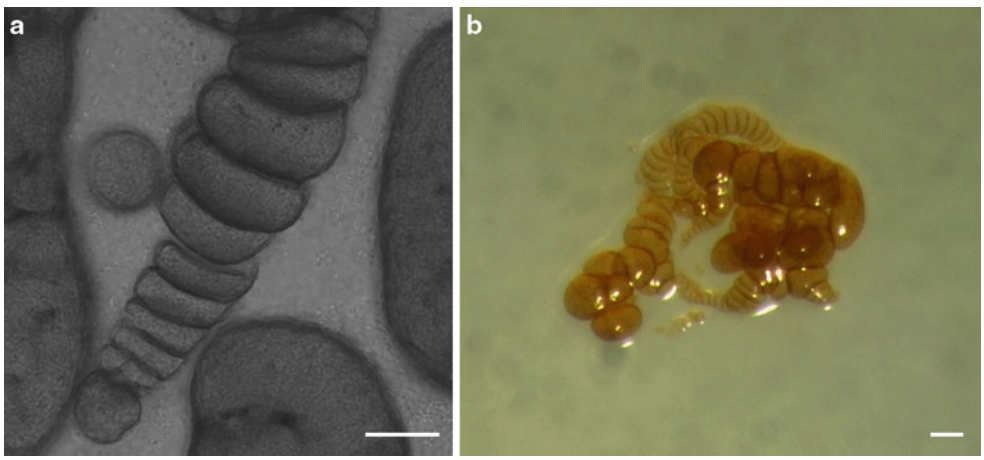

Fig. 18.3
Phaselicystis flava **unusual chain arrangement of sporangioles on agar. Bar, 40 µm (a) and 70 µm (b)**

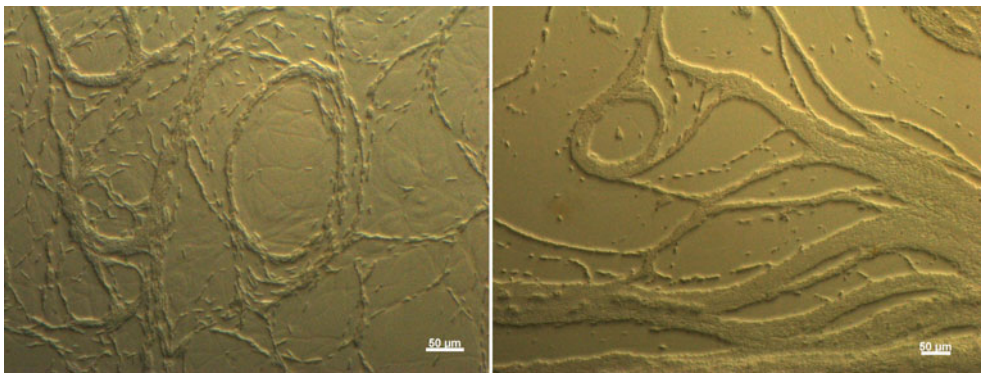

Fig. 18.4
Stereophotomicrographs of ***Phaselicystis flava*** **swarming showing the network arrangement of vegetative cells and tracks produced by the cells on agar**

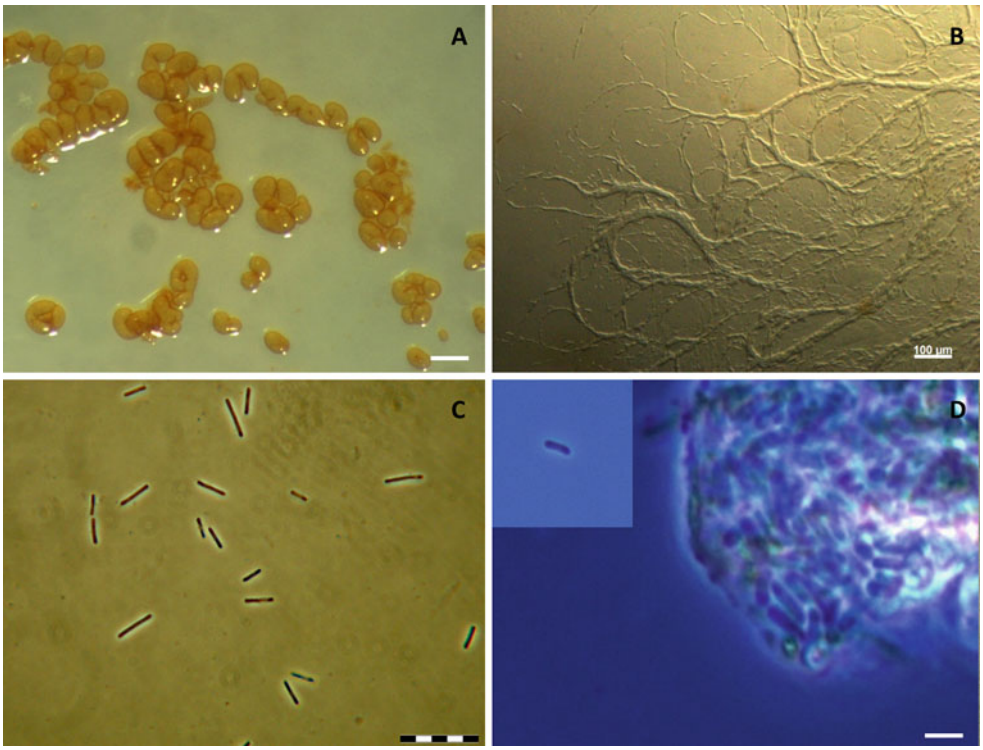

Fig. 18.5
Growth developmental stages of *Phaselicystis*. Bean-shaped fruiting bodies (a). Radial vein swarming on agar (b). Cigar-shaped vegetative cell with blunt rounded ends (c). Short rod myxospores packed inside the sporangiole and single myxospore (inset) (d). Bar, 70 µm (a), 100 µm (b), 20 µm (c), and 5 µm (d). Stereophotomicrographs (a–b), phase-contrast photomicrographs (c–d)

Fig. 18.6
Stereophotomicrograph of swarming and bacterial cell (*Escherichia coli*) lysis of *Phaselicystis flava* on agar

Set-up for Cultivation, Isolation

Members of *Phaselicystidaceae* (genus *Phaselicystis*) were isolated by cultivation in standard mineral salt ST21 agar (g per liter, Solution A: K_2HPO_4 1, Difco yeast extract 0.02, agar 10, dissolved in two-thirds water volume; Solution B: KNO_3 1, $MgSO_4{\cdot}7H_2O$ 1, $CaCl_2{\cdot}2H_2O$ 1, $FeCl_3$, 0.2, $MnSO_4{\cdot}7H_2O$ 0.1, dissolved in remaining water volume. After separate autoclaving, both solutions are combined with supplementation of 1 ml trace element solution) (Shimkets et al. 2006). The modified version MS21 (g per liter, KNO_3 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, $CaCl_2{\cdot}2H_2O$ 0.5, $FeCl_3$, 0.02, $MnSO_4{\cdot}7H_2O$ 0.1, HEPES 2.38, trace element solution (Drews 1974) 100 µL/L, yeast extract 0.02, Bacto agar 10, pH adjusted to 7.0 with KOH. After autoclaving, supplement with $K_2HPO_4$0.4) is preferred, as it produces less precipitate, allowing easier detection of the organism on agar. Although the isolated members of this group are considered non-cellulose degraders, supplementation with small rectangular filter paper strips on agar appears important for keeping the water and maintaining a moist sample. This allows rapid germination of myxospores and promotes faster swarming. The microbial baiting method (Singh 1947) using *Escherichia coli* appears satisfactory and useful for the bacteriolytic lifestyle of *Phaselicystis*. Cultures could be incubated for 2–4 weeks at room temperature or 28–30 °C, or in a humidity incubator. Petri dishes can be kept incubated at a dark environment to prevent the growth of cyanobacteria, plants, and other photosynthetic organisms. The use of 25–100 µg/mL cycloheximide and levamisole may be helpful in preventing molds and amoeba, respectively.

Members of the *Phaselicystidaceae* could be isolated either in the swarming and fruiting stage. Gliding swarm cells could be purified by cutting the farthest swarm edge and inoculating

Table 18.1
Diagnostic characteristics of *Phaselicystis flava* SBKo001[Ta]

Vegetative cell	
Shape	Long slender rod with rounded ends
Size	1.0–1.5 × 3.5–10.5 μm
Gram stain	Gram-negative
Myxospores	
Shape	Short slender rod with rounded end
Size	1.0–1.2 × 3.2–4.0 μm
Sporangiole	
Arrangement	Solitary, chain, cluster
Color	Yellow to gold
Shape	Bean, coiled, ovoid, sausage-like
Ovoid sporangiole	20–25 × 49–56 μm
Sorus size	32–52 × 86–193 μm
Swarm	
Colony	Tough and slimy radial veins
Edge	Flame-, flare-like
Congo red stain	Negative
pH cultivation	7.0–7.2
Temperature (°C)	
18	–
30	++
37	+
Salt tolerance (%)	ND
Oxygen requirement	Aerobic
Lysis of organism	
Escherichia coli	+
Micrococcus luteus	–
Saccharomyces cerevisiae	–
Hansenula anomala	–
Degradation	
Agar	+ (partially depressed)
Agarose	+
Cellulose	–
Chitin	–
Hydrolysis	
Xylan	+ (weak)
Casein	+
Skim milk	+
Starch	+ (weak)
Catalase	+
Antibiotic resistance (50 μg/mL)	
Apramycin	+
Ampicillin	–
Gentamicin	+
Kanamycin	+
Hygromycin B	+
Tobramycin	+
Spectinomycin	+
Streptomycin	+
Oxytetracycline	–
Tetracycline	–
Mol% G+C	69.2
Plasmid and Phage	ND
Fatty acids	
SCFA-BCFA ratio	Higher SCFA
Major	*i*-$C_{15:0}$, $C_{17:1}$ 2-OH, $C_{20:4}$ ω6 (arachidonic acid)
PUFA	$C_{20:4}$ (arachidonic acid)

ND not determined, *SCFA* straight-chained fatty acids, *BCFA* branch- chained fatty acids, *PUFA* polyunsaturated fatty acid
[a]Garcia et al. (2009b)

it into fresh medium supplemented with microbial bait, preferentially non-spreading bacteria (e.g., *Escherichia coli*). Fruiting bodies could be lifted out of the substrate and inoculated on new medium with bait. The resulting swarm colony is further purified by repeated transfers of the swarm edge into new medium. Pure culture isolate can be maintained in buffered VY/2 agar (g per liter, fresh Baker's yeast 5, $CaCl_2 \cdot 2H_2O$ 0.5, HEPES 1.19, Bacto agar 10, pH 7.0, adjusted with KOH) (Garcia et al. 2009a). Yeast medium enables fruiting body reproduction and may help maintain the original colony morphology over cultivation time. Tests for purity were performed in a high-nutrient medium that supports growth of contaminating bacteria. This could be achieved, for example, by transferring a small inoculum of the culture into a commercially available nutrient broth or Müller-Hinton liquid medium. In addition, purity tests could also be determined by inoculating on MD1 (Behrens et al. 1976) and Amb lm (Ringel et al. 1977) media, which support the simultaneous growth of both the contaminants and myxobacteria. Bacterial contaminants are often highly motile and usually turn the medium turbid overnight or after days of incubation, whereas molds appear small and as filamentous clumps. *Phaselicystidaceae* can be differentiated from the contaminants by its slender long rod shape and appear to be carried by the current of the medium.

Growth Cultivation, Maintenance, and Preservations

Pure isolates of *Phaselicystidaceae* can be maintained in buffered VY/2 (Garcia et al. 2009a), MD1 agar supplemented with 0.35 % arabinose – MD1A (Garcia et al. 2009b), PM12 (g per liter, Casein peptone 0.4, $MgSO_4 \cdot 7H_2O$ 1.5, Trizma Base 2, pH 7.3, after autoclaving supplement with $CaCl_2 \cdot 2H_2O$ 1, NaFe-EDTA 0.008, KNO_3 2, K_2HPO_4 0.062, glucose 3.5, dithionite 0.1) (Kopp et al. 2004), and MCA (g per liter, Bacto Casitone 1,

$CaCl_2 \cdot 2H_2O$ 1, $MgSO_4 \cdot 7H_2O$ 1, Bacto agar 11.2, supplemented with Vitamin B_{12} 10 µg/mL) (Garcia et al. 2009b). Vegetative cells normally do not survive long on agar and, in the absence of fruiting body formation, die after reaching the edge of the plate.

Permanent storage was achieved by desiccation of matured fruiting bodies and cryopreservation of vegetative cells. Mature fruiting bodies are lifted from the agar and placed on sterile filter paper for 2–4 weeks desiccation. Deep-freezing of small agar blocks from the culture or cell pellet could be cryopreserved in −80 °C freezer or liquid nitrogen with 20 % DMSO or glycerol. Either agar plugs or cell biomass was suspended in liquid medium (e.g., MD1) before the cryoprotectant is added. Desiccated and cryo-cultures can be reactivated by simply inoculating on semi-soft medium (0.8–1.0 % agar) to allow spore germination of the dried spores and prevent the delicate cryo-vegetative cells from drying out.

Ecology

Phaselicystidaceae appears to be distributed in soil samples and decomposing plant materials. They inhabit the warm environment of the Southeast Asian tropics and the African continent represented by the type and reference strain, respectively. *Phaselicystis* could always be identified in the same soil originally used for isolation. They appear to be common microbiota of Philippine forest soil, but seem less frequent when compared to *Sorangium*, *Polyangium*, and *Chondromyces* in the overall samples studied. Their occurrence in Europe has also been documented in one of the soil samples from Germany.

Symbiosis, Pathogenicity, and Clinical Relevance

No symbiotic or associated organisms are known to *Phaselicystidaceae*. They have not been implicated in any diseases related to plants and animals and considered a risk group 1 organism.

Application

Although *Polyangiaceae* are famous for diverse and interesting bioactive compounds (Reichenbach 2001; Reichenbach and Höfle 1993, 1999; Gerth et al. 2003; Bode and Müller 2006, 2008; Weissman and Müller 2009; Wenzel and Müller 2009; Weissman and Müller 2010), *Phaselicystidaceae* has yet not been well explored for this application but could potentially be an interesting new source. Considering the phylogenetic relatedness of these families, *Phaselicystidaceae* is also likely to produce novel compounds.

To date, the most significant application of this family is the production of polyunsaturated fatty acids (PUFAs). Omega-6 arachidonic acid has been identified in high amounts in both *P. flava* type and reference strains (Garcia et al. 2009b). An analysis of cellular fatty acids covering the whole order of myxobacteria revealed that arachidonic acid is also produced by some members of the *Polyangiaceae* (*Sorangiineae*) and *Nannocystaceae* (*Nannocystineae*) (Garcia et al. 2011; Stadler et al. 2010). Production of PUFAs in *Phaselicystidaceae* would not be unusual, as they have also been discovered in the novel *Sorangiineae* (Stadler et al. 2010.). In general, long-chained PUFAs are valuable for commercial and medical applications (Calder 2001; Fan and Chapkin 1998; Horrocks and Yeo 1999; Warude et al. 2006).

Concluding Remarks and Perspectives

Phaselicystidaceae belong to the suborder *Sorangiineae*, known for the production of many structurally unique bioactive compounds and other applications. Although only one species and two strains have been validly published, the family appears widely distributed in the environment. As this is a novel taxon, growth cultivation and optimization need to be established for secondary metabolite screening and other important medical-industrial applications.

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19 The Family *Polyangiaceae*

Ronald Garcia · Rolf Müller
Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland – Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology, Saarland University, Saarbrücken, Germany

Abstract
Polyangiaceae belong to the suborder *Sorangiineae* in the order *Myxococcales* and comprise the genera *Polyangium*, *Sorangium*, *Byssovorax*, *Chondromyces*, and *Jahnella*. Members of the *Polyangiaceae* family are commonly terrestrial isolates, mainly from soil and decaying plant material. So far, this is the only family of myxobacteria which include cellulose-degrading strains. They are recognized by distinct morphological and chemo-physiological features, from which genus and species can be delineated. Some members of the family produce most sophisticated and complex fruiting bodies resembling treelike structure. The genomes of representatives belong to the largest in the prokaryotes and contain a high percent of G+C. All genera within the family are coherent in the 16S rRNA gene phylogeny, which appears to be correlated with phenotypic characteristics. Cellular fatty acid analysis revealed strong support for each strain's affiliation to the corresponding taxon. *Polyangiaceae* are of interest for a wide range of applications. The microbial predatory lifestyle exhibited by most members has implications for environmental biocontrol. In addition, diverse novel antimicrobial and cytotoxic secondary metabolites are produced by this group. Further compounds act as anti-inflammatory, antitumor, and antiviral agents (e.g., anti-HIV). The anticancer agent epothilone was isolated from this family and is currently considered as the most successful pharmaceutical derived from the entire myxobacterial order. Recently, in some novel isolates of the *Polyangiaceae*, steroids and commercially valuable omega-3 and omega-6 polyunsaturated fatty acids were found.

Taxonomy, Historical, and Current

Short Description of the Family

Po.ly.an.gi.a'ce.ae. M. L. neut. n. *Polyangium* type genus of the family; -aceae ending to denote a family; M. L. fem. pl. n. *Polyangiaceae* the *Polyangium* family.

Based on phylogeny, *Polyangiaceae* cluster as a distinct clade in the *Myxococcales* (Garcia et al. 2010; Spröer et al. 1999), class

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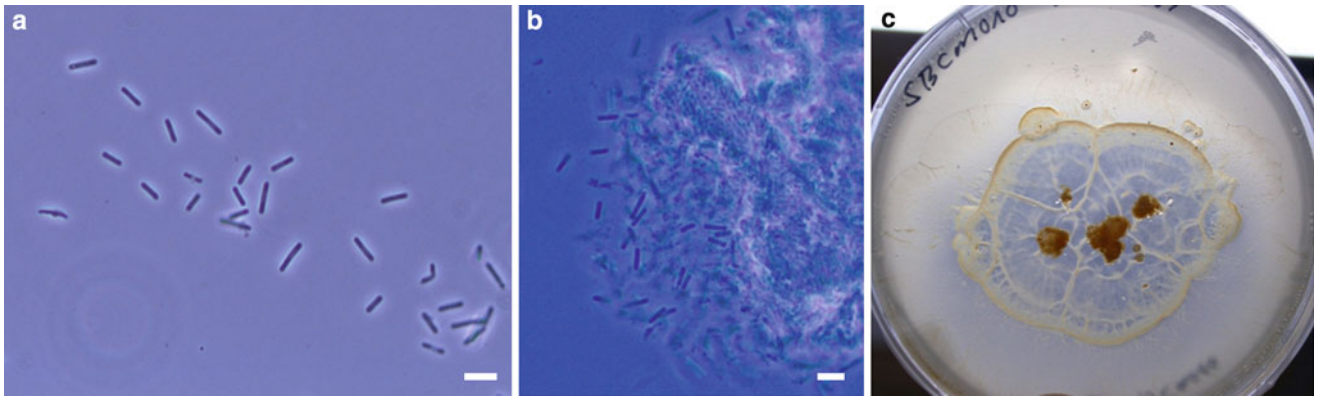

Fig. 19.1
Phenotypic characteristics of *Polyangiaceae*. Long slender rods with blunt rounded ends in *Chondromyces robustus* Cm a13^T (a). Rod-shaped myxospores released from *Jahnella thaxteri* sporangiole (b). Deeply agar-penetrating colony of *Chondromyces crocatus* (c). Bar, 10 μm (a), 5 μm (b). Petri dish diameter, 9 cm (c)

Table 19.1
Isolation sources for *Polyangiaceae*

Source	*Byssovorax*	*Chondromyces*	*Sorangium*	*Polyangium*	*Jahnella*
Soil, mud, sand	++	++	++	++	++
Decaying plant	++	++	++	++	++
Bark of trees	–	++	–	–	–
Plant leaf (phyllosphere)	–	+	–	–	–
Herbivore dung	+	++	+	+	+
Freshwater	–	–	+	+	–

Legend: ++, more frequent; +, less frequent; –, not encountered

Deltaproteobacteria, and phylum Proteobacteria (Shimkets and Woese 1992; Kaiser 1993). The family covers the type genus *Polyangium* (Reichenbach 2005) and includes genera of *Sorangium* (Jahn 1924), *Byssovorax* (Reichenbach et al. 2006), *Chondromyces* (Berkely and Curtis 1874), and *Jahnella* (Reichenbach 2005). Members of this family share common growth-stage characteristics. Vegetative cells are Gram-negative, phase-dark, long slender rods with rounded blunted ends appearing as rigid and non-flexuous. Cells are motile by gliding on substrate producing pseudoplasmodia or soft radial vein swarm. Fruiting bodies often arrange as sori and can be recognized by the yellow, red, orange, brown, and black colors of sporangioles, either produced directly on surface, borne with a stalk, or supported by a slime cushion. Myxospores are partially refractile slender rods with blunted ends and are known to be temperature and desiccation resistant. They are found tightly packed in sporangioles. A typical colony shows agar depression and corrosion. In a lean medium, swarming cells produce filmlike and thin colonies on the surface but often sunken into the agar. For some members, the swarm takes the shape of veins. Pink, peach, orange, red, and yellow are typical colony shades and vegetative cell colors. The swarm edge is characterized by meandering ridge, roll, band, curtain, and sometimes flare architecture. *Figure 19.1* shows the characteristic growth pattern of this family.

Polyangiaceae might be regarded as aerobes or micro-aerophiles. Members of this family commonly grow at a mesophilic temperature range, tolerate low amount of salt, and resist many antibiotics. In general, straight-chain fatty acids are found in much higher amount than the branched-chained type, with $C_{16:1}\omega7c$, $C_{17:1}$ 2OH, and iso-$C_{15:0}$ as the major components. Mol percent G+C of determined genera (*Byssovorax*, *Chondromyces*, and *Sorangium*) ranges from 69 to 70 (Reichenbach 2005). The family is widely distributed in terrestrial samples and appears commonly isolated from soil, decaying plant material, bark of trees, and herbivore dung (*Table 19.1*). However, they may also be found in some unexplored and extreme environments.

Comments on *Polyangiaceae* Genera

The genus *Byssovorax* bears the name *Byssophaga* in the third edition of this book. Since the generic name *Byssophaga* has been previously assigned to an alga, the genus was then renamed into *Byssovorax* (Reichenbach et al. 2006). The strain was first seen on cow dung and on a wood substrate where it was described

to resemble the ascomycete *Nectria* (Thaxter 1897). Unfortunately, Thaxter could not cultivate the strain, hence rendering the vegetative cells and swarm colony description missing. Based on a previous study (Thaxter 1904), *Byssovorax* myxospores' size (0.9–1.0 × 1.2–1.4 μm) is apparently much smaller (1.5–1.7 × 3.3–5 μm) than the rediscovered strain (Reichenbach et al. 2006). The taxonomic transfer of *Myxococcus cruentus* (Thaxter 1897) to *Byssovorax* by Reichenbach appears to be justified by the morphology of the fruiting body, vegetative cells, and swarming. All of these growth-stage characteristics did not match with *Myxococcus*. The shape of vegetative cells, myxospores, swarming pattern, and fruiting body of *Byssovorax* are clearly different from *Myxococcus*. Taking into account the phylogenetic clade (Reichenbach et al. 2006), the transfer into a novel genus within the *Polyangiaceae* appears justified.

Jahnella represents a genus name given in honor to a German scientist (Eduard Adolf Wilhelm Jahn, 1871–1942) who made great contributions on the first monograph and synopsis of myxobacteria (Reichenbach 2005). The bacterium was first discovered in 1924 and classified as *Archangium thaxteri* (Jahn 1924). Although *Jahnella* may look like *Archangium* during fruiting body development, this species can be differentiated after maturity. The latter genus never forms a sporangiole-type fruiting body, while the former does. In the first edition of this book, *Jahnella* (previously referred to as *Polyangium thaxteri*) was clearly illustrated in the colored figure plate containing sporangioles (Reichenbach and Dworkin 1981). The strain's novel branch position in *Polyangiaceae* has been demonstrated early in the phylogenetic analysis (Spröer et al. 1999), although it bears the name *Polyangium thaxteri*. The bacterium was then transferred into a new genus called *Jahnia* (Shimkets et al. 2006; Reichenbach 2005). The creation of this novel genus appears to be supported by the phenotypic differences with *Polyangium* and a novel phylogenetic branch diverging from the *Chondromyces* cluster (Garcia et al. 2010). The correction in the generic name from *Jahnia* to *Jahnella* follows the taxonomic rule of International Code of Bacterial Nomenclature after its valid publication (Euzéby 2007). The distinction of *Jahnella* to other genera in *Polyangiaceae* was not only supported by its phenotypic characteristics, 16S rRNA gene sequence and phylogenetic analysis (Garcia et al. 2010), but also in agreement with the fatty acid content (Garcia et al. 2011).

Jahn has established the genus *Sorangium* based on strains with sorus fruiting body (Jahn 1924). However, not all "sorus-type" fruiting bodies are bound to *Sorangium*, as other genera such as *Polyangium* and *Byssovorax* are also of the sorus type. The cellulose degradation ability of *Sorangium* and *Byssovorax* differentiates them from the genus *Polyangium* (Reichenbach 2005). Except for the recently cultivated genus *Byssovorax*, the classification of *Sorangium* and *Polyangium* in the past was often interchanged. The uniqueness of *Sorangium* in comparison to *Polyangium* is shown by its characteristic ability to degrade cellulose and its inability to attack and prey on microorganisms.

In *Sorangium*, the characteristic shape and color of sporangioles have been suggested as possible species delineation (Reichenbach 2005). Strains with black fruiting bodies and

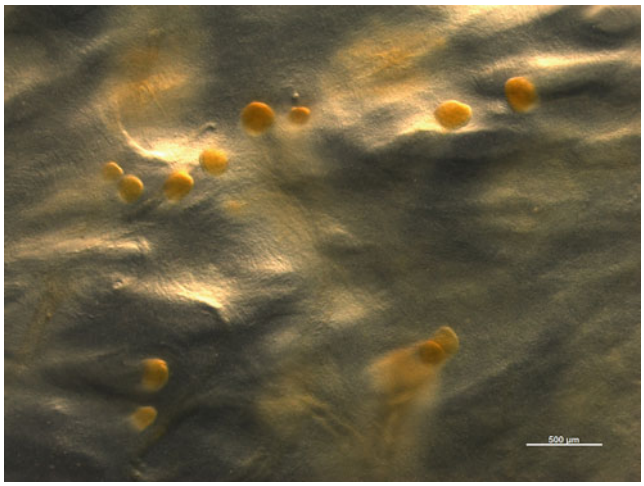

Fig. 19.2
Sporangiole-like cell aggregates of *Kofleria flava* Pl vt1^T

sporangioles were previously hypothesized to represent a novel species of *Sorangium* (e.g., "*S. nigrum*") (Reichenbach 2005). However, the phenotypic characteristic does not seem enough to justify the proposal for a new species. *Sorangium* occupies a homogeneous group in the 16S rDNA phylogenetic tree and shows less than 3 % evolutionary distance among its strains (Yan et al. 2003). The proposal to erect the novel species "*Sorangium nigrum*" may appear supported by additional molecular and phylogenetic data.

On morphological ground based on yellow fruiting-body-like aggregates, strain Pl vt1 appears to be a species of *Polyangium vitellinum* (Fig. 19.2). Its transfer to *Kofleria* in *Kofleriaceae* family (Reichenbach 2005) based on 16S rRNA gene sequence and its phylogenetic affiliation to the suborder *Nannocystineae* (Spröer et al. 1999; Garcia et al. 2010) suggests that strain Pl vt1 does not belong to the genus *Polyangium*. To date, there is no available live-type culture of *P. vitellinum* to verify its taxonomic position. Perhaps the species does not really belong to the genus *Polyangium* but to *Kofleria*. Besides *Polyangium vitellinum*, two additional species with yellow/golden sporangioles (*P. aureum*, *P. luteum*) have been described in this genus, but unfortunately, no viable-type strains are available. Based on the color of the fruiting body and an almost overlapping sporangiole size, the authors speculate that they might be the same species. Some of the recent isolated strains are perhaps representatives of these species, as they also produce yellow sporangioles. These strains were verified by 16S rRNA gene analysis to belong to the *Polyangium* cluster. A proposal for a neotype strain is envisaged for the future after a complete taxonomic description and deposition of the strain to an open culture collection.

Several *Cystobacter* (*Cystobacteraceae* family) strains that used to be classified in *Polyangium* have been discussed for their placement (Reichenbach 2005). Since *Cystobacter* strains also form a sporangiole-type fruiting body on agar, they often cause confusion to inexperienced investigators. A careful microscopic examination of the vegetative cells reveals a distinction

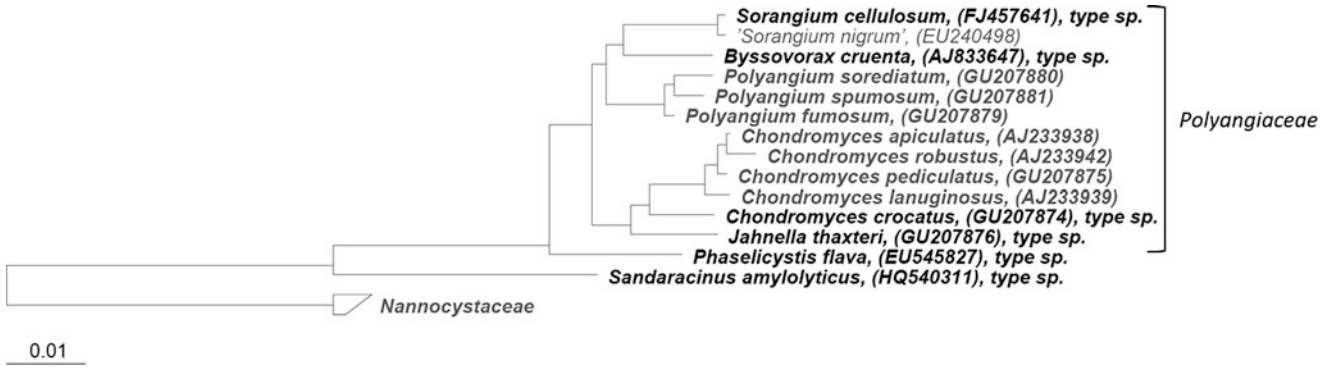

Fig. 19.3

Phylogenetic reconstruction of the family *Polyangiaceae* 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 50 % maximum frequency filter was applied to remove hypervariable positions from the alignment. Scale bar indicates estimated sequence divergence

between these two genera. *Cystobacter* are obviously long, thin, and needle-shaped, whereas *Polyangium* cells are shorter and thicker and exhibit blunt, rounded ends, close to being cigar-shaped. In addition, *Cystobacter* vegetative cell seems to be more flexible (flexuous type) and easily bent, while *Polyangium* appears to be more slender and robust.

Some species of *Chondromyces* appear to be ambiguous and confusing. Among the five species of *Chondromyces*, only *C. catenulatus* has no available viable strain deposited in the culture collection. The type strain described by Thaxter is currently housed in the Farlow herbarium (Reichenbach 2005). Based on the beautiful illustration drawn by the author (Thaxter 1904), and the scanning electron photomicrograph of the rediscovered strain in Australia (McNeil and Skerman 1972), it is clear that the bacterium must exist. To our knowledge, only these two records affirmed the occurrence of this strain in nature. The existence of this bacterium was further confirmed after its recent isolation, but its classification appears to be more complicated than expected based on phylogenetic studies. Since a novel taxon will be proposed, the authors decided not to include this bacterium in this chapter. A second species, *Chondromyces lanuginosus*, bears several names which were already used in a short period of time after its discovery. The strain was first discovered in 1913 (Kofler 1913), but after 3 years, the bacterium was described again and named *Chondromyces thaxteri* (Faull 1916). Later, the genus *Synangium* was created which includes *Synangium thaxteri* (Jahn 1924). Since there are really not many differences between the taxonomic descriptions of *Synangium* and *Chondromyces*, the proposal for *S. thaxteri* as a novel species in a new genus appears to be unjustified (Krzemieniewska and Krzemieniewski 1946).

Haploangium was proposed to be a genus with solitary sporangioles (Peterson 1959). The generic name was erected recently to include the two herbarial-type species deposited in the University of Missouri Herbarium, Columbia, MO, USA (Reichenbach 2005). Despite their inclusions in the latest edition of *Bergey's Manual of Systematic Bacteriology* (Reichenbach 2005), there is still no defined nomenclature to validly include these strains in *Polyangiaceae*. Based on morphological data, *Haploangium* fits in *Polyangiaceae*. However, its real identity and affiliation within the family cannot be confirmed with the absence of a live-type strain.

Several strains described to cluster within the *Polyangiaceae* appearing to represent novel genera were discovered recently (Garcia et al. 2010, 2011). Since they are not yet validly published, their full inclusion in this chapter is not yet possible. However, the significant and potential applications of these isolates are highlighted here.

Phylogenetic Structure of the Family and Related Taxa

Polyangiaceae cluster coherently based on the previous 16S rRNA gene phylogenetic tree (Garcia et al. 2010) and analysis using RaxML 16S rRNA gene tree of the Living Tree Project – LTPs (Yarza et al. 2010) (*Fig. 19.3*). The family is closely related to *Phaselicystidaceae* (Garcia et al. 2009b) and to the recently inaugurated *Sandaracinaceae* (Mohr et al. 2012), both found in *Sorangiineae* suborder. The phylogenetic position of *Polyangiaceae* in *Sorangiineae* has already been shown in previous studies despite of the inclusion of only few representative species (Spröer et al. 1999; Shimkets and Woese 1992; Ludwig et al. 1983). Currently, two major clades compose the *Polyangiaceae*, and the distinction between them appears supported by phenotypic and physiological characteristics. The first clade consists of *Sorangium*, *Byssovorax*, and *Polyangium*, all of which form sporangiole-type fruiting bodies directly attached to the substrate. The delineation between the clustering of the cellulose-degrading (*Sorangium* and *Byssovorax*) and the non-cellulose-degrading genera (*Polyangium*, *Chondromyces*, *Jahnella*) and between stalked (*Chondromyces*) and stalklike

slime-cushioned (*Jahnella*) clusters is clearly noticed in the phylogenetic tree. All type or proposed neotype species in their respective genera seem coherent to allow vivid delineation.

Recently, many 16S rRNA gene sequence data derived from clones of uncultured bacteria have shown high similarity with myxobacteria (Brinkhoff et al. 2012; Jiang et al. 2010), and they appear phylogenetically clustered or related to *Polyangiaceae*. These clones may possibly represent viable but not culturable (VBNC) organisms. Although myxobacteria are distinguished by the fruiting body development, a previous study has shown evidence for the existence of non-fruiting groups, and they seem to occupy a much larger portion of the myxobacteria than the known fruiting taxa (Jiang et al. 2007). In addition, the study revealed that currently uncultured myxobacteria are highly represented in the non-fruiting clusters. The diversity of myxobacteria in a marine environment was also documented and determined to be phylogeographically distinct from the terrestrial myxobacteria at high levels of classification (Jiang et al. 2010). Nevertheless, the occurrence of marine myxobacteria is not surprising, as such were previously reported from Pele's Vents, Hawaii (Moyer et al. 1995).

The coherency of *Polyangiaceae* and its affiliation to *Sorangiineae* has also been established by fatty acid-related phylogeny (Garcia et al. 2011). The presence of hydroxy fatty acids affirmed that the family does not belong to *Nannocystineae*, since this suborder is remarkable for the absence of hydroxy-type fatty acids. In addition to *Phaselicystidaceae*, the clustering of the *Polyangiaceae* appears to be supported by the presence of $C_{17:1}$ 2-OH fatty acid. Moreover, the predominance of the straight-chain- over the branched-chain-type fatty acid in this family has also been correlated to the 16S rRNA gene phylogenetic tree (Garcia et al. 2011).

Molecular Analysis

To date, there are no records of DNA-DNA hybridization studies, multi-locus sequencing, riboprinting-ribotyping, genome-based taxonomy, genomic signatures, and mass spectrometric MALDI-TOF analyses, at least, in *Polyangiaceae*. In eleven-type and proposed neotype strain members of this family, these methods appeared to be not required but may be useful in the future to clearly classify taxa. These analyses may also be important in strains which cannot be identified phenotypically, if, for example, they lose their ability to form fruiting bodies or show a degenerate structure in a particular life stage. In general, the generic members of *Polyangiaceae* could be distinguished by mass spectrometric identification of their secondary metabolites. In the application part of this chapter, the list of known compounds in this family is summarized. Compounds produced in this family rarely overlap with other genera, and so far only one secondary metabolite (thuggacin) was determined to be produced by two taxa (*Sorangium*, *Chondromyces*). Thus, mass spectrometry may provide a significant clue towards their identification.

Genome Analysis

Among the five valid genera of *Polyangiaceae*, only *Sorangium* has a published genomic sequence. In this view, the genomic comparison among related taxa in this family is not possible at this time, thus only the genome structure of *Sorangium* is presented here.

The genus *Sorangium* exemplified by a strain So ce56 exhibits the largest (13,033,779 base pairs) myxobacterial genome sequenced to date and is also considered one of the largest among the prokaryotes. Currently, several myxobacteria are under sequencing process in our laboratory, and their genomes appear to be larger than many sequenced bacterial strains. The complete genome of So ce56 was obtained by shotgun sequencing, and a fosmid library was used to validate its assembly (Schneiker et al. 2007). A large part of the genome from this strain is associated with regulation (specifically posttranslational modification) and appears to be correlated to the complex social lifestyle of the bacterium. Interestingly, 17 loci were found in the genome encoding for secondary metabolism and for many important industrial enzymes. Some additional important features of the genome are summarized as follows: (1) contains 9,367 predicted protein coding sequences (CDS); (2) is GC-rich, accounting to 71.38 %; (3) contains 60 tRNA, 4 ribosomal RNA operons, and 28 insertion sequences; and (4) contains 70 proteases which are perhaps involved in biodegradation of complex molecules (Schneiker et al. 2007).

Complex regulatory networks were revealed in *Sorangium cellulosum* genome, and these include the extracytoplasmic function sigma factor family, two-component regulatory systems, serine/threonine/tyrosine protein kinases (eukaryotic-like protein kinases (ELKs)), and enhancer-binding proteins. In addition, few one-component regulators which show similarity to those of many soil bacteria were found, with LysR-type transcriptional regulators appearing as the largest group. ELKs (317) are encoded by DNA equivalent to 6.2 % of the complete genome and found distributed throughout the chromosome (Schneiker et al. 2007). They are to date considered to represent the largest number of eukaryotic-like protein kinases in any prokaryote (Schneiker et al. 2007; Pérez et al. 2008; Leinenbach et al. 2009). Although their functions are largely unknown (Leinenbach et al. 2009), they seem to be involved in sophisticated and novel signal transduction systems (Pérez et al. 2008). In addition, ELKs are hypothesized to play an important role in the modulation of prokaryotic behavior (Kannan et al. 2007). Based on phosphoproteome analysis, approximately 40 % of the proteins analyzed are indeed phosphorylated (Schneiker et al. 2007). Interestingly, a previous study revealed that a large number of hypothetical proteins (e.g., sce0725, sce1064, sce5938, sce8432) including such not showing any similarities to known proteins are expressed (Leinenbach et al. 2009).

Phages and Plasmids

No naturally occurring plasmids have been found among the cultured-type species of *Polyangiaceae* and even on other strains

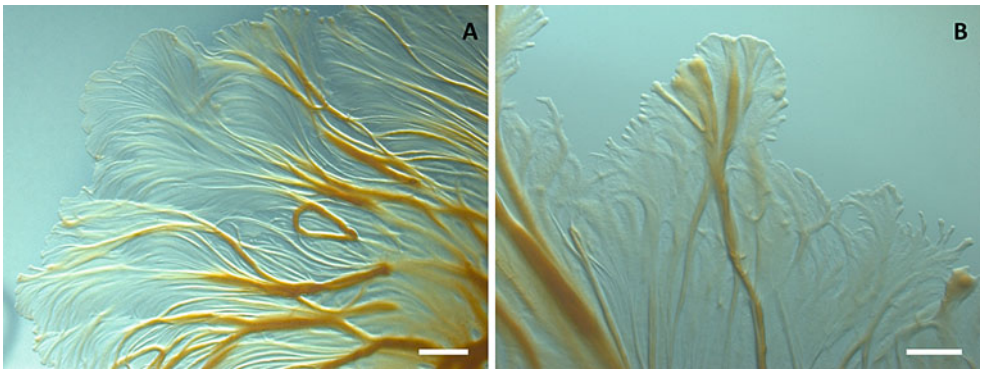

Fig. 19.4
Colony of *Byssovorax cruenta* DSM 14553^T. Long-radial vein swarming on agar (a). Characteristic fan-shaped edges (b). Bar, 2,000 μm (a), 1,000 μm (b)

in this family. Perhaps the methods to optimize the plasmid extraction should be developed for members of *Polyangiaceae* and for the whole myxobacterial order. However, an exogenous plasmid (pRP-GFP) can stably be maintained as an extrachromosomal element in some strains of *Sorangium cellulosum* (Tu et al. 2007). On the other hand, there were also no available studies on phages, superintegrons, and genomic islands in this family. The analysis for the absence of phages and plasmids has been discussed in the complete genome study of *Sorangium cellulosum* strain So ce56 (Schneiker et al. 2007).

Phenotypic Analysis

Polyangiaceae consists of five validly described genera composed of the genus *Polyangium*, *Sorangium*, *Byssovorax*, *Chondromyces*, and *Jahnella* (Reichenbach 2005). The family is currently the largest group in terms of genus and species diversity among other recognized valid families (*Phaselicystidaceae*, *Sandaracinaceae*) in *Sorangiineae*. *Polyangiaceae* are phenotypically characterized by cylindrical-shaped vegetative cells and myxospores, deep swarming ability, and colony-depressing growth on agar. These phenotypic characteristics seem not only exclusive to *Polyangiaceae* but also appear shared with other families in *Sorangiineae* (e.g., *Phaselicystidaceae*, *Sandaracinaceae*) and *Nannocystineae* (e.g., *Kofleriaceae*, *Nannocystaceae*).

Byssovorax Reichenbach 2006, gen. nov.

Bys.so.vo'rax. Gr. n. *byssos*, cotton, fine linen (for cellulose); L. adj. *vorax*, voracious, devouring; N.L. fem. n. *Byssovorax*, devourer of cellulose.

Fruiting bodies are composed of large and few polyhedral sporangioles (60–80 μm wide, 80–150 μm length) packed in sorus (220–560 μm diameter). Swarm spreads as pseudoplasmodia with fanlike head and tapering tail and glides independently into the agar and on cellulose filter paper. Large and long veins often may be produced after repeated subcultivations (Fig. 19.4). Slime trails or tracks resembling to a wrinkled parchment may be produced too. Large cell mounds resembling spherical knobs (200–500 μm diameter) on colony surface may be found. Vegetative cells are long and slender rods with blunted ends, measuring 0.9–1.1 × 3.5–7.5 μm. Myxospores are short fat rods (1.5–1.7 × 3.3–5 μm) and refractile. Exhibit bacteriolytic and cellulolytic type of nutrition (Fig. 19.5). Congo-red negative. Table 19.2 shows additional and differentiating characteristics versus other genera.

The mol% G+C of DNA is 69.9. The type species is *Byssovorax cruenta*, and the type strain is DSM 14553^T = CIP108850^T = JCM 12614^T. Source was soil sample with decaying plant material collected in 1996 in sagebrush steppe, a mile away, south of Holbrook, Arizona, USA (Reichenbach 2005).

Sorangium (*Ex* Jahn 1924) Reichenbach 2007, gen. nov., nom. rev. (Type Genus of the Suborder *Sorangiineae* Corrig. Reichenbach 2007)

So.ran'gi.um. Gr. n. *soros*, heap, pile; Gr. neut. n. *angeion* (Latin transliteration *angium*), vessel; N. L. neut. n. *Sorangium*, piled up vessel.

Fruiting bodies (Figs. 19.6 and 19.7) are composed of appressed polyhedral sporangioles (15–40 μm diameter), arranged as a cluster, or a linear chain in cellulose filter paper and crystalline powder. Often, they are produced on surface of the substrate but sometimes also within the agar. Fruiting bodies may appear as brown, black, orange, or yellow depending on the strains. *Sorangium* swarm (Fig. 19.8) could appear as light yellow to orange on cellulose paper and as transparent soft veins on lean agar. Swarm may also burrow into the agar to produce curtain-shaped appearance of the edges. In a nutrient-rich medium, intense yellow to orange colonies are produced. Vegetative cells are phase-dark slender rod with blunted ends and measure 0.8–1.2 × 3–8 μm. Myxospores are almost of the same shape as vegetative cells. They are refractile slender rods

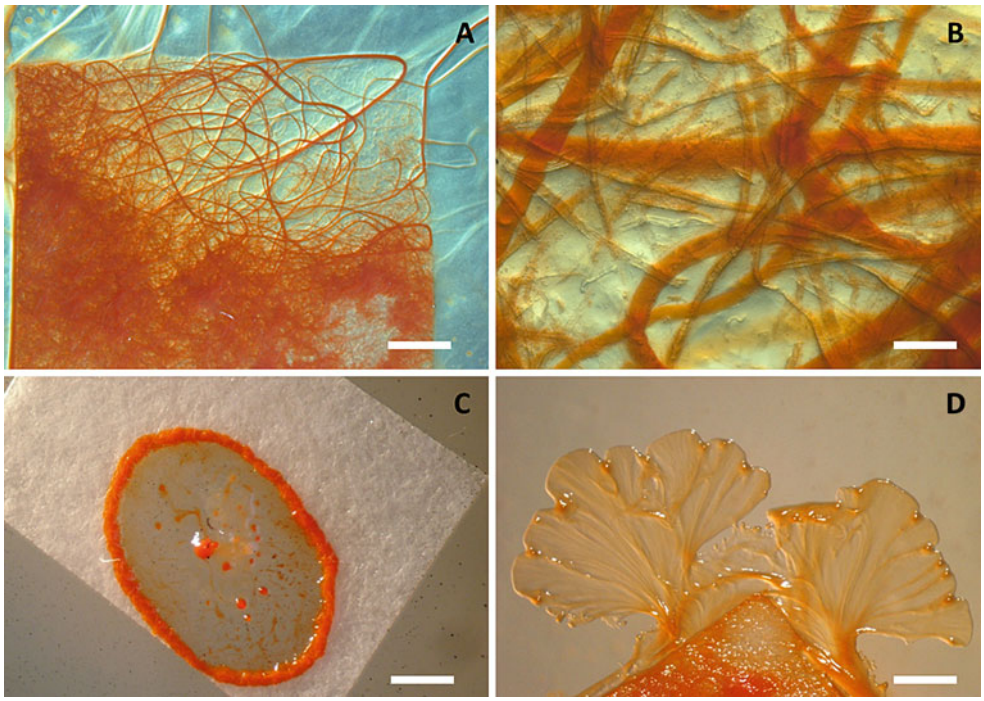

Fig. 19.5
Stereophotomicrographs of *Byssovorax cruenta* DSM 14553^T on filter paper. Decomposed edge of the filter paper and long undulating red veins produced by the strain (a). Magnified view of figure A showing the peculiar growth of the bacterium in filter fibers. Decomposed filter fibers are clearly differentiated from the large and anastomosing swarm veins (b). Decomposition pattern of filter paper on mineral salt agar and red colony swarming. Red mounds at the center of colony show shape similarity to *Myxococcus* fruiting body (c). Developing fan-shaped swarms at the edge of a filter paper (d). Bar, 2,000 μm (a), 200 μm (b), 1.5 × 1.0 cm filter paper (c–d)

(2–4 μm), enclosed in sporangiole, and desiccation and heat resistant. Strongly degrade cellulose. Bacteria and yeast cells are not lysed. Agar could be depressed, cut, and cleaved, but not liquefied. Stains negative in Congo red. Diagnostic characteristics to its related genera are shown in ❯ *Table 19.2*.

The mol% G+C of DNA is 69. The type species is *Sorangium cellulosum*, while the type strain is So ce1871^T = DSM 14627^T = JCM 12641^T, isolated in 2001. Source was from soil sample with decaying plant material, collected in 2000 in Rhodes Island, Greece (Reichenbach 2005).

Polyangium Link 1809 (Type Genus of the Family *Polyangiaceae* Jahn 1924)

Po.ly.an'gi.um. Gr. adj. *polu*, many, much; Gr. neut. n. *angeion* (Latin transliteration *angium*) vessel, container; N.L. neut. n. *Polyangium*, many vessels.

Fruiting bodies are composed of oval to polyhedral sessile sporangioles, arranged in a cluster or sometimes solitary. The fruiting body color varies from shades of peach, brown, gray, black, and yellow depending on the species and often found adhering in the agar. Sometimes, sporangioles may be born in a stalklike slime. Produce pseudoplasmodial swarm with band- or fan-shaped head and long pointed tail in the agar. Deep and long corroded agar tracks with globular, ridge-, band-, or mushroom-shaped pseudoplasmodium may also arise on the surface of the substrate. Rounded and solitary aggregates are often produced in the agar. Soft and slimy swarm may also be produced on agar. Swarm color varies from rose pink, peach, to yellowish depending on the species. ❯ *Figures 19.9*, and ❯ *19.10* show swarming, aggregation, and fruiting stage. Vegetative cells are long, slender, cylindrical rods with blunted ends measuring 0.7–0.9 × 4–10 μm. Myxospores are short slender rods with blunted ends and are partially refractile. Exhibits bacteriolytic type of nutrition. Stains negative in Congo red. Synthesis of lanosterol has been implicated among strains of this genus (Zeggel 1993; Bode et al. 2003).

The mol% G+C of DNA is not determined. The type species is *Polyangium vitellinum*. The type strain is Acc No. 4564, Thaxter collection, Farlow Herbarium, Harvard University, Cambridge, MA, USA (Reichenbach 2005). This strain was never isolated but described from wetwood and tree barks, swamps, ditches, and pools.

In seven described species of *Polyangium* (*P. vitellinum*, *P. luteum*, *P. aureum*, *P. parasiticum*, *P. sorediatum*, *P. spumosum*, *P. fumosum*), only the last three live (proposed neotype) strains (Reichenbach 2005) are available, which allow systematic studies. ❯ *Table 19.3* summarizes the characteristics of these species.

Table 19.2
Diagnostic characteristics among *Sorangium*, *Byssovorax*, and *Jahnella*

Characteristics	*S. cellulosum* So ce1871^T =DSM 14627^T	*B. cruenta* By c2^T =DSM 14553^T	*J. thaxteri* Pl t4^T =DSM 14626^T
Sporangiole: Shape	Ovoid to polyhedral	Ovoid to polyhedral	Coils
Arrangement	Sori, cluster, chain	Sori, cluster	Cluster
Size (μm)	20–30 (diameter)	80–140 (diameter)[b]	60–90 × 80–120[a]
Slime cushion	−	−	+
Swarm: Pattern	Soft radial veins	Pseudoplasmodium, Long-radial veins	Deep penetrating, Scattered long veins
Edge	Curtain-like in agar; Flame-like on surface	Fan-shaped; Ridge, band on paper	Bands in agar
Growth at pH 7.0	Good	Good	Good
Growth at 30 °C	Good	Good	Good
Salt tolerance (%)	1.0	ND	1.0
Oxygen requirement	Aerobic	Aerobic	Aerobic
Lysis of organism:			
Gram− *Escherichia coli*	−	+	+
Pseudomonas syringae	−	+	+
Gram + *Bacillus subtilis*	−	+	+
Micrococcus luteus	−	+	+
Mycobacterium chitae	−	+	+
Yeast: *Saccharomyces cerevisiae*	−	+	+
Degradation: Agar	+	+	+
Cellulose	+	+	−
Chitin	+	+	+
Hydrolysis: Xylan	+	+	+
Casein	ND	ND	+
Skim milk	+	+	+
Starch	+	+	+
Catalase	+	+	+
Antibiotic resistance (μg/mL):			
Apramycin	50	50	50
Ampicillin	−	−	−
Carbenicillin	10	50	10
Cephalothin Na salt	10	50	−
Gentamicin	50	50	50
Kanamycin	50	25	50
Hygromycin	10	50	10
Tobramycin	50	25	50
Spectinomycin	50	50	10
Oxytetracycline	−	25	−
Streptomycin	−	50	50
Rifamycin	−	10	−
Mol% G+C	69[a]	69.9[b]	ND
Plasmid	−	−	−
Fatty acids[c]: SCFA:BCFA ratio	Higher SCFA	Higher SCFA	Higher SCFA

Table 19.2 (continued)

Characteristics	*S. cellulosum* So ce1871^T =DSM 14627^T	*B. cruenta* By c2^T =DSM 14553^T	*J. thaxteri* Pl t4^T =DSM 14626^T
Major Fatty acids	$C_{16:0}$, *i*-$C_{17:0}$, *i*-$C_{15:0}$	$C_{16:1}\omega5c$, $C_{16:1}\omega7c$,	$C_{16:1}\omega7c$, $C_{18:1}$,
		$C_{18:1}\omega9c$, *i*-$C_{15:0}$	*i*-$C_{17:0}$, $C_{16:0}$
PUFAs	$C_{18:2}$	–	–

BCFA branched-chained fatty acids, *SCFA* straight-chained fatty acids, *PUFAs* polyunsaturated fatty acids, *ND* not determined
[a]Reichenbach (2005)
[b]Reichenbach et al. (2006)
[c]Garcia et al. (2011)

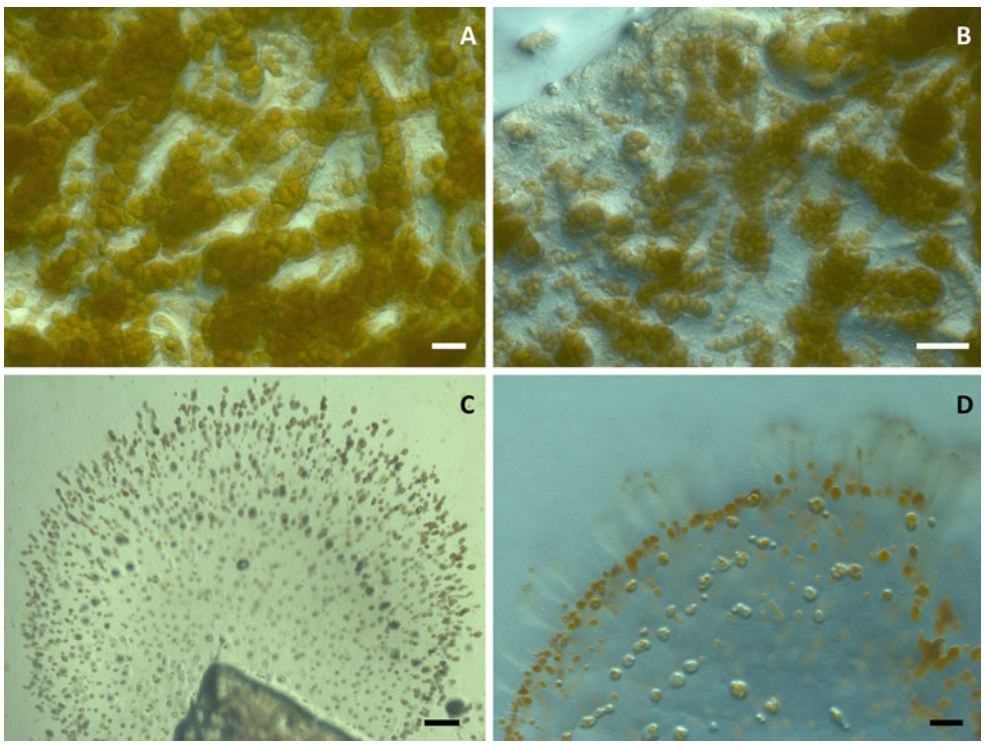

Fig. 19.6
Fruiting bodies of *Sorangium cellulosum* DSM 14627^T. Chains of sporangioles developed in filter paper fibers (a). Clumps and chains of sporangioles on decomposed filter paper (b). Radiating pattern of sporangioles and developing fruiting bodies in agar (c–d). Bar, 50 μm (a), 100 μm (b), 1,000 μm (c), 500 μm (d)

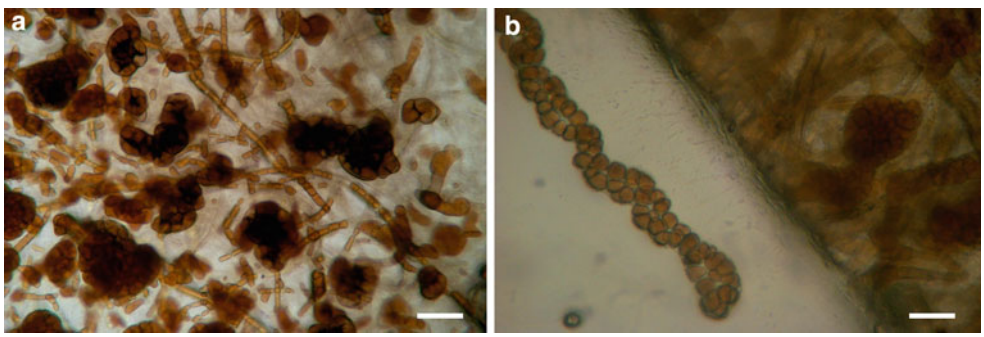

Fig. 19.7
Bright-field photomicrographs of *Sorangium cellulosum* fruiting bodies. Long chains and clusters of black polyhedral sporangioles in decomposed filter paper (a). Developed sporangioles at the edge of a filter paper (b). Bar, 300 μm (a), 150 μm (b)

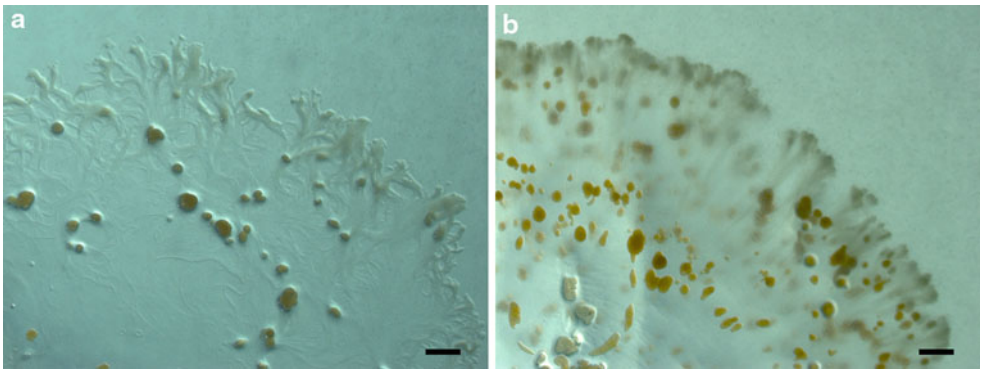

Fig. 19.8
Stereophotomicrographs of *Sorangium cellulosum* DSM 14627^T colony. Swarming veins with flare-like edges (a). Curtain-like pattern (colony edge) in agar and yellow aggregates of differentiating fruiting bodies. Bar, 500 μm

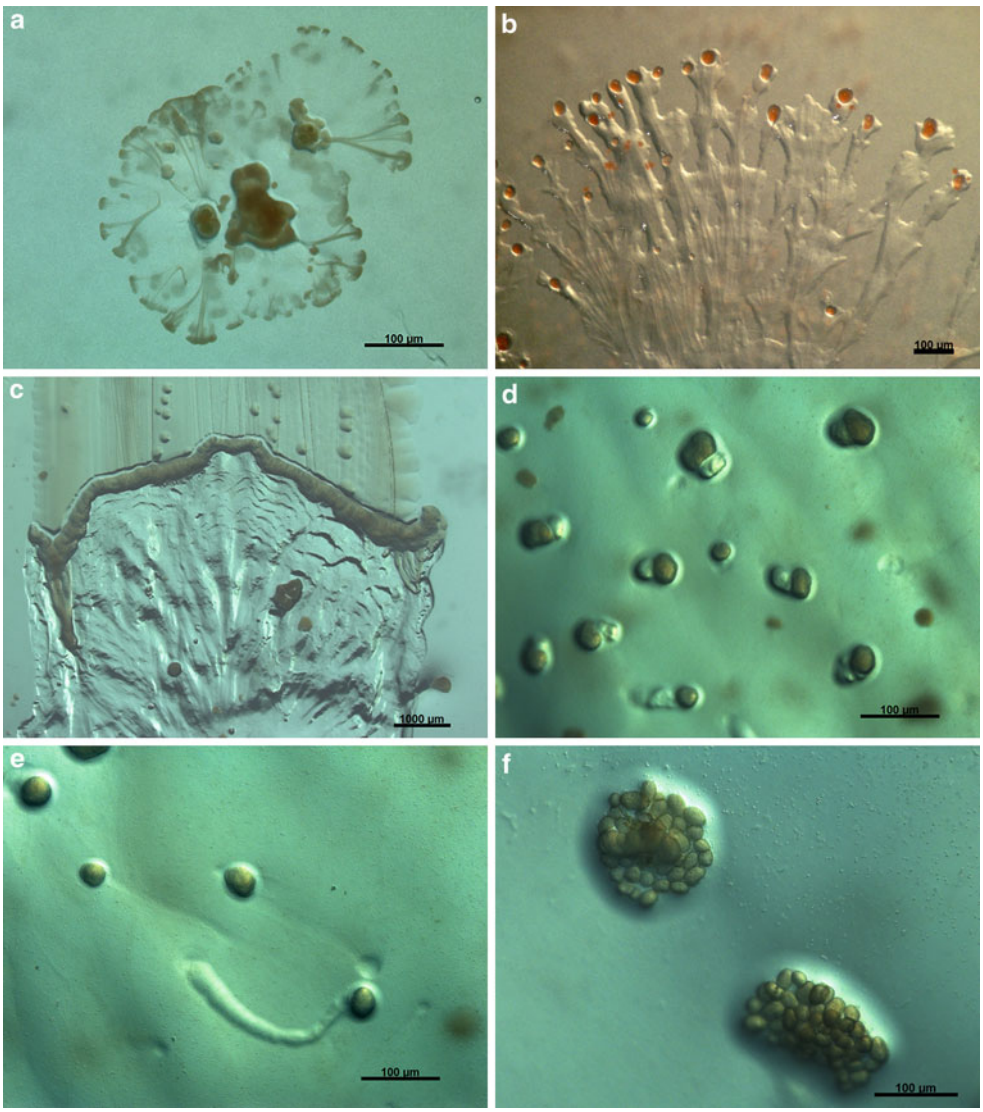

Fig. 19.9
Stereophotomicrographs of *Polyangium sorediatum* DSM 14670^T. Radiating pseudoplasmodial swarm with band edges and fan-shaped with long tails (a). Swarming tracks on agar produced by the migrating cell aggregates (b). Swarm roll lysing *Escherichia coli* (c). Cell aggregates appearing as mushroom- and comet-shaped forms (d–e). Cluster of oval fruiting bodies on agar (f)

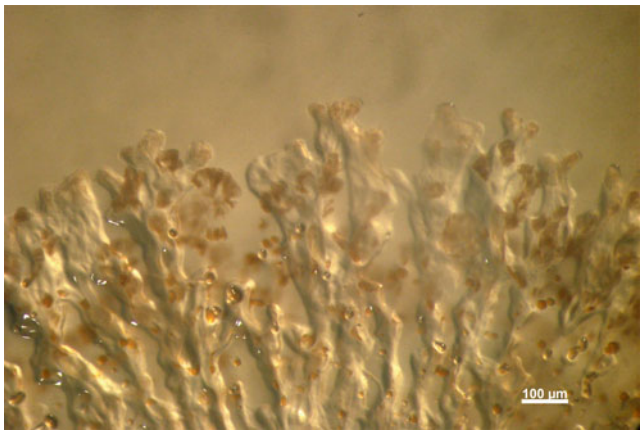

Fig. 19.10
Stereophotomicrographs of *Polyangium spumosum* DSM 14734^T showing the swarm agar tracks and cell aggregates

Chondromyces Berkeley and Curtis 1874

Chon.dro.my'ces. Gr. n. *chondros*, cartilage; Gr. masc. n. *mukês*, fungus N.L. masc. n *Chondromyces*, cartilaginous fungus.

Develop a sessile, spectacular, complex, and elegant miniature tree- or flowerlike fruiting body. The whole structure can be as high as 1,000 µm, or even bigger, and appears in shades of yellow to golden-orange color. A fruiting body consists of slime stalk and sporangioles. This unique and sophisticated structure has only one known counterpart with *Stigmatella* in *Cystobacteraceae* of suborder *Cystobacterineae* (see Chap. 2). Sporangioles may vary from ovoid-, barrel-, to turnip-shaped structures and can be impressively decorated with projecting hairlike structure or tails at the tip. All species in this genus are differentiated into species level by fruiting structure (*Figs. 19.11*, *19.12*, *19.13*, *19.14*, and *19.15*). Swarm colony appears thin, filmlike, and transparent to light orange and burrow in the medium (*Fig. 19.16*). The swarm is soft, non-stingy, easy to cut, and typically produce agar depressions and corrosions. Vegetative cells are composed of long and slender rods with blunted or rounded ends. Rod-shaped myxospores are tightly packed in the sporangioles. They are smaller than vegetative cells and more or less cylindrical with blunted ends too. Myxospores are slightly refractile, nonmotile, and heat and desiccation resistant. Exhibit bacteriolytic type of nutrition and are Congo-red negative.

The mol% G+C of DNA is 69–70. The type species is *Chondromyces crocatus*, isolated in 1988, while the type strain is Cm c5^T = DSM 14714^T = JCM 12616^T. The strain was isolated from a soil with decaying plants, collected in 1988 from a tropical rain forest, close to Iguacu, Brazil (Reichenbach 2005).

Chondromyces is composed of six validly described species, *C. crocatus*, *C. lanuginosus*, *C. apiculatus*, *C. robustus*, *C. pediculatus*, and *C. catenulatus*. Among them, only *C. catenulatus* has no designated live-type or neotype species; thus the discussions in this chapter is limited to the remaining five species. Despite the absence of live-type strain, *C. catenulatus* is still enlisted in the approved bacterial names (Skerman et al. 1980). Differentiating characteristics of *Chondromyces* species are shown in *Table 19.4*.

Jahnella Corrig. Reichenbach 2007, gen. nov.

Jah'ne.lla. N.L. fem. dim. n. *Jahnella*, named in honor of Eduard Adolf Wilhelm Jahn (1871–1942) who wrote the first synopsis (1911) and first monograph (1924) on myxobacteria.

Fruiting bodies (500–600 µm height or higher) consist of coils of sporangioles (*Fig. 19.17*), often elevated by means of slime cushions. Sporangioles may vary from yellowish to reddish brown shades and typically arranged as a cluster or, sometimes, solitary in agar. Colony burrows in the medium to produce a curtain-like or band appearance at the edge (*Fig. 19.18*). Swarm appears soft, and colors may vary from yellow, orange, golden-orange, to rusty brown. Furthermore, large, slimy, and mesh veins with partial agar depressions could also be produced. Swarm stains negative in Congo red. Vegetative cells are long and almost cylindrical rod with blunted ends (0.7–0.9 µm × 3–8 µm), phase dark, and move by gliding. Myxospores are short rods with blunted ends. *Jahnella* exemplifies the bacteriolytic but non-cellulolytic type of nutrition. *Table 19.2* shows the additional and diagnostic characteristics.

The mol% G+C of DNA is not determined. The type species is *Jahnella thaxteri*, isolated in 1988, with the type strain Pl t4^T = DSM 14626^T = JCM 12631^T. The strain was isolated from a soil sample collected in South Africa (Reichenbach 2005).

Isolation, Enrichment, and Maintenance Procedures

Sources of Myxobacteria

Members of the *Polyangiaceae* are widely spread in nature, and the choice of material for the isolation among members in this family depends on the target taxa. Soil containing decaying plant material appears to be an excellent source for the isolation of cellulose-degrading strains belonging to the genus *Sorangium* and *Byssovorax*. Often, not only these genera grow and colonize the substrates, however, members of *Jahnella*, *Polyangium*, and many *Chondromyces* species can also be isolated. In general, almost all members of *Polyangiaceae* can be isolated from soil and decaying plants. Using soil samples from tropical regions, fruiting bodies of some *Chondromyces* strains (e.g., *C. apiculatus*) could be found on surface of bracket fungi, phyllosphere (e.g., maize leaves), and tree barks (Garcia et al. 2009a). On the other hand, herbivore (e.g., rabbit) dung provides a good baiting substrate for many *Chondromyces* and *Polyangium* strains.

Table 19.3
Diagnostic characteristics among *Polyangium* species

Characteristics	*P. fumosum* PI fu5^T = DSM14668^T	*P. sorediatum* PI s12^T = DSM14670^T	*P. spumosum* PI sm5^T = DSM14734^T
Sporangiole color	Gray to black	Reddish brown	Brown
Growth at 30 °C	Good	Good	Good
Growth at pH 7.0	Good	Good	Good
NaCl tolerance (%)	ND	ND	0.5
Oxygen requirement	Aerobic	Aerobic	Aerobic
Lysis of organism:			
Gram− *Escherichia coli*	+	+	+
Pseudomonas syringae	−	−	−
Gram + *Bacillus subtilis*	+	+	+
Micrococcus luteus	−	−	−
Mycobacterium chitae	−	−	−
Yeast: *Saccharomyces cerevisiae*	−	−	−
Degradation: Agar	+ (partial)	+ (partial)	+ (partial)
Agarose	+ (partial)	+ (partial)	+ (partial)
Cellulose	−	−	−
Hydrolysis: Skim milk	+	+	+
Starch	+	+	+
Catalase	+	+	+
Antibiotic resistance (μg/mL):			
Apramycin	50	ND	50
Ampicillin	50	10	−
Carbenicillin	50	ND	−
Cephalothin Na salt	50	−	−
Gentamicin	50	10	50
Kanamycin	50	ND	50
Hygromycin	50	50	10
Tobramycin	50	ND	25
Spectinomycin	25	ND	10
Oxytetracycline	−	−	−
Streptomycin	25	ND	−
Rifamycin	25	−	10
Mol% G+C	ND	ND	ND
Plasmid	−	−	−
Fatty acids[a]: SCFA:BCFA ratio	Higher SCFA	Higher SCFA	Higher SCFA
Major Fatty acids	$C_{16:1}\omega7c$, *i*-$C_{15:0}$	$C_{16:1}\omega7c$, *i*-$C_{15:0}$	$C_{16:1}\omega7c$, *i*-$C_{15:0}$
PUFAs	−	Linoleic acid	Linoleic acid

All *Polyangium* species listed in this table represent the neotype/type strain (Reichenbach 2005)
BCFA branched-chained fatty acids, *SCFA* straight-chained fatty acids, *PUFAs* polyunsaturated fatty acids, *ND* not determined
[a]Garcia et al. (2011)

Collection, Processing, Treatment, and Storage of Samples

Freshly collected samples may be processed directly for isolation. If samples (e.g., soil, bark of trees, decaying plant material, and herbivore dung) cannot be processed immediately, they should be kept air-dried at room temperature for about 2–4 weeks. Samples could be capped in plastic containers or tubes. Moist and wet samples promote the growth of a variety of organisms including the unwanted and difficult-to-treat filamentous fungi.

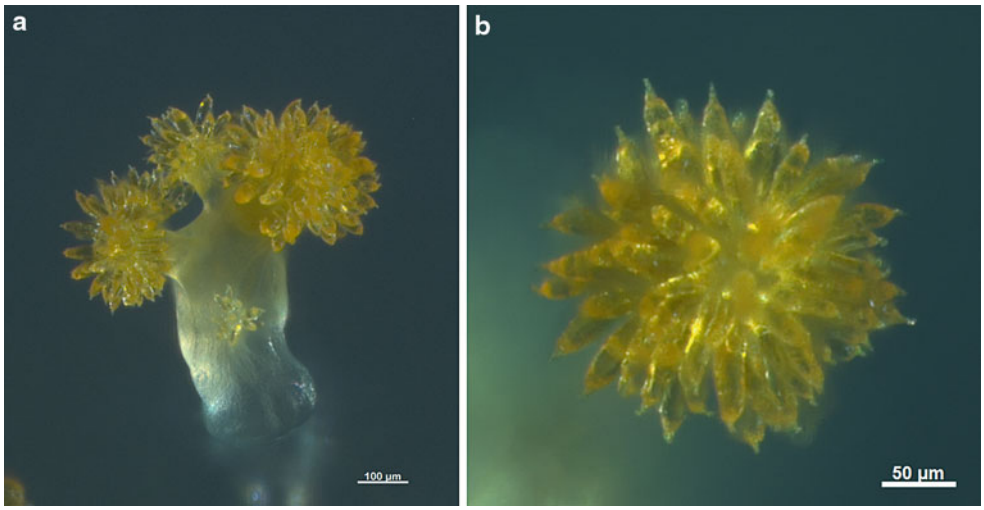

Fig. 19.11
Stereo photomicrographs of *Chondromyces apiculatus* Cm a14^T fruiting body. Branched-stalk showing clusters of sporangioles (a). Top view of a cluster of sporangiole showing its arrangement (b)

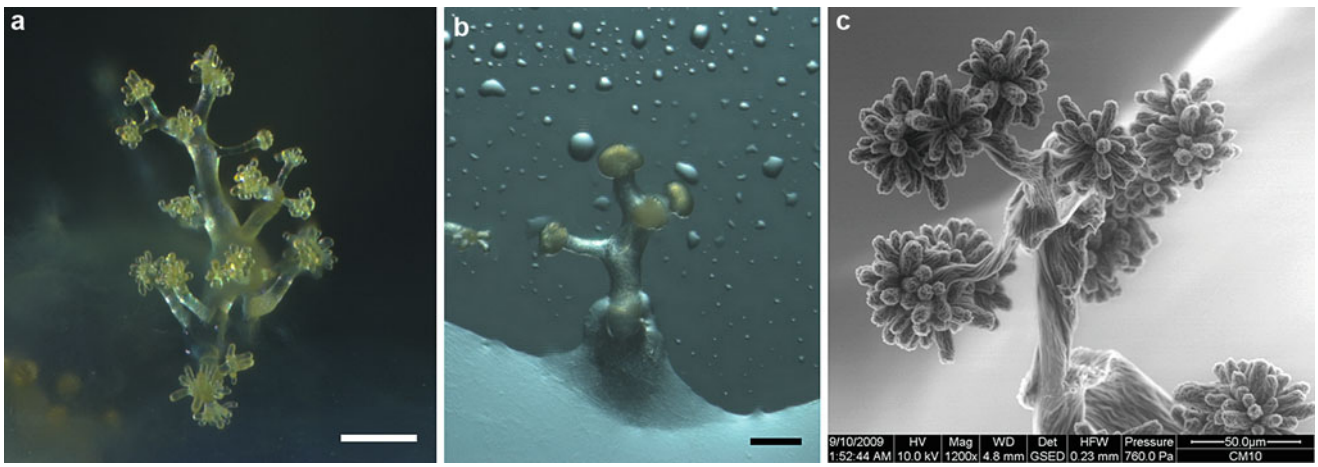

Fig. 19.12
***Chondromyces crocatus* fruiting body. Matured fruiting body bearing the ovoid cluster of sporangioles (a). Developing fruiting body with differentiating sporangioles on surface of Petri dish (b). Environmental scanning electron photomicrograph showing a branch of the fruiting body (c). (a–b) stereophotomicrographs. Bar, 200 µm (a), 100 µm (b)**

Dried samples can be permanently stored in the cupboard. In completely dried samples, *Polyangiaceae* could still be isolated even after years of storage. This is not surprising as members of this family have developed fruiting bodies encasing myxospores. Since myxospores are known to be resistant to desiccation and extreme temperatures, the adaptive survival mechanism of the organism to cope with the environmental conditions is secured. New population of cells is ensured upon germination of packed myxospore cells. The remaining unprocessed samples can be kept and stored dry for future isolation or could be used as a backup source for the re-isolation of lost isolates and those strains that cannot be revived after long years of storage in a freezer.

Setups for Cultivation

To isolate members of the *Polyangiaceae*, simple and lean media are required. Most members of this family grow in mineral salts ST21 agar (g per liter, solution A (K_2HPO_4 1, Difco Yeast extract 0.02, Agar 10, dissolved in two-thirds water volume); solution B (KNO_3 1, $MgSO_4{\cdot}7H_2O$ 1, $CaCl_2{\cdot}2H_2O$ 1, $FeCl_3$, 0.2, $MnSO_4{\cdot}7H_2O$ 0.1, dissolved in remaining water volume). After separate autoclaving, both solutions are combined with supplementation of 1 ml trace element solution, Shimkets et al. 2006), while other strains prefer simple buffered water agar medium—bufWA (g per liter, $CaCl_2{\cdot}2H_2O$ 0.5, HEPES 2.38, Agar 10, pH 7.0, adjusted with KOH). To avoid much

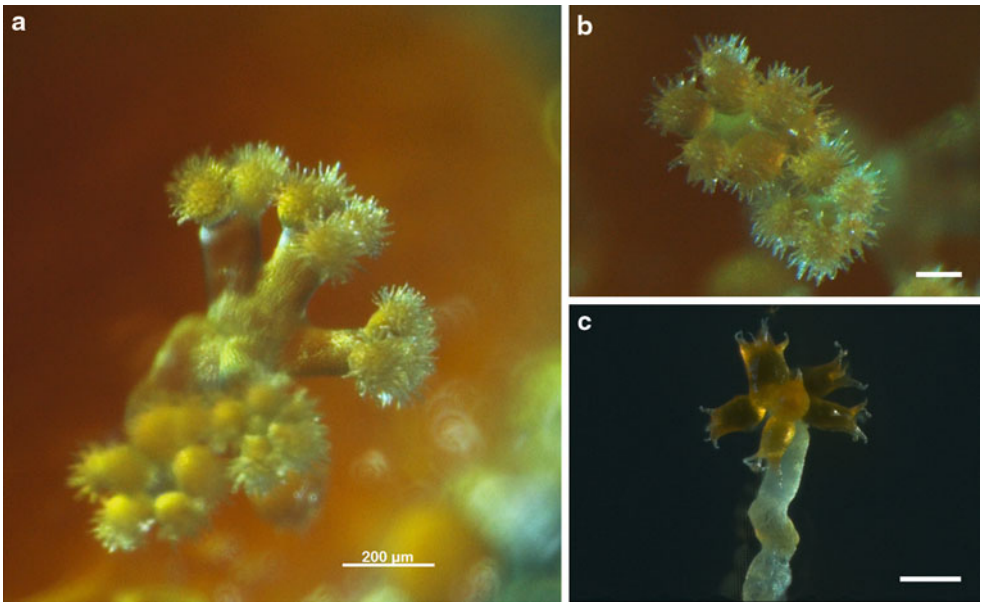

Fig. 19.13
Stereophotomicrographs of *Chondromyces lanuginosus* Sy t2^T (DSM 14631^T). Cluster of fruiting bodies (a). Top view of sporangioles showing the white and fine tails around the sporangiole (b). Cup-shaped sporangioles bearing few tails at the tip (c). Bar, 200 µm (a), 100 µm (b–c)

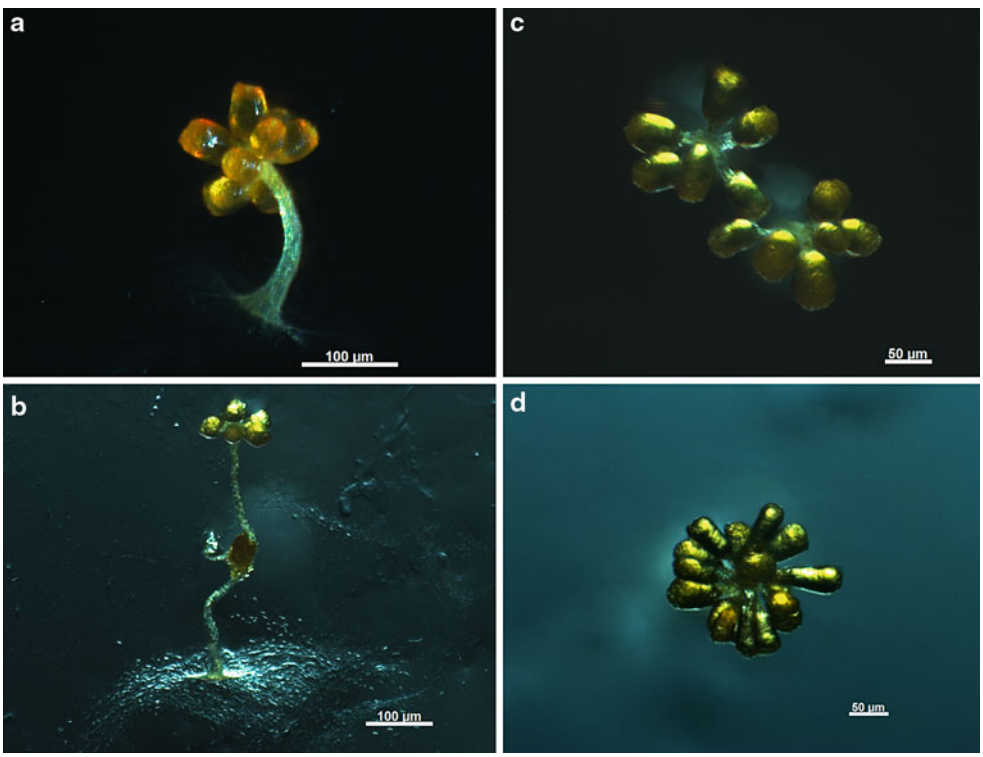

Fig. 19.14
Stereophotomicrographs of *Chondromyces pediculatus*. Fruiting body on a Petri dish (a). Secondary fruiting body developed on degenerated sporangioles (b). Barrel- and bell-shaped sporangioles connected to slime pedicels (c–d)

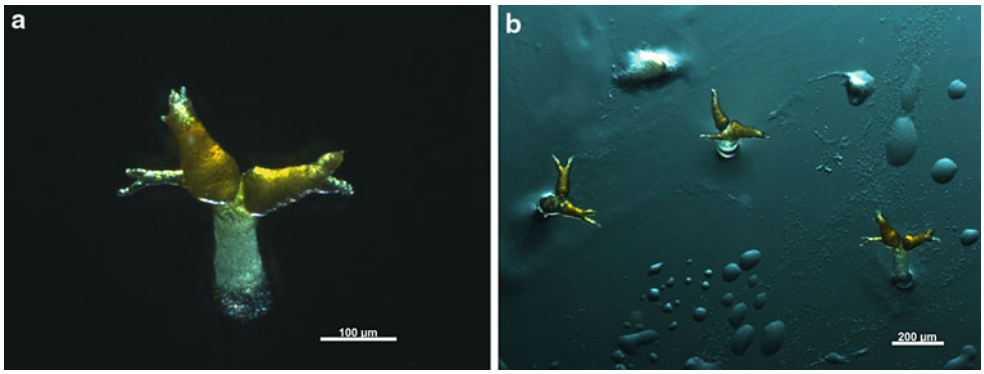

Fig. 19.15
Stereophotomicrographs of *Chondromyces robustus* Cm a13^T fruiting body in Petri dish showing the characteristic tails at the end of sporangiole (a–b). Background shows scattered vegetative cells and water droplets (b)

Fig. 19.16
Stereophotomicrographs of swarm colony surface. *Chondromyces lanuginosus* Sy t2^T (a). *C. apiculatus* Cm a14^T (b). *C. crocatus* Cm c5^T (c). Bar, 500 µm

precipitation of salts and to stabilize the pH, ST21 agar (Reichenbach and Dworkin 1992) was modified—MS21 (g per liter, KNO_3 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, $CaCl_2{\cdot}2H_2O$ 0.5, $FeCl_3$, 0.02, $MnSO_4{\cdot}7H_2O$ 0.1, HEPES 2.38, trace element solution (Drews 1974) 100 µL/L, yeast extract 0.02, Bacto agar 10, pH adjusted to 7.0 with KOH. After autoclaving, supplement with K_2HPO_4 0.4). In general, lean medium supports swarming and fruiting body formation, which are considered important in the isolation of most members in this family. Although complex and nutrient-rich medium may provide growth for members in this family, however, fastidious microorganisms are likely to outgrow them. Bacteriolytic-proteolytic-type strains in this family can be isolated by a microbial baiting method. Except for *Sorangium* which degrades filter paper or cellulose powder, all species known to date in this family are of the bacteriolytic-proteolytic type. Isolation setups are incubated at 28–30 °C. The baiting method using live bacterium (Singh Method, Singh 1947) either spotted or streaked on water agar supports the growth of many *Polyangium* but also other species belonging to *Nannocystineae* and *Cystobacterineae* suborders (see Chaps. 2, 14, 16). Since *Escherichia coli* do not spread over the agar and appear to be prey for most members of the family, they are used as the most preferred bait. All *Polyangiaceae* bacteriolytic-type members could also be isolated using this method.

Chondromyces and *Sorangium* appear mainly on mineral salts agar. Since the latter genus is considered cellulolytic, isolation could be performed by baiting with a small rectangular paper overlaid on the agar or by spotting with cellulose powder solution. The natural bacterial community present in the sample and moist environment provided by filter paper to hold liquid seem to be perfect conditions to promote growth of many *Chondromyces* species. *Byssovorax* is unique because it exhibits cellulolytic- and bacteriolytic-proteolytic-type nutrition and thus can be isolated in both bacterial and cellulose baiting methods.

Baiting using rabbit dung and animal herbivore on soil sample (Krzemieniewska and Krzemieniewski method, Krzemieniewska and Krzemieniewski 1946) exemplifies the early and classical method of isolation. This setup often yields species of *Chondromyces*, *Polyangium*, and *Byssovorax*. In our experience, many *Chondromyces* species (e.g., *Chondromyces apiculatus*, *C. lanuginosus*, and *C. robostus*) can be isolated using this method. In this setup, Petri dish is half filled with soil, and approximately six to eight rabbit dungs are partly buried and spread equidistantly in a circular manner.

Table 19.4

Diagnostic characteristics among *Chondromyces* species

Characteristics	*C. apiculatus* Cm a14^T = DSM14605^T	*C. crocatus* Cm c5^T = DSM14714^T	*C. lanuginosus* Sy t2^T = DSM14631^T	*C. pediculatus* Cm p51^T = DSM14607^T	*C. robustus* Cm a13^T = DSM14608^T
Isolation sources[a]	Dung, bark, rotting wood, soil	Decaying plant, bark, soil	Soil, dung	Soil, dung	Soil
Fruiting body: Stalk	Often unbranched	Often branched	Often branched	Unbranched	Unbranched
Height (μm)	230–700	600–>1,000	600–>1,000	350–1,000	500–>1,000
Sporangiole: Shape	Turnip	Ovoid	Cup-shaped	Bell-barrel	Globular-turnip
Number	20– > 70	> 100	2–5	4–15	3–20
Color	Orange	Yellow	Orange	Orange	Orange
Size:L × W (μm)	65–115 × 19–22	20–35 × 10–15	135–175 × 70–80	80–90 × 30–40	140–170 × 40–75
Arrangement	Cluster	Cluster	Cluster	Cluster	Cluster
Hairlike/tail No.	1	–	4–ca.60	–	1–3
Pedicel	+/–	–	–	+	–
Earthy smell	–	+	–	–	–
Growth at pH 7.0	Good	Good	Good	Good	Good
Growth at 30 °C	Good	Good	Good	Good	Good
Salt tolerance (%)	ND	1.0	0.5	0.5	0.5
Oxygen requirement	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Lysis of organism:					
Gram– *Escherichia coli*	+	+	+	+	+
Pseudomonas syringae	+	–	+	+	+
Gram+ *Bacillus subtilis*	+	+	+	+	+
Micrococcus luteus	+	+	+	+	+
Mycobacterium chitae	+	–	+	+	+
Yeast: *Saccharomyces cerevisiae*	+	+	+	+	+
Degradation: Agar	Partial	Partial	Partial	Partial	Partial
Agarose	Partial	Partial	Partial	Partial	Partial
Cellulose	–	–	–	–	–
Chitin	–	–	–	–	–
Hydrolysis: Skim milk	+	+	+	+	+
Starch	+	+	+	+	+
Catalase	+	+	+	+	+
Antibiotic Resistance (μg/mL)					
Apramycin	50	50	50	ND	ND
Ampicillin	50	50	–	ND	–
Carbenicillin	50	50	–	ND	ND
Cephalothin Na salt	50	50	–	ND	ND
Gentamicin	50	50	50	ND	10
Kanamycin	50	50	50	ND	ND
Hygromycin	50	50	10	ND	–
Tobramycin	50	50	25	ND	ND
Spectinomycin	25	50	10	ND	ND
Oxytetracycline	–	–	–	ND	–

Table 19.4 (continued)

Characteristics	*C. apiculatus* Cm a14^T = DSM14605^T	*C. crocatus* Cm c5^T = DSM14714^T	*C. lanuginosus* Sy t2^T = DSM14631^T	*C. pediculatus* Cm p51^T = DSM14607^T	*C. robustus* Cm a13^T = DSM14608^T
Streptomycin	25	50	–	ND	ND
Rifamycin	25	50	10	ND	ND
Mol% G+C[b]	69-70	70	ND	ND	ND
Plasmid	–	–	–	–	–
Fatty acids[c]: SCFA: BCFA ratio	Higher SCFA	Higher SCFA	Higher SCFA	Higher SCFA	Higher SCFA
Major	$C_{16:1}\omega7c$, *i*-$C_{15:0}$, $C_{17:1}$2OH	$C_{16:1}\omega7c$, *i*-$C_{15:0}$, $C_{17:1}$2OH	$C_{16:1}\omega7c$, *i*-$C_{15:0}$, $C_{17:1}$2OH	$C_{16:1}\omega7c$, C $_{16:0}$, *i*-$C_{15:0}$, $C_{17:1}$2OH	$C_{16:1}\omega7c$, $C_{18:1}\omega9c$, $C_{17:1}$2OH, *i*-$C_{15:0}$, $C_{18:2}\omega6,9c$
PUFAs	–	GLA, LA, ALA	–	–	–

All *Chondromyces* species listed in this table represent the neotype/type strain (Reichenbach 2005)
BCFA branched-chained fatty acids, *SCFA* straight-chained fatty acids, *PUFAs* polyunsaturated fatty acids, *ND* not determined, *GLA* gamma linolenic acid, *LA* linoleic acid, *ALA* alpha linolenic acid
[a,b]Reichenbach (2005). Species- rather than strain-associated
[c]Garcia et al. (2011)

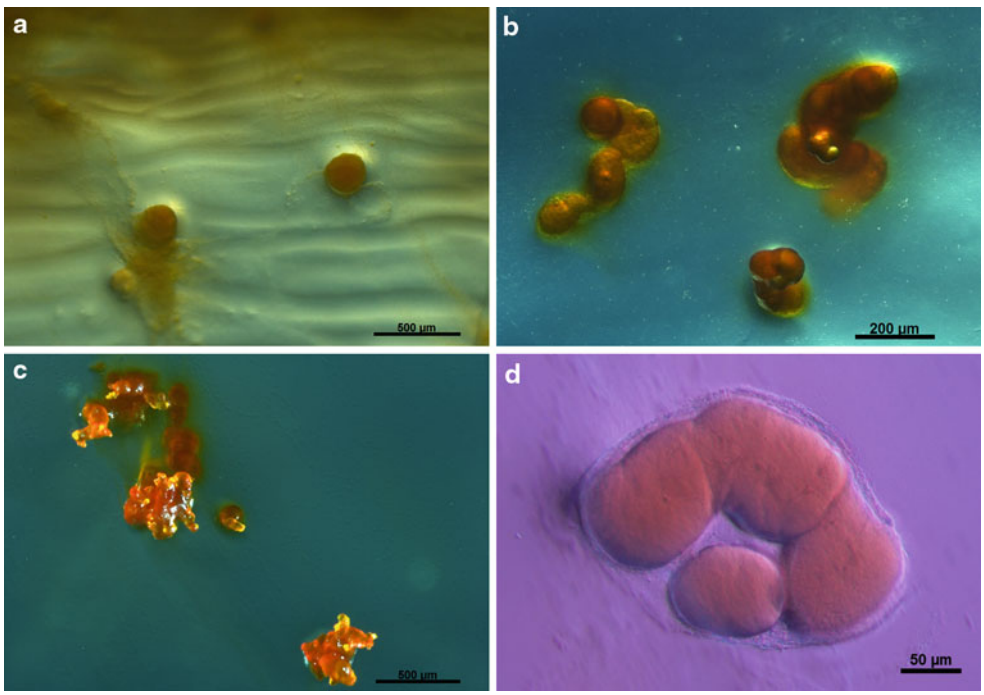

Fig. 19.17
Stereophotomicrographs of *Jahnella thaxteri* DSM 14626^T fruiting body. Mounds of cellular aggregate on surface of an agar (a). Coils of rusty orange-brown sporangioles in agar (b). Cluster of towering sporangioles on agar showing its resemblance to fruiting body of some *Cystobacter* strains (c). Phase-contrast photomicrograph of sporangiole showing an enclosing slimy envelope (d)

Soil is moderately moistened (not flooded or soaked) to its water-holding capacity to allow the *Polyangiaceae* growth and colonization on the bait. As this method is not selective to members of this family, other myxobacteria and non-myxobacterial strains are also expected to grow.

Moist (humidity) chamber method (Peterson 1969; Brock Neil et al. 2005) is a direct way of isolating myxobacteria from nature. Samples such as decaying plants, decomposing wood, bark of trees, and herbivore dung can be incubated directly in a Petri dish laid with filter paper moistened with water.

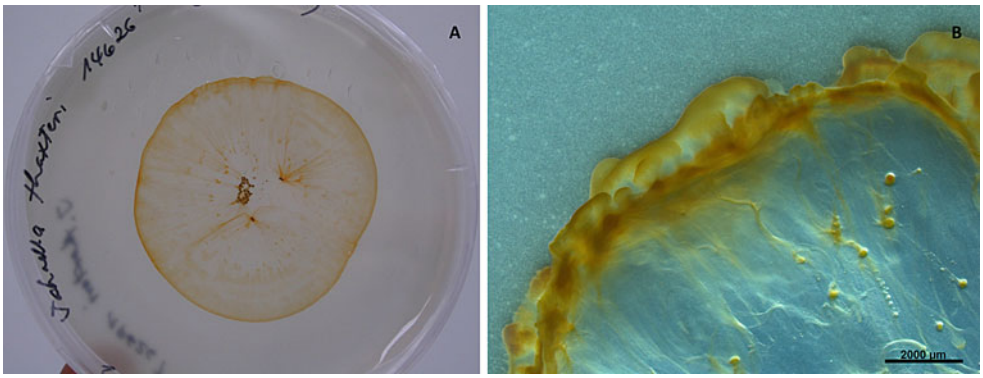

Fig. 19.18
***Jahnella thaxteri* DSM 14626^T. Colony on agar grown in 9 cm Petri dish (a). A rusty yellow-orange color of a swarm with long inner radiating veins (b)**

Chondromyces crocatus and *Chondromyces apiculatus* are among the most commonly isolated *Chondromyces* species in this setup, as well as in mineral salts agar. Fruiting bodies may sometimes develop scattered on the paper and sometimes even at the surrounding wall of the chamber.

Among other myxobacteria, *Polyangiaceae* grow much slower, and therefore, longer incubation time is required. Generally, members of myxobacterial families belonging to *Myxococcaceae* and *Cystobacteraceae* colonize within the first 2 weeks of incubation. To prevent growth of photosynthetic organisms, setups are commonly incubated in the dark. The use of cycloheximide (actidione) and nystatin to suppress fungal growth as well as levamisole (tetramisole) to inhibit amoeba and helminths may help in heavily contaminated samples. The latter two organisms create nuisance in the isolation of *Polyangiaceae* as they can spread easily together with bacterial contaminants and other microorganisms in agar medium. Cycloheximide is commonly added at 25–100 µg/mL concentration (Shimkets et al. 2006), while levamisole could also be added with the same concentration. However, both do not warrant total inhibition of the contaminants. A large amount of inoculum has the disadvantage that it also contains a huge load of contaminants. An observation on samples collected after rain usually is much easier to process for isolation due to lesser contaminants and may not need antibiotic supplementation.

Regardless of the method, isolation of *Polyangiaceae* does not only depend on the setup. Geographical location and the type of sample are the most important issues during the isolation. The tropical samples are far more diverse than those from temperate countries. Given the same method, *Chondromyces* and *Polyangium* species are, for example, more commonly found in equatorial countries. This observation has also been validated in the global study of myxobacterial distribution (Dawid 2000). On one hand, samples collected in a forest or in some unexplored environment often yield diverse and novel isolates (Garcia et al. 2009a, b). Last, the success of isolation in this family appears to be highly attributed to their growth appearance on the substrate and the ability to be recognized under the microscope.

Isolation and Purification

Isolation of *Polyangiaceae* deviates from the standard bacterial streaking method on agar plates. A colony derived from single cell often does not support growth of *Polyangiaceae*. Purification commonly starts from a swarm colony or fruiting body appearance on the medium. A small population of cells is needed to start the growth and is usually achieved by cutting a small piece of agar containing swarming cells at the farthest and cleanest colony edge. Transfer can be carried out in the same medium on a fresh agar plate, a lean medium (water agar with inoculated *E. coli* spots), or directly to buffered yeast medium. A streak of *Escherichia coli* on agar medium gives a satisfactory result in the isolation and purification of *Polyangium*, as this bacterial bait is lysed, and with development of pseudoplasmodial swarm. In the case of *Sorangium*, swarming and fruiting material can be inoculated on filter paper overlaid in modified mineral salt agar (MS21) and buffered yeast agar—bufVY/2 (g per liter, fresh Baker's yeast 5, $CaCl_2 \cdot 2H_2O$ 0.5, HEPES 1.19, Bacto agar 10, pH 7.0, adjusted with KOH) (Garcia et al. 2009b). To isolate from fruiting body elevated high above the substrate, sporangioles are typically plucked off (e.g., *Chondromyces*) using sterile fine glass or syringe needles. Fruiting bodies commonly adhering on agar substrates (e.g., *Polyangium*, *Jahnella*, *Sorangium*, and *Byssovorax*) could be isolated by careful lifting on the agar with fine needles. Since members of the *Polyangiaceae* bear sporangiole fruiting body, partial pressure to break the wall is sometimes necessary to allow myxospores to have contact with the agar. Cutting the farthest colony edge is often required to obtain pure isolate. Sometimes this process is

tedious and very time-consuming. The last step in the isolation is checking the purity of the isolated strain, which could be performed in clear media such as MD1 (grams per liter, Difco Casitone 3, $CaCl_2{\cdot}2H_2O$ 0.7, $MgSO_4{\cdot}7H_2O$ 2, Cyanocobalamin 0.5 μg/mL, trace element solution, Drews 1974) (Behrens et al. 1976) and Amb (grams per liter, Soluble starch 5, Difco Casitone 2.5, $MgSO_4{\cdot}7H_2O$ 0.5, K_2HPO_4 0.25) (Ringel et al. 1977).

Since *Polyangiaceae* grow slower than most other myxobacteria, isolation and purification plates should be incubated at a longer duration time (1–4 months). To prevent too much water loss in the medium, cultures are kept sealed with parafilm. Plates are normally stored in a dark environment to prevent the growth of photosynthetic organisms that may invade to cover the whole plate.

Contaminants and Control

The isolation of slow-growing *Polyangiaceae* is generally not straightforward. Tedious and hard-to-eliminate contaminants slow the process of isolation and purification. Large animals like spiders, mites, insects, and nematodes crawl on the isolation setup, and they carry and spread microbial contaminants all over the plate. They can be manually eliminated by lifting them up using fine forceps and needles. Alternatively, they can be killed by hot needle incineration. Microorganisms including amoeba, filamentous fungi, and other bacteria are the most difficult to eliminate contaminant, but they could be suppressed with antibiotics. In *Sorangium* crude culture, bacterial contaminants can be eliminated by treatment with kanamycin. This genus is remarkable for its high tolerance (1,000 μg/mL) to this antibiotic (Reichenbach et al. 2006). Many antibiotics may be helpful in the purification process. However, their incorporation in the isolation setup can restrict growth of *Polyangiaceae* and therefore is not recommended to be added unless a specific group or taxon is targeted.

In two-membered mixed cultures, dilution and spread-plating methods may possibly separate organisms into single colonies. However, these methods appear more applicable for a strain that can grow homogeneously in broth medium (e.g., *Sorangium*). Spread-, pour-, or streak-plate technique in a nutrient-rich medium (e.g., M-medium) could yield single-colony isolates. *Sorangium* can be easily identified by its orange or yellow color in the agar, which can then microscopically be verified by vegetative cell morphology. In some strains of *Chondromyces crocatus*, cocultured bacteria have been discovered (Jacobi et al. 1996, 1997). Although not all species of *C. crocatus* or other species of *Chondromyces* grow in the presence of associated bacteria, the cocultured bacteria could be eliminated by antibiotic treatment. It was also demonstrated that cocultured bacteria cannot survive after several generations (Jacobi et al. 1996); therefore, *Chondromyces* could be purified by simple repeated subcultivations.

Growth Cultivation and Maintenance

Pure culture isolates can be maintained in various media. For most *Sorangium* strains, they can be cultivated in M-medium (g per liter, Soy peptone 10, maltose monohydrate 10, $CaCl_2{\cdot}2H_2O$ 1, $MgSO_4{\cdot}7H_2O$ 1, ethylenediaminetetraacetic acid, iron (III)-sodium salt 0.008, HEPES 12) (Müller and Gerth 2006), P-medium (g per liter, Peptone 2, soluble starch 8, Probion 4, yeast extract 2, $CaCl_2{\cdot}2H_2O$ 1, $MgSO_4$ 1, HEPES 24, and glucose 2, supplemented the latter ingredient after autoclaving) (Pradella et al. 2002), and modified P-medium (grams per liter, Peptone 2, soluble starch 8, Probion 2, Yeast extract 1, $CaCl_2{\cdot}2H_2O$ 1, $MgSO_4$ 1, ethylenediaminetetraacetic acid, iron (III)-sodium salt 0.008, HEPES 23.8, pH7.5) (Garcia et al. 2009a). In many cases, *Sorangium* grows as orange clumps in M-medium, while a yellow to orange color could be observed in P-medium. Homogeneous suspension is often obtained in HS (g per liter, $MgSO_4{\cdot}7H_2O$ 1, KNO_3 1, peptone from casein 1.5, TRIZMA Base 2, NaFe-EDTA 0.008, pH 7.2 with KOH. After autoclaving, supplemented with K_2HPO_4 0.062, Glucose 4, and $CaCl_2{\cdot}2H_2O$ 0.075) and PM12 (grams per liter, casein peptone 0.4, $MgSO_4{\cdot}7H_2O$ 1.5, TRIZMA Base 2, pH 7.3, after autoclaving supplement with $CaCl_2{\cdot}2H_2O$ 1, NaFe-EDTA 0.008, KNO_3 2, K_2HPO_4 0.062, glucose 3.5, dithionite 0.1) (Kopp et al. 2004). Technical E-medium (grams per liter, skim milk powder 4, soy flour 4, starch 10, yeast extract 2, Glycerol 5 mL, $CaCl_2.2H_2O$ 1, $MgSO_4{\cdot}7H_2O$ 1, ethylenediaminetetraacetic acid, iron (III)-sodium salt 0.008, HEPES 12) has also been shown to provide satisfactory growth for cultivation of many *Sorangium* strains (Müller and Gerth 2006). Shaken broth cultures (180 rpm) can be kept for a maximum of 2 weeks or perhaps 4 weeks in a static condition. For longer storage, cultures can be inoculated on buffered yeast (bufVY/2) and CNS agar (g per liter, KNO_3 0.5, Na_2HPO_4 0.25, $MgSO_4{\cdot}7H_2O$ 1, $FeCl_2$ 0.01, HEPES 2.4, pH adjusted to 7.0–7.2 before autoclaving, Bollag et al. 1995) overlaid with small filter paper strips. The supplementation of a sugar source such as soluble starch, maltose, or glucose improves the growth of the strains.

Chondromyces, *Jahnella*, and *Polyangium* strains can also be maintained in VY/2 agar. Strains grow fast in this medium that the culture needs to be subcultivated (approximately 2 weeks) before the colony reaches the edge of the agar. In addition to yeast medium, *Chondromyces* can also be maintained in probion-based P-medium and with a maximum subcultivation interval of 2–3weeks. The addition of sterile filter paper strips, maltose, or both in VY/2 agar provides good carbon sources for maintenance of *Byssovorax* for a period of 4–6 weeks. Complex RG2 medium (grams per liter, fresh Baker's yeast 2.5, potato starch 2.5, soya meal 2, oat meal 1, $CaCl_2{\cdot}2H_2O$ 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, HEPES 2.38, Agar 15, pH adjusted to 7.0 with KOH before autoclaving) also supports the growth of many *Polyangiaceae*. Since myxobacteria are known for their gliding motility on surfaces, Petri dish cultivation is much preferred over the standard bacterial agar slant method as the

former gives wider space for swarming. Except for some moderately thermophilic strains which grows optimally at 42–44 °C (Gerth and Müller 2005), majority of the validly described *Polyangiaceae* known to date can be incubated and optimally growing at 30 °C.

Preservations and Reactivation of Cultures

Several methods have been employed to preserve *Polyangiaceae*, and these include cryopreservation, lyophilization, and desiccation. For cryopreservation, a concentrated amount of vegetative cells are important. Concentrated cells from 50 to 100 mL cultures are ideal for cryopreservation. Cells are suspended in small aliquots of fresh medium and re-suspended before being dispensed to cryo vials. A 20 % glycerol or DMSO concentration is added to the cell suspension before freezing in liquid nitrogen. Homogeneous and clumpy growing cultures can be stored this way. A concentrated cell pellet quickly frozen (with liquid nitrogen) without preservative can also be used as backup cryo culture. However, it has the disadvantage that the whole cryo culture can only be used once after thawing. Frozen cultures can be kept in −80 °C freezer or in a nitrogen tank. Liquid nitrogen storage has the advantage that myxobacteria could be stored for several decades. To revive the culture, a small amount of the concentrated cryo cultures is needed to inoculate in liquid medium or agar. *Polyangiaceae* strains (e.g., *Byssovorax*) can also be stored as agar blocks mixed with fresh liquid medium and cryoprotectant (e.g., glycerol, DMSO). Preferentially, agar plugs are sliced out from the colony edge with actively growing cells and containing a higher-cell density.

High-cell density and actively growing cells could be ideally preserved by freeze-drying. Although cells could be revived in this condition, however, no statistical data is yet available on their length of storage and percentage viability. Although lyophilization is not a reliable method for preservation (Reichenbach 1984) and not used by us for longtime storage of *Polyangiaceae* and other myxobacteria, the method may still be suitable after several trials of preservation optimizations. Most of our cultures are stored in −80 °C freezer and in liquid nitrogen tanks.

In general, newly isolated *Polyangiaceae* strains are capable of forming fruiting bodies. Completely developed mature fruiting bodies are ideal for desiccation using silica gel as they contain matured myxospores. In *Sorangium*, a fruiting body could easily be induced in filter paper overlaid to a yeast agar or on a mineral salt medium. Although *Byssovorax* could also form fruiting bodies on filter paper overlaid in yeast medium, this developmental stage appears to be rare (Reichenbach et al. 2006). *Jahnella*, *Chondromyces*, and *Polyangium* can also develop fruiting bodies in yeast medium even without cellulose source. If a strain has developed a fruiting body on paper, it could easily be lifted out and desiccated to around 2–4 weeks. In the case of fruiting bodies embedded in agar, desiccation could still be possible by agar incision and by mounting it to a sterile filter paper. Fruiting bodies can also be scraped using a loop or could be adhered to filter paper by wiping on culture surface. Desiccated fruiting bodies are stored at room temperature. Desiccation appears to be the cheapest way of preserving members of *Polyangiaceae*. However, it has the disadvantage of being dependent on matured fruiting bodies. To revive the desiccated culture, the filter paper containing the dried fruiting bodies can be simply inoculated by placing on a semisoft solid medium containing 0.8–1.0 % agar. Filter paper adsorbs water, which makes the fruiting body soft and allows the myxospores to break the sporangiole wall. Moreover, the semisoft agar also provides an easy access of the organism to nutrients and promotes faster swarming. Buffered yeast medium supports the germination and eventual vegetative colony swarming. Dried myxobacterial fruiting bodies can be brought to live culture even after 22 years of storage at room temperature (Reichenbach 2005).

Morphology and Developmental Stages

To date, phenotypic characteristics still serve as a very important and valuable marker to recognize myxobacteria belonging to *Polyangiaceae*. The family shares common characteristics in their life cycle. The most intricate and spectacular fruiting body formed after fascinating unicellular to multicellular development is doubtlessly found in the *Polyangiaceae*, specifically produced by the genus *Chondromyces*.

Vegetative Cells

Polyangiaceae vegetative cells are slender rods and could be considered bacilli type. Unlike many members of other myxobacterial family, *Polyangiaceae* vegetative cells glide much slower on surfaces and in substrate. Cell size varies according to the taxonomic group. *Sorangium* seems more variable in size (3–8 μm), while *Chondromyces*, *Polyangium*, and *Jahnella* appear much longer (8–10 μm). Newly isolated *Polyangiaceae* strains typically do not grow in suspension. In *Chondromyces crocatus*, a fine structure study of the vegetative cells performed by negative staining revealed the presence of fimbria (MacRae and McCurdy 1975). They are believed to be important in the swarming motility of the bacterium on the substrate. In *Myxococcus xanthus* (*Myxococcaceae* family), pili and fibrils were shown to be involved in contact-mediated cell-cell interactions (Dworkin 1996), whereas in *Chondromyces crocatus*, polar pili and amorphous slime layer have been postulated to provide an organized gliding movement (MacRae and McCurdy 1975). No studies have shown that flagella and capsules are found in *Polyangiaceae*. However, in many other bacteria, these structures are associated with the virulence and pathogenicity of the strain.

Swarm

All members of the *Polyangiaceae* share a typical swarm pattern. Colony produced by its members appears partly or deeply sunken in the medium, suggesting an agarolytic

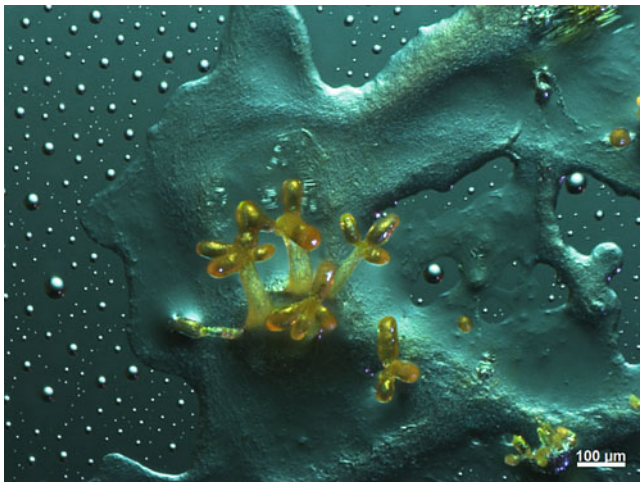

Fig. 19.19
Stereophotomicrograph of *Chondromyces* on surface of Petri dish showing the thin layer of swarm and clusters of fruiting bodies

characteristic. In *Chondromyces*, sometimes, a moist condition is enough for the strain to glide on the edge of a Petri dish or solid surface and is even satisfactory in producing fruiting bodies (*Fig. 19.19*). In contrast to *Cystobacteraceae* and *Myxococcaceae* which can cover the whole agar plate in couple of days, members of the *Polyangiaceae* glide much slower and take several weeks and even months to reach the edge of the plate. *Jahnella* and *Chondromyces* are also distinguished not only by the orange color of the swarm but also with few scattered veins in the colony. In *Jahnella*, the veins are more defined, thicker, penetrate deeper in the agar, and may appear teased at the edge. This pattern differs from almost transparent veins produced by *Sorangium* on surface of the agar. In semisoft agar, *Sorangium* swarm can produce a curtain-like appearance. Although almost all members of the *Polyangiaceae* can produce pseudoplasmodial swarm, however, this could be more defined in *Polyangium* and *Byssovorax*. Except for *Sorangium*, all members can create a cell roll or ridge swarm while prey-lysing other bacteria.

Fruiting Body

Among the myxobacteria, the *Polyangiaceae* could be considered the most structurally complex organisms. Fruiting bodies are normally developed in mineral salts, yeast agar, and lean medium, which are sometimes induced by microbial bait. A huge number of cells ($\sim 10^4$–10^6) seem to be required in the fruiting body development (Reichenbach 2001). All fruiting bodies in this family are composed of sporangioles produced either directly on substrate or developed above by means of stalk and slime cushion. *Chondromyces* are distinctively unique as it exhibits a tree- or floret-like structure fruiting body. A time-lapse video of *Chondromyces crocatus* illustrates the multicellular development from a partly elevated cell mound to a matured and complex treelike fruiting body (See online supplementary data). The process to complete the whole development may take 10 h, but it may take longer or shorter depending on the cell population (*Fig. 19.20*). In addition, the size, height, and complexity of the fruiting body may also depend on the population of cells destined to become fruiting bodies. During morphogenesis, a cell mound differentiates to form the stalk, branches, sporangioles, and myxospores. This intricate and fascinating process in a prokaryote is remarkable in myxobacteria but has a counterpart in slime molds.

Myxospores

Unlike *Myxococcaceae*, where a vegetative rod cell shortens to become a rounded refractile myxospore, *Polyangiaceae* vegetative cell transforms only to become a shorter and slightly refractile rod. During the fruiting body development, vegetative cells destined to become myxospores are assembled and packed in a protected case called a sporangiole. *Polyangiaceae* myxospores are characterized by being both desiccation and temperature resistant, ensuring protection from the external environment to allow the survival of the next cell population. Although the germination process in *Polyangiaceae* is not well understood, it seems that lytic enzymes play a role during the process.

Pigments and Unusual Bacterial Features

The major type of pigment in *Polyangiaceae* appears to be carotenoids. Most of the studies on their pigment have been described decades ago and have not been followed up since then. In *Polyangium*, spirilloxanthins and its ß-glucoside derivative, as well as glucoside fatty acid esters of myristic acid, have been identified (Jansen et al. 1995). In *Sorangium*, however, a nonesterified carotenoid rhamnoside has been found (Kleinig et al. 1971; Reichenbach and Kleinig 1984). *Chondromyces*, *Jahnella*, and *Byssovorax* may perhaps contain different carotenoid pigments since their culture vary from orange to a rusty brown and red color.

Byssovorax: The strain described in this genus appears to be unusual for the development of bloodred knobs (250–600 µm) and deep-red ring structures (350–500 µm outside diameter, 60–120 µm inner diameter), and often, these result from pseudoplasmodial contraction (Reichenbach et al. 2006). In some occasions, an unknown bowl- or cuplike-shaped structure (800 µm wide, 300 µm high) may be produced. Scattered, roundish, and colorless shade to whitish crystals may be seen around or within the colony (*Fig. 19.21*). This shows similarities to degenerated fruiting bodies of some myxobacterial strains. Unusual rings, such as a halo around the colony, may also be formed (*Fig. 19.22*). The former is believed to be resulted from pseudoplasmodial contraction (Reichenbach 2005), while the latter appears to be associated to secondary metabolites. In old cultures, bright-red oily droplets may also be produced (Reichenbach et al. 2006).

Polyangium: Mounds and long transparent slime ridges, assuming the shape of a mushroom or a comet, could be

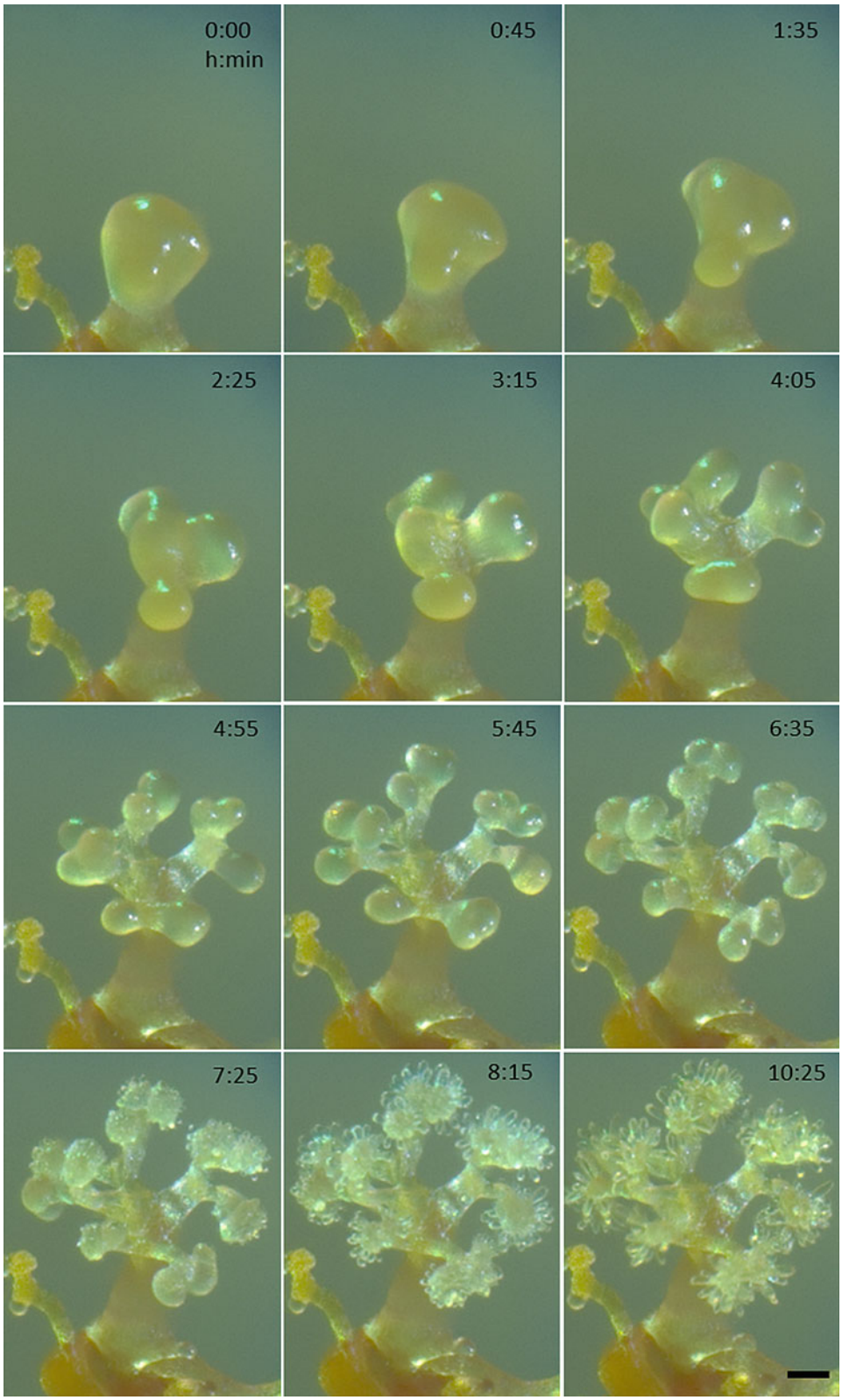

Fig. 19.20
Time-lapse stereophotomicrographs of *Chondromyces crocatus* SBCm016 fruiting body morphogenesis. Bar, 50 μm

produced in *Polyangium* culture. Often the slime is produced during cellular aggregation and in fruiting body development. In some cases, the slime can be converted to a stalk bearing the sporangioles (◉ *Fig. 19.23*).

Chondromyces: Strains that belong to this genus may produce secondary or even tertiary fruiting bodies on top of each other (◉ *Fig. 19.24*). In *Chondromyces lanuginosus*, a stalk can be highly branched and kinked after a series of fruiting body development. The stalk seems to be robust and hard that it remains standing on the agar even after the culture has died (◉ *Fig. 19.25*). In some species, basal plate at the stalk and umbels connected to a pedicel are remarkable. *Chondromyces crocatus* strains and some other myxobacteria are unusual for their earthy odor, which seems to be associated with the volatile compound geosmin (Schulz et al. 2004; Trowitzsch et al. 1981).

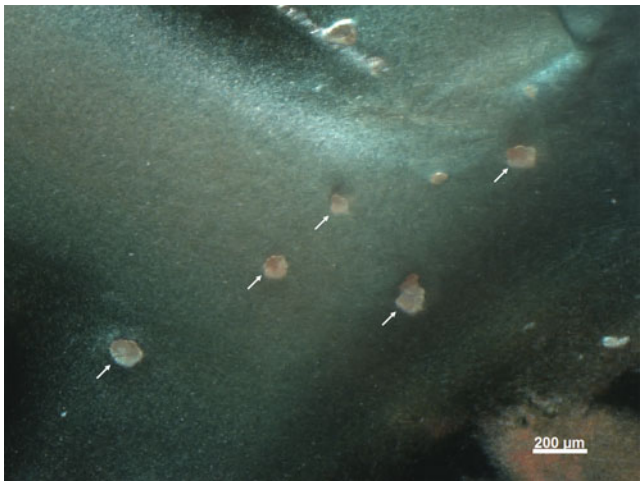

Fig. 19.21
Crystal-like structures (*arrows*) appearing in older culture of *Byssovorax cruenta* DSM 14553^T

Ecology

Myxobacteria have been found to be globally distributed in all continents and can be isolated from all types of vegetations. Similar results were observed in the worldwide search for this group of bacteria (Dawid 2000). Tropical regions and semiarid places appear to be the richest and most diverse sources. A small amount of soil sample (milligram scale) appears to be enough to isolate a variety of representative *Polyangiaceae*. In a soil environment, nearly all kinds of *Polyangiaceae* may appear, with *Sorangium* and *Polyangium* being the most common. *Polyangium* accounts to 42.1 %, while *Sorangium* tallied to about 5.2 % in global occurrence (Dawid 2000). Although *Chondromyces* were reported to be rarely found (Reichenbach 2005), our findings in tropical samples contradict this statement as this genus occurred in many samples. *Jahnella* and *Byssovorax* are among the rarely seen genera in our isolation program.

In soil samples containing decaying plant materials, diverse species of *Sorangium*, *Polyangium*, and *Chondromyces* could be isolated. *Sorangium* is almost always noticeable by the orange and yellow swarm degrading the filter paper bait. Since *Sorangium* is associated with the decomposition of plant material (as known cellulose degraders), they could easily be transported by wind, water current, and flood after heavy rain.

Bark of trees and decaying wood are additional ideal sources for some *Chondromyces* strains. Moreover, phyllosphere and the surface of bracket fungi represent unexplored habitat for the discovery of *Chondromyces apiculatus* and related species.

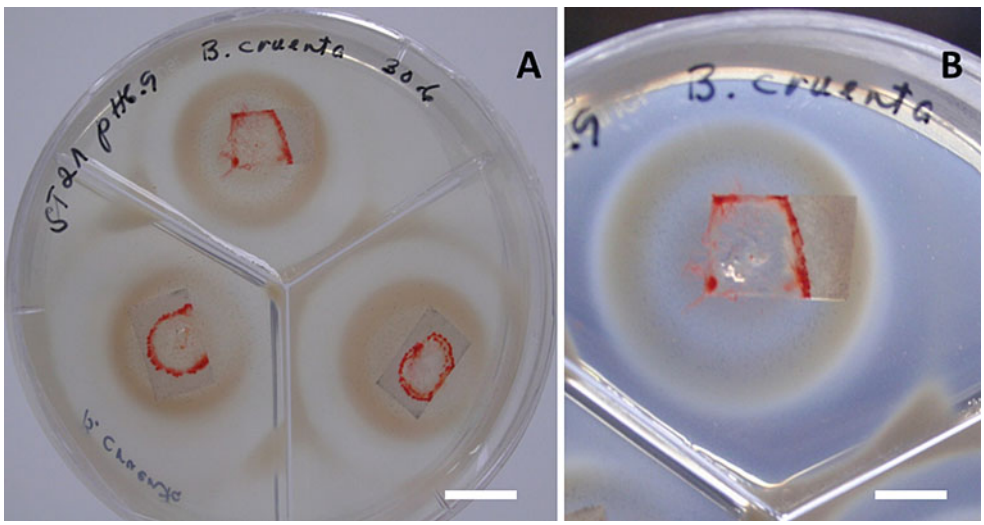

Fig. 19.22
Halo around the colony in older culture of *Byssovorax cruenta* DSM 14553^T grown in mineral salt agar. Petri dish diameter, 9 cm

The appearance of several fruiting bodies on the latter source could be seen after the rain as they appear to be flushed from tree bark. Some *Chondromyces* species are also often encountered on the rabbit dung bait. The widespread distribution of the *Chondromyces* seems to be associated to its inherent fruiting body structure, as they can easily get dispersed in nature. Since sporangioles are simply connected by a delicate slime pedicel (at least, in some strains), they could easily and accidentally be plucked off by many small animals like mites, spiders, worms, centipedes, and many other insects. In some species (e.g., *C. lanuginosus*, *C. apiculatus*), they could perhaps be easily dispersed by air or water due to their taillike structure at the sporangiole.

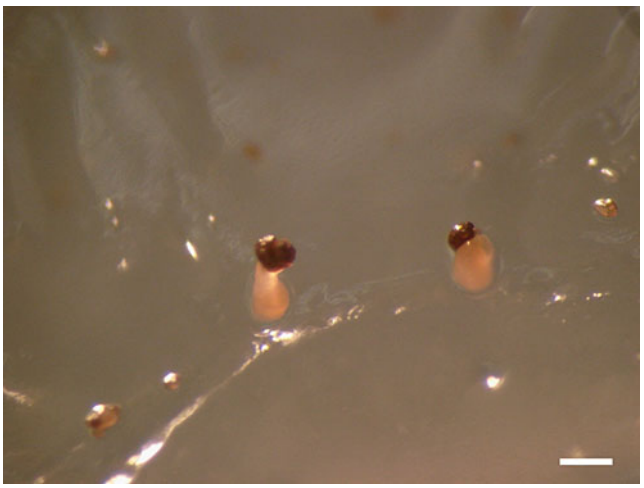

Fig. 19.23
Stereophotomicrograph of *Polyangium* sp. fruiting body on an agar showing the cluster of sporangioles produced on a slimy stalk. Bar, 400 μm

Although *Polyangium* is widely spread in soil samples, some strains of *Polyangium luteum* were reported to be inhabiting rabbit dung (Kühlwein and Schlicke 1971), while others (e.g., *Polyangium parasiticum*) have been described to parasitize green alga (Geitler 1925).

Extreme environments have also been documented for the presence of *Polyangiaceae*. Many *Sorangium* strains that can grow optimally above 40 °C were isolated in the Mediterranean and from desserts (Gerth and Müller 2005). Some strains discovered from the hot springs of Japan also appear to be in the *Polyangiaceae* cluster (Iizuka et al. 2006). Although these strains have not been isolated, they seem to represent the thermophilic group of *Polyangiaceae*.

A high-altitude environment appears to be also a reservoir for *Polyangiaceae*. *Sorangium* strains can also been found and isolated in soil samples containing plant material collected in the Alps. Studies on Antarctic (Dawid et al. 1988; Dawid 2000), Canadian, and Alaskan Arctic soils (Brockman and Boyd 1963) support the presence of psychrophilic myxobacteria, which most likely belong to *Polyangiaceae*. Phenotypically, some of the Arctic strains described by Dawid and coworkers (1988) show similar growth characteristics to *Polyangiaceae*. Not all strains that could be isolated from cold environment could be considered psychrophilic. It is also possible that some mesophilic strains may grow at a lower temperature (Menne and Ruckert 1988; Zhukova 1963). *Polyangiaceae* are a unique group of organisms that appear more adapted to growth at a wide temperature range.

Unlike some of the halophilic and halotolerant members of the *Kofleriaceae* (Fudou et al. 2002) and *Nannocystaceae* (Iizuka et al. 1998, 2003a, b, 2012; Schäberle et al. 2010), as of now, no *Polyangiaceae* has been isolated from the marine environment. However, previous metagenomic and phylogenetic studies revealed their presence in marine sediments and additionally, identified their unique position in the *Sorangiineae* clade (Brinkhoff et al. 2012; Moyer et al. 1995). Evidence for

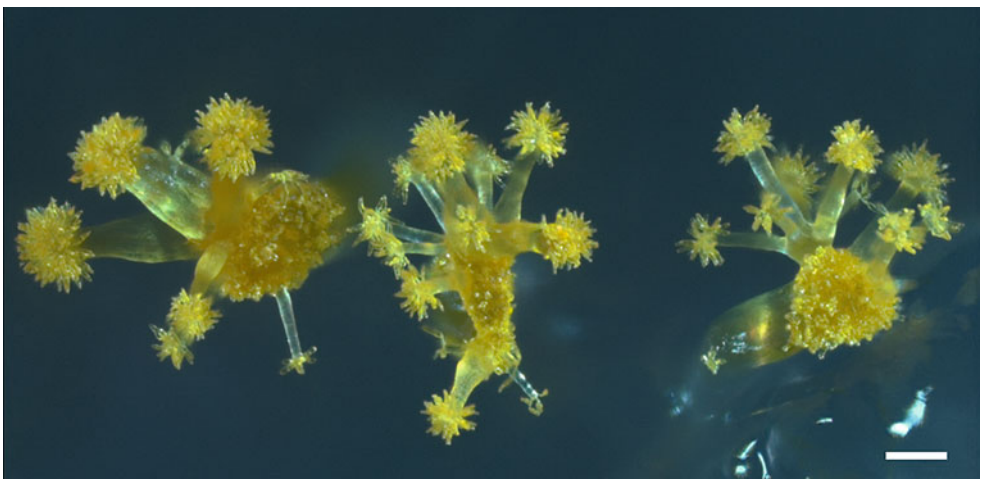

Fig. 19.24
Stereo photomicrographs of *Chondromyces apiculatus* Cm a14^T showing the secondary fruiting bodies. Bar, 350 μm

Fig. 19.25
Stereophotomicrograph of *Chondromyces lanuginosus* Sy t2^T-degenerated fruiting bodies showing only the remaining stalk and cell aggregate at the tip

cultivation among this group has already been shown in early studies on South Carolina coast beaches yielding a strain called "*Chondromyces cylindricus*" (Brockman 1967). However, this may not be a true halophile. In the future, a barophilic strain may be discovered in a marine environment.

Physiology and Metabolism

The five genera in this family are considered aerobic to microaerophilic. To date, no anaerobic member of *Polyangiaceae* has been discovered that could counterpart *Anaeromyxobacter* in *Cystobacterineae* (Sanford et al. 2002), although some uncultured strains seem to exist in hot spring environments (Iizuka et al. 2006). Unlike other families of myxobacteria, in general, members of the *Polyangiaceae* grow much slower. In *Sorangium*, for strains cultivated in a Casitone-based medium, the generation time was measured between 22 and 24 h (Sarao et al. 1985). In other *S. cellulosum* strains, generation time may reach 11 h at 30 °C incubation (Gerth et al. 1994).

Growth temperature optimal for most *Polyangiaceae* appears to be in the mesophilic range (26–34 °C). Since many of these bacteria were originally isolated from the tropics, mesophilic growth is expected. Some *Sorangium* and *Polyangium* strains can tolerate growth at 40 °C (McCurdy 1969), while other strains optimally grow at 37–42 °C (Gerth and Müller 2005). These strains could be considered moderately thermophilic and appear to be correlated to their adaptation to the environment they are isolated from (e.g., warm and semiarid habitat).

The optimal pH for members of the *Polyangiaceae* appears to be in the neutral or slightly alkaline range (pH 6.8–7.5). In most cases, the culture medium is adjusted to this range using a buffer solution. HEPES, a common buffer in media, maintains a stable pH at a neutral to slightly alkaline range. Most members of this family grow better in the slightly alkaline condition (pH 7.2), however, with an exception of a *Polyangium* strain documented to be growing in pH 3.2–3.3 from undisturbed peat bog sample collected in Belgium (Dawid 2000). Cellulose-degrading strains can tolerate a slightly acidic pH (~6.2) but at a reduced growth rate (Reichenbach 1999a). Poor growth can be observed below pH 5.0 and above pH 8.0. So far, no isolated strains considered truly acidophile and alkalinophile have been isolated in this family. Pressure tolerance in *Polyangiaceae* has never been determined, and no studies have shown that members of this family can tolerate extreme pressure. This characteristic is perhaps an adaptation to an environment, such as the deep sea ocean floor.

In general, terrestrial *Polyangiaceae* isolates tolerate only a minor amount of sodium chloride (~0.5–1.0 % w/v). At 0.5 % salt, many *Chondromyces* species could still be able to swarm on the agar; however, some strains of *Sorangium* and *Jahnella* can resist up to 1 % NaCl, but at a much slower growth. Moreover, with increased salt concentration, fruiting body development appears to be affected. Similar observations have been reported in non-*Polyangiaceae* isolates from marine samples collected in Shandong province, China (Li et al. 2002). The salt tolerance of *Polyangiaceae* could perhaps also be reflected on the exposure and adaptation of the strain to the saline environment. Truly halophilic member of this family has never been described but may also be interesting for novel secondary metabolites, just like their marine neighbors in *Nannocystineae* (Ojika et al. 2008).

Degradation of complex bio-macromolecules is strongly associated with *Polyangiaceae*. *Sorangium* and *Byssovorax* completely decompose filter paper and cellulose materials. In addition, *Sorangium* has also been implicated in the degradation of DNA and RNA (McCurdy 1969). Interestingly, almost all species in *Polyangiaceae* are able to hydrolyze gelatin, skim milk, dextrin, xylan, and starch (Reichenbach 2005). Breakdown of chitin has been observed among strains of *Byssovorax*, *Jahnella*, and *Sorangium*. Holes, depressions, and even partial liquefaction of solid medium are suggestive that all members of the *Polyangiaceae* are potential agar degraders. Hydrolytic enzymes, cellulases (e.g., exocellulase), proteases, etc., appear to be responsible for the degradation of many complex molecules and so far have not been well studied.

Physiological characterization using commercial bacterial identification kits does not appear to be useful for *Polyangiaceae*. The recommended media for these kits normally do not support the growth of strains belonging to this family. In addition, the kit recommends short incubation time which does not meet the requirement for the cultivation of slow-growing *Polyangiaceae*. In addition, the miniature kit has the disadvantage of drying fast before the bacteria grow or the reaction could be observed. In general, the physiological characterization of members of this family is performed individually according to the tested parameter.

Except for *Sorangium*, all genera belonging to *Polyangiaceae* are regarded as microbial predators capable of lysing a variety of live bacteria and eukaryotic organisms (e.g., yeast and unicellular algae). Gram-negative bacteria appear to be the most preferred prey of most members. Lysis of cyanobacteria has also

been implicated in *Polyangiaceae* and also appears to be a nutrient and vitamin source. Many *Chondromyces* species are noted to completely degrade single cell *Methylomonas clara* (Probion) and yeast as well.

Many strains in this family utilize enzymatically digested casein (e.g., Casitone) as an organic nitrogen source but require only a minimal amount (0.1–0.3 %). Although *Sorangium* can grow on a medium supplemented with potassium nitrate or ammonium sulfate as inorganic nitrogen sources, improved growth conditions can be achieved after supplementation of a small amount of enzymatically digested casein (e.g., Casitone). Sugars such as maltose, cellobiose, starch, and glucose appear to be an important carbohydrate source for cellulose-degrading *Byssovorax* and *Sorangium*. Maltose appears to be much preferred by *Byssovorax cruenta* (Reichenbach et al. 2006). Many *Chondromyces* strains grow better if starch is incorporated in the medium. Other carbon sources such as glycerol have also been successfully used for cultivation of many *Sorangium* strains.

Symbiosis, Pathogenicity, and Clinical Relevance

So far, microbial symbiosis in *Polyangiaceae* has only been described in *Chondromyces* strains. Previous studies have shown that *Chondromyces crocatus* grows in the presence of a symbiont (Jacobi et al. 1996, 1997). Based on the 16S rRNA gene sequence and molecular phylogenetic analysis, the symbiont was identified as a Gram-negative *Sphingobacterium*. This symbiont seems to be crucial as in its absence *Chondromyces* would not grow.

Apart from the algal freshwater parasite *Polyangium parasiticum* (Geitler 1924, 1925), none of the isolated and validly described members of the *Polyangiaceae* are considered pathogenic. Members of this family are classified as WHO Risk Group 1 organism, meaning that there is either no risk or low individual and community risk. Recently, a clone of an uncultured bacterium which appears to be the epizootic bovine agent (EBA) causing abortion in cattle (foothill abortion) has been identified to be distantly related (89.4 % 16S rRNA gene sequence similarity) to *Sorangium* (*Polyangium*) *cellulosum* (King et al. 2005). Based on myxobacterial 16S rRNA gene phylogeny, the EBA agent was determined as clustered with the *Sorangiineae* suborder but not found within a *Polyangiaceae* clade.

Application

Myxobacteria are one of the most proficient sources for many biotechnological applications. They belong to the few bacterial groups producing bioactive small molecules which appear to be used by them to kill or prey on other organisms and defend their ecological niche (Reichenbach 1999b). Since myxobacterial colonies, including *Polyangiaceae*, grow in a common slime sheet (Reichenbach 2001), the need to produce antibiotics seems justified to protect themselves from other organisms. The diverse and structurally unique secondary metabolites produced by this group of bacteria, with over 100 basic core structures elucidated to date, have led them to be recognized as one of nature's outstanding prokaryotic producers of bioactive compounds (Reichenbach 2001; Reichenbach and Höfle 1993, 1999; Gerth et al. 2003; Bode and Müller 2006, 2008; Weissman and Müller 2009, 2010; Wenzel and Müller 2009). Recently, their intriguing potential for new pharmaceutical leads has come to an industrial realization by approving a derivative of epothilone as an anticancer drug (Mulzer 2009). A total of 50 unique basic structures (no overlapping with the genus in the family) or 50 % of the myxobacterial compounds are derived from *Polyangiaceae* (*Table 19.5*). Most of them are polyketides, non-ribosomal peptides, or hybrids thereof (Weissman and Müller 2010). The overwhelming number of compounds in *Polyangiaceae* appears to be associated with the high number of strains isolated in this family (*Fig. 19.26*). Although not all genera are equally well explored in this family, the genus *Sorangium* produces most of the compounds (37), which is followed by *Chondromyces*, with eight novel scaffolds known. The number of substances discovered in *Sorangium* appears proportionally correlated to its large number of strains (23 %) at our collection. The compound diversity in *Polyangiaceae* is overwhelming and is predicted to increase in the future as more members in this family are continuously being discovered.

Recent reviews on myxobacterial secondary metabolites have identified 32 major classes of cytotoxic compounds (Weissman and Müller 2010), 11 of which are derived from the genus *Sorangium*. Some of the most interesting compounds from the *Polyangiaceae* family are briefly discussed here and shown in *Fig. 19.27*. The most prestigious among these compounds which has been developed as a pharmaceutical drug is epothilone. The compound was isolated at the German Research Centre Biotechnology (GBF, now Helmholtz Centre for Infection Research) by Reichenbach, Höfle, and colleagues in 1987 from *Sorangium cellulosum* strain So ce90 (Gerth et al. 1996; Höfle 2009). The discovery was driven by its strong antifungal activity towards *Mucor hiemalis* (Reichenbach and Höfle 1999). Epothilones were then rediscovered at Merck (from *Sorangium cellulosum* SMP 44, Peterson collection) as microtubule-stabilizing agents with a Taxol-like bioactivity (Bollag et al. 1995). This effect appears to be rare as a similar search in a large screening program performed by the Upjohn Company utilizing about 67,000 synthetic compounds and 70,000 natural product extracts did not reveal any hits (Lavelle 1995). After 20 years of subsequent research, epothilone was finally FDA approved as an anticancer drug (brand name, Ixempra^{R}; generic name, Ixabipelone) in 2007. The drug is currently marketed by Bristol-Myers Squibb for the treatment of locally advanced or metastatic breast cancer.

Disorazol, a macrocyclic compound bearing two oxazole rings, was also found acting on tubulins. However, destabilization of microtubule is caused by the compound. The compound was discovered from *Sorangium cellulosum* strain So ce12.

Table 19.5

Secondary metabolites in *Polyangiaceae* and their biological activities

Genus	Compound	Structural class	Biological activity	Mode of action	No.
Polyangium	Phenoxan	γ-pyrone	Antifungal, anti-HIV	Inhibits complex I respiration	
	Thiangazole	Tris-thiazolonine	Cytotoxic, antifungal, insecticidal, antiparasitic, anti-HIV	Inhibits complex I respiration	2
Chondromyces	Apicularen	Benzalactone enamide	Cytotoxic, antibacterial	Inhibits vacuolar-type ATPases, disruption of microtubule architecture, induces mitochondria-independent apoptosis	
	Ajudazol	Isochromanone	Antifungal, antibacterial	–	
	Chondramide	Depsipeptide	Cytotoxic, antifungal	Inhibits actin polymerization	
	Chondrochloren	Styrene	Antibacterial, antifungal	–	
	Crocacin	*N*-Acylpeptide	Antifungal, cytotoxic	Inhibits respiration	
	Crocapeptin				
	Pedein	Cyclic peptide	Antifungal, cytotoxic	Disrupts membrane integrity	
	Thuggacin	Macrolide	Antibacterial	Inhibits respiration	8
Sorangium	Ambruticin		Antifungal	Interferes with osmoregulation via the HOG pathway	
	Carolactone	Macrolactone	Antibacterial (anti-biofilm in *Streptococci*)	Inhibits kinase	
	Chivosazol	Macrolactone	Cytotoxic	Inhibits actin polymerization	
	Chlorotonil	Macrolactone	–	–	
	Disorazol	Macrodilactone	Cytotoxic, antifungal	Inhibits tubulin polymerization	
	Eliamide				
	Epothilone	Macrolactone	Cytotoxic, anticancer, antifungal	Induces tubulin polymerization	
	Etnangien	Macrolactone	Antibacterial, antiviral (HIV-1)	–	
	Eudesmadien				
	Icumazol		Antifungal		
	Invictolid				
	Jerangolid	Lactone	Antifungal	Alters membrane permeability	
	Leupyrrin	Macrodilactone	Antifungal, cytotoxic	Inhibits DNA, RNA, and protein synthesis	
	Maracen	Vinyl ether	Antibacterial (Mycobacteria)		
	Maracin	Vinyl ether	Antibacterial (Mycobacteria)		
	Noricumazol			Inhibits potassium-ion channel	
	Pellasoren		Cytotoxic	–	
	Pentacaronic acid			–	
	Phoxalone	Macrolide	Cytotoxic	–	
	Pyrrolnitrin	Phenypyrrole	Antifungal, antibacterial	Inhibits complex I respiration	
	Ratjadon	α-Pyrone	Cytotoxic	Inhibits the formation of nuclear export complex	
	Ripostatin	Macrolactone	Antibacterial, antifungal	Inhibits bacterial RNAP	
	Socein		Antifungal, cytotoxic	–	
	Sorangicin	Macrolactone	Antibacterial	Inhibits bacterial RNAP	
	Sorangiadenosine	Nucleoside	Antibacterial	–	
	Sorangiolid	Macrolactone	Antibacterial	Disrupts membrane integrity	
	Soraphen	Macrolactone	Antifungal, anticancer, antimetabolic syndrome	Inhibits acetyl-CoA carboxylase, BC domain	

Table 19.5 (continued)

Genus	Compound	Structural class	Biological activity	Mode of action	No.
	Soraphinol	Indole alkaloid	Radical scavenger	–	
	Spirangien	Spiroketal	Cytotoxic, antifungal	–	
	Spirodienal	Spiroketal	Cytotoxic	–	
	Sulasoren			–	
	Sulfangolid	Macrolide	Antibacterial	–	
	Tartrolon	Macrodiolide	Antibacterial, cytotoxic	Disrupts membrane integrity	
	Thuggacin	Macrolide	Antibacterial (Mycobacteria)	Inhibits respiration	
	Trichangion		–		
	Tuscolid	Macrolide	–		
	Tuscoron	Furanone	–		37
Byssovorax	Cruentaren	Benzalactone enamide	Cytotoxic, antifungal	Inhibits mitochondrial F_0F_1-ATPase	
	Byssochloren		Antibacterial (Mycobacteria)		
	Haprolid		Anti-HCV	–	3
Jahnella	–				0
Aetherobacter[a]	Aetheramide		Anti-HIV, cytotoxic, antifungal	–	1
Total					51

[a]Not yet validly published genus. Compound printed in bold represents an overlap with other genera

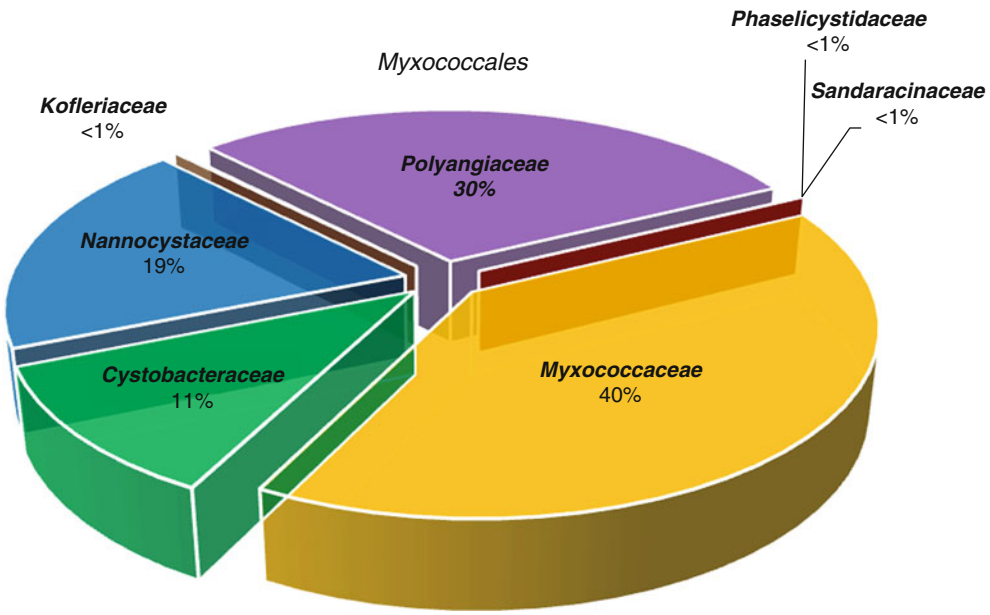

Fig. 19.26
Distribution of Myxobacterial families in the combined HIPS-MWIS (HZI, former GBF) collection. *Polyangiaceae* represents almost one third of the overall collection

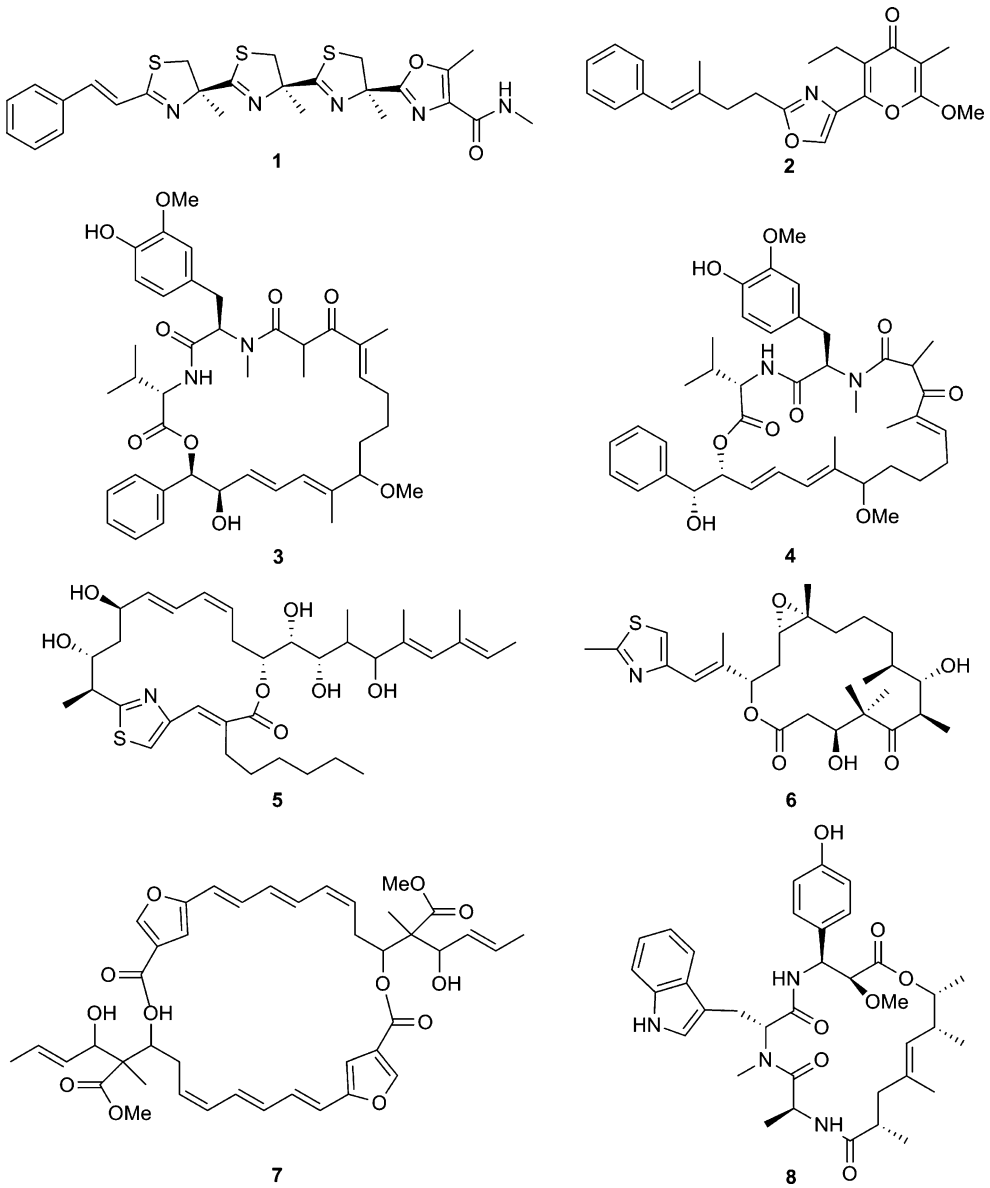

Fig. 19.27

Few examples of the bioactive compounds isolated from *Polyangiaceae*. Thiangazole A (1) and Phenoxan (2) produced by *Polyangium*. Aetheramide A (3) and aetheramide B (4) derived from the novel genus "*Aetherobacter*." Thuggacin A (5), a compound shared by both *Sorangium* and *Chondromyces*. Epothilone B (6) and Disorazol Z (7), highly active compounds from the genus *Sorangium*. Chondramide A (8) synthesized by many *Chondromyces* strains

Disorazol A shows impressive activity against mammalian cell culture at approximately 3–30 pg/mL (Irschik et al. 1995). Currently, the derivative disorazol Z is developed as peptide conjugate to become an anticancer lead due to its enormous activity (Aicher et al. 2012).

Chondramides are depsipeptide compounds produced by *Chondromyces crocatus* (Kunze et al. 1995; Sasse et al. 1998) and other related species. Interestingly, these compounds are structurally closely related to the jaspamides/jasplakinolide of the marine sponge genus *Jaspis* (Zabriskie et al. 1986; Crews et al. 1986), raising the question whether jaspamides might be made by a myxobacterium-like organism in the sponge (Rachid et al. 2006). Chondramides exhibit antimicrobial and cytostatic activity (Kunze et al. 1995). Investigations on the mode of action revealed that chondramides also act like jasplakinolide by inhibiting the actin polymerization.

Although many myxobacterial compounds are regarded as cytotoxic, the bulk of them are antimicrobial. Approximately

54 % of the myxobacterial compounds known today are antifungals, which often target the mitochondrial respiratory chain, specifically, complexes I and III (Gerth et al. 2003). Examples of these compounds in *Polyangiaceae* are thiangazole, phenoxan (both *Polyangium* derived), and crocacin from *Chondromyces*.

Antibacterial metabolites account for nearly one-third of the total myxobacterial compounds (Gerth et al. 2003). The antibacterial modes of action are much broader in scope spanning a large number of different targets. Etnangien, ripostatin, and sorangicin, all *Sorangium*-derived compounds, inhibit the eubacterial RNA polymerase. Interestingly, compounds acting against acid-fast mycobacteria have also been discovered in *Sorangium* species: maracin and maracen (Herrmann et al. 1998). The compound family thuggacin also acts as an antitubercular drug and is produced by both *Chondromyces* and *Sorangium* (Irschik et al. 2007a; Steinmetz et al. 2007).

Members of *Polyangiaceae* are also gaining recognition as producers of novel antivirals. Thiangazole (Kunze et al. 1993) and Phenoxan (Jansen et al. 1991; Kunze et al. 1992) are two of the anti-HIV compounds produced by the genus *Polyangium*. In addition to antibacterial activity, etnangien was also discovered to inhibit the reverse transcriptase HIV-1 (Irschik et al. 2007b). The first isolated depsipeptide compound class (aetheramides) in the proposed novel genus *Aetherobacter* (Garcia et al. 2010) was also determined to inhibit HIV-1 infection at an IC_{50} 0.015 μM (Plaza et al. 2012).

Steroids and Fatty Acids

The potential biotechnological application of myxobacteria is not only restricted to antibiotics, cytotoxic compounds, and antivirals; they have also been implicated in the production of steroids (Bode et al. 2003; Gawas et al. 2011). In *Polyangiaceae*, steroids (e.g., lanosterols) have been found distributed among *Polyangium* strains (Zeggel 1993), *Chondromyces*, and *Sorangium* (Garcia et al., unpublished data). Surprisingly, commercially valuable polyunsaturated fatty acids (PUFAs) are also produced by members of this family (Garcia et al. 2011; Stadler et al. 2010). PUFAs were previously thought to be absent in bacteria (Erwin and Bloch 1964) until strains living in high-pressure and low-temperature environments were revealed as producers (DeLong and Yayanos 1986; Nichols et al. 1993; Nichols and McMeekin 2002; Yano et al. 1997). *Polyangiaceae* are a fascinating myxobacterial family known to synthesize diverse fatty acids and PUFAs. In the comprehensive fatty acid-phylogenetic study in myxobacteria, members of the *Polyangiaceae* were discovered to synthesize omega-3 and omega-6 PUFAs (Garcia et al. 2011). High amounts of commercially valuable omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been identified in *Aetherobacter* (Stadler et al. 2010). For some members of the *Polyangiaceae*, omega-6 arachidonic acid, linoleic, γ-linolenic as well eicosadienoic acids have also been found (Garcia et al. 2011). These long-chained PUFAs have also been sought in many organisms due to their associated health applications and high commercial value (Nichols et al. 1993, 1999; Nichols and McMeekin 2002; Singh et al. 1996; Ward and Singh 2005; Warude et al. 2006).

Concluding Remarks and Perspectives

Polyangiaceae represent the most interesting family of myxobacteria in terms of morphology, physiology, and diversity. Although this group is difficult to work with and not easily isolated, efforts to cultivate them may lead to valuable applications in the biotech industry, as pharmaceuticals, and in the medical field.

From a metagenomics point of view, *Polyangiaceae* appear to be more diverse than anticipated. Evidence suggests that clones of currently uncultured myxobacteria can be brought to growing condition. In fact, several of these strains have already been isolated in our laboratory and characterized as belonging to this family but are not validly published yet. Their morphology and molecular affiliation were previously introduced in the updated and comprehensive myxobacterial phylogenetic tree (Garcia et al. 2010). These novel isolates are also comparably interesting in terms of their industrial and natural product applications.

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20 The Family *Syntrophaceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract
Syntrophaceae, a family within the order *Syntrophobacterales*, embraces four genera *Syntrophus, Smithella, Desulfobacca, and Desulfomonile*. Their 16S rRNA gene sequence phylogeny defines this family for the delineation of other genera and species. Strictly anaerobic, having either a respiratory type or fermentative type of metabolism, or grow only in the presence of H_2/formate-utilizing partners in syntrophic associations. All members are mesophilic. Members of the family are found in various, predominantly freshwater habitats. All described species are chemoorganoheterotroph with the exception of members of the genus has to be kursive which are described to be chemolithoautotroph. Substrates are oxidized either incompletely to acetate or completely to carbon dioxide. Electron acceptors used are crotonate, sulfate, and other sulfur compounds or halogenated benzoates, depending on the genus.

This contribution is a modified and updated version of a previous family description (Kuever et al. 2005a).

Taxonomy, Historical, and Current

Short Description of the Family

Syn.troph.a.ce'ae. N.L. masc. n. *Syntrophus*, type genus of the family; suff. *-aceae*, ending to denote family; N.L. fem. pl. n. *Syntrophaceae*, the Syntrophus family. (The description is an emended version of the description given in *Bergey´s Manual*, 2nd edition (Kuever et al. 2005a).

The family belongs to the order *Syntrophobacterales* within the *Deltaproteobacteria*. The family *Syntrophaceae* contains four genera *Syntrophus* (Mountford et al. 1984) which is the type genus of the family (Kuever et al. 2005a), *Smithella* (Liu et al. 1999), *Desulfobacca* and *Desulfomonile* (DeWeerd et al. 1991). Gram-staining negative. Morphological forms are always rod-shaped cells. Spore formation is absent. Most members are not motile; some are motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory or fermentative type of metabolism, or grow only in the presence of H_2/formate-utilizing partners in syntrophic associations. Simple organic molecules are used as electron donors. Members oxidize organic substrates incompletely to acetate or completely to carbon dioxide. All members are mesophilic. All described species are chemoorganoheterotrophs, whereas *Desulfomonile* spp. are described to grow as autotrophs on H_2 and CO_2.

Members of the genera *Syntrophus* and *Smithella* can use crotonate as electron acceptor or for fermentative growth, whereas members of the genera *Desulfobacca* and *Desulfomonile* can use sulfate, sulfite, and thiosulfate as electron acceptors which are reduced to sulfide. *Desulfomonile* spp. can also use *m*-halogenated benzoate as electron acceptor which is reductively dehalogenated to their corresponding benzoate derivative.

Members have been isolated from various sources, like freshwater, sewage sludge, and marine habitats.

Phylogenetic Structure of the Family

The phylogenetic structure of the family and its neighboring families within the order *Syntrophobacterales* is shown in ❯ *Fig. 20.1*. The borders of the family are primarily based on the phylogenetic tree as framework and their unique properties (physiology, chemotaxonomic markers) which are present in all members (see ❯ *Table 20.1*).

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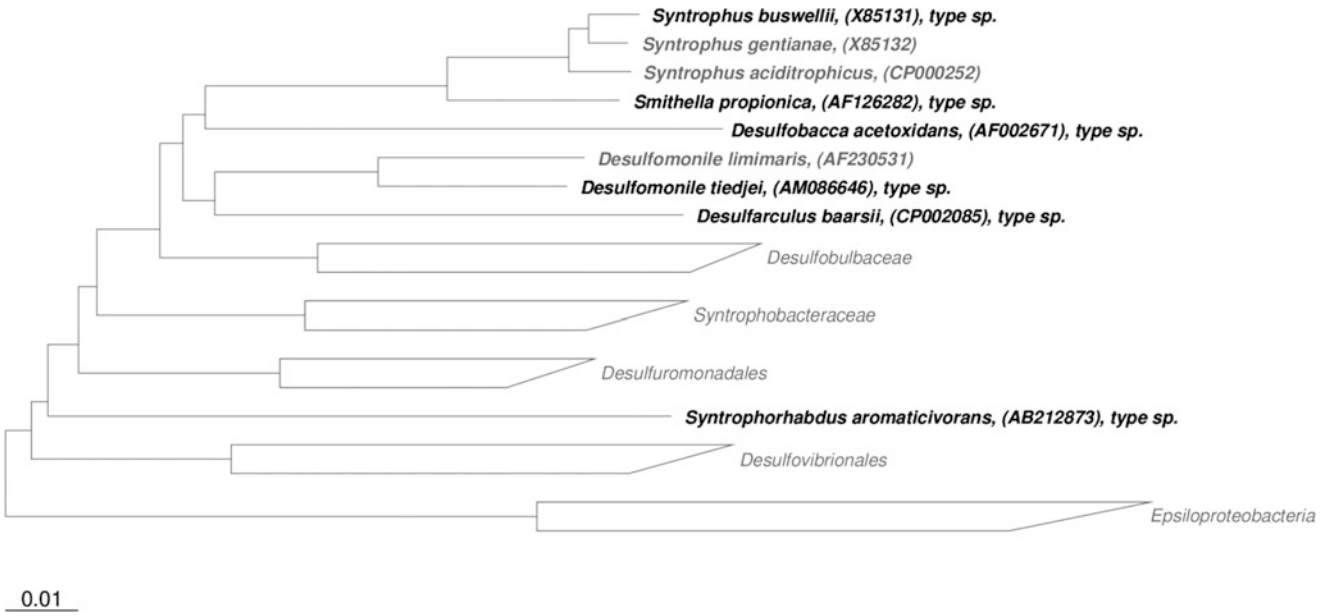

Fig. 20.1

Phylogenetic reconstruction of the family *Syntrophaceae* 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 archeal 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

Taxonomic Comment

The family *Syntrophaceae* is an artificial family which was established to build some kind of structure for the Deltaproteobacteria. As indicated in *Fig. 20.1*, the genera *Desulfobacca* and *Desulfomonile* are deep branching lineages and should be regarded each as separate families. Phylogenetic trees based on the dsrAB gene (Klein et al. 2001) and on the aprBA gene (Meyer and Kuever 2007) strongly supports this suggestion. In addition, the physiological character would be more uniform, because the family *Syntrophus* would then only contain syntrophic bacteria which are not able to use sulfate or other sulfur compounds as electron acceptors.

Molecular Analyses

DNA-DNA Hybridization Studies

Are absent.

Bioprinting and Ribotyping

Are absent.

MALDI-TOF

Are absent.

Genome Comparison

The complete genome sequence of three members of this family has been analyzed.

The genome of the type strain of *Syntrophus aciditrophicus*, strain SB, is 3,179,300 bp long and contains 3,217 genes with 3,166 coding for proteins. The mol% G+C of the DNA is 51.5 (McInerney et al. 1999).

The genome of the type strain of *Desulfobacca acetoxidans*, strain ASRB2, is 3,282,536 bp long and contains 3,022 genes with 2,969 coding for proteins and 54 coding for RNA genes. The mol% G+C of the DNA is 52.9 (Göker et al. 2011).

The genome of the type strain of *Desulfomonile tiedjei*, strain DCB-1, is 6,500,104 bp long (one chromosome and a 26,923 bp plasmid) and contains 5,664 genes with 5,494 coding for proteins. The mol% G+C of the DNA is 50.1 (a publication is absent).

In the genomes of *Desulfobacca acetoxidans* and *Desulfomonile tiedjei*, single copies of the dissimilatory adenylylsulfate reductase (AprBA) linked to the membrane-bound Qmo complex (QmoABC) and the dissimilatory sulfite

reduction (DsrAB) linked to the DsrMKJOP complex are present. The aprBA of *Desulfobacca acetoxidans* is closely related to the aprBA sequences of green sulfur bacteria, whereas the aprBA of *Desulfomonile tiedjei* resembles the gram-positive type (Meyer and Kuever 2007). All are absent in the genome of *Syntrophus aciditrophicus*.

Phages

Phages might be present, but are not reported so far.

Phenotypic Analyses

The main features of all members of the family *Syntrophaceae* are listed in ❯ *Table 20.1*.

Syntrophus Mountfort et al. 1984, 216[VP]

Syn´tro.phus. Gr. adj. *syntrophos*, having grown up with one, living with; N.L. masc. n. *Syntrophus*, one living syntrophically with another so that each produces a nutrient required by the other.

The genus *Syntrophus* contains three validly described species. Their characteristics are summarized in ❯ *Table 20.2*.

Smithella Liu et al. 1999, 553[VP]

Smi.thel´la N.L. fem. dim. n. *Smithella*, named for Paul H. Smith in honor of his many contributions to the understanding of methanogenic propionate degradation.

The genus *Smithella* contains a single validated species. Its characteristics are summarized in ❯ *Table 20.3*. The metabolism of propionate involves the reductive carboxylation to butyryl-CoA.

Table 20.1
Morphological and chemotaxonomic characteristics of the genera of *Syntrophaceae*

	Syntrophus	*Smithella*	*Desulfobacca*	*Desulfomonile*
Morphology	Rod shaped	Rod shaped	Oval to rod shaped	
Gram stain	Negative	Negative	Negative	Negative
Motility	+/–	Weak	–	–
Metabolism	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Major fatty acids	$C_{16:0}$, $C_{16:1\ c9}$, $C_{18:1\ c11}$	Nr	Nr	$C_{14:0}$, $C_{16:1\ w7c}$, $C_{16:0}$, Cyc-$C_{17:0}$[a]
Menaquinone	Nr	Nr	Nr	Nr
G+C content (mol%)	51.5–54.4	Nr	52.9	50.1
Substrate oxidation	Incomplete	Incomplete	Complete	Complete
CODH activity	Nr	Nr	Present	Present
Typical electron donors	Crotonate, benzoate	Crotonate, propionate, butyrate, fumarate, malate	Acetate	Benzoate, butyrate, pyruvate, formate, H_2, methoxylated aromatic acids
Chemolithoautotrophic growth	No species	No species	No species	All species
Fermentative growth	All species	All species	No species	All species
Growth by disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr
Typical electron acceptors	Some species can use crotonate; in general dependent on H_2/formate-utilizing partners	Dependent on H_2/formate-utilizing partners	Sulfate, sulfite, thiosulfate	Sulfate, sulfite, thiosulfate, *m*-halobenzoates
Optimal growth temperature (°C)	28–37	37	37	37
Habitat	Freshwater, brackish water, waste water treatment plant; might occur also in marine sediments	Freshwater, water treatment plants, might occur also in marine sediments	Freshwater, waste water treatment plants	Freshwater, water treatment plants, marine sediment

[a]Data from Kohring et al. 1994

Desulfobacca Oude Elferink et al. 1999, 348[VP]

De.sul.fo.bac´ca L. pref. *de-*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. fem. n. *bacca*, berry, especially olive; N.L. fem. n. *Desulfobacca*, a sulfate-reducing olive-shaped bacterium.

The genus *Desulfobacca* contains a single validated species. Its characteristics are summarized in ❯ *Table 20.4*.

Table 20.2
Comparison of selected characteristics of members of the *Syntrophus*

Characteristic	*Syntrophus buswellii*	*Syntrophus aciditrophicus*	*Syntrophus gentianae*
Type strain	Strain DM-2, DSM 2612A (syntrophic co-culture with *Desulfovibrio* sp. G11), DSM 2612B (syntrophic tri-culture with *Methanospirillum hungatei* DSM 864 and *Desulfovibrio* sp. G11), 2612M (monoculture isolated from *Syntrophus buswellii* coculture DSM 2612B).	Strain SB, ATCC 700169	Strain HQGö1, CIP 105111, DSM 8423.
Accession number of the 16S rRNA gene sequence of the type strain	CP000252	X85131	X85132
Morphology	Rod shaped	Rod shaped	Rod shaped
Cell size (μm)	0.8 × 1.0–2.0	0.5–0.7 × 1.0–1.6	0.8–1.0 × 1.3–1.6
Motility	+/–	–	–
Mol% G+C content	54.4	51.5	53.2
Major menaquinone	Nr	Nr	Nr
Optimal pH	7.2	7.2	7.1–7.4
Optimal temperature (°C)	37	35	28
Optimal NaCl concentration (g/l)	0–3	0–3	0–1
Growth factor requirement	Vitamins, clarified rumen fluid	Clarified rumen fluid	Vitamins, yeasts extract
Oxidation of substrate	Incomplete	Incomplete	Incomplete
Electron donors used			
H_2/CO_2	–	–	–
Formate	–	–	–
Acetate	–	–	–
Fatty acids	–	C_4[c], C_6 [c], C_7 [c], C_{16} [c], C_{18} [c]	–
Ethanol	Nr	Nr	Nr
Other n-alcohols	Nr	Nr	Nr
Lactate	Nr	Nr	Nr
Pyruvate	Nr	Nr	Nr
Fumarate	Nr	Nr	NR
Succinate	Nr	Nr	Nr
Malate	Nr	Nr	Nr
Other	Crotonate[a], cinnemate[a], benzoate[b], phenylpropionate[b]	Crotonate[a], unsaturated hexanoates and octanoates[c], methyesters of butyrate and hexanoate[c]	Crotonate[a], gentisate[a], hydroquinone[a], benzoate [b]
Fermentative growth	+	+	+
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr

Table 20.2 (continued)

Characteristic	*Syntrophus buswellii*	*Syntrophus aciditrophicus*	*Syntrophus gentianae*
Electron acceptors used			
Sulfate	–	–	–
Sulfite	–	–	–
Thiosulfate	–	–	–
Sulfur	–	–	–
Other	Crotonate	–	–
Literature	Mountfort et al. 1984, gen. nov. sp. nov.; Kuever and Schink 2005	Jackson et al. 2001, sp. nov.; Jackson et al. 1999	Wallrabenstein et al. 1996, sp. nov.; Wallrabenstein et al. 1995

[a]Used by fermentative growth
[b]Used with crotonate as electron acceptor or with H_2/formate-utilizing partners
[c]Used only with H_2/formate-utilizing partners

Desulfomonile DeWeerd et al. 1991, 178[VP] (Effective Publication DeWeerd et al. 1990, 28)

De.sul.fo.mo.ni´le. L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing procaryote); L. neut. n. monile, a necklace, a collar; N.L. neut. n. *Desulfomonile*, a "collared" sulfate-reducer.

The genus *Desulfomonile* contains two validly described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 20.5*. Both species can use benzoates halogenated in the *meta*-position as electron acceptor which are reductively dehalogenated to the corresponding benzoate. Acetate might be used as carbon source.

Biolog

Biolog dat are not available for sulfate-reducing bacteria.

Isolation, Enrichment, and Maintenance Procedures

All members of the family *Syntrophaceae* require anoxic media for growth. The media are prepared under specific conditions and the addition of a reductant is required, in general sulfide, to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992) and might be modified by other authors as listed in the original descriptions of various taxa.

For enrichment of *Syntrophus* and *Smithella*, the best strategy would be to establish syntrophic cultures degrading the aromatic compounds or fatty acids. Later pure culture can be obtained using crotonate (see Wallrabenstein et al. 1995; Liu et al. 1999). For the selective enrichment of *Desulfomonile* spp., it might be useful to use m-halobenzoate as electron acceptors for selective enrichment.

Most sulfate-reducing bacteria of this family have been enriched using batch cultures; other options are serial dilution techniques of natural samples. For isolation in general, roll-tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992; Kuever et al. 2005b).

For short-term preservation stock cultures can be stored at 2–6°C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. For long-term storage cultures can be kept freeze dried, at −80°C, or in liquid nitrogen.

Ecology

Habitat

Members of this family were isolated from various habitats including freshwater, brackish, and marine systems.

The type strain of *Syntrophus buswellii*, strain SB, was obtained as a syntrophic coculture with H_2/formate-utilizing partners (methanogens or *Desulfovibrio* sp.) using benzoate as substrate from anaerobic digester sludge obtained from the municipal sewage sludge treatment plant (Urbana, Illinois, USA) (Mountfort and Bryant 1982). Later, a pure culture was obtained using crotonate as substrate for fermentative growth (Wallrabenstein et al. 1995). Strain SB, the type strain of *Syntrophus aciditrophicus*, was isolated with crotonate as substrate from a syntrophic benzoate-degrading enrichment culture obtained from secondary anaerobic digester sludge from the municipal sewage treatment plant in Norman, Oklahoma, USA (Jackson et al. 1999). Strain HQGö1, the type strain of *Syntrophus gentianae*, was isolated from anoxic sewage sludge

Table 20.3
Comparison of selected characteristics of the only member of the genus *Smithella* (for easier comparison a table format is used)

Characteristic	*Smithella propionica*
Type strain	Strain LYP, OCM 661
Accession number of the 16S rRNA gene sequence of the type strain	AF126282
Morphology	Rod shaped
Cell size (μm)	0.5 × 3.0–5.0 (some cells up to 10.0)
Motility	Weak
Mol% G+C content	Nr
Major menaquinone	Nr
Optimal pH	6.5–7.5
Optimal temperature (°C)	33–35
Optimal NaCl concentration (g/l)	0–5
Growth factor requirement	Nr
Oxidation of substrate	Incomplete
Electron donors used	
H_2/CO_2	Nr
Formate	Nr
Acetate	–
Fatty acids	C_3–C_4[a]
Ethanol	Nr
Other n-alcohols	Nr
Lactate	Nr
Pyruvate	Nr
Fumarate	+[a]
Succinate	Nr
Malate	+[a]
Other	Crotonate[b]
Fermentative growth	+
Disproportionation of reduced sulfur compounds	Nr
Electron acceptor used	
Sulfate	–
Sulfite	–
Thiosulfate	–
Sulfur	–
Other	–
Literature	Liu et al. (1999), gen. nov., sp. nov.; Sobieraj and Boone 2005

[a]Only utilized in the presence of H_2/formate-scavenging methanogenic partners
[b]Fermented slowly in pure culture to butyrate and acetate

Table 20.4
Comparison of selected characteristics of the only member of the genus *Desulfobacca* (for easier comparison a table format is used)

Characteristic	*Desulfobacca acetoxidans*
Type strain	Strain ASRB2, DSM 1109
Accession number of the 16S rRNA gene sequence of the type strain	CP002629
Morphology	Oval to rod shaped
Cell size (μm)	1.3 × 1.9–2.2
Motility	–
Mol% G+C content	51.1
Major menaquinone	Nr
Optimal pH	7.5–7.7
Optimal temperature (°C)	37
Optimal NaCl concentration (g/l)	0–1
Growth factor requirement	Nr
Oxidation of substrate	Complete
Electron donors used	
H_2/CO_2	–
Formate	–
Acetate	+
Fatty acids	–
Ethanol	–
Other n-alcohols	–
Lactate	–
Pyruvate	–
Fumarate	–
Succinate	Nr
Malate	Nr
Other	–
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptor used	
Sulfate	+
Sulfite	+
Thiosulfate	+
Sulfur	–
Other	–
Literature	Oude Elferink et al. 1999, gen. nov., sp. nov

(Göttingen, Germany) using hydroquinone or gentisate as substrates for fermentative growth (Wallrabenstein et al. 1995).

Strain LYP, the type strain of *Smithella propionica*, was isolated with crotonate from a syntrophic propionate-degrading enrichment culture which was inoculated with anoxic sewage sludge (Oregon, USA) (Liu et al. 1999).

Members of the *Syntrophus/Smithella* cluster seem to be important for the syntrophic anaerobic degradation of oil compounds, in particular alkanes, coupled to methane formation (Zengler et al. 1999; Gray et al. 2011).

Table 20.5

Comparison of selected characteristics of members of the *Desulfomonile*

Characteristic	*Desulfomonile tiedjei*	*Desulfomonile limimaris*
Type strain	DCB-1, ATCC 49306, DSM 6799	DCB-M, ATCC 700979
Accession number of the 16S rRNA gene sequence of the type strain	AM086646	AF230531
Morphology	Rod shaped	Rod shaped
Cell size (μm)	0.8–1.0 × 5–10	0.4–0.8 × 6.0–8.0
Motility	–	–
Mol% G+C content	50.1	Nr
Major menaquinone		Nr
Optimal pH	6.8–7.0	Nr
Optimal temperature (°C)	37	37
Optimal NaCl concentration (g/l)	0–1	12.5
Growth factor requirement	Vitamins, 1,4-naphtoquinone	Vitamins
Oxidation of substrate	Incomplete/complete[a]	Nr
Electron donors used		
H_2/CO_2	+	+
Formate	+	+
Acetate	+[b]	–
Fatty acids	C_4[b]	C_3–C_4
Ethanol		Nr
Other n-alcohols		Nr
Lactate	+	+
Pyruvate	+	+
Fumarate		–
Succinate		–
Malate		Nr
Other	Benzoate, methoxy groups of aromatic compounds	Benzoate, *m*-halobenzoates
Fermentative growth	+	Nr
Disproportionation of reduced sulfur compounds	Nr	Nr
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	+
Sulfur	Nr	Nr
Other	*m*-Halobenzoates	*m*-Halobenzoates, fumarate, nitrate
Literature	DeWeerd et al. 1991, gen. nov. sp. nov.; DeWeerd et al. 1990	Sun et al. 2001

[a]Carbon-monoxide dehydrogenase activity indicates complete oxidation
[b]Poor growth

The type strain of *Desulfobacca acetoxidans*, strain ASRB2, was isolated from granular sludge of an laboratory-scale upflow anaerobic sludge reactor using acetate as only electron donor and carbon source (Oude Elferink et al. 1999).

The type strain of *Desulfomonile tiedjei*, strain DCB-1, was isolated from a consortium degrading 3-chlorobenzoate as only electron donor and carbon source which was obtained from sewage sludge Adrian, MI, USA Shelton and Tiedje (1884). Strain DCB-M, the type strain of *Desulfomonile limimaris*, was isolated in sulfate-free medium with 3-chlorbenzoate as electron acceptor and pyruvate or lactate as electron donor and carbon source from anoxic marine sediment obtained from Gulf Breeze, Florida, USA (Sun et al. 2001).

Pathogenicity, Clinical Relevance

No clinical relevance known.

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21 The Family *Syntrophobacteraceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract
Syntrophobacteraceae, a family within the order *Syntrophobacterales*, embraces the genera *Syntrophobacter*, *Desulfacinum*, *Desulfoglaeba*, *Desulforhabdus*, *Desulfosoma*, *Desulfovirga*, and *Thermodesulforhabdus*. Besides their 16S rRNA gene sequence phylogeny, all members of the family are defined by a wide range of morphological and chemotaxonomic properties for the delineation of genera and species. Strictly anaerobic, having a respiratory or fermentative type of metabolism. Syntrophic association with H_2/formate-utilizing partners might occur. Members are either mesophilic or moderately thermophilic sulfate-reducing bacteria. Members of the family are mainly found in freshwater, sewage sludge, or marine habitats. Most described species are chemoorganoheterotroph; some are chemolithoheterotroph or chemolithoautotroph. Most members perform a complete oxidation of organic substrates, except member of the genus *Syntrophobacter* which shows an incomplete oxidation to acetate.

This contribution is a modified and updated version of a previous family description (Kuever et al. 2005a).

Taxonomy, Historical and Current

Short Description of the Family

Syn.tro.pho.bac.te.ra'ce.ae. N.L. masc. n. *Syntrophobacter*, type genus of the family; suff. -aceae, ending to denote family; N.L. fem. pl. n. *Syntrophobacteraceae*, the *Syntrophobacter* family. The description is an emended version of the description given in *Bergey's Manual*, 2nd edition (Kuever et al. 2005a).

The family belongs to the order *Syntrophobacterales* (Kuever et al. 2005c) within the *Deltaproteobacteria*. The family *Syntrophobacteraceae* contains the genera *Syntrophobacter* (Boone and Bryant 1984), which is the type genus of the family (Kuever et al. 2006), *Desulfacinum* (Rees et al. 1995), *Desulfoglaeba* (Davidova et al. 2006), *Desulforhabdus* (Oude Elferink et al. 1995), *Desulfosoma* (Baena et al. 2011), *Desulfovirga* (Tanaka et al. 2000), and *Thermodesulforhabdus* (Beeder et al. 1996). Gram-staining negative. Morphological forms vary from rod- to oval-shaped cells of varying. Spore formation is absent. Most members are motile by means of one or two polar flagella. Strictly anaerobic, having a respiratory type of metabolism. Some species can grow by fermentation or are growing in syntrophic association with H_2/formate-utilizing partners. Simple organic molecules are used as electron donors; some species can use also H_2. Except *Syntrophobacter* all members oxidize organic substrates completely to carbon dioxide and water. Mesophilic and thermophilic members.

Most described species are chemoorganoheterotroph; some are chemolithoheterotroph or chemolithoautotroph. Sulfate and thiosulfate are used as electron acceptor and reduced to sulfide; some species can also use sulfite.

Members have been isolated from various sources, sewage sludge, freshwater, brackish, or marine sediment. The origin of members growing at elevated temperature are marine hydrothermal vents or hydrothermally influenced sediments.

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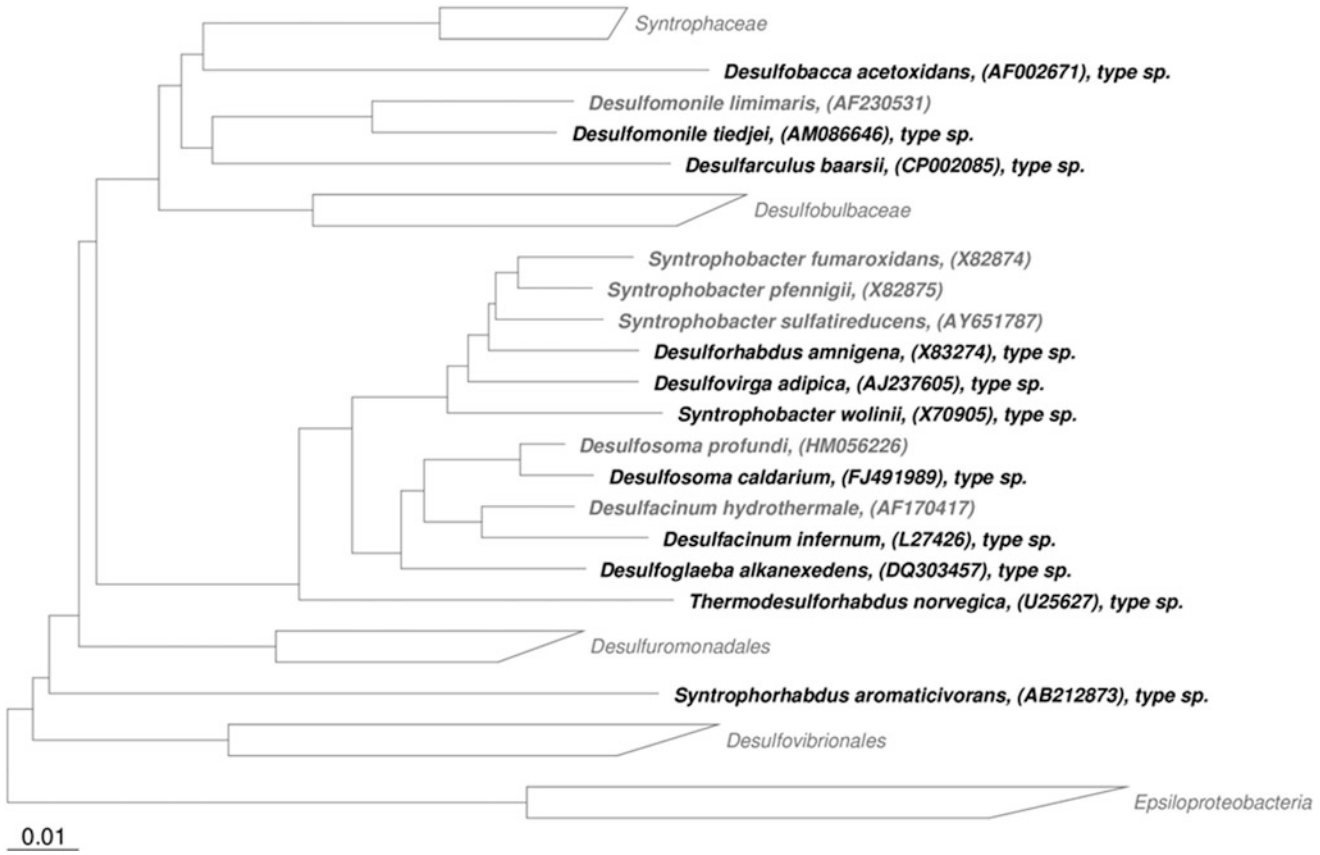

Fig. 21.1

Phylogenetic reconstruction of the family *Syntrophobacteraceae* 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

Phylogenetic Structure of the Family and Its Genera

The phylogenetic structure of the family and its neighboring families within the order *Syntrophobacterales* is shown on *Fig. 21.1*. The borders of the family are primarily based on the phylogenetic tree as framework; other unique properties (physiology, chemotaxonomic markers) which are present in all members are not found (see *Table 21.1*).

Molecular Analyses

DNA-DNA Hybridization Studies

Some DDH data are summarized in *Table 21.2*.

The mean DNA-DNA reassociation value between the type strains of *Desulfosoma profundi* and *Desulfosoma caldarium* was 16.9 % (Gregoire et al. 2012).

Bioprinting and Ribotyping

Are absent.

MALDI

Are absent.

Genome Comparison

The complete genome sequence of a single member of this family has been analyzed. The genome of the type strain of *Syntrophobacter fumaroxidans*, *strain MPOB*, is 4,990,251 bp long and contains 4,162 genes with 4,064 coding for proteins and 64 coding for RNA genes. The mol% G + C of the DNA is 59.9. A publication about the genome is absent. In the genome, single copies of the dissimilatory

Table 21.1

Morphological and chemotaxonomic characteristics of genera of *Syntrophobacteraceae*

	Syntrophobacter	*Desulfacinum*	*Desulfoglaeba*	*Desulforhabdus*	*Desulfosoma*	*Desulfovirga*	*Thermodesulforhabdus*
Morphology	Rod shaped	Oval to rod shaped	Rod shaped	Rod shaped	Oval to rod shaped	Rod shaped	Rod shaped
Gram stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+/−	+/−	−	−		+	+
Metabolism	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Major fatty acids	Nr	Nr	Nr	Nr	Iso-$C_{15:0}$, $C_{15:0}$, $C_{16:0}$	Nr	Nr
Menaquinone	MK-6 and MK-7	Nr	Nr	Nr	Nr	Nr	Nr
G + C content (mol%)	57.3–59.9	59.5–64.0	53.6	52.5	56.0–57.6	59.9	51.0
Substrate oxidation	Incomplete	Complete	Complete	Complete	Complete	Complete	Complete
CODH activity	Nr	Nr	Nr	Present	Nr	Nr	Nr
Typical electron donors	Propionate, pyruvate	H_2, formate, fatty acids (C_2 to C_6, C_8, C_{10}, C_{12}, C_{16}, C_{18}), isobutyrate, isovalerate, alanine, lactate, pyruvate, alcohols	Alkanes (C_6–C_{12}), pyruvate, fatty acids (C_4, C_6), 4-methyloctanoate	H_2, formate, fatty acids (C_2–C_4), isobutyrate, alcohols, lactate, pyruvate	H_2 and formate (only in the presence of acetate or yeast extract), short-chain fatty acids (C_3 to C_6), lactate, pyruvate	Formate, short-chain fatty acids (C_2 to C_{12}), iso-fatty acids (C_4–C_6), alcohols (C_2 to C_{10}), lactate, pyruvate, adipate, 2-hexenedioate, 3-hexenedioate, phenylpropionate	Fatty acids (C_2, C_4–C_{10}, C_{13}–C_{18}), lactate, pyruvate, fumarate, succinate, malate
Chemolithoautotrophic growth	No species	All species	No species	All species	No species[a]	No species	No species
Fermentative growth	Some species	All species	Nr	No species	All species	Nr	No species
Growth by disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr	Nr	Nr	Nr
Typical electron acceptors	Sulfate	Sulfate, thiosulfate, sulfite	Sulfate, thiosulfate	Sulfate, thiosulfate, sulfite	Sulfate, thiosulfate, sulfite	Sulfate, thiosulfate, sulfite, sulfur	Sulfate, sulfite
Optimal growth temperature (°C)	35–37	60	31–37	37	55–57	35	60
Habitat	Freshwater, anaerobic sewage sludge	Marine, hydrothermal vents	Freshwater, brackish water, marine	Freshwater, anaerobic sewage sludge	Terrestrial hydrothermal spring, hot aquifer	Freshwater, anaerobic sewage sludge	Marine, marine oil field

[a]Data from Gregoire et al. 2012 showed that *Desulfosoma caldarium* was not able to grow chemolithoautotrophically with H_2 and CO_2

Table 21.2
Percentage of DNA-DNA hybridization of different strains belonging to the family *Syntrophobacteraceae* (Harmsen et al. 1998; Chen et al. 2005)

	Syntrophobacter wolinii	*Syntrophobacter fumaroxidans*	*Syntrophobacter pfennigii*	*Syntrophobacter sulfatireducens*	*Desulforhabdus amnigena*
Syntrophobacter wolinii (filter bound)	100	23.2	16.3	Nr	26.0
Syntrophobacter fumaroxidans (filter bound)	9.4	100	16.4	12.5	24.7
Syntrophobacter pfennigii (filter bound)	5.0	19.8	100	Nr	13.0
Syntrophobacter sulfatireducens (filter bound)	Nr	12.5	Nr	100	Nr
Desulforhabdus amnigena (filter bound)	2.3	14.4	8.2	Nr	100

adenylylsulfate reductase (prBA) linked to the membrane-bound Qmo complex (QmoABC) and the dissimilatory sulfite reduction (DsrAB) linked to the DsrMKJOP complex were found. Interesting is that there is a second nonfunctional (crippled) set of QmoABC genes present in the genome.

The position of the family is also reflected by phylogenetic analysis of the dsrAB gene and the aprba genes (Klein et al. 2001; Meyer and Kuever 2007). The aprAB gene of all analyzed members of the family is closely related to gram-positive SRB (Meyer and Kuever 2007).

Phages

Phages might be present, but are not reported so far.

Phenotypic Analyses

The main features of all members of the family *Syntrophobacteraceae* are listed in ❯ *Table 21.1* and the tables featuring the genera (❯ *Tables 21.3*–*21.8*).

Syntrophobacter Boone and Bryant 1984, 355^{VP} (Effective Publication: Boone and Bryant 1980, 631) emend. Chen et al. 2005

Syn.tro.pho.bac´ter. Gr. adj. *syntrophos*, having grown up with one; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Syntrophobacter*, a rod which feeds together with (another species).

The genus *Syntrophobacter* contains four described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 21.3*.

Taxonomic comment: The phylogenetic tree (❯ *Fig. 21.1*) clearly indicates that *Syntrophobacter fumaroxidans* (kursive), *Syntrophobacter pfennigii* (kursive), and *Syntrophobacter sulfatireducens* (kursive) should not be regarded as a member of the genus *Syntrophobacter* and should be regarded as a new genus within this family.

Desulfacinum Rees et al. 1995, 88^{VP} emend. Sievert and Kuever 2000

De.sul.fa.ci´num: L. pref. *de*, from; L. n. *sulfur*, sulfur; L. neut. n. *acinum*, a berry, especially a grape; N.L. neut. n. *Desulfacinum*, a berry-shaped, sulfate-reducing bacterium.

The genus *Desulfacinum* contains two validly described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 21.4*. *Desulfacinum subterraneum* would be another member of this genus, but is not validated so far (Rozanova et al. 2001).

Desulfoglaeba Davidova et al. 2006, 2741^{VP}

De.sul.fo.glaeba. L. prep. *de*, from; N.L. pref. *sulfo-*, prefix used for N.L. masc. n. *sulfas -atis* (sulfate) in genus names of sulfate-reducing prokaryotes; L. fem. n. *glaeba*, clump, crumb, aggregate; N.L. fem. n. *Desulfoglaeba*, sulfate-reducing clump/aggregate.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 21.5*.

Desulforhabdus Oude Elferink et al. 1997, 1274^{VP} (Effective Publication: Oude Elferink et al. 1995, 123)

De.sul.fo.rhab´dus. L. pref. *de*, from; L. n. *sulfur*, sulfur; Gr. fem. n. *rhabdos*, rod; N.L. fem. n. *Desulforhabdus*, a rod-shaped sulfate reducer.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 21.6*.

Table 21.3

Comparison of selected characteristics of members of the genus *Desulfohalobium*

Characteristic	*Syntrophobacter wolinii*	*Syntrophobacter fumaroxidans*	*Syntrophobacter pfennigii*	*Syntrophobacter sulfatireducens*
Type strain	Strain DB (maintained as a coculture with *Desulfovibrio* sp. strain G-11; coculture, DSM 2805), DSM 2805 M (monoculture isolated from Syntrophobacter wolinii coculture DSM 2805)	Strain MPOB, DSM 10017	Strain KoProp1, CIP 105305, DSM 10092	Strain TB8106, AS 1.5016, DSM 16706
Accession number of the 16S rRNA gene sequence of the type strain	X70905	X82874	X82875	AY651787
Morphology	Rods, sometimes long filaments	Lemon-shaped rods	Lemon-shaped rods	Oval shaped
Cell size (μm)	0.6–1.0 × 1.0–4.5	1.1–1.6 × 1.8–2.5	1.0–1.2 × 2.2–3.0	1.0–1.3 × 1.8–2.2
Motility	–	–	+	–
Mol% G + C content	Nr	60.6	57.3	58.5
Major menaquinone	Nr	MK-6 and MK-7	MK-7	Nr
Optimal pH	7.0	7.0	7.0	7.0–7.6
Optimal temperature (°C)	35	37	37	37
Optimal NaCl concentration (g/L)	0.4	0–1	0–1	0–1
Growth factor requirement	Nr	Nr	–	Nr
Oxidation of substrate	Incomplete	Incomplete	Incomplete	Incomplete
Compounds used as electron donors and carbon sources				
H_2/CO_2	–	+[a]	–	–
Formate	–	+	–	–
Acetate	–	–	–	–
Fatty acids	C_3	C_3	C_3	C_3
Ethanol	Nr	–	–	–
Other *n*-alcohols	Nr	–	*N*-propanol[b]	–
Lactate	Nr	–	+	–
Pyruvate	+	+	–	+
Fumarate	+	+	–	–
Succinate	Nr	+	–	–
Malate	Nr	+	–	–
Other	Nr	Aspartate	–	–
Fermentative growth	+	+	+[b]	+
Disproportionation of reduced sulfur compounds	Nr	Nr	Nr	Nr
Electron acceptors used				
Sulfate	+	+	+	+
Sulfite	Nr	+	+	+
Thiosulfate	Nr	+	+	+
Sulfur[b]	Nr	Nr	Nr	–
Other	Nr	Fumarate	–	–
Literature	Boone and Bryant (1980), gen. nov., sp. nov.; McInerney et al. (2005); Wallrabenstein et al. (2004)	Harmsen et al. (1998), sp. nov.; McInerney et al. (2005)	Wallrabenstein et al. (1995), sp. nov.; McInerney et al. (2005)	Chen et al. (2005), sp. nov.

[a]Poor growth with sulfate as electron acceptor

[b]Only in coculture with a H_2/formate-utilizing partner

Table 21.4
Comparison of selected characteristics of members of the genus *Desulfacinum*

Characteristic	*Desulfacinum infernum*	*Desulfacinum hydrothermale*
Type strain	Strain BαG1 = ACM 3991 = DSM 9756	Strain MT-96 = DSM 13146
Accession number of the 16S rRNA gene sequence of the type strain	L27426	EU296539
Morphology	Oval-shaped rod	Oval-shaped rod
Cell size (μm)	1.0 × 2.5	0.8–1.0 × 1.5–2.5
Motility	–	+
Mol% G + C content	64.0	59.5
Major menaquinone	Nr	Nr
Optimal pH	7.1–7.5	7.0–7.3
Optimal temperature (°C)	60	60
Optimal NaCl concentration (g/L)	10.0	32–36
Growth factor requirement	Vitamins	–
Oxidation of substrate	Complete	Complete
Compounds used as electron donors and carbon sources		
H_2/CO_2	+	+
Formate	+	+
Acetate	+	+
Fatty acids	C_3–C_6, C_8, C_{10}, C_{12}, C_{16}, C_{18}	C_3–C_5, C_8, C_{10}, C_{12}, C_{16}, C_{18}
Ethanol	+	+
Other *n*-alcohols	*n*-propanol, *n*-butanol, *n*-hexanol, glycerol	*n*-propanol, *n*-butanol, *n*-hexanol
Lactate	+	+
Pyruvate	+	+
Fumarate	+	–
Succinate	+	–
Malate	+	–
Others	Isobutyrate, isovalerate, alanine	Isobutyrate, isovalerate, alanine
Fermentative growth	+	+
Disproportionation of reduced sulfur compounds	Nr	Nr
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	+
Sulfur	–	–
Other	–	–
Literature	Rees et al. (1995), gen. nov., sp. nov.	Sievert and Kuever (2000), sp. nov.

Desulfosoma Baeana et al. 2011, 735[VP]

De.sul.fo.so´ma.: L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, prefix used to characterize a dissimilatory sulfate-reducing prokaryote; Gr. neut. n. *soma*, body; N.L. neut. n. *Desulfosoma*, sulfate-reducing body.

The genus *Desulfosoma* contains two described species. Their chemotaxonomic and physiological properties are listed in ❯ *Table 21.7*.

Desulfovirga Tanala et al. 2000, 643[VP]

De.sul.fo.vir´ga.: L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing prokaryote); L. fem. n. *virga*, twig, rod; N.L. fem. n. *Desulfovirga*, a sulfate-reducing rod.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in ❯ *Table 21.8*.

Table 21.5
Selected characteristics of the only member of the genus *Desulfoglaeba* (a table is used for easier comparison with other genera)

Characteristic	*Desulfoglaeba alkanexedens*
Type strain	Strain ALDC, ATCC BAA-1302, JCM 13588
Accession number of the 16S rRNA gene sequence of the type strain	DQ303457
Morphology	Slightly curved rods
Cell size (μm)	1.0–1.4 × 2.5–3.0
Motility	–
Mol% G + C content	53.6
Major menaquinone	Nr
Optimal pH	6.5–7.2
Optimal temperature (°C)	31–37
Optimal NaCl concentration (g/L)	1.7–36 (NaCl is not required)
Growth factor requirement	–
Oxidation of substrate	Complete
Compounds used as electron donors and carbon sources	
H_2/CO_2	–
Formate	Nr
Acetate	–
Fatty acids	C_4, C_6
Ethanol	Nr
Other *n*-alcohols	Nr
Lactate	–
Pyruvate	+
Fumarate	–
Succinate	–
Malate	Nr
Others	4-Methyloctanoate, *n*-alkanes (C_6–C_{12})
Fermentative growth	Nr
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	–
Thiosulfate	+
Sulfur	–
Other	–
Literature	Davidova et al. (2006), gen. nov., sp. nov.

Table 21.6
Selected characteristics of the only member of the genus *Desulforhabdus* (a table is used for easier comparison with other genera)

Characteristic	*Desulforhabdus amnigena*
Type strain	Strain ASRB1 = ATCC 51979 = DSM 10338
Accession number of the 16S rRNA gene sequence of the type strain	X83274
Morphology	Rod shaped, sometime long chains
Cell size (μm)	1.4–1.9 × 2.5–3.4
Motility	–
Mol% G + C content	52.5
Major menaquinone	Nr
Optimal pH	7.2–7.6
Optimal temperature (°C)	37
Optimal NaCl concentration (g/L)	0–1
Growth factor requirement	Nr
Oxidation of substrate	Complete
Compounds used as electron donors and carbon sources	
H_2/CO_2	+
Formate	+
Acetate	+
Fatty acids	C_3–C_4
Ethanol	+
Other *n*-alcohols	*n*-propanol, *n*-butanol
Lactate	+
Pyruvate	+
Fumarate	–
Succinate	–
Malate	–
Others	Isobutyrate
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	+
Sulfur	–
Other	–
Literature	Oude Elferink et al. (1995), gen. nov. sp. nov.; Oude Elferink et al. (1995)

Table 21.7

Comparison of selected characteristics of members of the genus *Desulfosoma*

Characteristic	*Desulfosoma caldarium*	*Desulfosoma profundi*
Type strain	*Strain USBA-053, USBA 53, DSM 22027, KCTC 5670*	*Strain SPDX02-08, DSM 22937, JCM 16410*
Accession number of the 16S rRNA gene sequence of the type strain	FJ491989	HM056226
Morphology	Oval to rod shaped	Oval
Cell size (μm)	1.5 × 2.0	1.0–2.0 × 2.0–6.0
Motility	+	–
Mol% G + C content	57.6	56.0
Major menaquinone	Nr	Nr
Optimal pH	6.8	7.2
Optimal temperature (°C)	57	55
Optimal NaCl concentration (g/L)	25	2
Growth factor requirement	–	Yeast extract
Oxidation of substrate	Complete	Complete
Compounds used as electron donors and carbon sources		
H_2/CO_2	+[a]	+[a]
Formate	+	+
Acetate	–	–
Fatty acids	C_3–C_6	C_3–C_6
Ethanol	+[b]	–
Other *n*-alcohols	Glycerol	–
Lactate	+	+
Pyruvate	+	+
Fumarate	–	–
Succinate	–	–
Malate	–	–
Others	Isovalerate, serine	Isobutyrate, isovalerate
Fermentative growth	+	+
Disproportionation of reduced sulfur compounds	Nr	Nr
Electron acceptors used		
Sulfate	+	+
Sulfite	+	+
Thiosulfate	+	+
Sulfur	–	–
Other	–	–
Literature	Baena et al. (2011), gen. nov., sp. nov.; Gregoire et al. (2012)	Gregoire et al. (2012), sp. nov.

[a]Only in the presence of yeast extract; might also account for formate metabolism. Data for *Desulfosoma caldarium* corrected using data from Gregoire et al. (2012)
[b]Varying information in the text on this substrate

Thermodesulforhabdus Beeder et al. 1996, 625[VP] (Effective Publication: Beeder et al. 1995, 335)

Ther.mo.de.sul.fo.rhab´dus. Gr. adj. *thermos*, warm, hot; L. pref. *de*, from; L. n. *sulfur*, sulfur; N.L. pref. *desulfo-*, desulfuricating (prefix used to characterize a dissimilatory sulfate-reducing prokaryote); Gr. fem. n. *rhabdos*, rod; N.L. fem. n. *Thermodesulforhabdus*, thermophilic, rod-shaped, sulfate reducer.

The genus is monospecific. The chemotaxonomic and physiological properties are summarized in Table 21.9.

Table 21.8
Selected characteristics of the only member of the genus *Desulfovirga* (a table is used for easier comparison with other genera)

Characteristic	*Desulfovirga adipica*
Type strain	Strain TsuAS1, DSM 12016
Accession number of the 16S rRNA gene sequence of the type strain	AJ237605
Morphology	Rod shaped
Cell size (μm)	0.8–2.0 × 2.2–4.0
Motility	+
Mol% G + C content	59.9
Major menaquinone	Nr
Optimal pH	7.0
Optimal temperature (°C)	35
Optimal NaCl concentration (g/L)	0–1
Growth factor requirement	–
Oxidation of substrate	Complete
Compounds used as electron donors and carbon sources	
H_2/CO_2	–
Formate	+
Acetate	+
Fatty acids	C_3–C_{12}
Ethanol	+
Other *n*-alcohols	Primary alcohols (C_3–C_{10})
Lactate	+
Pyruvate	+
Fumarate	–
Succinate	–
Malate	–
Others	Adipate, 2-hexenedioate, 3-hexenedioate, iso-fatty acids (C_4–C_6), phenylpropionate[a]
Fermentative growth	Nr
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	+
Sulfur	+[b]
Other	–
Literature	Tanaka et al. (2000), gen. nov., sp. nov.

[a]Oxidized to benzoate
[b]Weak growth

Table 21.9
Selected characteristics of the only member of the genus *Desulfovirga* (a table is used for easier comparison with other genera)

Characteristic	*Thermodesulforhabdus norvegica*
Type strain	Strain A8444, DSM 9990
Accession number of the 16S rRNA gene sequence of the type strain	U25627
Morphology	Rod
Cell size (μm)	1.0 × 2.5
Motility	+
Mol% G+C content	51.0
Major menaquinone	Nr
Optimal pH	6.9
Optimal temperature (°C)	60
Optimal NaCl concentration (g/l)	16
Growth factor requirement	–
Oxidation of substrate	Complete
Compounds used as electron donors and carbon sources	
H_2/CO_2	–
Formate	–
Acetate	+
Fatty acids	C_4–C_{10}, C_{13}–C_{18}
Ethanol	+
Other *n*-alcohols	–
Lactate	+
Pyruvate	+
Fumarate	+
Succinate	+
Malate	+
Others	–
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Electron acceptors used	
Sulfate	+
Sulfite	+
Thiosulfate	–
Sulfur	–
Other	–
Literature	Beeder et al. 1996, gen. nov., sp.nov. Beeder et al. 1995

Biolog

Biolog data are not available.

Isolation, Enrichment, and Maintenance Procedures

All members of the family *Syntrophobacteraceae* require anoxic media for growth like all other sulfate-reducing bacteria. The media are prepared under specific conditions and the addition of a reductant is required, in general sulfide, to keep the medium oxygen-free. A detailed description is provided by Widdel and Bak (1992) and might be modified by other authors as listed in the original descriptions of various taxa.

For enrichment the used electron donor might be highly selective and will have a strong influence on what kind of sulfate-reducing bacteria will grow in the medium. As usual electron acceptor sulfate is used. Most sulfate-reducing bacteria of this family have been enriched using batch cultures; other options are serial dilution techniques of natural samples. For isolation in general, roll-tube techniques or deep agar serial dilution techniques are favored against plating techniques in combination with anoxic chambers (Widdel and Bak 1992; Kuever et al. 2005b).

For short-term preservation, stock cultures can be stored at 2–6 °C for 4–6 weeks. The transfer interval varies from strain to strain and depends on the tendency to lyse under suboptimal conditions. For long-term storage, cultures can be kept freeze-dried, at −80°C or in liquid nitrogen.

Ecology

Habitat

All members of this family were isolated from freshwater, sewage sludge, or marine systems of moderate or elevated temperature caused by hydrothermal activity.

The type strain of *Syntrophobacter wolinii*, strain DB, was isolated as a syntrophic coculture with propionate as electron donor from anoxic digester sludge from Urbana-Champaign, IL, USA (Boone and Bryant 1980). Later a pure culture was isolated using propionate and sulfate as electron acceptor (Wallrabenstein et al. 2004). The type strain of *Syntrophobacter fumaroxidans*, strain MPOB, was isolated as a syntrophic propionate-oxidizing bacterium from granular sludge from an anaerobic sludge bed reactor treating sugar-beet processing wastewater (Harmsen et al. 1998). The type strain of *Syntrophobacter pfennigi*i, strain KoProp1, was enriched with propionate as substrate from anoxic sewage sludge from the municipal sewage plant in Konstanz, Germany (Wallrabenstein et al. 1995). The type strain of *Syntrophobacter sulfatireducens*, strain TB8106, was isolated with propionate from a UASB reactor treating brewery wastewater in Beijing, China (Chen et al. 2005).

The type strain of *Desulfacinum infernum*, strain BαG1, was isolated from produced formation water collected directly from well A08(10) on the Beatrice field platform (British sector of the North Sea, close to the coast of Scotland)using lactate as electron donor and carbon source (Rees et al. 1995). The type strain of *Desulfacinum hydrothermale*, strain MT-96 T, was isolated with acetate as substrate from most probable number (MPN) dilution series inoculated with anoxic sediment from a shallow, submarine hydrothermal vent located in Palaeochori Bay, island of Milos in the Aegean Sea, Greece (Sievert and Kuever 2000).

The type strain of *Desulfoglaeba alkanexedens*, strain ALDC, was isolated using an alkane mixture as electron donor and carbon source from produced water obtained from a naval wastewater-storage facility at the US Navy Craney Island Fuel Depot in Portsmouth, VA, USA (Davidova et al. 2006).

The type strain of *Desulforhabdus amnigena*, strain ASRB1, was isolated with acetate as electron donor and carbon source from granular sludge of a pilot-scale UASB reactor treating paper mill wastewater (Balk, the Netherlands) (Oude Elferink et al. 1995).

Lactate was used as electron donor and carbon source for the isolation of strain USBA-053, the type strain *of Desulfosoma caldarium*, from a terrestrial hot spring located in Paipa, Colombia (Baena et al. 2011). The same substrate was used for the isolation of the type strain *of Desulfosoma profundi*, strain SPDX02-08, from a deep hot aquifer in south west France (Saint-Paul-Les-Dax, Aquitaine basin) (Gregoire et al. 2012).

The type strain of *Desulfovirga adipica*, strain TsuAS1, was isolated with adipate as electron donor and carbon source from anoxic mud of an anaerobic digester of the municipal wastewater treatment center, Tsuchiura, Ibaraki, Japan (Tanaka et al. 2000).

For the isolation of strain A8444, the type strain of *Thermodesulforhabdus norvegica*, immunomagnetic beads against a *Desulfovibrio* sp. were used for isolation with acetate from an anoxic sample of hot oil–water from a Norwegian oil platform in the North Sea (Beeder et al. 1995).

Pathogenicity, Clinical Relevance

There is no clinical relevance known.

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22 The Family *Syntrophorhabdaceae*

Jan Kuever
Department of Microbiology, Bremen Institute for Materials Testing, Bremen, Germany

Abstract

Syntrophorhabdaceae, a family within the order *Syntrophobacterales* (Kuever J, Rainey FA, Widdel F (2005) Order VI *Syntrophobacterales ord. nov.* In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) *Bergey's Manual of Systematic Bacteriology*, vol 2, 2nd edn, The Proteobacteria, part C (The *Alpha-*, *Beta-*, *Delta-*, and *Epsilonproteobacteria*). Springer, New York, p. 1021; Kuever et al. 2006), embraces a single genus *Syntrophorhabdus*. Besides their 16s rRNA gene sequence phylogeny, the only member of this family is defined by a wide range of morphological and chemotaxonomic properties for the delineation of genera and species. Strictly anaerobic. Growth only in the presence of an H_2-scavenging partner organism in a syntrophic mode. Anthraquinone-2,6-disulfonate (AQDS) is used as electron acceptor although growth in pure culture was not observed. The only described member is mesophilic. Members of the family are found predominantly in freshwater habitats like sewage sludge. The only described species is chemoorganoheterotroph and oxidizes organic substrates incompletely to acetate in syntrophy with a H_2 scavenger (e.g., *Methanospirillum hungatei* or a *Desulfovibrio* sp.) or with AQDS as electron acceptor (Qiu et al. 2008).

Taxonomy, Historical and Current

Short Description of the Family

Syn.tro.pho.rhab'da.ce.ae. N.L. masc. n. *Syntrophorhabdus*, type genus of the family; suff. *-aceae*, ending to denote family; N.L. fem. pl. n. *Syntrophorhabdaceae*, the *Syntrophorhabdus* family (Qiu et al. 2008).

The family belongs to the order *Syntrophobacterales* within the *Deltaproteobacteria*. The family *Syntrophorhabdaceae* contains a single genus *Syntrophorhabdus* (Qiu et al. 2008) which is the type genus of the family (Qiu et al. 2008). Gram-staining negative. Morphological forms are always rod-shaped cells. Spore formation is absent. Nonmotile. Strictly anaerobic, having an obligate syntrophic type of metabolism. Simple aromatic compounds are used as electron donors. The only member oxidizes the aromatic compounds incompletely to acetate. The only member is mesophilic. The only described species is chemoorganoheterotroph and can use only a few substrates in coculture with H_2-scavenging partners. Sulfate or other sulfur compounds cannot be used as electron acceptor. The only electron acceptor used so far is anthraquinone-2,6-disulfonate (AQDS). Members have been enriched as methanogenic or sulfate-reducing cocultures from sewage sludge treatment plants (Qui et al. 2004; Qiu et al. 2008).

Phylogenetic Structure of the Family

The phylogenetic structure of the family and its neighboring families within the order *Syntrophobacterales* is shown on ❯ *Fig. 22.1*. The borders of the family are primarily based on the phylogenetic tree as framework and their unique properties (physiology, chemotaxonomic markers) which are present in all members (see ❯ *Table 22.1*).

Molecular Analyses

DNA-DNA Hybridization Studies

Are absent.

Bioprinting and Ribotyping

Are absent.

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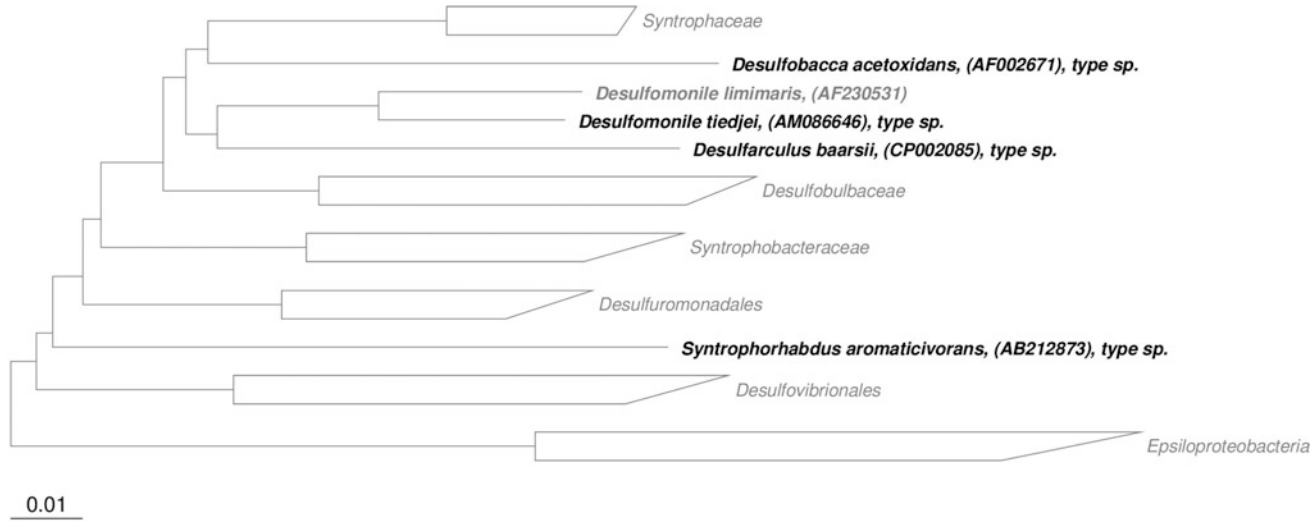

Fig. 22.1

Figure 22.1 **shows the phylogenetic reconstruction of the family *Syntrophorhabdaceae* 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**

MALDI-TOF

Are absent.

Genome Comparison

The complete genome sequence of the only described species of the genus *Syntrophorhabdus* which is also the only member of this family has been analyzed, but not published so far. The genome of the type strain UI of *Syntrophorhabdus aromaticivorans* is 3,230,684 bp long and contains 3,691 genes with 3,632 coding for proteins and 59 coding for RNA genes. The mol% G+C of the DNA is 51.9. In the genome genes for the dissimilatory adenylylsulfate reductase (aprBA) and the dissimilatory sulfite reduction (DsrAB) are absent.

Phages

Phages might be present, but are not reported so far.

Phenotypic Analyses

The main features of the single member of the family *Syntrophorhabdaceae* are listed in *Table 22.1*. The only species are gram-negative nonmotile rods. All are strictly anaerobic. There no data on the cellular fatty acid composition published.

Syntrophorhabdus Qiu et al. 2008, 1511^{VP} (Effective Publication Qiu et al. 2008, 2057)

Syn.tro.pho.rhab'dus. Gr. adj. *syn*, together with; Gr. n. *trophos*, one who feeds; Gr. fem. n. *rhabdus*, rod; N.L. fem. n. *Syntrophorhabdus*, rod which feeds together with (another species).

The genus *Syntrophorhabdus* contains one validly described species. Their chemotaxonomic and physiological properties are listed in *Table 22.1*.

Taxonomic comment: As can be seen in the phylogenetic tree published in the original description, there are several clone sequences which will fall into the same genus or family forming separate clusters (Qiu et al. 2008). So far other isolates belonging to this cluster are not described.

Biolog

Biolog data are not available.

Isolation, Enrichment, and Maintenance Procedures

The only member of this family was enriched with phthalate as electron donor for a methanogenic enrichment culture inoculated with sewage sludge from wastewater treatment plant (Qui et al. 2004). The media are prepared under specific conditions, and the addition of a reductant, in general sulfide, is required to keep the medium oxygen-free. A detailed description of the medium used for enrichment is provided by Sekiguchi et al. (2000).

Table 22.1
Selected characteristics of the only member of the genus *Syntrophorhabdus*

Characteristic	*Syntrophorhabdus aromaticivorans*
Type strain	Strain UI, DSM 17771 (in coculture with *Desulfovibrio* sp. strain UIS), JCM 13376 (in coculture with *Methanospirillum hungatei* DSM 864)
Accession number of the 16S rRNA gene sequence of the type strain	AB212873
Morphology	Rod
Cell size (μm)	0.4–0.8 × 1.2–3.0
Motility	–
Mol% G+C content	Nr
Major menaquinone	Nr
Optimal pH	7.0
Optimal temperature (°C)	25–37
Optimal NaCl concentration (g/l)	Nr
Growth factor requirement	–
Oxidation of substrate	Incomplete
H_2/CO_2	–
Formate	–
Acetate	–
Fatty acids	–
Ethanol	–
Other n-alcohols	–
Lactate	–
Pyruvate	–
Fumarate	–
Succinate	–
Malate	–
Other[a]	Phenol, p-cresol, isophthalate, 4-hydroxybenzoate, benzoate
Fermentative growth	–
Disproportionation of reduced sulfur compounds	Nr
Sulfate	–
Sulfite	–
Thiosulfate	–
Sulfur	–
Other	AQHS
Literature	Qiu et al. 2008, gen. nov., sp. nov.; Qiu et al. 2008

[a]Growth only in syntrophic coculture with a H_2-scavenging partner

For enrichment simple aromatic compounds like phenol, phthalate, 4-hydroxybenzoate, and benzoate should be used as substrates.

For short-term preservation, stock cultures can be stored at 2–6 °C for 4–6 weeks. For long-term storage no data are available.

Ecology

Habitat

Members of this might be present in anoxic areas of sewage sludge treatment plants or other anoxic habitats where a syntrophic degradation of aromatic compounds or more complex organic compounds depending on methanogenic or sulfate-reducing partners might occur.

Pathogenicity, Clinical Relevance

No clinical relevance documented.

References

Kuever J, Rainey FA, Widdel F (2005) Order *VI Syntrophobacterales ord. nov. In*: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) *Bergey's Manual of Systematic Bacteriology*, vol 2, 2nd edn, The Proteobacteria, part C (The *Alpha-*, *Beta-*, *Delta-*, and *Epsilonproteobacteria*). Springer, New York, p. 1021.

VALIDATION LIST NO 107 (2006) Int J Syst Evol Microbiol 56:1–6

Qiu YL, Hanada S, Ohashi A, Harada H, Kamagata Y, Sekiguchi Y (2008) *Syntrophorhabdus aromaticivorans* gen. nov., sp. nov., the first cultured anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen. Appl Environ Microbiol 74:2051–2058

VALIDATION LIST NO 122 (2008) Int J Syst Evol Microbiol 58:1511–1512

Qui YL, Sekiguchi Y, Imachi H, Kamagata Y, Tseng IC, Cheng S-S, Ohashi A, Harada H (2004) Identification and isolation of anaerobic, syntrophic phthalate isomer-degrading microbes from methanogenic sludges treating wastewater from terephthalate manufacturing. Appl Environ Microbiol 70:1617–1626

Sekiguchi Y, Kamagata Y, Nakamura K, Ohashi A, Harada H (2000) *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. Int J Syst Evol Microbiol 50:771–779

Yarza P, Ludwig W, Euzeby J, Amann R, Schleifer K-H, Glöckner FO, Rossello-Mora R (2010) Update of the all-species living tree project based on 16S and 23 S rRNA sequence analyses. Syst Appl Microbiol 33:291–299

Epsilonproteobacteria

23 The Family *Campylobacteraceae*

Albert J. Lastovica[1] · *Stephen L. W. On*[2] · *Li Zhang*[3]
[1]Extraordinary Professor (Food Microbiology), Department of Biotechnology, University of the Western Cape, Bellville, South Africa
[2]Leader of the Food Programme, Institute of Environmental Science and Research (ESR), Christchurch Science Centre, Ilam, Christchurch, New Zealand
[3]Senior Lecturer (Medical Microbiology and Immunology), School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia

Abstract
The *Campylobacteraceae* is the largest and most diverse family in the phylogenetically distinct *Epsilonproteobacteria*, presently comprising the genera *Campylobacter* (30 taxa), *Arcobacter* (17 taxa), and *Sulfurospirillum* (7 taxa). Individual species may be able to grow in microaerobic, anaerobic, and/or aerobic conditions, in temperatures from 25 °C to 42 °C; free-living, commensal, or pathogenic; motile or aflagellate; and able to colonize the oral cavity, intestine, stomach, or reproductive tracts of humans, large production animals (such as sheep, cattle, and deer), birds, and reptiles. Some species are known to be among the most frequent causes of human gastroenteritis, others are significant threats to bovine and ovine fertility, and many pose an as-yet unknown, or no, role in human or animal diseases. The taxonomy of the *Campylobacteraceae* has evolved extensively since its beginnings in 1963. This chapter outlines key events in the family's taxonomic history; reviews general phenotypic traits of each genus, including their isolation; outlines the clinical (including pathogenicity studies and antimicrobial resistance traits) and/or ecological significance of constituent species; and describes current approaches and challenges for species identification and epidemiological subtyping.

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Introduction

The family *Campylobacteraceae* is a diverse group of pathogenic, commensal, and environmental Gram-negative bacteria, currently comprising 3 genera: *Campylobacter* (30 taxa), *Arcobacter* (17 taxa), and *Sulfurospirillum* (7 taxa). These bacteria are found in surface water, groundwater, and food animals such as cattle, pigs, and sheep and their products such as milk. *Campylobacter* and *Arcobacter* colonize the mucosal surfaces of the intestinal tracts, urogenital tracts, and oral cavities of humans and a broad range of animal and bird hosts. *Campylobacter* is an important causative agent of enteritis in humans, particularly in young children. *Campylobacter* can also cause diverse extraintestinal infections such as bacteremia, meningitis, abortion, cellulitis, abscesses, and mycotic aneurysms in humans. This pathogen may cause the Guillain-Barré syndrome and reactive arthritis. *Campylobacter* is also responsible for enteritis, spontaneous abortion, infertility, and other illnesses in a variety of animals. *Arcobacter* is less well studied, but the pathogenicity of this organism has been demonstrated. *Sulfurospirillum* species are very fastidious and few strains have been studied. These organisms have been isolated from contaminated soil, surface water, and groundwater. There is no recognized association with humans or animals.

Taxonomy of the Family *Campylobacteraceae*

The family *Campylobacteraceae* presently contains three genera—*Campylobacter*, *Arcobacter*, and *Sulfurospirillum*. *Campylobacter* is the type genus and certain species in each of the other genera have, at some time or other, been previously classified as *Campylobacter* species, illustrating the substantive changes to the taxonomy of *Campylobacter* since its description in 1963. In fact, the changes to this particular phylogenetic group are even more far-reaching than the family *Campylobacteraceae* denotes. The changes have reflected major developments in bacterial taxonomic methodologies and the significant interest in these bacteria as clinically, economically, and ecologically important organisms. This section outlines the development of the taxonomy of the family *Campylobacteraceae* and descriptions of the taxa included. A phylogenetic reconstruction of the family *Campylobacteraceae* based on 16S rRNA data is illustrated in *Fig. 23.1*.

Developments in Taxonomy

Spiral organisms had been observed by microscopic examination of clinical specimens from diarrheal, gastric, and abortion cases since the late 1800s (e.g., Escherich 1886; Salomon 1898), but the study of many largely precluded due to an inability to culture them. Moreover, taxonomic methods were not advanced and bacterial classifications prior to even the most basic of genomic assays applied from the 1960s were predominantly based on cell morphology, growth requirements, and biochemical and immunological tests. *Vibrio* is a genus of curved- or spiral-shaped bacteria that resemble the causative agent of cholera. Until 1963 the genus was highly diverse and contained aerobic species ("true" *Vibrio* species including *V. cholerae*) and more fastidious microaerobic and anaerobic taxa from such diverse habitats as bovine and ovine abortions ("*V. fetus*," "*V. bubulus*": Smith and Taylor 1919; Florent 1953; Costas et al. 1987), the human oral cavity ("*V. sputorum*": Prévot 1940), pig feces ("*V. coli*": Doyle 1948), and bovine intestinal contents ("*V. jejuni*": Jones et al. 1931). In a seminal study, Sébald and Véron (1963) used the relatively new criteria of the G+C ratio in genomic DNA, and Hugh and Leifson's test for fermentative metabolism, to show that "*V. fetus*" and "*V. bubulus*" were notably different from other *Vibrio* species, proposing a new genus—*Campylobacter*—to encompass these taxa (Sébald and Véron 1963). The other microaerobic and/or anaerobic "*Vibrio*" taxa mentioned above (as well as "*V. faecalis*": Firehammer 1965) were reclassified as *Campylobacter* spp. in a subsequent study that used various biochemical and serological tests and the G+C ratio in DNA (Véron and Chatelain 1973).

Interest in *Campylobacter* dramatically increased with the findings of Butzler et al. (1973) and Skirrow (1977) that showed *Campylobacter* spp. frequently associated with human diarrhea. The isolation methods described facilitated studies of a wide range of sources, many yielding novel taxa. These included *C. mucosalis* (first designated "*C. sputorum* subsp. *mucosalis*"—from pig intestinal contents: Lawson et al. 1975), *C. concisus* (oral cavity: Tanner et al. 1981), *C. hyointestinalis* (pig intestine: Gebhart et al. 1983), "*C. nitrofigilis*" (plant roots: McClung et al. 1983), *C. lari* (gulls, first named "*C. laridis*": Benjamin et al. 1983), "*C. pylori*" (human gastric mucosa, first named "*C. pyloridis*" by Skirrow 1983), "*C. cryaerophilus*" (pig and cattle abortions: Neill et al. 1985), "*C. cinaedi*" and "*C. fennelliae*" (human intestine: Totten et al. 1985), "*C. mustelae*" (ferret gastric mucosa: first named "*C. pylori* subsp. *mustelae*" by Fox et al. 1988), *C. jejuni* subsp. *doylei* (human enteritis and gastritis: Steele and Owen 1988), "*C. intracellularae*" (porcine proliferative enteritis: McOrist et al. 1990), *C. upsaliensis* (dog feces: Sandstedt and Ursing 1991), and "*C. butzleri*" (human diarrhea: Kiehlbauch et al. 1991). The proposed new species *C. hyoilei* (Dep et al. 2001) was retracted as it was found to be a synonym of *C. coli* (Vandamme et al. 1997). Along with the increased interest in *Campylobacter* species, distribution in various hosts, and ecological niches, taxonomic methods were also advancing (Priest and Austin 1993; Gupta 1998; Vandamme et al. 1992c). Species delineations were accompanied by an increasingly sophisticated array of assays and approaches, including chemotaxonomic methods such as cellular fatty acid and isoprenoid quinone analysis, protein profiling, and, critically, DNA-DNA hybridization, which to this day remains a defining criterion for a bacterial species (Wayne et al. 1987). However, the key goal for classifications to reflect their "natural" evolutionary pathways remained elusive until the concept of the "molecular chronometer" was advocated by Carl Woese (1987), involving the comparative study of widely distributed, but functionally conserved, genes (notably the 16S subunit of bacterial rRNA) that change

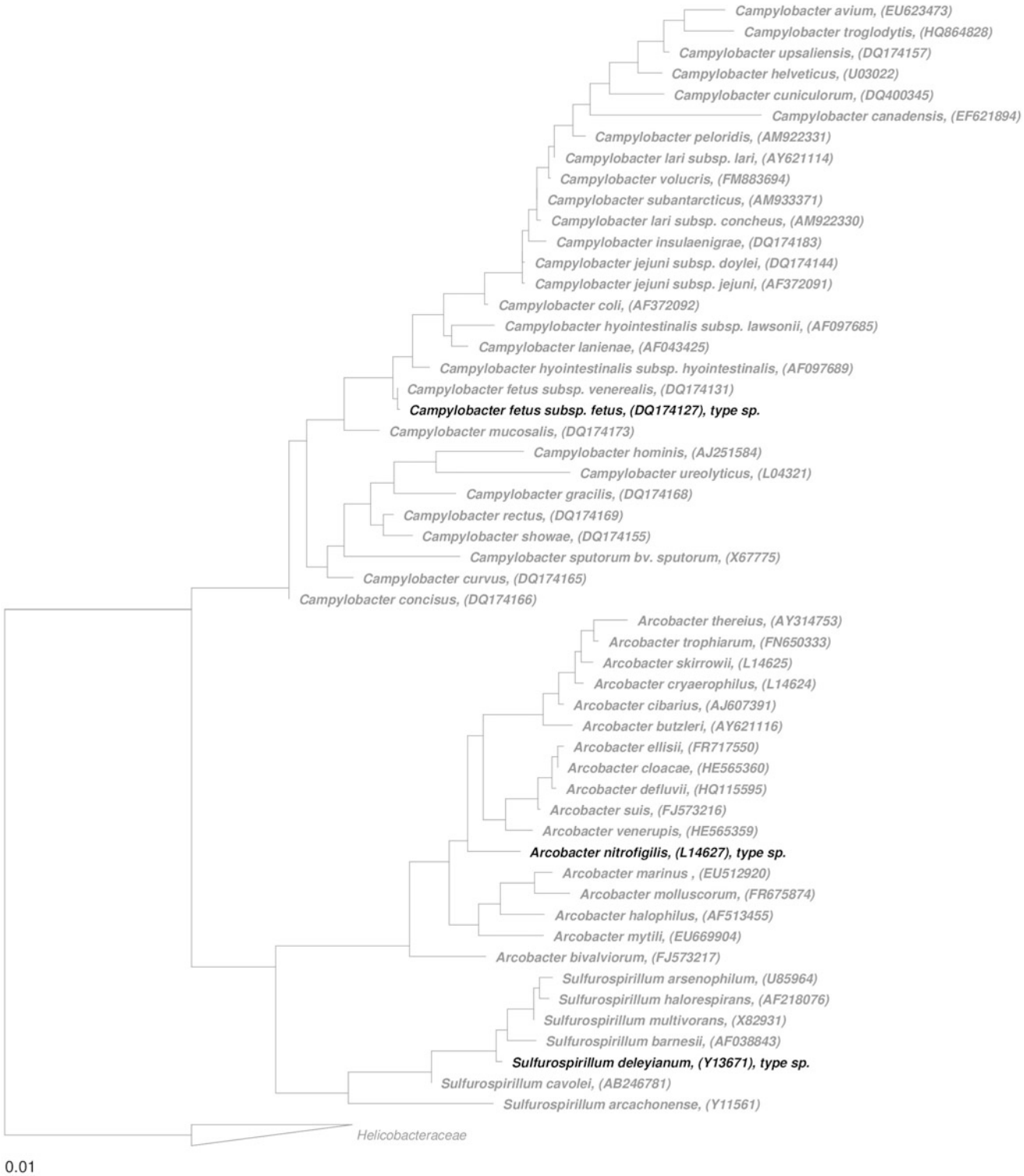

Fig. 23.1

Phylogenetic reconstruction of the family *Campylobacteraceae* 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

slowly over time to match changes in their surroundings (for a review, see, Woese 1987). This proposal was widely adopted and several studies of 16S rRNA gene sequence divergence in *Campylobacter* (Romaniuk et al. 1987; Lau et al. 1987; Paster and Dewhirst 1988) demonstrated significant phylogenetic diversity between several species, with one study clearly delineating three major groups (Thompson et al. 1988). The marked phylogenetic distinction of "*C. pylori*" and "*C. mustelae*," combined with some particular phenotypic traits including sheathed flagella (Han et al. 1989), and differences in cellular fatty acid composition (Goodwin et al. 1989a) led to the reclassification of "*C. pylori*" and "*C. mustelae*" to a new genus, *Helicobacter* (Goodwin et al. 1989b). Additional major changes were proposed in an authoritative study by Vandamme et al. (1992c) who used rRNA-DNA hybridization for phylogenetic placements and various polyphasic taxonomic data (including protein profiles, phenotypic, and immunotyping results) for closer genetic relationships. This proposal saw aerotolerant campylobacters assigned to a new genus, *Arcobacter*, as *A. nitrofigilis* and *A. cryaerophilus*, respectively, with *A. butzleri* reassigned later (Vandamme et al. 1992c). *Helicobacter* expanded with the enteric species *H. cinaedi* and *H. fennelliae*. One free-living strain (Laanbroek et al. 1977) was not immediately placed, but was later assigned to a novel genus, *Sulfurospirillum*, thus far containing only species of environmental origin (Schumacher et al. 1992). The remaining *Campylobacter* species were seen to belong to a single phylogenetic group; "*Bacteroides ureolyticus*" was formally assigned to *Campylobacter* later (Vandamme et al. 2005). Two oral anaerobic species, "*Wolinella curva*" and "*W. recta*," were also shown to belong to *Campylobacter*, but *W. succinogenes* remains distinct. The shared characteristics of *Campylobacter*, *Arcobacter*, and *Sulfurospirillum* led to the proposal of these taxa to be considered in the same family, *Campylobacteraceae* (Vandamme and de Ley 1991; Vandamme et al. 2005). In contrast, the unculturable "*C. intracellularae*" was later renamed *Lawsonia intracellularis*, a *Deltaproteobacteria* (McOrist et al. 1995). Similarly, a "*C. gracilis*-like" organism from intestinal tract infections was assigned to a new genus as *Sutterella wadsworthensis* (Wexler et al. 1996).

The family *Helicobacteraceae* includes *Helicobacter* and *Wolinella* species. The *Campylobacteraceae* and *Helicobacteraceae* belong to a distinct phylogenetic lineage first described as rRNA superfamily VI (Vandamme et al. 1991) and subsequently the *Epsilobacteria* (Cavalier-Smith 2002) and now most commonly referred to as the *Epsilonproteobacteria*, which also includes several genera found so far only as free-living organisms, including those assigned to the family *Nautilaceae*. Detailed descriptions of the *Helicobacteraceae* and *Nautilaceae* are outside the scope of this chapter.

Interest in *Campylobacter* and related organisms remains high as a consequence of continued and increasing awareness of the role they play in the pathogenesis of various diseases, and in the environment. The historical development of their taxonomy shows consistent development and expansion, and there is no reason to believe that this trend will cease. This presents particular challenges to frontline workers in the diagnosis of disease. Clinical regimens can differ for different species, and accurate species identification is needed to inform epidemiological studies that may lead to major public health efforts. Given the complexities of the identification process (see below) and the shortcomings of even widely accepted molecular tests such as PCR for accurate speciation (On and Jordan 2003; On et al. 2013), it is incumbent on those proposing new species to do so using good quality methods and in accordance with internationally recognized standards (Ursing et al. 1994). Increased access to, and advances in, whole-genome analysis may help in improved species delineations, but a polyphasic taxonomic approach remains necessary for best practice and to facilitate diagnostics for all.

Identification of Species of the Family *Campylobacteraceae*

The practice of identification generally forms the microbiologist's most common experience with taxonomy as a discipline. Irrespective of which area of microbiology one may be involved in—clinical, ecological, and fundamental—the identification of a strain to species level and sometimes subspecies, variant, or strain (where the practice is most commonly referred to as epidemiological subtyping) level is required. Strain identification may inform diagnoses, interventions, epidemiological, or population genetic studies and many more besides. The complexity of identification is often overlooked.

Identification involves matching data from an unknown isolate to those of known taxa. An isolate is considered to be identified when phenotypic (e.g., biochemical tests, fatty acid, or protein profiles) or genotypic (e.g., DNA fingerprints, sequences, PCR primers) data from the unknown strain matches that of a known taxon to an acceptable level. From first principles, the quality of the identification depends on (i) a sound classification in the first place, (ii) the quality of the database to which the unknown's characteristics are compared, and (iii) effective methods. In publicly available databases, misclassified strains may cause problems where others use the data for further analyses and base their identifications on erroneous data. Ideally, taxonomic descriptions should be based on polyphasic (multidisciplinary) studies to provide the most assurance for effective classification. Equally, new identification methods should be validated against a set of isolates that properly represent the diversity of the target species.

The clinical and economic importance of *Campylobacter* species particularly coupled to their taxonomic complexity has led to a wide range of phenotypic and genetic methods being developed to identify them. The most commonly used methods, and recent developments, are briefly reviewed below. There is an important caveat. Differentiation of taxa at the genus level is essentially achieved at the phylogenetic level. With the exception of *Arcobacter* that may be distinguished by their propensity to grow in aerobic conditions, and at temperatures lower than 30 °C, there are no easily discerned phenotypic features that characterize *Campylobacter* from the *Helicobacteraceae* or *Sulfurospirillum*

from the *Nautilaceae*. In certain situations this difference may be important, for example, common biochemical tests would not differentiate *C. coli* from *H. pullorum*, organisms found in both human diarrhea and poultry, a major source of infection.

Phenotypic Analysis of *Campylobacter*

All *Campylobacter* species are microaerophiles and have an optimal O_2 concentration of 5–10 % and a CO_2 concentration of 3–5 %. *C. gracilis*, *C. hyointestinalis*, *C. showae*, and *C. sputorum* bv. *faecalis* will grow under anaerobic conditions. *C. concisus*, *C. curvus*, *C. mucosalis*, *C. rectus*, and *C. gracilis* require H_2 or formate for growth. Campylobacters have an optimal growth temperature of 30–42 °C. All members of the *Campylobacter* genus have a low G+C content of 29–47 mol%. Bacterial cells (0.2–0.8 × 0.5–5.0 μm) are slender, curved, or helical rods often displaying "gull wing" morphology (Fig. 23.2). *C. showae* cells are straighter rods. In old cultures, or cultures exposed to stress in the environment, cells can change to spherical or coccal forms. Campylobacters have a characteristic darting, corkscrew-like movement attributed to a single non-sheathed flagellum. *C. showae* has a unipolar bundle of flagella, while *C. gracilis* and *C. hominis* are nonmotile as these species lack a flagellum (Vandamme and De Ley 1991; Lawson et al. 2001). *Campylobacter* species form circular, domed colonies 1–2 mm in diameter, beige or beige grey in color on blood agar. All *Campylobacter* species are nonspore-forming, reduce fumarate to succinate, are oxidase positive (except for *C. gracilis*), and are indole negative. Except for *C. jejuni* subsp. *doylei*, campylobacters will reduce nitrate. Oxidase activity is present in all species except for *C. gracilis*. No lecithinase or lipase activity is present, and starch, gelatin, and casein are not hydrolyzed. *C. fetus* and *C. rectus* cells have a microcapsule external to the bacterial outer membrane, which consists of paracrystalline S-layer proteins (Blaser et al. 1988). Biochemical characteristics of *Campylobacter* species are detailed in Table 23.1.

Phenotypic tests are commonly used to identify campylobacters. Tables, or dichotomous key-type schema, are frequently used in routine laboratories, but their use is far from straightforward, especially when evaluating test results described for so many taxa, and potentially with organisms from related genera (*Arcobacter*, *Helicobacter*) to consider too. Often, specific tests will assume more importance than others when determining whether an unknown belongs to one species or another. A classic example in *Campylobacter* is the use of the hippurate hydrolysis test to distinguish *C. jejuni* from other species (Morris et al. 1985); however, as with many other taxa, atypical (hippuricase-negative) strains exist and the recent description of a novel species, *C. avium*, that also hydrolyzes hippurate further obfuscates matters. Moreover, test outcomes can differ markedly depending on the methodologies used (On and Holmes 1991a, b, 1992). The use of tables or dichotomous tree schema (where results from a single test lead the user to different branches in the scheme and ultimately to a single named taxon) must therefore be undertaken with care.

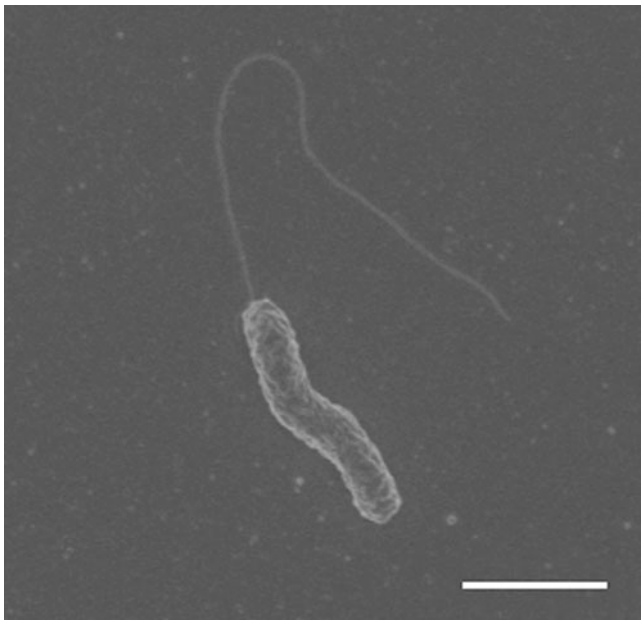

Fig. 23.2
Electron micrograph of a *Campylobacter concisus* cell. Bar maker = 1.0 μm

The limitations of manual assessment of phenotypic test data to identify *Campylobacter* species have led some investigators to explore the use of numerical, computer-assisted approaches to attain a more objective and potentially accurate result. Probability matrices (comprising lists of tests for which the percentage of strains giving a positive result is recorded) are commonplace in commercial biochemical identification systems such as API or Vitek, where test results from an unknown strain are statically evaluated with respect to taxa in the database and the "goodness of fit" of the results for each given. A predetermined threshold limit should be reached to consider the unknown isolate identified. This approach has been shown to be very effective for many *Campylobacter* and clinically related species (On et al. 1996, 1998b), but a large number of tests are required for best results (On 1996b). For particular groups of strains, "special purpose" matrices could be considered (On 1996b; On and Harrington 2001). Despite the advantages of probabilistic identification approaches, it is not infallible. The identification of highly atypical strains remains problematic (On et al. 1998b; On and Harrington 2001). There is also a risk of misidentification if the unknown isolate belongs to a taxon not present in the database, particularly if a species closely resembling that taxon is already present, although this issue exists with genetic assays such as PCR as well (On et al. 2013).

An alternative to conventional phenotypic analysis is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). MALDI-TOF-MS identifies bacterial species by mass spectrometry analysis of ions directly desorbed from intact bacteria (Marvin et al. 2003). A number of recent studies have examined the use of MALDI-TOF-MS in identification of *Campylobacter* species including *C. jejuni*,

Table 23.1
Biochemical characteristics of *Campylobacter* species

Characteristics	[a]1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Oxidase	+	+	+	v	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	v	+	+	+
Catalase	+	v	+	–	–	–	+	+	(+)	(–)	–	+	–	(+)	+	+	+	–	+	(–)	+	v[e]	+	–
Hippurate hydrolysis	–	–	–	–	–	(–)	–	–	–	–	–	–	–	(+)	–	–	–	–	–	–	–	–	–	–
Indoxyl acetate	–	–	+	–		v	–	–	–	(+)	+	–	–	+	–	ND	–	–	ND	+	v	–	–	+
Nitrate reduction	+	v	+	(–)		+	–	+	(+)	(+)	+	+	v	v[d]	+	ND	+	(–)	ND	+	+	(+)	+	+
Growth at 42 °C	+	+	+	(+)		v	–	+	–	(+)	+	+	v	v[d]	+	+	+	+	+	(–)	v	+	+	(+)
Alpha-hemolysis	ND	–	(–)	(–)		(–)	+	–	(–)	–	+	v	–	(+)	+	ND	+	(–)	ND	+	+	+	+	+
MacConkey agar	+w	+	v	–	–	(+)	–	(+)	v	(+)	–	(–)	–	(–)	+	v	(+)	(+)	–	–	+	v	v	–
Nutrient agar	–	–	+	(–)		+	v	+	(+)	+	(+)	+	(+)	+	–	+	(–)	+	+	(+)	v	+	–	+
NaCl (2.0 %)	–	ND	–	(–)		v	–	–	–	(–)	–	–	v	–	–	+	v	(+)	(+)	v	+	+	+	–
Glycine (1.0 %)	–	v	(+)	(–)		+	–	+	(–)	+	v	v	+	v[d]	–	(+)	+	(–)	+	+	v	+	+	+
Safranin (0.02 %)	–	ND	+	v		+	(–)	+	(+)	+	–	+	–	v[d]	v	–	+	+	–	–	–	(+)	–	+
Sodium deoxycholate (0.1 %)	+w	ND	+	–		(+)	–	+	(+)	(+)	v	v	–	v[d]	v	v	+	–	v	–	–	(–)	–	v
Nalidixic acid (32 mg/mL^{-1})	+	v	–	(+)	+	+	+	+	(+)	v	–	+	(+)	–	v	–	(+)	(+)	(+)	(+)	–	v	+	–
Cephalothin (32 mg/mL^{-1})	+	–	+	–	–	–	+	–	–	–	–	(–)	–	v[d]	+	+	(+)	(+)	(–)	–	–	–	–	(–)
Metronidazole (4 mg/mL^{-1})	+	ND	(+)	(–)		–	+	(+)	v	–	–	v	–	v	+	+	+	(+)	(+)	–	+	(–)	v	(+)
Carbenicillin (32 mg/mL^{-1})	+	ND	(+)	–	–	–	+	–	–	–	v	–	–	v	+	+	+	–	–	–	–	–	–	–
Cefoperazone (64 mg/mL^{-1})	–	ND	+	–		(+)	+	+	(–)	–	v	(–)	(–)	v[d]	+	ND	+	v	ND	–	–	–	–	(–)

[a]Species characterized: 1. *C. volucris* sp. nov. (n = 3), 2. *C. canadensis*, 3. *C. coli*, 4. *C. concisus*, 5. *C. cuniculorum*, 6. *C. curvus*, 7. *C. insulaenigrae*, 8. *C. fetus* subsp. *fetus*, 9. *C. fetus* subsp. *venerealis*, 10. *C. gracilis*, 11. *C. helveticus*, 12. *C. hyointestinalis*[b], 13. *C. hominis*, 14. *C. jejuni*[c], 15. *C. lanienae*, 16. *C. lari* subsp. *concheus*, 17. *C. lari* subsp. *lari*, 18. *C. mucosalis*, 19. *C. peloridis*, 20. *C. rectus*, 21. *C. showae*, 22. *C. sputorum*, 23. *C. subantarcticus*, 24. *C. upsaliensis*. Data taken from On et al. (1995b, 1996, 1998a, b), On (unpublished data), Lawson et al. (2001), Inglis et al. (2007)

[b]Data merged for *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii*

[c]Data merged for *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*

[d]Test results differ between *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*

[e]Test results differ between *C. sputorum* biovars *sputorum*, *paraureolyticus* (catalase negative), and *faecalis* (catalase positive)

Notation: + positive, (+) most strains positive, – negative reaction, (–) most strains negative, v variation in reaction depending on strain, w weak reaction, ND not determined

C. coli, *C. helveticus*, *C. lari*, *C. sputorum*, *C. upsaliensis*, and *C. fetus* (Alispahic et al. 2010; Bessede et al. 2011; Mandrell et al. 2005; Martiny et al. 2011; Winkler et al. 1999). Results from these studies showed that MALDI-TOF-MS accurately identified these *Campylobacter* species. However, MALDI-TOF-MS was not always able to identify two different species present in the same sample (Bessede et al. 2011). MALDI-TOF-MS analysis holds great potential for bacterial identification (Bright et al. 2002a). In practice, rigid standardization is required for optimal results (Bright et al. 2002b). Thus far, studies have shown that *C. jejuni*, *C. coli*, and *C. fetus* (and the related helicobacters, *H. pylori* and *H. mustelae*) display species-specific markers when analyzed by MALDI-TOF-MS (Mandrell and Wachtel 1999; Winkler et al. 1999). Hrabák et al. (2013) demonstrated that MALDI-TOF-MS is a relevant tool for the detection of antibiotic resistance.

Phenotypic Analysis of *Arcobacter*

Arcobacter spp. can be differentiated from *Campylobacter* spp. by their ability to grow in air and at lower temperatures, 15–30 °C (Vandamme et al. 1992b). Cells of the genus *Arcobacter* are morphologically similar to those of *Campylobacter*; they are Gram-negative rods, curved, S-shaped, or helical. They move with a darting, corkscrew-like movement by means of a single unsheathed polar flagellum at one or both ends of the cell (Vandamme et al. 1992b). Several *Campylobacter* spp. or a combination of *Campylobacter* and *Arcobacter* species may be present on the initial isolation plate as seen in a routine diagnostic laboratory (Lastovica 2006). Differentiation of *Arcobacter* and *Campylobacter* species may be difficult using phenotypic or biochemical methods (Yan et al. 2000). Biochemical tests routinely used for the identification of clinical bacteria often give variable results. Misinterpretation can easily be avoided by checking aerotolerance at 25 °C and 37 °C, as *Arcobacter* is aerotolerant, while *Campylobacter* is not. The most useful biochemical tests for the differentiation of *Arcobacter* are outlined in *Table 23.2* and Collado and Figueras (2011).

Cells of the genus *Arcobacter* grow under aerobic, microaerobic, or H_2-enriched microaerobic conditions. Hydrogen is not essential for growth. Growth is optimal between 15 °C and 37 °C, while some strains can grow at 42 °C. Colonies of *Arcobacter* are circular, domed, cream or off-white colored, and usually 1 mm in diameter. *Arcobacter* species do not ferment or oxidize carbohydrates. Energy is obtained from amino acids or tricarboxylic acid cycle intermediates, not carbohydrates.

Phenotypic Analysis of *Sulfurospirillum*

Species belonging to the genus *Sulfurospirillum* have, to date, been found only as free-living organisms in environments such as anoxic mud, anaerobic sludge, and contaminated freshwater and groundwater sources. They have not been found in association with animal hosts or with any known disease. Like other *Epsilonproteobacteria*, cells are curved to spiral in shape and motile through use of a single polar flagellum. Most species are capable of growth under microaerobic conditions, although *S. multivorans* is a strict anaerobe (John et al. 2009). The temperature range for species appears wide (from 8 °C to 40 °C) with optimal temperatures from 20 °C to 30 °C. The optimal pH range enabling growth of *Sulfurospirillum* species is from 7.0 to 7.5 (Holliger et al. 1993; Kodama et al. 2007). The DNA G+C content ranges from 32 % to 42.7 %. Metabolically, most species can utilize arsenate, sulfur, thiosulfate, nitrate, and oxygen as electron acceptors and fumarate and lactate as electron donors. Features allowing the discrimination of extant *Sulfurospirillum* species are listed by Kodama et al. (2007), Luijten et al. (2003), and Finster et al. (1997).

Molecular Analysis

A variety of techniques is available for the identification and characterization of *Campylobacter*, *Arcobacter*, *and Sulfurospirillum* species. While some of these techniques such as cellular fatty acid analysis (Rosseel et al. 1998; Sasser 2001; Steinbrueckner et al. 1998) have been useful for the analysis of *Campylobacter* and *Arcobacter*, these techniques are less frequently used than others described below.

Amplified Fragment Length Polymorphism (AFLP)

AFLP profiling involves digesting cellular DNA with two restriction enzymes and then detecting a subset of the resulting fragments by a PCR technique (Savelkoul et al. 1999). This profiling technique provides excellent resolution of genomic polymorphisms for species- and indeed strain-level identification. Numerical analysis of AFLP profiles assists with data interpretation and strain identification (Duim et al. 2001; On and Harrington 2000).

DNA-DNA Hybridization Studies

DNA-DNA hybridization (DDH) studies have been commonly used in the description of species in the family *Campylobacteraceae*. The reported DDH values ranged from no detectable homology to 57 % between the type strains of *Campylobacter* species, 4–55 % between the type strains of *Arcobacter* species, and 29–66 % between the type strains of *Sulfurospirillum* species. All reported DDH values were below the threshold (70 %) suggested for species delineation (Stackebrandt and Goebel 1994).

rRNA Sequence Analysis

Identification of the RNA subunits as phylogenetic markers, advent of PCR and more frequent use of gene sequencing,

Table 23.2
Biochemical and growth characteristics of *Arcobacter* species

	Species																
	[a]1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Growth conditions																	
37 °C, O_2	+	+	–	+	v	+	+	+	+	+	+	v	+	–	–	–	–
37 °C, mO_2	+	+	+	+	v	+	+	+	+	+	+	–	+	–	–	–	+
42 °C, mO_2	–	v	–	–	–	+	+	–	–	+	+	–	–	–	–	–	–
2 % NaCl	+	v	–	+	v	+	+	+	+	+	+	+	+	–	+	+	+
4.0 % NaCl	+	–	–	–	–	–	–	+	+	+	+	+	+	–	–	v	–
1.0 % glycine	–	–	–	–	–	–	–	–	+	–	+	–	–	–	+	v	–
0.04 % TTC	–	+	v	–	+	–	–	–	–	–	–	–	v	–	v	+	–
0.05 % safranin	–	+	+	+	+	+	–	–	+	+	–	–	+	–	+	v	–
0.1 % sodium deoxycholate	–	+	+	+	v	+	+	–	–	+	+	v	+	+	v	+	–
1.0 % ox bile	–	+	+	+	v	+	–	–	–	+	+	–	+	–	v	+	–
Minimal medium	–	+	+	v	–	+	+	–	–	–	–	–	–	+	+	–	+
MacConkey	–	+	+	+	v	+	v	–	–	+	+	–	–	+	v	v	+
CCDA	–	+	v	+	+	+	+	–	–	–	–	–	+	–	v	+	+
Cefoperazone (64 mg/L)	–	+	+	–	+	v	–	–	–	+	–	–	+	–	+	+	–
Biochemical activity																	
Catalase activity	+	v	v	+	+	+	+	–	–	+	+	+	+	+	+	+	+
Urease activity	–	–	–	–	–	+	v	–	–	–	–	+	–	–	–	–	+
Nitrate reduction	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Indoxyl acetate hydrolysis	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+
Alpha-hemolysis	–	–	–	–	–	–	–	–	–	–	–	–	+	–	v	–	–

Data from *A. bivalviorum* ($n = 3$) (Levican et al. 2012), *A. butzleri* ($n = 12$) (On et al. (1996), *A. cibarius* ($n = 15$) (Houf et al. 2005), *A. cloacae* ($n = 2$) (Levican et al. 2013), *A. cryaerophilus* ($n = 19$) (On et al. (1996), *A. defluvii* ($n = 8$) (Collado et al. 2011), *A. ellisii* ($n = 3$) (Figueras et al. 2011b), *A. halophilus* ($n = 1$) (Donachie et al. 2005, Figueras et al. 2011b), *A. marinus* ($n = 1$) (Figueras et al. 2011b, Kim et al. 2010), *A. molluscorum* ($n = 3$) (Figueras et al. 2011a), *A. mytili* ($n = 3$) (Collado et al. 2009a), *A. nitrofigilis* ($n = 4$) (Collado et al. 2009b, On et al. (1996), *A. skirrowii* ($n = 9$) (On et al. (1996), *A. thereius* ($n = 8$) (Houf et al. 2009), *A. trophiarum* ($n = 11$) (De Smet et al. 2011), *A. venerupis* ($n = 1$) (Levican et al. 2012). Additional data from Collado and Figueras (2011)
Notation: + (≥95 %), – (≤11 %), v (12–94 %), TTC triphenyl tetrazolium chloride, CCDA, *Campylobacter* charcoal-deoxycholate agar, O_2 aerobic conditions, mO_2 microaerobic conditions
[a]Species characterized: 1. *Arcobacter bivalviorum*, 2. *A. butzleri*, 3. *A. cibarius*, 4. *A. cloacae*, 5. *A. cryaerophilus*, 6. *A. defluvii*, 7. *A. ellisii*, 8. *A. halophilus*, 9. *A. marinus*, 10. *A. molluscorum*, 11. *A. mytili*, 12. *A. nitrofigilis*, 13. *A. skirrowii*, 14. *A. suis*, 15. *A. thereius*, 16. *A. trophiarum*, 17 *A. venerupis*

led to massive expansion of gene sequences in public databases such as GenBank for ready comparison. For many routine laboratories seeking to identify an unknown strain, this approach is highly attractive and the method has the advantage of being able to place a strain soundly in taxonomic space. The phylogenetic resolution of the 16S and 23S subunits is limited, and identification to subspecies level (i.e., for *C. fetus*, *C. lari*, *and C. jejuni*), biovar level (i.e., *C. sputorum*), and even genomospecies level (i.e., *C. concisus*) is not possible. Even with some genetically distinct species (including *C. jejuni* and *C. coli*, and *C. helveticus* and *C. upsaliensis*), there is insufficient sequence divergence to allow for entirely specific identification. In contrast, sequence divergence between *C. hyointestinalis* subspecies is so great that reliance on this method alone could lead to their misclassification as distinct species (Harrington and On 1999). As a result 16S rRNA gene sequence comparisons should be used with caution for speciation of *Campylobacteraceae*.

Genome Analysis

Among the species of *Campylobacter* genus, full genomes of *C. jejuni*, *C. fetus*, *C. concisus*, *C. curvus*, *C. lari*, and *C. hominis* have been sequenced. The first genome sequence of *Campylobacter* species, *C. jejuni* NCTC 11168, was published in the year 2000 and re-annotated in 2007 (Gundogdu et al. 2007; Parkhill et al. 2000). In addition to *C. jejuni* NCTC 11168, the genome

sequences of a number of other *C. jejuni* strains are now available—http://www.ncbi.nlm.nih.gov (Cooper et al. 2011; Fouts et al. 2005; Friis et al. 2010; Hofreuter et al. 2006). *Campylobacter* species have small genomes compared to other bacterial species. The genome sizes of *C. jejuni* strains were 1.63–1.85 Mb. *C. concisus* strain 13826 has a genome size of 2.1 Mb, which is the largest genome among the fully sequenced *Campylobacter* species. The full genome-sequenced *Campylobacter* strains have a G+C content less than 40 % (29.6–39.2 %) except for *C. curvus* strain 525.92 which has a G+C content of 44.5 %. Proteins encoded by these *Campylobacter* strains were from 1,580 to 2,092. Plasmids were identified in all full genome-sequenced *Campylobacter* species. However, not all *C. jejuni* strains contain a plasmid. A recent study by Ali et al. (2012) conducted comparative genomic analysis of 15 full genome-sequenced *Campylobacter* strains of six *Campylobacter* species (Pearson et al. 2007). This study identified the core-genome of the 15 *Campylobacter* genomes consisting of 552 gene families and the pan-genome consisting of 7,059 gene families. Virulence factors involved in adherence, invasion, motility, and toxins were found to be commonly present in all strains. Pathogenicity islands were identified in *C. fetus* but not in the other *Campylobacter* species (Ali et al. 2012).

Among the species of the *Arcobacter* genus, the whole genomes of *A. butzleri* and *A. nitrofigilis* have been sequenced (Miller et al. 2007; Pati et al. 2010; Toh et al. 2011). *A. butzleri* strain RM4018 and *A. butzleri* strain ED-1 have a similar genome size (2.3 Mb and 2.2 Mb); both strains have a G+C content of 27 % (Miller et al. 2007; Toh et al. 2011). Protein-coding genes identified in *A. butzleri* strain RM4018 and *A. butzleri* strain ED-1 were 2259 and 2158, respectively. Despite being a member of *Campylobacteraceae*, the proteome of *A. butzleri* is most similar to *Sulfurimonas denitrificans*, a member of the *Helicobacteraceae* (Miller et al. 2007). A substantial proportion of the *A. butzleri* genome is related to growth and survival under different environments (Miller et al. 2007). A plasmid was detected in *A. butzleri* ED-1 strain (Toh et al. 2011). *A. nitrofigilis* has a genome size of 3.2 Mb and a G+C content of 28.4 %, with 3,154 genes being protein-coding genes (Pati et al. 2010).

For the genus of *Sulfurospirillum*, the genome of *S. deleyianum* strain 5175^T was sequenced in the year 2010 (Sikorski et al. 2010). The full genome sequence of *S. barnesii* is now available (http://www.ncbi.nlm.nih.gov).

Multilocus Sequence Typing (MLST)

Multilocus sequence typing (MLST) is a technique for determining the sequence diversity of multiple loci. The procedure characterizes isolates of microbial species using the DNA sequences of internal fragments of multiple housekeeping genes. Internal fragments of approximately 450–500 bp of each gene are used, and these can be accurately sequenced using an automated DNA sequencer. For each housekeeping gene, the different sequences present within a bacterial species are assigned as distinct alleles, and for each isolate, the alleles at each of the loci define the allelic profile or sequence type (ST) (Dingle et al. 2001). This technique has been used successfully to determine the sequence types of *C. coli* in pig liver (von Altrock et al. 2013); *C. jejuni* in human, poultry, and bovine isolates (de Haan et al. 2013); and *C. hyointestinalis* and other emerging campylobacters (Miller et al. 2012). The MLST genotyping approach is less susceptible to factors causing genomic instability than earlier genomic methods using restriction site polymorphisms or single genetic markers (Wassenaar et al. 2000).

Phages

Campylobacter phages have been isolated from excreta of chickens, retail poultry, and other sources including feces of pigs, cattle, and sheep, abattoir effluence, and sewage (Connerton et al. 2011). The majority of *Campylobacter* phages are double-stranded DNA viruses belonging to the *Myoviridae* family (Connerton et al. 2011). A study by Sails et al. (1998) divided 16 *Campylobacter* phages commonly used in a bacteriophage typing system into three groups based on genome sizes and viral head diameters (Sails et al. 1998). In addition to lytic phages, recent full genome sequencing analysis showed that lysogenic phages are present in some *Campylobacter* species including *C. jejuni* (Fouts et al. 2005).

Whole genomes of *Campylobacter*-specific phages have been sequenced recently (Carvalho et al. 2012; Hammerl et al. 2012; Kropinski et al. 2011; Timms et al. 2010). These bacteriophages showed adaptations to their host and possess genes that may enhance *Campylobacter* metabolism, potentially benefiting both the bacteriophage and its host. Timms et al. (2010) demonstrated that two phages belonging to group II are capable of lysing both *C. jejuni* and *C. coli*. They had a very high level of sequence conservation (>96 %) despite separation in time and place of isolation. The genome sizes of these two phages were 177.5 kb (CP220) and 175.7 kb (CPt10).

CP220/CPt10 phages contained a large number of radical S-adenosylmethionine genes. Following this, the genomes of three other *Campylobacter* phages including two *C. jejuni* phages (CP81 and NCTC 12673) and a *C. coli* phage (vB_CcoM-IBB_35) were sequenced (Carvalho et al. 2012; Hammerl et al. 2012; Kropinski et al. 2011; Timms et al. 2010). The genomes of both CP81 and NCTC 12673 differ greatly from that of CP220/CPt10. The genome of NCTC 12673 showed the closest homology to the cynobacterial T4-related myophages (Kropinski et al. 2011).

A phage-typing scheme for *C. jejuni* and *C. coli* was previously developed (Frost et al. 1999; Grajewski et al. 1985; Khakhria and Lior 1992). Use of *Campylobacter* phage therapy in poultry has been investigated. It was found to reduce

Campylobacter numbers in chickens that received phages (Carrillo et al. 2005; Carvalho et al. 2010; El-Shibiny et al. 2009; Wagenaar et al. 2005).

Polymerase Chain Reaction (PCR)

The PCR process amplifies specific marker genes through annealing DNA "primers" present on either side of the target and then synthesizing this sequence using a DNA polymerase enzyme, commonly *Taq*. The procedure is relatively simple and widely available routinely. Assays for many *Campylobacter* species have been described, including *C. coli*, *C. concisus*, *C. jejuni*, *C. fetus* (both species and subspecies level), *C. hyointestinalis*, *C. lari*, *C. helveticus*, *C. upsaliensis*, *C. concisus*, *C. sputorum*, *C. mucosalis*, *C. hominis*, and *C. lanienae* (Eyers et al. 1993; Bastyns et al. 1995a, b, c; Harrington et al. 1999; Hum et al. 1997; Lawson et al. 1998, 1999; Lawson and Gebhart 2000; Linton et al. 1994, 1996; Logan et al. 2000; On and Harrington 2001; On and Jordan 2003; Misawa et al. 2000). Care must be taken to ensure that the basis of the specificity and sensitivity of the PCR assay is sound. For example, many tests for *C. coli*, *C. jejuni*, and closely related species are compromised one way or the other (Linton et al. 1996; On and Jordan 2003; On et al. 2013). It is important that PCR assays are appropriately evaluated and regularly reevaluated for their efficacy.

In addition to PCR assays that aim to detect specific taxa, restriction fragment length polymorphism analysis of 16S (Cardarelli-Leite et al. 1996) or 23S (Hurtado and Owen 1997; Fermer and Engvall 1999) rRNA genes has been described. These approaches aim to help routine laboratories to identify a broad range of species but will inevitably lack the specificity for differentiation of especially closely related taxa. A similar approach using degenerate primers to detect 16S rRNA genes of *Epsilonproteobacteria*, and subsequently resolving them using Degrading Gradient Gel Electrophoresis (DGGE), has been found useful to detect multiple species in a single sample (Petersen et al. 2007; Cornelius et al. 2012). Identification is best attained by excising, and subsequently sequencing, amplicons from gels, with the same caveats as for full-length 16S rRNA gene sequencing as mentioned above.

Detection by molecular methods has been developed for *Arcobacter*, resulting in increased sensitivity and a reduction in time for identification. A widely used method is the m-PCR that targets the 16S or 23S rRNA gene developed by Houf et al. (2000). Additional real-time PCR assays have been developed (Abdelbaqi et al. 2007a; Brightwell et al. 2007; González et al. 2010). Detailed summaries of molecular methods used to identify *Arcobacter* species have been provided by Houf (2010) and Collado and Figueras (2011).

While genus-specific PCR assays are available (Bastyns et al. 1995a), which target the 23S rRNA gene, false-negative reactions may be present (Scullion et al. 2006). A DNA microarray with excellent sensitivity and specificity has been developed for the detection of *C. jejuni*, *C. coli*, and *A. butzleri* (Quinones et al. 2007).

Ribotyping and RiboPrinting

Ribotyping discriminates bacterial species based on the nucleotide differences in ribosomal operons, which involves digestion of bacterial genomic DNA followed by hybridization of the digested DNA fragments with a ribosomal operon probe (Bouchet et al. 2008; Grimont and Grimont 1986). An automatic ribotyping system named RiboPrinter System was developed by DuPont Qualicon. In the family *Campylobacteraceae*, ribotyping was used mainly in the analysis of *C. jejuni*. Most of these studies used the automatic RiboPrinter System (RiboPrinting), with *Pst*I and *Hae*III being the commonly used restriction enzymes for DNA digestion.

A number of studies compared the discriminatory ability of different genotyping methods including ribotyping/RiboPrinting in typing *C. jejuni* isolates. In a study conducted by O'Reilly et al. (2006), 84 *C. jejuni* isolates were genotyped. Pulsed-field gel electrophoresis (PFGE) (*Sma*I digestion) revealed 53 subtypes, multilocus sequence typing (MLST) identified 33 subtypes, RiboPrinting (*Pst*I digestion) identified 28 subtypes, and fragment length polymorphism (*Dde*I digestion) of the *flaA* gene (*flaA*-RFLP) identified 16 subtypes (O'Reilly et al. 2006). Nielsen et al. (2000) examined 90 *C. jejuni* isolates and showed that random amplified polymorphic DNA typing (RAPD) and PFGE were the most discriminatory methods in genotyping *C. jejuni*, followed by *flaA*-RFLP (*Dde*I and *Alu*I digestion) and RiboPrinting (*Hae*III digestion). De Boer et al. (2000) examined 50 *C. jejuni* isolates and showed that amplified fragment length polymorphism (AFLP) fingerprinting was most discriminatory, identifying 41 distinct genotypes. This was followed by PFGE, *flaA*-RFLP, and RiboPrinting, which revealed 38, 33, and 26 genotypes, respectively (de Boer et al. 2000). Ge et al. (2006) examined 75 *C. jejuni* isolates and 45 *C. coli* isolates and identified 44 PFGE patterns and 22 RiboGroups. However, a study from Hänninen et al. (2001) examined 35 *C. jejuni* isolates and showed that the discriminatory power of PFGE, AFLP, and *Hae*III ribotyping is the same. These studies showed that despite the advantages in speed and reproducibility, the automatic RiboPrinting system is less discriminatory in genotyping *C. jejuni* isolates when compared to other molecular typing methods such as PFGE, MLST, and AFLP.

Isolation, Enrichment, and Maintenance

Isolation, Enrichment, and Maintenance of *Campylobacter*

Isolation of clinically relevant thermophilic campylobacters from stool samples is usually based on selective antibiotics used to suppress growth of contaminating flora. Growth at 42 °C under microaerobic conditions has been the standard protocol. The isolation of *Campylobacter* spp. from food or environmental samples, usually present in low numbers, often involves pre-enrichment procedures, followed by culture on antibiotic-containing selective media. However, inhibition and

overgrowth by competing bacteria may occur during the pre-enrichment step. Different isolation techniques may recover different *Campylobacter* species. Isolation procedures and media for *Campylobacter* have been reviewed by Corry et al. (1995). Ugarte-Ruiz et al. (2012) compared three isolation protocols (culture-dependent, direct plating, Bolton and Preston enrichment) and real-time qPCR for the detection and isolation of thermophilic *Campylobacter* from poultry feces, neck skin, and fresh packed meat. They found direct plating on Campyfood agar is optimal for the isolation of *Campylobacter* from highly contaminated samples. When enrichment is required, Bolton broth is the best choice. Real-time PCR yielded the highest number of positive samples in this study.

In a recently published study of 411 laboratories testing on-site for *Campylobacter* spp., most laboratories used culture-based methods, but procedures differed widely. These laboratories did not adhere to established guidelines, most likely resulting in underdiagnosis (Hurd et al. 2012). In a separate study, M'ikanatha et al. (2012) found similar results in a survey of 176 clinical laboratories in Pennsylvania. Enrichment media for *Campylobacter* are based on the use of selective antimicrobials such as vancomycin, cefoperazone, and cycloheximide. Campylobacters are sensitive to peroxides and may benefit from media with free radical scavengers such as charcoal or horse blood. *Campylobacter* isolation protocols traditionally have been done at 42 °C and under microaerophilic growth conditions. These protocols are suitable for thermophilic *Campylobacter* species such as *C. jejuni* and *C. coli*, but some *Campylobacter* species such as *C. concisus* and *C. upsaliensis* grow poorly or not at all at 42 °C, or have an essential requirement for a H_2-enriched atmosphere. Also, some *Campylobacter* isolates may be sensitive to the antibiotics present in selective media currently used.

The Cape Town Protocol (Lastovica 2006) overcomes these difficulties by utilizing membrane filtration onto antibiotic-free Tryptose Blood Agar media for the isolation of species of the *Campylobacter* genus as well as those of the related genera *Arcobacter* and *Helicobacter*. As this protocol utilizes a H_2-enriched atmosphere and growth at 37 °C, essentially all species of *Campylobacter*, *Arcobacter*, and *Helicobacter* can be isolated. The Cape Town Protocol has been used successfully to isolate *Campylobacter* spp. from human, chicken, and gull stools (Lastovica and le Roux 2003; Lastovica 2006; Kinzelman et al. 2008; Jacob et al. 2011). The complete protocol is outlined in Lastovica (2006).

Enrichment culture is often used to isolate *Campylobacter* species that may be present in low numbers from a variety of substrates. In a study comparing isolation of *Campylobacter* from broiler chicken environments, Williams et al. (2012) found that modified Exeter (m-Exeter) enrichment broth was significantly more effective in isolating *Campylobacter* spp. than Preston or modified Bolton (m-Bolton) broths. These authors conclude that the enrichment method used affects both the number and species of *Campylobacter* isolated from naturally contaminated samples. In a separate study, Vidal et al. (2013) found that enrichment in Exeter broth significantly increased the sensitivity of swab and cecal samples for the detection of *Campylobacter* spp. in broiler flocks. Jokinen et al. (2012) compared enrichment in Bolton broth and subsequent plating on charcoal-cefoperazone-deoxycholate agar (CCDA) against enrichment in Bolton broth and membrane filtration. While the conventional and filtration methods yielded similar number of campylobacters from water and fecal samples, the membrane method had higher specificity, making it a cost-effective procedure for the isolation of *C. jejuni* and *C. coli* from water and fecal samples. A range of *Campylobacter* species were recovered from a meat matrix using Campylobacter Enrichment Broth and membrane filtration onto Anaerobe Basal Agar (Lynch et al. 2010).

Various media such as Tryptose Blood Agar, Columbia agar, and Mueller-Hinton broth and agar can be used to isolate and maintain *Campylobacter* spp., and plates are usually subcultured every 3 or 4 days. The optimum atmosphere for *C. jejuni* and other thermophilic species is 85 % N_2, 10 % CO_2, and 5 % O_2. Other species of *Campylobacter* such as *C. concisus* with an essential requirement for H_2 are best grown in an atmosphere of 75–78 % N_2, 7–10 % H_2, 10 % CO_2, and 5 % O_2 at a temperature range between 37 °C and 42 °C.

Isolation, Enrichment, and Maintenance of *Arcobacter*

A variety of media and procedures has been used to isolate *Arcobacter* from a diversity of samples (Collado and Figueras 2011). A standardized isolation protocol for the isolation of *Arcobacter* is currently not available. A commonly used isolation protocol uses enrichment with CAT (cefoperazone, amphotericin B, and teicoplanin) broth followed by passive filtration through a 0.45 μm filter over blood agar (Atabay and Corry 1997). Merga et al. (2011) compared five methods for the isolation of *Arcobacter* from cattle, sheep, and badger feces. They found the most sensitive and specific method tested was an *Arcobacter*-specific broth in conjunction with modified charcoal-cefoperazone-deoxycholate agar (mCCDA) with additional added antibiotics. Other isolation protocols utilizing enrichment and media with selective supplements have been proposed by Johnson and Murano (1999), Houf et al. (2001), Van Driessche et al. (2003), and Houf and Stephan (2007). Methods for recovering *Arcobacter* spp. are very diverse, and there is no consensus about which method is the most useful (it depends on the sample being tested). Problems for recovery may include inhibition of some *Arcobacter* spp. with antibiotics (Atabay et al. 2006; Houf et al. 2001) as well as insufficient inhibition of contaminating microflora (Andersen et al. 2007; Fera et al. 2004). An enrichment step could reduce the diversity of *Arcobacter* species as it may favor the growth of faster growing species (Ho HT et al. 2006). Direct molecular detection may also be affected if enrichment broth is used, as amplification is favored for templates with a higher concentration in the PCR mixtures (Ho HT et al. 2006). Discordant results have been published between culturing and molecular detection of *Arcobacter*. Possible reasons for this may include different protocols that use a shorter enrichment time, plating media that is

not optimal for the suppression of contaminating bacteria, and missing coexisting strains or species that have variable growth rates (Fera et al. 2004; González et al. 2006). Microaerophilic and aerobic conditions for the isolation of *Arcobacter* spp. are used; these have been detailed by Collado and Figueras (2011).

An enrichment broth developed by Johnson and Murano (1999) proved to be significantly more efficient in isolating *Arcobacter* spp. from beef than three other enrichment broths tested concurrently by Hamill et al. (2008). In addition to providing superior growth for *Arcobacter*, culture plates utilizing Johnson and Murano broth exhibited a deep red color around the *Arcobacter* colonies, aiding in identification. Collado and Figueras (2011) summarize media and procedures used for the isolation of *Arcobacter*.

Arcobacter spp. will grow under aerobic, microaerobic, and H_2-enriched microaerobic atmospheric conditions. Maintenance is easy, as *Arcobacter* spp. will grow at room temperature (25 °C) under an aerobic atmosphere; cultures plates of *Arcobacter* can be left on the laboratory bench and subcultured every 3 or 4 days.

Preservation

Campylobacter cultures age and lose viability rapidly. Cultures may be stored for up to a week under microaerobic conditions at 4 °C. For longer-term storage, cultures can be frozen in a cryopreservative, such as 10 % skim milk (Cody et al. 2008) or 5 % glycerol, and stored at −80 °C or in liquid nitrogen. Cultures can last for years but may require enrichment for recovery of damaged cells. Freeze-drying is another method of preservation, but *Campylobacter* is susceptible to damage during the freeze-drying process. Freeze-dried cells can be successfully revitalized years later. Survival of freeze-dried cells can be significantly increased by optimizing the age of the cultures, the lyoprotectant, and the storage and rehydration conditions (Gorman and Adley 2004; Portner et al. 2007). The use of commercially available cryovials containing beads in a cryopreservative solution is another method of preservation.

Ecology

Ecology of *Campylobacter*

Campylobacter spp. are ubiquitous in the environment and have been isolated from soil (Chai et al. 2009), mud (Stuart et al. 2010), bathing beach sand (Bolton et al. 1999), and environmental water (Kemp et al. 2005). Campylobacter has been associated with soil amebas that may provide a protected environment for the bacteria (Baré et al. 2010; Axelsson-Olsson et al. 2010). *Campylobacter* has also been detected in untreated tap water, consumption of which resulted in outbreaks of campylobacteriosis (Jakopanec et al. 2008; Vogt et al. 1982). *Campylobacter* spp. are capable of surviving in bovine manure for a 10-month period (Inglis et al. 2010).

Campylobacter species can colonize the mucosal surfaces of the gastrointestinal tracts of humans and a wide variety of wild and domesticated birds and mammals, including food animals such as cattle, pigs, sheep, and especially poultry (Table 23.3). Thermophilic *C. jejuni* subsp. *jejuni* seems to have evolved to colonize the intestinal mucosa and cecum of birds whose body temperature is 42 °C, where they act as commensals. They have been isolated from the feces of cats, dogs, hamsters, rodents, and cattle (Inglis and Kalischuk 2003). Shellfish, meat, and unpasteurized milk are recognized as reservoirs for these organisms (Table 23.3, Moore et al. 2006; Koziel et al. 2012). *C. hyointestinalis* has been isolated from pigs, rusa deers, and reindeers (Hänninen et al. 2002; Hill et al. 1987; Gebhart et al. 1985). In immunocompromised humans survival and growth at extraintestinal sites can occur (Lastovica and Allos 2008). Some *Campylobacter* species are frequently (but not exclusively) host associated. *C. lari* is often isolated from wild birds and shellfish. *C. hyointestinalis*, *C. mucosalis*, and *C. coli* are frequently isolated from pigs. *C. upsaliensis* and *C. helveticus* are found in the intestinal tracts of cats and dogs. *C. fetus* subsp. *fetus* colonizes the intestinal tracts of sheep and cattle, and occasionally humans and turtles (Lastovica 2006; Harvey and Greenwood 1985). A variant that colonizes reptiles has also been described. *C. fetus* subsp. *fetus* is capable of causing bacteremia, abortion, cellulitis, endocarditis, and meningoencephalitis in humans (Lastovica and Allos 2008). *C. fetus* subsp. *venerealis* specifically colonizes the vagina of venereally infected cows and the prepuce of bulls. *C. sputorum* bv. *bubulus* is also found in these bovine urogenital sites. *C. concisus*, *C. sputorum* bv. *sputorum*, *C. curvus*, *C. rectus*, *C. gracilis*, and *C. showae* have been recovered from the human oral cavity (Kamma et al. 1994, 2000). Some of these species may be isolated from the human intestinal tract.

Ecology of *Arcobacter*

Arcobacter is widespread in the environment and has been isolated from diverse environments (Table 23.4). They are present in wetland soil (Han et al. 2009), piggery effluent-irrigated soil (Chinivasagam et al. 2007), and drinking water (Jacob et al. 1993; 1998). Water could be a reservoir for transmission to humans and animals (Ho HT et al. 2006). *Arcobacter* species have been isolated from groundwater, rivers, lakes, and seawater (Collado et al. 2008; Fera et al. 2004; Morita et al. 2004; Musmanno et al. 1997; Rice et al. 1999). *A. cryaerophilus*, *A. butzleri*, and *A. skirrowii* are significantly more prevalent in fecally contaminated water than in uncontaminated water (Collado et al. 2008). *Arcobacter* spp. have been isolated from the feces of livestock animals in significant numbers (Van Driessche et al. 2003) and could account for surface water contamination. *A. marinus* and *A. halophilus* have been isolated from seawater (Kim et al. 2010; Donachie et al. 2005) and *A. defluvii* from sewage (Collado et al. 2011). *Arcobacter* can remain viable in water for up to 250 days at 4 °C (Van Driessche and Houf 2008).

Table 23.3

Sources and disease associations of *Campylobacter* species[a]

Species or subspecies	Recognized sources	Human disease association	Animal disease association
C. avium	Chickens, turkeys	?	?
C. canadensis	Whooping and sandhill cranes	?	?
C. coli	Humans, dogs, cattle	Enteritis, septicemia	
C concisus	Humans, dogs, cats	Inflammatory bowel disease, periodontal disease, enteritis, septicemia,[b] Barrett's esophagus	Enteritis—canine
C. cuniculorum	Rabbits	?	?
C. curvus	Humans	Gastroenteritis; abscesses	?
C. fetus subsp. *fetus*	Cattle, sheep, dogs, turtles	Septicemia, meningitis, vascular infection, abortion	Spontaneous abortion—bovine, ovine
C. fetus subsp. *venerealis*	Cattle	Septicemia	Infectious fertility—bovine
C. gracilis	Dogs, humans	Abscesses	?
C. helveticus	Dogs, cats	?	?
C. hominis	Humans	?	?
C. hyointestinalis subsp. *hyointestinalis*	Pigs, cattle, hamsters	Enteritis, septicemia[b]	?
C. hyointestinalis subsp. *lawsonii*	Pigs, poultry, birds	?	?
C. insulaenigrae	Seals, porpoises	Enteritis, septicemia	?
C. jejuni subsp. *jejuni*	Humans, dogs, cattle, birds, poultry, cattle, sheep, milk, seafood, water	Enteritis, septicemia, abortion, appendicitis, colitis, myocarditis, reactive arthritis, Reiter's syndrome, Guillain-Barré syndrome	Spontaneous abortion (bovine, ovine); gastroenteritis (canine, feline)
C. jejuni subsp. *doylei*	Humans, dogs	Enteritis, septicemia	?
C. lanienae	Humans, cattle	?	?
C. lari subsp. *lari*	Cats, dogs, chickens, seals, mussels, oysters	Enteritis, septicemia	?
C. lari subsp. *concheus*		?	?
C. mucosalis	Pigs, dogs	?	
C. peloridis	Humans, molluscs	?	?
C. rectus	Humans	Abscesses	?
C. showae	Humans, dogs	Septicemia, cholangitis	?
C. sputorum bv. *paraureolyticus*	Cattle, humans	?	?
C. sputorum bv. *faecalis*	Cattle	?	?
C. sputorum bv. *sputorum*	Humans, cattle, pigs, sheep	Abscesses	?
C. subantarcticus	Penguins, albatrosses	?	?
C. troglodytis	Chimpanzees	?	?
C. upsaliensis	Cats, dogs, ducks, monkeys	Enteritis, septicemia, abortion, abscesses	Gastroenteritis—canine, feline
C. ureolyticus	Humans, milk, bovine feces	Ulcerative colitis	?
C. volucris	Black-headed gulls	?	?

[a]Data from Aabenhus et al. (2002), Bolton et al. (1987), Chaban et al. (2010), Debruyne et al. (2009, 2010), Engberg et al. (2000), Etoh et al. (1993), Flores et al. (1990), Foster et al. (2004), Fox et al. (1989), Garcia et al. (1983), Gebhart et al. (1985), Harvey and Greenwood (1985), Inglis et al. (2007), Kamma et al. (1999), Kaur et al. (2011), Koziel et al. (2012), Lastovica et al. (1989, 2006, 2009), Lastovica and Allos (2008), Lastovica and Skirrow (2000), Lawson et al. (2001), Lindblom et al. (1995), Macuch and Tanner (2000), Macfarlane et al. (2007), Mahendran et al. (2011), Man et al. (2010b), Mukhopadhya et al. (2011), Nielsen et al. (2013), On et al. (1995a), On and Vandamme (1997), Roop II et al. (1985), Rossi et al. (2009), Sandstedt and Ursing (1991), Sasaki et al. (2003), Stanley et al. (1992), Steele et al. (1985), Suzuki et al. (2013), Tanner et al. (1981), Taylor et al. (1986, 1987), Vandamme et al. (1989, 1992a, b, 1995), Van Etterijck et al. (1996), Zanoni et al. (2009), Zhang et al. (2009)

[b]HIV patients and children

? unknown

Table 23.4
Sources and disease associations of *Arcobacter* species

Species	First recognized	Recognized sources	Human disease association	Animal disease association
A. bivalviorum	Levican et al. (2012)	Mussels, clams	?	?
A. butzleri [a]	Kiehlbauch et al. (1991), Yan et al. (2000)	Pigs, bulls, horses, cattle, chicken, dogs, cats, primates, ostriches, ducks, water, sewage	Enteritis, bacteremia	Gastroenteritis—porcine, bovine; abortion—primate, porcine
A. cibarius	Houf et al. (2005)	Chicken	?	?
A. cloacae	Levican et al. (2013)	Sewage, mussels	?	?
A. cryaerophilus [a]	Neill et al. (1985)	Pigs, bulls, chicken, sheep, horses, dogs, cats, sewage	Enteritis, bacteremia	Abortion—bovine, porcine, ovine, equine
A. defluvii	Collado et al. (2011)	Sewage	?	?
A. ellisii	Figueras et al. (2011b)	Mussels	?	?
A. halophilus	Donachie et al. (2005)	Hypersaline lagoon water	?	?
A. marinus	Kim et al. (2010)	Seawater	?	?
A. molluscorum	Figueras et al. (2011a)	Mussels, oysters	?	?
A. mytili	Collado et al. (2009a)	Mussels	?	?
A. nitrofigilis [a]	McClung et al. (1983)	Estuarine plant roots	?	?
A. skirrowii	Vandamme et al. (1992b)	Sheep, bulls, pigs, chicken, ducks	Enteritis, bacteremia	Abortion—porcine, equine; gastroenteritis—bovine, ovine
A. suis	Levican et al. (2013)	Pork meat	?	?
A. thereius	Houf et al. (2009)	Pigs, ducks	?	?
A. trophiarum	De Smet et al. (2011)	Pigs	?	?
A. venerupis	Levican et al. (2012)	Mussels, clams	?	?

Additional data from Collado and Figueras (2011)
[a]Originally described as a *Campylobacter* spp., ? unknown

Food products of animal origin have been suggested as important reservoirs of *Arcobacter* for potential transmission to humans (Ho TK et al. 2006). *Arcobacter* may have a high prevalence in the intestinal tract and feces of food animals and retail meat products (Ho TK et al. 2006). On et al. (2002) demonstrated that there is a high prevalence of several species of *Arcobacter* in Danish pigs. *Arcobacter* contamination of meat products most likely occurs when the feces of contaminated animals come in contact with carcasses in abattoirs. *Arcobacter* has the highest prevalence on poultry, followed by pork and beef products, as well as raw milk (Scullion et al. 2006). Shellfish are another potential reservoir for *Arcobacter* infection, and a variety of *Arcobacter* species has been isolated from clams and mussels (Collado et al. 2009b). *A. mytili* and *A. molluscorum* were originally isolated from mussels (Collado et al. 2009a; Figueras et al. 2011a). *A. bivalviorum*, *A. venerupis*, and *A. ellisii* have recently been isolated from mussels and clams (Figueras et al. 2011a, b; Levican et al. 2013). These findings are of public health concern, as seafood is often eaten raw or undercooked. *A. butzleri*, followed by *A. cryaerophilus*, and then *A. skirrowii* are the most prevalent species in foods (Lehner et al. 2005). González and Ferrús (2011) demonstrated by culture and molecular methods the presence of *Arcobacter* spp. in 20 % of fresh Spanish lettuce samples.

Contact with the saliva or feces of pets may provide potential routes of transmission of *Arcobacter*. Fera et al. (2009) reported detecting by m-PCR a high prevalence of *A. butzleri* and *A. cryaerophilus* in oral swab specimens taken from pet cats. In contrast, in a separate study, Houf et al. (2008) could not detect by m-PCR (multiplex polymerase chain reaction) *Arcobacter* spp. in oral swabs or feces of 61 cats, but did detect *Arcobacter* spp. in dogs. These contrasting results for the detection of *Arcobacter* in cats may reflect differences in methodology between laboratories.

Arcobacter spp. have been detected in raccoons (Hamir et al. 2004), monkeys (Russell et al. 1992), rhinoceroses, gorillas, alpacas, and rheas (Wesley and Schroeder-Tucker 2011). At present, there is no information on the carriage of *Arcobacter* species in wild birds.

Clinical Significance

Significance of *C. jejuni* and *C. coli* in Human Enteritis

As well as causing a variety of animal diseases (*Table 23.3*, Butzler 1984), *Campylobacter* is universally recognized as an important cause of human enteritis, particularly in children.

Campylobacter was identified as an animal pathogen in the early twentieth century (Skirrow 2006); however, the organism was recognized as a human pathogen only in the late 1970s (Fourgeaux et al. 1977). Hoffmann et al. (2012) estimated the annual cost of illness at $1.7 billion and quality-adjusted life year losses at 13,300 for *Campylobacter* infections in the USA. Historically, most strains of *Campylobacter* isolated and identified in cases of human disease have been *C. jejuni* subsp. *jejuni* or *C. coli*.

The main sources of *Campylobacter* infection in humans are raw, undercooked, or contaminated meat, especially poultry, as well as contaminated water and milk (Hermans et al. 2012; Jacobs-Reitsma et al. 2008; Jay-Russell et al. 2013; Sahin et al. 2012; Sears et al. 2011; Taylor et al. 2012). The bulk of infections are reported sporadically, with common-source outbreaks relatively uncommon, but not unimportant. A recent CDC study (Taylor et al. 2012) of 252 *Campylobacter* outbreaks in the USA, which occurred during 1997–2008, involved 9, 135 illnesses, 159 hospitalizations, and 3 deaths. This study indicated that the main vehicles of *Campylobacter* outbreaks were foodborne (86 %), of which dairy products were implicated in 29 %, poultry in 11 %, and produce in 5 % of the cases. Recently Gardner et al. (2011) documented an outbreak of *C. jejuni* campylobacteriosis due to the consumption of raw peas. Person-to-person transmission or transmission by pet animals to humans may also occur infrequently (Allos and Lastovica 2011; Jacobs-Reitsma et al. 2008; Taylor et al. 2012). International travel to regions such as Africa and Latin America carries a risk of acquiring a *Campylobacter* infection. Results of a FoodNet survey indicated that of 8,270 reported travel-associated infections, 42 % were due to *Campylobacter* (Kendall et al. 2012). The infective dose of *Campylobacter*, as determined by human volunteer studies, can be as low as 500 bacteria (Black et al. 1988). The epidemiology of *Campylobacter* infections is markedly different in tropical and temperate countries (Allos and Lastovica 2011). In tropical countries (i.e., South Africa, Gambia, Bangladesh), infections occur year-round and primarily affect children, especially those <2 years old. Repeated infections often result in acquired immunity, as adult infections are usually asymptomatic (Glass et al. 1983). In temperate countries (e.g., UK, USA, Canada, France, New Zealand), infection has summer and fall peaks and is almost always symptomatic, and adults as well as children are at risk (Glass et al. 1983; Oberhelman and Taylor 2000) In tropical countries outbreaks are rare, while in temperate countries they are common. Watery diarrhea is usual in tropical countries, while inflammatory diarrhea with leucocytes and blood in stools is common in temperate countries (Megraud et al. 1990). Infection among children <5 years old in tropical areas is common, with up to eight episodes per year, and asymptomatic infections can outnumber symptomatic ones by two to one. In temperate areas, *Campylobacter* infection usually produces an acute gastrointestinal illness which is indistinguishable from illness caused by *Salmonella* or *Shigella*. A definitive diagnosis is only possible by detecting *Campylobacter* in the patient's stool. The essential lesion in *Campylobacter* enteritis is acute inflammatory enteritis that can affect the colon and rectum. Serum antibodies to *Campylobacter* antigens appear about the fifth day of illness, peak within 2–4 weeks, and decline over several months (Black et al. 1988). The mean incubation period of *Campylobacter* enteritis is 3.2 days, with a range of 1–7 days (Blaser and Engberg 2008).

The onset of the disease depends on the susceptibility of the patient, the virulence of the strain, and the infective dose. The beginning of *Campylobacter* enteritis is abrupt, often with cramping abdominal pains and then diarrhea; other symptoms may include fever, headache, myalgia, rigors, and vomiting (Allos and Lastovica 2011; Skirrow and Blaser 2000). Diarrhea is commonly profuse (up to 10 bowel movements per day). After 1 or 2 days of diarrhea, frank blood appears in the stools of up to 30 % of patients, indicating progression of the infection to the colon and rectum. Large variations in invasion frequency have been observed (Blaser and Engberg 2008). A particular aspect of *Campylobacter* enteritis is abdominal pain, which may mimic acute appendicitis. After about 4 days, the patient's condition improves, but patients may excrete *Campylobacter* in their stools for up to 69 days (Black et al. 1988). Recurrent enteritis, often with septicemia, caused by *Campylobacter*, is a typical problem of patients with immune deficiencies such as hypogammaglobulinemia and AIDS (Blaser and Engberg 2008).

During pregnancy, infection due to *Campylobacter* is rare in humans, but some incidents have been reported (Simor et al. 1986; Fujihara et al. 2006). *Campylobacter* outbreaks in neonatal nurseries have occurred where there has been a nosocomial spread of infection from communally used items such as thermometers (van Dijk and van der Straaten 1988).

Extraintestinal Infections Associated with *C. jejuni* and *C. coli*

Campylobacter has been associated with appendicitis, colitis, toxic megacolon, and extraintestinal infections such as meningitis, pancreatitis, endocarditis, and nephritis. These conditions are described in detail by Blaser and Engberg (2008).

Bacteremia due to *Campylobacter* enteritis is underreported, as blood cultures are rarely taken early in the disease, and not all methods of detecting bacteremia are equally sensitive. Skirrow et al. (1993) identified 257 blood culture isolates and found that 89 % were *C. jejuni* or *C. coli* and 8.6 % were *C. fetus*, *C. lari*, and *C. upsaliensis*. The remainder were either *Helicobacter fennelliae* or *H. cinaedi*. In their study, Skirrow et al. (1993) documented an average bacteremia rate of 1.5 per 1,000 intestinal infections for patients in England and Wales. The highest rate (5.9 per 1,000) was for patients aged 65 years or more and the lowest for children aged 1–4 years (0.3 per 1,000). *C. jejuni*, *C. coli*, *C. upsaliensis*, and other *Campylobacter* spp. have been repeatedly isolated in significant numbers from pediatric blood cultures in South Africa (Lastovica et al. 1989, 2002; Lastovica 2006). *Campylobacter sputorum* has also been implicated as a causative agent of bacteremia (Tee et al. 1998).

Post-infective Sequelae Associated with *C. jejuni* and *C. coli*

A post-infection complication of *Campylobacter* infection is the Guillain-Barré syndrome (GBS), a neurological disease causing ascending paralysis that may result in death. *C. jejuni* appears to be the only *Campylobacter* spp. associated with GBS. Koga et al. (1999) isolated *C. curvus* and *C. upsaliensis* from the stools of GBS patients, but there was no evidence to suggest these organisms were involved in GBS.

Poropatich et al. (2010) present data indicating that 31 % of 2,502 GBS cases studied were due to *Campylobacter* infection. Israeli et al. (2012) have recently reviewed the Guillain-Barré syndrome. A particularly severe form of GBS, acute motor axonal neuropathy, occurs in seasonal epidemics among children in rural China (Ho TW et al. 1995). Reactive arthritis (ReA) may also occur after *Campylobacter* infection. Bremell et al. (1991) documented that 2.9 % of 35 patients with gastrointestinal symptoms developed reactive arthritis after eating *Campylobacter*-contaminated chicken at a party. Pope et al. (2007), in a systematic review of the literature, indicated that 5 % of *Campylobacter* ReA might be chronic or relapsing (with respect to musculoskeletal systems).

Significance of *C. fetus* in Human Disease

The first report of *C. fetus* infection was that of King (1957). The clinical features of diarrheal disease due to *C. fetus* subsp. *fetus* (hereafter, *C. fetus* unless otherwise noted) infection are similar to *C. jejuni* infection (Rennie et al. 1994). *C. fetus* isolates from gastroenteritis cases that grew at 42 °C were reported by Harvey and Greenwood (1983). This is unusual, as this organism does not usually grow at this temperature. Although *C. fetus* infection may occur at any age, most infections occur in the elderly. In an analysis of 111 *C. fetus* strains isolated in Quebec, Canada, Trembley et al. (2003) found that 53 % occurred in persons older than 70 years. *C. fetus* infection usually occurs in compromised patients, more than 75 % of whom are men with serious medical conditions (e.g., diabetes mellitus, atherosclerosis, liver cirrhosis, chronic alcoholism, AIDS), and patients being treated with immunosuppressive agents (Guerrant et al. 1976; Monno et al. 2004; Sakran et al. 1999). *C. fetus* accounts for more bacteremia in adults than any other *Campylobacter* species except *C. jejuni* (Lastovica 1996; Skirrow et al. 1993). *C. fetus* may cause a prolonged relapsing illness that is characterized by chills, fever, and myalgia and that lacks an identified focus of infection (Guerrant et al. 1976). Secondary seeding to an organ may occur, which can lead to complications and occasionally death (Viejo et al. 2001).

Diarrhea precedes or accompanies bacteremia in nearly half the cases recorded by Guerrant et al. (1976), Farrugia et al. (1994), and Lastovica (1996). *C. fetus* exhibits a distinct affinity for vascular tissue, and infections have been associated with thrombophlebitis, cellulitis, and mycotic aneurysms (Cone et al. 2003; Montero et al. 1997). A 52-year-old man developed *C. fetus* endocarditis when he ate raw meat after having a tooth extracted (Miki et al. 2005). *C. fetus* infection resulting in abortion in pregnant women has been reported (Steinkraus and Wright 1994). *C. fetus* may infect the central nervous system, with meningoencephalitis being the most common manifestation (Ozeki et al. 2002).

C. fetus may remain latent in an immunocompromised host, after bacteremic seeding in a bony focus, only to be reactivated years later (Neuzil et al. 1994). *C. fetus* subsp. *venerealis* infections in humans are rare and thus far confined to episodes of bacteremia.

Significance of *C. concisus* in Human Disease

C. concisus is being increasingly isolated from clinical samples (Lastovica and le Roux 2000; Lastovica 2006; Engberg et al. 2005; Matsheka et al. 2002; Zhang et al. 2009). The disease potential of these non-*jejuni*/*coli Campylobacter* species is beginning to be appreciated (Lastovica and Allos 2008; Zhang et al. 2009). The virulence properties of very recently described *Campylobacter* species such as *C. avium* and *C. cuniculorum* (*Table 23.3*) are unknown at present.

It has been suggested that *C. concisus* may play a role in a number of gastrointestinal disorders, including inflammatory bowel disease (IBD), enteritis, periodontal disease, and Barrett's esophagus disease. The association between *C. concisus* and human IBD was reported, with a significantly higher prevalence of *C. concisus* being detected in intestinal biopsies and fecal samples collected from patients with IBD as compared to controls (Mahendran et al. 2011; Man et al. 2010b; Mukhopadhya et al. 2011; Zhang et al. 2009). *C. concisus* has been frequently isolated from, or detected in, diarrheal stool samples (Aabenhus et al. 2002; Cornelius et al. 2012; Engberg et al. 2000; Lastovica 2006, 2009; Lindblom et al. 1995; Nielsen et al. 2013; Van Etterijck et al. 1996). However, when *C. concisus* isolation rates from diarrheal and non-diarrheal stool samples were compared, no significant difference was found (Engberg et al. 2000; Van Etterijck et al. 1996). Currently, the role of *C. concisus* in human diarrheal disease (enteritis) remains inconclusive. PCR detected a 9 % occurrence of *C. concisus* in canine diarrheal stools and none in non-diarrheal stools (Chaban et al. 2010).

Isolation of *C. concisus* from subgingival plaque samples of patients with gingivitis and periodontitis has been reported (Kamma et al. 1999; Macuch and Tanner 2000; Tanner et al. 1981). However, most of these studies did not include samples collected from healthy controls. A study from Macuch and Tanner (2000) reported that *C. concisus* was more often isolated from individuals with initial periodontitis compared to healthy controls (67 % vs. 39 %); this difference was not statistically different (Macuch and Tanner 2000). Further investigations are needed to ascertain the relationship between *C. concisus* and periodontal disease.

Macfarlane et al. (2007) reported isolation of *C. concisus* from 57 % (4/7) of esophageal biopsies collected from patients with Barrett's esophagus and none of the controls (0/7). Whether *C. concisus* contributes to the development of Barrett's esophagus requires further investigation.

In addition to samples collected from the gastrointestinal tract, *C. concisus* has been isolated from blood samples of HIV patients and children (Lastovica and Allos 2008), the foot ulcer of a patient with diabetes (Johnson and Finegold 1987), and brain abscess of a patient with maxillary sinus carcinoma (de Vries et al. 2008). Although rare, isolation of *C. concisus* from these infected body sites that are normally sterile justifies their clinical significance and further investigation.

A significant complicating factor in evaluating the pathogenic potential of *C. concisus* is that strains may belong to one of several genetically distinct but phenotypically indistinguishable taxa known as genomospecies, that some have suggested demonstrate differences in pathogenic potential (Aabenhus et al. 2005). Extrachromosomal virulence genes have also been suggested as necessary for strains to be pathogens (Kaakoush et al. 2011).

Significance of *C. upsaliensis* in Human Disease

The organism was originally described as a catalase-weak or catalase-negative *Campylobacter* and was first isolated from the stools of healthy and diarrhetic dogs (Burnens and Nicolet 1992; Bourke et al. 1998; Sandstedt et al. 1983).

DNA-DNA hybridization studies indicated that this was a new species (Sandstedt and Ursing 1991). Using pulsed-field gel electrophoresis, Bourke et al. (1996) demonstrated genomic heterogeneity in seven serogroups of *C. upsaliensis*. Analysis of 96 *C. upsaliensis* strains from three continents showed that 84 % belonged to one of five serotypes (Lentzsch et al. 2004). These data point to clonal expansion of *C. upsaliensis* and indicate a high degree of antigen conservation during evolution (Bourke et al. 1996, 1998).

C. upsaliensis is a recognized human pathogen, causing acute or chronic diarrhea and bacteremia. *C. upsaliensis* has been consistently isolated in significant numbers from diarrhetic stools and blood cultures of South African pediatric patients (Lastovica et al. 1989; Lastovica 2006; Lastovica and Allos 2008). It has been associated with hemolytic uremic syndrome (Carter and Cimolai 1996) and with spontaneous human abortion (Gurgan and Diker 1994).

Significance of Campylobacters in Animal Disease

In cattle and sheep, the distinction of the two *C. fetus* subspecies is critical, since *C. fetus* subsp. *fetus* is associated with spontaneous abortion, but *C. fetus* subsp. *venerealis* causes infertility, a condition that is of far greater economic significance. The distinction and definition at the genetic and phenotypic level is not trivial (Moolhuijzen et al. 2009), but some useful approaches have been described (On and Harrington 2001). *C. hyointestinalis* has been associated with bovine diarrhea (Diker et al. 1990) and digestive tract infection in cattle (Bryner et al. 1964; Hum 1996).

Campylobacter fetus subsp. *fetus* may cause bovine venereal campylobacteriosis. This organism asymptomatically colonizes the prepuce and penis of bulls. The organism is transferred to cows during coitus, with the sites of infection being uterine glands, resulting in infertility or abortion. The organism is capable of persisting in the vagina for several years, during this time new bulls may be infected (Jimenez et al. 2011; Osburn and Hoskins 1970; Corbeil et al. 1981). *C. fetus* subsp. *fetus* is frequently isolated from the bovine gut (Atabay and Corry 1998). Should bacteremia develop from this organism, then abortion may follow. Ovine abortion may develop from bacteremia due to *C. fetus* subsp. *fetus*. The transmission route is thought to be fecal-oral, and asymptomatic carriage does occur. Infection of susceptible pregnant ewes may result in pathology to the placenta and subsequent abortion (Lindenstruth et al. 1949; Robertstad and Tucker 1956). *C. jejuni* can also cause abortion in cattle and sheep and, together with *C. upsaliensis*, can cause enteritis in cats and dogs, which may become reservoirs for human infection (*Table 23.1*). *C. jejuni* may occasionally cause fatal enteritis or hepatitis in ostriches (Verwoerd 2000; Stephens et al. 1998).

Significance of *Arcobacter* in Human Disease

Arcobacter has been isolated from asymptomatic people (Vandenberg et al. 2004; Samie et al. 2007). *A. butzleri* can cause diarrhea and associated gastrointestinal symptoms in humans (*Table 23.4*, Lastovica and le Roux 2000; Samie et al. 2007; Mansfield and Forsythe 2000). The clinical features of *Arcobacter* and *Campylobacter* infection in humans are similar, except *Arcobacter* usually has a watery persistent diarrhea, while that of *Campylobacter* tends to be bloody (Vandenberg et al. 2004). *A. butzleri* has been isolated from human diarrheic stools, blood cultures, and the peritoneal fluid of appendicitis patients (Kiehlbauch et al. 1991). Samie et al. (2007), using molecular methods, examined 322 human diarrhetic stools specimens in South Africa and found *A. butzleri* to be the 3rd most prevalent (6.2 %) after *Helicobacter pylori* (50.6 %) and *C. jejuni* (10.2 %). In gastroenteritis studies, *A. butzleri* tends to have the highest prevalence, followed by *A. cryaerophilus* and *A. skirrowii*, and the values reported by Samie et al. (2007), using molecular detection methods, were 6.2 %, 2.9 %, and 1.9 %, respectively. *A. butzleri* has been found to be an etiological agent of travelers' diarrhea acquired by US and European travelers to Mexico, Guatemala, and India (Jiang et al. 2010). *A. skirrowii* has been linked to gastroenteritis on a few occasions (Wybo et al. 2004). Samie et al. (2007) recovered *Arcobacter* spp. from 55 % of the patients, demonstrating coinfection by two to four pathogens such as *C. coli*, *C. concisus*, or another species of *Arcobacter*. Up to 17 % of South African pediatric stool samples may have up to five distinct species of *Campylobacter* or *Arcobacter* identified on the primary isolation plate (Lastovica 2006). The dynamics and effects of coinfections with multiple strains or species are poorly understood at present.

Arcobacter bacteremia has occasionally been reported (On et al. 1995c; Lau et al. 2002). It is thought that consumption of contaminated food or water is a route of transmission for *Arcobacter* diseases of humans and animals (Ho HT et al. 2006). In an outbreak of recurrent abdominal cramps due to *A. butzleri* in an Italian school, person-to-person transmission was suggested (Vandamme et al. 1992b). Another possible transmission route could be contact with pet cats or their feces (Fera et al. 2009). Host characteristics such as age and immune status may play a role in *Arcobacter* infections, as Samie et al. (2007) reported a lower prevalence of *Arcobacter* (3 %) in healthy school children aged 3–15 than age-matched hospitalized children (10.4 %).

Supporting the role that *Arcobacter* species may have in the development of diarrhea are the studies of Vandenberg et al. (2004) who examined 67,599 stool samples collected over 8 years with detailed clinical information. *Arcobacter* infections may be underestimated because of inappropriate detection and identification methods (Vandenberg et al. 2004). Optimum growth conditions for *Arcobacter* (30 °C) are not often applied with clinical specimens, and 42 °C is usually the only temperature used for isolation of *Campylobacter* and related organisms.

Significance of *Arcobacter* in Animal Disease

Arcobacter has been isolated from the intestinal tracts and feces of various farm animals and can cause disease in some of them (▶ *Table 23.4*, Ho HT et al. 2006). The most serious effects of *Arcobacter* on animals include abortions, mastitis, and diarrhea. *A. butzleri* has been associated with enteritis in cattle, pigs, and horses (de Oliveira et al. 1997). *A. skirrowii* has been associated with diarrhea and hemorrhagic colitis in sheep and cattle (Ho HT et al. 2006; Vandamme et al. 1992c). Fecal shedding of *Arcobacter* has been recognized in chicken, ducks, and turkeys by Atabay et al. (2006), but concomitant disease has not been identified in these animals.

A. butzleri has been isolated from primates with diarrhea (Anderson et al. 1993; Kiehlbauch et al. 1991). *A. cryaerophilus* was isolated from naturally infected rainbow trout (*Oncorhynchus mykiss* Walbaum). Experimental infection with other rainbow trout caused deaths with gross clinical abnormalities such as degenerated opercula and gills, liver damage, hemorrhagic kidney, and serous fluid in swollen intestines (Yildiz and Aydin 2006).

The pathogenicity and virulence mechanisms of *Arcobacter* are poorly understood. The capacity for in vitro invasion of cell lines has been demonstrated for *A. cryaerophilus* (Fernandez et al. 1995) and for *A. butzleri* (Ho et al. 2007).

Pathogenicity

Pathogenicity of *C. jejuni* and *C. coli*

C. jejuni and *C. coli* cause disease by colonization, attachment, invasion, and toxin production; these have been reviewed by Cróinín and Backert (2012). Recent research into the molecular mechanisms of virulence of *C. jejuni* has indicated that *C. jejuni* is a unique pathogen, capable of executing N-linked glycosylation of more than 30 proteins related to colonization, adherence, and invasion (Dasti et al. 2010). Colonization of the gut mucosa depends on the ability of the bacteria to attach to the gut mucosa, and the rapid motility and spiral shape of *Campylobacter* allows it to penetrate the mucus layer of intestinal cells. Functional flagella are important as virulence factors, as aflagellate bacteria do not colonize in vivo (Aguero-Rosenfeld et al. 1990).

The *Campylobacter* flagellum is composed of two homologous flagellins, FlaA and FlaB. The FlaA is responsible for adherence, colonization of the gastrointestinal tract, and invasion of host cells. The flagellum is responsible not only for motility but secretion of *Campylobacter* invasive antigen (Cia) (Dasti et al. 2010). Poly and Guerry (2008) proposed that flagella have the ability to secrete non-flagellar proteins that may be virulence associated. Larson et al. (2008) and Neal-McKinney and Konkel (2012)) provide evidence that in *C. jejuni*, Cia is exported from the flagella and is delivered to the cytosol of host cells, thus enabling *Campylobacter* to attach to epithelial cells. Fimbriae-like elements and cell surface proteins (PEB1, CadF) of *C. jejuni* appear to function in attachment and subsequent colonization and invasion (Doig et al. 1996).

Cytolethal distending toxin (CDT) is widely distributed in Gram-negative bacteria (Smith and Bayles 2006). It is the best described *Campylobacter* toxin and it is considered an important virulence factor. This toxin causes eukaryotic cells to arrest in the G2/M phase of the cell cycle, leading to cell apoptosis. All *C. jejuni* isolates possess genes that code for CDT; however, not all isolates produce this toxin (Pickett et al. 1996), as deletions and point mutations occur naturally (Abuoun et al. 2005). Jain et al. (2008) demonstrated that CDT (+) *C. jejuni* strains adhere to and invade epithelial cells more efficiently than CDT (−) strains. Smith and Bayles (2006) have written an extensive review of CDT. Host factors may play a substantial role in the *Campylobacter* pathogenesis of humans (Dasti et al. 2010).

Pathogenicity of *C. fetus*

C. fetus is an important veterinary pathogen with host tissue specificity for both the gastrointestinal and genital tracts in cattle and sheep and can cause abortion and infertility in these animals (Garcia et al. 1983; Skirrow 1994). *C. fetus* and *C. rectus* cells have a microcapsule external to the bacterial outer membrane, which consists of paracrystalline S-layer proteins and which confers protection for *C. fetus* strains (Blaser et al. 1988). The S-layer confers resistance to complement-mediated killing in nonimmune serum by preventing the binding of complement factor C3b to the *C. fetus* cell surface (Thompson 2002). *C. fetus* can also avoid antibody-mediated killing by high-frequency antigenic variation of the S-layer proteins. *C. rectus* is implicated as a pathogen in periodontal disease. It possesses an S-layer (Miyamoto et al. 1998) which confers resistance to

complement-mediated killing and causes downregulation of proinflammatory cytokines (Thompson 2002).

Pathogenicity of *C. concisus*

A number of studies have examined the virulence factors of *C. concisus*. Results from these studies suggest that multiple virulence factors are involved. *C. concisus* is motile and has a single polar flagellum (Mahendran et al. 2011; Man et al. 2010a). Motility is a recognized virulence factor. Using in vitro cell line models, specific oral and enteric *C. concisus* strains showed adhesion and invasion to intestinal epithelial cells and induced epithelial apoptosis (Ismail et al. 2012; Kalischuk and Inglis 2011; Man et al. 2010a; Nielsen et al. 2011). S-layer RTX proteins and their genes were detected in both oral and enteric *C. concisus* strains (Kaakoush et al. 2010; Kalischuk and Inglis 2011; Mahendran et al. 2011). *C. concisus* strains revealed hemolytic activities (Istivan et al. 2004; Kalischuk and Inglis 2011).

Pathogenicity of *C. upsaliensis*

C. upsaliensis may produce both inflammatory and non-inflammatory diarrhea in humans, and the organism's virulence may depend on invasion of the host cells. *C. upsaliensis* is capable of invading cell lines of gastrointestinal origin more efficiently than nonintestinal cell lines (Mooney et al. 2003). Sylvester et al. (1996) demonstrated that *C. upsaliensis* is capable of binding to CHO and HEp-2 cells in tissue culture. Surface proteins in the range 50–90 kDa on the *C. upsaliensis* isolates could bind to phosphatidylethanolamine, a putative cell membrane receptor. Biotin-labeled *C. upsaliensis* strains also bind in a concentration-dependent fashion to human small intestine mucin, implying that *C. upsaliensis* expresses an adhesin(s) capable of recognizing a specific mucin epitope(s) (Sylvester et al. 1996). Binding to mucins may influence bacterial access to cell membrane receptors, influencing host resistance to infection. Mooney et al. (2001) demonstrated that *C. upsaliensis* releases a cytolethal distending toxin that affected HeLa and T lymphocyte cells. These authors also described invasion of cultured epithelial cells, and electron microscopy revealed *C. upsaliensis* within Caco-2 cytoplasmic vacuoles (Mooney et al. 2003). Fouts et al. (2005) described a novel putative virulence locus, *lic*ABCD, for *C. upsaliensis*, similar to genes present in *Neisseria* (Serino and Virji 2002) These *lic*ABCD genes encode proteins involved in the acquisition of choline, the synthesis of phosphorylcholine, and the transfer of phosphorylcholine to lipooligosaccharide to facilitate attachment to host cells (Serino and Virji 2002).

Pathogenicity of *Arcobacter*

A. butzleri adhesion molecules have been demonstrated by their ability to agglutinate human, sheep, and rabbit erythrocytes, and a 20 kDa lectin-like hemagglutinin has been examined by Western immunoblotting (Tsang et al. 1996). This hemagglutinin can interact with erythrocyte receptors containing D-galactose (Tsang et al. 1996). *Arcobacter* can also adhere to epithelial cells in vitro (Carbone et al. 2003). *A. cryaerophilus* induced the accumulation of fluid and electrolytes in an ileal loop assay, as well as demonstrating in vitro invasion of Hep-2 cells (Fernandez et al. 1995). The induced expression of proinflammatory cytokine interleukin-8 (IL-8) is a major virulence factor of *Campylobacter* spp. and has been reported for *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, and *A. cibarius* (Ho et al. 2007). Bücker et al. (2009) demonstrated that *A. butzleri* induces barrier dysfunction in human intestinal HT-29/B6 cells. Johnson and Murano (2002) demonstrated a lack of cytolethal distending toxin among *Arcobacter* species from various sources. Very little is known about *Arcobacter* genes involved with virulence. The recently published genome sequence of an *A. butzleri* strain indicated that some putative virulence determinants homologous to those of *C. jejuni*, such as the genes coding for fibronectin-binding proteins CadF and Cj1349, the invasin protein CjaB, the phospholipase PldA, and others, are present (Miller et al. 2007).

These findings are suggestive that some *Arcobacter* spp. could be enteropathogens. The available gene sequence of *A. butzleri* lacks genes that encode for the cytolethal distending toxin (CDT) gene found in the genomes of various *Campylobacter* spp. (Miller et al. 2007). CDT induces cytotoxicity on eukaryotic cells by breaking double-stranded DNA.

Antibiotic Resistance

Campylobacter: Antibiotic Resistance

Antibiotic resistance in *Campylobacter* is a global trend and is recognized as a public health concern by the World Health Organization (WHO) (Moore et al. 2006). Antibiotic resistance in *Campylobacter* has been recognized for decades (Flores et al. 1985). A comprehensive review of antibiotic resistance in *Campylobacter* has been done by Luangtongkum et al. (2009) and Moore et al. (2006). Most patients infected with *Campylobacter* spp. will recover, usually by replacing lost fluids and electrolytes. Antibiotics, usually tetracycline, macrolides, and fluoroquinolones, are reserved for more severe cases. However, increasing resistance to fluoroquinolones, tetracycline, and erythromycin in *C. jejuni* and *C. coli* could compromise treatment. Clinical isolates of emerging *Campylobacter* spp. such as *C. upsaliensis* and *C. concisus* are also showing large increases in antibiotic resistance of erythromycin and ciprofloxacin (Moore et al. 2006; Vandenberg et al. 2006). Antibiotics have been used indiscriminately in animal production for decades to control or prevent infections and as growth promoters. There is ample evidence supporting the hypothesis that the unregulated use of antibiotics in food animal production has led to the emergence and spread of antibiotic resistance in *Campylobacter*

spp. (Aarestrup et al. 1997; Silva et al. 2011). On the increase are *Campylobacter* strains isolated from food, animals, and clinical specimens, which are resistant to more than one class of antibiotic (Silva et al. 2011).

Arcobacter: Antibiotic Resistance

There have been only a few antimicrobial susceptibility studies with clinical or veterinary strains of *Arcobacter*, and there is essentially no data on the very recently described *Arcobacter* species such as *A. defluvii*, *A. marinus*, and others. Similar to *Campylobacter*, most cases of *Arcobacter* infection are self-limiting and do not require antimicrobial treatment. When justified, antibiotic treatment (usually fluoroquinolones and tetracyclines) is advised (Son et al. 2007). In their study of 17 strains of *A. butzleri* and 13 strains of *A. cryaerophilus*, Fera et al. (2003) found all strains were highly resistant to penicillins, macrolides, chloramphenicol, trimethoprim, and vancomycin. There appears to be a tendency for strains of *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii* to be resistant to nalidixic acid, metronidazole, cephalothin, cefoperazone, and carbenicillin (On 1996). Strains resistant to ciprofloxacin and nalidixic acid have been described (On et al. 1995c). Abdelbaqi et al. (2007b) demonstrated that *A. butzleri* and *A. cryaerophilus* strains were resistant to ciprofloxacin and showed a mutation in the quinolone resistance-determining region of the *gryA* gene. Miller et al. (2007) found that the complete genome sequence data of *A. butzleri* strain RM4018 indicated high antibiotic resistance with the presence or absence of specific genes regulating antibiotic sensitivity.

Concluding Remarks

The understanding of the epidemiology, pathogenicity, clinical, and veterinary significance of *Campylobacteraceae* has grown dramatically in recent years. With the recognition of new species of *Campylobacter* and *Arcobacter*, our knowledge of the prevalence, diversity, and population biology of these microorganisms has increased significantly. A better awareness of the genomic diversity of *Campylobacter* and *Arcobacter* is due to a great extent to improved technology, allowing comparative analysis of these organisms. The first *C. jejuni* strain was sequenced in the year 2000. Subsequently, additional complete *Campylobacter* and *Arcobacter* genome sequences have been published, allowing new insights into these organisms. Although there has been progress in the understanding of the pathogenic potential of *Campylobacter* and *Arcobacter*, a considerable amount of research remains to be done (Gaynor and Szymanski 2012). *Campylobacter* infections and complications of infection, such as septicemia, abortion, meningitis, reactive arthritis, and the Guillain-Barré syndrome, may cause an increasing morbidity among susceptible individuals.

As *Campylobacter* and *Arcobacter* are foodborne diseases, there is an increased awareness of the prevalence and persistence of these organisms in the food chain and the transmission of antibiotic-resistant campylobacters and arcobacters from food animals to humans.

Antimicrobial resistance continues to increase globally. Government regulatory bodies have recognized this public health concern, and the use of antimicrobials in food animals has been regulated or even banned.

Ultimately, continuing research will contribute to the improvement of human and animal health. Research must provide protocols for reducing infection and find ways to implement improved food safety and effective control strategies.

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24 The Family *Helicobacteraceae*

Hazel M. Mitchell[1] · *Gifone A. Rocha*[2] · *Nadeem O. Kaakoush*[1] · *Jani L. O'Rourke*[1] · *Dulciene M. M. Queiroz*[2]
[1]School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, NSW, Australia
[2]Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

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Abstract

The family *Helicobacteraceae*, which lies within the Epsilon subdivision of the Proteobacteria, consists of five genera, *Helicobacter, Sulfuricurvum, Sulfurimonas, Sulfurovum, Thiovulum* and *Wolinella*. This family is dominated by the *Helicobacter* genus which currently consists of 33 validated named species and several Candidatus and unclassified organisms. Its type species, *Helicobacter pylori*, is recognized as a significant human pathogen responsible for a spectrum of gastrointestinal diseases. Bacteria from the *Helicobacter* and *Wolinella* genera are commonly associated with animal and/or human hosts, and in general colonize the oral and/or gastric and intestinal cavities. Interestingly, a number of bacteria recently classified within the *Helicobacteraceae* family have been isolated from environmental sources including iron- and sulfur-rich freshwater nature reserves, sulfidic caves and springs, hydrothermal vents, and deep-sea sediments. These bacteria belong to the genera *Thiovulum*, *Sulfuricurvum*, *Sulfurimonas* and *Sulfurovum*. While these bacteria are classified in the *Helicobacteraceae* family, the significant differences in their niches and lifestyles to *Helicobacter* species, and their similarities with bacteria belonging to the *Nautiliaceae* family, may in the future result in their reclassification.

Taxonomy, Historical and Current

The family *Helicobacteraceae* lies within the Order *Campylobacterales* of the Epsilon subdivision of the Proteobacteria (Garrity et al. 2005; Validation List 2006a). It consists of five genera, *Helicobacter, Sulfuricurvum, Sulfurimonas, Sulfurovum, Thiovulum* and *Wolinella*. From a clinical perspective the genus *Helicobacter* is the most important with many species implicated in gastrointestinal disease in humans and animals (Solnick et al. 2006). A phylogenetic reconstruction of the family *Helicobacteraceae*, based on 16S rRNA, is shown in ❯ *Fig. 24.1*.

Spiral-shaped bacteria were first observed in human gastric mucosa beginning early in the twentieth century (Krienitz 1906) and were subsequently described by several investigators (reviewed by Marshall 1989). Early observations of similar organisms in the gastric mucosa of dogs, cats, and a variety of other animals were also recorded around this time (Rappin 1881; Bizzozero 1892; Salomon 1896; Doenges 1939). The significance of these organisms was alternately debated and forgotten until 1982, when Robin Warren and Barry Marshall, Australian researchers, isolated a spiral bacterium from the gastric mucosa of patients with gastritis and peptic ulcer. As the morphological and physiological characteristics of the bacterium were very similar to members of the *Campylobacter* genus (Marshall and Warren 1984) the bacterium was named *Campylobacter pyloridis*. However, detailed studies of the bacterium's ultrastructure, fatty acid composition and 16S rRNA gene sequence demonstrated that the organism did not belong to the genus *Campylobacter* thus a new genus was proposed into which the bacterium was included under the name of *Helicobacter pylori* (Goodwin et al. 1989a). *Helicobacter pylori*, the type species, has been shown to persistently colonize the gastric mucosa of more than 50 % of the human population (Blaser and Atherton 2004) where it causes gastritis. In most infected individuals the infection is asymptomatic and does not result in more serious clinical outcomes. However, a small percentage of infected individuals will develop severe diseases including peptic ulcer disease, gastric carcinoma and gastric MALT (mucosa associated lymphoid tissue) lymphoma.

Currently the *Helicobacter* genus consists of a range of organisms that colonize the mucus layer covering the epithelial surface of the gastrointestinal tract of humans and a variety of animal species. Of the currently validated species 12 have been isolated from gastric tissue (❯ *Table 24.1*) and 21 isolated from the intestinal and/or hepatobiliary tract (❯ *Table 24.2*). A number of *Helicobacter* species may commonly (*Helicobacter aurati*) or occasionally (*Helicobacter bilis*, *Helicobacter muridarum*) be isolated from both gastric and enterohepatic sites. In addition there is a growing list of candidate and unvalidated species (❯ *Table 24.3*).

There is only one species in the genus *Wolinella*, the type species *Wolinella succinogenes*, which was initially classified as *Vibrio succinogenes* (Wolin et al. 1961). In 1991 the species formerly known as *Wolinella recta* and *Wolinella curva* were reclassified into the *Campylobacter* genus (Vandamme et al. 1991). In 2003, Bohr et al. detected *Wolinella* DNA in esophageal mucosa biopsies of patients suffering from stenosing squamous cell carcinoma (Bohr et al. 2003). This species was named "*Candidatus* Wolinella africanus," however, it is yet to be isolated.

The sulfide-oxidizing bacterium *Thiovulum majus* was identified in 1786 by Müller as cited by Wirsen and Jannasch (1978)

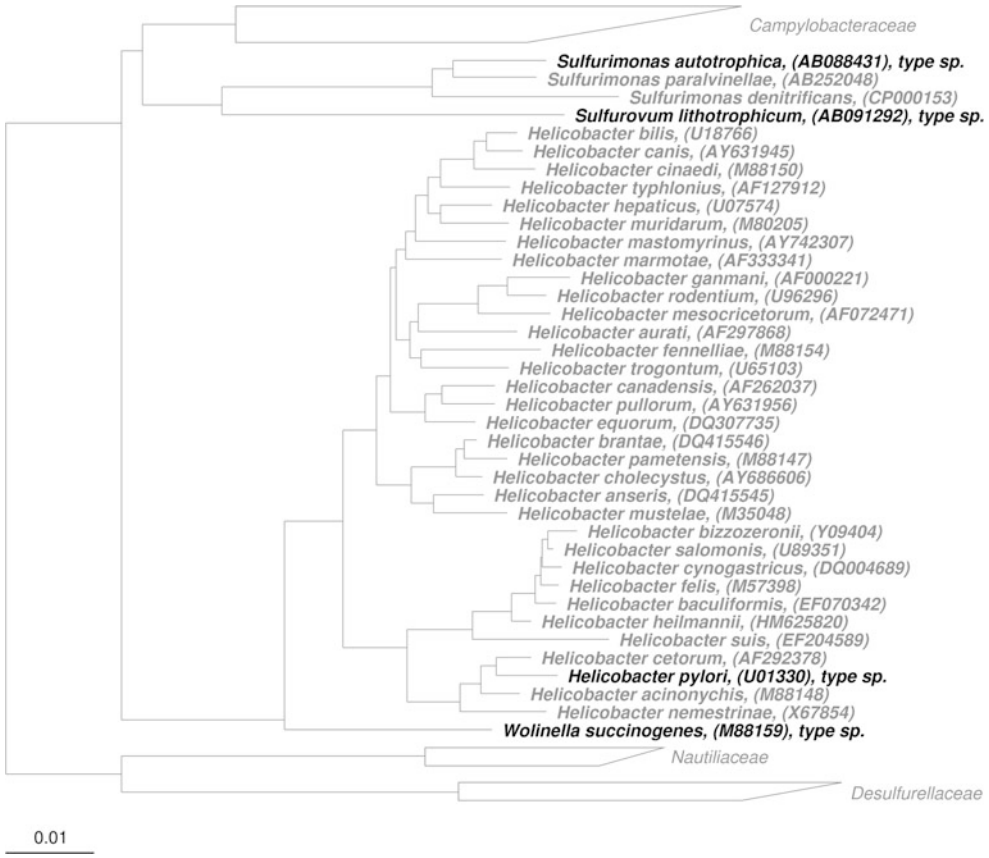

Fig. 24.1

Phylogenetic reconstruction of the family *Helicobacteraceae* 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

who initially named it *Volvox punctum*. Since then, this bacterium has undergone several name changes including *Monas mülleri*, *Thiovulum mülleri*, and finally, *Thiovulum majus* in 1913 (Hinze 1913; Wirsen and Jannasch 1978). In 1992, Lane et al. were the first to report the affiliation of *Thiovulum* with *Campylobacter*, *Helicobacter* and *Wolinella* species (Lane et al. 1992). More recently three additional environmental genera, *Sulfuricurvum*, *Sulfurimonas* and *Sulfurovum*, have been included in the *Helicobacteraceae* family.

Genus *Helicobacter*

Genus *Helicobacter* Goodwin et al. 1989a, gen.nov. emend Vandamme et al. 1991. (Type genus of the family *Helicobacteraceae* Garrity et al. 2005). (Gr. adj. *helix -îkos*, twisted, curved, spiral; N.L. masc. n. *bacter*, a rod, a staff; N.L. masc. n. *Helicobacter*, a spiral rod).

Type species: *Helicobacter pylori* (Marshall et al. 1985; Goodwin et al. 1989a).

Molecular Analyses

Phylogeny

The 16S rRNA gene has been employed extensively for identification and phylogenetic analyses of *Helicobacter* species (Dewhirst et al. 2005). This is due to the fact that this gene is of considerable length, and that specific regions of the 16S rRNA gene are highly conserved. However, 16S rDNA analysis may not differentiate closely related strains or species within the *Helicobacter* genus due to their high sequence similarity. Moreover, analysis based on the 16S rRNA gene does not necessarily correspond to the results of polyphasic taxonomy (Dewhirst et al. 2000b; Vandamme et al. 2000; Suerbaum et al. 2002; O'Rourke et al. 2004). Indeed, gene analysis of the 60 kDa heat-shock protein (HSP60) has been demonstrated to have a higher resolution than the conventional 16S rRNA gene for species identification of gastric and enterohepatic *Helicobacter* species (Mikkonen et al. 2004). However, phylogenetic studies employing genes such as

Table 24.1
Gastric *Helicobacter* taxa

Taxon	Original/Natural host	Strain/clone	GenBank 16S rRNA accession number	Reference
Helicobacter acinonychis	Cheetah	ATCC 51101^T	M88148	Eaton et al. 1993a
Helicobacter baculiformis	Cat	CCUG 53816^T	EF070342	Baele et al. 2008b
Helicobacter bizzozeronii	Dog	CCUG 35545^T	Y09404	Hanninen et al. 1996
Helicobacter cetorum	Whales, Dolphins	ATCC BAA-540^T	AF292378	Harper et al. 2002a, b
Helicobacter cynogastricus	Dog	LMG 23188^T	DQ004689	Van den Bulck et al. 2006
Helicobacter felis	Cat, Dog	ATCC 49179^T	M57398	Paster et al. 1991
Helicobacter heilmannii	Cat	DSM 24751^T	HM625820	Smet et al. 2012a
Helicobacter mustelae	Ferret	ATCC 43772^T	M35048	Fox et al. 1988; Goodwin et al. 1989
Helicobacter nemestrinae	Pig tailed macaque	ATCC 49396^T	X67854	Bronsdon et al. 1991
Helicobacter pylori	Human	ATCC 43505^T	U01330	Goodwin et al. 1989a
Helicobacter salamonis	Dog	CCUG 37845^T	U89351	Jalava et al. 1997
Helicobacter suis	Swine	DSM 19735^T	EF204589	Baele et al. 2008a

Table 24.2
Enterohepatic *Helicobacter* taxa

Taxon	Original/Natural host	Strain/clone	GenBank 16S rRNA accession number	Reference
Helicobacter anseris	Canada geese	ATCC BAA-1299^T	DQ415545	Fox et al. 2006
Helicobacter aurati	Hamster	ATCC BAA-1^T	AF297868	Patterson et al. 2000a
Helicobacter bilis	Mice	ATCC 51630^T	U18766	Fox et al. 1995
Helicobacter brantae	Canada geese	ATCC BAA-1298^T	DQ415546	Fox et al. 2006
Helicobacter canadensis	Human, Birds	ATCC 700968^T	AF262037	Fox et al. 2000
Helicobacter canis	Dog	ATCC 51401^T	AY631945	Stanley et al. 1993
Helicobacter cholecystus	Syrian hamster	ATCC 700242^T	AY686606	Franklin et al. 1996
Helicobacter cinaedi	Human, Hamster	CCUG 18818^T	M88150	Totten et al. 1985; Vandamme et al. 1991
Helicobacter equorum	Horse	CCUG 52199^T	DQ307735	Moyaert et al. 2007b
Helicobacter fennelliae	Human, Hamster	ATCC 35684^T	M88154	Totten et al. 1985; Vandamme et al. 1991
Helicobacter ganmani	Mice	CCUG 43526^T	AF000221	Robertson et al. 2001
Helicobacter hepaticus	Mice	ATCC 51448^T	U07574	Fox et al. 1994
Helicobacter marmotae	Marmote (woodchuck)	ATCC BAA-546^T	AF333341	Fox et al. 2002
Helicobacter mastomyrinius	Mastomys	ATCC BAA-1046^T	AY742307	Shen et al. 2005
Helicobacter mesocricetorum	Syrian hamsters	ATCC 700932^T	AF072471	Simmons et al. 2000
Helicobacter muridarum	Mice, Rats	ATCC 49282^T	M80205	Lee et al. 1992
Helicobacter pamatensis	Birds	ATCC 51478^T	M88147	Dewhirst et al. 1994
Helicobacter pullorum	Chicken	ATCC 51801^T	AY631956	Stanley et al. 1994
Helicobacter rodentium	Mice	ATCC 700285^T	U96296	Shen et al. 1997
Helicobacter trogontum	Rats	ATCC 700114^T	U65103	Mendes et al. 1996
Helicobacter typhlonius	Mice	ATCC BAA-367^T	AF127912	Franklin et al. 2001

Table 24.3
Candidatus and unvalidated species

Taxon	Original/Natural host	Strain/clone	GenBank 16S rRNA accession number	References
"*Candidatus* Helicobacter bovis"	Cattle	Not yet cultured	AF127027	De Groote et al. 1999
"*Helicobacter callitrichis*"	Marmoset	R-204	AY192526	Won et al. 2007
"*Helicobacter colifelis*"	Cat	'Feline isolate'	AF142062	Foley et al. 1998
"*Helicobacter macacae*"	Monkeys (rhesus and cynomolgus)	MIT 99-5501	AF333338	Fox et al. 2001
"*Helicobacter magdeburgensis*"	Mice	HM007-1	EF990624	Traverso et al. 2010
"*Helicobacter muricola*"	Mice	w-06	AF264783	Won et al. 2002
"*Helicobacter suncus*"	Musk Shrews	Kaz-1	AB006147	Goto et al. 1998
"*Helicobacter westmeadii*"	Human	*Helicobacter* sp.	U44756	Trivett Moore et al. 1997
"*Helicobacter winghamensis*"	Human	*NLEP 97-1090*	AF246984	Melito et al. 2001

hsp60 examine only a small region of 590 bases with only approximately 120 informative bases (Dewhirst et al. 2005). Thus, other studies have looked to larger molecules such as the 23S rRNA gene for differentiation and identification of *Helicobacter* species.

In order to investigate the discrepancy between the 16S rRNA gene analyses and those from other genotypic and phenotypic methods Dewhirst et al. obtained sequence data for the 23S rRNA gene of 55 *Helicobacter* strains representing 41 *Helicobacter* taxa (Dewhirst et al. 2005). They found that 16S rRNA and 23S rRNA gene analyses were discordant with the 23S rRNA gene analyses, in contrast to the 16S rRNA gene analyses, concurring with other molecular and phenotypic methods. Based on these findings Dewhirst et al. concluded that analyses employing the 23S rRNA gene were more reliable than those using the 16S rRNA gene for identification, classification, and phylogenetic analysis of *Helicobacter* species (Dewhirst et al. 2005). Although, the authors conceded that the information extracted from the sequence of the 16S rRNA gene remains useful and cost-effective (Dewhirst et al. 2005).

While these phylogenetic methods remain employed to date, formal characterization of bacterial isolates involves a more global approach including rRNA gene analyses, phenotypic analyses, enzymatic analyses and other genotypic analyses. Moreover, due to the reduction in costs for bacterial genome sequencing, researchers have resorted to this technique for characterization of their isolates.

Detection of *Helicobacter* species

In addition, variable regions within the ribosomal RNA genes and other conserved genes with an overall high sequence similarity have been commonly employed for the design of specific targets to identify bacterial species. Several oligonucleotide probes and assays for the detection and differentiation of *Helicobacter* species have been developed for *H. pylori* (On 1996; Jiang et al. 1998; Ozpolat et al. 2000; Gill et al. 2007; Guimaraes et al. 2007) and other *Helicobacter* species such as *H. bilis*, *H. bizzozeronii*, "*Candidatus H. bovis*," *H. canis*, *H. felis*, *H. heilmannii* strains 1–4, *H. hepaticus*, *H. pullorum*, *H. salomonis*, *H. suis* and *H. trogontum* (Stanley et al. 1993, 1994; Dewhirst et al. 1994; Battles et al. 1995; Fox et al. 1995, 1998b; Mendes et al. 1996; Germani et al. 1997; De Groote et al. 2000; Roosendaal et al. 2000; Shen et al. 2000; Choi et al. 2001; Ge et al. 2001; Bridgeford et al. 2008). However it should be noted that the sensitivity and specificity of these probes and assays have not been validated against all species currently described in this genus. With the increase in sequence similarity among species and the advent of next generation sequencing, researchers are gradually shifting to genomic sequencing for a more accurate characterization of bacterial isolates.

Genomes

In an attempt to gain a deeper understanding of the pathogenesis of *Helicobacter* species, over the last 16 years an increasing number of species have been subjected to genomic analyses. Currently, complete genomes of eight gastric *Helicobacter* species, including *H. pylori* (39 strains), *H. acinonychis, H. bizzozeronii, H. cetorum* (2 strains, unpublished data), *H. felis, H. heilmannii, H. mustelae* and *H. suis* (2 strains) have been published (*Table 24.4*). In addition, the complete genomes of three enterohepatic helicobacters (*H. canadensis, H. cinaedi* and *H. hepaticus*) are available (*Table 24.5*). Genome sequence projects of a further six *Helicobacter* species (*H. bilis* ATCC 43879, *H. bizzozeronii* CCUG 35545, H. *canadensis* MIT 98–5491, *H. cinaedi* CCUG 18818, *H. pullorum* MIT 98–5489 and NCTC 12824 and *H. winghamensis* ATCC BAA-430) as well as further strains of *H. bilis, H. canadensis* and *H. cinaedi* are currently underway. A summary of the characteristics of these genomes is shown in *Table 24.6*.

Table 24.4

Summary of genome characteristics for the gastric *Helicobacters*

Organism	BioProject	Source	Chromosomes (Plasmid)[a]	Size (Mb)	G+C % content	Gene No.	Protein No.	rRNA	tRNA	Pseudogene
H. acinonychis Sheeba G	PRJNA58685, PRJNA17251	Stomach of a cheetah with gastritis	1	1.55	38.2	1,655	1,613	6	36	–
			1 (pHac1)[a]	(0.003661)	(34.6)	(6)	(6)	–	–	–
H. bizzozeronii CIII-1	PRJNA68141, PRJEA65019	Human gastric mucosa isolate	1	1.76	46	1,935	1,894	6	35	–
			1 (phbz1)[a]	(0.052076)	(42.2)	(77)	(77)	–	–	–
H. cetorum MIT 00-7128	PRJNA162217, PRJNA97501	Gastric isolate from a beluga whale	1	1.95	34.5	1,761	1,717	6	38	–
			1 (pHCW)[a]	(0.012465)	(34.5)	(14)	(14)	–	–	–
H. cetorum MIT 99-5656	PRJNA162215, PRJNA97499	Gastric isolate from Atlantic white-sided dolphin.	1	1.83	35.6	1,716	1,674	6	36	–
			1 (pHCD)[a]	(0.014124)	(32.7)	(15)	(15)	–	–	–
H. felis ATCC 49179	PRJNA61409, PRJEA61189	Gastric mucosa of a cat	1	1.67	44.5	1,702	1,654	5	29	14
H. heilmannii ASB1.4	PRJNA182935 PRJEB367	Gastric isolate from a kitten with severe gastritis	1	1.8	47.8	–	1,928	9	41	–
H. mustelae 12198	PRJNA46647, PRJEA40677	Gastric mucosa of a ferret	1	1.58	42.5	1,474	398	8	38	28
H. pylori 26695	PRJNA57787, PRJNA233	Human gastric mucosa isolate	1	1.67	38.9	1,627	1,573	7	36	
H. suis HS1	PRJNA62531, PRJNA43415	Gastric mucosa of swine	1	1.64	40	1,215	1,161	6	38	10
H. suis HS5	PRJNA62533, PRJNA43417	Gastric mucosa of swine	1	1.67	40	1,221	1,136	5	38	42

Note: Data obtained from the National Center for Biotechnology Information (NCBI) – Genome. https://www.ncbi.nlm.nih.gov/genome

[a]Data in brackets relates to plasmids

Table 24.5

Summary of genome characteristics for the enterohepatic *Helicobacters*

Organism	BioProject	Source	Chromosomes (Plasmid)[a]	Size (Mb)	G+C % content	Gene No.	Protein No.	rRNA	tRNA	Pseudogene
H. canadensis MIT 98-5491	PRJNA55359, PRJNA30719	Human with diarrhea	1	1.62	33.7	1,603	1,535	9	40	19
H. cinaedi PAGU611	PRJDA162219 PRJDB88	A clinical isolate from human bacteremia	1	2.08	38.6	1,939	1,894	6	39	–
			1 (Phci1)[a]	(0.023054)	(31.5)	(29)	(29)	–	–	–
H. hepaticus ATCC 51449	PRJNA57737, PRJNA185	Colony of mice with liver tumors	1	1.8	35.9	1,916	1,876	3	37	–

Note: Data obtained from the National Center for Biotechnology Information (NCBI) – Genome. https://www.ncbi.nlm.nih.gov/genome

[a]Data in brackets relates to plasmids

Helicobacter pylori

The great genetic diversity observed in *H. pylori* due to point mutations, mosaicisms, non-conserved genes, genomic rearrangements, insertions and deletions, mobile DNA as well as considerable variation in gene content, led Covacci and Rappuoli to consider *H. pylori* a "quasi species" (Covacci and Rappuoli 1998). The complete genome sequencing of *H. pylori* strains has increased rapidly over recent years. Among the 39 strains deposited in Genbank (www.ncbi.nlm.nih.gov), 43.5 % contain at least one plasmid that also contributes to interstrain diversity. The circular genome of *H. pylori* strains ranges from 1.55 MB to 1.67 MB with G+C contents ranging from 38.3 mol% to 39.3 mol%, comprising 1,480–1,749 genes that encode

Table 24.6
Partially annotated genome summaries

Organism	BioProject	Source	Chromosomes	Size (Mb)	G+C % content	Gene No.	Protein No.	rRNA	tRNA
H. bizzozeronii CCUG 35545	PRJNA180975, PRJEA65021	Canine gastric mucosa	1	1.78	45.7	2,512	2,512	–	–
H. bilis ATCC 43879	PRJNA55617, PRJNA32489	Human feces	1	2.48	35.0	2,300	2,265	–	35
H. canadensis MIT 5491	PRJNA55289, PRJNA30071	Feces of a human with diarrhea	1	1.61	33.6	1,663	1,627	–	36
H. cinaedi CCUG 18818	PRJNA55291, PRJNA30073	A rectal swab taken from a homosexual man	1	2.18	38.5	2,409	2,373	3	36
H. cinaedi ATCC BAA-847	PRJDB113	A case of human bacteremia in Japan	1	2.24	38.34	2,322		2	40
H. pullorum MIT 98-5489	PRJNA55293, PRJNA30075	A human with gastroenteritis	1	1.92	34.1	2,044	2,008	–	36
H. pullorum NCTC 12824	PRJNA55293, PRJNA30075	A healthy broiler chicken in Switzerland	1	1.9	34.1	–	–	–	–
H. winghamensis ATCC BAA-430	PRJNA55619, PRJNA32491	Patients with gastroenteritis	1	1.65	35.5	1,674	1,639	–	35

Note: Data obtained from the National Center for Biotechnology Information (NCBI) – Genome. https://www.ncbi.nlm.nih.gov/genome

1,405–1,707 putative ORFs (Tomb et al. 1997; Alm et al. 1999). A core genome consisting of 1,091–1,281 genes (mean 1,150 genes) is present in all strains (Salama et al. 2000; Gressmann et al. 2005; Han et al. 2007). Approximately 60 % of the ORFs encode proteins with predictable function, whereas 24 % are conserved in other bacterial species, but with unknown function, and 17 % are *H. pylori* specific (Tomb et al. 1997; Alm et al. 1999). The genome contains two copies of 23S-5S and 16S rRNA genes. Although not present in all *H. pylori* strains, the *H. pylori* genome contain a variable number of insertion sequences (IS), IS605 and IS606 elements, that increase intragenomic recombination. These IS elements are not only integrated into the core chromosome, but are also encoded on plasmids in some *H. pylori* strains (Tomb et al. 1997; Hofreuter and Haas 2002).

There is also a repertoire of genes required for colonization and survival in the acid gastric environment. Of them, more than 30 *omp* (outer membrane protein) genes belong to the group of *hop* (*Helicobacter* outer membrane porins) and *hor* (*hop*-related groups) genes, including all those that encode *H. pylori* adhesins. These genes are thought to undergo phase variation, the functional status of these genes being regulated by a slipped number of CT dinucleotide repeats in the gene 5′ region (Tomb et al. 1997). Also identified in the *H. pylori* genome are the *fla*A and *fla*B genes that encode the FlaA and FlaB subunits of the flagellum, that are required for *H. pylori* to cross the gastric mucus layer (Eaton et al. 1996b) as well as σ^{28} and σ^{54} factors, that are required for FlaA and FlaB expression.

In addition *H. pylori* contains a series of genes encoding urease, an enzyme that is essential for bacterial colonization and survival in the highly acidic gastric environment (Hazell and Lee 1986). *H. pylori* produces an abundant amount of urease which can be detected in the cytoplasm and on the bacterial surface, which associates with urea naturally present in the gastric juice (Bode et al. 1989; Bauerfeind et al. 1997). Under acidic conditions, *H. pylori* expresses UreI, a proton-gated urea channel that rapidly accelerates the entrance of urea into the cytoplasm and catalyzes the efflux of the urea products NH_3, NH_4 and CO_2 to the periplasm (Weeks et al. 2000). Not only ammonia, but also CO_2 that is converted to HCO_3^- by α-carbonic anhydrase present in the periplasmic membrane, enables rapid periplasmic pH control without changes in the pH of the cytoplasm (Scott et al. 2002). Moreover, the urease apoenzyme consisting of UreA and UreB is activated by the insertion of nickel, which is required for the activity of urease accessory genes *ure*E/*ure*F and *ure*G/*ure*H (Voland et al. 2003).

It has been shown that nickel binds to the nickel receptor (NikR) an outer membrane receptor and is transported across the cellular membrane into the cytoplasm by the nickel permease, NixA. TonB/ExB/ExD machinery, involved in iron transport, facilitates nickel uptake by *H. pylori* (Schauer et al. 2007). The response of *H. pylori* to acid is also modulated by the two-component systems HP0165/HP0166 and HP0244 as well as by Fur, the iron-responsive regulator (Bury-Mone et al. 2004). A change in environmental pH leads to phosphorylation of HP0165, a sensor kinase protein. The phosphate from the activated sensor kinase is then transferred to the HP0166 response regulator, which binds to DNA activating or repressing target genes (Pflock et al. 2006). The second sensor kinase, HP0244, is required for periplasmic buffering at low pH (Wen et al. 2009). Other *H. pylori* genes are required for response to acid and iron uptake and storage. Two of these are analogous to those found in *E. coli*, the *fec* system and *feo*B that are required for iron uptake. *nap*A and *pfr* coding ferritin like proteins are required for iron

storage and the *fur* gene encoding the Fur protein is required for ferric uptake regulation (Tomb et al. 1997). Transcriptome analysis has contributed to the clarification of the mechanisms by which *H. pylori* resists acid. Among the 95 genes found to be aberrantly expressed in Fur knockout *H. pylori* strains as compared with wild-type strains, include those involved in ammonia production (amidase and asparaginase), in detoxification (*sod*B and the *kat*A catalase), pathogenicity (*cag*-PAI and *nap*A) and transcriptional regulation (de Reuse and Bereswill 2007).

In addition *H. pylori* possesses genes encoding ammonia-producing enzymes, amidases, AmiE and AmiF, that hydrolyze short-chain amides in ammonia and their corresponding organic acid. ORFs for all enzymes required for the biosynthesis of biotin, folate, molybdopterin, pantothenate, pyridoxal phosphate and riboflavin are also present in the *H. pylori* genome. In addition there are a series of genes encoding virulence factors that will be described below. Of note, *H. pylori* contains a type 4 secretion system (T4SS) which is located on the cag-pathogenicity island (cag-PAI). The T4SS is responsible for translocating bacterial protein and peptidoglycan into the gastric epithelial host cell (Kwok et al. 2007). Genes encoding Com systems, ComB, ComEC and ComH, have been detected in the majority of *H. pylori* strains (Hofreuter et al. 2001) and act in natural bacteria transformation. The *H. pylori* transcriptome is a very complex and compact structure (Sharma et al. 2010) comprising massive antisense transcription, with more than 60 small RNAs (sRNAs) and potential regulators of *cis* and *trans*-coded mRNA targets. It has been proposed that *H. pylori* uses riboregulation for control of its gene expression (Pernitzch and Sharma 2012). Small hydrophobic proteins may also participate in the transcription regulation (Pernitzch and Sharma 2012). *H. pylori* contains several RNases, including RNase H, RNase H-II, RNase J, RNase N, RNase P, RNase R and RNase III, but as observed in all *Epsilonproteobacteria*, it lacks RNase E. RNase J and RNase III are thought to regulate virulence related genes (Tsao et al. 2009), reviewed in (Pernitzch and Sharma 2012). Cytosine methylation of *H. pylori* DNA may also modulate the expression of virulence factors by inhibiting genes that encode OMPs, CagA, VacA and genes controlling motility (Kumar et al. 2012). Combined genomic and proteomic analyses in *H. pylori* has contributed to the identification of functionally active genes, subcellular proteins and disease specific proteins. For example the proteome of *H. pylori* strain 26695 showed 1,000–1,800 spots, with the majority of these present in the pH range 4–8 (Jungblut et al. 2000). At present, 567 proteins have been identified and distributed into 17 major functional categories and numerous subcategories (Jungblut et al. 2010). The data are available in the Proteome Database System for Microbial Research (http://www.mpiib.berlin.de/2D-PAGE). PROMPT Database allows the access to the protein mapping as well as comparisons with other functional aspects (http://webclu.biowzw.tum.de/prompt).

The *H. pylori* response to nitric oxide (NO) stress involves high expression of proteins participating in protein processing including the ribosomal protein S1, aminopeptidase and an ATP-dependent protease binding subunit. Also, proteins involved in oxidative stress and detoxification are up-regulated in response to NO, including AlpC, NapA, SodB, however virulence factors including CagA and paralyzed flagellar protein are down regulated (Qu et al. 2009). The Fur regulon and thioredoxin have been shown to be essential for *H. pylori* survival under NO stress (Qu et al. 2009).

When cultured in vitro, at pH 5.0, at least 10 proteins of *H. pylori* are differentially expressed. For example *H. pylori* ferritin undergoes a conformational change and binds to bacterial DNA, thus protecting the DNA from oxidative damage caused by free radicals in the presence of metal ions such as iron and copper (Huang et al. 2010). Protein phosphorylation has been considered the most important post-translational modification in bacteria, and dys-regulated phosphorylation is associated with the pathogenesis of human diseases induced by the microorganism. In *H. pylori*, 84 phosphopeptides from 67 proteins and a total of 126 sites have been identified. Among these are phospho-serine, phospho-threonine and phospho-tyrosine that are mainly located in the cell membrane and which participate in a series of bacterial metabolic processes, including enzymatic or catalytic activity and molecular interactions. Phosphoproteins linked to transporter, structural, molecular transducer and electron carrier activities have also been detected in *H. pylori*. Notably, the *H. pylori* phosphoproteome exhibits a high abundance of tyr-phosphorylation (18.5 %) that seems to be related to bacterial pathogenicity and a high level of membrane proteins (Ge et al. 2011).

Helicobacter acinonychis

The genome sequence of *H. acinonychis* strain Sheeba G, isolated from the stomach of a cheetah with gastritis, has been sequenced by Eppinger et al. and compared with the genome of *H. pylori* (Eppinger et al. 2006). It comprises a circular chromosome of 1,553,928 bp as well as a plasmid (pHac1) of 3,661 bp. The average G+C content of the chromosome is 38.2 mol% and that of the plasmid 34.6 mol%. The chromosome contains 1,611 predicted CDSs in a coding area of 89.7 % with an average length of 865 bp. The chromosome contains 36 tRNAs and 6 rRNA loci.

The size and G+C content of the Sheeba genome have been shown to be very similar to those of *H. pylori* 26695 (1,667,867 and 39 mol%) and *H. pylori* J99 (1,643,831 and 39 mol%). All three genomes share numerous orthologous coding sequences (CDSs). The proteins encoded by the 612 orthologous CDSs present in all three genomes do not have internal gaps and differ at only a few of their amino acids. Based on these findings and other comparisons, *H. acinonychis* has been described as very closely related to *H. pylori*, being almost as closely related to the two *H. pylori* genomes (26695 and J99) as they are to each other.

Helicobacter bizzozeronii

The genome of *H. bizzozeronii* CIII-1, isolated from a corpus gastric biopsy specimen obtained from a Finnish female patient with severe gastric symptoms (Kivisto et al. 2010), has been

sequenced by Schott et al. (2011b). The genome is composed of a circular chromosome of 1,755,458 bp and a 52,076 bp circular plasmid. The G+C content of the chromosome is 46 mol%. There are 1,894 CDSs in a coding area of 93 % with an average length of 851 bp (1,079 genes per kilobase). A putative function could be predicted for 1,280 (67.7 %) CDSs, while 614 (32.4 %) of the CDSs were annotated as hypothetical proteins. The plasmid contains 77 CDSs, 21 of which (27.3 %) have been assigned a putative function. The genome also contains 36 tRNAs and 2 rRNA loci. Comparison of this genome with ten *H. pylori* genomes found 562 CDSs unique to *H. bizzozeronii*, however a putative function was only ascribed to 147 (26 %) of these CDSs (Schott et al. 2011a). The majority of these functions were linked to metabolism and chemotaxis as described below.

In common with the other gastric helicobacters *H. bizzozeronii* was found to contain a number of *fla* gene families required for flagella functionality, a complete urease gene cluster (UreAB) and a number of orthologs for others enzymes that are known to be important for survival in acidic environments. The genome however lacked the second urease cluster, UreAB2, which has been described in *H. felis* and *H. mustelae*, although two copies of a putative allophanate hydrolase, similar to that detected in *H. felis*, were found. One possible function of this ortholog could be to contribute to the cytoplasmic degradation of urea in conjunction with urease (Schott et al. 2011a). A further similarity of this genome to the *H. felis* genome is the high prevalence of membrane chemotaxis proteins (MCPs) with five times the number seen in *H. pylori* being observed. It is believed that MCPs are important for organisms to respond to a range of environmental signals, thus allowing them readily colonize and transfer between a number of different hosts.

A high level of genome plasticity was also observed in *H. bizzozeronii* with the description of 43 phase variable/contingency genes, five IS elements (two belonging to the IS200/IS605 family and three to the IS607 family), 22 mini-IS elements and a putative genomic island of ~70 kb and a prophage. Compared to *H. pylori* it was also found to contain genes that would allow for greater metabolic flexibility which led Schott et al. to propose that this combination of flexibility and plasticity makes it easier for these organisms to adapt to different hosts (Schott et al. 2011a).

Helicobacter canadensis

The genome of *H. canadensis* Type strain NCTC 13241, isolated from a human with diarrhea, has been sequenced by Loman et al. (2009). It consists of a single circular chromosome of 1,623,845 bp with a G+C content of 34 mol%. Within the genome 1,535 predicted CDSs, 40 tRNAs and 3 rRNA loci were identified. One hundred and seventeen CDSs from *H. canadensis* had no detectable ortholog in any other genome-sequenced *Epsilonproteobacteria*. Twenty-nine coding regions with variations in homopolymeric tract length, indicative of phase variation, were identified in the genome sequence of *H. canadensis* strain NCTC 13241. Further investigation of these 29 coding regions showing variations in homopolymeric tract length by Snyder et al. (2010) showed that 16 of the 29 coding regions had homopolymeric tracts characteristic of translational phase variation, while five were potentially associated with promoter regions, which lead to transcriptional phase variation. The remaining eight annotated coding sequences were shown to have long poly-G tracts ($\geq$10 bp) towards the end of the annotated coding region. Snyder et al. suggested that such changes in the repeat tract length would alter the protein sequence at the *C*-terminus, but not stop the expression of the protein. Based on this latter finding, the authors concluded that this mechanism of *C*-terminal phase variation has implications for stochastic switching of protein sequence in bacterial species that already undergo transcriptional and translational phase variation (Snyder et al. 2010). Of these 29 phase-variable genes, a number may be implicated in virulence, including an Immunoglobulin A (IgA) protease, and two homologues of the vacuolating cytotoxin of *H. pylori* (Loman et al. 2009).

Helicobacter cinaedi

The genome of *H. cinaedi* strain PAGU611, isolated from a patient with bacteremia, has recently been sequenced by Goto et al. (2012). It was found to contain a 2,078,348 bp chromosome as well as a 23,054 bp plasmid, (pHci1), with average G+C contents of 38.6 mol% and 31.6 mol%, respectively. The chromosome was shown to contain 2,096 predicted protein-coding sequences (CDSs), 39 tRNA genes, and 2 rRNA operons. The plasmid was shown to encode 29 predicted CDSs, of which 27 were hypothetical proteins. No prophage or insertion sequence elements were observed in the genome. Reciprocal best hit analysis of PAGU611 chromosomal CDSs against four sequenced *Helicobacter* species showed homologies of 66.1 % to *H. hepaticus* (Suerbaum et al. 2003), 49.6 % to *H. mustelae* (O'Toole et al. 2010), 47.4 % to *H. felis* (Arnold et al. 2011), and 47.3 % to *H. pylori* 26695 (Tomb et al. 1997). Based on synteny plots of all orthologs *H. cinaedi* was found to be most similar to *H. hepaticus*.

In addition, the genome of the type strain of *H. cinaedi* (ATCC BAA-847), isolated from a rectal swab of a homosexual man (Totten et al. 1985), has been sequenced by Miyoshi-Akiyama et al. (Miyoshi-Akiyama et al. 2012). It was found to consist of a single circular chromosome of 2,240,130 bp with an average G+C content of 38.34 mol%. The chromosome was shown to contain a total of 2,322 protein coding genes, 40 tRNA genes for all amino acids, and two RNA operons. The chromosome also contains three prophage-like elements. In addition, in comparison to the genome of PAGU611, the *H. cinaedi* ATCC BAA-847 genome contains two unique regions spanning 1.4–1.6 Mbp, the latter half (from 1.54 to 1.56 Mbp) of which corresponds to a putative prophage.

Helicobacter felis

The genome of *H. felis* CS1, isolated from a cat, has been fully sequenced by Arnold et al. in an attempt to generate novel

insights into its molecular pathogenesis (Arnold et al. 2011). It contains 1,672,681 bp, which includes a 6,700 bp episomally replicating plasmid. The genome of *H. felis* is comparable in size to the sequenced genomes of *H. pylori* (1.59–1.67 Mb), slightly larger than that of the *H. mustelae* and *H. acinonychis* genomes (1.58 and 1.55 Mb) but smaller than that of the *H. hepaticus* genome (1.8 Mb). Its G+C content is 44.5 mol%. There are 1,671 genes and 1 pseudogene located in the coding area (92 %). The average length of an *H. felis* gene is 921 bp (0.998 genes per kb). In addition, the genome contains 35 tRNA genes as well as the *H. felis* plasmid, previously reported by De Ungria et al. (1998).

The *H. felis* genome contains a complete urease gene cluster (*ure*ABIEFGH). An ortholog of the *H. pylori* nickel transporter NixA and a predicted high-affinity nickel transport protein were shown to be encoded directly downstream of the urease gene cluster. As in *H. mustelae* (O'Toole et al. 2010) the *H. felis* genome was found to have an additional *ure*AB2 operon. The *H. felis* genome also contains at least 40 motility/chemotaxis-related genes encoded by the *fla, flg, flh,* and *fli* gene families, which in *H. pylori* and other flagellated bacteria have been shown to play a role in the regulation, secretion, and assembly of the flagellum (O'Toole et al. 2000).

H. felis also possesses a large number of chemotaxis genes including up to 20 predicted methyl-accepting inner MCPs. In other bacteria binding of chemo-attractants such as urea, bicarbonate, or amino acids to MCPs has been observed to be associated with a change in direction of flagellar motor rotation. One copy of *cheA, cheW,* and *cheY* orthologs were also present in the *H. felis* genome. Like *H. pylori H. felis* lacks *cheB* and *cheR* which, in other bacteria, have been shown to be responsible for modulating the chemotactic response, by addition and removal of methyl groups to/from MCPs. It has been hypothesized that the large number of predicted MCP-like chemotaxis sensors present in *H. felis*, suggests "an elaborate spatial orientation in a diverse habitat" (Arnold et al. 2011). In addition, a type III secretion system that exports flagellar subunit components across both membranes (encoded in *H. felis* by predicted orthologs of the *H. pylori* genes *flhA*, *flhB* and *fli* H,I,P,Q,R) has also been detected.

Helicobacter heilmannii

The genome of *H. heilmannii sensu stricto* type strain ASB1 isolated from the gastric mucosa of a kitten with severe gastritis, has recently been reported by Smet et al. (2012b). The genome comprises a chromosome 1,804,623 bp in length with a G+C content of 47.4 mol%. The genome contains 1,918 protein-coding sequences (CDSs) with an average length of 933 bp. A putative function was predicted for 1,183 (62 %) of the CDSs, whereas 735 (38 %) CDSs were annotated as hypothetical proteins. In addition, the genome of *H. heilmannii* contains 41 tRNA genes, and 9 rRNA genes.

Helicobacter hepaticus

The genome of *H. hepaticus* ATCC 51449, sequenced by Suerbaum et al. (2003), was the first enterohepatic *Helicobacter* genome to be sequenced. It has a circular chromosome of 1,799,146 bp and a G+C content of 35.9 mol%. Of the 1,875 predicted proteins, a function was assigned to 713 (38 %) with a high level of confidence, 673 (35.9 %) were conserved hypothetical proteins (309 with some evidence of function and 364 were without assignment of function) and 489 proteins (26.1 %) had no significant database match. A total of 938 and 941 (50.2 %) of the *H. hepaticus* ORFs have orthologs in the genomes of *H. pylori* strains 26695 and J99, respectively, and 953 have an ortholog in *C. jejuni* NCTC 11168. Of these ORFs 821 were found in both *H. pylori* and *C. jejuni*, 109 in *H. pylori* only and a further 130 in *C. jejuni* only.

The H. hepaticus genome contains a urease gene cluster (*ureABIEFGH*) similar to that found in *H. pylori* and downstream of this cluster, transcribed in the opposite direction, is a cluster of homologs of *E. coli* nickel transport genes *nikABCDE*, that are not present in *H. pylori* or *C. jejuni*. In animal models the urease gene has been shown to promote hepatic inflammation but not essential for cecal colonization (Ge et al. 2008a). The flagellar biosynthesis system of *H. hepaticus* is similar to that of *H. pylori*, with genes encoding two flagellin types, FlaA and FlaB, which are under control of respective σ^{28} and σ^{54} promoters.

Helicobacter mustelae

The genome of *H. mustelae* ATCC 12198, isolated from the gastric mucosa of a ferret, was sequenced by O'Toole et al. (2010). It was shown to comprise a single circular chromosome of 1,578,097 bp with a G+C content of 42.47 mol%, which is one of the highest G+C percentages in the *Helicobacter* genus (Solnick and Schauer 2001). Of the 1,403 predicted proteins (CDSs), 91.9 % is coding area. The mean predicted CDS length is 995, which is slightly higher than other related bacteria. The *H. mustelae* genome does not contain plasmids, insertion elements, or genomic islands. It does however have 4 phage genes, as well as a number of regions of deviating G+C content that have been associated with auto-transporter genes and flagellin modification surface proteins (O'Toole et al. 2010).

The motility genes *fla/flg/fli/flh*, which have been shown to be required for gastric colonization in other *Helicobacter* species, have also been detected in the *H. mustelae* genome. Further a collagenase secretion gene, shown in *H. pylori* to be essential for colonization of the gastric mucosa of Mongolian gerbils (Kavermann et al. 2003), was also observed. Of the 27 genes shown to be essential for *H. pylori* colonization of gerbils all but three had orthologs in *H. mustelae*, suggesting a shared set of core proteins for gastric persistence (O'Toole et al. 2010).

In addition, the genome of *H. mustelae* ATCC 12198 includes both the *ure*AB and *ure*AB2 loci that have previously

been reported to play a role in resistance to gastric acid and facilitate colonization of the gastric mucosa (Stoof et al. 2008; Carter et al. 2011). Comparison of these two urease systems has shown that while UreAB is regulated by nickel UreAB2 is regulated by iron. It has been suggested that this second urease system is important for bacterial survival in low nickel environments such as that found in carnivores.

Helicobacter suis

The genomes of *H. suis* (HS1) and *H. suis* (HS5), both isolated from the gastric mucosa of swine, have been sequenced by Vermoote et al. (2011). Based on comparative analysis, the genome sequences of two *H. suis*, type 1 (HS1) and type 5 (HS5) strains are 1,635,292 bp and 1,669,960 bp, respectively, with an average of G+C content of 40 mol%. As found in *H. pylori* *H. suis* carries three copies of the 5S rRNA gene however it possesses only one copy of each of the 16S and 23S rRNA genes, compared to the two found in *H. pylori*. The genome of HS1 comprises 1,266 ORFs and that of HS5, 1,251 ORFs. *H. suis* shares several genes that are identified in other *Helicobacter* species including *ureA*, *ureB*, *ureI*, *ureE*, *ureF*, *ureH* and *ureG* encoding urease that is required for acid acclimation and the flagellar biosystem. Genes encoding putative outer membrane proteins required for adhesion to the gastric epithelial cells and lifelong maintenance of the infection were also detected. Genes, homologous to members of the major *H. pylori* OMP families (coding Hop and Hor proteins, iron-regulated and efflux pump OMPs), are also observed. The *H. suis* genome possesses genes encoding a number of predicted OMPs similar to porins and a series of genes related to oxidative stress resistance, including homologues of the *katA* of *H. acinonychis*, the *sodB* of *H. pylori*, the mismatch repair ATPase (*mutS*) and bacterioferritin co-migratory protein of *H. hepaticus*, and the genes encoding and the NAD(P)H (*mdaB*) quinone reductase of *Campylobacter fetus* as well as the gene coding peroxiredoxin of *H. pylori*.

Phenotypic Analyses

The general phenotypic characteristics regarding biochemical reactivity, morphology and growth of the validated *Helicobacter* taxa are listed in *Tables 24.7* and *24.8*. The basic biochemical tests used for the identification and differentiation of all *Campylobacter*-like organisms are commonly used to identify *Helicobacter* strains. The lack of application of highly standardized procedures and the well-known biochemical inertness of *Campylobacter*-like organisms render biochemical identification of all of these bacteria very difficult. There are no biochemical characteristics that can separate members of the genus *Helicobacter* from the genus *Campylobacter* (*Arcobacter* strains can be differentiated from campylobacters and helicobacters by their ability to grow in air and at low temperatures). It should be noted that minimal standards for the description of new *Helicobacter* species have been described (Dewhirst et al. 2000b). These minimal standards outline tests (biochemical tests and others) and test procedures that are recommended for the description and hence, differentiation, of helicobacters.

Helicobacter pylori

Helicobacter pylori ([L. n. *pylorus* (from Gr. n. *pulôros*, gate keeper)], the lower orifice of the stomach, the pylorus; L. gen. n. *pylori*, of the pylorus) was initially isolated from the gastric mucosa of humans presenting with dyspepsia (Marshall et al. 1984, 1985). While originally thought to be a *Campylobacter* species and given the name *Campylobacter pyloridis*, which was later corrected grammatically to *Campylobacter pylori*, subsequent studies of its 16S rRNA gene showed that it was sufficiently distant from other campylobacters to warrant it being placed in its own genus (Romaniuk et al. 1987). It was formally named *Helicobacter pylori* and it became the type species of the *Helicobacter* genus (Goodwin et al. 1989a). *H. pylori* has also been detected in certain primate species, particularly rhesus monkeys (Dubois et al. 1994; Solnick et al. 1999), sheep (Dore et al. 2001), a closed colony of barrier maintained cats (Handt et al. 1994) and dogs (Buczolits et al. 2003). Morphologically the bacterial cells are curved to S-shaped rods, 0.5 µm × 2.5–5 µm (*Fig. 24.2*). They are motile by means of 4–8 unipolar or bipolar sheathed flagella. The bacteria grow as small, translucent colonies (1–2 mm) or a spreading film with increased periods of incubation. Optimum growth is obtained under microaerobic conditions at 37 °C with no growth obtained at 42 °C or under anaerobic conditions. For primary isolation 4–5 days incubation is required, which can be reduced to 2 days for subsequent subculture.

All strains have been reported to be urease, catalase and oxidase positive. They do not reduce nitrate and do not hydrolyze indoxyl acetate or hippurate. Activities for γ-glutamyl transpeptidase, leucine arylamidase and alkaline phosphatase are present. *H. pylori* is sensitive to 1 % glycine, 1.5 % NaCl, and cephalothin. It is resistant to nalidixic acid with variable resistance to metronidazole. The major fatty acid of *H. pylori* is $C_{14:0}$ and $C_{19:0}$ cyclic with smaller amounts of $C_{18:0}$, $C_{18:1}$ and the major isoprenoid is MK-6 (Goodwin et al. 1989b; Moss et al. 1990). *H. pylori* was originally thought to be asaccharolytic, due to the use of standard methods to detect carbohydrate metabolism. However, recently it has been shown to utilize glucose via the pentose phosphate and Entner-Doudoroff pathways. Glycolysis is also utilized with fermentation of glucose possibly leading to the production of mixed acid products or the provision of metabolites for the Krebs cycle (Mendz et al. 1993, 1994; Chalk et al. 1994). *H. pylori* also has a requirement for amino acids whose deamination could lead to the production of nitrogen, carbon and energy for the cell (Nedenskov 1994; Reynolds and Penn 1994). Features of both aerobic and anaerobic respiration have also been reported (Hazell and Mendz 1997). *H. pylori* is resistant to cefsulodin, nalidixic acid, trimethoprim and

Table 24.7
Characteristics of validated gastric *Helicobacter* species

Taxon	Catalase production	Nitrate reduction	Alkaline phosphatase reduction	Urease	Indoxyl acetate hydrolysis	γ - glutamyl transferase	Growth at 42 °C	Growth with 1 % glycine	Susceptibility to		Periplasmic fibrils	No. flagella	Distribution flagella
									Nalidixic acid 30ug disc	Cephalothin 30ug disc			
Gastric													
H. acinonychis	+	–	+	+	–	+	–	–	R	S	–	2–5	M
H. baculiformis	+	+	+	+	–	+	–	–	I	R	+	11	B
H. bizzozeronii	+	+	+	+	+	+	V	–	R	S	–	10–20	B
H. cetorum	+	–	–	+	–	+	+	–	V	S	–	2	B
H. cynogastricus	+	+	+	+	–	+	–	–	ND	ND	+	6–12	B
H. felis	+	+	V	+	–	+	+	–	R	S	+	14–20	B
H. heilmannii	+	+	–	+	–	+	–	–	ND	ND	–	10	B
H. mustelae	+	+	+	+	+	+	+	–	S[e]	R	–	4–8	L & B
H. nemestrinae	+	–	+	+	–	ND	+	–	R	S	–	4–8	M
H. pylori	+	–	+	+	–	+	–	–	R	S	–	4–8	M
H. salomonis	+	+	+	+	V	+	–	–	R	S	–	10–23	B
H. suis	+	–	+	+	–	+	–	–	ND	ND	–	4–10	B

ND Not Determined, *M* Monopolar, *V* Variable, *B* Bipolar, *R* Resistant, *L* Lateral, *I* Intermediate, *ST* Sub Terminal, *S* Sensitive, + Majority of strains

Table 24.8

Characteristics of validated enterohepatic *Helicobacter* species

Taxon	Catalase production	Nitrate reduction	Alkaline phosphatase reduction	Urease	Indoxyl acetate hydrolysis	γ - glutamyl transferase	Growth at 42 °C	Growth with 1 % glycine	Susceptibility to		Periplasmic fibrils	No. flagella	Distribution flagella
									Nalidixic acid 30ug disc	Cephalothin 30ug disc			
H. anseris	−[a]	−	−	+	+	−	+	W	S	R	−	2	ST
H. aurati	+	−	−	+	+	+	+	−	S	R	+	7–10	B
H. bilis	+	+	−	+	−	+	+	V	R	R	+	3–14	B
H. brantae	−[a]	−	−	−	+	−	+	W	S	I	−	2	ST
H. canadensis	+	V	−	−	+	−	+	+	R	R	−	1–2	B
H. canis	−	−	+	−	+	ND	+	−	S	V	−	2	B
H. cholecystus	+	+	+	−	−	−	+	+[b]	I	R	−	1	M
H. cinaedi	+	+	−	−	−	−	−	+	S	I	−	1–2	M/B
H. equorum	+	+	+	−	−	−	−	−	R	R	−	1	M
H. fennelliae	+	−	V	−	+	−	−	+	S	S	−	2	B
H. ganmani	−/V	+	−	−	−	ND	−	−	S	R	−	2	B
H. hepaticus	+	+	−	+	+	−	−	+	R	R	−	2	B
H. marmotae	+	−	+	+	−	−	+	+	R	R	−	2	B
H. mastomyrinus	+	−	−	+	−	−	+	+	R	R	−	2	B
H. mesocricetorum	+	+	+	−	ND	−	+	−	S	R	−	2	B
H. muridarum	+	−	+	+	−	+	−	−	R	R	+	10–14	B
H. pametensis	+	+	+	−	−	−	+	+	S	S	−	2	ST
H. pullorum	+	+	−	−	−	ND	+	−	S	R	−	1	M
H. rodentium	+	+	−	−	−	−	+	+	R	R	−	2	B
H. trogontum	+	+	−	+	−	+	+	+	R	R	+	5–7	B
H. typhlonius	+	V	−	−	−	−	+	W	S	R	−	2	B

[a]Note: catalase results incorrect in species description, negative results confirmed (JG Fox, personal communication)

[b]Only 87 % human isolates positive. All animal isolates positive

+ Majority of strains, *ND* Not Determined, *M* Monopolar, *B* Bipolar, *V* variable, *L* Lateral, *R* Resistant, *I* Intermediate *S* Sensitive, *ST* Sub Terminal

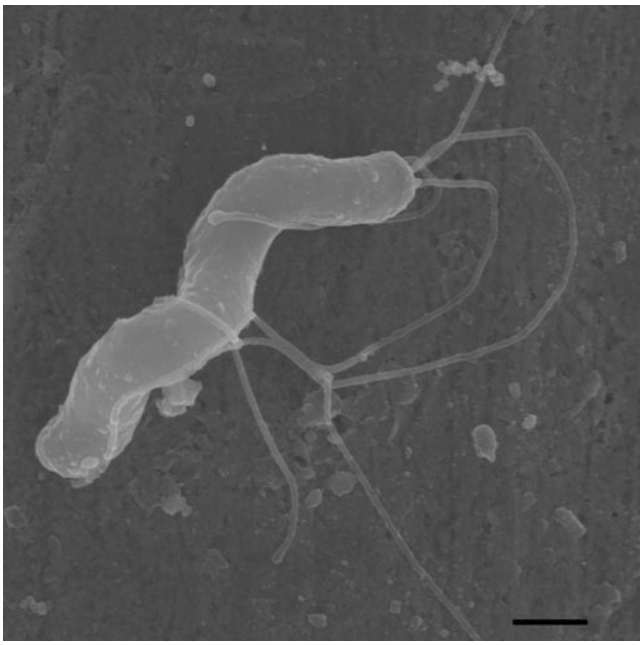

Fig. 24.2
Transmission electron micrograph of a freeze-dried preparation of *Helicobacter pylori* showing its S-shaped morphology, sheathed flagella and terminal bulb (bar = 0.5 μm)

vancomycin and highly susceptible to amoxicillin, bismuth salts, quinolones, rifabutin and tetracycline. The susceptibility to clarithromycin is variable and within the taxa the resistance to metronidazole is high.

Type strain: Royal Perth Hospital 13487^T = ATCC 43504^T = CCUG 15815 B^T = CIP 103995^T = DSM 4867^T = JCM 12093^T = LMG 18041^T = NCTC 11637^T.

Genbank accession number (16S rRNA): U01330.

G+C content of DNA: 39 mol%.

Helicobacter acinonychis

Helicobacter acinonyx (N.L. gen. n. *acinonychis*, of *Acinonyx*), referring to the feline species *Acinonyx jubatus*, cheetah was first isolated from the gastric mucosa of a cheetah (Eaton et al. 1993a). The original spelling of the specific epithet "acinonyx" was later corrected by Truper et al. to *acinonychis* (Truper and DeClari 1997). *H. acinonychis* has also been isolated from two Sumatran tigers (Schroder et al. 1998) and detected in cheetahs from a zoo in the United States and in lions, tigers and tiger/lion hybrids from a European circus (Dailidiene et al. 2004). Morphologically the bacterial cells are short, spiral rods 0.3 μm wide by 1.5–2.0 μm in length. The cells are motile by means of tufts of two to five sheathed monopolar flagella. The bacteria grow under microaerobic conditions at 37 °C but not at 25 °C or 42 °C. Growth does not occur aerobically or anaerobically. The cells have oxidase, catalase, urease, alkaline phosphatase, acid phosphatase, phosphohydrolase, γ-glutamyl transpeptidase and arginine β-naphthylamide aminopeptidase activity. Leucine and histidine β-napthylamides are weakly hydrolyzed. Hippurate and indoxyl acetate are not hydrolyzed. H_2S is not produced. Nitrate is not reduced. The bacteria do not grow in the presence of 1.0 % glycine or 1.5 % NaCl. All strains of *H. acinonychis* are sensitive to cephalothin and metronidazole but resistant to nalidixic acid. In addition, they are sensitive to penicillin, ampicillin, nitrofurazone, erythromycin, gentamicin, chlortetracycline and chloramphenicol but resistant to sulfamethoxazole plus trimethoprim and vancomycin.

Type strain: 90-119^T (= ATCC 5110^T = CCUG 2926^T = CIP 10424^T = NCTC 12686^T.)

Genbank accession number (16S rRNA): M88148.

G+C content of DNA: 30 mol%.

Helicobacter anseris

Helicobacter anseris (*L. gen. n. anseris, of a goose*) was first isolated from the feces of Canada geese (Fox et al. 2006; Validation List 2006b). Morphologically the bacterial cells are slender, slightly curved rods, 0.5 μm in diameter by 1.8 μm in length. The cells are motile by means of single sheathed flagella that are located sub-terminally at each end of the cell. This latter feature is only found in two other *Helicobacter* species, *H. brantae* and *H. pamentensis*, both of which are also found in birds (Dewhirst et al. 1994; Fox et al. 2006). The bacteria grow as a spreading layer under microaerobic conditions at both 37 °C and 42 °C, but do not grow under aerobic or anaerobic conditions. All strains isolated have oxidase and urease activity but do not have catalase, alkaline phosphatase or γ-glutamyl transpeptidase activity. All strains hydrolyze indoxyl acetate and grow in 1 % glycine. They do not reduce nitrate. *H. anseris* is sensitive to nalidixic acid and resistant to cephalothin.

Type strain: MIT 04-9362^T (= ATCC BAA-1299^T = CCUG 52421^T).

Genbank accession number (16S rRNA): DQ415545.

Genbank accession number (23S rRNA): DQ418749.

Helicobacter aurati

Helicobacter aurati (L. adj. *auratus*, adorned with gold, covered with gold, gilded, golden; N.L. gen. masc. n. *aurati*, of the golden one, named after the golden Syrian hamster, *Mesocricetus auratus*) was first isolated from the stomach and cecum of adult Syrian hamsters (Patterson et al. 2000b; Validation List 2002b). Morphologically the bacterial cells are fusiform rods with periplasmic fibers and are 0.6 μm in diameter and 4–8 μm in length. They are motile by means of bipolar, multiple (7–10) sheathed flagella. The bacteria grow as a thin spreading film on agar under microaerobic conditions at 37 °C and 42 °C, but not at 25 °C. No growth is observed under anaerobic conditions. All five strains isolated have urease, catalase, oxidase and γ-glutamyl transpeptidase activities. All strains hydrolyze indoxyl acetate, and lack alkaline phosphatase activity. They do not reduce nitrate or grow on Brucella agar containing 1 %

glycine, 1.5 % NaCl, 2.0 % NaCl or 3.0 % NaCl. All strains are sensitive to nalidixic acid but resistant to cephalothin.

Type strain: MIT 97-5075c^T (= ATCC BAA-1^T = CCUG 47791^T).

Genbank accession number (16S rRNA): AF297868.

Helicobacter baculiformis

Helicobacter baculiformis (L. n. *baculus*, rod; L. suff. *-formis* from L. n. *forma*, figure, shape, appearance), -like, in the shape of; N.L. masc. adj. *baculiformis*, rod-shaped) was first isolated from the gastric mucosa of a cat (Baele et al. 2008b). Morphologically the bacterial cells are large, slender to slightly spiral rods, approximately 1 µm wide by 10 µm long. The cells possess four periplasmic fibrils running along the external side of the helix and are motile by means of tufts of up to 11 sheathed bipolar flagella. The flagella are blunt ended with the terminal diameter being wider than the average diameter of the flagellar body. The bacteria grow on agar as a spreading layer under microaerobic conditions as well as in a 5 % CO_2-supplemented atmosphere. Optimal growth is obtained by using biphasic culture plates (continually adding broth to the surface of the plate) or very moist plates. Weak growth occurs under anaerobic incubation but the bacteria do not grow under aerobic conditions. Growth occurs at 37 °C, but not at 25 °C or 42 °C. The bacteria have oxidase, catalase, urease, esterase, γ-glutamyl transpeptidase, L-arginine arylamidase and alkaline phosphatase activity. They do not have pyrrolidonyl arylamidase or L-aspartate arylamidase activity. The cells are able to reduce nitrate and triphenyltetrazolium chloride (TTC). Hippurate and indoxyl acetate are not hydrolyzed. The bacteria do not grow in the presence of 1 % glycine, 1.5 % NaCl or 1 % Ox bile. The type strain, strain M50^T, is resistant to cephalothin and has intermediate sensitivity to nalidixic acid.

Type strain: M50^T (=LMG 23839^T = CCUG 53816^T).

Genbank accession number (16S rRNA): EF070342.

Helicobacter bilis

Helicobacter bilis (L. n. *bilis -is*, bile; L. gen. n. *bilis*, of the bile, referring to the bodily fluid from which it was isolated) was first isolated from the colon and cecum of mice and the bile and livers of mice with hepatitis (Fox et al. 1995; Validation List 1997), and subsequently from healthy and diarrheic cats and dogs (Eaton et al. 1996a; Hanninen et al. 2005; Rossi et al. 2008), rats, hamsters and gerbils (Dewhirst et al. 2000a). Strains from human bile, liver and gallbladder samples have also been identified as *H. bilis* by 16S rRNA gene sequence comparisons (Fox et al. 1998a; Tolia et al. 2004). Polyphasic analysis of the phenotypic and genotypic characteristics of what was previously referred to as *Flexispira* taxa 2, 3 and 8, isolated from humans, sheep and pigs, have shown them also to be members of the species *H. bilis* (Dewhirst et al. 2000a; Hanninen et al. 2005).

Morphologically the cells are fusiform to slightly spiral in shape and are 0.5 µm wide by 4–5 µm in length. The cells are motile by means of tufts of sheathed bipolar flagella (3–14). The bacteria grow under microaerobic conditions as pinpoint colonies, although cultures often appear as a thin spreading layer, at both 37 °C and 42 °C. They do not grow at 25 °C. The bacteria have urease, catalase and oxidase activities. They can grow in 20 % bile and 0.4 % TTC and display variable growth in 1 % glycine. They do not grow in 1.5 % NaCl. Nitrate is reduced. H_2S is detected on lead acetate discs. Indoxyl acetate and hippurate are not hydrolyzed. *H. bilis* is resistant to cephalothin and nalidixic acid but sensitive to metronidazole.

In 2010, Rossi et al. reported that *H. bilis* strains could be divided into two distinct and divergent genomic groups (Rossi et al. 2010). In the absence of a specific phenotype or pathotype to distinguish these groups it has been suggested that they may be referred to as two genomospecies *Helicobacter bilis sensu stricto* and *Helicobacter* sp. FL56.

Type strain: Hb1^T (= ATCC 51630^T = CCUG 38995^T = CIP104752^T).

Genbank accession number (16S rRNA): U18766.

Helicobacter bizzozeronii

Helicobacter bizzozeronii (N.L. gen. n. *bizzozeronii*, of Bizzozero, named in honor of Guilio Bizzozero, an Italian pathologist who was one of the first scientists to describe spiral organisms in the canine gastric tract) (Bizzozero 1892), were first isolated from the gastric biopsies of dogs (Hanninen et al. 1996). Isolates have also been cultivated from two human patients (Andersen et al. 1999; Jalava et al. 2001; Kivisto et al. 2010). Morphologically the bacterial cells are spiral, do not have periplasmic fibrils and are 0.3 µm wide and 5–10 µm in length. The cells are motile by means of tufts bipolar sheathed flagella (10–20). The bacteria grow on agar as spreading films under microaerobic conditions at 37 °C and 42 °C but not at 25 °C. All strains are oxidase, catalase, and urease positive. All strains have γ-glutamyl transpeptidase, alkaline phosphatase and DNase activity, hydrolyze indoxyl acetate but do not undertake hippurate hydrolysis. The bacteria do not grow on media containing 1 % ox bile, 1 % glycine, or 1.5 % NaCl. They reduce nitrate and TTC. *H. bizzozeronii* is resistant to nalidixic acid and susceptible to cephalothin, cefoperazone, and metronidazole.

Type strain: Hanninen 1^T (= Storkis^T = CCUG 35545^T = CIP 105233^T).

Genbank accession number (16S rRNA): Y09404.

Helicobacter brantae

Helicobacter brantae (N.L. gen. n. *brantae*, of *Branta*, the zoological genus name of Canada geese (*Branta canadensis*)) was first isolated from the feces of Canada geese (Fox et al. 2006; Validation List 2006b). Morphologically the bacterial cells are slender, slightly curved rods, and are 0.5 µm wide and 1.8 µm in length. The cells are motile by means of single sheathed flagella that are located sub-terminally at each end. The bacteria grow on

solid agar as a spreading layer under microaerobic conditions at both 37 °C and 42 °C, but not under aerobic or anaerobic conditions. All strains have oxidase activity but not catalase, urease, alkaline phosphatase and γ-glutamyl transpeptidase activity. All strains grow weakly in 1 % glycine but do not reduce nitrate. Five of seven isolates hydrolyzed indoxyl acetate. *H. brantae* is sensitive to nalidixic acid and shows intermediate resistance to cephalothin.

Type strain: MIT 04-9366^T (= ATCC BAA-1298^T = CCUG 52420^T).

Genbank accession number (16S rRNA): DQ415546.

Genbank accession number (23S rRNA): DQ418750.

Helicobacter canadensis

Helicobacter canadensis (N.L. masc. adj. *canadensis*, pertaining to Canada, the country of original isolation) was first isolated from the feces of Canadian patients with diarrhea (Fox et al. 2000; Validation List 2002b), and from blood cultures of an Australian patient with bacteremia (Tee et al. 2001). *H. canadensis* has also been detected in wild geese in Sweden (Waldenstrom et al. 2003, 2007), Guinea fowl from a French farm (Nebbia et al. 2007) and isolated from cecal samples of intensively reared Guineafowl and pheasants in North-Western Italy (Robino et al. 2010). *H. canadensis* has also been isolated from wild rodent feces in China (Goto et al. 2004). *H. canadensis* and atypical *H. canadensis* isolates (unable to hydrolyze indoxyl acetate), have been reported in porcine feces from Denmark and The Netherlands. Interestingly, these porcine isolates grouped separately from *H. canadensis* strains from humans and geese (Inglis et al. 2006). Further *H. canadensis/H. pullorum*-like organisms have been detected in the stomachs of three laboratory rabbits (Van den Bulck et al. 2006a).

Morphologically the bacterial cells are slender, curved to spiral rods, 0.3 μm wide by 1.0–4 μm in length, and have one to three spirals. They are motile by means of non-sheathed, single unipolar or bipolar flagella. The bacteria grow as spreading layer under microaerobic conditions at 37 °C or 42 °C. They do not grow under aerobic or anaerobic conditions. All four strains have catalase and oxidase activity but are negative for urease, alkaline phosphatase, and γ-glutamyl transpeptidase activity. It is most closely related to *H. pullorum* phenotypically and phylogenetically but it can be distinguished by its ability to hydrolyze indoxyl acetate. Two of four strains reduce nitrate. *H. canadensis* is resistant to nalidixic acid and cephalothin.

Type strain: NLEP-16143^T (= ATCC 700968^T = CCUG 47163^T = MIT 98-5491^T).

Genbank accession number (16S rRNA): AF262037.

Helicobacter canis

Helicobacter canis (L. gen. n. *canis*, of the dog) was first isolated from the feces of diarrheic or healthy domestic dogs (Stanley et al. 1993; Validation List 1994a) although it was first reported in 1993 in a boy with gastroenteritis (Burnens et al. 1993). *H. canis* has also been isolated from a dog with hepatitis (Fox et al. 1996a), cats (Rossi et al. 2008), and a colony of Bengal cats with and without diarrhea (Foley et al. 1999) and a *H. canis*-like organism was isolated from a harp seal (Harper et al. 2003). *H. canis* DNA has also been detected in the feces of children shown to be transiently positive for *H. pylori* (Haggerty et al. 2005). It has also been isolated from a number of immuno-deficient patients with bacteremia, including a patient with X-linked hypogammaglobulinemia (Gerrard et al. 2001), a patient with gastric lymphoma (Alon et al. 2010) and a woman with common variable immune deficiency and liver failure (Abidi et al. 2013). *H. canis* has also been isolated from two immune-competent patients, one with bacteremia (Leemann et al. 2006), and the other a 7-month old child with bacteremia (Prag et al. 2007). In addition, *H. canis* has been detected in chronic duodenal ulcerations in a patient with Crohn's disease (Tankovic et al. 2011).

Morphologically the cells are helically curved, slender rods, 0.25 μm wide and 4 μm long with one to two spiral turns. The cells are motile by means of single bipolar, sheathed flagella and exhibit darting motility in broth cultures. The bacteria grow on blood agar as pinpoint, non-pigmented, translucent colonies that appear α-hemolytic after 48 h, when incubated under microaerobic conditions at 37 °C or 42 °C. No growth is observed under aerobic or anaerobic conditions or at 25 °C. The bacteria are oxidase-positive but catalase- and urease-negative. Glucose is not fermented. Hydrogen sulfide is not produced in triple sugar iron medium. Neither nitrate nor selenite is reduced and hippurate is not hydrolyzed. The cells can grow in the presence of 1.5 % bile, but not in the presence of safranin O. *H. canis* is sensitive to nalidixic acid but resistant to polymyxin B.

Type strain: ATCC 51401^T (= CCUG 32756^T = CIP 104753^T = NCTC 12739^T).

Genbank accession number (16S rRNA): AY631945.

G+C content of DNA: 48 mol%.

Helicobacter cetorum

Helicobacter cetorum (L. gen. pl. n. *cetorum*, of cetaceans (whales, dolphins)) was first isolated from the stomachs and feces of adult captive cetaceans (whales, dolphins) (Harper et al. 2000, 2002a, b; Validation List 2006b). And has since been detected in South American fur seals and dolphins (Goldman et al. 2009, 2011), Atlantic spotted dolphins (Suarez et al. 2010). Interestingly, a *Helicobacter* species that was shown to be closely related to *H. cetorum* was detected in analyses of lymph node metatranscriptomes of healthy mule deer (*Odocoileus hemionus*) (Wittekindt et al. 2010). In addition, *H. cetorum* has been reported to be common in the upper digestive tract of an asymptomatic Venezuelan adult population (García-Amado et al. 2007).

Morphologically the cells are fusiform in shape and are 0.6 μm wide and 4 μm in length. They do not have periplasmic fibers. The cells are motile by means of single bipolar flagella.

The bacteria grow under microaerobic conditions and form a thin spreading film on agar plates at 37 °C and 42 °C. They do not grow under aerobic or anaerobic conditions or at 25 °C. All isolates have urease, catalase, oxidase, and γ-glutamyl transpeptidase activities. They do not hydrolyze indoxyl acetate or have alkaline phosphatase activity. They do not reduce nitrate or grow on plates containing 1 % glycine. All five strains of *H. cetorum* are sensitive to cephalothin. Sensitivity to nalidixic acid varied among the strains (1/5 sensitive, 1/5 intermediate sensitivity and 3/5 resistant).

Type strain: MIT 99-5656^T (= ATCC BAA-540^T = CCUG 52418^T).

Genbank accession number (16S rRNA): AF292378.

G+C content of DNA: 30 mol%.

Helicobacter cholecystus

Helicobacter cholecystus (from. Gr. n. *cholê*, bile, and Gr. n. *kustis*, bladder; gallbladder; N.L. masc. adj. *cholecystus*, related to the gallbladder) was first isolated from the gallbladder of Syrian hamsters with cholangiofibrosis (Franklin et al. 1996; Validation List 1997). Morphologically they are curved or straight fusiform shaped rods, with the filamentous form being 0.5–0.6 μm wide by 3.0–5.0 μm in length. The cells are motile by means of a single sheathed polar flagellum. The bacteria grow on solid agar as pinpoint colonies under both microaerobic and anaerobic conditions, at both 37 °C and 42 °C. They do not grow aerobically or at 25 °C. Both strains of *H. cholecystus* had oxidase, catalase, alkaline phosphatase, arginine aminopeptidase, and L-arginine arylamidase activities but not urease, histidine aminopeptidase, leucine aminopeptidase or γ-glutamyl transferase activity. The bacteria can grow in the presence of 1 % glycine and 5 % bile salts, but not in the presence of 1.5 % NaCl. Nitrate is reduced. Indoxyl acetate and hippurate are not hydrolyzed. The one *H. cholecystus* strain tested was resistant to cephalothin and exhibited intermediate sensitivity to nalidixic acid.

Type strain: Hkb-1^T (=ATCC 700242^T = CIP 105596^T).

Genbank accession number (16S rRNA): AY686606.

Helicobacter cinaedi

Helicobacter cinaedi (L. gen. n. cinaedi, of a homosexual) was first isolated from a rectal swab collected from a homosexual man attending a Sexually Transmitted Disease Clinic in Seattle, WA, USA (Totten et al. 1985). It has also been occasionally isolated from the blood of homosexual men, as well as blood, cerebrospinal fluid and feces of adults and children, with and without, risk factors for HIV infection and the intestinal tract of healthy and diarrheic hamsters, cats, dogs and foxes (Vandamme et al. 1991, 2000; Kiehlbauch et al. 1995; Rossi et al. 2008). Asymptomatic captive rhesus monkeys (*Macaca mulatta*) are also commonly infected (Fernandez et al. 2002), in some cases infection being associated with colitis (Fox et al. 2001a). It was originally identified as a novel *Campylobacter* species and named *Campylobacter cinaedi* (Totten et al. 1985). When the *Campylobacter* genus underwent reclassification in 1991, *C. cinaedi* was moved into the *Helicobacter* genus (Vandamme et al. 1991). More recent polyphasic taxonomic studies have shown that "*Helicobacter westmeadii*" and *Helicobacter* sp. strain Mainz are in fact *H. cinaedi*, rather than novel species as originally proposed (Vandamme et al. 2000).

Morphologically the bacterial cells are S-shaped rods 0.3–0.5 μm wide by 1.5–5 μm in length, with a characteristic gull winged appearance. They display rapid motility by means of single sheathed bipolar flagellum. When grown on agar *H. cinaedi* may form pinpoint colonies, but more typically they form a translucent spreading film. All strains grow at 37 °C under microaerobic conditions but do not grow under aerobic or anaerobic conditions or at 42 °C. All strains have oxidase and catalase activity but have no urease activity. They can reduce nitrate, but do not hydrolyze indoxyl acetate or hippurate and have little or no alkaline phosphatase activity. H_2S is not produced in triple sugar iron agar. All strains can grow in the presence of 1 % glycine and 0.04 % TTC but do not grow in the presence of 2 % NaCl. *H. cinaedi* is resistant to nalidixic acid and has intermediate sensitivity to cephalothin.

Type strain: Fennell 165^T (= CCUG 18818^T = CIP 103752^T = DSM 5359^T = LMG 7543^T = NCTC 124230^T).

Genbank accession number (16S rRNA): M88150.

G+C content of DNA: 37–38 mol%.

Helicobacter cynogastricus

Helicobacter cynogastricus (Gr. n. kuon, dog; N. L. masc. adj. pertaining to the stomach; N.L.masc. adj.cyanogastricus pertaining to a dog's stomach) was first isolated from the gastric mucosa of a dog (Van den Bulck et al. 2006b). Morphologically the bacterial cells are tightly coiled spirals that are up to 1 μm wide × 10–18 μm in length. The cells possess one periplasmic fibril, which runs along the external side of the helix. The cells are motile by means of tufts of 6–12 sheathed flagella at one or both ends of the cell, and display a movement similar to that of *H. felis* and *H. bizzozeronii*. The bacteria grow as a spreading film on moist agar plates, or as an oily layer on biphasic culture media when incubated under microaerobic and anaerobic conditions at 30 °C and 37 °C. The bacteria do not grow at 25 °C or 42 °C. The cells have oxidase, catalase, urease, esterase, γ-glutamyl transpeptidase, L-arginine arylamidase and alkaline phosphatase activities. They do not have pyrrolidonyl arylamidase or L-aspartate arylamidase activities. The cells can reduce nitrate and TTC. They do not hydrolyze hippurate or indoxyl acetate. The bacteria do not grow on media containing 1 % ox bile, 1 % glycine or 1.5 % NaCl. *H. cynogastricus* JKM4 was sensitive to metronidazole, ampicillin, clarithromycin, enrofloxacin, lincomycin, tylosin, neomycin, streptomycin and gentamicin.

Type strain: JKM4^T = (LMG 23188^T).

Genbank accession number (16S rRNA): DQ004689.

Helicobacter equorum

Helicobacter equorum (L. gen. pl. n. *equorum*, of horses) was first isolated from the feces of two healthy horses from Ghent University, Belgium (Moyaert et al. 2007b). Recently an *H. equorum*-like organism was detected by PCR and in situ hybridization in a 35-year-old man with X-linked agammaglobulinemia who had refractory chronic pleurisy (Funato et al. 2011). Morphologically the bacteria are curved rods, 0.3 μm wide by 1.5–4 μm in length. The cells are motile by means of a single, sheathed, mono-polar flagellum. They do not possess periplasmic fibrils. The cells grow as a thin transparent spreading film on 10 % horse blood agar under microaerobic conditions at 37 °C, but not at 42 °C or 25 °C. The bacteria do not grow under aerobic or anaerobic conditions. The bacteria have oxidase, catalase, alkaline phosphatase, esterase and L-arginine arylamidase activities. They have no urease, DNase, γ-glutamyl transferase, hippuricase, pyrrolidonyl arylamidase or L-aspartate arylamidase activity. Indoxyl acetate is not hydrolyzed. They can reduce nitrate, however TTC is not reduced. No growth is obtained on media containing 1 % glycine, 1 % Ox bile, 1.5% NaCl. Neither H_2S nor acid is produced from glucose fermentation. *H. equorum* is resistant to cephalothin and nalidixic acid.

Type strain: EqF1^T (=LMG 23362^T = CCUG 52199^T).

Genbank accession number (16S rRNA): DQ307735.

G+C content of DNA: 38.5 mol%.

Helicobacter felis

Helicobacter felis (L. gen. n. *felis*, of a cat) was first isolated from the gastric mucosa of a cat (Lee et al. 1988) and later further isolates were cultured from dogs (Paster et al. 1991) and rabbits (Van den Bulck et al. 2005a). In addition there have been a number of reports of morphologically similar bacteria in humans (Germani et al. 1997; Van den Bulck et al. 2005b; De Bock et al. 2007; Yakoob et al. 2012). Morphologically the bacteria are rigid, spiral-shaped cells, 0.4 μm wide and 5–7.5 μm in length with five to seven spirals per cell (*Fig. 24.3*). The cells are surrounded by periplasmic fibers, which appear as concentric helical ridges, either in pairs, threes, or singly on the surfaces of the cells. The bacteria are motile by means of tufts of 10–17 bipolar, sheathed flagella that are positioned slightly off center at the end of the cell. The cells move with a rapid corkscrew-like motion. The bacteria are fastidious and grow as a spreading film only on media enriched with blood or serum. Growth occurs under both microaerobic and anaerobic conditions at 37 °C and 42 °C (1/7 strains tested did not grow at 42 °C). They do not grow at 25 °C. All strains have urease, oxidase, catalase, alkaline phosphatase, arginine aminopeptidase, and γ-glutamyl transpeptidase activities and the majority (6/7 strains) histidine aminopeptidase and leucine aminopeptidase activity. Nitrate is reduced. Hippurate is not hydrolyzed. Indole and H_2S are not produced. No growth occurs in the presence of 1 % glycine and 1.5 % NaCl. *H. felis* is susceptible to cephalothin, ampicillin, erythromycin, metronidazole and resistant to nalidixic acid.

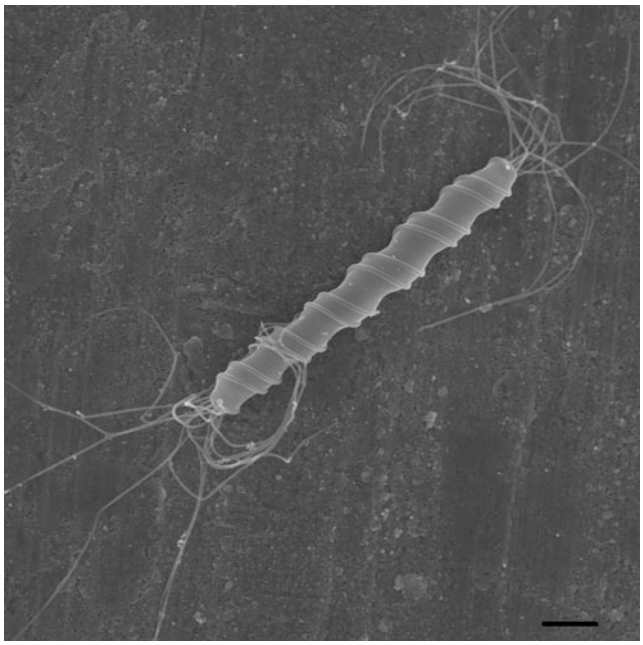

Fig. 24.3
Transmission electron micrograph of a freeze-dried preparation of *Helicobacter felis* showing its helical/spiral morphology, bipolar tufts of sheathed flagella and periplasmic fibers arranged in pairs (bar = 0.4 μm)

Type strain: CS1^T (= ATCC 49179^T = CIP 104382^T = NCTC 12436^T).

Genbank accession number (16S rRNA): M57398.

G+C content of DNA: 42.5 mol%.

Helicobacter fennelliae

Helicobacter fennelliae (N.L. gen. fem. n. *fennelliae*, of Fennell, named after C.L. Fennell, the person who first isolated this organism from rectal swabs of homosexual males) was first isolated from rectal swabs of homosexual men (Totten et al. 1985) and has since been associated with bacteremia in immunocompromised and immunocompetent patients and pediatric diarrheic stools (Orlicek et al. 1994; Hsueh et al. 1999; Lastovica and Smuts 2007). Along with *H. cinaedi* it was transferred to the *Helicobacter* genus in 1991 and renamed *Helicobacter fennelliae* (Vandamme et al. 1991). Morphologically the bacteria are S-shaped rods 0.3–0.5 μm wide and 1.5–5 μm in length. The cells are rapidly motile by means of single sheathed bipolar flagella. The bacteria grow as pinpoint, translucent colonies, which may spread on moist blood agar when grown under microaerobic conditions at 37 °C. They have a distinctive hypochlorite odor. They do not grow under aerobic or anaerobic conditions or at 25 °C or 42 °C. Hydrogen is required for growth. All strains have oxidase and catalase activity but not urease activity. They do not reduce nitrate. They hydrolyze

indoxyl acetate but not hippurate and there is limited or no alkaline phosphatase activity. H_2S is not produced in triple sugar iron agar. The bacteria can grow in the presence of 1 % glycine, 0.04 % triphenyltetrazolium chloride but not 2 % NaCl. *H. fennelliae* is sensitive to cephalothin, nalidixic acid, metronidazole, erythromycin and ampicillin.

Type strain: Fennell 231^T (= ATCC 35684^T = CCUG 18820^T = CIP 103758^T = DSM 7491^T = NCTC 11612^T).

Genbank accession number (16S rRNA): M88154.

G+C content of DNA: 37–38 mol%.

Helicobacter ganmani

Helicobacter ganmani (N.L. gen. n. *ganmani*, of ganman which, in the language of the Gadigal people (indigenous Australians who live in the Sydney Harbour area), means "snake finder.") Thus ganmani is intended to refer to both the spiral, snake-like morphology of the organism and to the area in Australia from which it was first described. It was first isolated during routine screening of intestinal scrapings from conventional and specific pathogen-free mice (Robertson et al. 2001). It has also been detected in a number of wild rodent species (gerbils, jerboas and bandicoot rats) in China (Goto et al. 2004). In addition, *H. ganmani* was detected in pediatric liver biopsies by PCR and was confirmed by denaturing gradient gel electrophoresis (DGGE) and DNA sequence analysis (Tolia et al. 2004). Morphologically the cells are curved to spiral rods, 0.3 µm wide and 2.5 µm in length with two turns per cell (Fig. 24.4). They are motile by means of single, unsheathed bipolar flagella. Single colonies are rarely seen and are less than 1 mm in diameter, irregular, non-pigmented and translucent on 5 % horse blood agar incubated under anaerobic conditions only at 37 °C, a feature which makes then unique in the *Helicobacter* genus. No growth occurs in microaerobic or aerobic conditions, or at 42 °C. All strains have oxidase activity but have no urease, alkaline phosphatase or DNase activity. Weak catalase activity was detected in 2 of 7 strains. All strains reduce nitrate and TTC but not selenite. Indoxyl acetate is not hydrolyzed. *H. ganmani* is resistant to nalidixic acid and cephalothin.

Type strain: CMRI H02^T (= CCUG 43526^T = CIP 106846^T).

Genbank accession number (16S rRNA): AF000221.

Helicobacter heilmannii

Helicobacter heilmannii (N.L. gen. masc. n. *heilmannii*, of Heilmann, in honour of Konrad Heilmann who described the first large case study of gastrospirilla infections in humans) (Heilmann and Borchard 1991), previously known as "*Gastrospirillum hominis*" (McNulty et al. 1989) and "*Candidatus* Helicobacter heilmannii" (Solnick et al. 1993; O'Rourke et al. 2004), was first isolated from the feline gastric mucosa (Smet et al. 2012a). *H. heilmannii*-like organisms have been seen in a number of primate species, 'big cats' (O'Rourke et al. 2004), lynx and foxes (Morner et al. 2008), wild boar

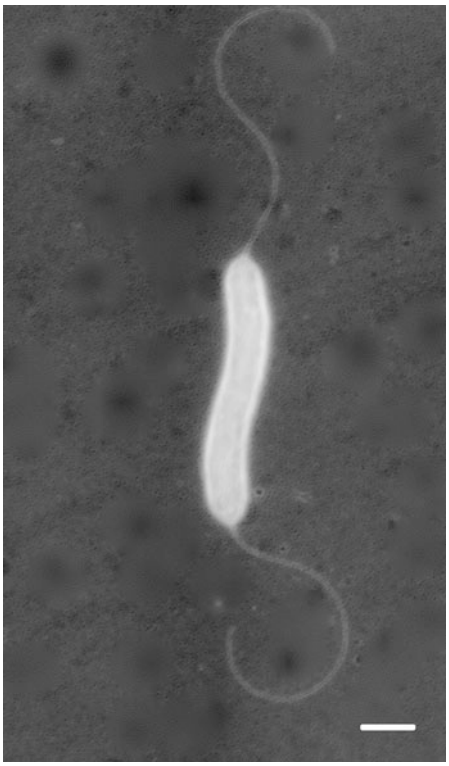

Fig. 24.4
Transmission electron micrograph of a negatively stained preparation of *Helicobacter ganmani* showing its S-shaped morphology and single unsheathed bipolar flagella (bar = 0.5 µm)

(Fabisiak et al. 2010) etc. The cells are tightly coiled spirals with up to nine turns. They are approximately 3–6.5 µm long and approximately 0.6–0.7 µm wide (Fig. 24.5). The cells are motile by means of tufts of up to 10 sheathed, blunt ended bipolar flagella. Optimal growth and primary isolation are best obtained by using biphasic culture plates under microaerobic conditions at 37 °C. The cells grow as a spreading film and can also grow as distinct colonies on dry agar. No growth at 42 °C or 25 °C. The cells grow weakly under anaerobic conditions. All strains are oxidase, catalase and urease positive, have γ-glutamyl transferase and L-arginine arylamidase activity but no alkaline phosphatase activity. All strains reduce TTC and nitrate. They hydrolyze hippurate but not indoxyl acetate. No growth occurs on media supplemented with 1 % bile, 1.5 % NaCl or 1 % glycine.

Due to the high level of phenotypic and genotypic similarity of a number of gastric helicobacters including *H. suis, H. felis, H. bizzozeronii, H. salomonis, H. cynogastricus, H. baculiformis* and *H. heilmannii* and the fact that these organisms are very difficult to cultivate, Haesebrouck et al. have recently proposed that to simplify matters in clinical situations, these organisms should be referred to as *H. heilmannii sensu lato* (*H. heilmannii* s. l.) (Haesebrouck et al. 2011). This term would also include organisms previously described, based on their morphologies, as "*Gastrospirillum hominis*" (McNulty et al. 1989) and

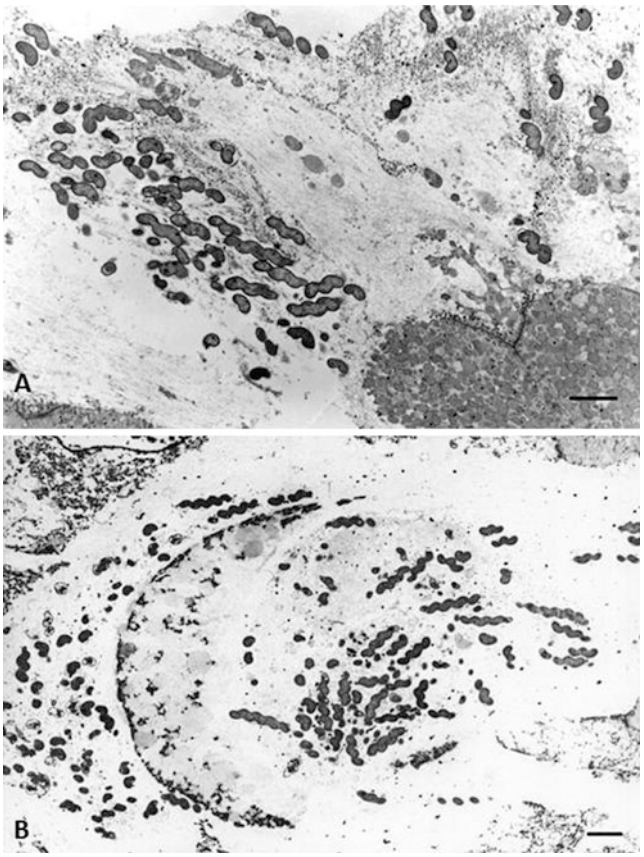

Fig. 24.5
Transmission electron micrograph of a thin section of gastric tissue obtained from (a) a cat and (b) a mandrill monkey showing *H. heilmannii* s.l.-like bacteria with their characteristic helical/spiral morphology (bar = 0.5 μm)

"*Gastrospirillum suis*" (Queiroz et al. 1990). If the organisms are successfully identified to the species level then the name *H. heilmannii sensu stricto* (*H. heilmannii* s.s.) or other species name should be used.

Type strain: ASB1^T (=DSM 24751^T = LMG 26292^T).

Genbank accession number (16S rRNA): HM625820.

Helicobacter hepaticus

Helicobacter hepaticus (L. masc. adj. *hepaticus*, belonging to the liver) was first isolated from livers and intestinal mucosal scrapings of mice (Fox et al. 1994; Validation List 1994b), and has been detected in humans by PCR (Hamada et al. 2009). Morphologically the cells are slender curved-to-spiral rods, 0.2–0.3 μm wide and 1.5–5.0 μm long, with one to three spiral turns. The cells are motile by means of sheathed, single, bipolar flagella. The cells grow as distinct pinpoint colonies, however cultures often appear as a thin spreading layer. Growth occurs under both microaerobic and anaerobic conditions at 37 °C, however there is no growth under aerobic conditions or at 25 °C or 42 °C. *H. hepaticus* has urease, catalase, and oxidase activity. The cells grow anaerobically in the presence of 1.5 % NaCl, 1 % glycine, 0.1 % trimethylamine N-oxide and 0.4 % TTC. Nitrate is reduced and H_2S can be detected on lead acetate discs. The bacteria hydrolyze indoxyl acetate but not hippurate. *H. hepaticus* is resistant to cephalothin and nalidixic acid but sensitive to metronidazole.

Helicobacter ulmiensis was originally designated in Genbank (AJ007931) as a new species but subsequent investigations have confirmed the strain to be an incorrectly classified *H. hepaticus* isolate (On 2009).

Type strain: Hh-2^T (=ATCC 51448^T = CCUG 33637^T = CIP 104100^T = LMG 16316^T).

Genbank accession number (16S rRNA): U07574.

Helicobacter marmotae

Helicobacter marmotae (N.L. n. *Marmota*, scientific name of a genus of rodents; N.L. gen. n. *marmotae*, of *Marmota*, isolated from *Marmota monax*, relating to a short-tailed burrowing rodent species found in North America) was isolated from the livers of woodchucks and from the feces of clinically normal cats (Fox et al. 2002; Validation List 2006b). They have recently been detected in the intestines and livers of prairie dogs (Beisele et al. 2011). The bacteria are slender, curved to spiral rods (0.2 μm by 1.5–5 μm), which have one to three spirals. The cells are motile by means of sheathed, single unipolar or bipolar flagella. Growth occurs on solid agar media under microaerobic conditions as a spreading layer at both 37 °C and 42 °C. They do not grow under aerobic or anaerobic conditions. The cells have oxidase, catalase, urease and alkaline phosphatase activity, but not γ-glutamyl transpeptidase activity. The bacteria do not hydrolyze indoxyl acetate, or reduce nitrate. *H. marmotae* is resistant to nalidixic acid and cephalothin.

Type strain: MIT 98-6070^T (= ATCC BAA-546^T = CCUG 52419^T).

Genbank accession number (16S rRNA): AF333341.

Helicobacter mastomyrinus

Helicobacter mastomyrinus (N.L. masc. adj. *mastomyrinus*, pertaining to *Mastomys*, a rodent genus (the type strain was isolated from *Mastomys natalensis*)) was isolated from the liver and feces of mastomys and the feces a colony of laboratory mice (Shen et al. 2005; Validation List 2006b) Morphologically the bacterial cells are curved or spiral shaped rods measuring 0.28 μm wide and 5.25 μm long and lack periplasmic fibrils. The cells are motile by means of bipolar, sheathed flagella. Cultures grow under microaerobic conditions at both 37 °C and 42 °C on solid agar and appear as a spreading film after 4–7 days of incubation. They do not grow aerobically or anaerobically, or at 25 °C. The bacteria are oxidase, catalase and urease positive, but γ-glutamyl transferase and alkaline phosphatase negative. They do not hydrolyze indoxyl acetate or reduce nitrate. *H. mastomyrinus* is resistant to both nalidixic acid and cephalothin.

Type strain: MIT 97-5574^T (= ATCC BAA-1046^T = CCUG 52417^T).

Genbank accession number (16S rRNA): AY742307.

Helicobacter mesocricetorum

Helicobacter mesocricetorum (N.L. n. *Mesocricetus*, generic name of the Syrian hamster (*Mesocricetus auratus*); N.L. gen. pl. n. *mesocricetorum*, of *mesocriceti*, of Syrian hamsters) was first isolated from the feces of asymptomatic Syrian hamsters (Simmons et al. 2000; Validation List 2000). Morphologically the cells are spirally curved rod-shaped bacteria 0.4–0.6 µm wide by 2–3 µm in length. The cells are motile by means of singular non-sheathed bipolar flagella. The bacteria grow as pinpoint colonies or as a thin spreading film on solid agar under microaerobic conditions at both 37 °C and 42 °C. No growth occurs at 25 °C or under aerobic or anaerobic environments. The bacteria have both catalase and alkaline phosphatase activity but lack urease and γ-glutamyl transferase activity. No growth occurs in the presence of 1 % glycine or 1.5 % NaCl. They do not produce H_2S or hydrolyze hippurate, but can reduce nitrate. *H. mesocricetorum* is resistant to cephalothin but sensitive to nalidixic acid.

Type strain: MU 97-1514^T (= ATCC 700932^T = CCUG 45420^T).

Genbank accession number (16S rRNA): AF072471.

Helicobacter muridarum

Helicobacter muridarum (N.L. pl. n. *Muridae*, family name for Old World rats and mice; N.L. gen. pl. n. *muridarum*, of the *Muridae)* was first isolated from the intestinal mucosa of rats and mice (Lee et al. 1992) and have subsequently been found in older animals to colonize the stomach (Lee et al. 1993). Morphologically they are helical in shape, 0.5–0.6 µm wide and 3.5–5.0 µm long and have two to three spiral turns (*Fig. 24.6*). The cells are motile by means of bipolar tufts of 10–14 sheathed flagella and have a rapid corkscrew-like motion. Each cell is surrounded by 9–11 periplasmic fibers, which appear as concentric helical ridges on the surface of the cell. *H. muridarum* is nutritionally fastidious, growing only on media enriched with blood or serum, optimal growth occurring on a moist agar surface. The bacteria grow as a fine, translucent, spreading film following 2–3 days incubation under microaerobic conditions at 37 °C. Good growth occurs in atmospheres containing 1–16 % O_2 and 5–10 % CO_2. No growth occurs under aerobic or anaerobic conditions or at 25 °C or 42 °C. All strains are urease, oxidase, catalase, alkaline phosphatase and arginine aminopeptidase positive. Hippurate is not hydrolyzed and nitrate is not reduced. Indole and H_2S are not produced. No growth occurs in the presence of 2 % bile salts, 1 % glycine, or 1.5 % NaCl. *H. muridarum* is resistant to nalidixic acid and cephalothin.

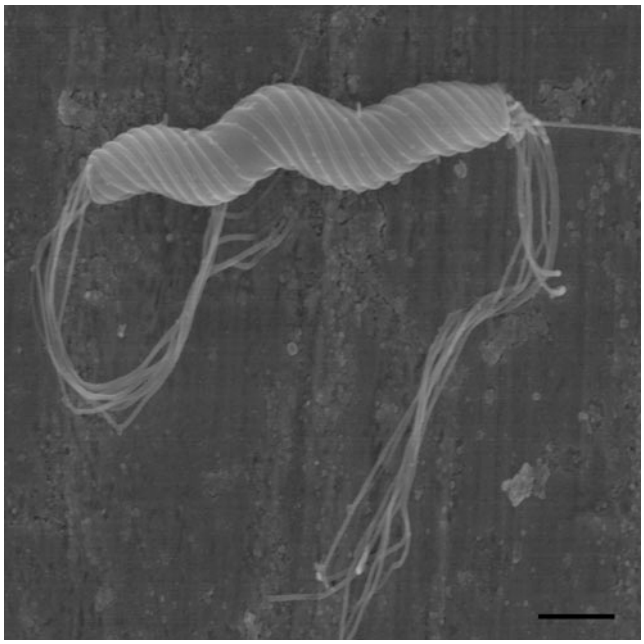

Fig. 24.6
Transmission electron micrograph of a freeze-dried preparation of *Helicobacter muridarum* showing its spiral morphology, bipolar tufts of sheathed flagella and periplasmic fibers entwining the cell (bar = 0.4 µm)

Type strain: STI^T (= ATCC 49282^T = CCUG 29262^T = CIP 104248^T = LMG 13646^T = NCTC 12714^T).

Genbank accession number (16S rRNA): M80205.

G+C content of DNA: 34.6 ± 0.6 mol%.

Helicobacter mustelae

Helicobacter mustelae (L. n. *mustela*, a weasel, and also a generic name (*Mustela*); L. gen. n. *mustelae*, intended to mean of a ferret (*Mustela putorius furo*) was first isolated from normal and inflamed gastric mucosa of ferrets and named *Campylobacter* subsp. *mustelae* (Fox et al. 1988). It was later reclassified as member of the *Helicobacter* genus (Goodwin et al. 1989a). It has also been isolated from the gastric mucosa of mink (Taylor et al. 1994). Morphologically the bacteria are straight or curved rods 0.5–0.65 µm wide and 2–5 µm in length (*Fig. 24.7*). The cells are motile by means of multiple, peritrichous, sheathed flagella. The bacteria form non-pigmented, translucent colonies (1 mm in diameter) on blood agar under microaerobic conditions at 37 °C. They grow at 42 °C but not 25 °C and in anaerobic conditions in the presence of 10 % CO_2. The cells have catalase, oxidase and urease activity but do not have leucine arylamidase activity. They reduce nitrate and hydrolyze indoxyl acetate but not hippurate. Growth in the presence of 1 % glycine is variable (10 % positive). All strains fail to grow in the presence of 1.5 %, 2 % and 3 % NaCl but can grow in the presence of 0.04 % TTC. All *H. mustelae* strains were shown to be sensitive to nalidixic

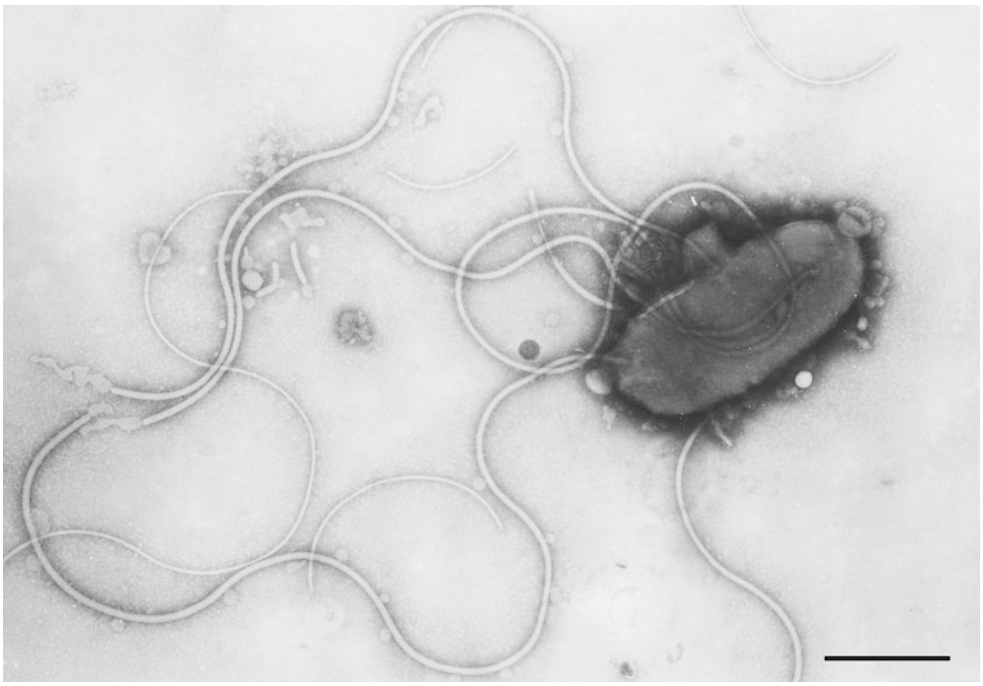

Fig. 24.7
Transmission electron micrograph of a negatively stained preparation of *Helicobacter mustelae* showing its rod-shaped morphology and polar and lateral sheathed flagella (bar = 0.5 μm)

acid but resistant to cephalothin. The majority of strains (91 %) were sensitive to metronidazole.

Type strain: R85-13-6^T (= ATCC 43772^T = CCUG 25715^T = CIP 103759^T = NCTC 12198^T).

Genbank accession number (16S rRNA): M35048.

G+C content of DNA: 36–41 mol%.

Helicobacter nemestrinae

Helicobacter nemestrinae (N.L. gen. n. nemestrinae, of *nemestrina*, of the macaque species *Macaca nemestrina*) was isolated from the gastric mucosa of a pigtailed macaque (Bronsdon et al. 1991). Morphologically the cells are helically curved, rod-shaped bacteria 0.2–0.3 μm wide and 2.0–5.0 μm long. The cells are motile by means of multiple sheathed unipolar flagella. Growth of small, colorless, translucent colonies 0.5–1.0 mm in diameter with irregular edges occurs on moist enriched blood or chocolate agar after 3–5 days, following incubation under microaerobic conditions at both 35 °C and 42 °C, but not at 25 °C. The cells have oxidase, catalase, and urease activity but no DNase activity. They do not reduce nitrate or hydrolyze hippurate or esculin. H_2S is not produced on TSI agar. The bacteria do not grow in the presence of 1 % glycine, 1.5 % NaCl, or 1 % bile. *H. nemestrinae* is sensitive to penicillin, ampicillin, erythromycin, gentamicin, cephalothin, furoxone, rifampin, metronidazole, and chloromycetin and resistant to nalidixic acid, trimethoprim, sulfamethoxazole and vancomycin.

Type strain: T81213-NTB^T (=ATCC 49396^T = CIP 104754^T = DSM 7492^T = LMG14378^T = NCTC 12491^T).

Genbank accession number (16S rRNA): X67854.

G+C content of DNA: 39 mol%.

Comment: *H. nemestrinae* differs from *H. pylori* by virtue of growth at 42 °C and its cellular fatty acid profile (Bronsdon et al. 1991). DNA-DNA hybridization and 16S rRNA studies suggested that this was a novel species, thus it was designated *H. nemestrinae*. As the G+C content was markedly lower than all other known *Helicobacter* species (24 mol%) a re-appraisal was undertaken. Sequence analysis of seven housekeeping genes and two flagellin genes from the single strain of *H. nemestrinae* (ATCC 49396^T) showed that all sequences clustered with those from multiple isolates of *H. pylori* (Suerbaum et al. 2002). Repeat sequence analysis of the 16S rRNA gene of *H. nemestrinae* showed that it differed by 38 bp from that previously reported and was less than 1 % different from multiple *H. pylori* strains. In addition, repeat analyses of the G+C content of *H. nemestrinae* type strain yielded a reproducible value of 39 mol% (P.V., unpublished observations). These results show that the single strain, previously classified as *H. nemestrinae*, is a junior heterotypic synonym for *H. pylori*. As such the subcommittee on the taxonomy of *Campylobacter* and related bacteria have commented that it may be necessary to re-examine the taxonomic status of *H. nemestrinae* (On 2000).

Helicobacter pametensis

Helicobacter pametensis (N.L. masc. adj. *pametensis*, pertaining to the Pamet River, Truro, Massachusetts) was initially isolated

from tern, gull and swine feces, with the species name derived from the origin of the isolates, the Pamet River in Truro, Massachusetts (Dewhirst et al. 1994). A similar organism was later isolated from a pet cat and identified as *H. pametensis* using dot blot DNA hybridization (Neiger et al. 1998). Morphologically the bacteria are curved rods 0.4 μm wide and 1.5 μm long, with rounded ends. The cells are motile by means of a single sheathed flagellum at each end, which is inserted sub-terminally. Occasionally cells may have a third flagellum adjacent to one of the others. The bacteria grow on blood agar as translucent, colorless, pinpoint, colonies following incubation under microaerobic conditions at 37 °C and 42 °C. Growth is weak under anaerobic conditions, and there is no growth in air at 37 °C. All strains have oxidase, catalase, alkaline phosphatase, and arginine β-naphthylamide aminopeptidase activities but do not have urease, glycine β-naphthylamide aminopeptidase and γ-glutamyl transpeptidase activities. Proline, pyroglutamate, tyrosine, alanine, phenylalanine activities are not detected. Hippurate and indoxyl acetate are not hydrolyzed. H_2S is not produced on a triple sugar iron slant. Lysine and ornithine decarboxylase activity are not detected. Nitrate is reduced. Growth occurs in the presence of 1 % glycine but not in the presence of 3.5 % NaC1. *H. pametensis* is sensitive to nalidixic acid and cephalothin.

Type strain: B9^T (=ATCC 51478^T = CCUG 29255^T = CIP 10429^T = LMG 12678^T).

Genbank accession number (16S rRNA): M88147.

G+C content of DNA: 38 mol%.

Helicobacter pullorum

Helicobacter pullorum (L. n. *pullus*, a young fowl, a chicken; L. gen. pl. n. *pullorum*, of chickens) was isolated from the cecum of asymptomatic broiler chickens, the liver and intestinal content of hens exhibiting lesions suggestive of vibrionic hepatitis, and the feces of patients with gastroenteritis (Stanley et al. 1994; Validation List 1995). In addition, Laharie et al. have reported the presence of *H. pullorum* and/or *H. canadensis* DNA in intestinal biopsy specimens from patients with Crohn's disease and in matched controls (Laharie et al. 2009). Morphologically the cells are gently curved, slender, rod-shaped cells, 3–4 μm in length. The cells are motile by means of an unsheathed monopolar flagellum. They have a typical darting motility. The bacteria grow under microaerobic conditions forming pinpoint non-pigmented, translucent α-hemolytic colonies on 5 % horse blood agar at 37 °C and 42 °C. No growth occurs under aerobic or anaerobic conditions. All strains have oxidase activity and the majority of strains (14/16) have catalase activity. They do not have urease or alkaline phosphatase activity or hydrolyze indoxyl acetate. The majority of strains (13/16) can grow in the presence of 1 % bile. None of the strains grow in the presence of safranin O. The majority of *H. pullorum* strains (15/16) are sensitive to nalidixic acid and polymyxin B and all strains are resistant to cephalothin and cefoperazone.

Type strain: ATCC 51801^T (= CIP 104787^T = DSM 7492^T = NCTC 12824^T = CCUG 33873).

Genbank accession number (16S rRNA): AY631956.

G+C content of DNA: 33–35 mol%.

Helicobacter rodentium

Helicobacter rodentium (N.L. plur. gen. n. *rodentium* (from L. part. adj. *rodens -entis*, gnawing), of rodents, of gnawing animals) was initially isolated from the intestinal mucosa and feces of mice (Shen et al. 1997). The cells are slender curved to spiral rods 0.3 μm in diameter by 1.5–5 μm in length, which have one to three spiral turns. They are motile by means of non-sheathed, single, bipolar flagella. The bacteria grow under microaerobic conditions or anaerobic conditions at 37 °C as individual colonies 1–2 mm in diameter, however often cultures appear as thin spreading layers. The bacteria do not grow under aerobic conditions. The majority of strains (including the type strain) grow at 42 °C but not 25 °C. The bacteria have oxidase activity, weak catalase activity but no urease activity. They reduce nitrate but do not hydrolyze indoxyl acetate or hippurate. Growth occurs in the presence of 1.5 % NaCl, 1 % glycine, and 0.04 % TTC. *H. rodentium* is resistant to cephalothin and nalidixic acid.

Phenotypic, protein electrophoretic and phylogenetic analyses have shown that *H. rodentium* and *H. ganmani* are closely related, but distinct species. These species, along with *H. canadensis, H. mesocricetorum* and *H. pullorum* are atypical from all other *Helicobacter* species in lacking flagellar sheathing. The significance of this is unknown.

Type strain: MIT 95-1707^T (= ATCC 700285^T).

Genbank accession number (16S rRNA): U96296.

Helicobacter salomonis

Helicobacter salomonis (N.L. gen. n. *salomonis*, of Salomon, named in honor of Hugo Salomon, a German scientist who was one of the first workers to describe three morphologically distinct spiral organisms in canine gastric mucosa) was isolated from a gastric biopsy of a healthy pet dog (Jalava et al. 1997). More recently, similar organisms have been detected by PCR in red foxes (*Vulpes vulpes*) (Morner et al. 2008), rabbits (Van den Bulck et al. 2005a) and humans (Trebesius et al. 2001; Van den Bulck et al. 2005b). Morphologically, the bacterial cells are loose spirals that are 0.8–1.2 μm wide by 5–7 μm long. They do not have periplasmic fibrils. They are motile by means of tufts of 10–23 sheathed flagella that occur at one or both ends of the cell. The bacteria grow under microaerobic conditions on fresh moist agar plates as a spreading film at 37–40 °C, but not at 25 °C or 42 °C. All strains have oxidase, catalase, urease, γ-glutamyl transpeptidase, and alkaline phosphatase activity and hydrolyze indoxyl acetate. Four of the five strains have DNase activity. They reduce nitrate and TTC. They are negative for hippurate hydrolysis, pyrrolidonyl arylamidase, L-arginine arylamidase, and L-aspartate arylamidase activity. They do not grow on media

containing 1 % ox bile, 1 % glycine, or 1.5 % NaCl. They are resistant to nalidixic acid and susceptible to cephalothin and cefoperazone. One of five strains tested was resistant to metronidazole. Three *H. salomonis* strains tested were found to be highly susceptible to ampicillin, clarithromycin, tetracycline, tylosin, enrofloxacin, gentamicin and neomycin (Van den Bulck et al. 2005b).

Type strain: Inkinen^T (=CCUG 37845^T = CIP 105607^T).

Genbank accession number (16S rRNA): U89351.

Helicobacter suis

Helicobacter suis (L. n. *sus*, pig; L. gen. n. *suis*, of a pig), first observed in stained histological sections of antral gastric mucosa of pigs and by Queiroz and colleagues in 1990, was initially given the name "*Gastrospirillum suis*" (Queiroz et al. 1990). Almost one decade following the discovery of the bacterium it was named "*Candidatus* Helicobacter suis" (De Groote et al. 1999a). The recent isolation of the microorganism in vitro allowed the characterization of *H. suis* as a species (Baele et al. 2008a). This organism has also been detected in humans (De Groote et al. 2005; Van den Bulck et al. 2005b). Morphologically the bacteria are helical shaped, with 3–8 (mean 5) complete spiral turns, 1.5–5.2 (mean 4.0) μm long and 0.4–0.7 (mean 0.6) μm wide with truncated and flattened ends (Fig. 24.8). They are motile by means of tuffs of four to 10 bipolar, sheathed flagella. Growth occurs under microaerobic conditions at 37 °C, but not at 25 °C or 42 °C, with weak growth under anaerobic conditions. The organisms grow as a thin spreading film with optimal growth occurring with the use of biphasic growth plates. All strains produce urease, catalase and oxidase. They reduce TTC and have activities for γ-glutamyl transferase, L-arginine arylamidase and alkaline phosphatase. Activity of pyrrolydonyl arylamidase and L-aspartate arylamidase is not detected. No growth is observed in presence of 1.5 % NaCl, 1 % glycine, 1 % ox bile or 5 μg/mL metronidazole. Nitrate is not reduced and there is no hydrolysis of hippurate or indoxyl acetate.

Type strain: HS1^T (= LMG 23995^T = DSM 19735^T).

Genbank accession number (16 s rRNA): EF204589.

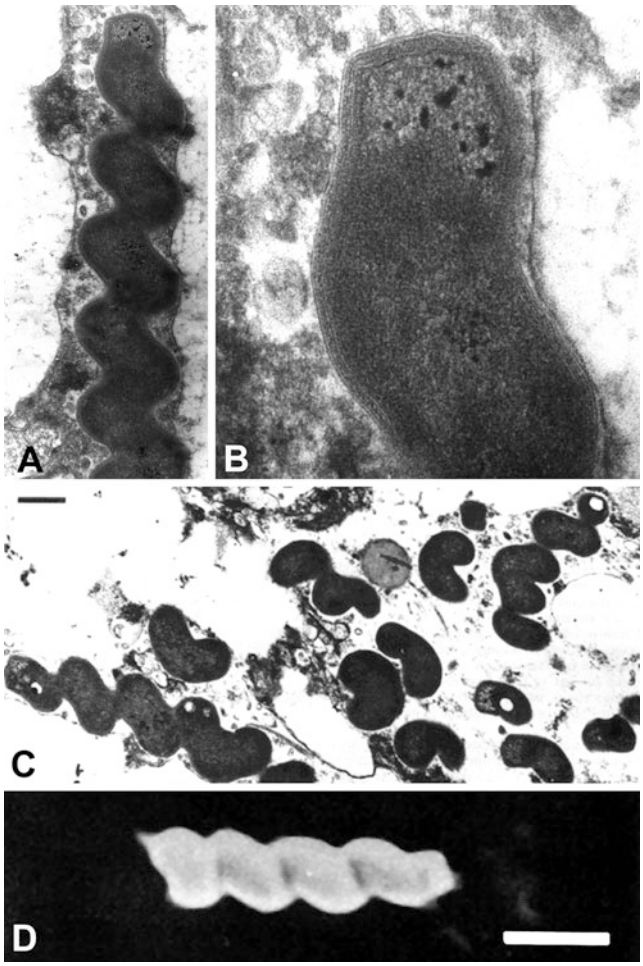

Fig. 24.8
(a) A thin section of *Helicobacter suis*. The plasma membrane is present (bar = 0.1 μm). (b) Transmission electron micrograph of an extremity of *Helicobacter suis* showing: non-membrane bound granules polar membrane continuous over the whole pole of the cell, flagella, plasma membrane and cell wall (bar = 0.1 μm). (c) Transmission electron micrograph showing *Helicobacter suis* in the antral region of pig stomach (bar, 0.5 μm). (d) Scanning electron micrograph of *Helicobacter suis* (bar = 0.5 μm). Reproduced with permission of J. Med. Microbiol. 1990, 33:61–66

Helicobacter trogontum

Helicobacter trogontum (Gr. part. adj. *trogon trogontos* (from Gr. v. *trôgô*, to gnaw), gnawing; N.L. gen. pl. n. *trogontum*, of gnawing animals) was isolated from the colonic mucosa of rats (Mendes et al. 1996). Subsequent polyphasic characterisation of a number of morphologically similar organisms, provisionally named "*Flexispira*" *taxa* 1, 4 and 5, have shown them to belong to the species *H. trogontum* thus expanding their host range to include sheep and pigs (Dewhirst et al. 2000a; Hanninen et al. 2003). Morphologically the cells are fusiform to slightly spiral rods with pointed ends, 0.6–0.7 μm × 4–6 μm in size. They are motile by means of tufts of 3–7 bipolar, sheathed flagella. Ultrastructurally the cells are characterized by the presence of periplasmic fibers coiled around the protoplasmic cylinder, giving a crisscross appearance to the bacterial surface. Bacteria initially grow as punctiform colonies and then as a fine spreading translucent film on moist blood agar plates after 3–5 days at 37 °C or 42 °C under microaerobic conditions. No growth occurs at 25 °C or under aerobic or anaerobic conditions. All strains have urease, catalase and oxidase activity. They do not reduce nitrate nor hydrolyze hippurate. Ornithine decarboxylase and γ-glutamyl transpeptidase activities are present, with variable activity for alkaline phosphatase. Growth occurs in the presence of 1 % glycine. *H. trogontum* is resistant to nalidixic acid and cephalothin but sensitive to 1.5 % NaCl and metronidazole.

Type strain: LRB 8581^T (= ATCC 700114^T = CCUG 49050^T).

Genbank accession number (16S rRNA): U65103.

Helicobacter typhlonius

Helicobacter typhlonius (Gr. n. *tuphlôn*, cecum; L. masc. suff. *-ius*, suffix used with the sense of belonging to; N.L. masc. adj. *typhlonius*, belonging to the cecum, site of original isolation) was first isolated from enteric lesions of severe combined immune deficient (SCID) mice and originally named *Helicobacter typhlonicus*, (Franklin et al. 1999). However, the name was later changed to *Helicobacter typhlonius* (Franklin et al. 2001; Validation List 2002a). *H. typhlonius* DNA has been reported to be common in fecal samples of research mice and has also been detected in the sex organs of three mouse strains (Franklin et al. 2001; Scavizzi and Raspa 2006).

Morphologically the cells are filamentous curved to spiral rods, 0.3 μm in diameter and 2–3 μm in length, with no periplasmic fibers. They are motile by means of single, bipolar sheathed flagella. The bacteria grow on agar as pinpoint colonies under microaerobic conditions at 37 °C or 42 °C. They do not grow under anaerobic or aerobic conditions or at 25 °C. They are oxidase and catalase positive but urease negative, with no alkaline phosphatase or γ-glutamyl transferase activity. They can grow in the presence of 1 % glycine, however the majority of cells harvested have a coccoid morphology. They do not grow in the presence of 1.5 % NaCl. Nitrate is reduced. Indoxyl-acetate and hippurate are not hydrolyzed. *H. typhlonius* is resistant to cephalothin and sensitive to nalidixic acid.

Type strain: MIT 97-6810^T (=ATCC BAA-367^T = CCUG 48335^T = CIP 107792 T = LMG 12678^T).

Genbank accession number (16S rRNA): AF127912.

Candidatus and Unvalidated Species

"*Candidatus* Helicobacter bovis"

In 1999, De Groote et al. proposed the name "*Candidatus* Helicobacter bovis" for "*Helicobacter*-like organisms" detected in the gastric crypts of the abomasal of healthy Belgian and Dutch cattle based on cross reactivity with a polyclonal antibody to *H. pylori* (De Groote et al. 1999b). Ultrastructural studies revealed the presence of large groups of multiple spiral bacteria with 1–3 turns. The cells are 0.3 μm in diameter and 1.5–2.5 μm in length and have at least four flagella at one pole. The presence of a flagellar sheath was not determined. Pyloric biopsy samples were shown to be urease positive. Cloning and sequencing of the 16S rRNA gene from DNA obtained from seven individual cattle showed them to have more than 99 % similarity. Based on a 16S rRNA similarity matrix the closest taxonomic relative was shown to be *H. bilis* (92.8 %). A "*Candidatus* Helicobacter bovis"-specific probe confirmed the presence of these bacteria in the cattle abomasum by in situ hybridization.

A high prevalence of "*Candidatus Helicobacter bovis*" has also been reported in cattle from Venezuela (85 %) and Europe (80 %) however they were not detected in the abomasums of goats (Braun et al. 1997; De Groote et al. 1999b; Gueneau et al. 2002; Gustafsson et al. 2006). The involvement of "*Candidatus* Helicobacter bovis" in gastric disease in cattle remains unknown (Haesebrouck et al. 2009).

Genbank accession number (16S rRNA): AF127027.

"*Helicobacter callitrichis*"

A novel *Helicobacter* species, "*Helicobacter callitrichis*" has been isolated from the stools of five of 60 marmosets, none of which, including the five in which *Helicobacter* species were isolated, showed clinical symptoms (Won et al. 2007). The five strains were reported to vary in shape, from curves to loosely coiled spirals, and in size, being 0.20–0.65 μm in width by 2.3–4.3 μm in length. The bacteria were motile via one pair of bipolar-sheathed flagella. The cells lacked periplasmic fibers. All isolates form a thin spreading film on agar plates and grow under microaerobic conditions, at either 37 °C or 42 °C. The bacteria grow well on 1 % glycine plates and on Mueller–Hinton agar containing 5 % sheep's blood, as well as on brain heart infusion agar containing 5 % sheep's blood, but not on nutrient agar containing 5 % sheep's blood. All isolates have oxidase, catalase and alkaline phosphatase activities, but are negative for γ-glutamyl transpeptidase and urease activity and for nitrate reduction. All isolates are susceptible to nalidixic acid, but resistant to cephalothin.

Based on phylogenetic analysis all selected strains fell within the cluster of *Helicobacter* species. Their 16S rRNA sequences were identical and showed relatively high similarity with a number of "*Flexispira rappini*" isolates, some of which have now been reclassified as *H. bilis*.

Proposed type strain: R-204.

Genbank accession number (16S rRNA): AY192526.

G+C content of DNA: 39.6 mol%.

"*Helicobacter colifelis*"

"*Helicobacter colifelis*" was observed in fecal smears from an 8-week-old kitten with severe diarrhea (Foley et al. 1998). In these kittens large numbers of spiral-shaped bacteria were observed in the crypts and surface mucus layer of the cecum and colon. The association with diarrhea was not proven since inoculation of pathogen-free cats with infected feces did not produce symptoms, although feces of the cats were positive by PCR using primers specific for the *Helicobacter* genus. Ultrastructurally "*H. colifelis*" is spiral shaped with two coils, 0.67 μm in width by 4–5.9 μm in length, with bipolar tufts of flagella that are connected to basal plates at the point of insertion. Genetically "*H. colifelis*" is most closely related to *H. canis* (98.3 %), *H. pullorum* (96.9–96.4 %), *H. hepaticus* (96.7 %) and *H. cinaedi* (96.5 %). Since "*H. colifelis*" is currently uncultivated and was

identified on the basis of 16S rRNA sequence from a single isolate, it remains unclear whether it represents a novel enterohepatic *Helicobacter* species.

Genbank accession number (16S rRNA): AF142062.

"Helicobacter macacae"

"*Helicobacter macacae*" was isolated from five monkeys with endemic diarrhea and three monkeys without diarrhea located in two colonies of rhesus and cynomolgus monkeys in which diarrhea from chronic idiopathic colitis was enzootic (Fox et al. 2001b, 2007). "*H. macacae*" has also been cultured from the liver and intestine of a baboon with islet cell amyloidosis, focal hepatitis, and colitis (Garcia et al. 2006) and detected by PCR in diarrheic stools of children shown to be transiently positive for *H. pylori* (Haggerty et al. 2005). The cells are slender slightly curved rods 0.2 μm wide and 2–3 μm in length. The organism is motile, by means of a single sheathed flagellum located at both ends of the cell. The bacteria grow slowly on solid agar under microaerobic conditions forming small, pinpoint colonies at 37 °C and 42 °C. It does not grow under aerobic or anaerobic conditions. The bacteria are catalase and oxidase positive, but urease, alkaline phosphatase, and γ-glutamyl transpeptidase negative. The bacteria hydrolyze indoxyl acetate and grow in 1 % glycine, but do not reduce nitrate to nitrite. It is resistant to both nalidixic acid and cephalothin.

Sequencing of the 16S and 23S genes and phenotypic analysis of the "*H. macacae*" has shown they are significantly different from other *Helicobacter* species to warrant their inclusion as a novel *Helicobacter* species. Note: Since writing this Chapter, *H. macacae* has been classified as a validated species.

Proposed Type strain: MIT 99–5501.

Genbank accession number (16S rRNA): AF333338.

"Helicobacter magdeburgensis"

"*Helicobacter magdeburgensis*" (isolated at the University of Magdeburg) is an enterohepatic *Helicobacter* species which has been isolated from the intestines of specific pathogen-free laboratory mice (Traverso et al. 2010). The bacteria were characterized using a polyphasic approach incorporating both phenotypic and phylogenetic analysis. Morphologically, the bacterial cells are straight spiral or curved rod-shaped and 0.3–0.6 μm wide by 1–6 μm long. These bacteria are highly motile and contain single monopolar or single bipolar flagella. These flagella were commonly, but not always, sheathed and about 28–32 nm in diameter. The bacteria grow under anaerobic conditions on Columbia agar plates containing 5 % sheep blood at 37 °C. *H. magdeburgensis* was positive for nitrate reductase, tetracoleum reductase and alkaline phosphatase activity but negative for urease, hippurate and γ-glutamyl transferase.

Analysis of chromosomal DNA revealed that this bacterium has an approximate genome size of 1.7–1.8 Mbp. The organism also contained an IVS of 179 bp in its 16S rRNA gene. A *H. magdeburgensis*-specific PCR was developed based on the amplification of a 750 bp product.

Genbank accession number (16S rRNA) of strain HM007-1: EF990624.

Genbank accession number (23S rRNA) of strain HM007-1: HM222564.

"Helicobacter muricola"

"*Helicobacter muricola*" was isolated from the cecum and feces of Korean wild mice (*Mus musculus molossinus*) (Won et al. 2002). The organism measures 0.4–0.5 μm in width by 2–5.5 μm in length, with the same morphology as other enterohepatic helicobacters such as *H. typhlonius*, *H. hepaticus* and *H. rodentium*. It is motile by means of bipolar unsheathed flagella. Isolation requires 7–15 days, which is longer than usual for most helicobacters. The bacteria grow as pinpoint colonies or a thin spreading film under microaerobic conditions. It is catalase and urease positive but negative for alkaline phosphatase and γ-glutamyl transpeptidase activity and indoxyl acetate is not hydrolyzed. No growth occurs in 1 % glycine or at 42 °C. "*H. muricola*" is sensitive to nalidixic acid but resistant to cephalothin. It is most closely related to another murine *Helicobacter* species, *H. muridarum* (96.7 % similarity).

Proposed type strain: w-06

Genbank accession number (16S rRNA): AF264783.

"Helicobacter suncus"

"*Helicobacter suncus*" was isolated from the gastric mucosa of house musk shrews (*Suncus murinis*) with gastritis (Goto et al. 1998). It is a curved rod 0.5 μm in width and 3.55 μm in length. The bacteria are motile by means of bipolar, sheathed flagella. They grow as transparent mucoid colonies on moist blood agar plates after incubation at 37 °C under microaerobic and anaerobic conditions. It does not grow under aerobic conditions. Strains of "*H. suncus*" have catalase, oxidase and urease activity. They can reduce nitrate but do not hydrolyze indoxyl acetate or hippurate. "*H. suncus*" has alkaline phosphatase and arginine aminopeptidase activity but not **γ**-glutamyl transpeptidase. The bacteria are sensitive to tetracycline, erythromycin and chloramphenicol and resistant to nalidixic acid and cephalothin. Phylogenetically and biochemically "*H. suncus*" is most closely related to *H. mustelae* and the unnamed *Helicobacter* sp. "Bird-B and Bird-C."

Proposed type strain: Kaz-1

Genbank accession number (16S rRNA): AB006147.

"Helicobacter westmeadii"

Helicobacter westmeadii was first isolated from blood cultures of HIV-positive males with AIDS (Trivett Moore et al. 1997) and similar organisms have also been cultivated from the blood of

other HIV-infected patients (Weir et al. 1999). The bacteria can be either rod or spiral shaped and are 0.5 μm wide by 1.5–2 μm in length. The cells are motile by means of a single, unipolar, sheathed flagellum. The bacteria grow as a fine, translucent, spreading film after 4 days incubation at 35–37°C with no growth at 25 °C or 42 °C. The bacteria grow in a microaerobic atmosphere containing hydrogen or anaerobically. It does not grow under aerobic conditions. The bacteria have oxidase and catalase activities but not urease activity. Alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, acid phosphatase, and naphthol- AS-BI-phosphohydrolase are all produced. It does not produce lysine decarboxylase, ornithine decarboxylase, or arginine decarboxylase. The cells can reduce nitrate to nitrite. Hippurate is hydrolyzed, however, indole and H_2S are not produced. The organisms are resistant to cephalothin, but sensitive to nalidixic acid.

In a later study by Vandamme et al. whole-cell protein electrophoresis and a panel of 64 biochemical tests were used to further characterize *H. westmeadii* and a number of other isolates. The results, confirmed by DNA-DNA hybridization, indicated that "*H. westmeadii*" is a junior synonym of *H. cinaedi* (Vandamme et al. 2000).

Proposed type strain: none named.

Genbank accession number (16S rRNA): U44756.

"*Helicobacter winghamensis*"

"*Helicobacter winghamensis*" was isolated from stools of children and adults with gastroenteritis (Melito et al. 2001). The organism is a curved to spiral rod (0.3–0.6 μm × 2 μm) that is motile by one or two bipolar, unsheathed flagella. Colonies show a mixture of spreading and non-spreading morphology. Growth occurs under microaerobic conditions at 37 °C but not at 42 °C or in aerobic or anaerobic conditions. "*H. winghamensis*" is negative for urease and alkaline phosphatase activities, and it does not reduce nitrate. Catalase activity is also absent, which among helicobacters has only been described for *H. canis* and taxa 7 and 8 of *Helicobacter* sp. "*flexispira*," the latter now classified as *H. bilis* (Dewhirst et al. 2000a). "*H. winghamensis*" is bile tolerant, hydrolyzes indoxyl acetate and is variably resistant to nalidixic acid and cephalothin.

Proposed type strain: strain NLEP 97–1090.

Genbank accession number (16S rRNA): AF246984.

In addition to the above there have been several reports of other possible unique *Helicobacter* species isolated from prairie dogs (Beisele et al. 2011), pinnipeds (Harper et al. 2003) and from Australian marsupials including the brushtail possum, ring tail possum, koalas and kangaroos ((Coldham et al. 2011) + T. Coldham unpublished data). Furthermore, screening of 154 vertebrate species representative of Mammalia, Aves, Reptilia and Amphibia for *Helicobacter* DNA by PCR for the 16S rRNA gene showed positive samples in 93 species, including 80 new hosts highlighting the diversity of this genus and its broad host range (Schrenzel et al. 2010).

Physiology

Studies on the physiology of *Helicobacter* species are primarily on the well-established pathogen *H. pylori* and little is known about other species within this genus.

Atmospheric Requirements

H. pylori is a capnophile that can grow under microaerobic and aerobic conditions (supplemented with CO_2) at high bacterial concentrations; however, at low cell densities *H. pylori* behaves like an oxygen-susceptible microaerophile (Bury-Mone et al. 2006). *H. pylori* cells grown in vitro under microaerobic conditions appear to mimic better the physiology of organisms grown in their natural niche in the human stomach (Bury-Mone et al. 2006). While the bacterium possesses an active fumarate reductase (major end product succinate), it does not grow anaerobically in the presence of fumarate. Due to this obligate requirement for O_2 and its niche within the host, *H. pylori* possesses several enzymes such as alkyl hydroperoxide reductase, superoxide dismutase, catalase, and thioredoxin-linked thiol peroxidase to counteract the effects of reactive oxygen and nitrogen species. Recently, an enzymatic NO detoxifying system (HP0013) for the in vivo microbial protection against nitrosative stress was characterized within *H. pylori* (Justino et al. 2012).

Owing to its capnophilic nature, CO_2 occupies an essential position in the physiology of *H. pylori*. Carbonic anhydrases catalyze the interconversion of CO_2 and bicarbonate, and also function in CO_2 transport and pH homeostasis (Bury-Mone et al. 2008). *H. pylori* possesses two non-essential carbonic anhydrases, a cytosolic form and one that resides in the periplasm which have been shown to contribute to the urease-dependent response to acidity in *H. pylori* (Bury-Mone et al. 2008).

Energy Metabolism

Energy metabolism within *H. pylori* occurs through aerobic respiration with significant ATP yields via oxidative phosphorylation (Kelly et al. 2001). It has been established that in *H. pylori* and *H. hepaticus* and possibly in *H. acinonychis* and *H. mustelae*, H_2 can also be used as a source of energy (Benoit and Maier 2008). Nitrogen metabolism plays a central role in the physiology of gastric *Helicobacter* species, in particular *H. pylori*. The two major sources of nitrogen available in the gastric environment are urea and amino acids. In addition to its role in acid resistance, the nickel-dependent *H. pylori* urease, and more recently the iron-dependent urease of *H. mustelae* (Carter et al. 2011), are key enzymes in nitrogen metabolism. It has been shown that nitrogen from urea can be used for the synthesis of amino acids such as glutamate, glutamine, phenylalanine, aspartate and alanine (Williams et al. 1996). Two amidases within *H. pylori* allow the bacterium to obtain

nitrogen from other sources besides urea and amino acids (De Reuse and Skouloubris 2001).

Citric Acid Cycle, Fatty Acid Metabolism and Nucleic Acid Metabolism

H. pylori possesses a complete citric acid cycle but one that deviates from the standard cycle in three steps (Kather et al. 2000). The only enzyme of the citric acid cycle for which no open reading frame is detected in the genome is the NAD-dependent malate dehydrogenase, which in this organism is substituted by malate:quinone oxidoreductase (Kather et al. 2000).

Investigation of the fatty acid profiles of eight *Helicobacter* species has shown that they can be differentiated into two groups (Haque et al. 1996). The first group consists of mostly gastric colonizers including *H. pylori*, and contains a high percentage of tetradecanoic (14:0) fatty acids and 19-carbon cyclopropane (19:0cyc) fatty acids, and a low proportion of octadecanoic (18:1) fatty acids. Moreover, unlike *Campylobacter* and *Wolinella* species, 11 out of 13 *Helicobacter* species contained cholesteryl glucosides (Haque et al. 1996). Phosphatidylethanolamine, cardiolipin, and phosphatidylglycerol were identified as the predominant phospholipids in *H. pylori*, while phosphatidylserine was detected at lower abundance (Hirai et al. 1995).

H. pylori synthesizes both pyrimidine and purine nucleotides *de novo*. The pyrimidine pathway is essential for the growth and survival of the bacterium which is supported by its limited capacity to salvage pyrimidines and the lack of key pyrimidine salvage enzymes from the bacterium's genome (Mendz 2001). *H. pylori* incorporates preformed purines, and pathways for purine salvage, which are proposed to be essential for the organism's survival (Mendz 2001), are clearly identifiable in its genome.

Metal Uptake

Comparison of iron uptake of four gastric-colonizing *Helicobacter* species (*H. pylori*, *H. felis*, *H. acinonychis*, and *H. mustelae*) with five intestinal-colonizing species (*H. fennelliae, H. cinaedi, H. muridarum, H. bilis,* and *H. hepaticus*), has shown that all gastric species except for *H. pylori*, which obtains iron from human lactoferrin, are nonsiderophore-producing organisms that are only able to use iron from heme and hemoglobin (Dhaenens et al. 1999). In contrast, intestinal *Helicobacter* species produce siderophores and are able to use a wide range of iron sources for growth (Dhaenens et al. 1999). In addition to iron uptake, in 2010, Stoof et al. identified an ABC transporter (FeuDE and CeuE) involved in nickel and cobalt acquisition in *H. mustelae* which works independently from the nickel transporter NixA (Stoof et al. 2010). Moreover, it was determined that TonB2 is required primarily for nickel acquisition, while TonB1 was required for heme utilization (Stoof et al. 2010).

Isolation, Enrichment and Maintenance Procedures

Specimens

For the isolation of *H. pylori* from human gastric biopsies the specimens may be transported to the laboratory using a range of transport media, including thyoglicolate, Brain Heart Infusion (BHI) broth, Brucella broth supplemented or not with 20 % glycerol, Stuart's transport medium supplemented with 20 % glycerol and Skim Milk supplemented with 17 % glycerol (Han et al. 1995). Maintenance of the transport media at a low temperature of 4 °C or less is a crucial for successful isolation of *H. pylori*. Other gastric *Helicobacter* species are generally cultured from mucus scrapings or biopsies obtained from gastric mucosa that has been washed in saline (Lee et al. 1988) or to minimize contamination porcine and feline mucosa were submersed in an acid bath (1 % HCl) for 1 hour (Van den Bulck et al. 2006b; Baele et al. 2008a; Smet et al. 2012a). For some species large amounts of inoculum are required for primary isolation (Baele et al. 2008a; Smet et al. 2012a). The lower bowel enterohepatic *Helicobacter* species are most commonly cultivated from feces (fecal slurries) or mucosal biopsies/scrapings from the colon or cecum, again washed prior to inoculation onto agar plates. Isolation from homogenized liver samples and gall bladders is also possible (Stanley et al. 1994; Fox et al. 1995, 2002; Franklin et al. 1996; Shen et al. 1997, 2005). Occasionally enterohepatic *Helicobacter* species may migrate to the stomach (Lee et al. 1993) or cause bacteremia, particularly in persons infected with the human immunodeficiency virus or other immunocompromised hosts (Mammen et al. 1995; Weir et al. 1999; Cuccherini et al. 2000). It should be noted that optimum cultivation of the helicobacters is achieved by placing fresh specimens onto moist agar plates as soon as possible. However in some instances organisms have been isolated from tissue samples that have been frozen in a cryoprotective agent such as glycerol and stored at −80 °C prior to cultivation (Coldham et al. 2011).

Selective and Non-Selective Media

The primary isolation of *Helicobacter* species is generally performed with solid media supplemented with 5–10 % horse or sheep blood, or 20 % fetal calf serum (FCS). Enriched agar bases such as Blood agar base, Brucella, Columbia, brain heart infusion agar (BHI), trypticase soy agar (TSA) and others are routinely used. It has been noted however that *H. bizzozeronii* does not grow on Brucella agar (Jalava et al. 1998). Recently the cultivation of some of the more fastidious gastric bacteria has been achieved by the addition of growth factors, such as those found in the Vitox supplement (Oxoid, Thermo Scientific), the addition of charcoal to remove toxic compounds and lowering the pH of the media to 5 (Van den Bulck et al. 2006b; Moyaert et al. 2007b; Baele et al. 2008a, b; Smet et al. 2012a).

The yield from culture may be improved if both selective and non-selective agars are used, the latter in combination with

a filter technique. Commonly used antibiotic supplements are Skirrow's (TVP) (Skirrow 1977) and CVA (Burnens et al. 1993):

Skirrow's Supplement	
Vancomycin	10 mg/L
Trimethoprim	5 mg/L
Polymyxin B	2,500 IU/L

In addition Amphotericin B (5 mg/L) or cycloheximide (100 mg/L) is generally added to inhibit fungal growth.

CVA supplement:	
Cefoperazone	20 mg/L
Vancomycin	10 mg/L
Amphotericin B	2 mg/L

To facilitate identification of *H. pylori* Belo Horizonte Medium can also be used. This is a selective and indicator medium composed of BHI agar supplemented with 10 % whole sheep blood, vancomycin, nalidixic acid, amphotericin B and triphenyl-tetrazolium chloride which confers a unique golden aspect to the colonies (Queiroz et al. 1987; *Fig. 24.9*). An alternative medium that has been used for the isolation of a number of other helicobacter species is the one developed in the Fox laboratory. This medium consists of blood agar base (Oxoid; Remel), 5 % horse blood, 50 μg/mL of amphotericin B, 100 μg/mL of vancomycin, 3.3 μg/mL of polymyxin B, 200 μg/mL of bacitracin, and 10.7 μg/mL of nalidixic acid. The use of this medium has resulted in the successful cultivation of *H. anseris, H. brantae, H. cetorum* and *H. marmotae* (Fox et al. 2002, 2006; Harper et al. 2002a).

When enterohepatic helicobacters are isolated from feces or intestinal content, the slurry is usually passed through a 0.45 or 0.65 μm with the filtrate placed directly onto either selective or non-selective agar. An alternative method involves placing a mucus scraping directly onto the surface of a 0.65 μm filter which has already been placed onto non-selective agar. The plates are incubated for approximately 2 h in a CO_2 incubator. The filter is then removed and the plates incubated under the appropriate conditions. These methods have been adapted from methods described for the cultivation of *Campylobacter* species (Steele and McDermott 1984; Robertson et al. 2001).

Growth Conditions

For primary isolation of new species agar plates are often incubated under both microaerobic and anaerobic conditions however it has been show that nearly all *Helicobacter* species prefer microaerobic conditions, exhibiting growth in 3–7 % O_2. No growth is observed under aerobic conditions, though occasional species will grow in anaerobic as well as microaerobic conditions (Fox et al. 1994; Shen et al. 1997; Robertson et al. 2001).

Fig. 24.9
***Helicobacter pylori* colonies appear as a unique golden aspect on the surface of Belo Horizonte medium**

An exception to this is *H. ganmani* which only grows under anaerobic conditions (Robertson et al. 2001). Hydrogen is required for some species and may promote growth of others, sometimes in a strain-dependent fashion. For example, isolates originally described as "*H. westmeadii*" were subsequently identified as hydrogen-requiring *H. cinaedi* (Vandamme et al. 2000). Microaerobic conditions can be achieved either by the use of anaerobic jars with commercial gas generating envelopes with or without a catalyst (CampyPak Plus, Becton Dickinson, Cokeysville, MD, Campylobacter Gas Generating Kits, Oxoid, Thermo Fisher) or by the use of vented jars or mixed gas incubators. For the latter various mixtures of gases have been utilized with the most common being 10 % H_2, 10 % CO_2, 80 % N_2 (Dewhirst et al. 1994; Patterson et al. 2000b; Harper et al. 2002a; Shen et al. 2005; Fox et al. 2006); 5 % H_2, 5 % CO_2, 90 % N_2 (Fox et al. 1995, 2002; Franklin et al. 1996; Mendes et al. 1996); 8 % H_2, 8 % CO_2, 88 % N_2 (Van den Bulck et al. 2006b; Baele et al. 2008a) and some that have included a small amount of oxygen (5 %) (Stanley et al. 1993, 1994; Moyaert et al. 2007b; Smet et al. 2012a).

The majority of *Helicobacter* species are fastidious with respect to isolation and cultivation and require freshly poured plates incubated with lids uppermost to maintain a moist environment. *H. pylori* is less demanding in this regard than many of the other species. A high degree of humidity during incubation, obtained by placing wet paper towel in anaerobe jars, is often essential for the culture of many different species. Plates are incubated for a minimum of 3 days and up to 10 days with them being checked every few days for growth. The need for moist plates has been highlighted by the successful growth of *H. bizzozeronii* and *H. salomonis* on agar plates to which BHI broth was added every few days (Jalava et al. 1998). This concept

has been taken further in the recent successful cultivation of a number of gastric species that had previously resisted numerous attempts at cultivation. A team from the University of Ghent, Belgium, have developed this method further by the continual addition of BHI broth (every 2 days), in some instances supplemented with FCS or horse serum, to keep the plates wet during incubation thus providing biphasic growth conditions. The organisms were initially detected in the broth on the surface of the plates (Van den Bulck et al. 2006b; Baele et al. 2008a, b; Smet et al. 2012a).

Culture is routinely performed at 37 °C although a number of species have been shown to grow at 42 °C. Most *Helicobacter* species grow poorly or not at all in liquid media, though *H. pylori* and some others can often be adapted to grow in liquid culture supplemented with serum or β-cyclodextrin. The routine cultivation of a number of *Helicobacter* species (including *H. pylori*) has been detailed in a number of recent method-based publications (Blanchard and Nedrud 2012; Whitmire and Merrell 2012). These methods however would not be suitable for the more fastidious helicobacters such as *H. bizzozeronii, H. salomonis, H. suis and H. heilmannii.*

Identification

Helicobacter species grow as small (0.5–2 mm) translucent colonies or as a thin watery film. Experience is often required to recognize the film-like growth of some species. Organisms are gram-negative and often pleomorphic. After prolonged incubation most helicobacters assume a coccoid morphology (*Fig. 24.10*), the significance of which remains controversial (Cellini et al. 1994; Eaton et al. 1995; Kusters et al. 1997; Nilsson et al. 2002). Routine identification of many *Helicobacter* species relies on their positive tests for urease, catalase, and oxidase, though many enterohepatic species are urease negative. Definitive identification requires a polyphasic approach to taxonomy, but there is an obvious role for molecular diagnostic tests provided their specificity and sensitivity has been evaluated using balanced and well-chosen sets of reference strains. 16S rRNA sequence analysis may be insufficiently sensitive to distinguish different species, while in other cases it may exaggerate differences that are not confirmed by other methods.

Preservation

Helicobacter species can be preserved in an enriched broth (e.g. brucella, brain heart infusion) or skim milk supplemented with a cyroprotective agent such as glycerol (10–20 %) or DSMO and then stored at −80 °C or in liquid nitrogen for up to 5 years (Fox et al. 1988; Hanninen et al. 1996). An alternative solution for freezing is 25 mL BHI, 75 mL inactivated horse serum plus 7.5 g glucose (Van den Bulck et al. 2006b; Baele et al. 2008a; Smet et al. 2012a). These organisms generally do not freeze-dry successfully.

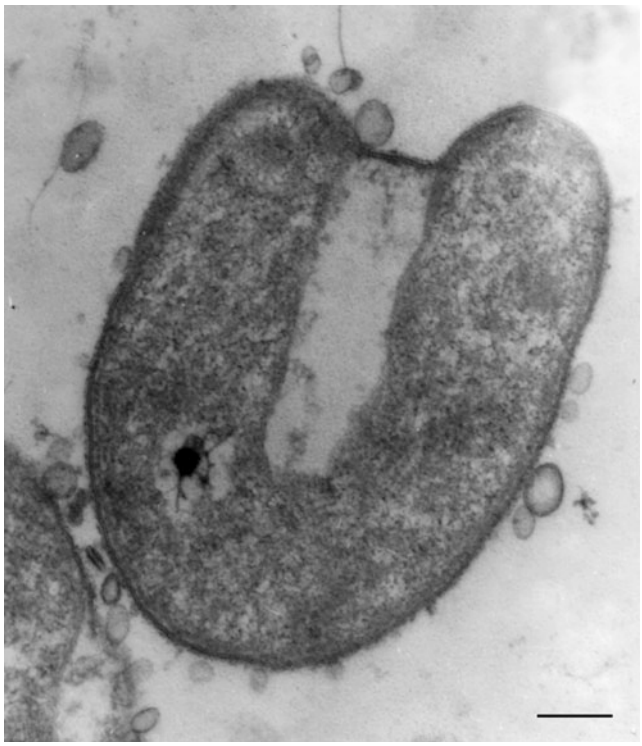

Fig. 24.10
Transmission electron micrograph of a thin section of the coccoid form of *Helicobacter pylori* (bar = 0.2 μm)

Pathogenicity, Clinical Relevance

Helicobacter species are ubiquitous colonizers of the gastrointestinal tract of humans and other mammalian species. In most cases these bacteria do not appear to have a pathogenic relationship with their host. However, there are important exceptions to this generalization, most notably *H. pylori*, which is associated with the development of peptic ulcers and gastric cancer in humans. Even with *H. pylori*, however, it should be remembered that the relationship between pathogen and host is complex, often commensal, and perhaps even beneficial in some cases (Blaser 1999).

Disease Association in Humans

Helicobacter pylori

H. pylori colonizes the gastric mucosa of more than half of the world's population being more prevalent in developing countries (Megraud et al. 1989; Graham 1991). The infection is mainly acquired in early childhood and persists lifelong (Mitchell et al. 1992a). The apparent increase in infection prevalence with increasing age is explained by a cohort effect, i.e., the elevated prevalence of infection among older persons reflects greater exposure of these individuals to infection during childhood (Mitchell et al. 1992a; Sipponen 1995). Socio-economic status

in childhood is a determinant risk factor for acquisition of infection with low family income, poor hygiene, crowded household conditions and a number of cultural habits (Malaty et al. 2001; Mitchell 2001) increasing the risk of infection. Transmission of infection is primarily intra-familial, person-to-person by oral-oral and/or fecal-oral routes, infected mothers and infected siblings being the most common risk factors for the transmission of *H. pylori* (Mitchell et al. 1987, 1992b; Malaty et al. 1991; Rothenbacher et al. 1999; Queiroz et al. 2012). During the first years of life children frequently gain and lose the infection.

The course of the infection is highly variable and depends on the pattern of distribution of the gastritis, as well as host, environmental and bacterial virulence factors. Patients with predominantly antral gastritis have a normal or increased acid output and hypergastrinemia and are at increased risk of developing duodenal ulcer disease (Queiroz et al. 1993; Gillen et al. 1998; McColl et al. 2000). *H. pylori* also causes gastric ulceration however in contrast with duodenal ulceration, it is associated with decreased acid output, normal or diminished parietal cell mass as well as a decrease in gastric acid as a consequence of long lasting chronic atrophic gastritis (Schubert and Peura 2008). In a small percentage of infected patients, atrophy may progress to intestinal metaplasia, spreading from the non-secreting epithelium to the proximal stomach. Association between *H. pylori* and gastric cancer was initially based on two extensive cohort epidemiological studies that demonstrated that individuals previously infected by *H. pylori* were at an increased risk of gastric cancer in comparison with the non-infected ones (Nomura et al. 1991; Parsonnet et al. 1991). In 1994, the International Agency for Research on Cancer classified *H. pylori* as a Class I carcinogen (IARC 1994). Further studies have strengthened these findings and described bacterial virulence factors (Blaser et al. 1993, 1995; Evans et al. 1998) and host factors associated with the disease that is the second leading cause of mortality due to cancer worldwide (El-Omar et al. 2000, 2003; Peek and Blaser 2002; Machado et al. 2003; Rocha et al. 2005; Piazuelo et al. 2010). Chronic inflammation has been considered a hallmark of several types of cancer. In the case of gastric cancer, long-term severe inflammation induced by *H. pylori* infection may lead to loss of glandular tissue resulting in multifocal atrophic gastritis that may evolve to intestinal metaplasia that in some individuals progresses to dysplasia/neoplasia (Correa and Piazuelo 2012).

The causal relationship between *H. pylori* infection and MALT lymphoma was established based on epidemiological studies and on the fact that eradication of the microorganism could lead to cure of the neoplasia (Wotherspoon et al. 1991; Ahmad et al. 2003; Farinha and Gascoyne 2005). Although the mechanisms of tumorigenesis in MALT lymphoma have not been clearly demonstrated, *H. pylori* flavodoxin A has been associated with an increased risk of developing gastric MALT lymphoma (Chang et al. 1999; Yang et al. 2004).

It has also been suggested that *H. pylori* infection plays a role in the development of multifactorial conditions including hematological disorders such as iron deficiency anemia (Ashorn et al. 2001; Baggett et al. 2006; Qu et al. 2010), cobalamin deficiency (Marino et al. 2007) and chronic immune thrombocytopenic purpura (Gasbarrini et al. 1998; Takahashi et al. 2004; Stasi et al. 2009). Due to the chronic inflammatory nature of the *H. pylori* infection, it has been associated with some neurological conditions, including, Alzheimer's (Kountouras et al. 2009) and Parkinson's (Charlett et al. 2009) diseases, and ischemic heart disease (Longo-Mbenza 2009; Izadi et al. 2012); however, currently there is insufficient data to prove a causal role for *H. pylori* in the pathophysiology of these diseases. Of interest however is a recent report, based on multiple logistic regression analysis, that showed that seropositivity to *H. cinaedi*, but not to *H. pylori* or *Chlamydia pneumoniae*, was an independent risk factor for atrial arrhythmia (Khan et al. 2012). Further it has been reported that *H. pylori* infection may protect against gastroesophageal reflux disease (Queiroz et al. 2002; Blaser 2010), asthma (Chen and Blaser 2007), allergic (Chen and Blaser 2007) and atopic diseases (Reibman et al. 2008; Holster et al. 2012), however further studies are required to support this contention.

Other Helicobacters

Gastric infections. Other than *H. pylori*, the only significant gastric *Helicobacter* seen in humans is *Helicobacter heilmannii sensu lato.* These organisms have an overall prevalence of approximately 0.5 % among patients, including children, undergoing endoscopy (Oliva et al. 1993; O'Rourke et al. 2001; Iwanczak et al. 2012). When identification of the individual *Helicobacter* species has been undertaken *H. suis* is shown to be the most prevalent organism with a recent study involving 123 patients showing prevalence rates of 36.6 % for *H. suis*, 21 % for *H. salomonis*, 15 % for *H. felis* and 8 % for *H. bizzozeronii* (Trebesius et al. 2001; De Groote et al. 2005; Van den Bulck et al. 2005b). However, of these the only organism successfully cultured from a human patient is *H. bizzozeronii* (Andersen et al. 1999; Jalava et al. 2001). It has been suggested that these infections may be zoonoses (Meining et al. 1998; Baele et al. 2009).

As compared with *H. pylori*, *H. heilmannii* s.l. infection is more frequently focal, has fewer organisms, is usually restricted to the antral gastric mucosa, is associated with a mild chronic gastritis which is typified by a small number of neutrophils and the presence of lymphoid aggregates and lymphoid follicles (Morgner et al. 2000). Peptic ulcers have also been seen in a small number of cases (O'Rourke et al. 2001). *H. heilmannii* s.l. has also been associated with gastric MALT lymphoma and the risk of developing tumors is higher in *H. heilmannii* s.l. infection than in *H. pylori* infection (Stolte et al. 1997; Morgner et al. 2000). Of note, a homologus *fld*A encoding the flavodoxin A of *H. pylori* that has been associated with an increased risk of gastric MALT lymphoma has been detected in *H. suis* (Chang et al. 1999; Yang et al. 2004). The eradication of *H. heilmannii* s.l. may lead to the cure of the MALT lymphoma (Stolte et al. 1997; Morgner et al. 2000; Okiyama et al. 2005).

Apart from the above infections the only other report to date of the presence of a gastric *Helicobacter* in humans has been the finding of DNA related to the 16S rRNA gene of *H. cetorum*, an organism normally found in marine mammals,

along with the recently described "*Candidatus* Wolinella africanus" in the gastric juice of asymptomatic Venezuelan subjects (García-Amado et al. 2007).

Enterohepatic Infections

Soon after the initial descriptions of *H. pylori* and its potential as a human pathogen the first reports of non-gastric helicobacters potentially implicated in human disease were made by Quinn et al. (1983, 1984). They described the isolation of three different types of bacteria, two of which were subsequently characterized as *H. cinaedi* and *H. fennelliae* (Totten et al. 1985; Vandamme et al. 1991), from homosexual men presenting with anorectal and intestinal symptoms including proctitis. Subsequent to this there have been numerous reports of enterohepatic *Helicobacter* species (EHS) associated with gastroenteritis however a direct causal link has yet to be established. The additional species implicated include *H. canis, H. pullorum, H. canadenesis,* "*H. winghamensis*" and *H. bilis* (previously referred to as flexispira taxon 8) (Romero et al. 1988; Burnens et al. 1993; Stanley et al. 1994; Fox et al. 2000; Melito et al. 2001; Hanninen et al. 2005).

These discoveries led to the examination of a possible role for these mucus-associated bacteria in the etiology of inflammatory bowel disease (IBD). Potential evidence to support a causal role of EHS in IBD has come from both animal and human studies [recently reviewed in (Fox et al. 2011; Hansen et al. 2011; Mukhopadhya et al. 2012)]. *H. macacae* and isolates of Flexispira taxon 10 have been implicated in the development of colitis in two primate species, the cotton-top tamarin monkey (*Saguinus oedipus*) and the rhesus monkey (*Macaca mulatta*). In addition, a number of EHS including *H. hepaticus* and *H. bilis* have been shown to induce IBD-like disease in animal models utilizing immunodeficient animals. While initial reports found no evidence of EHS in IBD human patients (Bell et al. 2003; Grehan et al. 2004; Huijsdens et al. 2004; Zhang et al. 2011) in several subsequent studies EHS DNA, detected by PCR, Southern blot and fluorescent in situ hybridization, has been consistently found in a higher proportion of IBD patients compared to control patients (Bohr et al. 2004; Oliveira et al. 2004, 2006; Laharie et al. 2009; Thomson et al. 2011). In addition, there have been a number of reports of the findings of *Helicobacteraceae* DNA in children presenting with IBD (Zhang et al. 2006; Man et al. 2008; Kaakoush et al. 2010). Attempts to cultivate these organisms from IBD patients have, to date, been unsuccessful however this problem may be overcome as our knowledge of these bacteria increases and improved cultivation methodologies are developed. One interesting finding as a consequence of these studies was the idea that colonization by *H. pylori* actually prevented individuals from developing IBD. This may be attributed to the notion that antibodies produced during gastric infections from *H. pylori* cross react with many other *Helicobacter* species thus preventing the EHS from establishing a foothold in the intestinal tract. The organisms detected include a number of EHS and also other members of the Proteobacteria phylum, including the closely related *Campylobacter* and *Wolinella* species (Mukhopadhya et al. 2012). It has been proposed that the role of EHS in IBD is not as a direct initiator of inflammation but rather as a contributing factor that results in a change to a person's intestinal microbiota, dysbiosis, thus making them more susceptible to the development of the disease (Hansen et al. 2011; Mukhopadhya et al. 2012).

In common with other intestinal pathogens, EHS have been found to translocate resulting in bacteremia and other secondary infections such as cellulitis and arthritis (reviewed in O'Rourke et al. 2001; Fox 2002; Solnick 2003; Freeman and Holland 2007; Minauchi et al. 2010; Kikuchi et al. 2012; Rimbara et al. 2012). While *H. cinaedi* is the predominant organism identified in these infections other cases have been attributed to *H. bilis* (previously referred to as flexispira), *H. fennelliae* and more recently *H. canis* (Leemann et al. 2006; Alon et al. 2010; Turvey et al. 2012). Predisposing factors including HIV, alcoholism, end-stage renal failure, carcinoma, diabetes, primary immunodeficiencies such as X-linked agammaglobulinemia and hypogammaglobulinemia are often reported however there have been a number of cases in immunocompetent individuals.

Studies in animals of EHS indicated they can also translocate into the hepato-biliary system leading to the development of liver disease and hepatocellular carcinoma (HCC) in susceptible animals (Fox et al. 1994, 2001b; Ward et al. 1994). This raised the possibility that these organisms could also be implicated in hepatobiliary disease in humans. Early reports of such an association were made by Fox et al. in 1998 in which they detected *H. bilis* and *H. pullorum* DNA in Chilean patients presenting with chronic cholecystitis (Fox et al. 1998a) and by Matsukura et al. who reported an association of *H. bilis* and biliary tract and gall bladder cancers in high risk populations (Matsukura et al. 2002). This was followed by a number of other studies however the most common finding was *H. pylori* DNA (reviewed in Pellicano et al. 2008; Tu et al. 2009; Fox et al. 2011) with only two instances of successful cultivation of a bacterium from a liver sample. Again these were both identified as *H. pylori* (Queiroz and Santos 2001; Xuan et al. 2006). While a possibility, more research is required to show a causative role for helicobacters in hepatobiliary disease in humans.

Disease Association in Animals

Helicobacter species are ubiquitous along the gastrointestinal mucosal surface of a broad range of domestic and feral animals. Nevertheless, there are very few well-documented instances in which infection of an immunocompetent, natural host results in clinical disease (Solnick and Schauer 2001).

Gastric infections. Gastric *Helicobacter* infections in animals are associated with a histologic gastritis that resembles that seen in humans, though the polymorphonuclear component is usually minimal or absent. Cats and dogs generally show high levels of colonization by gastric bacteria with at least six different *Helicobacter* species now identified (Haesebrouck et al. 2009). The main colonizers are those classified within *H. heilmannii* s.s. with very little currently known about the two recently described

new species, *H. baculiformis* and *H. cynogastricus*. A study in Beagle dogs showed a differential distribution of *H. bizzozeronii* and *H. felis* in their gastric mucosa and intracellular localization of organisms in parietal cells and macrophages (Lanzoni et al. 2011). The dominant histology seen in cats and dogs is a mild chronic gastritis characterized by lymphocytic and lymphoplasmacytic infiltrates in the lamina propria with occasional formation of lymphoid follicles (Geyer et al. 1993; Hermanns et al. 1995; Eaton et al. 1996a; Happonen et al. 1998; Rossi et al. 2008). The American College of Veterinary Internal Medicine (ACVIM) issued a consensus statement that concluded that most cats and dogs are infected with gastric bacteria irrespective of health status. Histological changes are detected but there is no simple association of bacteria with disease (Simpson et al. 2000). Interestingly, Bridgeford et al. have recently postulated an association between gastric *Helicobacter* species and feline gastric lymphoma (Bridgeford et al. 2008).

H. suis has been reported to have high prevalence rates in pigs (up to 60 %) and has been associated with gastritis in experimentally and naturally infected pigs and ulceration of the *pars oesophagea* (Queiroz et al. 1990, 1996; Mendes et al. 1991; Haesebrouck et al. 2009). On histological examination, the ulcer is found to extend through the epithelium and lamina propria into the muscularis mucosae. The lumen of the ulcer contains an exudate consisting of neutrophils, eosinophils and mononuclear cells. Areas of necrosis and granulation tissue consisting of proliferating fibroblasts and capillaries are detected (Queiroz et al. 1996). A variable degree of antral gastritis, ranging from a very mild to an intense mononuclear cell infiltration is observed in the stomach of all infected pigs with a low degree of oxyntic gastritis in approximately half of them. Neither atrophy nor intestinal metaplasia is generally observed, but lymphoid aggregates and follicles are frequently seen in the gastric mucosa of infected pigs (Queiroz et al. 1996). A decrease in daily weight gain has also been demonstrated in experimentally infected pigs (De Bruyne et al. 2012). It has been proposed that the bacteria may have an indirect effect on ulcer formation via induction of increased levels of acid secretion (Yeomans and Kolt 1996).

A number of larger felid species, including cheetahs, lions and tigers, have been shown to be colonized by *H. acinonychis* and *H. heilmannii* s.l. and it is known that chronic gastritis is a significant clinical problem in captive cheetahs (Eaton et al. 1993a, b; Munson 1993; Jakob et al. 1997; Munson et al. 1999). Cheetahs that presented with chronic vomiting and weight loss had severe lymphocytic gastritis characterized by variable numbers of neutrophils, gland abscesses, lymphoid follicles and epithelial erosions (Eaton et al. 1993b; Munson 1993; Munson et al. 1999). A treatment study in cheetahs suggested that more severe disease might be associated with *H. acinonychis* infection rather than with *H. heilmannii* s.l. (Wack et al. 1997). A long-term prospective study on the effects of various antimicrobial therapies in captive cheetahs showed only short-term reduction in *Helicobacter* infection and gastritis and that such treatment is only warranted for symptomatic relief in moderate to severe clinical presentations (Citino and Munson 2005). In contrast treatment of two tigers infected with *H. acinonychis* resulted in the eradication of the bacterium and resolution of their gastric lesions (Cattoli et al. 2000).

Some animal hosts are colonized by more than one *Helicobacter* species, such as *H. pylori* and *H. acinonychis* in cheetahs and *H. pylori* and *H. heilmannii* s.l. in non-human primates (Euler et al. 1990; Eaton et al. 1993b; Dubois et al. 1994). In non-human primates, gastritis appears to be less severe in animals infected with *H. heilmannii* s.l. than in those infected with *H. pylori* (Dubois et al. 1991). *H. heilmannii* s.l. are found intracellularly in parietal cells, which may be linked to increased acid output levels (Dubois et al. 1991).

H. mustelae is the natural colonizer of ferret gastric mucosa where it is found in association with a diffuse antral chronic gastritis and gastric ulcers (Fox et al. 1986, 1990). The bacteria readily adhere to the epithelial surface with evidence of endocytosis, a feature not commonly seen with other helicobacters except for *H. pylori* (O'Rourke et al. 1992). Reports have also shown the presence of *H. mustelae* in ferrets suffering from pyloric adenocarcinoma and MALT lymphoma (Erdman et al. 1997; Fox et al. 1997). Inoculation of *H. mustelae* into uninfected ferrets results in an inflammatory response similar to that seen in naturally infected animals (Fox et al. 1991). However, an association between ulceration and malignancy in ferrets and *H. mustelae* infection requires further clarification.

H. aurati has been associated with chronic gastritis, intestinal metaplasia and one reported case of gastric adenocarcinoma in hamsters however role these organisms play in the etiology of these diseases requires further elucidation (Patterson et al. 2000a, b; Nambiar et al. 2005). The role of other gastric *Helicobacter* species in other animals such as *H. cetorum* in marine mammals and "*Candidatus* H. bovis" in ruminants is currently unknown though gastric ulcers are found in these animals (De Groote et al. 1999b; Harper et al. 2002a; Oxley et al. 2004; Haesebrouck et al. 2009).

Enterohepatic Infections

Fourteen *Helicobacter* species have been identified in rodents and while they are generally considered normal commensal intestinal microbiota some species have been associated with disease. Surveys of experimental and wild rodent species have shown that their intestinal mucosa is commonly colonized with one or more of the EHS. The organisms commonly found include *H. bilis, H. ganmani, H. hepaticus, H. mastomyrinus, H. muridarum, H. rodentium, H. trogontum* and *H. typhlonius* (Zenner 1999; Goto et al. 2000; Nilsson et al. 2004; Bohr et al. 2006; Comunian et al. 2006; Johansson et al. 2006; Taylor et al. 2007; Wasimuddin et al. 2012). There is little evidence to support a pathogenic role for these organisms but rather they form part of the normal intestinal microbiota in immunocompetent animals. However this commensal role is not the case when these species are experimentally introduced into immunodeficient animals. As such these organisms are often used as models of

typhlocolitis and IBD. Some examples of these models are described in the following reports and reviews (Franklin et al. 2001; Jiang et al. 2001; Whary et al. 2006; Hale et al. 2007; Chichlowski et al. 2008; Nell et al. 2010; Patterson et al. 2010; Eaton et al. 2011; Fox et al. 2011; Hansen et al. 2011).

An important feature of the EHS is that under normal conditions, in immunocompetent animals, many of them exhibit the ability to colonize gastric mucosa and/or to translocate to the hepatobiliary system and in these instances they can be associated with serious diseases in their normal hosts. One feature that should be noted is that this generally occurs in older animals indicating that there may be a change in their immune competence that allows these bacteria to relocate from their natural habitats. For example, *H. muridarum* and *H. aurati* have been found in gastric mucosa associated with gastritis, atrophy, intestinal metaplasia and adenocarcinoma (Lee et al. 1993; Patterson et al. 2000a; Nambiar et al. 2005). However the most significant evidence for the pathogenic and carcinogenic potential of these intestinal organisms came from the original description of *H. hepaticus* (Fox et al. 1994).

Of all the EHS, *H. hepaticus* has been shown to cause the most severe diseases. In the early 1990s, prior to the isolation of *H. hepaticus*, a novel form of active chronic hepatitis was noted in several barrier-maintained mouse strains. Examination of these animals revealed the presence of spiral organisms in their livers that were subsequently cultured and characterized as *H. hepaticus* (Fox et al. 1994, 1996b; Ward et al. 1994). Subsequent animal infection studies confirmed that *H. hepaticus* was capable of inducing hepatitis and hepatocellular carcinoma with factors including mouse strain, host immune response, sex and age and route of infection being shown to play an important role in disease progression (Fox et al. 1996b; Ihrig et al. 1999; Rogers et al. 2004, 2007; Diwan et al. 2008; Garcia et al. 2008, 2011; Theve et al. 2008). *H. hepaticus* has also been implicated in the development of intestinal carcinoma in immunodeficient mice (Fox et al. 2011).

H. bilis has also been shown to colonize the liver and, as its name suggests, the bile of aged mice (Fox et al. 1995). It has also been recently detected in aged hamsters diagnosed with a range of hepato-biliary diseases including hepatitis, hepatic dysplasia and biliary hyperplasia (Fox et al. 2009). Although the role of *H. bilis* in the above diseases and IBD requires clarification it has been suggested that *H. bilis* may be capable of altering the host's response to its own microbiota that could result in intestinal inflammation (Jergens et al. 2007). The recent reclassification of a number of the flexispira organisms as *H. bilis* s.s. (Hanninen et al. 2005; Rossi et al. 2010) has not only expanded their host range but has also identified it as a pathogen capable of inducing abortion in sheep (natural and experimental infections) and experimentally infected guinea pigs (Kirkbride et al. 1985, 1986; Bryner et al. 1987).

H. cinaedi, H. fennelliae and *H. mesocricetorum* are considered to be normal intestinal flora of hamsters (Gebhart et al. 1989; Zenner 1999; Simmons et al. 2000; Rossi et al. 2008) with *H. cinaedi* also being reported in asymptomatic captive rhesus monkeys (Fernandez et al. 2002). In contrast experimental infection of young pig-tailed macaques with *H. cinaedi* and *H. fennelliae* resulted in the development of bacteremia and diarrhea similar to that caused by *C. jejuni* (Flores et al. 1990). Unlike the other *Helicobacter* species isolated from hamsters *H. cholecystus* has shown a very strong correlation with cholangiofibrosis (20/22 animals) and centrilobular pancreatitis (22/22 animals) with only one of 10 uninfected animals showing signs of pancreatitis (Franklin et al. 1996). While the observation of hepatobiliary disease in hamsters was not new (Zenner 1999) Franklin et al. concluded that they could not rule out the possibility that the presence of these organisms was secondary to the presence of disease. There has also been a suggestion that a *H. cholecystus*-like bacterium could be associated with hepatobiliary disease in a ferret colony (Garcia et al. 2002).

The remaining EHS isolated from rodents is *H. marmotae*, which was cultured from the liver of a woodchuck diagnosed with hepatocellular carcinoma (HCC) (Fox et al. 2002). It has also been isolated from the feces of clinically healthy cats. The role of *H. marmotae* in HCC is however confounded by the fact the woodchucks, including the one from which the organism was isolated from, have a high incidence of HCC caused by the woodchuck hepatitis virus. Experimental infection of A/J mice, a strain susceptible to *H. hepaticus* induced hepatitis, has however been shown to result in inflammation in the cecum, proximal colon and liver, with 2/48 mice developing hepatitis. *H. marmotae* was also cultivated from the liver of these two animals (Patterson et al. 2010).

While originally isolated from dogs *H. canis*, along with *H. cinaedi* and *H. bilis*, are also commonly found in cats (Stanley et al. 1993; Kiehlbauch et al. 1995; Rossi et al. 2008; Recordati et al. 2009; Castiglioni et al. 2012). The association of these EHS with disease remains controversial. While Rossi et al. reported no significant colonization differences in healthy versus diarrheic animals (Rossi et al. 2008), Castiglioni et al. found that in symptomatic dogs younger animals had higher colonization levels of these *Helicobacter* species which correlated with higher levels of mucosal fibrosis and atrophy (Castiglioni et al. 2012). It has been suggested that transmission of these EHS to humans may be via a fecal-oral route rather oral-oral route as *Wolinella* spp. rather than *Helicobacter* spp. were found to be the predominant *Helicobacteraceae* in the oral cavity of dogs (Craven et al. 2011).

The equine *Helicobacter*, *H. equorum*, bas been reported to be more prevalent in young, < 6-month old, foals with only low numbers being detected in adult horses (Moyaert et al. 2009). Experimental infection of horses with *H. equorum* revealed that while it colonized the cecum, colon, and rectum of adult horses no apparent disease was induced (Moyaert et al. 2007a).

The remaining five enterohepatic *Helicobacter* species have been identified in a range of avian species. While two of these species, *H. canadensis* and *H. pullorum*, have been associated with human diseases currently there is no evidence for a pathogenic role in their natural avian hosts. The same can also be said for *H. anseris*, *H. brantae* and *H. pametensis*. Two experimental infection studies of chickens with *H. pullorum* have failed to provide evidence of the development of disease despite the fact that the organisms were able to colonize

the intestinal tracts in high numbers and were detected continuously in fecal samples (Neubauer and Hess 2006; Ceelen et al. 2007). This latter finding coupled with the high prevalence rates of *H. pullorum* in chickens and the recent detection of *H. pullorum* in chicken products (Miller et al. 2006; Gonzalez et al. 2008) does however raise public health concerns. Like other EHS, their role in disease in immunocompromised animals warrants further investigation as shown by the fact that in the original description of *H. pullorum* these organisms were detected in the liver, presumably having translocated from the gastrointestinal tract, of animals suffering from vibronic hepatitis (Stanley et al. 1994).

Animal Models of *Helicobacter* Infections

Numerous *Helicobacter* species, including mouse-adapted strains of *H. pylori*, have been utilized in natural and experimental models of infection to further elucidate the role of these organisms in gastrointestinal disease. These models have allowed for a greater understanding of the disease initiation and development, identification of colonization and virulence factors and have allowed for the testing of therapeutic treatments including vaccination. The information gathered from these studies is vast and not within the scope of this article however this material has been well reviewed in a number of publications (O'Rourke and Lee 2003; Whary and Fox 2004; Kodama et al. 2005; Nell et al. 2010; Fox et al. 2011; Hansen et al. 2011; Krueger et al. 2011).

Virulence Factors

Helicobacter pylori

To colonize the harsh gastric environment, *H. pylori* displays a series of virulence factors including spiral form, motility and flagella that enable the organism to cross the mucus layer. Further, a number of outer membrane proteins have been shown to be required for adhesion and a potent urease for survival in the gastric acid environment. Other *H. pylori* virulence factors, not present or not expressed in all strains, are associated with increased risk of more serious *H. pylori*-associated disease outcomes. These include the *cag*-PAI that comprises a DNA fragment of 40 kb and a G+C content of 35 %, that differs from that of the bacterial genome (Censini et al. 1996), suggesting horizontal acquisition. The *cag*-PAI contains 32 genes including orthologs of all 11 VirB proteins and VirD4 as well as various associated factors. The *cag*-PAI located on the *cag*-PAI encodes a T4SS (Backert et al. 2000; Odenbreit et al. 2000) that injects peptidoglycan and CagA encoded by the *cag*A gene into the host target cells. CagA, which was recently recognized as the first bacterial protein with oncogenic properties (Hatakeyama 2011), localizes to the inner surface of the plasma membrane and is phosphorylated by multiple members of the Src kinase family (Selbach et al. 2002; Stein et al. 2002; Azuma et al. 2004; Poppe et al. 2007; Tammer et al. 2007). Phosphorylated CagA forms a physical complex with SHP-2 phosphatase that results in the triggering of abnormal cellular signals leading to deregulation of cell growth, cell to cell contact, cell migration, elongation of epithelial cells and an increase in cell turnover, which enhances the risk of the damaged cells acquiring pre-cancerous genetic changes (Higashi et al. 2002a, b; Tsutsumi et al. 2003; Suzuki et al. 2005). Phosphorylation of CagA occurs within the tyrosine motifs in the carboxy-terminal variable region of the protein, defined as EPIYA (Glu-Pro-Ile-Tyr-Ala) A, B, C and D based on the amino acid sequences present. The CagA protein almost always possesses the EPIYA-A and -B segments and in strains circulating in Western countries one to three C motifs. In contrast, in East Asian countries a D motif is most common (Backert et al. 2001; Higashi et al. 2002a, 2005; Stein et al. 2002; Argent et al. 2004; Hatakeyama and Higashi 2005). EPIYA-C and -D are the main sites of phosphorylation upon entrance of CagA into gastric cells and are essential for interaction between the CagA protein and the host cell SHP-2 phosphatase (Higashi et al. 2002b). It is well known that the EPIYA-D or EPIYA with more C repeats is associated with increased SHP-2 phosphatase activity, a higher degree of atrophic gastritis and an increased risk of gastric cancer (Naito et al. 2006; Basso et al. 2008; Batista et al. 2011).

The *H. pylori* vacuolating cytotoxin (*vac*A) gene encodes a pro-toxin of approximately 140 kDa, including an amino-terminal signal peptide and a carboxyl-terminal domain (Telford et al. 1994; Harris et al. 1996). The cleavage of the protein during secretion results in toxin monomers of 88 kDa comprising two subunits, p33 required for membrane channel formation and p55 required for VacA binding to cells (Nguyen et al. 2001; Torres et al. 2004). After interacting with its target host cells receptor(s), the VacA evokes a cascade of events including induction of large cytoplasmic vacuoles, mitochondrial damage and inhibition of T-cell activation (Kuck et al. 2001; Gebert et al. 2003; Nakayama et al. 2004); all considered to be relevant events in the genesis of *H. pylori* associated diseases (Palframan et al. 2012; Leunk et al. 1988; Atherton and Blaser 2009; Wen and Moss 2009). There is accumulating evidence that VacA could be the prototype of a new class of monofunctional A-B toxins in which the A subunit exhibits pore-forming instead of enzymatic activity (Boquet and Ricci 2012). The *vacA* gene is highly polymorphic and sequence variations occur in the signal region encoding the signal peptide and mature protein *N*-terminus (s1 and s2), in the intermediate region encoding part of the p33 subunit (i1, i2), in the protein cleavage site (d1 and d2) and in the middle region encoding part of the p55 epithelial cell binding domain (m1 and m2) (Atherton et al. 1995; Rhead et al. 2007; Atherton and Blaser 2009; Ogiwara et al. 2009). Strains s1m1 produce large amounts of VacA toxin, s1m2 produce moderate amounts and s2m2 produce little or no toxin. *vac*A s1, i1, d1 and m1 have been associated with increased risk of gastric cancer and peptic ulcer (Blaser 1994; Atherton et al. 1995; van Doorn et al. 1999; De Gusmao et al. 2000; Ashour et al. 2002; Rhead et al. 2007; Ogiwara et al. 2009; Ferreira et al. 2012).

The duodenal ulcer promoting gene, *dup*A, was described as the first putative disease specific *H. pylori* marker due to its

association with an increased risk of developing duodenal ulcer and protection against gastric atrophy, intestinal metaplasia and gastric carcinoma (Lu et al. 2005; Moura et al. 2012). However, as observed for other *H. pylori* virulence markers, the association between *dupA* and disease is dependent on the geographic origin of the *H. pylori* strains (Argent et al. 2004; Hussein et al. 2008; Schmidt et al. 2009). The *dupA* consists of two *virB4* homologous genes, *jhp0917* and *jhp0918*, located in the plasticity region of the *H. pylori* genome, that form one continuous ORF by the insertion of a T or C base after the position 1,385 in the *jhp0917* 3′ region that results in a frameshift allowing the reading of the intact *virB4* gene.

All *H. pylori* adhesins coded by *omp* genes belong to the Hop group of proteins. *H. pylori* displays several mechanisms to generate diversity in the OMPs. BabA, blood group antigen binding adhesin, encoded by the *babA2* gene binds to fucosylated Lewis^b antigen (Le^b) on the surface of gastric epithelial cells (Boren et al. 1993, 1994; Ilver et al. 1998; Gerhard et al. 1999). Patients infected by *H. pylori* strains expressing polymorphisms in the promoter region of *babA2* usually in association with *cagA* and *vacA* s1 alleles are at increased risk of gastric cancer (Gerhard et al. 1999; Yamaoka 2012). Another mechanism relies on slipped-strand mispairing, which involves different numbers of CT dinucleotide repeats in homopolymeric motifs located in the gene promoter region or in the 5′ gene sequence (switch "on," functional and "off" nonfunctional). SabA (sialic acid binding adhesin) binds to sialylated carbohydrate structures, sialyl-Lewis^x, which are upregulated as complex gangliosides in inflamed gastric tissue. It has been postulated that SabA contributes to the persistence of the infection (Aspholm et al. 2006) and has been associated with an increased risk of gastric cancer (Yamaoka et al. 2006). An active OipA (outer inflammatory protein) "on" is associated with a more pronounced infiltration of neutrophils on the gastric mucosa. High density of *H. pylori oip*A "on" strains is associated with an increased risk of developing duodenal ulcer disease and gastric cancer (Yamaoka et al. 2006; Yamaoka 2012). *homB* and its paralogous *hom*A genes, observed as a single or double copy in the *H. pylori* genome, code a putative OMP adhesin. Strains harboring *homB* have been associated with peptic ulcer disease in children (Oleastro et al. 2006, 2008, 2009).

H. pylori strains from children with duodenal ulcer share a similar proteome profile in which motility-associated and anti-oxidant proteins and those involved in the metabolism of glucose, amino-acids and urease are over-expressed as compared with the profile of strains from children with gastritis alone (Vitoriano et al. 2011). Comparative analysis of the proteome of a non-carcinogenic *H. pylori* strain and its in vivo derived strain that induces gastric cancer in Mongolian Gerbils and INS-GAS mice has demonstrated downregulation of the expression of 26 proteins. Of note, the presence of an arginine instead of a cysteine residue at position 296 of the carcinogenic strain is associated with changes in flagellar adhesiveness and morphology, resulting in a higher motility in the carcinogenic as compared with the non-carcinogenic strain (Franco et al. 2009).

Other Helicobacters

Sequencing of the genomes of a number of *Helicobacter* species has allowed for further elucidation of potential virulence factors within this genus. This has allowed researchers to highlight similarities and differences of the other species with the well-recognized pathogen *H. pylori* and other closely related pathogens such as *C. jejuni*. In common with *H. pylori* all gastric helicobacters have been shown to possess similar colonization factors including those for motility/flagella and urease as described previously. By comparison, while flagella and motility are important for enterohepatic helicobacters to colonize their niche of intestinal mucus, urease is no longer critical and many of these organisms are urease negative. The following is a more detailed description of some of the virulence factors of the individual species.

While the genome of *H. acinonychis* is very similar to that of *H. pylori* Eppinger et al. found a number of differences which they suggest happened when the gastric bacterium 'jumped' from one host to another, i.e. from humans to felines (Eppinger et al. 2006). Two possible mechanisms have been described in *H. acinonychis* that may have helped it to avoid the immune responses of their 'new' host. Fragmented genes were frequently found with some of these encoding outer membrane proteins, restriction or modification enzymes, transport systems, transposases, fucosyl transferases and two VacA proteins. In addition, genes implicated in the sialylation of bacterial cell surface polysaccharides, which in other pathogens has been shown to facilitate the avoidance of complement-mediated phagocytosis, were found to be specific to *H. acinonychis*. Other differences to *H. pylori* included the finding of two prophages, three insertion sequence elements, a 3,661 bp plasmid (pHac1) and a genomic Island (HacGI integron).

The genome of *H. mustelae* has been reported to contain homologs of a number of putative virulence determinants of *H. pylori* (NapA, plasminogen adhesion and collagenase) and *C. jejuni* (invasion antigen CiaB and adhesion Peb4a) (O'Toole et al. 2010). In contrast, orthologs of *cagA, vacA, babA, sabA* and *oipA*, key *H. pylori* virulence determinants, were absent. *H. mustelae* was also found to encode a unique group of virulence-related genes, including a haemagglutinin/haemolysin protein, and a glycosyl transferase for producing blood group A/B on its lipopolysaccharide as well as four secretion systems. An unusual finding was the identification of 10 predicted autotransporter genes of which only one, the *hsr* gene, has a known function. The *hsr* gene encodes for a protein found in an array of surface rings, responsible for antigenic variation and unique to *H. mustelae*, which has been implicated in its ability to induce gastric inflammation (O'Toole et al. 1994; Patterson et al. 2003).

H. felis was shown to lack a *cag*-PAI, VacA, and a cytolethal distending toxin (Arnold et al. 2011). However, in addition to the previously mentioned type III secretion system associated with flagella, the *H. felis* genome was found to harbor only one other secretion system, the *com*B regulon. This encodes T4SS components *com*B2, 3, 4, 6, 8, 9, 10, which have been shown to be required for the natural competence of *H. pylori*

(Hofreuter et al. 1998, 2003; Karnholz et al. 2006). The organization of the *com*B components in *H. felis* differs from that in *H. pylori*, the *H. felis* orthologs being detected in three operons spread across the genome, as opposed to being organized in two operons, encoding *com*B2-4 and *com*B6-10, respectively in *H. pylori* (Arnold et al. 2011).

NapA, an *H. pylori* virulence factor involved in immunomodulation (Yoshida et al. 1993), has also been identified in *H. felis*. It has been reported that NapA protects *H. pylori* from oxidative stress damage, and that its production is influenced by the ferric uptake regulator (Fur) (Cooksley et al. 2003), which is also present in the *H. felis* genome. In addition to Fur, orthologs of the *Escherichia coli* Fec and Feo siderophore-mediated iron uptake systems, multiple copies of *frp* genes encoding heme- or lactoferrin-binding proteins and the non-heme iron storage protein ferritin have been detected in *H. felis* (Arnold et al. 2011). Additional transcriptional regulators identified in the *H. felis* genome include the nickel response repressor (NikR) and the carbon storage regulator (CsrA).

Three enzymes, a collagenase, secreted serine protease HtrA and g-glutamyl transpeptidase (Gong et al. 2010; Rossi 2012, p. 523; Hoy et al. 2010), also present in *H. pylori* and implicated in virulence, have also been detected in *H. felis*. Further, 52 outer membrane proteins belonging to the Hor, Hop, Hof, and Hom gene families have been detected.

The *H. felis* genome, like other sequenced *Helicobacter* genomes, has a limited number of transcriptional regulators, these being three sigma factors, **r**54/rpoN, **r**70/rpoD, and **r**28/FliA. FliA, as well as the anti-sigma factor FlgM of *H. felis*, contribute to the regulation of the *fla*/*fli*/*flg*/*flh* motility regulon. In addition the CstA regulator of the "stringent response" to carbon starvation has been identified in the *H. felis* genome.

The genome of *H. suis* (Vermoote et al. 2011) contains components of the *comB* transport apparatus and *virB* and *virD4* ATPases, homologous to the *H. pylori* T4SS. Only two genes homologous to the *cag*-PAI of *H. pylori* have been identified in *H.* suis, *cagE* and *cagX*, but *virB*- and *virD*-type ATPases, all members of the *H. pylori* type IV secretion system 3 (*tfs*3), are detected in the genome of *H. suis*. *fldA*, *napA*, *ggt* and the pyruvate oxidoreductase complex genes, homologous to *H. pylori*, were also detected in *H. suis*. The NapA protein that in *H. pylori* plays a role in neutrophil recruitment and stimulates the production of reactive oxygen species and gastric expression of IL-12 as well as playing a role in iron storage is also detected in the *H. suis* genome. A membrane-associated homolog of the virulence factor MviN in *H. acinonychis* was also detected in *H. suis*. Although the *H. suis* genome contains a *vacA* gene homologous to *H. pylori*, it lacks the signal sequence. In addition, it also lacks Bab and Sab adhesins.

Schott et al. and Smet et al. described many of these same virulence factors in *H. bizzozeronii* (Schott et al. 2011a) and *H. heilmannii* (Smet et al. 2012b) respectively. These included the γ-glutamyl transpeptidase, the immunomodulator NapA, the flavodoxin FldA, the plasminogen binding proteins PgbA and PgbB, the collagenase PrtC, the tumor necrosis factor alpha-inducing protein (Tipα), the proline oxidase PutA, the secreted serine protease HtrA and a peptidyl propyl-cis, trans-isomerase involved in macrophage activation. In common with the other closely related members of *H. heilmannii* s.l. these organisms were also found to lack the *cag*-PAI, as well as orthologs for VacA, BabA, BabB and SabA. They did however contain a secretion system associated with flagella and the T4SS components *com*B2, 3, 4, 6, 8, 9, and 10. A potentially new virulence factor, a polysaccharide lysase, which in other pathogens may be important in the early stages of infection (Makris et al. 2004), was also described in *H. bizzozeronii* however its role in helicobacter infections remains to be elucidated.

The H. hepaticus genome lacks an ortholog of the *H. pylori* vacuolating cytotoxin gene (*vacA)* but was shown to contain a cluster of genes (*cdtABC*) that encode a cytolethal distending toxin, which in *C. jejuni* has been associated with cell cycle arrest, chromatin fragmentation, and apoptotic cell death via a type I DNase-like activity. This toxin was shown to induce intestinal epithelial cell death via the mitochondrial pathway (Liyanage et al. 2010). Apart from three proteins with homology to basic components of a T4SS, *H. hepaticus* contains no orthologs of genes present in the *H. pylori* CagPAI and no evidence of a type III secretion system, apart from its flagellar apparatus (Suerbaum et al. 2003). While the Bab and Sab *H. pylori* adhesins were not detected an ortholog of a *C. jejuni* adhesion, PEB1, was detected.

The *H. hepaticus* genome contains one large region as well as numerous smaller regions that differ in their G+C content from the rest of the chromosome. The largest region, the *H. hepaticus* genomic island 1 (HHGI1) has a G+C content of 33.2 mol% and contains 70 ORFs. The majority of genes within the HHGI1 encode hypothetical proteins however three proteins with homology to structural components of type VI secretion systems (T6SSs) have been identified on the HHGI1 as well as a gene with homology to *Vibrio cholerae hcp*, which encodes a secreted protein coregulated with the *V. cholerae* hemolysin. In contrast to most other PAIs, HHGI1 is not associated with a tRNA gene, and is not flanked by direct repeats. However, HHGI1 contains a prophage P4-like integrase gene (HH269), which has been reported in several PAIs. Studies in mice have demonstrated a role for this PAI in the development of hepatitis in these animals, however it is not required for colonization (Boutin et al. 2005; Ge et al. 2008b).

Sequencing of the genome of *H. canadensis* has identified a range of virulence factors including an immunoglobulin A (IgA) protease, and two homologues of the vacuolating cytotoxin of *H. pylori* (Loman et al. 2009). In addition, a capsular polysaccharide export locus, similar to that in *C. jejuni*, was identified. This is the first evidence of a polysaccharide capsule in *Helicobacter* spp. In *C. jejuni* this export locus has been reported to encode orthologs of KpsS, KpsD, KpsE, KpsT, KpsM and KpsC (Parkhill et al. 2000). An annotated sialyltransferase (*siaD*) has been identified just outside this locus which, given the importance of capsule sialylation in the virulence of *Neisseria meningitidis and* group B *Streptococci* (Wessels et al. 1989; Estabrook et al. 1992), suggests that this

sialyltransferase may play an important role in *H. canadensis* virulence. In addition, two strong, frame-shift candidates for translational phase variation were observed to flank this sialyltransferase. These encoded a hypothetical protein and a polysaccharide deacetylase family protein, respectively. A region of the genome, homologous to the HHGI1 genomic island of *H. hepaticus* was also noted. Genes encoding an N-linked glycosylation pathway, including two copies of PglB similar to that found in *C. jejuni*, were also observed in *H. canadensis* (Loman et al. 2009). Genes for this glycosylation pathway have also been found in *H. winghamensis* and *H. pullorum* with studies of the latter highlighting similarities and differences of this system in the helicobacters compared to *C. jejuni* (Jervis et al. 2010).

A genomic island (HciGI1), reported to contain 12 genes involved in the assembly of a T6SS, was also identified in the genome of *H. cinaedi* PAGU611 (Goto et al. 2012). These T6SS genes were shown to be homologous to those found in *H. hepaticus* (33.7–83.4 % amino acid sequence identity). Interestingly the *H. hepaticus* T6SS was shown to limit intestinal colonization of germ free mice and prevent intestinal inflammation during colonization (Chow and Mazmanian 2010). The *H. cinaedi* chromosome was also shown to encode two clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (cas) systems that confer immunity to host cells against foreign DNA. Of these, one is encoded on HciGI1. The *H. mustelae* chromosome also encodes a CRISPR/cas system similar to that in the *H. cinaedi* HciGI1. In addition the *H. cinaedi* strain PAGU611 was shown to encode a number of known and potential virulence factors including a cytolethal distending factor (Shen et al. 2009), an alkyl hydroperoxide reductase (Charoenlap et al. 2012), NapA and a homolog of *C. jejuni* invasion antigen B (Dasti et al. 2010). Interestingly the latter was also detected in the *H. mustelae* genome (O'Toole et al. 2010).

Further elucidation of virulence factors associated with the EHS will follow as more genomes are sequenced and annotated with studies of the *H. bilis, H. pullorum* and *H. winghamensis* genomes currently underway. However the most prominent virulence factor for this group of organisms identified so far if the cytolethal distending toxin (CDT). In addition to being detected in *H. hepaticus* and in human and animal isolates of *H. cinaedi* it has also been characterized in *H. bilis, H. canis, H. pullorum* (both human and animal isolates), *H. marmotae* and *H. mastomyrinus* (Chien et al. 2000; Young et al. 2000a, b; Kostia et al. 2003; Taylor et al. 2003; Shen et al. 2005).

Genus *Wolinella*

Genus *Wolinella* Tanner et al. 1981, gen. nov. (N.L. fem. dim. n. *Wolinella*, named after M.J. Wolin, American bacteriologist who first isolated the type species).

Type species: *Wolinella succinogenes* (Wolin et al. 1961) Tanner et al. 1981.

Molecular Analyses

Phylogeny

The overall phylogenetic position of *W. succinogenes* relative to other bacteria was determined from 16S rRNA sequencing. This indicated that *Wolinella* falls in the family *Helicobacteraceae*.

Genome

The genome of *W. succinogenes* DSM 1740 is 2.11 Mb in size and has a G+C content of 48.5 mol%. The genome contains 2,092 predicted genes which encode 2,043 proteins (9 rRNA sequences; 40 tRNA sequences). The genome contains an insertion sequence IS*1302* which is 1,306 bp in size, with 36-bp imperfect terminal repeats and has a G+C content 33.8 mol%.

Transformation of *W. succinogenes* cells with plasmid DNA has been achieved by electroporation under anaerobic conditions (Krafft et al. 1995; Simon and Kröger 1998). The cells are washed with 0.3 M sucrose and suspended in growth medium. Cells are electroporated (25 μF capacity, 1.5 kV, 800 ω resistance) after addition of plasmid DNA (5–10 μg). Bacteria are incubated for 2 h at 37 °C, and subsequently plated on agar medium containing the appropriate selective antibiotic.

Phenotypic Analyses

Cells of *W. succinogenes* are non-spore forming Gram-negative rods with a rapid, darting motility via a single polar flagellum. The cells are helical, curved or straight, 0.5–1.0 μm in diameter and 2–6 μm in length, with rounded or tapered ends. Colonies are pale yellow opaque to gray translucent. When grown with nitrate as terminal electron acceptor, colonies appear reddish.

Cells of *W. succinogenes* grow at 37 °C, have oxidase activity, and produce hydrogen sulfide, but are asaccharolytic and are negative in many routine biochemical tests. Common characteristics for *W. succinogenes* are listed in ❯ *Table 24.9*.

Type strain: ATCC 29543^T = NCTC 11488^T = DSM 1740^T = FDC 602 W^T.

Genbank accession number (16 s rRNA): DQ174165.

Physiology

The cells grow only by anaerobic respiration and do not ferment carbohydrates. Fumarate, nitrate, nitrate, N_2O, polysulfide or DMSO can serve as terminal acceptor with formate as the electron donor. Molecular hydrogen and, at least in fumarate respiration, sulfide are alternative electron donor substrates. Sulfate, thiosulfate, organic disulfides (RSSR) and trimethylamine-N-oxide (TMAO) are not used as terminal electron acceptors. The ATP required for growth is synthesized by oxidative phosphorylation. The reactions sustaining growth of *W. succinogenes* are summarized in ❯ *Table 24.10*. Fumarate

Table 24.9
Characteristics common to *Wolinella succinogenes* (Simon et al. 2006)

Positive	Gram-negative rods; motile via a single polar flagellum; growth in broth supplemented with formate and fumarate; growth in anaerobic and microaerobic atmospheres; reduction of nitrate, nitrite, neutral red, and benzyl viologen; oxidase and benzidine positive; production of hydrogen sulfide, hydrogen, carbon dioxide and succinate; growth in the presence of NaF (0.5 g/l), Oxgall (10 g/l), deoxycholate (1 g/l), Janus green (0.1 g/l), basic fuchsin (0.032 g/l), crystal violet (0.005 g/l), Evans blue (0.05 g/l), malachite green (0.02 mg/l), brilliant green (0.0125 g/l), penicillin (4–16 μg/ml), polymyxin B (2–4 μg/ml), and kanamycin (1, 2 or 4 μg/ml); arginine aminopeptidase positive.
Negative	No growth in unsupplemented broth media or in media supplemented with only 0.2 % nitrate; no growth on agar surfaces in air or in air containing 10 % CO_2; no spores formed; not haemolytic; pH is not lowered when cells are incubated in formate- and fumarate-supplemented media with 1 % adonitol, 1 % amygdalin, 1 % arabinose, 1 % dextran, 1 % dulcitol, 1 % fructose, 1 % galactose, 1 % glucose, 1 % glycerol, 1 % glycogen, 1 % inositol, 1 % inulin, 1 % lactose, 1 % maltose, 1 % mannitol, 1 % mannose, 1 % melezitose, 1 % melibiose, 1 % raffinose, 1 % rhamnose, 1 % ribose, 1 % salicin, 1 % sorbitol, 1 % sucrose, 1 % trehalose, 1 % xylose, 0.25 % esculin, or 0.5 % starch; catalase negative; lysine, ornithine, and arginine not decarboxylated; starch, dextran, esculin, casein, DNA and gelatin not hydrolyzed; hydrogen peroxide, urease, lecithinase, lipase, indole, and acetylmethylcarbinol not produced; nitrous oxide, methane, formate, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, caproate, lactate, and pyruvate not detected; gas not formed under an agar layer over a broth culture; no growth in the presence of NaCl (20 or 40 g/l), indulin scarlet (0.5 g/l) or penicillin (64 μg/ml); negative or weak reactions in API ZYM and API AN-Ident tests, except for arginine aminopeptidase.

Table 24.10
Reactions sustaining growth of *Wolinella succinogenes* (Simon et al. 2006)

$HCO^-_2 + Fumarate + H^+ \rightarrow CO_2 + Succinate$
$HCO^-_2 + NO^-_3 + H^+ \rightarrow CO_2 + NO^-_2 + H_2O$
$3HCO^-_2 + NO^-_2 + 5H^+ \rightarrow 3CO_2 + NH^-_4 + 2H_2O$
$HCO^-_2 + N_2O + H^+ \rightarrow CO_2 + N_2 + H_2O$
$HCO^-_2 + [S] \rightarrow CO_2 + HS^-$
$HCO^-_2 + (CH_3)_2SO + H^+ \rightarrow CO_2 + (CH_3)_2S + H_2O$
$HS^- + Fumarate + H^+ \rightarrow [S] + Succinate$

reductase, hydrogenase, formate dehydrogenase, nitrite reductase and polysulfide reductase are the key enzymes of fumarate, nitrite and polysulfide respiration, with molecular hydrogen or formate serving as electron donor. Nitrate ammonification using formate or H_2 as electron donors is dependent on the periplasmic nitrate reductase NapA and the cytochrome *c* nitrite reductase NrfA (Simon et al. 2003). This mode of respiration is independent of NapC (NrfH and FccC in *W. succinogenes*) and depends on the gene cluster napAGHBFLD (Kern et al. 2007). More recently, NosH which is involved in N_2O reduction, has been shown to donate electrons to the respiratory chain of nitrate respiration, and thus, replace NapGH (Kern and Simon 2008). Kern et al. have found that *W. succinogenes* is able to grow by sulfite respiration with formate as electron donor (Kern et al. 2011a). Sulfite is reduced to sulfide by the periplasmic cytochrome *c* sulfite reductase MccA, while MccB, MccC and MccD are all essential for sulfite respiration. The expression of this system is induced by sulfite but repressed in the presence of fumarate or nitrate (Kern et al. 2011a).

Fumarate can be used as the sole source of carbon during growth by fumarate respiration (Bronder et al. 1982). It can be replaced by L-malate, L-aspartate or L-asparagine (Wolin et al. 1961; Kafkewitz 1975). The growth of the bacterium by fumarate respiration doubles in the presence of glutamate. When nitrate is employed as terminal acceptor, succinate is required as a carbon source (Bokranz et al. 1983). Succinate can be substituted by malate, fumarate or L-asparagine but not oxaloacetate, pyruvate, bicarbonate, acetate, propionate, butyrate, L-glutamate, D-aspartate or δ-aminolevulinic acid. Acetate and glutamate can be used as carbon sources during polysulfide respiration. As a source of sulfur, sulfate can be used for fumarate respiration, while sulfide or cysteine can be employed for nitrate respiration (Bokranz et al. 1983).

The components of the oxidative and nitrosative defence network of *W. succinogenes* have been identified by Kern et al. (2011b). The two periplasmic multihaem *c*-type cytochromes cytochrome *c* peroxidase and NrfA mediated resistance to hydrogen peroxide. Two AhpC-type peroxiredoxin isoenzymes were involved in protection against different organic hydroperoxides, while the two superoxide dismutases play a role in oxygen and superoxide stress defence. NrfA and the flavodiiron protein Fdp are as key components of nitric oxide detoxification, while NrfA mediates resistance to hydroxylamine stress.

Isolation, Enrichment and Maintenance Procedures

W. succinogenes can be cultivated using minimal media containing appropriate electron donor and electron acceptor substrates according to the various modes of anaerobic respiration. The most commonly used growth conditions consist of a broth medium containing formate and fumarate. Fumarate can be replaced by either nitrate or polysulfide. For growth with H_2, formate is left out of the medium. Further media include growth with formate and nitrous oxide (N_2O) (Yoshinari 1980),

formate and nitrite (Lorenzen et al. 1993), formate and dimethyl sulfoxide (DMSO) (Lorenzen et al. 1994), or sulfide and fumarate (Macy et al. 1986). Media may be supplemented with 0.5 % (w/v) yeast extract or 0.5 % (w/v) brain heart infusion broth which raises the growth rate slightly.

Ecology

W. succinogenes was isolated from an inoculum of bovine rumen fluid (Wolin et al. 1961). The bacterium has also been isolated from sewage (Yoshinari 1980; Tanner et al. 1984) and from humans (Radcliffe et al. 1979). *Wolinella* DNA has been detected in the oral cavity of humans (Silva et al. 2010), dogs (Petersen et al. 2007; Craven et al. 2011), and cats (Petersen et al. 2007). In 2006, Zhang et al. detected organisms closely related to *W. succinogenes* in the colon of children (Zhang et al. 2006). "*Candidatus* Wolinella africanus" DNA was detected in the upper digestive tract of 76 % of Venezuelan volunteers (aged 18–68 years, 41 females, 50 males) (García-Amado et al. 2007).

Pathogenicity, Clinical Relevance

While *W. succinogenes* is classified as a non-pathogen, the genome sequencing of this bacterium revealed that this organism shares 1,269 of its 2,046 genes with *H. pylori* and *C. jejuni*, many of which were identified as virulence factors (Baar et al. 2003).

W. succinogenes was detected in gastric biopsies from a sea lion with gastritis (Oxley et al. 2004). In 2003, Bohr et al. detected "*Candidatus* Wolinella africanus" DNA from esophageal mucosa biopsies of South African patients suffering from stenosing squamous cell carcinoma (Bohr et al. 2003). Hussey et al. also detected "*Candidatus* Wolinella africanus" DNA from one hyperplastic adenoid in a child with laryngopharyngeal reflux (Hussey et al. 2011).

Genus *Sulfuricurvum*

Genus *Sulfuricurvum* Kodama and Watanabe 2004, gen. nov. (L. neut. n. *sulfur -uris*, sulfur; L. adj. *curvus -a -um*, curved; N.L. neut. n. *Sulfuricurvum*, curved bacterium that utilizes sulfur).

Type species: *Sulfuricurvum kujiense* Kodama and Watanabe 2004.

Sulfuricurvum is a monotypic genus of the family *Helicobacteraceae* (Han et al. 2012). *Sulfuricurvum kujiense* (N.L. neut. adj. *kujiense*, referring to Kuji, Iwate Prefecture, Japan, where the bacterium was isolated) strain YK-1 was isolated from drain water from an underground crude-oil storage cavity at Kuji in Iwate, Japan (Kodama and Watanabe 2004). It is the type strain of the only species within this genus. Sequences with high homology to *S. kujiense* have also been detected in the iron- and sulfur-rich freshwater nature reserve De Bruuk in The Netherlands (Haaijer et al. 2008), an Athabasca oil sands reservoir in western Canada containing severely biodegraded oil (Hubert et al. 2012), meromictic lake sediments (Nelson et al. 2007), chemoclines (Hubert et al. 2012), sulfidic caves and springs (Porter and Engel 2008), anoxic bottom waters of a volcanic lake in Iceland, a water well field adjacent to the North Saskatchewan River in Canada (Medihala et al. 2012a), and other autotrophic denitrification conditions (Sahu et al. 2009; Zhao et al. 2011).

S. kujiense is a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing, motile, curved rod which has a single polar flagellum (Kodama and Watanabe 2004). Optimum growth occurs in a low-strength salt medium at pH 7.0 and 25 °C. The bacterium utilizes sulfide, elemental sulfur, thiosulfate and hydrogen as the electron donors and nitrate as an electron acceptor under anaerobic conditions (Kodama and Watanabe 2004). Oxygen may serve as the electron acceptor under microaerobic conditions (1 %). *S. kujiense* does not grow on sugars, organic acids or hydrocarbons as carbon and energy sources (Kodama and Watanabe 2004). The genome of *S. kujiense* YK-1 (DNA coding region: 93.04 %; G+C content: 44.56 mol%) consists of a circular chromosome 2,574,824 bp in size and four plasmids 118,585 bp, 71,513 bp, 51,014 bp, and 3,421 bp in size, harboring a total of 2,879 protein-coding and 61 RNA genes (Han et al. 2012).

Type strain: YK-1^T = JCM 11577^T = MBIC 06352^T = ATCC BAA-921^T

Genbank accession number (16 s rRNA): CP002355.

Genus *Sulfurimonas*

Genus *Sulfurimonas* Inagaki et al. 2003, gen. nov. (L. neut. n. *sulfur*, sulfur; Gr. n. *monas*, a unit, monad; N.L. fem. n. *Sulfurimonas*, sulfur-oxidizing rod).

Type species: *Sulfurimonas autotrophica* Inagaki et al. 2003.

In 2003, a novel mesophilic, sulfur- and thiosulfate-oxidizing bacterium, *Sulfurimonas autotrophica* (Gr. n. *autos*, self; Gr. adj. *trophikos*, nursing, tending or feeding; N.L. fem. adj. *autotrophica*, autotroph) strain OK10^T (= ATCC BAA-671^T = JCM 11897^T), was isolated from deep-sea sediments at the Hatoma Knoll in the Mid-Okinawa Trough hydrothermal field (Inagaki et al. 2003). *S. autotrophica* are motile short rods which possess a single polar flagellum and grow chemolithoautotrophically with elemental sulfur, sulfide and thiosulfate (oxygen as electron acceptor) at 10–40 °C (optimum 25 °C) and pH 4.5–9.0 (optimum pH 6.5) (Inagaki et al. 2003). *S. autotrophica* strain OK10 has a genome 2,153,198 bp in size with genome with 2,165 protein-coding and 55 RNA genes and a G+C content of 35.2 mol% (Sikorski et al. 2010) and its Genbank accession number (16 s rRNA) is CP002205.

Another novel mesophilic bacterium, *Sulfurimonas paralvinellae* (N.L. gen. n. *paralvinellae*, of *Paralvinella*, a genus of annelid polychaetes from which the organism was first isolated) strain GO25^T (= JCM 13212^T = DSM 17229^T), was isolated from a nest of hydrothermal vent polychaetes, *Paralvinella* sp., at the Iheya North field in the Mid-Okinawa

Trough (Takai et al. 2006). Similar to *S. autotrophica*, *S. paralvinellae* cells are motile short rods which possess a single polar flagellum; however, the bacteria grew chemolithoautotrophically with molecular hydrogen, elemental sulfur and thiosulfate (nitrate or oxygen as electron acceptor) at 4–35 °C (optimum 30 °C) and pH 5.4–8.6 (optimum pH 6.1) (Takai et al. 2006). The G+C content of the genomic DNA of *S. paralvinellae* GO25 is 37.6 mol% and its Genbank accession number (16 s rRNA) is AB252048.

In 2006 *Thiomicrospira denitrificans* (N.L. v. *denitrificare*, to denitrify; N.L. part. adj. *denitrificans*, denitrifying) strain DSM 1251^T (= ATCC 33889^T) was reclassified as *Sulfurimonas denitrificans* due to is phylogenetic relatedness to *S. autotrophica* and *S. paralvinellae* (Hoor 1975; Takai et al. 2006). In 2008, the genome of *S. denitrificans* DSM1251 (isolated from a tidal mud flat) was sequenced and found to be 2.2 Mbp in size (93.8 % coding, 34.5 mol% G+C, 4 rRNA operons, 2104 CDS) (Sievert et al. 2008). The bacterium had a complete, autotrophic reductive citric acid cycle and a branched electron transport chain, with genes encoding complexes for the oxidation of hydrogen, reduced sulfur compounds, and formate and the reduction of nitrate and oxygen. Its Genbank accession number (16 s rRNA) is CP000153.

With the inclusion of *S. denitrificans* into *Sulfurimonas*, bacteria within the genus were described as mesophilic and facultatively anaerobic straight to slightly short rods, elongated rods or spiral in different growth phases and under different growth conditions (Takai et al. 2006). Bacterial growth occurs chemolithoautotrophically with sulfide, S_0, thiosulfate and H_2 as electron donors and with nitrate, nitrite and O_2 as electron acceptors, using CO_2 as a carbon source (Takai et al. 2006). It has been suggested that the autotrophic denitrification activity of *Sulfurimonas* species may increase heavy metal mobility, and thus, their toxicity (Shao et al. 2009). The ecological niches of *Sulfurimonas* sp. are deep-sea hydrothermal environments and marine sulfidic environments, having been further detected in petroleum-contaminated groundwater discharged from underground crude oil storage cavities in Kuji, Iwate, Japan (Watanabe et al. 2000, 2002), pelagic and sulfidic areas of central Baltic Sea and Black Sea redoxclines (Brettar et al. 2006; Grote et al. 2007, 2008; Glaubitz et al. 2009, 2010; Fuchsman et al. 2012), the Veslefrikk and Gullfaks oil field in the North Sea (Bodtker et al. 2008), a water well field adjacent to the North Saskatchewan River in Canada (Medihala et al. 2012b), a lab-scale biotrickling filter treating high loads of hydrogen sulfide (Maestre et al. 2009, 2010), marine sediments in Victoria Harbor of Hong Kong (Zhang et al. 2009), the Jan Mayen vent field located on Mohn's Ridge in the Norwegian-Greenland Sea (Lanzen et al. 2011), the Enermark Medicine Hat Glauconitic C field in southeastern Alberta (Shartau et al. 2010), aquifers in Bitterfeld, Germany (Alfreider and Vogt 2012) the Southern Mariana Trough (Kato et al. 2012), and a chimney rock sample from the Mid-Atlantic Ridge (Muyzer et al. 1995).

In 2012, Grote et al. studied another putative *Sulfurimonas* species "*Sulfurimonas gotlandica*" strain GD1 which was isolated from the Baltic Sea oxic-anoxic interface where it plays a key role in nitrogen and sulfur cycling (Grote et al. 2012). *S. gotlandica* strain GD1 was shown to grow chemolithoautotrophically by coupling denitrification with oxidation of reduced sulfur compounds and dark CO_2 fixation (Grote et al. 2012). Whole genome analysis revealed a 2.95 Mb sequence (2,879 coding sequences, 4 rRNA operons, 47 tRNA) with 95.7 % 16S rRNA sequence identity to *S. denitrificans* DSM 1251, and high metabolic flexibility suggesting a considerable capacity for adaptation to variable redox conditions (Grote et al. 2012).

Genus *Sulfurovum*

Genus *Sulfurovum* Inagaki et al. 2004, gen. nov. (L. neut. n. *sulfur*, sulfur; L. neut. n. *ovum*, egg; N.L. neut. n. *Sulfurovum*, sulfur (−oxidizing) egg).

Type species: *Sulfurovum lithotrophicum* Inagaki et al. 2004.

Bacteria from the genus *Sulfurovum* have been detected at the Iheya North hydrothermal system in Japan (Inagaki et al. 2004; Nakagawa et al. 2005), the Jan Mayen vent field located on Mohn's Ridge in the Norwegian-Greenland Sea (Lanzen et al. 2011), the thermal springs Trollosen and Fisosen located on the High Arctic archipelago Svalbard (Reigstad et al. 2011), in sediments in the G11 pockmark at Nyegga in Norway (Roalkvam et al. 2011), in in situ reactor columns degrading benzene (Kleinsteuber et al. 2008), a granular sludge bed reactor (Chen et al. 2008), and in an aquifer in Rifle, Colorado (Handley et al. 2012).

In 2004, a mesophilic sulfur- and thiosulfate-oxidizing bacterium, *Sulfurovum lithotrophicum* (Gr. masc. n. *lithos*, stone; Gr. adj. *trophicos*, nursing; tending, feeding; N.L. neut. adj. *lithotrophicum*, feeding on inorganic substrates) strain 42BKT^T (= ATCC BAA-797^T = JCM 12117^T), was isolated from the gas-bubbling sediment at the Iheya North hydrothermal system in the mid-Okinawa Trough, Japan (Inagaki et al. 2004). The isolate grew chemolithoautotrophically with elemental sulfur or thiosulfate as a sole electron donor and oxygen or nitrate as an electron acceptor at 10–40 °C (optimum 28–30 °C) and in the pH range 5.0–9.0 (optimum 6.5–7.0) (Inagaki et al. 2004). *S. lithotrophicum* 42BKT is a non-motile and coccoid to oval-shaped bacterium which has a G+C content of 48.0 mol% (Inagaki et al. 2004) and its Genbank accession number (16 s rRNA) is AB091292.

Furthermore, in 2005, Nakagawa et al. reported the isolation of a bacterial species with phylogenetic relatedness to *S. lithotrophicum*, and subsequently, this isolate was named *Sulfurovum* sp. NBC37-1 (Nakagawa et al. 2005). The 16S rRNA gene sequence of strain NBC37-1 was 99.1 % similar with that of *S. lithotrophicum* 42BKT, however, the DNA-DNA hybridization value between these strains was ~42 %, indicating that strain NBC37-1 is a new species within this genus (Nakagawa et al. 2005). *Sulfurovum* sp. NBC37-1 is closely related to the endosymbiont of a hydrothermal vent gastropod *Alviniconcha* suggesting that it may have some symbiotic relationship with vent animals (Nakagawa et al. 2007). In 2007, the genome of strain NBC37-1 was analyzed and was found to have

a single circular chromosome 2,562,277 bp in size and a G+C content of 43.8 mol% (90.1 % coding, 3 rRNA operons, 2466 CDS) (Nakagawa et al. 2007). This strain is capable of growth via hydrogen and sulfur-compound oxidation under microaerobic and anaerobic conditions (Nakagawa et al. 2005), for which sulfur oxidation has been attributed to the *sox* pathway (Yamamoto et al. 2010).

Another sulfur-oxidizing *Sulfurovum* species, "*Candidatus* Sulfurovum sediminum" strain AR was isolated from a 78 m-deep marine sediment collected off Svalbard within the Arctic Circle (Park et al. 2012). The draft genome of this bacterium is 2.12 Mb (11 contigs, 2,248 predicted genes) in size, has a G+C content of 39.4 mol% and contains genes for sulfur oxidation and carbon fixation (Park et al. 2012). The average nucleotide sequence identity of strain AR contigs to *Sulfurovum* sp. strain NBC37-1 was ~73 %, suggesting strain AR may be a novel species of this genus (Park et al. 2012).

Genus *Thiovulum*

Genus *Thiovulum* Hinze 1913, genus. (Gr. n. *theion* (Latin transliteration *thium*), sulfur; L. n. *ovum*, egg; N.L. neut. dim. n. *Thiovulum*, small sulfur egg).

Type species: *Thiovulum majus* Hinze 1913 (Approved Lists 1980).

Thiovulum majus (L. neut. comp. adj. *majus*, larger) is the only species of this genus. Morphologically the cells are spherical with a diameter of 5–10 µm and flagella cover most of the cell surface (Fenchel 1994). The chemolithotrophic nature of *T. majus* was confirmed by Wirsen and Jannasch (Wirsen and Jannasch 1978) who grew the bacteria in open-flow cultures run with aerated seawater and a continuous supply of H_2S. However, these bacteria require the presence of both sulfide and oxygen (optimally ~4 % (Fenchel 1994)), a condition found in marine sulfidic sediments such as those in Golfo Dulce in Costa Rica (Kuever et al. 1996) and Nivå Bay in Denmark (Thar and Fenchel 2005), the active deep sea hydrothermal vent system Pele's Vents in Hawaii (Moyer et al. 1995), and granular sludge bed bioreactors (Scully et al. 2005). In order to remain at the preferred oxygen gradient, these bacteria have developed two mechanisms. *T. majus* can attach to a solid surface with a mucous stalk or remain free swimming and they are governed by a strong chemotactic response (Fenchel 1994), with mean cell speeds reported to reach 615 µm s^{-1} (Garcia Pichel 1989). Upon swimming outside of the preferred region, *T. majus* do not reverse their swimming direction but perform a "U-turn" by gradually changing their direction (Fenchel 1994). Moreover, *T. majus* has adapted to overcome the chemical oxidation of H_2S in the presence of O_2 (Jorgensen and Postgate 1982; Jorgensen and Revsbech 1983).

Further investigation of the motility of *T. majus* has shown the bacterium to swim a left-handed helix path (Fenchel 1994). This was established through the observations that *T. majus* is capable of orienting itself in oxygen gradients, and subsequently, the integration of the bacterium's phobic response as well as its chemotactic response into a single model of helical klinotaxis (Thar and Fenchel 2001; Thar and Kuhl 2005).

Type strain: no culture isolated.

Genbank accession number (16S rRNA): not available.

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25 The Family *Nautiliaceae*: The Genera *Caminibacter*, *Lebetimonas*, and *Nautilia*

Satoshi Nakagawa[1,2] · *Ken Takai*[2]
[1]Laboratory of Microbiology, Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan
[2]Subsurface Geobiology Advanced Research (SUGAR) Project, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Kanagawa, Japan

Abstract
Phylogenetically diverse chemoautotrophic *Epsilonproteobacteria* occur dominantly in various redoxclines such as deep-sea hydrothermal vents, stratified ocean, terrestrial sulfidic caves, and oil fields. Except the order *Nautiliales*, the taxonomy of chemoautotrophic *Epsilonproteobacteria* has not been well established. *Nautiliaceae*, the sole family within the order *Nautiliales*, consists of the genera *Caminibacter*, *Nautilia*, and *Lebetimonas*. Members of the family form deeply branched lineages in the 16S rRNA-based phylogenetic tree of *Epsilonproteobacteria*. They are Gram-negative motile rods with one or more polar flagella and do not form endospores. All members of the family are exclusively found in the close proximity to deep-sea hydrothermal vents. Members of the family are moderate thermophiles growing optimally between 40 °C and 60 °C. Under autotrophic conditions, *Nautiliaceae* members have an ability to grow anaerobically via respiratory S^0 reduction with H_2.

Taxonomy, Historical and Current

Short Description of the Family

Deep-sea hydrothermal vents host unique ecosystems fueled primarily by microbial chemosynthesis. In 1995, cultivation-independent studies revealed the dominance of diverse *Epsilonproteobacteria* in geographically separated deep-sea hydrothermal fields (Haddad et al. 1995; Moyer et al. 1995; Polz and Cavanaugh 1995). Comparison of the 16S rRNA gene sequences of deep-sea vent *Epsilonproteobacteria* identified six distinct clades, called groups A, B, D, E (relatives of marine *Sulfurospirillum* spp.), F, and G (Corre et al. 2001). To date, at least one pure culture within all these phylogenetic groups was obtained and characterized (Takai et al. 2003; Nakagawa et al. 2005a, b; Nakagawa and Takai 2008). Although there is massive diversity in the temperature range for growth, the phenotypes of members of deep-sea vent *Epsilonproteobacteria* are similar (Takai et al. 2003; Nakagawa et al. 2005a, b, c; Nakagawa and Takai 2008). All known deep-sea vent *Epsilonproteobacteria* have an ability to grow chemoautotrophically utilizing H_2 and/or sulfur compounds as an electron donor. Recent genome analyses of chemoautotrophic *Epsilonproteobacteria* have provided new insights into their versatile energy metabolism and evolution (Nakagawa et al. 2007; Sievert et al. 2008; Campbell et al. 2009; Sikorski et al. 2010; Anderson et al. 2011; Giovannelli et al. 2011; Grote et al. 2012).

Phylogenetic Structure of the Family and Its Genera

The family *Nautiliaceae* belongs to the *Epsilonproteobacteria* group D (Corre et al. 2001) and is the only validly described family of the order *Nautiliales* (Miroshnichenko et al. 2004). At present, this family is comprised of three genera based on the phylogeny of the 16S rRNA gene (*Table 25.1*). Cells of *Nautiliaceae* members are Gram-negative motile rods with one or more polar flagella and do not form endospores. Under autotrophic conditions, they grow anaerobically via respiratory S^0 reduction with H_2 (hydrogen sulfur autotrophy). The *Nautiliaceae* species exhibit phylogenetic similarities of 95.0–99.7 % to members within the family and 85.3–91.8 % to all other species of the *Epsilonproteobacteria*, indicating that the family can be clearly defined by 16S rRNA gene sequence comparisons (*Fig. 25.1*). All members of the family *Nautiliaceae* are moderate thermophiles which grow optimally between 40 °C and 60 °C and exhibit growth temperature maxima up to 70 °C (*Table 25.2*). Members of the family *Nautiliaceae*, together with other thermophilic deep-sea vent *Epsilonproteobacteria*

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(i.e., members of the genera *Hydrogenimonas* and *Nitratiruptor*), form deeply branched lineages in the 16S rRNA-based phylogenetic tree of *Epsilonproteobacteria*, and thermophily and chemoautotrophy may be primitive traits of *Epsilonproteobacteria* (Nakagawa and Takaki 2009).

Molecular Analyses

Genomic Features

The deep-sea vent epsilonproteobacterial genomes encode for multiple systems for respiration, sensing, and responding to environment and detoxifying heavy metals, reflecting their adaptation to the deep-sea vent environment (Nakagawa et al. 2007; Campbell et al. 2009; Sikorski et al. 2010; Anderson et al. 2011; Giovannelli et al. 2011). When compared to other deep-sea vent *Epsilonproteobacteria*, members of the family *Nautiliaceae* appear to have relatively small genomes. At present, the complete genome sequences are available for *Nautilia profundicola* (Campbell et al. 2009) and *Caminibacter mediatlanticus* (Giovannelli et al. 2011). Additionally, the genome of *Nautilia lithotrophica* is being sequenced, and draft sequences of *Lebetimonas* members from the Mariana Arc are also available. The genome of *N. profundicola* consists of a 1.68 Mb single circular chromosome containing 1745 protein-coding genes (CDSs). The genome contains genes for the reductive tricarboxylic acid (rTCA) cycle for CO_2 fixation, a diverse suite of hydrogenases, membrane-bound polysulfide reductase, and numerous stress response systems (Campbell et al. 2009). The genome of *Caminibacter mediatlanticus* consists of a 1.66 Mb single circular chromosome containing 1826 CDSs. These *Nautiliaceae* genomes, like another thermophilic deep-sea vent *Epsilonproteobacteria* (*Nitratiruptor* sp. SB155-2; Nakagawa et al. 2007), contain a gene encoding reverse gyrase (Rgy) which was thought to be a characteristic protein of hyperthermophiles (Campbell et al. 2009; Giovannelli et al. 2011). *Epsilonproteobacteria*-type rgy genes are present and expressed in bacterial communities residing in geographically distinct deep-sea vents (Campbell et al. 2009).

Except *Nautiliaceae*, the taxonomy of deep-sea vent *Epsilonproteobacteria* has not been well constructed, especially at family or higher taxonomic levels. As additional genome sequences become available, the comprehensive taxonomic framework of deep-sea vent *Epsilonproteobacteria* will be established.

Table 25.1
Taxa in the family *Nautiliaceae*

Genus	Species
Caminibacter	*hydrogeniphilus*[a]
	mediatlanticus[b]
	profundus
Lebetimonas	*acidiphila*[a]
Nautilia[a]	*abyssi*
	lithotrophica[a, c]
	nitratireducens
	profundicola[b]

Nomenclatural types are indicated with a superscript "a"
[b]Whole-genome sequence is available
[c]Whole genome is being sequenced

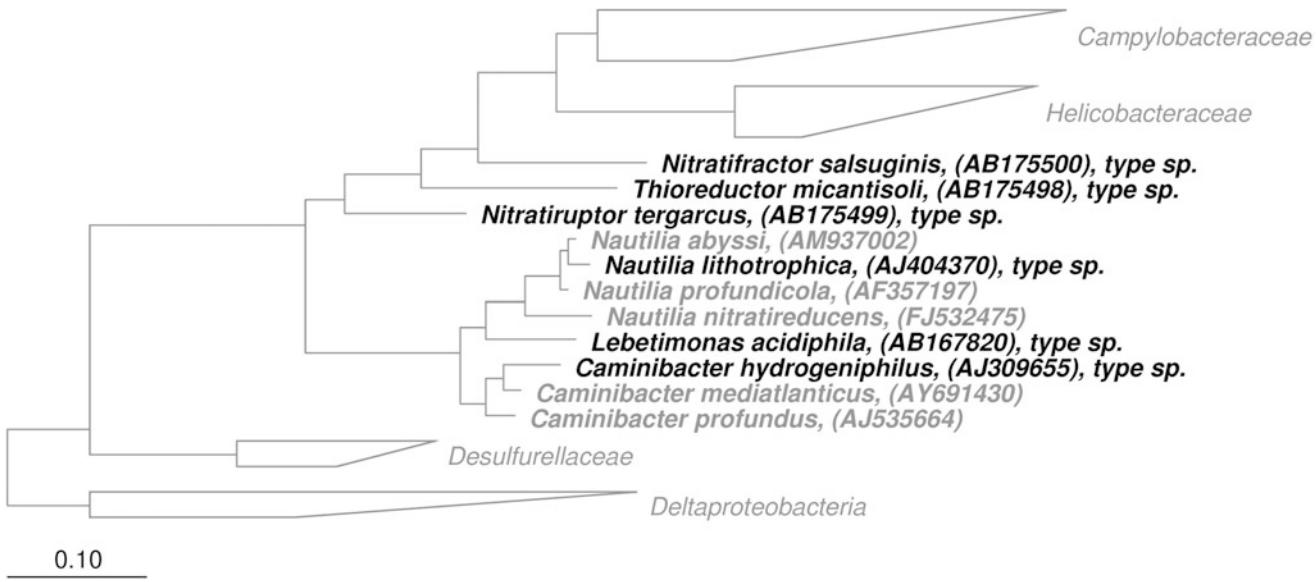

Fig. 25.1
Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis et al. 2008) of the sequences of all members of the family *Nautiliaceae* present in the LTP_106 (Yarza et al. 2010). The tree was reconstructed by using a subset of sequences representative of close relative genera to stabilize the tree topology. In addition, a 40 % conservational filter for the whole bacterial domain was used to remove hypervariable positions. The bar indicates 10 % sequence divergence

Phenotypic Analyses

Lebetimonas Takai et al. 2005

Le.be.ti.mo'nas. L. n. *lebes* cauldron; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Lebetimonas* cell from a cauldron.

The family *Nautiliaceae* was originally proposed to contain the genera *Nautilia* and *Caminibacter* (Miroshnichenko et al. 2004). Subsequently, Takai et al. (2005) isolated and described one additional genus, *Lebetimonas*. The genus *Lebetimonas* presently includes one species: *L. acidiphila*. This species, as implied by the name, is characterized by the acidic pH for its optimal growth (Table 25.2). Cells of *L. acidiphila* are highly motile rods with a mean length of 1.5–2.5 μm and width of approximately 0.6–0.8 μm. Their optimum temperature for growth is 50 °C (range, 30–68 °C); optimum pH, 5.2 (range 4.2–7.0); and optimum NaCl concentration, 2 % (range, 0.6–5.0 %) (Takai et al. 2005). The major cellular fatty acids are $C_{14:0}$ (4.3 %), $C_{14:0}$ 3-OH (9.8 %), $C_{16:0}$ (12.5 %), $C_{16:1}$ (9.7 %), $C_{17:0}$ (4.4 %), $C_{18:0}$ (26.5 %), $C_{18:1}$ (22.2 %), anteiso-$C_{19:0}$ (4.8 %), and $C_{19:1}$ (5.8 %). Nitrate, ammonium, or yeast extract is utilized as a nitrogen source. Under optimal growth conditions, the doubling time is about 120 min and the cell yield is 3–4 × 10^9 cells mL^{-1}. The type strain of the species is Pd55 (= JCM12420 = DSM 16356; Takai et al. 2005), which was isolated from an in situ colonization system deployed in a hydrothermal diffusing flow (maximum temperature of 78 °C) at the TOTO caldera in the Mariana Arc. The DNA G + C content is 34.0 mol%.

Caminibacter Alain et al. 2002

Ca'mi.ni.bac.ter. L. gen. n. *camini* of a chimney; N.L. n. *bacter* masc. equivalent of Gr. neut. n. *bakterion* rod, staff; N.L. n. *Caminibacter* rod from a hydrothermal chimney, relating to the origin of the type species.

The type genus of the family *Nautiliaceae* is *Caminibacter* (Alain et al. 2002), which includes three validly described species: *C. hydrogeniphilus* (Alain et al. 2002), *C. profundus* (Miroshnichenko et al. 2004), and *C. mediatlanticus* (Voordeckers et al. 2005). All validly described *Caminibacter* species have an ability to utilize nitrate as an electron acceptor (Table 25.2). *C. profundus* is the only facultative anaerobes within the family *Nautiliaceae* (Miroshnichenko et al. 2004), although other members are obligate anaerobes (Table 25.2). Cells of *C. profundus* are motile rods (1.2–1.5 × 0.5 μm). Optimum temperature for growth is 55 °C (range, 45–65 °C); optimum pH, 6.9–7.1 (range, 6.5–7.4); and optimum NaCl concentration, around 3 % (range, 0.5–5.0 %). Under optimal growth conditions, the doubling time is about 40 min and the cell yield is 7 × 10^8 cells mL^{-1}. The type strain of the species is CR (= DSM 15016 = JCM 11957; Miroshnichenko et al. 2004). The DNA G+C content is 32.1 mol %.

C. hydrogeniphilus is the type species of the genus *Caminibacter* (Alain et al. 2002). Cells are motile rods (1.0–1.5 × 0.5 μm). Optimum temperature for growth is 60 °C (range, 50–70 °C); optimum pH, 5.5–6.0 (range, 5.0–7.5); and optimum NaCl concentration, 2.0–2.5 % (range, 1.0–4.0 %). Poor heterotrophic growth was observed on complex organic substrates. Under optimal growth conditions, the doubling time is about 90 min and the cell yield is 2.5 × 10^8 cells mL^{-1}. The type strain of the species is AM1116 (= DSM 14510 = CIP 107140). The DNA G+C content is 29 ± 1 mol %.

Cells of *C. mediatlanticus* are rods approximately 1.5 μm in length and 0.75 μm in width. Optimum temperature for growth is 55 °C (range, 45–70 °C); optimum pH, 5.5 (range, 4.5–7.5); and optimum NaCl concentration, 3 % (range, 1.0–4.0 %). Colonies form on Phytagel plates. Acetate, lactate, formate, and peptone inhibit the growth. Under optimal growth conditions, the doubling time is about 7 h. The type strain of the species is TB-2 (= DSM 16658 = JCM 12641; Voordeckers et al. 2005). The DNA G+C content is 27.1 mol % (from genome sequence).

Nautilia Miroshnichenko et al. 2002

Nau.ti'li.a. N.L. fem. n. *Nautilia* from *Nautile*, the name of the French submersible used for the exploration and investigation of deep-sea hydrothermal areas.

The genus *Nautilia* (Miroshnichenko et al. 2002) harbors four validly described species: *N. lithotrophica* (Miroshnichenko et al. 2002), *N. profundicola* (Campbell et al. 2001; Smith et al. 2008), *N. abyssi* (Alain et al. 2009), and *N. nitratireducens* (Pérez-Rodríguez et al. 2010). When compared to members of the genera *Caminibacter* and *Lebetimonas*, *Nautilia* species are characterized by near-neutral optimal pH for their growth (Table 25.2). Additionally, *Nautilia* species exhibit a relatively high G+C content of their genomic DNA (Table 25.2). The type species of the genus *Nautilia* is *N. lithotrophica*. Cells of *N. lithotrophica* are motile rods and 0.4–0.75 μm × 1.1–2.0 μm in size. Optimum temperature for growth is 53 °C (range, 37–68 °C); optimum pH, 6.8–7.0 (range, 6.4–7.4); and optimum NaCl concentration, 3.0 % (range, 0.8–5.0 %). Milk-white colonies of 0.5–1.0 mm in diameter form on agar plates. Ammonium, nitrate, glutamate, yeast extract, gelatin, tryptone, and urea are used as nitrogen sources. Under optimal growth conditions, the doubling time is about 140 min. The type strain of the species is 525 (= DSM 13520; Miroshnichenko et al. 2002). The DNA G+C content is 34.7 mol%.

Cells of *N. profundicola* are motile rods approximately 0.4 μm in length and 0.3 μm in width. Optimum temperature for growth is 40 °C (range, 30–55 °C); optimum pH, 7.0 (range, 6.0–9.0); and optimum NaCl concentration, 3.0 % (range, 2.0–5.0 %). Major cellular fatty acids are $C_{18:1}$ (73.3 %), $C_{16:1}$ (12.7 %), and $C_{16:0}$ (12.3 %). Ammonium, nitrate, yeast extract, and peptone are used as nitrogen sources. Under optimal growth conditions, the doubling time is about 6 h. The type strain of the species is AmH (= DSM 18972 = ATCC BAA-1463; Smith et al. 2008). The DNA G+C content is 33.5 mol %.

Table 25.2

Comparison of physiological characteristics of members of the family *Nautiliaceae*

	C. hydrogeniphilus	*C. mediatlanticus*	*C. profundus*	*L. acidiphila*	*N. abyssi*	*N. lithotrophica*	*N. nitratireducens*	*N. profundicola*
Origin	Alvinella tube, East Pacific Rise (Elsa)	Chimney, Mid-Atlantic Ridge (Rainbow)	In situ growth chamber, Mid-Atlantic Ridge (Rainbow)	In situ colonization system, Mariana (TOTO)	Chimney, East Pacific Rise (13 °N)	Alvinella tube, East Pacific Rise (13 °N)	Chimney, East Pacific Rise (9 °N)	Alvinella body, East Pacific Rise (13 °N)
Temperature for growth (°C)								
Range	50–70	45–70	45–65	30–68	33–65	37–68	25–65	30–55
Optimum	60	55	55	50	60	53	55	40
NaCl concentaration for growth (g L^{-1})								
Range	10–40	10–40	5–50	6–50	20–40	8–50	10–35	20–50
Optimum	20–25	30	30	20	30	30	20	30
pH for growth								
Range	5.0–7.5	4.5–7.5	6.5–7.4	4.2–7.0	5.0–8.0	6.4–7.4	4.5–8.5	6.0–9.0
Optimum	5.5–6.0	5.5	6.9–7.1	5.2	6.0–6.5	6.8–7.0	7.0	7.0
Electron donors								
H_2	+	+	+	+	+	+	+	+
Acetate	–	–	–	–	–	–	+	ND
Formate	–	–	–	–	–	+	+	+
Yeast extract	–	–	–	–	–	–	+ (0.1 g L^{-1})	ND
Electron acceptors								
Elemental sulfur	+	+	+	+	+	+	+	+
O_2	–	–	+ (up to 2 %)	–	–	–	–	–
Nitrate (product)	+ ($NH4^+$)	+ ($NH4^+$)	+ ($NH4^+$)	–	–	–	+ ($NH4^+$)	–
Thiosulfate	–	–	–	–	–	–	+	–
Growth with formate as carbon source	–	–	–	–	–	+	+	+
DNA G + C content (mol %)	29.0 ± 1.0	27.1	32.1	34.0	35.0	34.7	36.0	33.5

ND not determined

Cells of *N. abyssi* are motile rods approximately 1.6 μm in length and 0.4 μm in width. Optimum temperature for growth is 60 °C (range, 33–65 °C); optimum pH, 6.0–6.5 (range, 5.0–8.0); and optimum NaCl concentration, 3.0 % (range, 2.0–4.0 %). *N. abyssi* is the only *Nautilia* species that is unable to utilize formate as a carbon source (Table 25.2). Alternatively, yeast extract and peptone can be used as a carbon source. Ammonium, glutamate, yeast extract, tryptone, gelatin, and urea are used as nitrogen sources. Under optimal growth conditions, the doubling time is about 2 h. The type strain of the species is PH1209 (= DSM 21157 = JCM 15390; Alain et al. 2009). The DNA G+C content is 35 mol %.

Cells of *N. nitratireducens* are motile rods, 1.0–1.5 μm in length and 0.3–0.5 μm in width. Optimum temperature for growth is 55 °C (range, 25–65 °C); optimum pH, 7.0 (range, 4.5–8.5); and optimum NaCl concentration, 2.0 % (range, 1.0–3.5 %). Colonies form on Phytagel plates. This species has an ability to utilize thiosulfate and selenate as an electron acceptor. *Nautilia nitratireducens* is the only facultatively chemoautotrophic *Nautilia*, which has an ability to utilize yeast extract as a carbon source (Pérez-Rodríguez et al. 2010). Under optimal growth conditions, the doubling time is about 45.6 min. The type strain of the species is MB-1 (= DSM 22087 = JCM 15746; Pérez-Rodríguez et al. 2010). The DNA G+C content is 36.0 mol %.

Isolation, Enrichment, and Maintenance Procedures

To improve the recovery of *Nautiliaceae* members, samples should be inoculated on board ship immediately after the recovery. If not applicable, samples should be stored at 4 °C under anaerobic and reducing conditions since all *Nautiliaceae* species are strict or facultative anaerobes. We usually store samples in 100 mL glass bottles tightly sealed with gas-impermeable butyl rubber stoppers under a N_2 atmosphere. Solid samples, e.g., chimney structures, are sectioned and slurried for inoculation with sterile seawater containing reducing agents such as sodium sulfide (usually used at 0.03–0.05 %, w/v). Samples processed as described above are usually kept refrigerated in the dark. If not applicable, short-time preservation at room temperature probably does not critically damage *Nautiliaceae* members.

All isolates of the family *Nautiliaceae* are moderate thermophiles. Thus, all *Nautiliaceae* members, except *Nautilia nitratireducens* for which primary enrichment was performed at 28 °C, were enriched at temperatures of at least 45 °C. Media are usually prepared anaerobically according to Balch et al. (1979). In general, the liquid media for *Nautiliaceae* contain artificial seawater supplemented with elemental sulfur and reducing agents with a gas phase H_2/CO_2 (80:20, v/v). Although some media previously used for the primary enrichment of *Nautiliaceae* members contained complex substrates, elimination of the organics did not affect the growth. To our knowledge, use of complex organics, such as yeast extract and peptone, sometimes leads to the enrichment of fermentative thermophiles, such as *Thermococcus* and *Thermosipho* species. Since these fermenters can be easily distinguished morphologically, the culture attempts should be checked routinely by microscopy. Pure cultures were obtained by serial dilution to extinction or by plating. Using solidified media, successful colony formation was reported for *Nautilia lithotrophica*, *N. nitratireducens*, and *Caminibacter mediatlanticus* (Miroshnichenko et al. 2002; Voordeckers et al. 2005; Pérez-Rodríguez et al. 2010). The purity should be checked also by sequencing the 16S rRNA gene using several PCR primers. Members of the family *Nautiliaceae* can generally be preserved at 4 °C for up to several years. For long-term preservation, cells in 5 % DMSO or 10 % glycerol were successfully preserved in glass capillaries in a liquid nitrogen container.

Strains of the family *Nautiliaceae* can be cultivated on various media typically used for marine hydrogen-oxidizing bacteria. In spite of the phylogenetic distance between the *Aquificales* and the *Epsilonproteobacteria*, these chemolithoautotrophs can be grown in the same media at different temperatures (Takai et al. 2003; Nakagawa et al. 2005a, b). Although reducing agents are usually added to the media for *Nautiliaceae* to remove dissolved oxygen completely, *C. profundus*, the only facultative anaerobes within the family, was isolated using a medium with no reducing agent (Miroshnichenko et al. 2004).

A useful medium formulation (designated SME medium; Stetter et al. 1983) for members of the family *Nautiliaceae* consists of (per liter):

NaCl	13.85 g
$MgSO_4 \bullet 7H_2O$	3.5 g
$MgCl_2 \bullet 6H_2O$	2.75 g
KCl	0.325 g
NaBr	50 mg
H_3BO_3	15 mg
$SrCl_2 \bullet 6H_2O$	7.5 mg
$(NH_4)_2SO_4$	10 mg
KI	0.05 mg
$Na_2WO_2 \bullet 2H_2O$	0.1 mg
$CaCl_2 \bullet 2H_2O$	0.75 g
KH_2PO_4	0.5 g
$NiCl_2 \bullet 6H_2O$	2 mg
Elemental sulfur (S^0)	30 g
$Na_2S \bullet 9H_2O$	0.5 g
Resazurin	1 mg
Trace mineral solution Balch et al. (1979)	10 mL

Dissolve the chemicals without S^0 and $Na_2S \cdot 9H_2O$, adjust the pH to 6.0–7.0, dispense 3 mL portions into test tubes, and autoclave. Under gas purging with H_2/CO_2 (80:20), add S^0 (sterilized by steaming for 1 h on each of 3 successive days) and 5–10 % (w/v) Na_2S solution (pH adjusted to 7.0 and autoclaved) to each tube. Keep flushing vigorously for at least 2–3 min, confirm resazurin turns colorless, cap the tubes with

butyl rubber stoppers, and secure with screw caps. In our experience, this medium enriches members of *Thioreductor* at 10–40 °C and *Desulfurobacteriaceae* at 65–75 °C (Takai et al. 2003; Nakagawa et al. 2005a, b). It should be noted that static cultures of the *Epsilonproteobacteria* sometimes show no apparent turbidity, since cells attach to S^0 particles. Thus, it is advisable to routinely check for growth by microscopy.

Removing Na_2S in the medium would allow the growth of chemolithoautotrophs preferring more oxic conditions. In our experience, medium lacking Na_2S but containing 0.1 % (w/v) $NaNO_3$ has enriched members of the *Epsilonproteobacteria* group A (at 40–60 °C), *Epsilonproteobacteria* group B or F (at 10–40 °C), or the *Persephonella* (at 65–75 °C) (Takai et al. 2004; Nakagawa et al. 2005b, c; Takai et al. 2005).

Ecology

Main Habitat

All *Nautiliaceae* members validly described to date have been isolated from microbial habitats in close proximity to hydrothermal vent emission (*Table 25.2*). Additionally, all environmental 16S rRNA gene sequences closely related to or belonging to the family *Nautiliaceae* were exclusively retrieved from deep-sea hydrothermal vents worldwide. More specifically, *Caminibacter hydrogeniphilus* and all *Nautilia* isolates were isolated from East Pacific Rise (Alain et al. 2002; Miroshnichenko et al. 2002, 2004; Smith et al. 2008; Pérez-Rodríguez et al. 2010), *Caminibacter mediatlanticus* and *Caminibacter profundus* from Mid-Atlantic Ridge (Miroshnichenko et al. 2004; Voordeckers et al. 2005), and *Lebetimonas acidiphila* from Mariana Arc (Takai et al. 2005). Although *Nautiliaceae* members appear to represent minor populations in deep-sea vents, they were detected more frequently in the association with colonization devices deployed into the deep-sea vents (Reysenbach et al. 2000; Corre et al. 2001; Takai et al. 2004; Nakagawa et al. 2005a, b). In addition, since *Nautilia lithotrophica*, *Nautilia profundicola*, and *Caminibacter hydrogeniphila* were isolated from *Alvinella*, these species potentially live in some symbiotic association with the polychaete. Members of the family *Nautiliaceae* probably play a significant role not only in sulfur cycling but in hydrogen and nitrogen cycling in deep-sea hydrothermal environments.

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Zetabacteria

26 The Family *Mariprofundaceae*

Ana Paula B. Moreira · Pedro M. Meirelles · Fabiano Thompson
Instituto de Biologia, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil

Abstract
Mariprofundaceae belongs to the order *Mariprofundales*, within the class *Zetaproteobacteria*. The family *Mariprofundaceae* embraces a single genus *Mariprofundus*, and a single species *Mariprofundus ferrooxydans*. Cells are motile curved rods and appear Gram-negative by transmission electron microscopy. It is obligatory lithotrophic; requires Fe^{2+}, marine salts; and is oxygen dependent. For decades, it had been known that neutrophilic Fe-oxidizing bacteria (FeOB) are associated with hydrothermal venting of Fe(II)-rich fluids associated with seamounts in the world's oceans. The evidence originated from the mineralogical remains of the microbes. Recent culture and culture-independent techniques were employed to study Fe-rich microbial mats associated with hydrothermal venting at a submarine volcano. For the first time two iron-oxidizing strains – PV-1 and JV-1 – were isolated and characterized phenotypically and phylogenetically, resulting in the description of ζ (*Zeta*)*-proteobacteria* class nov., *Mariprofundales* ord. nov., *Mariprofundaceae* fam. nov. and *Mariprofundus ferrooxydans* sp. nov.

Taxonomy: Historical and Current

Mariprofundaceae (Mar.i.pro.fund'a.ce.ae). L. masc. n. *Mariprofundus* the type genus of the family; N.L. *-aceae* ending denoting a family; N.L. masc. n. *Mariprofundaceae* the family of *Mariprofundus*. The description is that of the order (Emerson et al. 2007). The mol% G+C of the DNA of the type strain PV-1^T is 54. Type genus: *Mariprofundus*.

Mariprofundaceae is phylogenetically distant to the known classes of α-, β-, γ-, ε-, or δ-*Proteobacteria*. Isolates PV-1 and JV-1 show a deeply rooted phylogenetic position within the Proteobacteria, but form a distinct lineage from the other described classes. *Zetaproteobacteria* class nov., *Mariprofundales* ord. nov., *Mariprofundaceae* fam. nov. and *Mariprofundus ferrooxydans* sp. nov. were created to accommodate strain PV-1^T. *Zetaproteobacteria* class also includes several environmental clones. Description of *Mariprofundales* is that of the class and in addition is characterized by organisms coming from the deep ocean (Emerson et al. 2007). Physiological features are that of the single genus. Cells are motile curved rods that appear Gram-negative by transmission electron microscopy. Growth is obligately lithotrophic and requires Fe^{2+} as the energy source. Growth is oxygen dependent and requires marine salts. *Mariprofundus*, the type genus of *Mariprofundaceae* family, was described by Emerson, Rentz, Lilburn, Davis, Aldrich, Chan and Moyer in 2007, but the Validation List (N° 135) came up in 2010 (Euzeby 2010).

The members of this taxon were isolated, for the first time, from a sample collected in 1996 (PV-1), and again from a sample collected in 1998 (JV-1). The isolation followed attempts to grow and obtain pure culture of putatively lithotrophic representatives that constitute microbial mats at the hydrothermal vent sites of Loihi Seamount, a submarine volcano that is part of the Hawaiian archipelago. The vents are 1,100–1,325 m below the surface, temperature ranges from 10 °C to 167 °C. The vent fluid is characterized by high concentrations of CO_2 (up to 17 mM) and Fe(II) (up to 268 μM), but low H_2S. Most of the vents are surrounded by microbial mats that have a gelatinous texture and are heavily encrusted with rust-colored Fe oxides (Emerson and Moyer 2002). Previous reports had provided evidence for the presence and activity of Fe oxidizers associated with hydrothermal vents. These studies had demonstrated the presence of Fe oxide–encrusted sheaths and filaments, associated with both active and extinct vents (Cowen and Silver 1984; Alt 1988; Juniper and Fouquet 1988; Puteanus et al. 1991; Mandernack and Tebo 1993; Stoffers et al. 1993; Juniper and Tebo 1995; Bogdanov et al. 1997; Iizasa et al. 1998;

E. Rosenberg et al. (eds.), *The Prokaryotes – Deltaproteobacteria and Epsilonproteobacteria*, DOI 10.1007/978-3-642-39044-9_378,

Van Dover et al. 2001). Other evidences were microfossil remains of putative Fe-oxidizing bacteria (FeOB) associated with ancient hydrothermal vent sites as well as other ancient Fe-rich environments (Juniper and Fouquet 1988; Duhig et al. 1992; Little et al. 1999; Trewin and Knoll 1999; Preat et al. 2000). In addition, museum mineral collections and field work related the potential biogenic fossilized filaments with this novel bacteria (Hofmann and Farmer 2000). The signature structures resembled those of the Fe(III)-encrusted sheaths of *Leptothrix ochracea* and the helical stalk-like filaments of *Gallionella* spp. (van Veen et al. 1978; Hallbeck and Pedersen 1991; Hanert 1992). Abundant *Gallionella*-like stalk material microscopically identified as putative *G. ferruginea* cells had already been recovered from a shallow water volcanic system near Santorini Island in the Mediterranean Sea (Hanert 1981, 1992). Freshwater lithotrophic Fe oxidizers grow microaerobically at circumneutral pH, but do not form morphologically distinct Fe-oxide structures (Emerson and Moyer 1997; Emerson et al. 1999; Sobolev and Roden 2001). At Loihi, large mats of hydrous ferric oxides (HFOs) around the vent orifices were analyzed microscopically and showed abundant empty Fe-encrusted sheath casts of *L. ochracea*-like iron-oxidizing bacteria (Karl et al. 1989). Moyer et al. (1995) investigated the diversity of the bacterial community from a Loihi microbial mat via small-subunit rRNA bacterial clone library. In that study, a single clone sequence (PVB OTU4) detected from a vent-associated microbial mat dominated by *Epsilonproteobacteria* was absolutely unique. Subsequently, (Emerson and Moyer 2002) succeeded to grow and isolate putatively lithotrophic representatives from vent sites at the summit of Loihi. The isolates JV-1 and PV-1 formed filamentous stalk-like structures as they grew that resembled the types of oxides seen in the mats at Loihi. Both strains grew microaerobically with Fe^{2+} as the sole energy source and CO_2 as the only available carbon source, indicating the strains were obligate lithotrophic FeOB. The strains had an absolute requirement for marine salts and did not grow in a freshwater Fe-oxidizing medium. Both strains are motile. PV-1 came from a cool (23 °C) vent site and JV-1 was isolated from a hot (165 °C) vent. In laboratory conditions, both isolates grew between 10 °C and 30 °C. The pH range for growth was 5.5–7.2 and appeared optimal between 6.2 and 6.5 (Emerson and Moyer 2002; Emerson et al. 2007).

PV-1 and JV-1 SSU rDNA sequences were identical (1,488 bp). rep-PCR confirmed this genotypic similarity among strains; however, the banding patterns were distinct from freshwater FeOB. According to SSU rRNA gene phylogeny, PV-1 and JV-1 did not cluster with any of the known classes of α-, β-, γ-, ε-, or δ-*Proteobacteria*. Instead, they showed a deeply rooted phylogenetic position within the Proteobacteria. The novelty of the strain PV-1 within the Proteobacteria was confirmed by phylogenetic analysis of the proteins, gyrase beta-subunit (GyrB), and the recombination protein A (RecA). In both cases, PV-1 clustered within the Proteobacteria, but formed a distinct lineage (Emerson et al. 2007). The authors reported that a BLAST search of GenBank using the SSU rRNA gene sequence from PV-1^T retrieved environmental clones that clustered within the

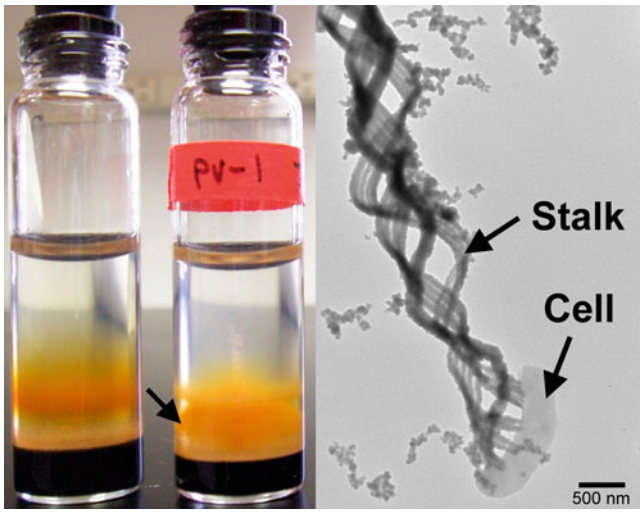

Fig. 26.1
***PV-1* cultures. *Left*: the *right* bottle (with the *red tag*) contains *PV-1* culture in log phase. The *arrow* indicates the orange biological iron oxide precipitates. The *left* bottle is the control (uninoculated). *Right*: transmission electron microscopy (TEM) of a *PV-1* cell (*lower arrow*) with twisted stalk made of iron oxides and organic matrix (*upper arrow*) (Extracted from Singer et al. (2011))**

PV-1/JV-1 lineage. All these clones were from deep-sea marine habitats associated with hydrothermal activity; including PVB OTU 4 (Moyer et al. 1995). Other clones were originated from Southern Mariana Trough hydrothermal fluid (Kato et al. 2009), Kebrit Deep brine-seawater interface, Red Sea (Eder et al. 2001), Cleft Segment hydrothermal vent, Juan de Fuca Ridge (accession DQ832638), Guaymas Basin hydrothermal sediment (Dhillon et al. 2003). The most similar sequence (85.3 % similarity) from a cultivated isolate came from *Methylophaga marina* (at the time of writing, 2007). Terminal restriction fragment length polymorphism (T-RFLP) analysis of Fe-rich microbial mats collected at several different vent sites around the summit of Loihi revealed that the PV-1 phylotype was consistently widespread and associated with lower-temperature vents <40 °C (median temperature = 23 °C). On the basis of phylogenetic and physiological data, it was proposed that isolate PV-1^T (= ATCC BAA-1019: JCM 14766) represents the type strain of a novel species in a new genus, *Mariprofundus ferrooxydans* gen. nov. sp. nov. The strain was the first cultured representative of a new monophyletic class of the Proteobacteria that is widely distributed in deep-sea environments, ζ (zeta)-*Proteobacteria* cl. nov. (*Fig. 26.1*). *M. ferrooxydans* is a chemoautotrophic, neutrophilic Fe-oxidizing bacterium that gains its energy for growth by oxidizing ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron, and fixes CO_2. Furthermore, as it grows, it produces a stalk-like structure composed primarily of HFOs, the waste product of the Fe-oxidation reaction that remains behind after the cells are gone to avoid encrustation (*Fig. 26.1*) (Emerson et al. 2007).

Despite their abundance, neutrophilic FeOB have been historically difficult to culture. This was the main reason the iconic

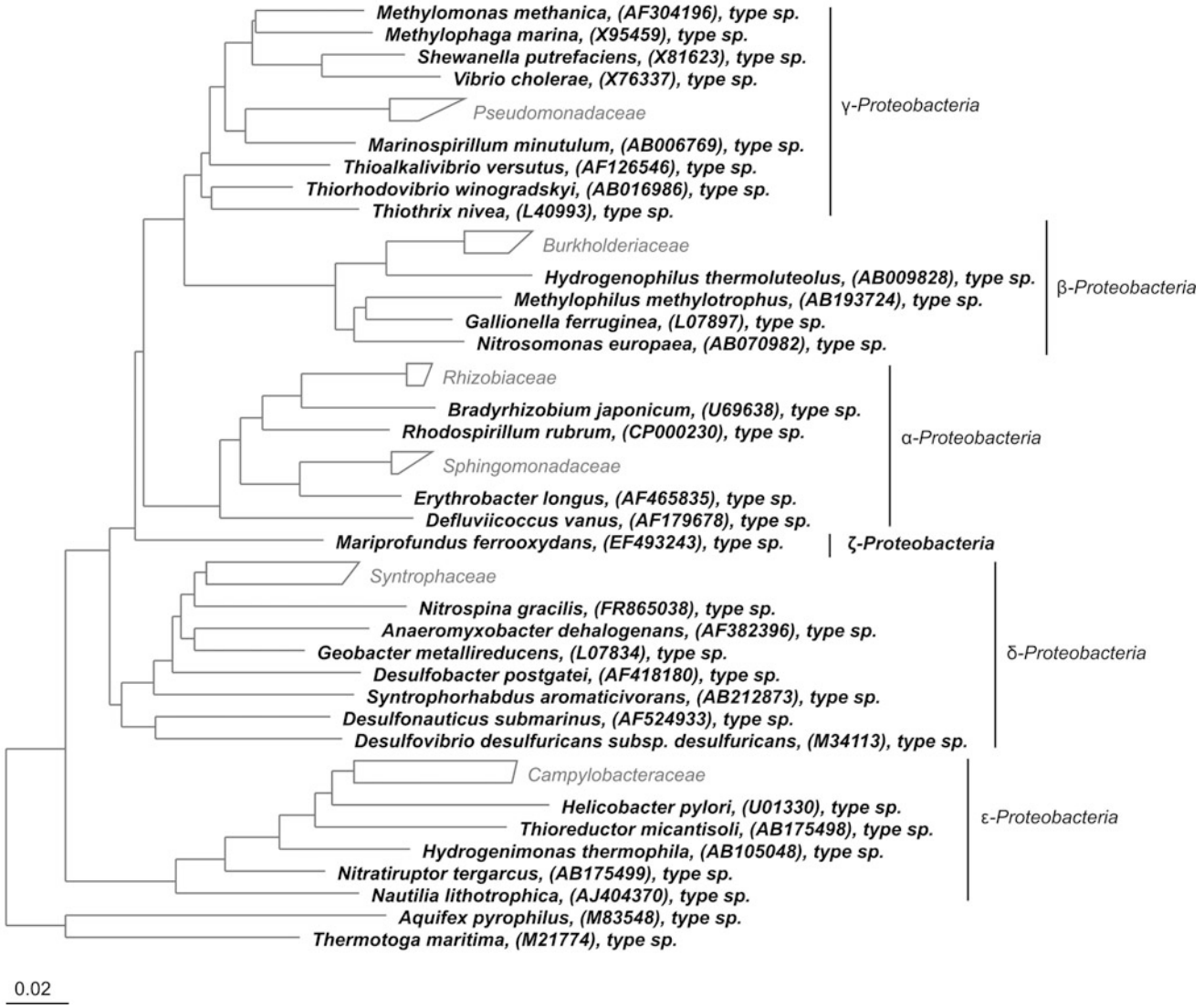

Fig. 26.2

Consensus phylogenetic tree of the family *Mariprofundaceae* based on 16S rRNA. Final topology is based on maximum-likelihood (RAxML, Stamatakis 2006) reconstruction. Branching of Alpha, Delta, and *Epsilonproteobacteria* was optimized by maximum parsimony (ARB, Ludwig et al. 2004). 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). In addition, a 50 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Representative sequences from *Thermotogae* and *Aquificae* were used as outgroups. Scale bar represents 0.02-bp substitutions per nucleotide position

structures were thought to belong to the freshwater *Gallionella* spp. or *L. ochracea* (both *Betaproteobacteria*), which produce similar structures. Until now, most works are based on culture-independent molecular techniques and rely on the coarse resolution of the SSU rRNA gene to improve the current knowledge on the diversity of the ζ-*Proteobacteria* (Rassa et al. 2009; Forget et al. 2010; McAllister et al. 2011). At the time of writing (Out/2012), a NCBI BLAST search using the type strain SSU rRNA gene sequence (PV-1^T), excluding clones, retrieved four sequences ranging from 93 % to 99 % sequence identities: *Mariprofundus* sp. SDT4S14 (JQ073806 – 93 %); *Mariprofundus* sp. SDT4S13 (JQ073805 – 95 %); *Mariprofundus* sp. GSB2 (HQ206653.1 – 97 %; McBeth et al. 2011); *Mariprofundus* sp. M34. (JF317957 – 99 %). Taxonomy studies with these strains are still lacking for reclassification or identification to the species level, which may result in new species descriptions.

Phylogenetic Structure of the Family and Its Genera

The phylogenetic reconstruction of the family *Mariprofundaceae* based on 16S rRNA gene sequence is represented in *Fig. 26.2*. PV-1^T is a distinct member of the phylum Proteobacteria.

It does not cluster with any of the classes of α-, β-, γ-, ε-, or δ- *Proteobacteria*. Instead, it branches deeply within the Proteobacteria as shown in a phylogenetic tree created by maximum-likelihood (RAxML, Stamatakis 2006) reconstruction. Branching of Alpha, Delta and *Epsilonproteobacteria* were optimized by maximum parsimony (ARB, Ludwig et al. 2004). 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). In addition, a 50 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Representative sequences from *Thermotogae* and *Aquificae* were used as outgroups. Scale bar represents 0.02-bp substitutions per nucleotide position.

Molecular Analyses

Rep-PCR

Since PV-1 and JV-1 SSU rDNA sequences were identical (1,488 bases), a rep-PCR approach was performed to compare both strains and the freshwater FeOB *Gallionella capsiferriformans*, *Sideroxydans lithotrophicus* and *S. paludicola*. In duplicated runs, PV-1 and JV-1 shared >98 % similarity in their rep-PCR profiles, where 95 % similarity usually indicates unrelated strains. These nearly identical profile shared by PV-1 and JV-1 was distinct from the other FeOB strains tested (Emerson et al. 2007).

Ribotyping

Terminal restriction fragment length polymorphism (T-RFLP) is a direct DNA fingerprinting technique that distinguishes bacterial strains as "ribotypes" in a bacterial community. It employs fluorescently labeled PCR primers (only one of the primers is labeled at the 5′ end) to amplify a selected region of bacterial 16S rRNA from total community DNA. The PCR product is digested with restriction enzymes, and the fluorescently labeled terminal restriction fragment is precisely measured by using an automated DNA sequencer (Liu et al. 1997). T-RFLP allowed Emerson and Moyer (2002) to verify the presence of the PV-1/JV-1 pure cultures' ribotype (a single 332-bp fragment) within the vents community DNA. The community DNA was retrieved from Loihi at distinct occasions: 1998, 1996 – post-eruption and pre-eruption. The 332-bp fragment was present in all samples but was dominant in pre-eruption communities. Later, the analysis of Fe-rich material collected at different vent sites around the summit of Loihi in 2004 also showed that PV-1 ribotype was present in all sites. The vents' temperature ranged from 10 °C to 65 °C, and there were two main T-RF clusters. One associated with vents <40 °C, and another with vents >40 °C. PV-1/JV-1 ribotype was associated with cooler vents (Emerson et al. 2007).

MLSA

*Gyr*B and *rec*A genes' nucleotide sequences were translated and employed to phylogenetic analysis. In both cases, phylogenetic trees showed the clustering of PV-1 within Proteobacteria but as a distinct lineage, confirming the novelty of the organism (Emerson et al. 2007). The genome sequencing allowed a robust MLSA analysis employing 10 evolutionary conserved genes. The phylogenetic reconstruction was performed with the translated proteins: FusA, GyrB, IleS, LepA, LeuS, PyrG, RecA, RecG, RplB, RpoB. The resulting concatenated maximum-likelihood tree showed PV-1 branching out as a distinct class within the Proteobacteria. The most closely related neighbor appeared to be the *Magnetococci* subdivision, which only comprises one sequenced genome, *Magnetococcus* sp. MC-1 (Singer et al. 2011), although these are not valid names, according to the last update of the "*List of Prokaryotic names with Standing in Nomenclature*" (LPSN, Feb/20/2013; http://www.bacterio.cict.fr/).

Genome Comparison

The unique genome sequence released for the *Mariprofundaceae* family is that of the type strain *Mariprofundus ferrooxydans* PV-1^T, available as draft (GenBank Accession: AATS01000001.1) (Singer et al. 2011). However, two other genome projects are ongoing to the *Mariprofundus* genus, relative to strains *Mariprofundus* sp. GSB2 (McBeth et al. 2011), GenBank Genome Bioproject: PRJNA169683; and *Mariprofundus* sp. M34 (McAllister et al. 2011); GenBank Genome Bioproject: PRJNA165245. Concerning these genome projects, no data is available yet.

Regarding organization and content, PV-1 genome comprises 32 scaffolds totalizing 2,867,087 bp, with average 54 % G+C content and including 2,866 protein coding sequences (CDSs). According to the authors, the draft might include 98.5 % of all CDSs, missing only 44 genes. Six phage integrases and 21 transposases are present. The distribution of the transposases is uniform, and the G+C content differs from that of the genome average. Other evidences of lateral gene transfer (LGT) acquisition are the neighboring genes, coding functions such as transduction mechanisms, posttranslational modification, and cell motility. No plasmids were found (Singer et al. 2011).

The metabolic processes highlighted by genome annotation are carbon acquisition and storage, energy acquisition and storage, and life cycle (Singer et al. 2011).

PV-1 genome contains both forms, I and II, ribulose bisphosphate carboxylase (RuBisCo) genes, possibly enabling CO_2 fixation under a variety of CO_2/O_2 concentration ratios. PV-1T also has three carbonic anhydrase–encoding genes. A large gene cluster includes homologues to bicarbonate uptake genes and a predicted urea carboxylase. A predicted operon encodes for a phosphoenolypyruvate-dependent sugar phosphotransferase system (PTS), the major carbohydrate

transport system in bacteria (Postma et al. 1993). A fructose/mannose-specific transporter is enclosed, but fructose metabolism requires 1-P-phosphofructokinase, which was missing. However, imported mannose-6-phosphate could be converted by the existent *man*A to fructose-6-phosphate, a substrate for glycolysis I. This raises the possibility of mixotrophically growth to PV-1. This behavior was not observed experimentally, but fructose and mannose were not tested. Other suggested destinies of mannose are the glycoproteins and glycolipids that extend into the extracellular space, required during stalk formation, and/or integral membrane proteins. Carbon storage did not appear to be encoded (Singer et al. 2011).

The PV-1 genome contains a cytochrome *cbb*3 oxidase regulon (ccoNOP), which should confer high fitness for microaerobic respiration. Nevertheless, the stabilizer non-cofactor-containing subunit, *cco*Q (Peters et al. 2008), was missing. Two cytochrome *bd* quinol oxidases may enable growth in oxygen-limited condition and also act as O_2-scavengers (D'mello et al. 1996). Some features such as a superoxide dismutase, several peroxidases, and alkyl hydroperoxide reductases may protect against oxygen radicals. Catalase and glutathione reductase were not found. Most redox carriers belonged to the cytochrome family (Singer et al. 2011).

The search for Fe(II) oxidation-related genes revealed that PV-1 genome harbors more than 70 genes for electron transport (identified with Pfam domains). Homologs to known iron redox genes in other organisms were not found (e.g., *pio* and *fox* operon in *Rhodobacter* sp. strain SW2 and *R. palustris*; *iro*, *cyc*1, *cyc*2, *cox* genes and *rus* in *Acidithiobacillus ferrooxidans*) (Yarzabal et al. 2002a, b; Jiao et al. 2007). The coding gene for the protein expressed in PV-1 cells oxidizing Fe(II), extracted and identified as molybdopterin oxidoreductase Fe_4S_4 was found in the genome (Singer et al. 2011). By topology prediction, it is located within the periplasm, outside either membrane. The neighborhood includes a cluster of 17 CDSs together with other cytochrome, succinate dehydrogenase, and ferredoxin encoding genes region. Orthologous gene neighborhood comparison suggested *G. capsiferriformans, S. lithotrophicus*, and, to a lesser extent, *Geobacter uraniumreducens*, *G. metallireducens*, and *Geobacter* sp. M18, as other possible gene hosts. An Fe(II) oxidation model was proposed as follows: "*The conversion of Fe(II) to Fe(III) may be catalyzed by an iron oxidase located in the outer membrane that is closely associated with a molybdopterin oxidoreductase Fe-S region located in the periplasm. The enzyme accepts electrons from ferrous iron and passes them on to an electron transport chain consisting of several oxygen sensitive cytochromes, which are predicted to be essential in the microaerobic* environment *PV-1 inhabits. Since the electrons obtained from the oxidation of Fe(II) with O_2 are low potential electrons, reverse electron transport and the concurrent consumption of proton motive force are required for NADH synthesis*" (*Fig. 26.3*) (Singer et al. 2011).

Two genes were related to glycogen/starch synthesis proteins whether several amylases (glycogen and starch hydrolysis) were found. Carbon-concentrating mechanisms encoding genes (e.g., *ccmKLMNOP*, *chpXY*, *cmpABCD*) were not identified. Candidate genes involved in polyphosphate (poly P) synthesis were identified in correlation to the poly P bodies observed in PV-1 cells (Singer et al. 2011). Moreover, the complete set of genes required for the uptake and conversion of poly P to ATP indicated the use of poly P as energy source as well as phosphate reserve during anaerobic conditions (Singer et al. 2011). Poly P was proven to serve as energy and/or phosphate reservoir in *Thiobacillus* strain Q and *Accumulibacter phosphatis* (Beudeker et al. 1981; Gommers and Kuenen 1988; Martín et al. 2006).

It was observed a relative abundance of regulatory and signaling proteins (9.35 %), predominating histidine kinases with various function domains, including PAS/PAC, GGDEF/EAL, and multisensors. Other abundant functional genes were diguanylate cyclases, sensory box proteins, and two-component transcriptional regulators. PAS domains are sensor modules, sensitive to changes in light, redox potential, oxygen, small ligands, and overall energy level of a cell. They are thought to enhance flexibility in complex redox environments (Taylor and Zhulin 1999). GGDEF/EAL are response modules, oftentimes coupled, displaying diguanylate cyclase activity. Cyclic diguanylate, ubiquitous in bacteria, is a signaling molecule, which may control the switch of lifestyle and trigger stalk formation (Galperin et al. 2001). In other known neutrophilic Fe(II) oxidizers, regulatory and signaling genes comprise 10.61 % (*G. capsiferriformans*) to 12.61 % (*S. lithotrophicus*) of all CDSs. In *Thiomicrospira crunogena* XCL-2, also a hydrothermal vents inhabitant, the proportion of these CDSs is 9.5 %. In contrast, the methyl-accepting chemotaxis protein-encoding (MCP) genes found are few (three CheY-like receivers and one CheW), compared to other Fe(II) oxidizers and hydrothermal vent inhabitants. A full complement of flagellar genes was found, in accordance with a motile cell cycle stage (Singer et al. 2011) (*Fig. 26.3*).

The genome analysis of PV-1 gives further information on the *Mariprofudaceae* family, especially where biochemical data is missing. Main features are the complete TCA cycle, the ability to fix CO_2, a sugar phosphotransferase system (PTS), and a two-component signal transduction system genes, the latter mostly nearby genes required for cell division, phosphate uptake and transport, exopolymer and heavy metal secretion and flagellar biosynthesis, indicating these are highly regulated functions. Genes that may enable sense and respond to oxygen gradients are well represented.

Phages

One phage cluster was found consisting of 32 CDSs flanked by a transposase and three hypothetical proteins upstream. G+C content differs from that of the genome average. According to nucleotide sequence alignment identity, the closely related phage gene clusters are *Pseudomonas* phage MP29 (30 %), Bacteriophage D3112 (29 %), and *S. lithotrophicus* ES-1 (24 %). The similarity with ES-1 led to the suggestion that this region provided a selective advantage to neutrophilic FeOB (Singer et al. 2011).

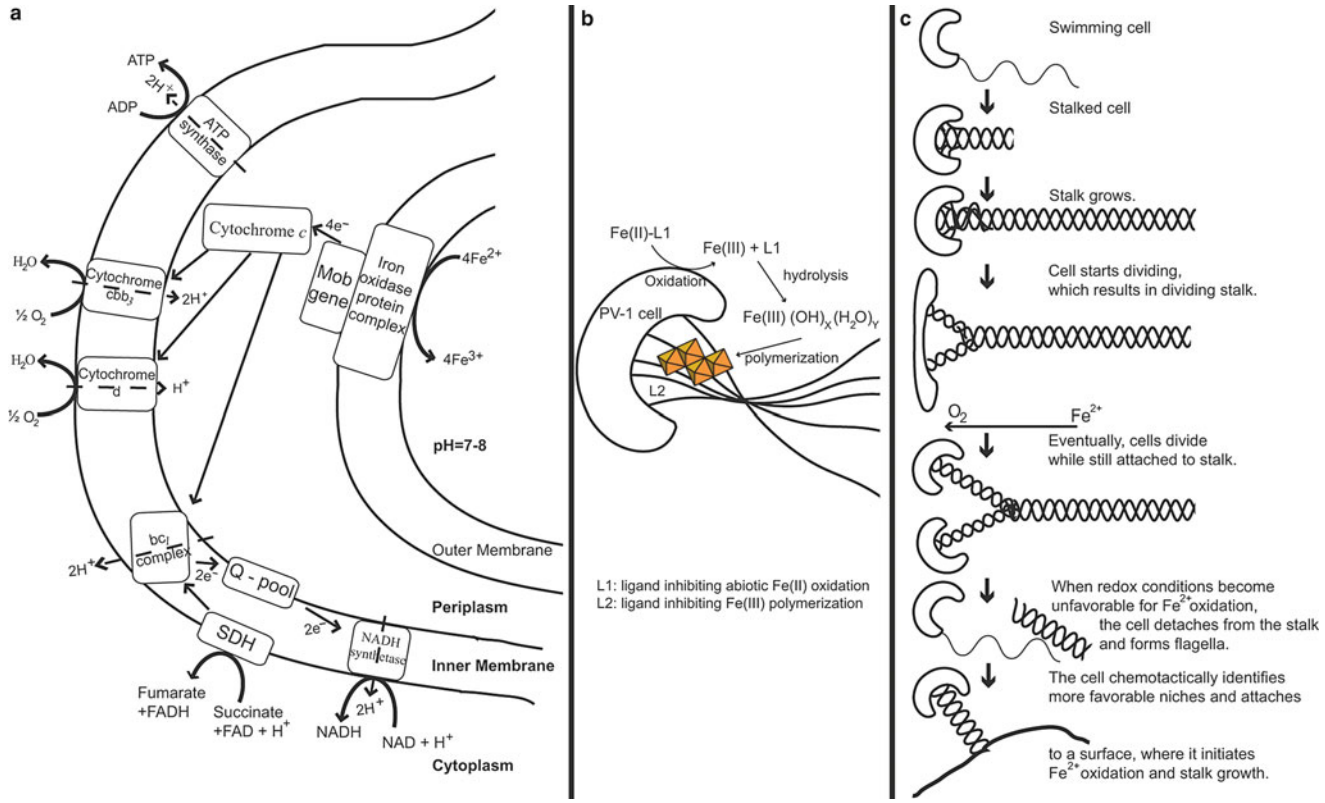

Fig. 26.3

Conceptual iron oxidation model in relation to the life cycle in *M. ferroxydans PV-1*. (a) Proteins potentially involved in the energy acquisition via Fe(II) oxidation through the outer and inner membrane as predicted from genomic analysis. The "*Mob gene*," possibly located in the periplasm, represents the experimentally identified molybdopterin oxidoreductase Fe_4-S_4 region. Its function may include the shuttling of electrons between outer and inner membrane. (b) Biologically formed iron oxides are stored in the stalk of *PV-1*. As the cell performs Fe(II) oxidation, it rotates, which results in a twisted, coiled stalk. (c) Schematic of the life cycle in *PV-1*. The cell moves in the environment until it identifies conditions suitable for Fe(II) oxidation. The flagella are discarded and stalk growth initiated. As the cell divides, the stalk becomes bifurcated, and each cell continues to form a stalk. When O_2 concentrations exceed the maximum tolerable by *PV-1*, the cell detaches from the stalk and forms flagella to move to a better suited niche, where the life cycle starts over (Extracted from Singer et al. (2011))

Phenotypic Analyses

Family *Mariprofundaceae* Emerson, Rentz, Lilburn, Davis, Aldrich, Chan and Moyer 2007

Mariprofundaceae (Mar.i.pro.fund'a.ce.ae). N.L. masc. n. *Mariprofundus* type genus of the family; *-aceae* ending to denote a family; N.L. fem. Pl. n. *Mariprofundaceae* the *Mariprofundus* family.

The family *Mariprofundaceae* was circumscribed on the basis of phylogenetic analysis of 16S rRNA sequences. The family is phenotypically, metabolically, and ecologically uniform. Includes one monospecific genus. The salient properties have been covered under "short description of genus" and "short description of type species."

Type genus: *Mariprofundus.*

Genus *Mariprofundus* Emerson, Rentz, Lilburn, Davis, Aldrich, Chan and Moyer 2007

Mariprofundus gen. nov. (Mar.is.pro.fund'us. L. masc. n. *maris* the sea; L. adj. *profundus* deep; L.masc. n. *Mariprofundus* a deep-sea organism).

Cells are motile curved rods that appear Gram-negative by transmission electron microscopy (TEM). Growth is obligately lithotrophic and requires Fe^{2+} as the energy source. Growth is oxygen dependent and requires marine salts.

The mol% G+C of the DNA is 54 %.

Type species: *Mariprofundus ferrooxydans.*

The genus currently contains one species effectively published: *Mariprofundus ferrooxydans* (Emerson et al. 2007). *M. ferrooxydans* displays the following properties in addition to those given by the genus description. Cells of strains PV-1 and

JV-1 are curved rods (approximately 0.5 × 2–5 μm), motile, and appear to be aerotactic, showing preference toward low oxygen concentrations. Genomic analysis confirmed motility by identifying flagellar genes (Singer et al. 2011). Cultures grow microaerobically with Fe^{2+} as the sole energy source and CO_2 as the only available carbon source. The cells do not grow on other reduced inorganic energy sources besides iron or on organic compounds. Polyphosphate (poly P) bodies were observed by Cryo-TEM in PV-1 strain (Singer et al. 2011). As a result of growth, filamentous stalk-like structures containing poorly crystalline iron oxyhydroxide are produced, being the most distinguishing characteristic of *Mariprofundaceae* (Emerson et al. 2007). The morphotypes found can be: amorphous particulate oxides, sheaths, twisted or helical stalks, and Y or V-shaped irregular filaments (Emerson and Moyer 2002). The major element in these structures is Fe, which gives an orange color to the cultures' tubes and the mats surrounding the hydrothermal vents (Emerson and Moyer 2002; Singer et al. 2011). The optimum growth temperature is 30 °C and the optimum pH range is 6.0–6.5. The type strain (PV-1) oxidizes reduced Fe from different substrates at pH 5.5–7.2 (e.g., FeS, $FeCO_3$, $FeCl_2$, $Fe(NH_4)_2(SO_4)_2$, $FeSO_4$, Fe^0), being an obligate chemolithoautotroph. A protein significantly expressed in PV-1 cells oxidizing Fe(II) was extracted and identified as molybdopterin oxidoreductase Fe_4S_4 region. The coding gene was detected in the genome (Singer et al. 2011). The G+C content of the DNA of PV-1 is 54 %. The cells are catalase negative. The dominant fatty acids are 11:0 iso 3OH, 17:0 iso 3OH, 16:0, and 18:1 iso-H. The type strain is PV-1^T, ATCC™ BAA-1019 (=JCM 14766) isolated from iron-rich microbial mats associated with regions of hydrothermal venting at Loihi Seamount in the Pacific Ocean.

Isolation, Enrichment, and Maintenance Procedures

PV-1 was isolated from an iron mat collected in 1996 associated with a cool (23 °C) diffuse vent field near the Naha vents site, at a depth of 1,325 m, on the south rift of Loihi Seamounts (Emerson and Moyer 2002). The strain JV-1 was isolated from the hot (165 °C) Ikaika Vents, located in a 300-m deep caldera, close to the summit of the same seamount, at a depth of 1,298 m, in 1998 (Emerson et al. 2007).

Sampling was done with a submersible, by suction, vacuuming up the Fe mats from the sea floor to collection bottles. The samples were allowed to settle for 1–2 h before the overlaying water was poured off, leaving a mixture of HFOs. This Fe oxide was stored at 4 °C for enrichment studies. PV-1 was isolated directly from an inoculum of undiluted mat material, while JV-1 was enriched from a mat sample initially diluted to 10^{-7}. After a sequential two dilution series to extinction, the morphologically homogeneous cultures obtained did not grow heterotrophically (Emerson and Moyer 2002).

Growth was achieved by employing two Fe oxidizer growth medium: gradient tube and liquid. As the pH changes from acid conditions toward neutrality, the chemical oxidation of Fe(II) becomes faster. Thereby, neutrophilic FeOB growth is limited to gradients of O_2 and Fe (II) at oxic-anoxic boundaries. The gradient tube method relies in opposing gradients of O_2 and Fe(II) to allow growth at the oxic-anoxic interface in a gel-stabilized medium containing a ferrous sulfide (FeS) plug (Emerson and Floyd 2005). The FeS settles to the bottom, simultaneously creating reducing conditions and releasing Fe(II) into the overlying liquid. Wolfe's minimal medium-artificial seawater (MWMM/ASW) mineral salts medium employed was composed of (per liter): 27.5 g of NaCl, 5.38 g of $MgCl_2 \cdot 6H_2O$, 6.78 g of $MgSO_4 \cdot 7H_2O$, 0.72 g of KCl, 0.2 g of $NaHCO_3$, 1.4 g of $CaCl_2 \cdot 2H_2O$, 1 g of NH_4Cl, and 0.05 g of $K_2HPO_4 \cdot 3H2O$. Wolfe's vitamins and trace element solutions (Wolin et al. 1963) were added (1 mL/L). Sodium bicarbonate served (10 mM) as a buffering agent and C source. The gradient tubes used 0.15 % (wt/vol) low melt agarose as the gel-stabilizing agent. Agarose is added to the overlaying medium, to prevent the cells from precipitating out of the oxic-anoxic transition zone as they become mired in Fe-oxide precipitates (Emerson and Floyd 2005). A liquid enrichment technique was also employed. To maintain low concentrations of Fe(II) and O_2, air and $FeCl_2$ were added to a liquid medium in a closed vessel. The same basal salts medium described above was used, without agarose. For the dilution series, either 10 mL of liquid in 25-mL tubes or 60-mL bottles with 40 mL of medium were used. The recipients were prepared by gassing the medium with N_2 (5 min), then with CO_2, until pH was reduced to 6.4–6.5. The medium was additionally gassed (2–3 min) with N_2 and autoclaved at 121 °C for 20 min. Prior to inoculation, approximately 200 μM $FeCl_2$ was delivered with a syringe, plus enough sterile air to yield approximately 1 % O_2 in the headspace. The sterile $FeCl_2$ stock solution was prepared by bubbling deionized water (dH_2O) with N_2 (15 min) followed by addition of $FeCl_2$ (final 100 mM). This solution was filter-sterilized into a pre-sterilized bottle that had been flushed with N_2 gas. The solution was bubbled with sterile N_2 gas (5 min) (Emerson and Moyer 2002).

Enrichment was performed by means of a dilution series with gradient tubes. A series of 10× fold dilutions of the original sample was done in tubes with sterile ASW (below). From these dilutions, gradient tubes were inoculated (10 μL) in 10× steps from 10^{-2} to 10^{-8}. In liquid medium, serial dilutions were done starting with a vent sample diluted to 10^{-2}, followed by a series of 10× dilutions until 10^{-7}. The inoculum is inserted just above the FeS layer with a pipette (Emerson and Floyd 2005). The tubes were incubated in the dark at varied temperatures (12–80 °C). Every 24 or 48 h, additional air and $FeCl_2$ were added. Growth is visible as the line of the inoculum spreads into a rust-colored band at the oxic-anoxic interface (Emerson and Floyd 2005). Cell growth was verified by epifluorescence microscopy with either acridine orange or Syto to check for cells bounded to the Fe oxides. To obtain purified enrichments, subsequent dilution series were done with an inoculum from the highest dilution tube where growth occurred. For pure cultures, at least two additional dilutions to extinction were

carried out until uniform cell morphology was achieved. To check for purity, heterotrophic medium (commercial R2A or nutrient agar) with NaCl (2.5 %) was inoculated and incubated, aerobically and microaerobically, at room temperature (Emerson and Moyer 2002).

The basal medium for studies was ASW amended with nitrogen and phosphate sources as follows (per L deionized-H_2O): 27.5 g NaCl, 6.78 g $MgCl_2$ $7H_2O$, 5.38 g $MgCl_2 \cdot 6H_2O$, 1.4 g $CaCl_2 \cdot 2H_2O$, 1.0 g NH_4Cl, 0.72 g KCl, 0.2 g $NaHCO_3$, 0.05 g $K_2HPO_4 \cdot 3H_2O$, 1.0 ml trace mineral supplement, and 1.0 ml vitamin supplement (both from American Type Culture Collection). Sterile aliquots of the vitamins and mineral solutions have to be added after the autoclavation at 121 °C for 20 min. Filter-sterilized sodium bicarbonate (10 mM final) is recommended as buffer and C source (Emerson et al. 2007). Growth conditions include limited, but constant, supply of Fe(II) and O_2 using gradient tubes or plates, or bottle cultures. These techniques share in common the provision of Fe(II) concentrations in the 50–300 μM range and O_2 concentrations below 15 % of ambient. Detailed step-by-step, such as how to prepare and add the FeS stock solution, is provided by Emerson and Floyd (2005).

Recently, Summers et al. (2013) have proven that the growth of PV-1 strain using a poised electrode as the sole energy source is feasible. Electrode-based growth was conducted in 80-mL three-electrode reactors containing graphite electrodes poised at −0.076 V (versus standard hydrogen electrode [SHE]) and flushed continuously with a mix of O_2–CO_2–N_2 (8:10:82). The chambers contained a platinum counter electrode and a calomel reference electrode, connected via a salt bridge, and were autoclaved with all electrodes in place before each use. Similar microbial electrolysis cells had been used to cultivate microbes capable of anode respiration where the electrode was poised at a potential to accept electrons from cells (Bond et al. 2002; Marsili et al. 2008). The opposite flow (from electrodes to cells) had been observed in anaerobes (e.g.,, *Shewanella* and *Geobacter* spp.) (Ross et al. 2011; Strycharz et al. 2011) and environmental enrichments (Pisciotta et al. 2012). Summers et al. (2013) succeeded to create a surface which was poised at a constant redox potential designed to mimic that of Fe(II) in the environment and supply cells (inoculated from classical iron-containing medium) with a stream of electrons at a known potential. Under these conditions, *M. ferrooxydans* PV-1 attached and accepted electrons from an electrode, being propagated autotrophically, onto new electrodes, three times. When electrodes from third-transfer were placed in sterile classical FeOB growth medium, a characteristic zone of Fe(III) precipitation was formed, and Y-shaped twisted stalks could be visualized via light microscopy. Cells attached to the electrodes did not produce the characteristic stalks but exhibited the Y- or V-shaped cell division morphology. The authors concluded this phenotype is not an artifact of Fe(III) precipitation, but is linked to the growth of the strain. This innovative method promises to overcome the main challenges involved in the laboring cultivation of Fe(II) oxidizing bacteria: (i) provide constant Fe(II) electrons supply; (ii) avoid the formation of Fe(III) too rapidly; and (iii) keep oxygen concentration at a micromolar range to minimize abiotic Fe(II) oxidation, all at a time. If so, electrochemical cultivation will favor studies on obligately lithotrophic bacteria, allowing a better comprehension of their metabolism (Summers et al. 2013).

Maintenance

It is recommended to transfer the cultures every 3–4 weeks. Refrigerated storage will prolong the useful life of a culture. Long-term storage is achieved by removing the growth band with a pipette and mixing it with sterile 1:1 glycerol (20 %). The glycerol stocks must be immediately frozen in either −80 °C or liquid nitrogen (Emerson and Floyd 2005).

Ecology

In all habitats that ζ-*Proteobacteria* lives, there is an interface of steep redox gradients of oxygen and iron (Glazer and Rouxel 2009). The first strains of the *Mariprofundaceae* (PV-1 and JV-1) were isolated from iron mats at deep marine environments. PV-1 was isolated from Naha Vents, a cool and diffuse vent site (23 °C), located below the summit of the Loihi seamount at a depth of 1,325 m. JV-1 was isolated from the hot (165 °C) Ikaika Vents, located in a 300-m deep caldera close to the summit of the same seamount at a depth of 1,298 m (Emerson et al. 2007). Experiments concerning microbiologically influenced corrosion in near-shore shallow waters (5–7 m) have led researchers to isolate another *Mariprofundus* sp. strain, GSB2, from an iron oxide mat in a salt marsh (McBeth et al. 2011). This strain shares 96 % SSU rRNA gene similarity with *M. ferrooxydans*, apparently being a new species.

Emerson et al. (2007) found that sequences derived from SSU rRNA gene clone libraries in some deep ocean environments were highly similar to PV-1^T: Kebrit Deep (depth 1,468 m), in the Red Sea (Eder et al. 2001), Loihi Seamount hydrothermal (Moyer et al. 1994, 1995), Guaymas Basin (Dhillon et al. 2003); Cleft Segment hydrothermal system off the coast of Oregon and the Mariana Trench (Davis et al. 2005), showing that FeOB are more widespread than previously thought (Jannasch and Mottl 1985; McCollom 2000; Amend and Teske 2005).

Some studies using an independent culture approach have found sequences related to ζ-*Proteobacteria* in different environments. In a biogeography study, McAllister et al. (2011) have found that this class encompasses 28 OTUs and some of these are endemic, suggesting ζ-*Proteobacteria* is much more diverse than expected. Sequences related with ζ-*Proteobacteria* were also found at arsenic-rich hydrothermal venting in a near-shore coral reef environment, at Ambitle Island in the Tabar-Feni island arc (5–10 m), east of Papua New Guinea (Meyer-Dombard et al. 2012). The presence of this class in different shallow-water habitats, like salt marsh, coral reefs, and temperate monsoon influenced coast, also was found in different studies (Dang et al. 2011; McBeth et al. 2011; Meyer-Dombard et al. 2012).

Pathogenicity: Clinical Relevance

No pathogenic bacteria are known within the *Mariprofundaceae* to date (Feb/2013).

Application

The ability to link Fe(II) oxidation to internal metabolism has suggested that cells could use electrical resources to power the synthesis of organic compounds. For example, cathodes have emerged as possible tools in biofuel synthesis serving as electron sources for the reductive microbial metabolism (Hallenbeck 2012).

Electrochemical cultivation of *Thiobacillus ferrooxidans* to yield high biomass has already been performed (Matsumoto et al. 2002). However, the bottleneck for electrochemical CO_2 reduction resides on reducing the external voltage to as low as 1.24 V, the thermodynamic limit for producing organic compounds via CO_2 reduction coupled with water oxidation [$CO_2 + H_2O$ to $(CH_2O) + O_2$, where (CH_2O) represents a carbohydrate] (Hori et al. 1994; Whipple and Kenis 2010; Peterson and Nørskov 2012). Mogi et al. (2013) used the voltage-multiplying circuits of the chemolitho-autotrophic Fe-oxidizing *M. ferrooxydans* PV-1 and described an integrated bioelectrochemical system that affords simultaneous CO_2 reduction and H_2O oxidation at an external low-voltage of less than 1.24 V. The system consists of a single chamber containing three-electrodes where an electrode potential of 0.1 V is generated. An optically transparent conductive glass, fluorine-doped SnO_2 (FTO), is placed at the bottom of the reactor as the working electrode. Ag/AgCl (KCl sat.) and a platinum wire are the counter and reference electrodes, respectively. The electrolyte is a 4.0 mL solution of ASW media containing $FeCl_2$ (60 mM), which is deaerated by bubbling with a mixture of CO_2 (20 %) and N_2 (80 %) gas. O_2 concentration in the reactor headspace is adjusted to 3 % and a cell suspension (0.1 mL) is injected. After 216 h of cultivation, the total charge that passed across the FTO electrode reached 119C, which is four times larger than the amount of Fe ions initially added into the reactor. The current generation was inhibited by increasing O_2 concentration in the headspace. It was concluded that, within the reactor, Fe ions are cycled between cells and the cathode through microbially mediated redox reactions (Mogi et al. 2013). The production of stalks during electrochemical cultivation was verified by staining FTO electrodes, after cultivation, with a rhodamine-conjugated specific lectin *Ulex europaeus* agglutinin I (UEA) (Chan et al. 2011). Bright areas in fluorescence images gave a positive result. Moreover, the selectivity of UEA allowed the suggestion of the molecular structure of the synthesized polysaccharides, including mainly α-L-fucose, β-D-galactose, and β-D-N-acetyl glucosamine sugar chains (Mogi et al. 2013). Cell number increase was confirmed by directly enumerating stained cells (4′,6- diamidino-2-phenylindole). Linear sweep (LS) voltammetry examination showed that the redox potentials of $E(O_2/H_2O)$ and $E(Fe^{3+/}Fe^{2+})$ under physiological conditions of PV-1 were estimated to differ by 0.19 V. Value that is one order of magnitude lower than the difference of the redox potentials of $E(O_2/H_2O)$ and $E(CO_2/CO_2^{\bullet -})$. Therefore, Mogi et al. (2013) developed a system that may proceed at an external low-voltage <1.24 V. The minimum input voltage required to trigger voltage-multiplier circuits in electronic devices is typically in the range of 0.3–1.2 V. The authors highlighted that the low input voltage (0.19 V) bioelectrochemical system described provides new opportunities to exploit low-voltage electricity for the production of chemical fuels.

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Erratum to Chapter 1: *Bdellovibrio* and Like Organisms

Or Rotem · Zohar Pasternak · Edouard Jurkevitch
Department of Plant Pathology and Microbiology, Faculty of Agriculture, Food and Environmental Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

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