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Editors

The Prokaryotes

Actinobacteria

Fourth Edition

Springer Reference

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With 786 Figures and 254 Tables

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Foreword

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

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

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

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

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

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

Happy hunting!

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Preface

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

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

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

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

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

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

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

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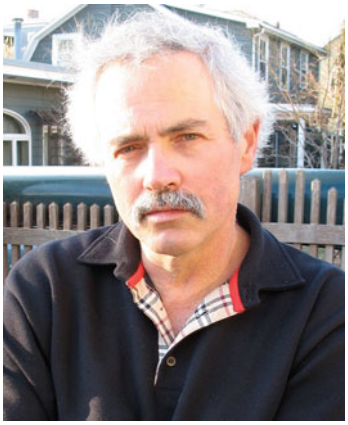
Edward DeLong received his bachelor of science in bacteriology at the University of California, Davis, and his Ph.D. in marine biology at Scripps Institute of Oceanography at the University of California, San Diego. He was a professor at the University of California, Santa Barbara, in the Department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



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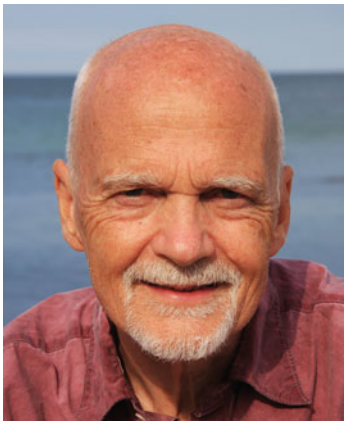
Stephen Lory received his Ph.D. degree in microbiology from the University of California in Los Angeles in 1980. The topic of his doctoral thesis was the structure-activity relationships of bacterial exotoxins. He carried out his postdoctoral research on the basic mechanism of protein secretion by Gram-negative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



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

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

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1 Harmonized Phylogenetic Trees for *The Prokaryotes*

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Abstract
The taxonomic outline of the current edition of *The Prokaryotes* is accompanied with the phylogenetic reconstruction of each respective taxon. In order to harmonize the reconstruction procedures and layout of over 350 trees, reconstructions have been produced using the LTP database (The All-Species Living Tree Project). This database is a compilation of 16S rRNA gene sequences representing the type strains of species with validly published names. The LTP team has applied a unified methodology and criteria for the harmonized reconstructions. In all cases, corresponding authors' instructions served to fine-tune the phylogenetic analysis and select the final tree topology.

Introduction

Volumes 06–11 of *The Prokaryotes, 4th Edition* (PK4), compile the taxonomy of Archaea and Bacteria up to year 2013. Taxonomic classification is based on objective criteria spanning phylogeny, genomic coherence, and a phenotypic property that guarantees the identification of the taxon (Tindall et al. 2010; Wayne et al. 1987). The advent of the sequencing technologies revealed the nucleotide sequence of highly conserved molecules such as ribosomal RNAs, enabling the reconstruction of phylogenies that reflect genealogic relationships of microorganisms (Woese 1987; Ludwig and Schleifer 1994). These phylogenies allowed as well the recognition of wrongly classified taxa (i.e., para and polyphyletic groups) promoting many reclassifications and also new classifications. Therefore, following primarily genealogical criteria, a major improvement of the Bergey's taxonomic outline was achieved at the beginning of the twenty first century (Garrity 2001). For practical reasons related to its informative content and availability, the 16S rRNA gene has been regarded as a priority in the classification of prokaryotes (Stackebrandt et al. 2002; Ludwig et al. 2011). Consequently, during the last decade of the twentieth century, the yearly rates in publishing descriptions of new species increased about sixfold (Tamames and Rosselló-Móra 2012), being the current rate between 600 and 700 new species per year.

Based upon the high rate of annual species descriptions, the exponential growth of environmental and non-type species 16S rDNA sequence entries in public repositories, the lack of a coordinated curation of type strain 16S rRNA gene sequences, and the lack of curated sequence-associated information, our group initiated "The All-Species Living Tree Project" or "LTP" (Yarza et al. 2008) to provide a reference tool for taxonomic purposes. The LTP project produces a highly curated 16S rRNA gene sequence database, alignment, and phylogenetic reconstruction for all type strains of Archaea and Bacteria with validly published names. All these resources are regularly updated and publicly available at the project's Web site (http://www.arb-silva.de/projects/living-tree/). The sequence selection process is based on stringent criteria to retain only high quality and of almost full-length sequences, both important prerequisites to achieve a reliable phylogenetic inference. Missing sequences from type strains without a good-quality SSU entry in former LTP releases (called "orphan" species) have recently been resolved by some international public microbial resource centers (mBRC) in a project called SOS, Sequencing Orphan Species (Yarza et al. 2013): the catalogue of the hitherto validly named species for which a type strain is available in at least one mBRC is now completed. All sequences included in LTP are automatically aligned, and manually optimized, according to the ARB-SILVA standards (Ludwig et al. 2004; Quast et al. 2013), which takes into account the 16S rRNA's secondary structure. In addition,

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curated sequence-associated metadata is associated to all LTP entries, including updated nomenclature and taxonomic classification according to LPSN (Euzéby 1997; Parte 2014; Yilmaz et al. 2014).

The PK4 editorial board has used the LTP as a basic framework to harmonize the phylogenetic reconstructions and tree layouts of almost all taxa included in the present edition. The reconstruction of the phylogenetic trees was based on general standard procedures and in accordance with the particular author's instructions when possible. Authors were initially supplied with several preliminary trees. A dialogue between the authors and tree-editor was then initiated to fix three parameters: sequence dataset, phylogenetic filter, and treeing algorithm (see below for details). The comparison of multiple topologies allowed understanding the topology stability and the selection of the best tree based on current sequence availability. In some cases multifurcations were manually drawn to indicate branching orders that could not be resolved. All figures provided by the tree-editors were finally validated by the respective authors of each chapter (~320 chapters). For practical reasons, the PK4 team decided that trees requiring the inclusion of sequences of uncultured organisms, candidatus taxa, or the use of highly specialized phylogenetic methodologies would not be object of harmonization (12 chapters).

Software Tools

The ARB software version 5.5 (Ludwig et al. 2004) was used for database management, alignment edition, and tree reconstructions. Implemented in the software package, the SINA alignment program was used for multiple sequence alignments (Pruesse et al. 2012). In addition, phylogenetic reconstructions were performed using two algorithms: neighbor-joining with the Jukes-Cantor model (Saitou and Nei 1987) and RAxML (version 7.0.3) with the GTRGAMMA correction (Stamatakis 2006) also implemented in ARB. For the final figure production, the program Xfig 3.2.5 was used.

Datasets

Sequences used corresponded to the LTP release 111. The dataset was complemented with the classified taxa up to December 2013 not included in the release and additional taxa requested by the authors. For maximum likelihood reconstructions, few representatives from closest families were selected as outgroups. On the other hand, for neighbor-joining reconstructions, 758 reference sequences of representative taxa were selected by means of quality criteria (almost complete length and low ambiguity rates) and taxonomic coverage (i.e., balanced representation of all orders included in the LTP); these sequences were included in the analyses to stabilize the topologies calculated by neighbor-joining. The reference sequences can be found in the latest LTP release (LTPs115) with the field "NJ_support_pk4_ltp." Most taxa analyzed would comprise some of these supporting sequences; hence, we refer to them as "nearly 750 supporting sequences."

Phylogenetic Filters

The use of sequence conservation profiles as phylogenetic filters is highly recommended to recognize branch attraction effects resulting from plesiomorphic sites (Tindall et al. 2010). These are designed to remove alignment columns according to positional variability. In addition, filters reduce the noise produced by sequencing errors, ambiguities, homopolymers, and low-confidence areas of the alignment with dubious positional orthology. A "termini" filter was used in all cases to remove sequence overhangs beyond the gene ends. This filter removes occasional plasmid vector fragments or gene stretches that had not been properly trimmed. In addition, several filters that improved stabilization and resolution of the branching orders were used. To better resolve deeper branches, a 40 % maximum frequency conservational filter was used to remove all hypervariable positions. Alternatively, depending on each specific sequences set divergence (indicated in their respective reconstruction), 10 %, 20 %, or 30 % conservational filters were applied. Occasionally, only termini and Domain positional filters were applied to be less restrictive and allow variable positions to solve short branches.

Figure Conventions

All sequences in the trees display species name and, in brackets, the corresponding accession number. The type strain representing a type species of a genus is tagged as "type sp." and highlighted with black color (*Fig. 1.1*). In order to focus the attention into the main chapter's taxa, neighboring groups are often presented as folded clades. All sequences in PK4 trees correspond to type strains unless the opposite is indicated.

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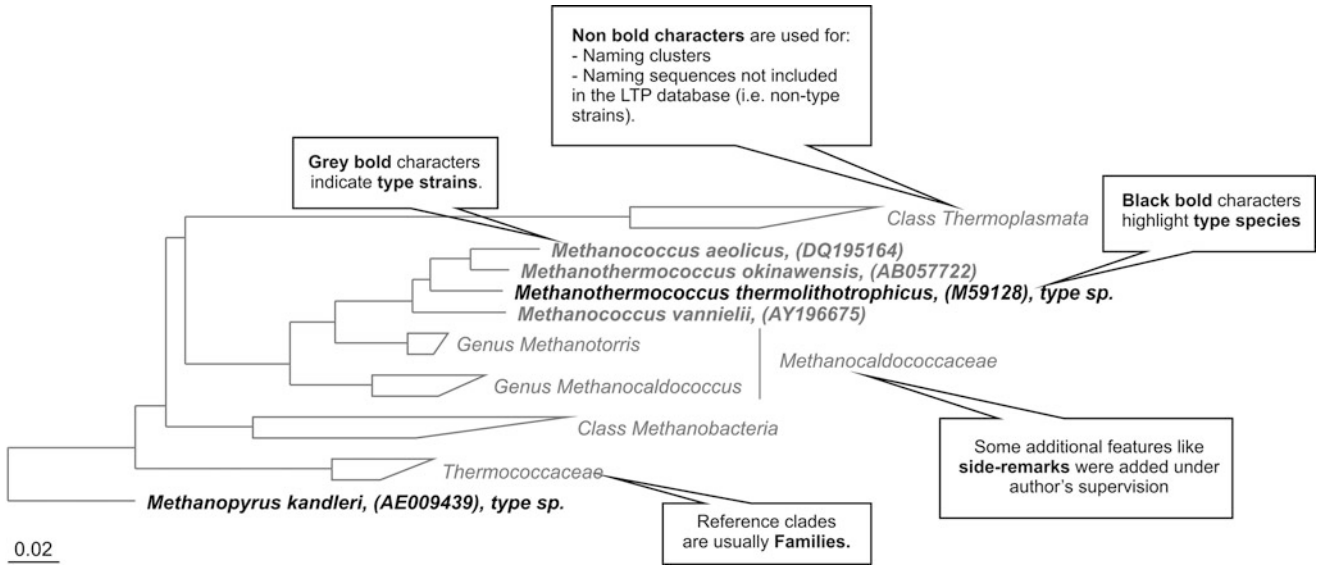

Fig. 1.1

Example of a standard PK4's phylogenetic tree layout. ***The tree in this figure is a draft, not a final version. Here it is used only as an illustration***

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2 The Family *Acidimicrobiaceae*

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Abstract
The order *Acidimicrobiales*, phylum Actinobacteria, is a phylogenetically well defined lineage that embraces 5 genera. While the family *Acidimicrobiaceae* harbors 5 genera, the family *Iamiaceae* is monogeneric. However, there is phylogenetic evidence that the genus *Ilumatobacter*, *Acidimicrobiaceae*, is more closely related to the genus *Iamia* than to other members of the family. While *Acidimicrobium*, *Ferrimicrobium, Ferrithrix and Aciditerrimonas* are obligate acidophilic, oxidize ferrous iron or reduce ferric iron and contain meso-diaminopimelic acid in their peptidoglycan, *Ilumatobacter* grows under neutral or slightly alkaline conditions, is and organotrophic and contains LL-diaminopimelic acid in its peptidoglycan. The iron oxidizing members are involved in uncontrolled pollution by heavy metals but are also used under controlled conditions for biomining.

Taxonomy, Historical and Current

The ranks subclass, order and family, *Acidimicrobidae*, *Acidimicrobiales* and *Acidimicrobiaceae*, respectively, were described by Stackebrandt et al. (1997) as ranks within the class *Actinobacteria*. The family has been emended by Zhi et al. (2009), by changing the set of 16S rRNA gene sequence signatures from 291 to 309 (U-A), 294–303 (C-G), 1118–1155 (C-G), 1311–1326 (A-U) and 1410–1490 (A-U), described in 1997 to 242–284 (U–G), 291–309 (U–A), 316–337 (C–G), 819 (U), 952–1229 (C–G) and 1115–1185 (U–G). The former set of signatures (Stackebrandt et al. 1997) were used for the affiliation of *Ferrimicrobium* and *Ferrithrix* to the family (Johnson et al. 2009).

The subclass was informally elevated to the rank of the class *Acidimicrobiia* (Garrity et al. 2005; Ludwig et al. 2012) and contains two families. The family *Acidimicrobiaceae* is composed of five monospecific genera, namely *Acidimicrobium* (Clark and Norris 1996), *Ferrimicrobium and Ferrithrix* (Johnson et al. 2009), *Aciditerrimonas* (Itoh et al. 2011) and *Ilumatobacter* (Matsumoto et al. 2009).

The family *Iamiaceae* comprises the monospecific genus *Iamia* and will be dealt with in a separate chapter (see chapter on ❯ *Lamiaceae*, Order, this volume). While the iron-oxiding taxa *Ferrimicrobium acidiphilum* $T23^T$ and *Ferrithrix thermotolerans* $Y005^T$ share 94.2 % 16S rRNA gene sequence similarity, *Acidimicrobium ferrooxidans* ICP^T is more distantly related (91–93 %). Even less close is *Ilumatobacter fluminis* $YM22^T$(<90 %), which clusters with *Iamia majanohamensis* in ❯ *Fig. 2.1*, though they are placed in separate families. The deep branching point of *I. fluminis* together with some markedly different features (❯ *Table 2.1*) cast doubt whether this organism should be retained in the family. The (tentative) decision to place this taxon into *Acidimicrobiaceae*, originally made solely upon its more closely relatedness to the iron-oxidizing taxa than to other members of the Actinobacteria (Ludwig et al. 2012), was adopted by Jean Euzeby (http://www.bacterio.cict.fr/).

The topology of the *Acidimicrobiaceae* lineage is not settled. As shown in a comparative study on 30 actinobacterial sequences (Rudi et al. 2006) only the tree based upon alignment-independent bilinear mathematical modeling showed a tight clustering of *Acidimicrobium* and *Rubrobacter* as a distinct subgroup of the Actinobacteria. A distinct lineage within Actinobacteria was also encountered in a phylogenetic analysis of ribosomal proteins (Lu and Zhang 2012). Maximum-parsimony and maximum-likelihood analysis of 16S rRNA gene sequences, on the other hand, showed a branching of *Acidimicrobium* and *Rubrobacter* outside the actinobacteria proper with low branch confidence (Rudi et al. 2006). This situation has also been encountered in a principal component analysis plot (Garrity and Holt 2001) in which *Acidimicrobium* was an outlier of the Actinobacteria, branching between Firmicutes and Actinobacteria. In the neighbor joining analysis of the LTP tree, the family appears as a sister clade of the family *Coriobacteriaceae*, distantly related *Rubrobacterales*. In the description of *Ilumatobacter* (Matsumoto et al. 2009) the family branches at the root of the order *Actinomycetales*.

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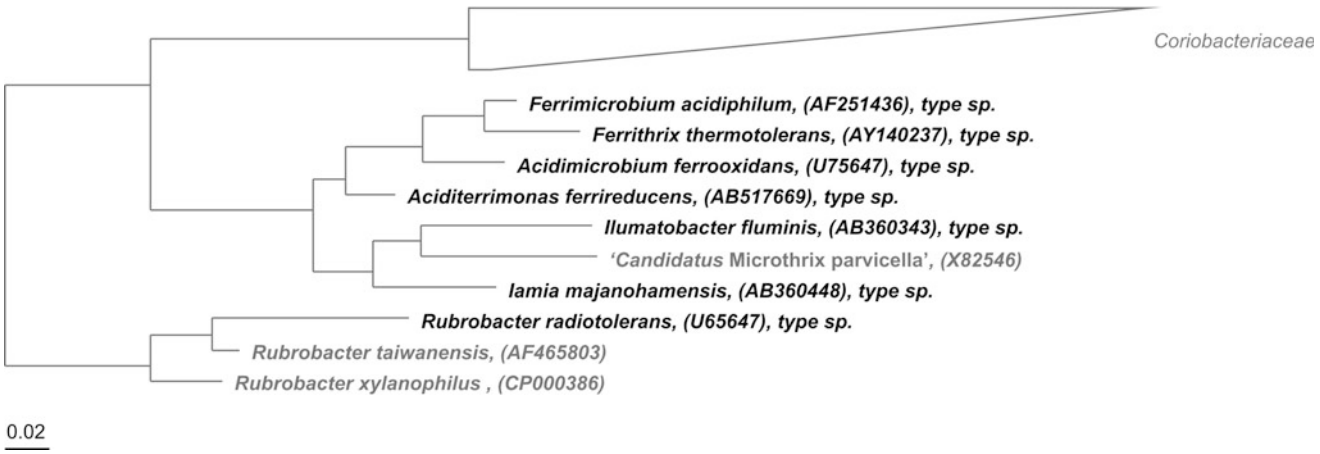

Fig. 2.1
Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the order *Acidimicrobiales* and some neighboring taxa present in the LTP_106 (Yarza et al. 2010). The tree was reconstructed by using a subset of sequences. List of type strains used for dendrogram construction: *Acidimicrobium ferrooxidans* strain ICP^T, *Aciditerrimonas ferrireducens* JCM 15389^T, *Ferrimicrobium acidiphilum* T23^T, *Ferrithrix thermotolerans* Y005^T, *Iamia majanohamensis* F12^T, *Ilumatobacter fluminis* YM22-133^T; no strain has been indicated for "*Candidatus* Microthrix parvicella." The bar indicates 1 % sequence divergence

The presence of additional members of *Acidimicrobiaceae*, one of them, even outnumbering A. ferrooxidans in nickel extraction from a mineral concentrate at 49 °C (Cleaver et al. 2007), has been mentioned before in several studies (Bond et al. 2000; Johnson et al. 2003; Cleaver et al. 2007). Norris et al. (2011) introduced the (as yet invalid) name "Acidithiomicrobium" for ferrous-iron organisms from geothermal habitats which were shown to grow autotrophically with sulfur as an energy source – a feature not expressed by any other member of the family (Clark and Norris 1996; Johnson et al. 2009). Two enrichment cultures, one from the island of Milos (strain P1), the other from a pyrite enrichment culture established with samples from several sources (Davis-Belmar and Norris 2009; Cleaver et al. 2007) (strain P2, also named strain sp. 2 by Cleaver et al. 2007), together with a uncultured organisms from acid volcanic soil (Mount Hood, USA), branches adjacent to *A. ferrooxidans* (see below).

As shown by Rheims et al. (1996) and later by Montalvo et al. (2005) on the basis of 16S rRNA gene sequences the nearest member of the family is "*Candidatus* Microthrix parvicella." This organisms is an uncultivated Gram-positive filamentous bacterium with filament of often <200 μm in length. It commonly occurs in low loaded domestic treatment plants where it is the most important cause of bulking sludge and frequently responsible for scum formation (Blackall et al. 1996; Rossetti et al. 2005).

Molecular Analyses

Due to the interest of bioleaching organisms, many strains of *Acidimicrobiaceae* have been enriched and identified by molecular means and several clone libraries have been generated which shows the abundance of these organisms in geothermal soil, acidic waters, sulfidic main waste and other mine environments as well as acidic biofilms (see Habitat). The most extensive compilation, based on 16S rRNA gene sequence comparison has been shown by Norris et al. (2011). While most clone libraries used extracted DNA for gene amplification, Militon et al. (2010) worked with reversely transcribed 16S rRNA.

Several probes for FISH and DNA dot blots were designed by Cleaver et al. (2007) to determine members of the *Acidimicrobium* group in bioreactor enrichment cultures: *Acidimicrobium* group (Am:654 CGATCYTCTACCGGACTC*)*, *A. ferrooxidans* (Amf:995 CTCTGCGGCTTTTCCCTC CATG) and "*Acidimicrobium*" species 2 (Am2:131 GTTGTCCCG-AACTATGGGG TAGG) (Bond and Banfield 2001) designed a FISH 16S rRNA gene probe to detect *Acidimicrobium ferrooxidans* and relatives from Iron Mountain, Ca (GTACCGGCCCAGATCGCTG). This probe was also used by Macalady et al. (2007) to identify the presence of these organisms in acidic, cave wall biofilms from the Frasassi cave system, Italy. Yet another 16S rRNA gene probe was designed by González-Toril et al. (2003) (CCT CCG AAT TAA CTC CGG) for the identification of *Acidimicrobium ferrooxidans* and *Ferrimicrobium* spp. in the Tinto river, Spain. The same environment was also probed by an oligonucleotide prokaryotic acidophile microarray (Garrido et al. 2008).

Among other Yellowstone isolates, the uniqueness of strain Y0018, closely related to *A. ferrooxidans* by 16S rRNA gene sequencing, was determined by Amplified ribosomal DNA restriction enzyme analysis (Johnson et al. 2003). ARDRA was also used to relate *Ferrimicrobium*-like organisms, originating from lithotrophic arsenite-oxidizing enrichment cultures (Sultana et al. 2012). Restriction fragment length polymorphism (RFLP) was used by Bond et al. (2000) to pre-screed the identity of isolated from a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid Mine drainage site and by Norris et al. (2011) for a pre-analysis of organisms from two enrichment sources cultures of different origin and by Xin et al. (2008) to

Table 2.1

Properties of members of the family *Acidimicrobiaceae*. *Acidimicrobium* (Clark and Norris 1996), *Ferrimicrobium* and *Ferrithrix* (Johnson et al. 2009), *Aciditerrimonas* (Itoh et al. 2011), and *Ilumatobacter* (Matsumoto et al. 2009)

Property	*Acidimicrobium*	*Ferrimicrobium*	Ferrithrix	Aciditerrimonas	Ilumatobacter
Morphology	Rods with variable length, filaments are noticeable in strain TH3	Rods, short chains during exponential phase	Long entangled filaments, flocs; sometimes single rods	Short rod	Rods
Motility	+, on yeast extract	+	–	+	–
CO_2 fixation	+	–	–	n.d.	n.d.
Presence of RuBisCo	+	–	–	n.d.	n.d.
Optimal growth temperature	45–50	35, not at 45	37–50	50	26–31
Optimal pH	2.0	2.0	1.8	3.0	7–11
Obligately acidophilic	+	+	+	+	–
Ferrous iron oxidation	+	+	+	–	n.d.
Autotrophic growth	+	–	–	+	
Heterotrophic growth on	Yeast extract	Glycerol, citric acid, glutamic acid	Glycerol, ethanol	Yeast extract or some sugars	Some enzymes for heterotrophic growth present
Peptidoglycan type	meso-DAP, A1γ[a]	meso-DAP, A1γ	meso-DAP, A1γ	meso-DAP, A1γ	LL-DAP, glycine, hydroxyglutamate
Major fatty acids (>5 % of total)	i-$C_{16:0}$, ai-$C_{17:0}$[b]	i-$C_{16:0}$, i-$C_{14:0}$, i-$C_{15:0}$, ai-$C_{15:0}$, $C_{17:1}\omega6c$	i-$C_{16:0}$	i-$C_{16:0}$, ai-$C_{17:0}$, i-$C_{18:0}$	i-$C_{16:0}$, i-$C_{17:1}\omega9c$, i-$C_{17:0}$, i-$C_{16:1}$, i-$C_{18:1}$, $C_{17:1}\omega8c$, $C_{18:1}\omega7c$
Major menaquinone	MK-9(H8)[b]	MK-10(H10)	Not determinable	MK-9(H8)	MK-(H8)
DNA mol% G + C	67–69	55	50	74	68

[a]Data from Normand (1996, 2006)
[b]Data from Kurahashi et al. (2009)

determine the presence of members of actinobacteria in marine sponges. The terminal restriction fragment length polymorphism (T-RFLP) method was used to determine the appearance of *Acidimicrobium* like organisms spp. in a granular sludge bed-anaerobic filter bioreactor which was dominated by *Methanosaeta*-like Archaea and beta- and gammaproteobacteria (Collins et al. 2005), by Wakeman et al. (2008) to study the dynamics in simulated-heap leaching of a polymetallic sulfide ore, by Jenkins et al. (2009) to determine that acidification of grassland results in the accumulation of acidophilic actinobacteria, including *Acidimicrobium*, *Streptomyces* and *Actinospica*, and by Zammit et al. (2012) in a recent proteomic study on the effect of chlorine ions on acidiphilic model organism. Single Strand Conformational Polymorphism Analysis (SSCP) analysis in addition to ARDRA and BOX analysis, as well as analysis of the V4-V5 region of the 16S rRNA gene by DNA pyrosequencing was applied by Köberl et al. (2011) to determine the shift in microbial populations due to long-term farming of desert soil. Among others, several extremophilic bacterial groups, e.g., members of *Acidimicrobium*, *Rubellimicrobium* and *Deinococcus-Thermus*, disappeared from desert soil after agricultural use.

Acidimicrobium ferrooxidans DSM 10331^T is the only strain of the family for which a complete genome (CP001631) is available (Clum et al. 2009). The circular chromosome with a DNA G+C content of 68.3 % mol% is 2,158,157 bp long, contains 2,092 predicted genes, 2,038 of which were protein coding genes, 54 RNAs and two rRNA operons. 76 % of the genes were assigned a putative function. Genes involved in amino acid transport and metabolism (174) exceeds those defined for carbohydrate transport and metabolism (87).

Phenotypic Analyses

The phenotypic diversity of the family is broad, due to the inclusion of *Ilumatobacter fluminis*. It contains autotrophic strains when grown on ferrous iron and heterotrophic organisms grown on yeast extract and/or organic compounds. Autotrophic and heterotrophic growth is shown by *A. ferrooxidans*. The family embraces acidiphilic and slightly alkaliphilic, as well as moderately thermophilic and mesophilic organisms. The iron-oxidizing members of the family oxidize iron (Fe^{2+}) under aerobic conditions or reduce Fe^{3+} under anaerobic conditions as shown by Bridge and Johnson (1998) and Johnson et al. (2009). With this capacity some of its members are involved in the geomicrobiological cycling of iron and CO_2 in acidiphilic ore-containing environments, as

exemplified in acidic environments (González-Toril et al. 2003) or in ore bioleaching (see sections Habitat and Application). Elementous sulfur is not oxidized by any validly named member of the family, though the hitherto invalidly named taxon "*Acidithiomicrobium*" oxidizes pyrite during autotrophic growth and growth well autotrophically on sulfur (Davis-Belmar and Norris 2009; Norris et al. 2011).

Acidimicrobium Clark and Norris 1996, Validation List N° 59

a.ci.di.mi.cro'bi.um ML.n. *acidum* acid, L. masc. adj. acidus; gr. adj. *micros* small; gr. masc. n. *bios* life; ML neuter n *Acidimicrobium*, referring to a bacterium from acidic environments.

The only species *A. ferrooxidans* was described for two strains, strain TH3 originally isolated from a test copper leaching dump (Brierley 1978) and strain ICP^T, isolated from a pyrite enrichment established with a sample from a geothermal site, Island. Strain TH3 was reisolated by Norris and Barr (1985) and the phylogenetic position of this facultatively thermophilic and Gram indeterminate strain determined to be a member of the Actinobacteria (Lane et al. 1992). The two strains show the same electrophoretic whole cell protein pattern, the same DNA G+C content, autotrophic growth on ferrous iron, heterotrophic growth on yeast extract, during which the cells are motile. The two strains differ from each other in the filamentous growth of strain TH3 on ferrous iron and yeast extract. Cells are gram-positive rods, 0.35–0.4 × 1–1.5 μm, which may be filamentous with variable length. Other properties are listed in *Table 2.1*.

The species is able to fix CO_2 at normal atmospheric concentration. It contains a CO_2 uptake system which is induced when CO_2 is limiting. A ribulose bisphosphate carboxylase/oxygenase (RuBisCO) is active during CO_2 fixation (Norris et al. 2011). As discussed by Clark and Norris (1996) the release of unidentified fixed carbon from strain ICP supports the mixotrophic growth of the Firmicutes *Sulfobacillus* species in mixed cultures. Gene sequence analysis indicates the presence of the large subunit of RuBisCO forms I and II (cbbL-1 and cbbL2). While cbbL-1 is present in *A. ferrooxidans* strains DSM 10331^T and strain P2, the cbbl-2 gene is only present in strain P2 (note, that strain P2 is a representative of the unnamed iron and sulfur-oxidizing "*Acidithiomicrobium*" taxon). The gcbbL-1 gene has probably been acquired from the proteobacterium *Acidithiobacillus ferrooxidans* or its ancestor, while the cbbL-2 gene of strain P2 is more similar to that of *Acidiphilium cryptum* and its relatives.

The type strain DSM 10331^T growth in the presence of iron (4.4 g/L) and zinc (3.3 g/L), but not in the presence of nickel and copper (1.5 and 0.6 g/L respectively). Another isolate of the species from a copper sulfide heap, however (Watkin et al. 2009), grows up to 40 g/L nickel and 45 g/L copper.

A proteomic study included *A. ferrooxidans* DSM 10331^T to investigate the effect of 6 g L^{-1} NaCl in brackish water bioleaching (Zammit et al. 2012). The highest number of 2.6 × 10^9 cells were found at 0 g L^{-1} NaCl while 2.0 × 10^9 cells L^{-1} were present at 20 g L^{-1} NaCl. Ferrous iron oxidation, however, was the same. Among others, upregulated proteins were those involved in membrane biosynthesis, α-subunit of the ATP synthase, amino acid biosynthesis genes, an oxidative stress protein peroxidase and the large chain of RuBisCO. Among the downregulated genes were AMP-dependent synthase and ligase (breakdown of complex fatty acids), a cell shape determining protein (actine-like filament forming).

Ferrimicrobium Johnson et al. 2009, 1087^{VL}

Fer.ri.mi.cro'bi.um. L. neut. n. *ferrum* iron; N.L. neut. n. *microbium* microbe; N.L. neut. n.

Ferrimicrobium iron microbe.

The type strain T23^T of *Ferrimicrobium acidiphilum* was among a group of five organisms originally obtained from acid mine drainage from pyrite and cobalt mines in Wales and Idaho (Bacelar-Nicolau and Johnson 1999). 16S rRNA gene sequence analysis indicated that these mesophilic heterotrophic acidophiles form a distinct cluster, related to the moderately thermophilic *Acidimicrobium ferrooxidans*. As they appeared to represent more than a single species, only strain T23^T was further characterized and described. In order to catalyze the oxidative dissolution of pyrite this heterotrophic iron-oxidizing bacterium required a source of organic carbon, provided in the study of Bacelar-Nicolau and Johnson (1999) in form of yeast extract. The authors also mention that the requirement for organic carbon is minimal and might, in nature, be satisfied by the organic carbon originating from autotrophic acidophiles, as well as from other sources.

The mode of action involves the oxidation of pyrite by producing ferric iron which oxidizes pyrite in an abiotic reaction, in which ferric iron is reduced back to ferrous iron (Bacelar-Nicolau and Johnson 1999). In mixed cultures with e.g. *Thiobacillus oxidans*, the reduced sulfur compound is oxidized to SO_4^{2-}. The authors mention the unpublished hydrophobicity of strain T23^T which makes it likely to attach to pyrite similar to the feature reported for *T. ferrooxidans*.

On solid ferrous iron and tryptone soya broth strain overlay plates displays gelatinous colonies (1–3 mm) with ferric iron deposits in the centre (fried-egg morphology). It grows poorly on solid ferrous iron medium and on solid ferrous iron, tryptone soya broth and potassium tetrathionate (Johnson et al. 2009). Under anaerobic conditions the type strain was able to reduce ferric iron, using glycerol as an electron donor. Tolerant to 200 mM of both ferrous and ferric iron, 150 mM copper, and less than 50 mM zinc. Neither cbbL-1 nor cbbL-2 forms of the RuBisCO genes were amplified.

Cells are gram-negative, rod-shaped (1–3 μm long) and motile. In liquid iron-containing medium the broth becomes turbid and orange colored. Growth in ferrous sulfate and 0.01 % yeast extract is supported by 5–15 mM glycerol, citric acid and glutamic acid, but not by glucose, fructose, ribose, glucosamine, glucuronic acid and ethanol. Acetic acid was highly toxic. Other properties are indicated in *Table 2.1*.

Ferrithrix Johnson et al. 2009, 1088[VL]

Fer.ri.thr'ix. L. neut. n. *ferrum* iron; Gr. fem. n. *thrix* hair, thread; N.L. fem. n. *Ferrithrix* iron thread.

The type strain Y005[T] of the only species, *Ferrithrix thermotolerans*, has been isolated from a geothermal site in the Beryl Spring/Gibbon river area of Yellowstone National Park, Wyoming, USA (Johnson et al. 2003). It shares with *Ferrimicrobium acidiphilum* several properties, such as obligate acidophily, phylogenetic similarity, absence of RuBisCO genes, lack of elemental sulfur oxidation, dissimilatory oxidation of ferrous iron, pyrite oxidation in yeast extract containing medium, reduction of ferric iron under anaerobic conditions using glycerol as electron donor, and heavy metal tolerance (<100 mM).

On ferrous iron, ferrous iron/tetrathionate and inorganic iron solid overly media colonies are small (1–2 mm), rhizoidal and iron-encrusted. In ferrous iron containing liquid medium growth occurs as flocs, approx. 1–3 mm in diameter. Flocs consist of long entangled filaments, 0.5 × 3–4 μm. cells are non-motile. Growth in ferrous sulfate and 0.01 % yeast extract is supported by 5–15 mM glycerol and ethanol, but by glucose, fructose, ribose, glucosamine, glucuronic acid, citric acid glutamic acid and ethanol. Acetic acid was highly toxic. Other properties are indicated in ● *Table 2.1*.

Aciditerrimonas Itoh et al. 2011, 1284[VL]

A.ci.di.ter.ri.mo'nas. L. neut. n. *acidum* an acid; L. n. terra soil; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Aciditerrimonas* acidic soil monad.

The type strain IC-180T, isolated from a sulfataric field at Ohwaku-dani in Hakone, Japan, is facultatively anaerobic and autotrophic, reducing ferric iron with H_2/CO_2 (4:1 v/v) under anaerobic conditions, but not under an $N_2/CO2$ (4:1 v/v) atmosphere. Does not oxidize ferrous iron. Heterotrophic growth occurs using yeast extract, glucose, lactose, mannose and xylose as carbon and energy sources. Yeast extract cannot be replaced by arabinose, fructose, galactose, ribose, sucrose, starch, acetate, butyrate, formate, fumarate lactate, malate, propionate or succinate. Cells are short rods, 0.5–0.6 × 0.8–1.1 μm, motile with peritrichous flagella. In addition to properties listed in ● *Table 2.1*, grows at 35–58 °C and pH 2.0–4.5; the phospholipid composition includes phosphatidyl-*N*-methylethanolamine and an unknown ninhydrin-positive phosphoglycolipid.

The 16S rRNA signature pattern is the same as that of the family *Acidimicrobiaceae* as indicated by Zhi et al. (2009), except for 952: 1229 (U–A).

Ilumatobacter Matsumoto et al. 2009, 204[NL]

I.lu.ma.to.bac'ter Gr. n. *iluma* -atos sediment deposited in water; N. L. masc. n. *bacter*, a rod, a bacterium; N. L. masc. n. *Ilumatobacter* a rod isolated from sediment.

The type strain of the monospecif genus, *I. fluminis* YM22-133[T], was isolated from sediment of the mouth of the Kuira river on the island Iriomote, Okinawa prefecture, Japan at the depth of 1 m. Colonies on Marine Agar were colorless and small (<1 mm). Aerobic, rod shaped 0.4–0.5 × 1.3–1.6 μm. As determined by the API ZYM (bio-Merieux) test panel, the following enzymes, among others, were active: alkaline phosphatase, esterase, (C4), esterase lipase (C8), leucin arylamidase, valin arylamidase, cystine arylamidase, acid phosphatase, α-glucosidase, and β-glucosidase. α-galactosidase, β-glucuroninidase, α-mannosidase and α-fucosidase were absent. Other properties are indicated in ● *Table 2.1*.

Isolation, Enrichment and Maintenance Procedures

The isolation protocols of ferrous iron-oxidizing actinobacteria differ widely. As indicated by Johnson et al. (2003) the main problem is the poor growth of these organisms on solid media and the separation from other strains exhibiting a similar metabolism. In the description of the type strain ICP[T] (Clark and Norris 1996) a Percoll gradient (Pertoft et al. 1978) was used to separate the larger *Sulfobacillus*-like organisms from the smaller *Acidimicrobium* cells. As the separation was not complete, the latter organisms was obtained in pure culture on solid medium after 35 serial autotrophic cultures under air. Another successful method includes the use of the overlay plating technique (Johnson and McGinness 1991; Johnson 1995). This method, leading to the isolation of a strain of the genus *Acidimicrobium* (Johnson et al. 2003) and the type strains of *Ferrimicrobium acidiphilum* (T23[T]), and *Ferrithrix thermotolerans* (Y005[T]) (Johnson et al. 2009), includes the use of overlaid solid media containing either ferrous sulfate (Feo) or ferrous sulfate plus potassium tetrathionate (FeSo); both media (pH ∼ 2.5), containing a small amount (0.025 %, w/v) of tryptone soya broth (TSB). *Aciditerrimonas ferrireducens* was isolated on *Sulfolobus* medium (Brock et al. 1972), supplemented with 1.0 g yeast extract (L^{-1}) at pH 2.2. and 55 °C. This medium was also used for maintenance, omitting $FeCl_3 \times 6H_2O$ (alternatively DSMZ medium 1189 (DSMZ 2001)).

Isolation of single colonies of "*Acidithiomicrobium*" was done on Phytagel plates (Sigma) supplemented with ferrous iron ($FeSO_4$. $7H_2O$, 13.9 g L^{-1}) and tetrathionate ($K_2S_4O_6$ 0.1 5 g L^{-1}) (Clark and Norris 1996), incubated at 47 °C. "*Acidithiomicrobium*" strain P2 were grown in shaken flasks with elemental sulfur as substrate (5 g L^{-1}) in a medium (Norris et al. 1996) initially at pH 3. This medium contained, per liter, $MgSO_4 \times 7$ H_2O, 0.5 g; $(NH_4)_2SO_4$, 0.4 g; K_2HPO4, 0.2 g; and KCl, 0.l g. With 50 mM ferrous iron as the substrate the pH was initially adjusted with H_2SO_4 to 1.7. Cultures growing autotrophically on ferrous iron, sulphur or pyrite were gassed with 5 % (v/v) CO_2, in air. All cultures were grown at 48 °C.

The establishment of enrichment cultures has been described by Johnson et al. (2003) and includes basically

a ferrous sulfate, yeast extract medium, pH 2.0 incubated, with intermittent shaking, at 45 °C. After 6 days (iron/yeast extract enrichments) or 35 days (pyrite enrichments), samples from these cultures were again streak inoculated onto Feo and FeSo solid media.

Isolates were purified by repeated single-colony isolation on Feo or FeSo solid media and maintained in ferrous iron/yeast extract liquid media or 10 mM ferrous sulfate/0.025 % Tryptic Soy Broth (pH 1.5), depending on the strain. Routine maintenance is recommended on DSM medium 709 consisting of $MgSO_4 \times 7\ H_2O$ 0.5 g, $(NH_4)_2SO_4$ 0.4 g, K_2HPO_4 0.2 g, KCl 0.1 g, distilled water 1,000 ml, pH adjusted to 2.0 with H_2SO_4. *Ferrimicrobium acidiphilum* and *Ferrithrix thermotolerans* are grown routinely in liquid media containing 10 mM ferrous sulfate and 0.02 % (w/v) yeast extract at pH 2.0. at 30 °C and 45 °C, respectively.

Ilumatobacter fluminis was obtained on medium "R" after cultivation at 25 °C for 30 days. Medium "R" is a mineral salt medium supplemented with antibiotics and Eagle's minimal essential medium. Enrichment was on Difco Marine Broth which can also be used for routine maintenance.

While *A. ferrooxidans*, *Aciditerrimonas ferrireducens* and *Ilumatobacter fluminis* are shipped as freeze dried cultures by the DSMZ, *F. acidiphilum* and *F. thermotolerans* are shipped as active cultures.

Habitat

Excluding *Ilumatobacter fluminis*, the range of habitat for the cultured acidiphilic autotrophic and heterophilic members of the family is rather narrow. The oldest representative, strain TH3, had been isolated from a Copper leach dump, Kennecott Chino Mine, New Mexico. Other strains originate from iron and pyrite mines or spent ore heaps (Bacelar-Nicolau and Johnson 1999; Cleaver et al. 2007; Readett et al. 2003; Yin et al. 2008; Davis-Belmar and Norris 2009), mine drainage water (Hallberg et al. 2006; Johnson et al. 2009), forested wetland impacted by reject coal (Brofft et al. 2002) and in geothermal sites in Yellowstone National Park (Johnson et al. 2009), Iceland (Clark and Norris 1996) or Milos, Greece (Norris et al. 2011).

The range of acidic habitats was verified and geographical sites expanded by non-culture studies. A large number of clones with BLAST similarity values above 95 %, even as high as 99 %, were found to be related to all acidophilic members of the family, with DNA retrieved from river water (González-Toril et al. 2003; Urbieta et al. 2012; Garrido et al. 2008; Garcia-Moyano et al. 2012; Souza-Egipsy et al. unpublished, EU370264), acid mine effluent (González-Toril et al. 2011), sulfidic mine waste dumps (Breuker et al. 2009), volcanic deposits (Fujimura et al. unpublished) and various hot springs (e.g., Korf et al. unpublished). Clone sequences of family members have also been found in acidic saline drain (Zammit et al. 2012), and in the soil environment, such as aliphatic hydrocarbon-contaminated soil (Militon et al. 2010), grassland (Jenkins et al. 2009), arsenic-contaminated soil (Sultana et al. 2012), desert soil (Köberl et al. 2011). They were also found in association with sponges (Montalvo et al. 2005; Xin et al. 2008) and in the biofilm on walls of sulfide mines (Bond et al. 2000; Bond and Banfield 2001) and cave environments (Macalady et al. 2007).

Not all sequences are highly related to the type strains of the three acidiphilic species, giving the impression of the occurrence novel species, even genera. Norris et al. (2011) and Itoh et al. (2011) compiled the phylogenetic position of many of the sequences of cultured strains and of clone sequences. Some of them cluster between *Ilumatobacter fluminis* and *Iamia majanohamensis* on the one side and members of *Acidimicrobium*, *Ferrimicrobium* and *Ferrithrix* on the other side, giving the impression that iron and heavy metal oxidation and reduction might be a widespread metabolic feature of members of the *Acidimicrobiales*. A few additional strains of *Ilumatobacter* have mainly been detected in the marine environment (e.g., AB286031, Kasai, unpublished).

Ecology and Application

Ferrous iron oxidizing organisms are widely present in appropriate environments in nature where they are involved in the oxidation of pyrite and other sulfidic minerals. As this also happens uncontrolled in ore waste and in active and shut-down mines and the environment, mainly streams and rivers, the environment is confronted with a serious pollution problem in terms of heavy loads of metals such as copper, nickel, cobalt, zinc and iron. Rowe and Johnson (2008) summarizes the main points of the microbial involvement in the iron cycle. Mainly bacteria are involved in biomining which is the controlled solubilization of sulfidic ores by acidophilic organisms, such as *Sulfobacillus* spp., *Thiobacillus* spp., *Leptospirillum* spp. and many other chemolithotrophic and heterotrophic acidiphilic strains that are either enriched by, or inoculated for, commercial bioleaching operations (Wakeman et al. 2008). The role of members of the family *Acidimicrobiaceae* to optimize such processes is acknowledged, though they are not considered to be the main players in iron and sulfur cycles. The ability of certain strains of *Acidimicrobium ferrooxidans* and other members of the family (as well as other moderately thermophilic iron-oxidizing bacteria) to reduce ferric to ferrous iron acid under oxygen limitation gives them an advantage to exploit environments, i.e., using reduced sulfur compounds as electron donors, when the oxygen content is lowered, such in thermal environments as seen in heap leaching, mineral waste dumps, tank reactor biomining and in geothermal sites (Bridge and Johnson 1998). Because it this potential, *A. ferrooxidans* is frequently used in experiments attempting to optimize conditions for commercial biomining (Bridge and Johnson 1998; Bacelar-Nicolau and Johnson 1999; Rowe and Johnson 2008; Wakeman et al. 2008; Watkin et al. 2009).

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3 The Family *Acidothermaceae*

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Abstract
Family *Acidothermaceae*, within the order *Actinomycetales*, contains thermophilic bacteria isolated from thermal springs and placed in genus *Acidothermus*. Genus *Acidothermus* was found and isolated from Yellowstone National Park, Wyoming, USA, in the course of a search for thermostable cellulases (Mohagheghi et al. Int J Syst Bacteriol 36:435–443, 1986). *Acidothermus cellulolyticus* is presently the sole species, in the sole genus of the family *Acidothermaceae*. A 16S rRNA-based phylogenetic study has found *Acidothermus* to be most closely related to *Frankia*; however, other genes have yielded different topologies. The genome of *A. cellulolyticus* strain 11B has been sequenced recently. Several genes for plant biomass degradation have been characterized; the thermostable properties of *A. cellulolyticus* enzymes for both cellulose and hemicellulose degradation have value for biotechnological applications.

The family *Acidothermaceae* was initially described as phylogenetically close to *Frankia* (*Frankiaceae*), *Cryptosporangium* (*Cryptosporangiaceae*), *Geodermatophilaceae*, (*Geodermatophilaceae*), *Nakamurella* (*Nakamurellaceae*), and *Sporichthya* (*Sporichthyaceae*) and grouped into suborder *Frankineae*. However, this suborder is now an order, *Frankiales*, and now considered to contain six families: besides the *Frankiaceae* the *Acidothermaceae*, the *Cryptosporangiaceae*, the *Geodermatophilaceae*, the *Nakamurellaceae*, and the *Sporichthyaceae* (Normand P et al. 2012).

Introduction

Thermal springs constitute highly unusual ecological islands, surrounded by mesophilic biotopes where forests and grasslands thrive, producing enormous amounts of cellulosic and lignin materials. Thermal springs are associated with fissures or faults in the earth's crust resulting from volcanic activity, as in the case of the Kamchatka Peninsula (Russia), or ancient caldera, as in the case of Yellowstone National Park (Wyo, USA). The resulting vertical circulation of water brings cold surface water in contact with hot deep layers with resulting geysers, pools, and more ephemeral thermal features. Microbial inhabitants of thermal features such as *A. cellulolyticus* have been the source of enzymes with thermostable properties that are useful in biotechnological applications.

Acidothermus strains were first isolated because they are capable of carrying out rapid degradation of cellulose, at relatively high growth temperatures (55 °C optimum, Mohagheghi et al. 1986). Cellulose is the most abundant form of carbon on earth, constituting the major fraction of plant structural matter that contributes to soil organic matter formation over centuries. Most animals cannot catabolize cellulose, except those that harbor cellulolytic bacteria in their caecum, rumen, or intestine.

The *Acidothermus* genome contains a gene cassette coding for highly efficient, thermostable enzymatic cellulose degradation (Tucker et al. 1989) that is now being employed in biotechnological applications. Cellulase enzymes from a few microorganisms are used industrially in paper and textile industries to modify fibers and improve textures and applications are increasingly possible during bioconversion of plant feedstock biomass to ethanol. However, to date, with the exception of the *A. cellulolyticus* E1 enzyme, the commercial cellulases are not thermostable.

The analysis of the complete genome of *A. cellulolyticus* 11B revealed the presence of genes predicted to encode xylanase (2), xylan esterase (3), and xylosidase (1) (Barabote et al. 2009), enzymes that degrade the hemicellulosic fraction of plant cell walls. Subsequent biochemical characterization has confirmed

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the thermostability and activity of one endoxylanase over a broad pH range (Barabote et al. 2010).

Recently, a cellulase from *A. cellulolyticus* has been incorporated into crop plants by genetic transformation and expressed to enhance digestibility during pretreatment of cellulosic biomass for biofuel production (Brunecky et al. 2011; Chou et al. 2011). The thermal adaptation of the enzyme prevents digestive activity until the feedstock is heated.

Taxonomy, Historical, and Current

Aci.do.ther.ma.ce'ae. masc. n. *Acidothermus* type genus of the family; suff. -aceae ending to denote a family; N.L. fem. pl. n. Acidothermaceae the *Acidothermus* family. This description is an emended version of the one given in the *Bergey's Manual*, 2nd edition (Normand et al. 2012).

The family *Acidothermaceae* was created by Rainey, Ward-Rainey, and Stackebrandt (in (Stackebrandt et al. 1997)) and emended by Zhi et al. (2009) to accommodate the single genus *Acidothermus* and the single species *Acidothermus cellulolyticus* (Mohagheghi et al. 1986). As its name implies, it grows in hot acidic springs (pH 4–6, 45–65 °C). Phylogenetic analyses based on the 16S rRNA gene have shown that *Acidothermus cellulolyticus* has as closest neighbors members of the genus *Frankia* (Normand et al. 1996).

Type genus: *Acidothermus* Mohagheghi, Grohmann, Himmel, Leighton and Updegraff 1986, 442^{VP}.

Genus I. *Acidothermus* Mohagheghi, Grohmann, Himmel, Leighton and Updegraff 1986, 442^{VP}.

A.ci.do.ther'mus. L. adj. *acidus* sour, acidic; Gr. adj. *thermos* hot; N.L. masc. n. *Acidothermus* acidic and hot (loving).

Slender rods and filaments, 0.4 × 5–20 μm, with rounded ends (Fig. 3.1a–d). Cell morphology in liquid culture depends on carbon source. When glucose or cellobiose is provided as sole carbon source, the organism grows as slender filaments, whereas on cellulose or xylan, the cells grow as short rods (Fig. 3.1a–d). No endospores are formed. No flagella have been reported, and no motility has been observed, although the genome of *Acidothermus cellulolyticus* contains the complete coding sequences for a flagellar apparatus (Barabote et al. 2009); expression of a *flaF* homolog has been detected in liquid culture in mid-log phase (M. Lee, Y.Y. Guo, R.D. Barabote, A.M. Berry unpublished). Gram-stain variable but generally Gram-stain negative. Thin sections show no outer cell membranes. The main constituents of purified cell walls are DAP, glucosamine, muramic acid, serine, and alanine. On LPBM mineral salts agar (Mohagheghi et al. 1986), colonies are creamy white, smooth, circular, entire, and 1–3 mm in diameter. In liquid culture, moderate turbidity is observed and cells may tend to flocculate and sediment out after 3 days. Obligate aerobes, prototrophic, grow on several carbon sources including D-glucose, cellobiose, and cellulose. Thermophilic with type species optimal temperature of 55 °C (range 37–70 °C) and acidophilic with optimal pH of 5.5 (pH range 3–7). Isolated from 45 °C to 65 °C acidic water and mud samples in Yellowstone National Park in the course of a screening program to obtain thermostable cellulases, but it has also been detected in soils by PCR (Talia et al. 2012) as well as a colonizer of plant tissues (Rezaei et al. 2011). Cells may be stored in 20 % (v/v) glycerol in LPBM medium at −80 °C and thawed on ice.

DNA G + C content (mol%): 66.9 (determined by complete genome sequencing).

Type species: *Acidothermus cellulolyticus* strain 11B Mohagheghi, Grohmann, Himmel, Leighton and Updegraff 1986, 442^{VP}.

1. *Acidothermus cellulolyticus* Mohagheghi, Grohmann, Himmel, Leighton and Updegraff 1986, 442^{VP}

cell.u.lo.ly'ti.cus. N.L. n. *cellulosum* cellulose; N.L. masc. adj. *lyticus* (from Gr. masc. adj. lutikos) able to loosen, able to dissolve; N.L. masc. adj. *cellulolyticus* cellulose-dissolving.

Morphology is as described for the genus. Grows on D-glucose, cellobiose, cellulose, xylan, D-galactose, maltose, sucrose, raffinose, D-mannose, D-mannitol, or D-sorbitol as sole carbon and energy sources. *A. cellulolyticus* 11B is auxotrophic and requires the addition of either yeast extract or Casamino acids to low-phosphate basal salts medium (LPBM) supplemented with a carbon source for growth. This organism could grow very well when all 20 proteinogenic amino acids were added to LPBM-cellobiose medium. Sequential elimination of each of the 20 amino acids revealed that the organism is unable to grow in the absence of either one of eight amino acids: arginine, aspartic acid, glutamic acid, histidine, lysine, proline, tryptophan, and tyrosine. The *A. cellulolyticus* 11B genome encodes all enzymes for the synthesis of arginine, glutamic acid, and proline from ornithine. When a pool of five of the eight amino acids (aspartic acid, histidine, lysine, tryptophan, tyrosine) were supplemented with ornithine, growth was restored (Fig. 3.2). This suggests that *A. cellulolyticus 11B* may be deficient in the biosynthesis of ornithine, an intermediate in arginine biosynthesis and the urea cycle.

No growth is observed on nutrient broth, acetate, lactate, citrate, or pectin. Citrate and acetate are inhibitory at 0.01 M. Resistant to penicillin G at 100 μg/ml; sensitive to vancomycin and lysozyme. Catalase-positive (Mohagheghi et al. 1986). Does not contain polyamines (Hamana et al. 1991). Actively digests cellulose and hemicellulose. Contains several genes for cellulose degradation (McCarter et al. 2002) and for hemicellulose degradation (Barabote et al. 2009). Contains a gene for *shc* (squalene-hopene cyclase) that may be involved in the ability to grow at high temperatures (Alloisio et al. 2005).

Source: isolated from 55 °C to 65 °C acidic water and mud samples in Yellowstone National Park in the course of a screening program to obtain thermostable cellulases (Mohagheghi et al. 1986).

DNA G + C content (mol%): 66.91 (determined by complete genome sequencing).

Type strain: ATCC 43068, 11B.

Sequence accession nos: AJ007290 (16S rRNA gene sequence of ATCC 43068 T); CP000481 (complete genome sequence of strain 11BT).

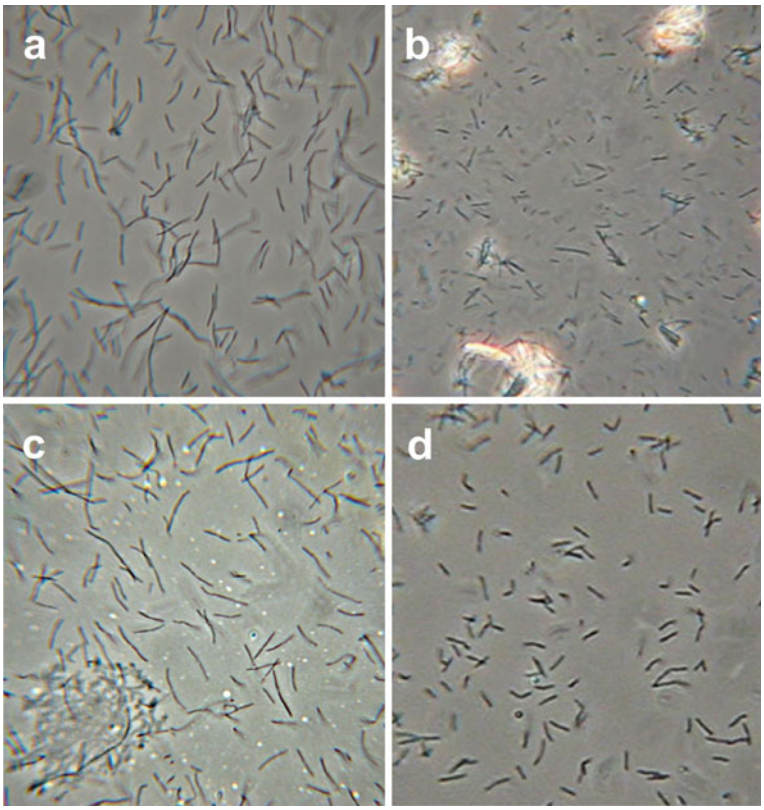

Fig. 3.1
Cell morphology of *Acidothermus cellulolyticus* 11B in shaking culture, with different carbon sources. (a) Glucose, 24 h; (b) cellulose 41 h; (c) cellobiose 24 h; (d) oat-spelt xylan 25 h (Y.Y. Guo, R.D. Barabote, A.M. Berry unpublished)

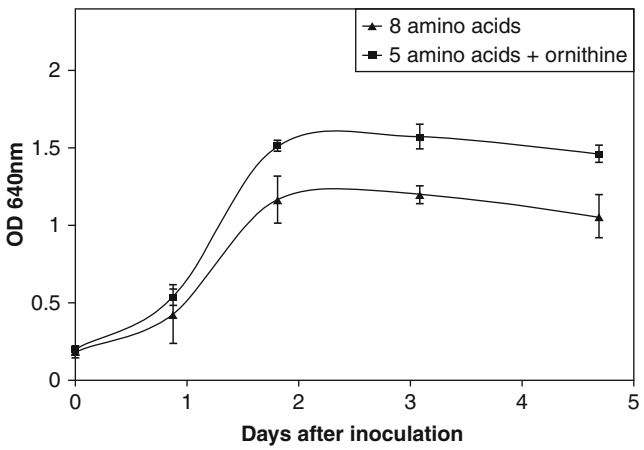

Fig. 3.2
Growth of *A. cellulolyticus* 11B in liquid culture, LPBM supplemented with eight amino acids (arginine, aspartic acid, glutamic acid, histidine, lysine, proline, tryptophan, and tyrosine) or with five amino acids (aspartic acid, histidine, lysine, tryptophan, tyrosine) plus ornithine (R.D. Barabote, S. Paliwal, A.M. Berry, unpublished)

Phylogenetic Structure of the Family and Its Genera

See *Fig. 3.3*

Analyses of 16S rRNA sequence have resulted in positioning genus *Acidothermus* in the Actinobacteria close to *Frankia* (Rainey and Stackebrandt 1993), despite a complete lack of common phenotypic features and their contrasted ecological biotopes. This proximity was confirmed by analysis of *recA* (Marechal et al. 2000) and *glnA* (Clawson et al. 2004), but the number of taxa sampled was in both studies limited. A more recent multi-locus genome-wide approach has not confirmed this proximity, positioning *Acidothermus* away from *Frankia* in a rake-like position with *Thermobispora bispora*, *Streptosporangium roseum*, *Thermomonospora curvata*, *Thermobifida fusca*, and *Nocardiopsis dassonvillei* (Wu et al. 2009).

These differences illustrate the difficulty of using a single marker to reconstruct the phylogeny of the genome as a whole. Each gene must thus be considered as having evolved independently from the others and to have moved from one microbe to the other. If *Nocardiopsis* is

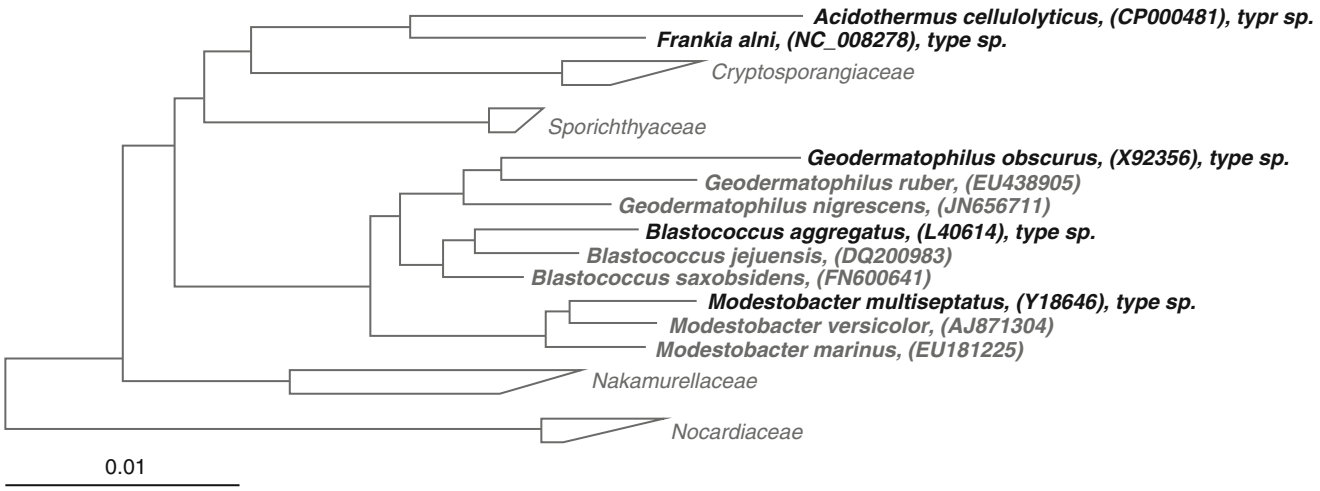

Fig. 3.3

Phylogenetic reconstruction of the family *Acidothermaceae* 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

Table 3.1

Characteristics of the *Acidothermus* genome compared with representative phylogenetic neighbors

Characteristic	*Ac*	*Fa*	*Go*
Genome size (nt)	2 443 540	7 497 934	5 322 497
G + C% of genome	66.9	72.8	74.0
G + C% of rRNA	60.8	58.3	59.4
# of genes	2,321	6,718	5,334
# of tRNA	46	46	48
# of rRNA operons	1	2	3
Protein coding density %	88.50	86.02	85.66
Genome accession number	CP000481	CT573213	CP001867

a skin pathogen and *Streptosporangium* is a soil inhabitant, it must be underlined that three of the neighbors mentioned (*Thermobispora*, *Thermomonospora*, *Thermobifida*) are thermophiles, which may have resulted in a high exchange rate to facilitate adaptation of an ancestral soil microbe to thermal springs life.

Dating the transition is not easy. It has been proposed that genes accumulate mutations at a constant rate, and by using some evolutionary events that have left fossil traces, it has been proposed that a 1 % difference in the 16S rRNA genes corresponded to 50 MY (Ochman et al. 1999). Since the distance between *Acidothermus* and *Frankia* spp. ranges from 4.6 % to 5.1 %, the emergence of *Acidothermus* from the *Frankia* clade would have occurred 230–250 MY ago at a time when higher land plants were becoming established over continents (Simon et al. 1993) but before the emergence of major actinorhizal lineages (Normand et al. 1996).

Molecular Analysis

The genome sequence of *Acidothermus*, a single chromosome with no separate plasmids, was published in 2009 (Barabote et al. 2009) and found to have features contrasted to those of its closest 16S-relatives, *Frankia* and *Geodermatophilus* (*Table 3.1*). The most striking feature is genome size, 2.44 Mb, less than half of that of its phylogenetic neighbors, a feature that is in all likelihood related to the habitat, thermal

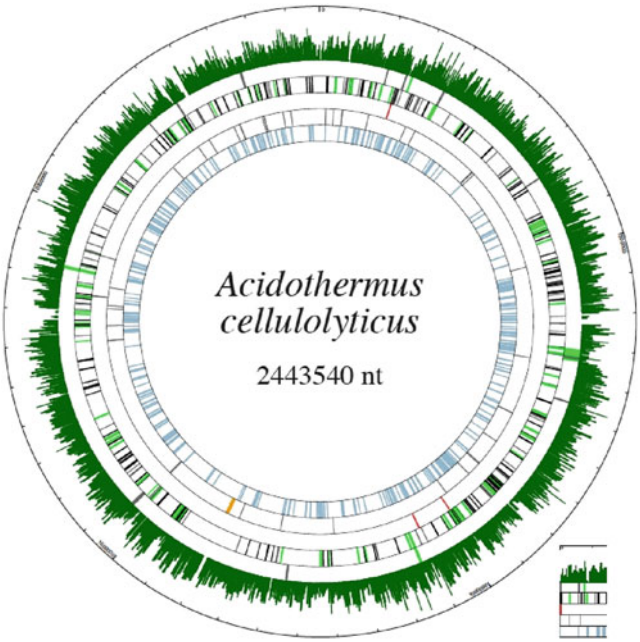

Fig. 3.4
***Acidothermus* circular map. 1-GC (range 55–80 %). 2-*Acidothermus* cellulase genes (*green*) and other glucanase genes (*gray*). 3-*Acidothermus* specific genes (unknown in *gray*; conserved in *black*; others in *green*). 4-Transposases (*gray*) and phage (*red*). 5-rRNA (*yellow*), and tRNA gen (*gray*). 6- *Acidothermus* Lgt genes (absent in *Frankia*, present in other actinobacteria, threshold 30 %)**

springs where high temperature likely imposes streamlining selection, and thus a reduced genome size (Sabath et al. 2013). The genome GC% is 66.9 %, markedly lower than those of its neighbors, while the GC% of the ribosomal genes is higher, illustrating that selection for adaptation to a thermophilic lifestyle is more targeted to rRNA than to DNA (Hurst and Merchant 2001).

Other potential genomic adaptations to a high temperature lifestyle such as revealed by principal component analysis (PCA) of global as well as synonymous codon usage, or ratios of IVYWREL amino acids in the *A. cellulolyticus* proteome and cytosolic subproteome, were investigated; however, no adaptational trend could be discerned (Barabote et al. 2009). These findings suggest that *A. cellulolyticus* may have only recently moved from a soil biotope to a hot spring lifestyle and is not yet fully optimized for it.

Several genomic islands with a lower G + C% and deviant dinucleotide signature were identified (Barabote et al. 2009); these carried no recognizable orthologs in close relatives but had their highest similarity to proteins from low G + C Gram-positives *Bacteroides, Nitrosococcus*, and *Thermoanaerobacter*. Furthermore, there were 21 secreted biomass-degrading enzymes whose genes are spread around the genome (◉ *Fig. 3.4*).

Fig. 3.5
Thermal features in the Norris Geyser Basin area, Yellowstone National Park, Wyoming, USA (Photo: A.M. Berry)

Phages: No phage has been described for *Acidothermus*; however, no specific work has been done for that purpose. A search in the genome for "phage" as keyword revealed only two occurrences; however, these genes are not conserved and are not close to one another.

Phenotypic Analysis

A. cellulolyticus utilizes plant biomass as carbon source, likely primarily cellulose and hemicellulose. This organism is not a hyperthermophile, but rather an intermediate thermophile, likely dwelling in acid thermal pools, streams, or other thermal features, which are surrounded by vegetation. In Yellowstone National Park, thermal features of the temperature range amenable to *A. cellulolyticus* typically occur in meadows or small riparian areas (◉ *Fig. 3.5*).

Plant cell walls are structurally highly complex. Even though cellulose and hemicellulose are primary components, pectins are also abundant in the matrix of primary cell walls. Lignin permeates plant secondary cell walls, e.g., in xylem (woody tissue). The genome of *A. cellulolyticus* does not contain homologs for pectin or lignin polymer degradation. Hence the plant biomass available as carbon source in the thermal features must be subject to additional mechanisms of processing. A comprehensive ecological study of *A. cellulolyticus* remains to be performed; however, it is reasonable to expect that both biotic community interactions and abiotic (thermal) factors may be involved in a coordinated process of biomass degradation. Pectin, for example, can be efficiently leached from plant cell walls simply by heating at low pH. Several fungal taxa degrade lignin effectively. Growth of *A. cellulolyticus* in liquid culture or on solid plant biomass substrate was strongly inhibited by a range of plant phenolic compounds, including monomers typical of lignin

formation or breakdown (Joh et al. 2011). Once the pectin (or lignin) is removed in the aqueous environment of a thermal pool, *A. cellulolyticus* would be capable of degrading the remaining hemicellulose and cellulose.

The endoglucanases are a class of enzymes used to recycle cellulose and generate glucose which can in turn generate fuels such as ethanol. One endoglucanase from *A. cellulolyticus* has thus been cloned into the fungus *Trichoderma reesei* to obtain hyper-expression (Zou et al. 2012). A codon-optimized synthetic gene encoding this thermostable enzyme was also transformed into rice (*Oryza sativa* L. ssp. *japonica*) under the control of the rice seed storage protein Gt1 promoter, with the enzymes produced in the seeds having an optimum pH of 5.0 and optimum temperature of 80 °C, which is similar to the enzymes produced by the native bacterium host, thus demonstrating that transgenic rice seeds could be used as a bioreactor for production of enzymes for cellulosic biomass conversion (Zhang et al. 2012). It is also a way to enhance cellulose digestion in the cow's rumen (Chou et al. 2011) or provide a pretreatment of plant feedstocks to facilitate subsequent bioconversion for biofuels (Brunecky et al. 2011).

Other thermostable determinants in the genome are interesting from an industrial point of view, such as the highly thermostable xylanases and related enzymes that can degrade hemicelluloses (Barabote et al. 2010), an alditol oxidase that can catabolize an array of polyols (Winter et al. 2012), or an aminopeptidase engineered into a transaminopeptidase by site-directed mutagenesis of catalytic Ser(491) into Cys to cause the formation of peptide bonds to yield linear homo-oligopeptides, hetero-dipeptides, and cyclic dipeptides (Usuki et al. 2011).

Conclusion

Acidothermus appears alone in its clade, with no close neighbor. It has been isolated from an acidic thermal spring but has also been detected directly from mesophilic soil and aquatic communities through 16S rRNA targeted studies (Talia et al. 2012). Its origin could be a recent evolutionary transition from a mesophilic soil ancestor and illustrates the speed at which bacteria can adapt to extreme conditions, presumably by taking up genes from bacteria already inhabiting the biotope.

Hyperthermophiles in some thermal springs have been well studied, especially in the emblematic Yellowstone National Park; however, aquatic ecosystems harboring organisms of moderate thermal adaptation are still waiting for an exhaustive study and may reveal the presence of *Acidothermus* relatives with varied metabolic or ecological properties. The expected massive arrival of metagenomic data, in particular from moderately acidic thermal features (streams, seeps, etc.) may contain genomes from *Acidothermus* relatives.

The study of the genomes of *Acidothermus* and relatives will likely continue to yield interesting thermostable enzymes for the conversion of plant biomass into fuel material. It may also help understand how genomes adapt to a range of extreme conditions.

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4 The Family *Actinomycetaceae*

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Abstract

Actinomycetaceae, the only family classified in the order *Actinomycetales* of the phylum Actinobacteria, embraces the genera *Actinomyces*, *Actinobaculum*, *Arcanobacterium*, *Mobiluncus*, *Trueperella*, and *Varibaculum*. Of these genera, the genus *Actinomyces* has been indicated to be quite diverse, and in a phylogenetic tree based upon 16S rRNA, it showed polyphyletic branching into a number of different clusters and individual lineges. Therefore, it is evident that the generic limits within the family are still uncertain, and although the genera *Actinobaculum*, *Arcanobacterium*, *Mobiluncus*, *Trueperella*, and *Varibaculum* have been studied in considerable detail, their relationship to the lesser known hypothetical genera is still unresolved. Members of the family are defined by a wide range of morphological and chemotaxonomic properties, such as polar lipids, fatty acids, amino acids of peptidoglycan, and whole-cell sugars which are used for the delineation of genera and species. Although many of the species of *Actinomycetaceae* have been extensively studied, using standard biochemical tests, the applicability of such tests have remained limited because the results are often irreproducible. The family harbors many important species which can be found in a wide range of habitats including human, animal, and environmental. Many of these species have been proven to be the casual agents of many human and animal infections.

Family *Actinomycetaceae* Buchanan 1918, 403 (emend. Stackebrandt, Rainey, and Ward-Rainey 1997, 484), emend. Zhi, Li, and Stackebrandt 2009, 594^{VP}.

Ac. ti. no. my. ce. ta' ce. ae. N. L. masc. n. *Actinomyces*, the type genus of the family; -*aceae*, ending to denote the family; N. L. fem. pl. n. *Actinomycetaceae*, the *Actinomyces* family.

Taxonomy, Historical and Current

The family *Actinomycetaceae* was created by Buchanan in 1918 and was originally used to accommodate many diverse organisms such as members of the genera *Actinobacillus*, *Leptotrichia*, *Actinomyces*, and *Nocardia*. After several revisions, membership of the family was restricted to bacterial species that appeared to be linked taxonomically by the following phenotypic characteristics, ability to produce Gram-positive, branching, and, later on, fragmenting filaments without aerial hyphae and spores; comparatively exacting nutritional requirements; facultatively anaerobic (capnophilic) to anaerobic growth; and fermentative carbohydrate metabolism (Slack 1974; Slack and Gerencser 1975). Taking into account these common features, the family *Actinomycetaceae* was thought to include the genera *Actinomyces*, "*Arachnia*," *Bifidobacterium*, "*Bacterionema*," and *Rothia* (Slack 1974). However, the validity of this family concept was increasingly questioned after modern and more relevant taxonomic techniques such as chemotaxonomic, numerical phenetic, and molecular genetic procedures had been applied to the respective organisms.

In the initial hierarchical classification of the class *Actinobacteria* using 16S rRNA gene sequence phylogenetic clustering and taxon-specific 16S rRNA signature nucleotides (Stackebrandt et al. 1997) and subsequent update of this classification by Zhi et al (2009), the family *Actinomycetaceae* Buchanan 1918 with the type genus *Actinomyces* Harz 1877 has been accommodated in the suborder *Actinomycineae* (Stackebrandt et al. 1997), emend. Zhi et al. 2009, order *Actinomycetales* Buchanan 1918 (Skerman et al. 1980), emend. Stackebrandt et al. 1997, emend. Zhi et al. 2009. This order has been included in the class *Actinobacteria* by Stackebrandt et al. (1997) and Zhi et al. (2009).

Additionally, in the current edition of *Bergey's Manual of Systematic Bacteriology*, a taxonomic modification of taxa with higher ranks has been proposed (Ludwig et al. 2012). Thus, with the elevation of the suborders of Zhi et al. (2009) to orders, the family *Actinomycetaceae* was classified as the only member of the order *Actinomycetales* of the phylum Actinobacteria. The family appears as an independent clade somewhat related to the family *Jonesiaceae* of the order *Micrococcales*. Though the current edition of *Bergey's Manual of Systematic Bacteriology* appeared in print later after the dissection of the genus *Arcanobacterium* to the genera *Arcanobacterium* and *Trueperella* (Yassin et al. 2011), the family comprises now the diffuse type genus *Actinomyces* and the five genera *Actinobaculum*, *Arcanobacterium*, *Mobiluncus*, *Trueperella*, and *Varibaculum*.

Molecular Analyses

Phylogenetic analysis using 16S rRNA sequence provided valuable insight into the classification and evolution of *Actinomycetaceae* (Fig. 4.1a). One major finding is that the family forms a monophyletic group within the Actinobacteria. Further, all tree construction methods agree that the family is divided into well-separated clades including the genera *Actinobaculum*, *Actinomyces*, *Arcanobacterium*, *Mobiluncus*, *Trueperella*, and *Varibaculum*. The monophyly of the genera *Mobiluncus* and *Trueperella* are strongly supported (bootstrap values = 96–100 %). On the other hand, the monophyly of the genus *Actinobaculum* is moderately supported (bootstrap value 73 %), whereas the monophyly of the genus *Arcanobacterium* is poorly supported (bootstrap value 63 %). The monophyly of the genus *Actinomyces* is not supported (bootstrap value 32 %). Interestingly, the monospecific genus *Varibaculum* is highly nested within the genus *Actinomyces* as sister to the species *Actinomyces neuii* (bootstrap value 98 %). The association of *Varibaculum* and *A. neuii* was recognized by Hall et al. (2003a). Strong support (bootstrap values = 96 %) for sister-clade relationship is found between the genus *Arcanobacterium* and the genus *Trueperella*. Noteworthy, all tree construction methods consistently indicated that the genus *Actinomyces* is polyphyletic, result which was foreshadowed by Pascual Ramos et al. (1997a).

Currently, the family *Actinomycetaceae* is delineated from other members of the phylum Actinobacteria solely on the basis of its branching position in 16S rRNA gene trees. With the exception of the genus *Actinomyces*, all genera of the family are primarily distinguished on the basis of genus-specific 16S rRNA signature nucleotides (Table 4.1). However, these signature nucleotides are based on published 16S rRNA sequences of type strains, and they change when new sequences are added to the databases.

Overview of Genomic Features of *Actinomycetaceae*

Genomic features of limited numbers of *Actinomycetaceae* have been described by various authors (Gorlas et al. 2012; Roux et al. 2012; Yasawong et al. 2010). Some features of the completed genomes are summarized in Table 4.2. The sequenced genomes varied in size from 1.71 Mb (*Actinomyces coleocanis*) to 2.39 Mb (*Mobiluncus mulieris*) with G+C contents varying from 49.6 % (*Actinomyces coleocanis*), 53.1 % (*Arcanobacterium haemolyticum*), and 55.6 % (*Mobiluncus curtisii*) to 68.73 % (*Actinomyces urogenitalis*) and possess a large number of tRNA molecules ranging from 46 (*A. coleocanis*) to 50 (*Arcanobacterium haemolyticum*). The genomes had similar global partition of protein functions, as measured by the distribution of COG families. COG analysis revealed that members of the *Actinomycetaceae* had significantly higher abundance of the proteins responsible for carbohydrate transport and metabolism (6.77–14.25 % of total gene count), translation (6.08–10.87 %), transcription (6.36–9.08 %), amino acid transport and metabolism (5.49–8.56 %), energy production and conservation (5.21–6.29 %), and inorganic ion transport and metabolism (4.04–6.72 %), but lower abundances of the proteins responsible for defense mechanisms (2.01–3.16 %), lipid transport and metabolism (1.85–2.93 %), and secondary metabolite biosynthesis (0.68–1.09 %). This profile indicates that the principal source for energy production and biosynthesis of cellular

components in this group of bacteria originates from the fermentation of polysaccharides or dietary fiber, which results in the production of short-chain fatty acids that are then used as energy sources. This probably explains their specific ecological success as commensals of the mucous membranes of the gastrointestinal tract, vagina, and dental plaque of humans and animals.

Furthermore, a number of conserved signature indels (CSIs) and whole proteins or conserved signature proteins (CSPs) that are specific for species have been identified in the genome of sequenced species of *Actinomycetaceae* (Gao and Gupta 2012). The latter authors reported that the enzyme deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), which is a part of the nonmevalonate pathway of isoprenoid biosynthesis, contains a 12-amino acid (aa) insert in a highly conserved region that is uniquely present in all available sequences of the genera *Actinomyces*, *Arcanobacterium*, and *Mobiluncus*. Another CSI consisting of a 6-aa insert that is specific for all sequenced *Actinomycetaceae* species is present in the integral membrane protein. The high degrees of conservation and specificity of these signatures for species of this family indicate that they provide good and reliable molecular markers for this family. Isoleucine tRNA synthetase (IleRS), which is essential for protein synthesis, also contains a 3-aa insert in a conserved region that is specifically present in all available sequences of the genera *Actinomyces* and *Mobiluncus* but which is lacking in *Arcanobacterium haemolyticum* as well as all other actinobacteria

Genome Properties of the Genus *Actinomyces*

Of the currently recognized 41 *Actinomyces* species, two type strains that belong to *A. coleocanis* DSM 15436^T and *A. urogenitalis* DSM 15434^T in addition to *A. odontolyticus* ATCC 17982 and a number of clinical isolates belonging to different *Actinomyces* species are at various stages of sequence completion (Table 4.2). The genomes size of *Actinomyces* species range from 1.7 to 3.04 Mb and possess a large number of tRNA molecules (from 46 to 51). Genomic G+C content ranging from 49.6 % to 68.73 %. The overall composition of COG supracategory was similar throughout the genus.

The genome of the type strain of *A. urogenitalis* DSM 15434^T (BCM-HGSC). (GOLD ID Gi02602) is 2, 614,023 bp long, contains 2,453 genes, including one copy for the 16S rRNA gene, and the mol% G+C of DNA is 68.73 %. Of the 2,453 gene predicted, 2,403 were protein-coding genes and 50 RNA genes were identified (Table 4.2). The majority of the protein-coding genes (68.2 %) were assigned with a putative function while the remaining ones were annotated as hypothetical proteins. The highest number of genes associated with general COG functional categories is found for carbohydrate transport and metabolism (263) followed by amino acid transport and metabolism (158) and transcription (145).

The genome of *A. coleocanis* DSM 15436^T (BCM-HGSC). (GOLD ID Gi02601) consists of 1,719,346 base pairs with

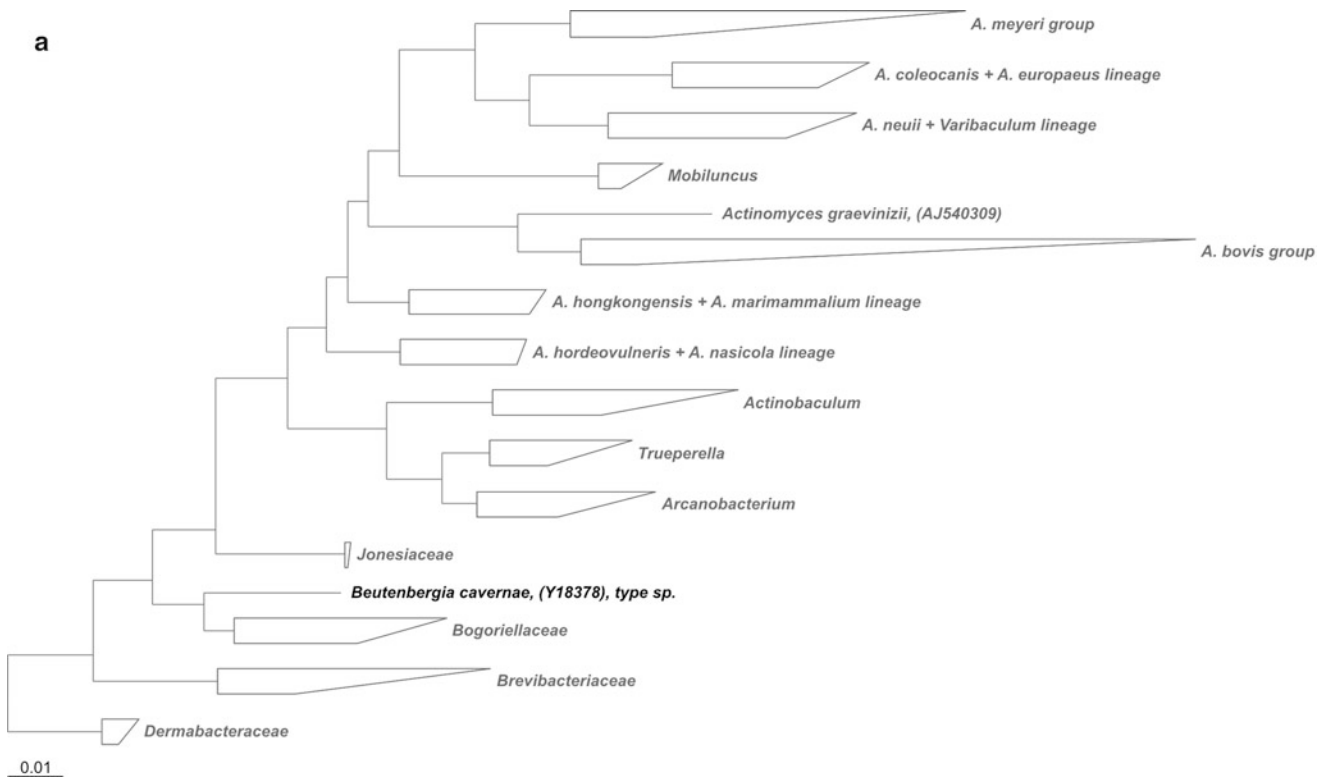

Fig. 4.1 (Continued)

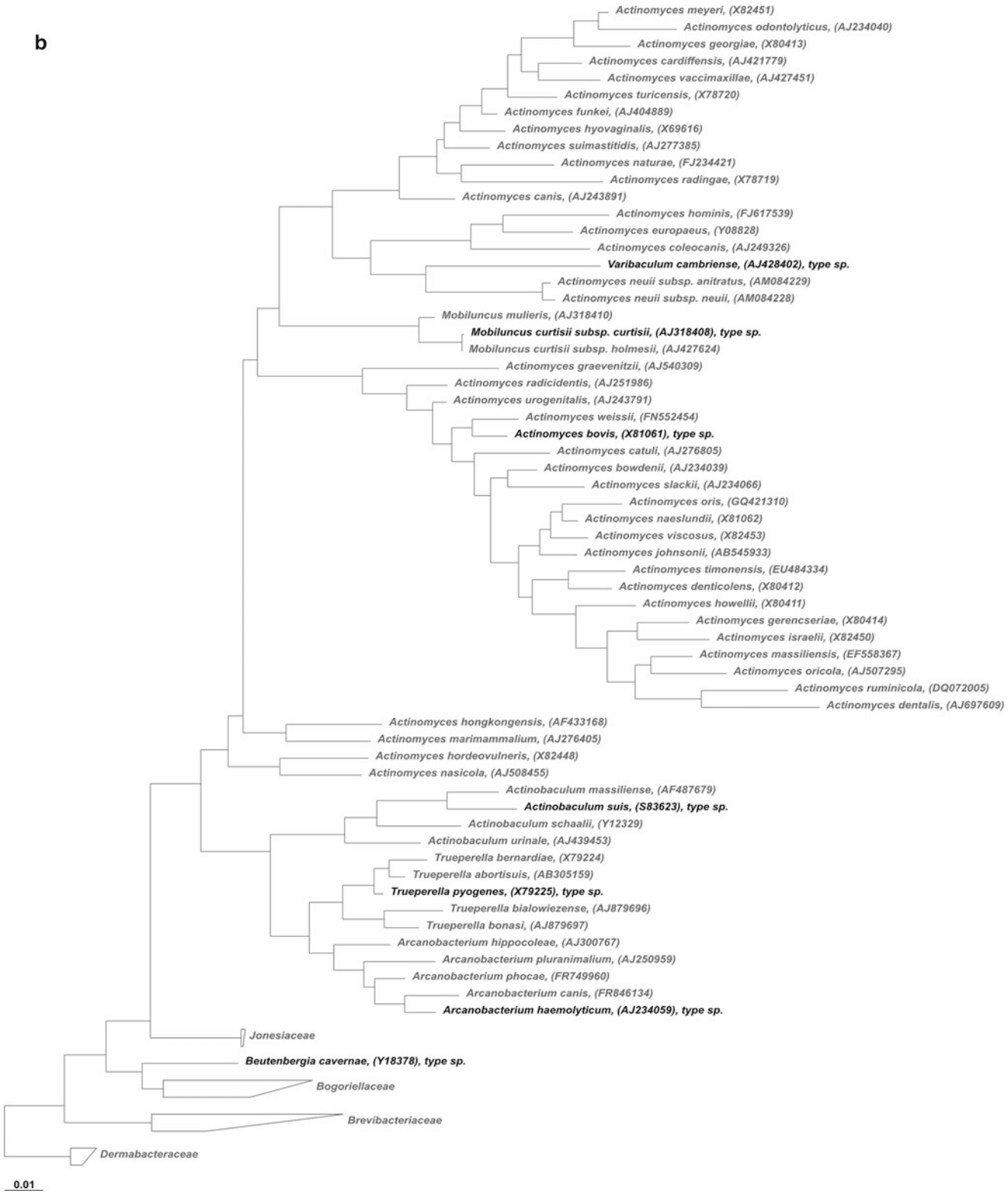

Fig. 4.1

Phylogenetic reconstruction of the family *Actinomycetaceae*, (a) folded tree and (b) unfolded tree, 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

Table 4.1

16S rRNA Signature nucleotides that define the two main clusters and the four lineages of the genus *Actinomyces* as well as the type species of the validly published genera of the family *Actinomycetaceae*

	Actinomyces main groups			Lineages									Genera			
E. coli position	Core cluster I	*A. graevinizii*	Core cluster II	*A. coleocanis*	*A. europaeus*	*A. hominis*	*A. marimmamalium*	*A. hongkongensis*	*A. hordeovulneris*	A. nasicola	*A. neuii*	*Varibaculum*	*Arcanobacterium*	*Trueperella*	*Actinobaculum suis*	Mobiluncus
70–98	G-A	A-A	Y-R	A-G	A-G	A-G	G-A	G-A	A-G	A-G	A-G	A-G	G-A	G-A	**A-U**	G-A
100	G/U	U	G/U	G	G	G	U	U	G	G	G	G	U	U	**C**	U
114–313	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	**C-G**
145–177	C-G	**U-G**	Y-G	U-A	U-G	U-G	U-G	U-G	U-G	U-G	U-G	U-U	U -R	U-G	U-G	U-G
146–176	**U-G**	U-G	**G-Y**	**U-A**	**U-A**	**U-A**	**G-U**	**G-U**	**G-C**	G-U	G-U	G-C	G-Y	G-Y	G-U	G-U
154–167	Y-R	U-A	G-U	U-G	C-G	C-G	C-G	U-G	G-U	U-G	C-G	C-G	C-G	C-G	C-G	**G-U**
157–164	Y/R-R/Y	U-G	**C-G**	**A-U**	**A-U**	**A-U**	U-G	C-G	C-G	C-G	G-U	C-G	**U-G**	**G-U**	**G-U**	U-G
166	G/Y	U	R/C	G	U	U	U	U	G	U	G	U	G	G	G	**C**
307	C	A	R	A	G	G	C	C	C	C	U	U	C	C	**U**	U
316–337	C-G	C-G	**U-G**	C-G	C-G	U-G	C-G	C-G	C-G	C-G	C-G	U-G	U-G	U-G	U-G	C-G
408	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	**A**
411	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	**G**
440	C	C	C	C	C	C	C	C	C	C	C	U	**U**	**C**	U	U
441	G/U	G	G	G	G	G	A	A	A	A	G	G	A	A	G	**U**
443	C	U	U	U	U	U	U	U	U	U	C	U	**C**	**U**	U	U
444–490	R-Y	A-U	**C-G**	A-U	G-U	G-U	A-U	A-U	A-U	A-U	C/U-G	C-G	A-U	A-U	G-U	C-G
446–488	U-G	**G-C**	U-G	G-A	G-A	G-A	G-C	G-C	G-C	G-C	C-G	U-G	**U-G**	**A-U**	A-U	Y-G
450–483	G-C	**C-G**	**C-G**	G-C	A-U	A-U	G-C	G-C	G-C	G-C	**A-U**	**C-G**	C-G	R/Y-Y/R	G-C	A-U

501–544	C-G	C-G	C-G	**U-A**	**U-A**	**U-A**	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G	C-G
502–543	G-C	G-C	G-C	**A-U**	**A-U**	**A-U**	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C
560	A	A	A/U	A	U	U	A	A	A	A	A	A	A	A	A	**U**
590–649	U-G	U-G	U-G	U-G	U-G	U-G	**G-U**	**G-U**	U-G	U-G	U-G	U-G	U-G	U-G	U-G	U-G
591–648	**G-Y**	**G-C**	**V-A,Y**	U-A	U-A	U-A	U-A	U-A	U-A	**U-U**	U-A	**G-U**	U-A	U-A	U-A	**C-G**
598–642	U-A	U-A	Y-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	**U-A**	**C-A**	**C-A**	U-A
613–627	Y-R	U-G	Y-R	C-G	U.G	C-G	**A-U**	C-G	C-G	**U-G**	Y-G	C-G	C-G	Y-G	C-G	**A-U**
614–626	C-G	C-G	C-G	A-U	G.U	A-U	A-U	A-U	A-U	**G-U**	C.G	A-U	G-C	G-Y	G-C	**G-U**
615–625	Y-G	**C-G**	Y-G	G-C	U-A	U.G	**U-G**	G-U	C-G	C-G	G-C	**U-G**	**G-C**	**G-U**	**G-U**	C-G
668–738	A-U	**U-A**	A-U	A-U	A-U	A-U	A-U	A-U	A-U	A-U	**U-A**	A-U	U-A	U-A	U-A	U-A
669–737	G-C	**A-U**	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	**A-U**	G-C	A-U	A-U	G-C	G-C
835–851	G-C	**A-U**	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C
838–848	G-Y	C-G	G-U	G-U	G-U	G.U	G-U	U-A	A-U	A-U	**G.U**	**G-C**	C-G	C-G	**U-G**	A-U
839–847	R-G,U	**U-G**	C-G	C-G	C-G	G.U	C-G	C-G	**A-U**	C-G	**C-G**	**G-U**	C-G	C-G	C-G	**U-G**
840–846	Y-G	C-G	C-G	U.G	C-G	C-G	U-G	U-G	U-G	C-G	U-G	C-G	U-R	U-G	C-G	**G-U**
929–1388	G-C	G-C	G-C	**A-U**	**A-U**	**A-U**	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C	G-C
1118–1155	**U-A**	U-A	**C-G**	U-A	U-A	U-G	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	U-A	**C-G**
1122–1151	**G-C**	G-C	**A-U**	G-C	G-C	G-C	G-C	A-U	A-U	A-U	A-U	A-U	G-C	G-C	G-C	**A-U**
1243–1294	C-G	C-G	Y-G	G.U	A-U	A-U	C-G	C-G	C-G	C-G	**U-G**	C-G	C-G	C-G	C-G	C-G
1244–1293	Y-R	U-G	U-R	C-G	U.G	U.G	**C-G**	**C-G**	U-G	C-G	**G-C**	**G-C**	**U-A**	R-G,U	C-G	C-G
1245–1292	G-C	**G-U**	G-Y	G-C	G.U	G.U	G-U	A-U	A-U	G-C	**U-G**	**U-G**	G-C	G-Y	G-C	**A-U**
1246–1291	G-C	G-C	G-C	G-C	G-C	G.U	G-C	G-C	G-C	G-C	**C-G**	**C-G**	G-C	G-Y	G-C	G-C
1308–1329	C-G	**U-A**	Y-R	C-G	C-G	A-U	C-G	U-A	U-A	U-A	C-G	C-G	U-A	U-A	C-G	U-A
1311–1326	**U-A**	U-A	**R-Y**	A-U	A-U	A-U	**U-A**	G-C	G-C	G-C	**A-U**	G-C	G-C	G-C	G-C	G-C

Abbreviations: *V* variable nucleotide composition, *Y* pyrimidine, *R* purine

Table 4.2
General genome features of some members of the family *Actinomycetaceae*

Organism	Genome size (Mb)	% G+C content	No. of protein-coding genes	No. of RNA genes	No. of rRNA genes	No. of 16S rRNA	No. of tRNA genes	No. of pseudogenes	Reference
Actinomyces coleocanis DSM 15436^T	1.71	49.6	1,546	52	4	1	48	–	BCM-HGSC
Actinomyces naselundii MG1	3.04	68.47	2,489	63	9	3	51	–	J. Craig Venter Institute
Actinomyces oris K20	2.87	67.8	2,939	53	3	1	50	–	Osaka University
Actinomyces odontolyticus ATCC 17982	2.39	65.45	2,159	60	10	3	48	–	Washington University, St. Louis
Actinomyces urogenitalis DSM 15434^T	2.61	68.73	2,403	50	4	1	46	–	BCM-HGSC
Arcanobacterium haemolyticum DSM 20595^T	1.98	53.1	1,821	64	12	4	50	90	Yasawong et al. (2010)
Mobiluncus curtisii subsp. *curtisii* ATCC 35241^T	2.13	55.66	1,894	52	3	1	46	–	Baylor College
Mobiluncus curtisii subsp. *holmesii* ATCC 35242	2.08	55.62	1,829	52	3	1	46	–	Baylor College
Mobiluncus mulieris ATCC 35243^T	2.39	55.14	2,300	48	3	1	45	–	Baylor College

49.6 % G+C content. Of the 1,598 genes predicted, 1,546 were protein-coding genes and 52 rRNA genes (*Table 4.2*). The majority of the protein-coding genes (68.9 %) were assigned a putative function, while the remaining ones were annotated as hypothetical proteins. The most represented classes of genes were those involved in carbohydrate transport and metabolism, followed by transcription (119 and 86, respectively) and amino acid transport and metabolism (80).

The genome of *Actinomyces odontolyticus* ATCC 17982^T (Washington University). (GOLD ID: Gi01705) consists of 2,393,758 bases with 65.45 % G+C content. Of the 2,219 genes predicted, 2,159 were protein-coding genes and 60 rRNA genes (*Table 4.2*). The majority of the protein-coding genes (66.7 %) were assigned a putative function, while the remaining ones were annotated as hypothetical proteins. The highest number of genes associated with general COG functional categories is found for carbohydrate transport and metabolism, followed by amino acid transport and metabolism (174 and 135, respectively) and transcription (113). Interesting from the perspective of pathogenicity, one gene annotated to COG category cell motility predicted to code for Flp pilus assembly protein TadC (COG2064). Pili are protein structures that extend from the surface of bacterial cells to allow the bacteria to adhere to their environment (Gerlach and Hensel 2007). Other *Actinomyces* species, for which whole-genome sequences currently available and its genome contains genes annotated to COG category cell motility are *A. naeslundii* MG1 and *A. oris* K20 (each containing three genes). One of these genes (COG3063) encodes for Tfp pilus assembly protein PiLF which enable bacteria to attach to host cells and other substrates (Burrows 2005; Skerker and Berg 2001).

Genome Properties of the Genus *Arcanobacterium*

The complete genome sequence of two species branching within the 16S rRNA gene tree of *Arcanobacterium* has been released. The genome of the type strain of *Arcanobacterium haemolyticum* DSM 20595^T (Yasawong et al. 2010). (GOLD ID Gco1291) is 1,986,154 bp long and contains 1,885 genes, including four copies for the 16S rRNA gene, and the mol% G+C of DNA is 53.1 %. The latter value falls into the range of 50–57 mol% determined for species of the genus *Arcanobacterium*. Of the 1,885 gene predicted, 1,821 were protein-coding genes and 64 RNAs; 90 pseudogenes were also identified (*Table 4.2*). The majority of the protein-coding genes (68.5 %) were assigned with a putative function while the remaining ones were annotated as hypothetical proteins. The highest number of genes associated with general COG functional categories is found for translation, ribosomal structure, and biogenesis (136), followed by carbohydrate transport and metabolism (125) and replication, recombination, and repair (119). In accord

with the genus description, a gene (GenBank:YP 003696699) specifying homologs of CAMP (Christie, Atkins, Munch-Petersen) factor family protein, which are typically found in pathogenic staphylococci, was also identified. CAMP factors have been shown to bind to immunoglobulins of the G and M classes and have long been known as pathogenic determinants. Interestingly, two genes, one encoding hemolysin A (GenBank: YP 003697287) and the other encoding phospholipase D (GenBank:YP 003697432), were also identified. Hemolysin A and phospholipase D are virulence factors involved in *A. haemolyticum* infections and both with cytotoxic effects.

Genome Properties of the Genus *Mobiluncus*

The complete genome sequence of two species branching within the 16S rRNA gene tree of *Mobiluncus* has been released. The genome of the type strain of *Mobiluncus curtisii* subsp. *curtisii* ATCC 35241^T (Baylor College of Medicine). (GOLD ID Gi03779) is 2,136,873 bp long and contains 1,946 genes, including one copy for the 16S rRNA gene, and the mol% G+C of DNA is 55.66 %. The latter value falls over the range of 49–54 mol% determined for species of the genus *Mobiluncus* (Hoyles et al. 2004). Of the 1,946 genes predicted, 1,894 were protein-coding genes and 52 RNAs were identified. The majority of the protein-coding genes (69.8 %) were assigned with a putative function while the remaining ones were annotated as hypothetical proteins. The highest number of genes associated with general COG functional categories is found for translation, ribosomal structure, and biogenesis (135), followed by amino acid transport and metabolism (116), followed by replication, recombination, and repair (105) and carbohydrate transport and metabolism (99). In accord with the genus description is the presence of genes (43) related to cell motility.

Phenotypic Analyses

The family encompasses non-spore-forming, nonmotile, ordinarily facultatively anaerobic bacteria that belong to the order *Actinomycetales* and contain Gram-positive, non-acid-fast, predominantly diphtheroid cells that tend to form branched filaments in tissue or in some stages of cultural development. The metabolism of these chemoheterotrophic bacteria is fermentative.

Examination of the chemical markers across the family *Actinomycetaceae* has not been thoroughly investigated enough to draw any firm conclusions regarding the delimitation and description of the family. Currently, extensive study has been performed to examine the chemical constituents of individual species assigned to genera of *Actinomycetaceae*. Table 4.3 (A. F. Yassin unpublished) provides a general overview of the distribution of particular chemical markers within the family.

The mode of cross-linkage and the amino acid composition of the tetrapeptide bridge of the peptidoglycan layer vary between members of the *Actinomycetaceae*. Generally, the type abbreviated A5 (Schleifer and Seidl 1985) is the only peptidoglycan type found, so far examined, in the genera *Actinomyces*, *Actinobaculum*, *Arcanobacterium*, and *Trueperella* (Table 4.3). Two subtypes have been identified: the one abbreviated A5α with L-lysine or L-alanine as the diamino acid in position 3 of the tetrapeptide subunit (L-Lys-L-Lys-D-Glu or L-Lys-L-Ala-L-Lys-D-Glu) was

Table 4.3
Chemotaxonomic characteristics of the genera of the family *Actinomycetaceae*

Organisms	Peptidoglycan type	Peptidoglycan	Cell-wall sugars	Acyl type	Menaquinones	Phospholipids	Fatty acids	Mol % G+C
Actinomyces bovis	A5α	L-Lys-L-Lys-D-Asp	Glucose + mannose + rhamnose + 6-deoxytalose	Acetly	MK-8 + MK- 9[a] + MK-10	DPG, PG, PC, PI, PIM	S, U	63.8
Arcanobacterium	A5α	L-Lys-L-Lys-D-Glu	Rhamnose + glucose	Acetyl	MK-8(H_4) + MK-9(H_4)[a]	DPG, PG, PI	S, U, I, A	50–52
Trueperella	A5α	L-Lys-L-Lys-D-Glu or L-Lys-L-Ala-L-Lys-D-Glu	Rhamnose + glucose	Acetyl	MK-9(H_4) + MK-10(H_4)[a]	DPG, PG, PI, PGL	S, U, I, A	56–58
Actinobaculum	A5α	L-Lys-L-Lys-D-Glu or L-Lys-L-Ala-L-Lys-D-Glu	Glucose + rhamnose + 6-deoxytalose	Acetyl	Abscent	DPG, PG, AbGL	S, U	55–57
Varibaculum	ND	Not determined	Galactose	Acetyl	MK-8(H4) + MK-9(H4)[a]	DPG, PG, PC, PI, PIM	S, U, I, A	51–55
Mobiluncus curtisii subsp. *holmesii*	ND	Not determined	Galactose + rhamnose	Acetyl	MK-8 + MK-9[a]	DPG, PG, PGL	S, U, I, A	49–52

[a]Major component; *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PC* phosphatidylcholine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PGL* phosphoglycolipid, *AbGL* choline-containig phosphoglycolipid. *S* straight-chain, saturated, *U* monounsaturated, *A anteiso*-methyl-branched, *I iso*-methyl-branched. Mol% G+C, guanine phus cytosine content of the DNA

found in the peptidoglycan of the genera *Actinobaculum*, *Arcanobacterium*, *Trueperella*, and some *Actinomyces* species. The second subtype abbreviated A5β with L-ornithine as the diamino acid in position 3 of the tetrapeptide subunit (L-Orn-L-Lys-D-Glu) is restricted to some species of the genus *Actinomyces* (Table 4.6).

The principal respiratory quinones distributed in the genera of the family *Actinomycetaceae*, except the genus *Actinobaculum*, are menaquinones with eight, nine, and ten isoprene units with varying degree of saturation (Table 4.3). Though the menaquinones of the majority of *Actinomyces* species have not yet been analyzed, two distinct patterns have been identified in the genus. The first pattern is characterized by fully unsaturated menaquinones MK-8 and MK-9 with the latter component being the major one. This pattern has also been found in the genus *Mobiluncus*. The second pattern consisted of MK-8(H_4) and MK-9(H_4) with the latter compound as the major component. This pattern was also present in the genus *Arcanobacterium*. The genus *Trueperella*, thus far, exhibits MK-10(H_4) as major menaquinone in addition to minor amount of MK-9(H_4). Species of the genus *Actinobaculum* differ from other genera of the *Actinomycetaceae* by their conspicuous lack of respiratory quinones.

A variety of phospholipid classes have been identified in members of the family *Actinomycetaceae*. For instance, diphosphatidylglycerol or cardiolipin (DPG) was found almost entirely in all members of the family. Generally, phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylinositol mannosides (PIMs) are variably distributed. The occurrence of phosphoglycolipids (PGL) is limited to the genus *Trueperella* (Yassin et al. 2011) and *Actinomyces naturae* (Rao et al. 2012). Noteworthy is the absence of phosphatidylethanolamine (PE) and monomethylphosphatidylethanolamines (PMME) and dimethylphosphatidylethanolamines (PDME). Of interest, members of the genus *Actinobaculum* are characterized by the presence of a choline-containing phosphoglycolipid resembling the compound isolated from *Mycoplasma fermentans*. However, the exact chemical structure and function of this compound remains obscure.

The cellular fatty acid profiles of the genera of *Actinomycetaceae* are very similar to each other and contain saturated normal and monounsaturated fatty acids from 12 to 20 carbon atoms. Striking, however, is the presence of one or two types of major fatty acids, which account for 60–90 % of the fatty acid mass. In particular, the dominant fatty acids are 18:1ω9c (accounting for 50–60 % of total fatty acids) and 16:0 (accounting for 12–18 % of total fatty acids). Traces of diunsaturated fatty acid 18:2 ω6,9c, *iso*-branched, and *anteiso*-branched fatty acids vary.

The following sugars were found as common constituents of the whole-cell hydrolysates of members of the family *Actinomycetaceae*: fucose, galactose, glucose, mannose, rhamnose, and 6-deoxytalose. However, differences in the cell wall sugar composition were observed in whole-cell hydrolysates of the different genera. Thus, the predominating cell wall sugar liberated from the whole-cell hydrolysate of *Varibaculum* is galactose, while galactose and rhamnose are the characteristic sugars released from the whole-cell hydrolysates of member of the genus *Mobiluncus*. The cell wall sugars of members of the genera *Arcanobacterium* and *Trueperella* are virtually identical, containing rhamnose and glucose. Species of the genus *Actinobaculum* are characterized by the presence of glucose, rhamnose, and 6-deoxytalose (except for *Actinobaculum suis* which do not contain 6-deoxytalose). In contrast, marked differences in the composition of cell wall sugars were observed between *Actinomyces* species. For instance, the cell wall sugars of *Actinomyces bovis* consists of glucose, mannose, rhamnose, and 6-deoxytalose, whereas the cell wall sugars of *A. meyeri* consists of galactose, glucose, mannose, fucose, and rhamnose and the cell wall sugars of *A. georgiae* consists of only galactose.

Thus, also the chemotaxonomic patterns that emerge strongly suggest that the genus *Actinomyces* is heterogenous and that some *Actinomyces* species should be excluded from the genus, even constituting the nuclei of new genera. In addition, certain variations concerning the peptidoglycan structure and cell wall sugars have been observed between *Actinobaculum suis* and the remaining members of the genus *Actinobaculum*. Apart from the genera *Actinomyces* and *Actinobaculum*, the distribution of a combined set of chemical markers have been considered typical for delineating the genera *Arcanobacterium*, *Trueperella*, *Mobiluncus*, and *Varibaculum*.

Isolation, Enrichment, and Maintenance Procedures

Most species of the genera of the *Actinomycetaceae* have been isolated from human and veterinary clinical materials. They are generally considered to be opportunistic pathogens, which seldom recovered as single isolates but found in association with other bacteria. The ability to isolate and distinguish these bacteria and to determine their sensitivity to antibiotics, could greatly assist treatment of diseases in which they are involved. The successful isolation of members of the *Actinomycetaceae* from clinical materials and environment depend on several factors such as oxygen requirements, incubation temperature, pH and provision of suitable medium. Members of the family vary considerably with respect to their oxygen requiremnets. Some are considered strict anaerobe growing well in the obsence of oxygen, while others are considered facultative anaerobe grown in an atmosphere containing 5%CO_2. The nutritional requirements of species within the family have been very little studied, and therefore, it is difficult to make generalization of the nutritional requirements. However, it is recommended that a combination of different media be used to maximize recovery rates from clinical materials.

Sampling Techniques

The specimens of choice for the isolation and enumeration of strains belonging to the genera *Actinobaculum*, *Actinomyces*, *Arcanobacterium*, *Mobiluncus*, *Trueperella*, and *Varibaculum* include deep-needle aspirates, draining sinus, body fluids, and

biopsy specimens. Inappropriate specimens for isolation of *Actinomyces* species include swabs, sputum, and urine. During sampling, precautions must be taken to avoid contamination with normal flora from skin or other body sources that may be introduced into the sample. An effective means of collecting material for microbiological examination is the use of fine-needle aspiration (Hong et al. 1993; Vera-Alvarez et al. 1993). Although some species are facultative anaerobes, samples obtained from sites of infection should be treated as anaerobic and exposure to atmospheric oxygen should be avoided to optimize recovery rates.

Transport Media

The most important parameter in the successful isolation of species of the genera *Actinobaculum*, *Actinomyces*, *Arcanobacterium*, *Mobiluncus*, *Trueperella*, and *Varibaculum* is minimizing the time between specimen collection and the incubation of the inoculated media. Therefore, prompt transport of clinical specimens to the microbiology laboratory is necessary for optimal isolation. In addition, techniques for collection and transport of clinical specimens should maintain anaerobiosis to enable satisfactory recovery of these anaerobic bacteria. The transport medium should also preserve the organisms at the same level and in the same properties they had at the sampling. To fulfil these requirements the transport medium should have low nutrients contents, no inhibitory factors, low oxidation–reduction potential, and physiological pH. Adequate transport media are commercially available, e.g., Port-A-Cul (Becton–Dickinson) and Portagerm (bioMérieux) which should be used according to the manufacturer's instructions. Apart from the commercially available media, a considerable variety of transport and culture materials have been designed for use in the clinical laboratory. Möller (1966) described a transport medium (VMG) which he found superior for demonstrating streptococci and anaerobic nonsporulating bacteria in endodontic sample. In contrast to non-nutrient transport medium such as Stuart medium, VMG contains ingredients which support growth and a bacteriostatic agent.

Formula of VMG II Medium (Möller 1966)

Part a	
Agar	0.1 g
Aq. dest.	900 mL

Dissolve the agar in distilled water by boiling.

Part b	
Salt stock solution II	
Phenylmercuric acetate	0.03 g
$CaCl_2 \cdot 6H_2O$	2.4 g
KC1	4.2 g

Part b	
NaCl	10.0 g
$MgSO_4 \cdot 7H_2O$	1.0 g
Sodium glycerophosphate	100.0 g
Distilled water	1,000 mL

Dissolve phenylmercuric acetate in about 800 mL of distilled water by gentle heating. Then add the other salts. Make up to volume with distilled water. Store at room temperature.

Part c	
Bacto-gelatin	10.0 g
Tryptose	0.5 g
Thiotone	0.5 g
Cysteine hydrochloride	0.5 g
Thioglycolic acid	0.5 mL
Bacteriological charcoal (Oxoid)	10.0 g
Stock salt solution II	100 mL

Dissolve the ingredients of part (c) in part (a) after cooling the latter to approximately 50 °C. Add part (b) and adjust pH to 7.5 with 1 M sodium hydroxide. Dispense in screw cap vials or small test tubes and autoclave at 121 °C for 20 min. Store at room temperature.

SBL Medium

This medium was designed by Gästrin et al. (1968) and recommended as a suitable transport medium in different types of bacteriological examinations.

Formula of SBL Medium (Gästrin et al. 1968)

Bacto agar	8–10 g
Thioglycolic acid	0.5 mL
Sodium glycerophosphate	10.0 g
$CaCl_2$ (1 % in H_2O)	10.0 mL
Cysteine hydrochloride	0.025 g
Methylene blue (0.1 % in H_2O)	2.0 mL
Distilled water	950 mL
pH	7.2

Reduced Transport Fluid (RTF) Medium

This medium was designed by Syed and Loesche (1972) for the transport and storage of oral specimens. This medium should best maintain the viability of microorganisms present within a clinical specimen at refrigeration temperature.

Formula of RTF Medium (Syed and Loesche 1972)

Mineral Salt Solution No. 1	
K_2HPO_4	0.6 %
Mineral Salt Solution No. 2	
NaCl	1.2 %
$(NH_4)_2SO_4$	1.2 %
KH_2PO_4	0.6 %
$MgSO_4$	0.25 %
Complete RTF Medium	
Stock solution no.1	75.0 mL
Stock solution no. 2	75.0 mL
0.1 M EDTA	10.0 mL
8 % Na_2CO_3	8.0 mL
1 % dithiothreitol (freshly prepared)	20.0 mL
0.1 % Resazurin (optional)	1.0 mL
Distilled water	814 mL

This medium was filter-sterilized by using a membrane filter (0.22 µm pore size) and dispensed into screw cap tubes. The pH of this medium was 8 ± 0.2 without adjustment, and it decreased to 7 after 48 h incubation in anaerobic atmosphere (85 % N_2, 10 % H_2, and 5 % CO_2).

General-Purpose Culture Media

For the recovery of *Actinomycetaceae*, several general-purpose media have been recommended and are usually satisfactory for primary isolation and subsequent cultivation from clinical specimens and from natural habitats. It is recommended to use several types of media supplemented with sheep, horse, or human blood. These media include fluid thioglycollate broth (THIO), possibly supplemented with 0.1–0.2 % sterile rabbit serum; peptone-yeast extract-glucose broth or agar (PYG, PYGA); Brain Heart Infusion broth or agar (BHIB, BHIA); Trypticase soy broth or agar (TSB, TSA); heart infusion agar or TSA or Columbia agar supplemented with 5 % defibrinated rabbit, sheep, or horse blood; Chocolate agar; Schaedler broth or agar (Slack and Gerencser 1975; Schaal and Pulverer 1981); Fastidious Anaerobe Agar (FAA) supplemented with 5 % horse blood; plate count agar (PCA); and CC-medium (Heinrich and Korth 1967). Generally, enhanced growth occurs under anaerobic conditions. Some strains may also grow well in an atmosphere containing 5 % CO_2 and others may grow poorly in ambient air. The optimum growth temperatures range between 35 °C and 37 °C. Colonies may appear after 3–7 days of incubation.

Methods for Reducing Oxygen Tension

In addition to appropriate media, successful isolation of members of the genera *Actinobaculum*, *Actinomyces*, *Arcanobacterium*, *Mobiluncus*, *Trueperella*, and *Varibaculum* requires an atmosphere of reduced oxygen and increased carbon dioxide concentrations (microaerophilic environment). Numerous methods, which do not necessitate the use of conventional anaerobic equipment, have been devised to provide this appropriate atmosphere. These include the use of candle jar, the use of the anaerobic jar technique such as the commercially available GasPak BBL Microbiology Systems, and the use of a method that apply the Fortner principle (Fortner 1928) of oxygen consumption by microorganisms in culture. The latter method is a simple biological technique for reducing oxygen tension, based on the Fortner principle which utilizes the ability of a rapidly growing facultative anaerobe to reduce the oxygen tension in a closed system, thus making possible the growth of oxygen-sensitive organisms. In this method the agar medium poured into a glass Petri dish and one-third of the plate was streaked with *Serratia marcescens*, and the left part of the plate was inoculated with the clinical material or with a pure culture of an anaerobic organism such as *Actinomyces* species. The plate was then covered with a sterile glass top and sealed with plasticine and incubated at 37 °C for at least 4 days.

Isolation of *Actinomyces* Species

Since most of the clinical specimens used for isolation are either from the mouth or from the female genital tract, the profuse commensal flora present make the isolation and recognition of *Actinomyces* spp. laborious. Therefore, sampling techniques that avoid contamination of the samples with normal microbiota and culturing media which appear to optimize the recovery of *Actinomyces* are recommended.

Processing of Samples

Plaque samples or pus were resuspended by being shaken in small screw-capped vials containing 1.0–2.0 mL of a reduced transport medium (RTF) (Syed and Loesche 1972) or Fastidious Anaerobe Broth (FAB) (LAB071) and sterile glass beads (diameter: 1–4 mm). The samples were vortexed for 15 s to disperse bacterial aggregates and to facilitate the extraction of bacteria from infected dentin tissue. Serial tenfold dilutions were made in MI broth (Boue et al. 1987) containing, per liter, biotrypcase, 10 g; neopeptone, 5 g; yeast extract, 1 g; NaCl, 5 g; sodium thioglycollate, 0.5 g; and 3-(*N*-Morpholino) propanesulfonic acid (MOPS), 2.5 g. The pH was adjusted to 7.0 and 100 µl aliquots of appropriate dilutions were spread onto a range of culture media.

In the same manner intrauterine contraceptive device (IUCD) was soaked in 15 mL of Brewer's modified thioglycollate broth (BD, 11716) or Brain Heart Infusion (BHI) broth (CM225, Oxoid) or FAB for 30 min. The broth was vortexed for 15 s and diluted. 100 µl aliquot was inoculated on to each of the following pre-reduced culture media: Columbia agar (CM0331, Oxoid)

supplemented with 5 % defibrinated horse blood, Columbia agar supplemented with 5 % defibrinated horse blood and gentamicin (14 mg/L), Mupirocin–metronidazole blood agar (MMBA, see below for composition), and Schaedler K-V agar with 5 % sheep blood (221556, BD). Columbia blood agar and MacConkey agar (CM7, Oxoid) were similarly inoculated and incubated in air plus 5 % CO_2 for 24–48 h. Similarly endocervical swabs collected from women with pelvic inflammatory disease (PID) are processed.

Media for Isolation and Subsequent Cultivation of *Actinomyces*

The isolation and enumeration of oral *Actinomyces* from dental plaque has previously been possible only when they represented a major component of the plaque flora. They have been isolated under relatively nonselective conditions, on infusion medium alone (Pine and Watson 1959), or on infusion medium supplemented with blood or serum (Sykes and Skinner 1973). However, from the large number of media available, it can be concluded that there is no standard medium for the detection of *Actinomyces*. In addition to the general-purpose media enriched with blood or serum, other media without blood such as CC-medium (Heinrich and Korth 1967) and Tarozzi's liver broth have been used for the isolation and cultivation of *Actinomyces* from the mouth and actinomycotic lesions.

Formula of CC-Medium (Heinrich and Korth 1967)

Sol. I Mineral and trace element solution	
$MgSO_4 \cdot 7H_2O$	20.0 g
$CaCl_2 \cdot 2H_2O$	2.0 g
$FeSO_4 \cdot 7H_2O$	0.4 g
$MnSO_4 \cdot 2H_2O$	15.0 mg
$Na_2MoO_4 \cdot 2H_2O$	15.0 mg
ZnSO4	4.0 mg
$CuSO_4 \cdot 5H_2O$	0.4 mg
$CoCl_2 \cdot 4H_2O$	0.4 mg
Boric acid	20.0 mg
Potassium iodide	10.0 mg

Dissolve in 1 L of distilled water and acidify with 10 mL of 10 % HCl.

Sol. II Vitamin Solution	
Thiamine HCl	20.0 mg
Pyridoxine HCl	20.0 mg
Biotin	1.0 mg
Folic acid	5.0 mg
Vitamin B_{12} (1 mg/100 mL)	1.0 mL
p-Aminobenzoic acid	20.0 mg

Sol. II Vitamin Solution	
myo-Inositol	20.0 mg
Nicotinamide	10.0 mg
Nicotinic acid	10.0 mg
Ca-Pantothenate	20.0 mg

Dissolve in 100 mL of distilled water.

Sol. III Amino Acid + Vitamin Solution	
Casein hydrolysate	12.0 g
Yeast extract	12.0 g
L-Cysteine HCl	500.0 mg
L-Asparagine	30.0 mg
DL-Tryptophan	20.0 mg
Sol. II (vitamin solution)	12.0 mL

Final CC-Medium

Dissolve 4.0 g of KH_2PO_4 in 250 mL of distilled water and adjust to pH 7.3 with NaOH. Add 10 mL of Sol. I, 500 mg of potato starch dissolved in 70 mL of boiling distilled water, about 20 g of agar (depending on quality), and distilled water to give a final volume of 900 mL. Sterilize by autoclaving at 121 °C for 15 min. After cooling to about 50 °C, add Sol. III under aseptic conditions. Adjust the final pH to 7.3. Pour the medium into glass Petri dishes.

Formula of Tarozzi's Liver Broth

Fresh beef liver	300.0 g
Bacto peptone	12.0 g
NaCl	3.0 g
K_2HPO_4	2.0 g
Distilled water	1,000 mL
pH	7.8

Firstly skin the liver and then boil in 1 L of distilled water for ca. 1 h. Cut the boiled liver into pieces measuring approximately 2 × 1 × 1 cm and distribute into test tubes (1 piece per tube). Dissolve the medium ingredient in the water used for boiling the liver and dispend 8 mL of the medium to each tube. Sterilize by autoclaving at 121 °C for 15 min. Inoculate when cool and then aseptically seal with a layer of sterile paraffin wax or with liquid Vaseline. Colonies growing on the surface of the liver slices appear cottony and white. This medium is also recommended for the short-term preservation of *Actinomyces* in the laboratory.

Partially Selective Culture Media

The isolation and enumeration of *Actinomyces* from dental plaque and actinomycotic lesions may be influenced by the presence of other microorganisms, particularly the acidogenic oral streptococci and other Gram-negative bacteria. The influence of

sodium fluoride on the growth and viability of streptococci was demonstrated by Bibby and van Kesteren (1940). They found that sodium fluoride at concentration >100 p.m. in medium exerted a bactericidal effect on streptococci. The observation that sodium fluoride at a concentration ≥250 μg/mL inhibits the growth of all commensal oral streptococci whereas all oral *Actinomycetaceae*, except for *Propionibacterium proprionicum*, grew without any or with only minimal inhibition (Beighton and Colman 1976) and that colistin has bactericidal activity against most Gram-negative bacteria without affecting the growth of Gram-positive bacteria (Garrod and O'Grady 1971) enabled the formulation of several media for selective isolation of *Actinomyces* species from clinical samples. Therefore, the strategy of choice was the use of selective media that simultaneously enable the proliferation of *Actinomyces* but inhibit the growth of oral streptococci and other interfering bacteria.

Several chemical agents, in varying concentrations, have been shown to suppress the growth of oral streptococci and a variety of Gram-positive and Gram-negative interfering bacteria but allow substantial proliferation of *Actinomyces* species. In particular, cadmium compounds, tellurite compounds, and flavine dyes have been used alone or in combinations. The ability of these substances to increase significantly the antimicrobial effect of many antibiotics has led to heightened interest in the formulation of selective media for improved recovery of *Actinomyces* species from dental plaque and actinomycotic lesions. However, these media are only partially selective as they may be inhibitory to some strains of *Actinomyces*.

CNAC-20 Medium (Ellen and Balcerzak-Raczkowski, 1975)

CNAC-20 is a partially selective medium based on Columbia CNA agar base supplemented with cadmium sulfate for detecting colonies of *Actinomyces viscosus* and *Actinomyces naeslundii* in dental plaque samples. The addition of the antimicrobial agents, colistin, and nalidixic acid renders the medium selective for Gram-positive microorganisms, especially streptococci and staphylococci, while they inhibit the growth of *Enterobacteriaceae* and *Pseudomonas*. Cadmium sulfate is added in a concentration sufficient to inhibit substantially full growth of many strains of dental plaque streptococci but insufficient to inhibit the growth of *A. viscosus* and *A. naeslundii* (Ellen and Balcerzak-Raczkowski 1975). Strains of *A. viscosus*, *A. naeslundii*, and *A. israelii* grew on CNAC-20 in characteristic round, white, smooth, and opaque colonies.

Formula of CNAC-20	
Columbia CNA agar base (BBL)	42.5 g
$3CdSO_4.8H_20$	20.0 mg
Distilled water	1,000 mL

Suspend 42.5 g of Columbia CNA agar base (BBL Cat. No. 212104) in 1 L distilled water. Sterilize by autoclaving at 121 °C for 12 min. Cool to 45–50 °C and add aseptically 50.0 mL sterile defibrinated sheep blood. Alternatively the medium could be prepared from the ingredients as follows:

Ingredient per 1 L distilled water	
Peptone from casein	12.0 g
Peptone from meat	5.0 g
Yeast extract	5.0 g
Beef extract	3.0 g
Corn starch	1.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Colistin	10.0 mg
Nalidixic acid	10.0 mg
$3CdSO_4{\cdot}8H_2O$	20.0 mg
Final pH	7.3 ± 0.2 at 25 °C

Sterilize in autoclave at 121 °C for 15 min. Cool to 50 °C and add aseptically 50.0 mL sterile defibrinated sheep blood.

Plates containing the CNAC-20 medium are incubated at 35 °C in 90 % air and 10 % CO2 to stimulate the growth of the microaerophilic species *A. viscosus* and *A. naeslundii* while impairing the growth of anaerobic A*ctinomyces* species and other Gram-positive bacteria, which prefer anaerobic conditions for primary isolation.

BYS Medium (Beighton and Colman 1976)

This medium was employed by Beighton and Colman (1976) for the isolation of *Actinomyces* from dental plaque. The inclusion of sodium fluoride (NaF) and colistin sulfate as selective agents enabled an increase in proportion of *Actinomyces* species recovered from dental plaque in the presence of small count of other oral flora. The medium composed of two parts, a basal culture medium (BYS medium) and enrichment medium (FS medium) for supplementation of the BYS medium.

Formula of BYS medium	
Brain Heart Infusion	37.0 g
Yeast extract	5.0 g
Polyvinylpyrrolidone	10.0 g
Cysteine HCl	1.0 g
Agar	15.0 g
Distilled water	1,000 mL

After sterilization at 121 °C for 15 min, the medium was cooled to 50 °C and 50.0 mL of sterile horse serum was added.

Formula of FS medium	
NaF solution	25.0 mg/mL
Colistin sulfate solution	1.0 mg/mL

The solutions are sterilized separately by autoclaving at 121 °C for 15 min.

The complete medium was prepared by aseptically adding 10.0 mL of a NaF solution plus 5.0 mL of colistin sulfate solution to 1,000 mL of sterile BYS medium.

Modification of FS Medium (Boue et al. 1987)

The selectivity of the medium designed by Beighton and Colman (1976) for the recovery of *Actinomyces* from dental plaque was further improved by the following modification of the FS medium as proposed by Boue et al. (1987):

Formula of modified FS medium	
NaF	100 μg/mL
Cadmium sulfate	20 μg/mL
Polymyxin	20 μg/mL
Oxolinic acid	30 μg/mL
Nystatin	30 μg/mL

Cadmium Sulfate Fluoride Acridine Trypticase (CFAT) Agar (Zylber and Jordan 1982)

CFAT medium was developed by Zylber and Jordan (1982) for the detection and selective isolation of *Actinomyces naeslundii* and *Actinomyces viscosus* from dental plaque. The medium contains neutral acriflavin and potassium tellurite in combination with the selective agents cadmium and fluoride to eliminate most of the competing plaque flora. Colonies of *Actinomyces* spp. appear cream to slightly greenish in color, entire edged, convex or raised, and opaque on this medium.

Formula per Liter of CFAT	
Tryptic soy broth	30.0 g
Glucose	5.0 g
Agar	15.0 g
Cadmium sulfate	13.0 mg
Sodium fluoride	80.0 mg
Neutral acriflavin	1.20 mg
Potassium tellurite	2.50 mg
Basic fuchsin	0.25 mg
Defibrinated sheep blood	50.0 mL
Distilled water	1000.0 mL
Final pH	7.0 ± 0.2

GMC Medium (Kornman and Loesche 1978)

GMC is a gelatin-based medium containing metronidazole (10 μg/mL) and cadmium sulfate (20 μg/mL) for selective isolation of *Actinomyces* species from dental plaque samples. Metronidazole was chosen as the primary selective agent which is relatively ineffective in vitro against most microaerophilic *Actinomyces* species but inhibits the growth of many anaerobic species in vitro. The combination of metronidazole and cadmium sulfate effectively suppressed anaerobes and facultative Gram-positive cocci while allowing good recovery of the microaerophilic *A. viscosus* and *A. naeslundii* strains. The addition of gelatin to the GMC medium allows differentiation of catalase-positive, gelatinase-positive organisms, e.g., propionibacteria, which might otherwise be confused with *A. viscosus*.

Formula of GMC	
Agar	15.0 g
Trypticase (BBL)	10.0 g
Gelatin	30.0 g
Sodium acetate	1.0 g
Sodium formate	1.0 g
Sodium lactate (60 %)	5.0 mL
Sodium succinate	1.0 g
Yeast extract (Difco)	1.0 g
Sodium chloride	2.0 g
Glucose	1.0 g
D-Mannitol	1.0 g
Potassium nitrate	0.5 g
$3CdSO_4 \cdot 8H_2O$	20.0 mg
Distilled water	874 mL
Final pH	7.2 ± 0.1

After sterilization at 121 °C for 15 min, the medium was cooled to 50 °C and added aseptically with the filter-sterilized solution containing the following ingredients:

Menadione (0.05 % stock solution)[a]	2.0 mL in the refrigerator
Dithiothreitol	0.1 g
Sucrose	0.5 g
Cysteine hydrochloride	0.5 g
Sodium phosphate dibasic	1.0 g
Sodium carbonate (anhydrous)	0.5 g
Distilled water	50 mL

[a]Prepared in 50 % ethanol in distilled water; filter-sterilized and stored in an amber glass bottle in the refrigerator.

Finally, add 1.0 mL of filter-sterilized metronidazole solution (0.5 g in 50.0 mL distilled water).

Columbia Agar Base Supplemented with Metronidazole (Traynor et al. 1981)

Traynor et al. (1981) described CBA containing 2.5 mg/L of metronidazole for the isolation of *Actinomyces* spp. from cervical specimens of women using IUCDs. Although the isolation rate of *Actinomyces* species was significantly increased, it should be noted that a time-consuming serial dilution technique of a kind not commonly used for routine specimens was used to remove competing flora from the primary inoculum prepared by soaking the specimens in thioglycollate broth.

Formula of CBA-metronidazole	
Columbia agar base (Oxoid CM0331)	39.0 g
Metronidazole	2.5 mg
Distilled water	1,000 mL
pH	7.3 ± 0.2 at 25 °C

Dissolve 39.0 g of Columbia agar in 1 L of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121 °C for 15 min. Cool to 50 °C and add 5 % sterile defibrinated horse blood. Add the corresponding amount of filter-sterilized metronidazole.

Mupirocin–Metronidazole Blood Agar (MMBA) (Lewis et al. 1995)
A selective culture medium contains two antibiotics, mupirocin (pseudomonic acid) and metronidazole, which are inhibitory for most overgrowing competing microorganisms found in dental plaque, cervical specimens from women using IUDs, and other medical specimens (Lewis et al. 1995). Mupirocin inhibits the growth of staphylococci, streptococci, *Haemophilus*, *Neisseria*, *Enterococcus*, *Escherichia coli*, and other enterobacteria, while metronidazole at a concentration of 2.5–5.0 mg/L inhibits most of the obligate anaerobes found in clinical material with only the genera *Actinomyces*, *Bifidobacterium*, and *Propionibacterium* being regularly resistant.

Formula of MMBA medium	
Columbia agar base (Oxoid CM0331)	39.0 g
Mupirocin	128.0 mg
Metronidazole	2.5 mg
Distilled water	1,000 mL
pH	7.3 ± 0.2 at 25 °C

Dissolve 39.0 g of Columbia agar in 1 L of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121 °C for 15 min. Cool to 50 °C and add 5 % sterile defibrinated horse blood. Add the corresponding amount of filter-sterilized mupirocin and metronidazole.

Media for Isolation of *Actinomyces* from Environmental Sources

For the isolation of *Actinomyces* species from oil-contaminated groundwater or oil-contaminated soils, the samples are serially diluted with distilled water, and 500 mL aliquots were spread on R2A plates or plate count agar (PCA, Difco Nr. 247940) or peptone/yeast extract/fructose (PYF) solidified with 15.0 g agar/L. According to Bowman et al. (2006), the R2A agar was supplemented with 1.0 mg/L resazurin (as a redox indicator) and 0.25 g/L-cysteine hydrochloride (as a reducing agent) and buffered at pH 5.0 with 20 mM acetate buffer prior to solidification with agar. All plates were incubated at 30 °C. Anaerobic plates were incubated in an anaerobic chamber supplied with gas comprised 90 % N_2, 5 % CO_2, and 5 % H_2.

Formula of supplemented R2A agar (Bowman et al. 2006)	
Yeast extract	0.5 g
Proteose peptone (Difco)	0.5 g
Casamino acids	0.5 g
Cysteine hydrochloride	0.25 g
Glucose	0.5 g
Soluble starch	0.5 g
Sodium pyruvate	0.3 g
K_2HPO_4	0.3 g
$MgSO_4 \cdot 7H_2O$	0.05 g
Resazurin	1.0 mg
Distilled water	1,000 mL
Agar	15.0 g
pH	5.0

Alternatively suspend 18.2 g of R2A agar (BD, Nr. 218263) in one liter of distilled water. Mix thoroughly and then add 1.0 mg/L resazurin and 0.25 g/L-cysteine hydrochloride. Sterilize by autoclaving at 121 °C for 15 min. Cool to 50 °C and adjust to pH 5.0 with 20 mM acetate buffer.

Formula of PYF medium (Engelmann and Weis 1985)	
Peptone	5.0 g
Tryptone	5.0 g
Yeast extract	10.0 g
Fructose	5.0 g
Na_2HPO_4	2.0 g
Tween 80	1.0 mL
Cysteine HCl	0.5 g
Distilled water	1,000 mL
Agar	15.0 g
Adjust pH to 7.0	

Isolation of *Arcanobacterium* Species

For primary isolation, complex media such as Columbia blood agar supplemented with 5 % horse, sheep, or human blood, BHI broth (or agar), or Trypticase soy broth (or agar) have been recommended and are usually satisfactory. *A. haemolyticum* and *A. hippocoleae* are more difficult to grow in liquid media. However, addition of serum may enhance growth of *A. haemolyticum* considerably. Thus, a suitable liquid medium for *A. haemolyticum* consists of BHI broth supplemented with 5 % horse serum (Collins and Cummins 1986). All of the other *Arcanobacterium* species apparently grow well on Columbia blood agar supplemented with 5 % horse or sheep blood and incubated under increased CO_2 tension (~5 %) at 36 ± 1 °C.

Recognition of *A. haemolyticum* in cultures from clinical specimens is often hampered by its delayed β-hemolysis and

the presence of additional pathogens (e.g., streptococci) or microbes from the indigenous flora of the affected mucous membranes. Thus, media for selective isolation or improved recognition of this organism were developed. A selective medium that greatly reduces the growth of commensal organisms and permitting easier recognition of *A. haemolyticum* was used by (Brenwald et al. 1990). This medium consisted of a blood agar base (Oxoid No 2) containing 5 % horse blood, 8 mg/L mupirocin, 4 mg/L aztreonam, and 1 mg/L amphotericin B. Mupirocin is highly active against commensal staphylococci and streptococci, whereas aztreonam and amphotericin B were used to inhibit the growth of Gram-negative bacteria and yeasts, respectively. Cummings et al. (1993) found that medium and atmosphere had measurable effects on the colonial and beta-hemolysis of *A. haemolyticum*. They tested Trypticase soy agar, Columbia agar, and heart infusion agar containing 5 % sheep blood, and cultures were incubated in ambient air, 6–8 % CO_2 for 24, 48, and 72 h of incubation times. They found that Trypticase soy agar was the superior medium and CO_2 was the superior atmosphere for beta-hemolysis. A minimum of 48 h was needed for expression of beta-hemolysis and pitting. Agar pitting was not significantly affected by variations in medium or atmosphere. Anaerobic incubation produces the smallest *A. haemolyticum* colonies and zones of hemolysis. Coman et al. (1996) used a medium containing 5 % sheep blood agar and 3.5 % NaCl for selective isolation of *A. haemolyticum* from pharyngeal swabs of children. Jurankova and Votava (2001) applied sheep blood agar with a streak of *Staphylococcus aureus* to recognize *A. haemolyticum* on the basis of the reverse CAMP (Christie, Atkins, and Munch-Petersen) phenomenon. The medium of Votava et al. (2000) containing Columbia blood agar base and 5 % washed sheep erythrocytes sensitized with equi factor (EF) of *Rhodococcus equi* is based upon the same principle.

Isolation of *Trueperella* Species

For isolation of *Trueperella* species from clinical specimens, it is recommended to use several types of media such as Columbia agar supplemented with 5 % sheep or horse blood, Chocolate agar, Brucella agar with 5 % sheep blood, *Brucella* lacked blood agar with kanamycin–vancomycin, and Phenylethyl alcohol agar with 5 % sheep blood. Growth is much enhanced by the addition of 1 % Tween 80 to nutrient agar or BHI agar. Good growth occurs under aerobic and strictly anaerobic conditions, but optimal growth is obtained in a 5–10 % CO_2-enriched atmosphere. The optimum temperature for growth is 37 °C and the temperature range of growth is from 20 °C to 40 °C.

The nutritional requirements of *Trueperella pyogenes* have been investigated by several authors (Reddy et al. 1977, 1980, 1982; Fraga and Reddy 1982). For these purposes they used semidefined (SDM) and chemically defined (CDM) media. They reported that the addition of hemin greatly increased the growth of *T. pyogenes* strains. All *T. pyogenes* strains showed an obligate requirement for CO_2/HCO_3^- for their growth. Peptides are required for growth even in the presence of a complete complement of 20 amino acids and $(NH_4)_2SO_4$. Inositol can replace the peptide requirement for growth. Furthermore, *T. pyogenes* strains require a fermentable carbohydrate for growth. All strains required riboflavin and nicotinic acid and most require adenine and uracil for optimal growth.

Isolation of *Actinobaculum* Species

Actinobaculum species are slow growing, obligate anaerobe requiring enriched culture media that contain blood or blood product for their isolation from clinical materials. Attempts to grow *A. suis* aerobically or under 10 % CO_2 were unsuccessful (Soltys 1961). The nutritional requirements and metabolic features of *A. suis* were studied by Wegienek and Reddy (1982). They employed a peptone–yeast extract–starch (PYS) medium which support optimal growth of *A. suis* and containing Trypticase, yeast extract, starch, minerals, cysteine, and sodium carbonate. The replacement of starch in PYS medium with maltose resulted in decreased growth, while the deletion of starch or maltose from the medium resulted in no growth of the organism, indicating that a fermentable carbohydrate is required for growth of *A. suis*. Deletion of Na_2CO_3 from PYS led to approximately 20 % decrease in growth. This suggests that *A. suis* fixes CO_2 released during the metabolism of starch or maltose and uses it for biosynthetic purposes such as production of acetate. The deletion of only yeast extract from PYS resulted in about 75 % decrease in growth but growth inhibited by the simultaneous deletion of yeast extract and Trypticase. Supplementation of a defined mixture of purine and pyrimidine bases, vitamins, and amino acids to PYS minus yeast extract effectively replaces yeast extract. Vitamins are essential for growth of *A. suis*. A mixture of pyridoxal, riboflavin, and nicotinic acid stimulates growth comparable to that obtained in the medium with the full complement of all 10 vitamins. Purine and pyrimidine bases are required for optimal growth. The deletion of Trypticase resulted in negligible growth even in the presence of amino acids, suggesting a peptide(s) in Trypticase is a requirement for the growth of *A. suis*.

The nutritional requirements of *Actinobaculum* species associated with human diseases, *A. massiliense*, *A. schaalii*, and *A. urinale*, are not known in detail. Media commonly used for cultivation include 5 % Columbia sheep blood agar, 5 % and 10 % horse blood agar, Chocolate agar, *Brucella* blood agar with hemin and vitamin K1 (BD), nutrient agar, and semisolid agar containing pepsin blood and thioglycollate. Generally, they require 3 days to produce good growth in an anaerobic atmosphere at 35 °C but grow poorly in air with 5 % CO_2 and not at all in ambient air. The selective use of anaerobic blood cultures, e.g., Bactec Lytic Anaerobic/F bottle, is useful for isolation of *Actinobaculum* from the blood of infected patients. Unlike *A. suis*, enhanced growth of *A. massiliense*, *A. schaalii*, and *A. urinale* is obtained in BHI broth supplemented with 1 % Tween 80 (Yassin unpublished).

Isolation of *Varibaculum* Species

Varibaculum cambriense requires enriched culture media such as Columbia agar or Fastidious Anaerobe Agar (LabM, Bury, United Kingdom) supplemented with 5 % horse blood or Chocolate agar for cultivation. Good growth was obtained under anaerobic conditions or in an atmosphere containing 5% CO_2 after 3–7 days incubation at 37 °C.

Isolation of *Mobiluncus* Species

Mobiluncus species are strict anaerobes and are extremely fastidious, requiring fresh moist media and extended anaerobic incubation for multiplication. The fastidious nature of these organisms has greatly impeded their recovery form vaginal fluid by conventional culture techniques (Spiegel et al. 1983b; Hjelm et al. 1984; Holst et al. 1984a). Because of the difficulty in isolating these organisms by culture, most researchers attempting to define the prevalence of this organism have used direct techniques such as Gram stain, wet smear, direct staining with monoclonal antibodies, or DNA probes. However these procedures may also detect other motile bacteria unrelated to the curved rods. Therefore, it was important to find a means of isolating *Mobiluncus* spp. selectively in primary culture, a difficult task because the anaerobic flora of the vagina is profuse (10^{12} bacteria/g of secretion). Approaches to this objective have concentrated on attempts to inhibit the growth of other more rapidly growing bacteria present in vaginal specimens by using antibiotics and other agents in solid media (Durieux and Dublanchet 1981; Holst et al. 1984a; Thomason et al. 1984a), alkaline pretreatment (Påhlson and Forsum 1985; Påhlson et al. 1986), and cold enrichment (Smith and Moore 1988) to improve the rate of recovery.

Nonselective Solid and Liquid Culture Media

Several commercial nonselective solid media were reported for recovery of *Mobiluncus* from clinical specimens. However, these media vary in their ability to support growth of fresh clinical isolates (Smith and Moore 1988). These include Columbia agar, Brain Heart Infusion agar, brucella agar, Chocolate agar, DST agar, peptone–yeast extract–glucose agar, Schaedler agar, and Trypticase soy agar (Vetere et al. 1987; Smith and Moore 1988; Spiegel 1992). An addition of 4–15 % horse, human, sheep, or rabbit blood enhances growth. A supplementation of culture media with one of the potential growth factors arginine-free base, sodium hippurate, sodium formate, sodium fumarate, bovine serum, and campylobacter growth supplement-FBP, however, did not result in any significant improvement of growth (Vetere et al. 1987; Thomason et al. 1984a; Taylor-Robinson and Taylor-Robinson 2002). From the 12 liquid culture media evaluated by Taylor-Robinson and Taylor-Robinson (2002), only Columbia blood broth (CBB) and peptone–starch–dextrose (PSD) supplemented with 10 % horse serum supported the growth of some strains of *Mobiluncus curtisii* and *Mobiluncus Mulieris* to 10^9 organisms/mL within 48 h, and viable bacteria persisted longer in some media (e.g., CBB) than in others. Such maintenance of viable organisms in liquid medium is particularly important in the production of soluble metabolic products, such as the cytotoxin.

Selective Culture Media

Due to the mixed nature of infections from which *Mobiluncus* species are usually isolated, it was necessary to develop selective growth media that enhance the growth of *Mobiluncus* and in the meantime suppress the growth of competing flora such as *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella* spp., and *Atopobium vaginae* involved in bacterial vaginosis (Holst et al. 1984a; Thomason et al. 1984a; Smith and Moore 1988; Spiegel 1989). For these purposes several media containing effective antimicrobial agents such as colistin plus nalidixic acid (Durieux and Dublanchet 1980, 1981), polymyxin plus metronidazole or tinidazole (Sturm 1989), and colistin plus nalidixic acid, tinidazole, and Nile blue A (Spiegel 1986) have been designed. For instance, Smith and Moore (1988) described two selective media, Rlk and SA, combined with cold enrichment at 4 to °C to aid the isolation of *Mobiluncus* species from vaginal specimens. The Rlk medium is based on Columbia CNA agar supplemented with 2 % peptone, 0.6 % yeast extract, and 5 % laked rabbit or sheep blood. Tinidazole (48 mg/L) and nalidixic acid (20 mg/L) were added to the medium after autoclaving. The SA medium is also based on Columbia CNA agar supplemented with 2 % rabbit serum, 1.6 % laked rabbit or sheep blood, nalidixic acid, and tinidazole. After pouring the Rlk and SA media, the plates are left to dry in room for 1–2 days and then pre-reduced for 1–7 days before use. A further selective medium referred to as Mobi agar was designed by Spiegel (1989) as follows:

Formula of Mobi Agar Spiegel (1989)	
Columbia broth (Difco)	35.0 g
Cysteine HCl	0.4 g
Soluble starch (Difco)	10.0 g
Resazurin solution (11 mg/44 mL)	4.0 mL
Colistin methane sulfonate	10 μg/mL
Nalidixic acid	15 μg/mL
Deionized water	950.0 mL
Bacto agar (Difco)	15.0 g

The components are boiled with frequent agitation to dissolve solid components. After autoclaving, the medium cooled to 45–50 °C. Add 20.0 mL rabbit serum, 0.6 μg/mL tinidazole, and 1.2 mL of Nile blue A solution (0.5 g/10 mL). The plates were reduced over night prior to inoculation in an atmosphere consisting of 80 % nitrogen, 10 % hydrogen, and 10 % carbon dioxide.

Maintenance and Preservation Procedures

Short-Term Maintenance

Satisfactory maintenance of the viability and physiological characteristics of strains belonging to the genera of the *Actinomycetaceae* involves serial transfer of the organisms to fresh medium. The majority of species grow well on Columbia agar supplemented with 5 % sheep blood and incubation under anaerobic conditions or under increased CO_2 tension (~5 %) at 35 °C. Furthermore, tryptose agar supplemented with 5 % sheep blood was used to maintain cultures of *Trueperella pyogenes* with subcultivation at 3-week intervals (Roberts 1968; Reddy et al. 1982). In addition, Fortner plates containing CC-medium without carbohydrates but supplemented with 0.2 % (v/v) rabbit or fetal bovine serum were used for the maintenance of *Actinomyces* species. In the latter case, care should be taken as contamination may be caused by the *Serratia* strain simultaneously grown on the plate especially when the agar surface is too wet upon inoculation. Nevertheless, the frequency of transfer varies with the organism, and repeated subculturing or leaving the culture in the incubator beyond the time at which subculturing should be made results in loss of viability. For example, culture of *Actinobaculum urinale* loses its viability when incubated for longer than 10 days at 35 °C in an atmosphere containing 5 % CO_2 on Columbia blood agar, while cultures of *Actinobaculum massiliense* and *Actinobaculum schaalii* lose their viability under the same conditions after 14–21 days. In contrast, the cells of *Actinobaculum suis* continue to proliferate after 40 days of incubation. Notably, it is advisable not to store living cultures of *Actinomycetaceae* strains in the refrigerators at 4 °C as the cells of many species lose viability.

Liquid media such as BHI broth or BHI broth supplemented with 1 % Tween 80 dispended in test tubes have been used successfully for maintaining *Actinomycetaceae* strains alive. After inoculation the tubes are incubated under anaerobic conditions either using the GasPack jars or they may be sealed using sterile liquid paraffin. The layer of paraffin prevents dehydration of the medium and ensures anaerobic conditions. For members of the genera *Arcanobacterium* and *Trueperella*, the tubes may be incubated under anaerobic conditions or under increased CO_2 tension (~5 %). An advantage of the use of liquid culture is the reduction of subculture frequency.

Long-Term Maintenance

The most efficient and reliable method for storing stock cultures of *Actinomycetaceae* strains is by freeze drying in a suitable stabilizer such as fetal calf serum or skim milk. A thick suspension of the strain scraped from agar was suspended in fetal calf serum or 10 % skim milk. This suspension is dispended into small glass vials and rapidly frozen at low temperature (−70 °C). Once frozen the vials are placed in the drying chamber of a freeze dryer and lyophilized by standard techniques. Lyophilized cultures should be rehydrated in 0.8 % NaCl and streaked directly on Columbia agar with 5 % sheep blood plates for routine use.

Alternatively, cryopreservation at low temperature (−70 °C) using cryoprotectant such as dimethylsulfoxide (DSMO) or glycerol has been used by some authors. Rao et al. (2012) used peptone/yeast extract/glucose (PYG) containing 15 % glycerol and 5 % dimethylsulfoxide as a cryoprotectant medium for storing environmental isolates of *Actinomyces* species. The suspension was then dispended into small vials and stored at −80 °C to reduce the metabolic activity of the strains. Microbank cryogenic beads (Prolab Diagnostics) has been used successfully by Hoyles et al. (2004) for maintaining stock cultures of *Mobiluncus* species stored at −70 °C according to the manufacturer's instructions.

Ecology

Members of the family *Actinomycetaceae* are found in a wide range of habitats varying from human and animal to environmental sources. They form a significant component of the indigenous microflora of humans and animals (Beighton and Miller 1977; Dent and Marsh 1981) and their distribution is very similar in these habitats. Therefore, they may be expected to be recovered from any body fluid or tissue contaminated, colonized, or infected by these endogenous bacteria. In addition, one member of the family has been recently detected and isolated from oil-contaminated sites (Bowman et al. 2006; Duarte et al. 2001; Rao et al. 2012).

Distribution of Members of the Genus *Actinomyces*

Distribution of *Actinomyces* in Human

Actinomyces species are frequently found as commensal members of the normal microflora of human mucous membranes of the oropharynx, gastrointestinal tract, and female genital tract. Naeslund (1925) was the first to show by cultural means that anaerobic species of actinomycetes existed in tonsils. *Actinomyces* are predominant members of the human oral commensal microbiota and are among the initial colonizers of oral surfaces. They contribute to the development of oral biofilm at early stages and may constitute up to 27% of the pioneer bacteria (Socransky et al. 1977; Theilade et al. 1982; Nyvad and Kilian 1987; Gibbons 1989; Li et al. 2004). They have evolved unique mechanisms that favor colonization and persistence in this microenvironment (Yeung 1999). Several species have been isolated from the human oral cavity. These include *A. georgiae*; *A. gerencseriae*; *A. israelii*; *A. meyeri*; *A. naeslundii* genospecies I, II, and III; *A. odontolyticus*; and *A. viscosus* (Tanner et al 1994). *A. odontolyticus*, *A. naeslundii*, and *A. viscosus* are the primary species in infants' mouths (Sarkonen et al. 2000) as well as in early dental plaque formation (Liljemark et al. 1993;

Mishra et al. 2010; Nyvad and Kilian 1987). *Actinomyces georgiae*, *Actinomyces gerencseriae*, and *Actinomyces meyeri* have been isolated from gingival crevices of periodontally healthy individuals (Cato et al. 1984; Johnson et al. 1990). Other *Actinomyces* species of oral origin include *A. radicidentis* isolated from infected root canals (Collins et al. 2000) and *A. graevenitzii* isolated from respiratory tract secretions (Pascual Ramos et al. 1997b) and infants' saliva (Sarkonen et al. 2000). In addition, the use of highly discriminatory genotypic methods facilitated the resolution of subspecies variations most pronounced in *A. naeslundii* and *Actinomyces viscosus* (Johnson et al. 1990; Bowden et al. 1999; Henssge et al. 2009). It is now recognized that *Actinomyces oris*, previously known as *A. naeslundii* genospecies II, is considered to be the most abundant *Actinomyces* species in the human oral cavity (Ton-That et al. 2011). Thus, the number and variety of periodontal species recognized seems to be continually increasing and undergoing taxonomic revision.

The occurrence of *Actinomyces* species as normal inhabitants of the intestinal tract has not been clearly documented. Although Naeslund (1931) failed to identify *Actinomyces* in any fecal samples from asymptomatic individuals, he suggested that they appear in the gut by transit from the oral mucosa. Indirect evidence for the view that these organisms may form a small but significant component of the intestinal flora can be derived from cases of abdominal actinomycosis thought to be induced endogenously after abdominal surgeries, appendicitis, bowel perforations, or neoplasm. The colonization is almost exclusively by *Actinomyces israelii*, and the most common sites of infections are the transverse colon and the cecum with the appendix predominating (Ferrari et al. 2000; Garner et al. 2007).

There are controversies as to whether *Actinomyces* are normal members of the vaginal flora. Curtis and Pine (1981) demonstrated *A. israelii* in the genital tract of virtually healthy women. In an extensive study performed on 1,108 samples from the cervix, vagina, and perineum of 15 women (5 IUD users, 5 using oral contraceptives, and 5 without contraception), *A. israelii* in varying frequencies was identified in all women (Persson and Holmberg 1984a). As a mean, *A. israelii* was recovered in 24 % of the perineal samples, 13 % of the vaginal, and 6 % of the cervical samples. From this study Persson and Holemberg concluded that *A. israelii* is a part of the indigenous genital flora of healthy women. In contrast to Persson and Holemberg, Dybdahl et al. (1991) regarded *Actinomyces* colonization of the female genital tract as a rare event in women who are not users of intrauterine devices (IUCDs). The frequent isolation of *Actinomyces* from the genital tract of women using intrauterine devices (IUDs) has been reported (Evans 1993; Fiorino 1996; Westhoff 2007). It is estimated that the prevalence of *A. israelii* in IUD users worldwide may range between 1.6 % and 11.6 % (Valicenti et al. 1982; Nayar et al. 1985; Cleghorn and Wilkinson 1989; Dybdahl et al. 1991). These findings were based on either the observations of *Actinomyces* species in the cervical smears of generally asymptomatic women with IUDs (Valicenti et al. 1982) or on cases of IUD-associated pelvic actinomycosis in women using the device (Fiorino 1996). However, it is noteworthy to mention that the specificity and sensitivity for identifying the organisms depends on the detection methods. It is suggested that the prevalence of *Actinomyces*-positive cervical smears range from 0 % to 31 % with an average of 7 % (Gupta 1982; Fiorino 1996). Because the Papanicolaou test (Pap test) lacks specificity and sensitivity, other diagnostic approaches have attempted to produce more consistent results and to clarify whether *Actinomyces* is a normal inhabitant of the female genital tract in the absence of IUD (Pine et al. 1981; Persson and Holmberg 1984b, 1985). Generally immunofluorescent staining of smears is most sensitive and specific. Although culture is the gold standard for identifying the organism, it is not routinely useful due to special handling and the time required; in addition, results of cultures are often not concordant with cytology or stained tissue. Therefore, culturing and immunofluorescent staining are often used as complementary methods. The most common *Actinomyces* species associated with pelvic colonization is *A. israelii*. However, apart from *A. israelii* and *Propionibacterium propionicum*, other *Actinomyces* species such as *A. gerencseriae*, *A. naeslundii*, *A. viscosus*, *A. odontolyticus*, *A. meyeri*, and *A. cardiffensis* were recovered from specimens of the female genital tract (Slack and Gerencser 1975; Schaal and Pulverer 1984; Schaal and Lee 1992; Hall et al. 2002).

Besides the well-known *Actinomyces* species mentioned above, a plethora of new *Actinomyces* species such as *A. dentalis* (Hall et al. 2005), *A. europaeus* (Funke et al. 1997a), *A. funkei* (Lawson et al. 2001b), *A. hominis* (Funke et al. 2010), *A. massiliense* (Renvoise et al. 2009), *A. neuii* (Funke et al. 1994), *A. timonensis* (Renvoise et al. 2010), *A. turicensis* (Wüst et al. 1995), and A. urogenitalis (Nikolaitchouk et al. 2000) have been identified from human sources, and there are indications that much new diversity still remains to be discovered. Although knowledge about their exact ecological function is still rudimentary, they are found to participate in many infections, such as actinomycosis, human bite wounds and abscesses at different body sites, eye infections, and oral, genital, and urinary tract infections (Sabbe et al. 1999; Schaal and Lee 1992).

Distribution of *Actinomyces* in Animals

Little efforts have been directed to study the establishment of different *Actinomyces* species as part of the indigenous microflora of healthy domestic and wild animals. However, they occur commonly on the buccal and nasopharyngeal mucous membranes of several animal species and thus assumed to represent part of the indigenous microflora of host animals. *A. bovis* has also been isolated from cattle, sheep, pigs, dogs, horses, and other mammals. *Actinomyces viscosus* has been isolated from gingival plaques of hamsters and from cervical plaque of rats (Howell 1963; Jordan et al. 1972). *A. denticolens*, *A. howellii*, and *A. slackii* were recovered from the dental plaque of dairy cattle (Dent and Williams 1984a, b, 1986). Similarly, *A. viscosus* and *A. naeslundii* were found among the major bacteria that contribute to the initial dental plaque formation of neonatal monkeys (Kilian and Rölla 1976; Beighton 1985). Love et al. (1990)

studied the anaerobic bacterial flora of the oral cavity of healthy feline and found that *A. viscosus*, *A. hordeovulneris*, and *A. denticoIens* comprised 12 % of the facultative anaerobic flora. *A. hyovaginalis* was found among the nasal and tonsillar flora of piglets before and after weaning (Baele et al. 2001).

Some other *Actinomyces* spp. recovered from animal sources such as *A. canis*, *A. bowdenii*, *A. catuli*, *A. coleocanis*, *A. bowdenii*, *A. suimastitidis*, *A. marimammalium*, *A. vaccimaxillae*, and *A. weissii* have been reported, to date, only in the original descriptions of these species. It is still disputed whether these organisms have a natural habitat outside the animal.

Distribution of *Actinomyces* in Nature

Actinomyces spp. have been demonstrated in nature outside of an animal or human host (Duarte et al. 2001; Rao et al. 2012). Molecular community profiling via soil DNA-based PCR-denaturing gradient gel electrophoresis (PCR-DGGE) and analysis of the sequences of three prominent bands of the profiles generated with the highly polluted soil samples suggested that the underlying organisms were related to *Actinomyces* sp., *Arthrobacter* sp., and a bacterium of uncertain affiliation (Duarte et al. 2001). Later, *Actinomyces naturae* was isolated from contaminated groundwater collected from a well at an area where petrochemical wastes were deposited (Rao et al. 2012).

Distribution of Member of the Genus *Arcanobacterium*

A. haemolyticum is generally thought of as an obligate human pathogen, being only isolated in association with clinical symptoms. Isolation of the bacterium from the nasopharynx of asymptomatic persons is rare. A number of studies have shown that carriage in the throats of healthy individuals was generally less than 0.2 % of the sampled populations (Banck and Nyman 1986; Wat et al. 1991; Mackenzie et al. 1995). Although 16S rRNA metagenomic sequencing of human skin microbiota has revealed that actinobacteria represent 51.8 % of the skin bacteria (Grice et al. 2009), *Arcanobacterium* spp. have not yet been identified. Carriage still may occur in other parts of the body, as no studies have examined whether *A. haemolyticum* is present on the skin or other mucosa, thus acting as either part of the normal flora or being a reservoir for disease. An interesting finding is that *A. haemolyticum* could be identified as one of the major secondary colonizers of leprosy skin ulcers (Sturm et al. 1996) and may contribute to the inflammatory reaction of these lesions. *A. haemolyticum* is currently thought to be spread via aerosolized droplets from infected persons (Banck and Nyman 1986; Gaston and Zurowski 1996), but direct contact may also spread the bacteria.

Although human beings are considered to be the main reservoir of *A. haemolyticum*, reports of sporadic isolations from animals, including horses (Hassan et al. 2009), sheep (Roberts 1969), cattle (Richardson and Smith 1968), and a pet rabbit (Tyrrell et al. 2002), have been published. In addition *A. haemolyticum* has been isolated from a female European badger, a wildlife host, for which the potential for direct human contact is minimal (Wragg et al. 2011). The likely role of *A. haemolyticum* in this single case is uncertain, and further study is necessary to determine how widespread this potentially zoonotic organism is among badgers.

The natural habitat of *A. phocae* is unknown. The bacterium was isolated from nasal, fecal, and vaginal swabs taken from healthy harbor seals in California and was often isolated from infected bite wounds, including those from conspecifics, as well as superficial wounds, suggesting that *A. phocae* is part of the normal oral or skin flora of marine mammals (Johnson et al. 2003). *A. canis* has been isolated from otitis externa of a 7-year-old female English bulldog (Hijazin et al. 2012b).

Distribution of Member of the Genus *Trueperella*

Trueperella species occur as commensals of the mucous membranes of many domestic animals such as dairy and beef cattle, sheep, swine, and goats. *Trueperella pyogenes* was isolated from up to 100 % of the udders, urogenital, upper respiratory, and gastrointestinal tracts of healthy animals (Timoney et al. 1988; Carter and Chengappa 1991; Quinn et al. 1994; Jost et al. 2002a; Billington et al. 2002a). *T. pyogenes* is one of the common tonsillar and nasal flora of piglets (Baele et al. 2001). The organism is part of the bacterial flora of the scrotal skin and the preputial cavity of rams (Gouletsou et al. 2004). In addition, this organism was isolated from semen of beef breed bulls (Sprecher et al. 1999) without a significant relationship between the cultural results and possible sperm abnormalities.

Distribution of Member of the Genus *Actinobaculum*

Actinobaculum is a well-recognized commensal flora of the mucosal surfaces and skin of animals and humans. *A. suis* is a common inhabitant of the preputial sac in the scrotum of boars (Jones and Dagnall 1984). It is occasionally isolated from the vagina of healthy sows (Jones 1984). Carr and Walton (1990) isolated *A. suis* from footwear of handlers working with boars. It may also be isolated from voided urine and pen floors of farrowing and nursery rooms.

A. schaalii, *A. massiliense*, and *A. urinale* are natural components of the human genitourinary tract. They have been recovered from human blood and urine (Lawson et al. 1997; Greub and Raoult 2002; Hall et al. 2003a). Although *A. massiliense* has been reported to cause skin infection (Waghorn 2004), it is not known whether the organism contributes to resident commensal skin flora or represents exogenous acquisition.

Distribution of Member of the Genus *Varibaculum*

Although its natural habitat remains unknown, *V. cambriense* is occasionally considered to be skin commensal organism

(Chu et al. 2009) and one of the vaginal anaerobic microflora (Verhelst et al. 2005). It has been isolated from intrauterine devices and human vagina and abscess specimens. In a study of the vaginal microflora, Verhelst et al. (2005) found that *Varibaculum cambriense* represent 2.2 % and 13.6 %, respectively, of cultured organisms in grade II and grade III specimens.

Distribution of Member of the Genus *Mobiluncus*

The normal ecological niche for *Mobiluncus* species is not known. However, they are most often found in vaginas with a disturbed ecology, primarily of women with BV, but rarely found in healthy women. Thus, they have been isolated from the vaginas of women with BV in up to 85 % of cases (Holst et al. 1987). The organisms have also been recovered in high rates from the rectal specimens of women with clinically evident BV, but in lower rates from the rectal specimens of women without vaginal infections, children, and men (Hallén et al. 1987, 1988; Holst et al. 1987). The difference in isolation rates between women with BV, on the one hand, and healthy women, on the other, might be due to the occurrence of the microorganisms in higher numbers in the rectums of women who develop this condition compared with those in the rectums of healthy subjects (Holst 1990). The observations of the simultaneous occurrence of *Mobiluncus* in the vaginas and rectum of women with BV led to the suggestion that rectal colonization may predispose to vaginal colonization (Holst et al. 1987).

Mobiluncus has been isolated from the vagina and cervical canal of pregnant women (Krohn et al. 1989; Kaneko et al. 1992). However, the rates of colonization of the organism differ according to the groups of women. Thus, Krohn et al. (1989) detected *Mobiluncus* morphotypes by Gram stain in 3 % of 471 pregnant women without clinical signs of BV compared with 28 % of 122 pregnant women with BV. On screening of *Mobiluncus* species in female lower genital tract, Kaneko et al. (1992) detected the organisms from the vagina in 2/280 cases (0.7 %) in healthy pregnant women and from the cervical canal in 3/278 cases (1.1 %).

Mobiluncus species were only infrequently recovered from genital samples from males (Holst 1990). They have been isolated from the seminal fluid, urethras, and/or coronal sulci of male consorts of women with bacterial vaginosis, but were never recovered from the male consorts of healthy women (Holst et al. 1984b; Holst 1990; Magnanelli et al. 1990).

Pathogenicity, Clinical Relevance

Members of the family *Actinomycetaceae* are generally considered to be of relatively low virulence and are especially prevalent in infections associated with predisposing or underlying conditions such as trauma, surgery, malignancy, immunodeficiency, and presence of foreign bodies. They are seldom recovered as single isolates but are often mixed with other anaerobic or aerobic bacteria.

Genus *Actinomyces*

The most frequent illness of humans and cattle that is caused by anaerobic species of the genus *Actinomyces* is called actinomycosis. The term "actinomycosis" was introduced by Bollinger (1877) to describe case of cervicofacial disease in cattle "lumpy jaw" followed by Israel (1878) in his accurate description of cervicofacial and thoracic cases of the disease and in humans. As currently defined, actinomycosis is a subacute-to-chronic, granulomatous inflammatory disease characterized by suppuration, abscess formation, and draining sinus tracts, which erupt to the skin or mucosal surfaces and drain pus that may contain sulfur granules (Slack and Gerencser 1975; Pulverer and Schaal 1984).

Histologic Findings

The visualization of actinomycotic granules in exudates or in histopathologic tissue sections is strongly supportive of the diagnosis. Multiple sections of a biopsy specimen from affected tissue should be used for histologic diagnosis. Sections of biopsy material typically reveal acute or chronic inflammatory granulation tissue with infiltration by neutrophils, foamy macrophages, and lymphocytes surrounding dense fibrosis (Slack and Gerencser 1975). Sulfur granules may comprise no more than 1 % of total tissue in a given lesion and hence are easily missed by routine tissue staining. Visualization is facilitated by special stains such as Grocott–Gomori methenamine silver, *p*-aminosalicylic acid, and McCallen–Goodpasture and Brown–Brenn Gram stain. The sulfur granules are approximately 0.1–1 mm in diameter and may be seen with the naked eye as yellowish particles, and because of their yellowish appearance, they are commonly referred to as "sulfur granules" even though there is no clear evidence that they contain sulfur at all (*Fig. 4.2*). The sulfur granules are nearly pathognomonic, but their absence does not exclude the diagnosis of actinomycosis. Similar granules may also be found in infections with other organism such as *Nocardia brasiliensis*, *Streptomyces somaliensis*, or *Actinomadura madurae*. Microscopically, the granules manifest a cauliflower-like shape at low magnification (*Fig. 4.2*), and at higher magnification a clump of filamentous actinomycete microcolonies surrounded by polymorphonuclear neutrophils can be observed (*Fig. 4.3*). Brown–Brenn Gram stain renders these microcolonies visible as Gram-positive, intertwined branching filaments, with radially arranged, peripheral hyphae. Coexisting with them are the companion bacteria, which are Gram-positive and Gram-negative cocci and rods.

Etiology

Actinomycosis generally is a polymicrobial infection that involves *Actinomyces* associated with different bacteria, so-called concomitant bacteria (Brown 1973; Schaal and Lee 1992). It has been suggested that *Actinomyces* species are pathogenic only

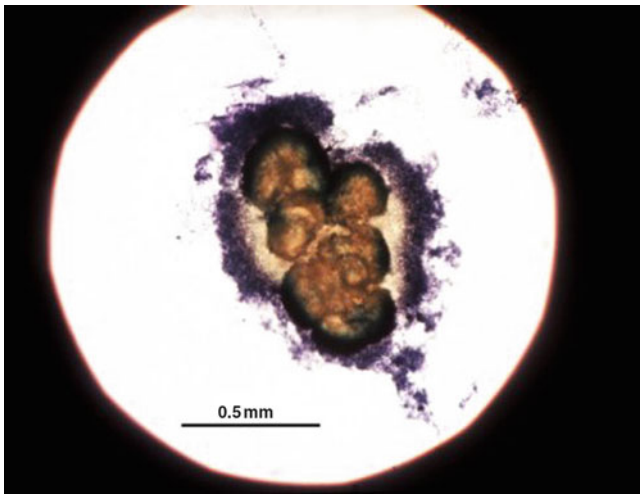

Fig. 4.2
Cauliflower like shape actinomycotic sulfur granule at low magnification.

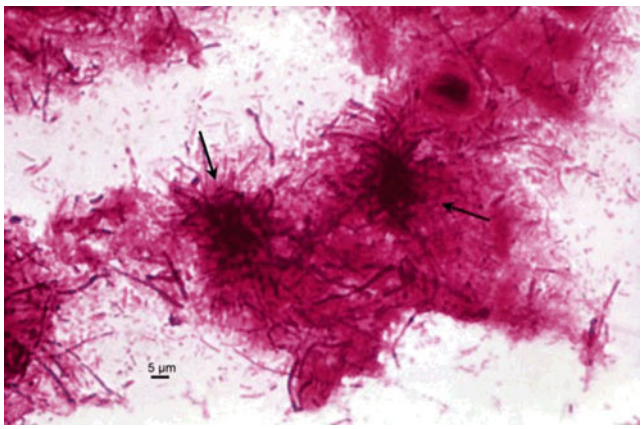

Fig. 4.3
Gram-stained smear of a crushed actinomycotic sulfur granule. Note the host of various Gram-positive and Gram-negative bacterial forms in addition to the nests → of Gram-positive, interwoven and branching filaments which represent the causative *Actinomyces* species.

with the synergistic action of concomitant bacteria. *A. israelii* and *A. gerencseriae* are by far the most common cause of infection, while *A. meyeri*, *A. naeslundii*, *A. odontolyticus*, *A. oris*, and *A. viscosus* are less common causes (Pulverer et al. 2003). In addition to these well-known actinomycotic species, some coryneform anaerobic bacteria capable of causing actinomycosis have also recently been assigned to the genus *Actinomyces*. Among these, *A. graevinitzii* an early colonizer of the oral cavity (Sarkonen et al. 2000) has been reported in a case of multiple pulmonary abscesses (Nagaoka et al. 2012) and in a case of pneumonia (Fujita et al. 2012). Another species of non-oral origin, *A. turicensis* was reported in a case of abdominal actinomycosis with clinical feature of multiloculated cystic mass in the left iliac fossa (Ong et al. 2012), in a case of hepatic abscess in a patient with previous dental manipulation (Riegert-Johnson et al. 2002), and in a case of soft tissue abscess of the breast (Attar et al. 2007) and to be associated with genital, skin-related, and urinary tract infections (Sabbe et al. 1999). Conversely, *A. funkei*, *A. radingae*, and *A. europaeus* have been isolated from soft tissue infections, usually in mixed anaerobic/aerobic infections, but have not been reported to be associated with cases of actinomycosis. Another species of related anaerobic Gram-positive, filamentous bacteria, *Propionibacterium propionicum* (formerly *Arachnia propionica* and *Actinomyces propionicus*) is a less common cause of actinomycotic infections (Wunderink et al. 2011).

The type and number of concomitant bacteria present in actinomycotic lesions varied according to the site of infection. The most commonly recovered concomitant bacteria include Gram-negative facultative anaerobe, e.g., *Aggregatibacter actinomycetemcomitans, Eikenella corrodens, Capnocytophaga, Enterobacteriaceae*; Gram-negative anaerobe, e.g. *Bacteroides* species, *Fusobacterium* species; as well as Gram-positive aerobe cocci such as *Staphylococcus* species and *Streptococcus* species. (Holm 1950; Fiorino 1996; Garner et al. 2007; Ghafghaichi et al. 2010; Ochiai et al. 1993; Simpson et al. 1996). The frequency of recovery of the different species found in an actinomycotic lesion varies from case to case, but an average of two to four and sometimes up to ten of these concomitant species are usually found in association with the causative actinomycete. Thus, in abdominal cases coliforms and Gram-negative bacilli, e.g., *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Bacteroides* spp. are predominated. Several observations reported the high frequency of *Peptostreptococcus* and *Bacteroides fragilis* associated with *Actinomyces israelii* in pelvic actinomycosis (Henderson 1973; Lomax et al. 1976; Valicenti et al. 1982). The bacteria that are involved in periodontitis comprises Gram-negative strict anaerobic rods such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Bacteroides* spp., and *Selenomonas* spp. (van Winkelhoff et al. 2002) as well as some anaerobic Gram-positive microorganisms such as *Peptostreptococcus micros* and *Eubacterium* species (Nonnenmacher et al. 2001). Their contribution to the pathogenesis in actinomycosis is unclear. It is possible that these concomitant bacteria may serve as copathogens, enhancing the progress of the disease by creating an anaerobic milieu in which *Actinomyces* thrives. This may be due to the reduction of oxygen tension in tissues and through anaerobiosis-induced inhibition of phagocytes Ochiai et al. (1993).

Clinical Manifestations

Infections are usually associated with the breakdown of normally physical barriers, such as disruption of mucosal membranes in the mouth and gastrointestinal tract. Infection spreads contiguously, ignoring tissue planes. Four clinical forms of actinomycosis, i.e., cervicofacial, thoracic, abdominopelvic, and cerebral, account for the majority of infections in humans (Slack and Gerencser 1975).

Cervicofacial Actinomycosis

Cervicofacial actinomycosis is the most common manifestation, comprising 50–70 % of reported cases. The face and neck are the most common sites of infection. The disease is characterized by soft tissue swelling of the perimandibular area, but it may spread into the orbita, sinuses, ear, or neck (Olson et al. 1989; Gazzano et al. 2010). The majority of cases have been observed in patients with poor oral hygiene and/or a history of invasive dental procedures or trauma. The most involved etiologic agents belonged to at least nine different species, among which *A. israelii* and *A. gerencseriae* predominated (Pulverer et al. 2003). The highest incidence was found in female patients aged 11–40 years and in male patients aged 21–50 years. Cervicofacial actinomycosis may extend to the underlying mandible or facial bones, leading to the development of periostitis or osteomyelitis.

Thoracic Actinomycosis

Thoracic actinomycosis accounts for 15–20 % of cases. Thoracic actinomycosis may involve the lungs, pleura, mediastinum, or chest wall. Aspiration of oropharyngeal secretions containing actinomycetes is the usual mechanism of infection. Occasionally, thoracic actinomycosis may result from the introduction of organisms via esophageal perforation, by direct spread from an actinomycotic process of the neck or abdomen, or via hematogenous spread from a distant lesion (Rose et al. 1982; Apothéloz and Regamey 1996). Poor oral hygiene and associated dental disease may increase the risk (Russo 2005). Misdiagnosis of the disease as tuberculosis, lung abscess, or bronchial carcinoma is common (Russo 1995). Untreated cases can spread to involve the pleura, pericardium, and chest wall, ultimately leading to the formation of sinuses that discharge sulfur granules.

Abdominal and Pelvic Actinomycosis

Abdominal actinomycosis accounts for approximately 10–20 % of reported cases (Postal et al. 2001). The disease has been recognized as one of the most misdiagnosed disease. It presents as an indolent chronic suppurative process with atypical symptoms that are often misinterpreted as malignant disease. Patients who have undergone appendectomy or have had a missed perforated appendicitis or women with a history of intrauterine contraceptive device use are at an increased risk (Garner et al. 2007). The ileocecal region is the most commonly affected abdominal site with the appendix predominating (Ohmagari et al. 1988; Thanos et al. 2004). Hepatic involvement is often associated with multiple small abscesses (Sharma et al. 2002; Lai et al. 2004). Other reported sites include the colon (Cowgill and Quan 1979; Heer et al. 1986), stomach (Van Olmen et al. 1984; Fernández-Aceñero et al. 2004), gallbladder (Freland et al. 1987), pancreas (Somsouk et al. 2008), small bowel (Belak et al. 2001), anorectal region (Smith 1992; Coremans et al. 2005), pelvis, and abdominal wall (Lau et al. 1986; Gupta et al. 1990). Involvement of retroperitoneal organs may result from hematogenous dissemination or direct extension (Berchtenbreiter et al. 1999; Olson et al. 1993).

Pelvic actinomycosis is associated with long-term use of an intrauterine contraceptive device (Henderson 1973; Fiorino 1996; Lely and van Es 2005). The incidence of actinomycotic infection increases with duration of IUCD use and the mean duration of coil usage in women with actinomycosis is 8 years (Fiorino 1996). Symptoms and signs of genital actinomycosis are nonspecific, and in the majority of cases, women may complain of abdominal pain, weight loss, vaginal discharge, and fever (Duguid 1983). The reason for the association with IUCD usage is unclear. Insertion of the device may traumatize the cervical and uterine mucosa, inducing mild inflammatory changes in the endometrium with necrosis that creates an anaerobic environment that favors growth of *A. israelii* and other anaerobics (Valicenti et al. 1982). When pelvic actinomycosis occurs, it usually causes endometritis, salpingo-oophoritis, or tubo-ovarian abscess, and a mass in the adnexa might be palpable, suggesting a pelvic malignancy (Hinnie et al. 1995; Perlow et al. 1991; Hwang et al. 2009). Ultimately, extension to the abdominal wall or deep pelvic structures can occur. Even involvement of the urinary bladder presenting with frequency, dysuria, hematuria, or a suprapubic mass and sulfur granules in the urine has been observed (Wajszczuk et al. 1984; Zakut et al. 1987).

Cerebral Actinomycosis

Actinomycosis of the central nervous system (CNS) is rare and is usually secondary to hematogenous spread from primary infection in the lung, abdomen, or pelvis. However, extension from foci of infection in the ears and cervicofacial regions may proceed along connective tissue planes or through foramina at the base of the skull, causing focal infection of the CNS or diffuse basilar meningitis. The disease may present as a brain abscess, meningitis or meningoencephalitis, a subdural empyema (Louie et al. 1979; Soto-Hermández et al. 1999), an actinomycoma, and a spinal and cranial epidural abscess (Albright et al. 1974; Brunon et al. 1980; Schwartz and Christoff 1960; Smego 1987; Wickbom and Davidson 1967; Yung et al. 2000). Actinomycotic cerebral abscesses are usually singular but may be multiple, unilocular or multilocular, and encapsulated or, less frequently, unencapsulated (Bolton and Ashenhurst 1964). The clinical features are indistinguishable from those of pyogenic infections of intracranial and spinal structures.

Cutaneous Actinomycosis

Cutaneous localizations of actinomycosis generally occur by contiguity of underlying foci of tooth or lung, by direct inoculation, or by spread through the bloodstream during a septicemic phase of the infection. In the latter case, there are often multiple lesions. Primary cutaneous actinomycosis of the extremities is uncommon and usually has an association with trauma and bites (Gupta et al. 2012; Reiner et al. 1987; Sardana et al. 2001). Skin, subcutaneous tissues, muscle, and bone (with periostitis or acute or chronic osteomyelitis) are involved alone or in various combinations (Blinkhorn et al. 1988; Rippey and Hendry 1988; Kargi et al. 2003; Mah et al. 2005).

Lacrimal Canaliculitis and Other Eye Infections

Infection of the eye with *Actinomyces* species has been recognized for many years. The most common site of infection involving the eye is the lacrimal system. Canaliculitis is more common in women. It is usually unilateral and more frequently involves the lower canaliculus. Epiphora, caused by obstruction, may be the only initial symptom, followed by localized swelling. The classic clinical features of *Actinomyces* canaliculitis are mild to severe swelling of the canaliculus, mucopurulent discharge from the punctum, inflamation of the medial canthus, epiphora, and a red pounting punctum (Fulmer et al. 1999; MacNab, 1994). Actinomycosis less frequently results in conjunctivitis, lacrimal sac involvement, keratitis, and endophthalmitis (Briscoe et al. 2004; Caretti et al. 2011; Hussain et al. 1993; Pine et al. 1960a; Roussel et al. 1991). Various microorganisms have been implicated as causal pathogens. Besides the most commonly identified species, *A. israelii*, *A. gerencseriae*, *A. naeslundii*, *A. viscosus*, and *A. odontolyticus*, another related organism, *Propionibacterium propionicum*, has also been frequently isolated (Seal et al. 1981). The incidence of isolation of the latter organism from lacrimal concretions was as frequent as was *A. israelii* (Hussain et al. 1993; Schütt-Gerowitt et al. 1999). These anaerobic bacteria may likely gain entry to the canaliculi indirectly from the saliva via the fingers or directly via the nasal passage and nasolacrimal duct (Pine et al. 1960a; Hussain et al. 1993). In addition to these common pathogens, *A. neuii* has been reported to be associated with acute postoperative endophthalmitis (Garelick et al. 2002).

Complications Associated with Actinomycosis

Systemic dissemination and potentially life-threatening complication can occur if *Actinomyces* infections are unrecognized and/or inadequately treated (Shay 2002). Hematogenous seeding from an oral source is a dominant cause of bacterial endocarditis (Cohen et al. 2007; Huang et al. 1998), pericarditis (Jánoskuti et al. 2004; Llenas-García et al. 2012), liver abscess (Lall et al. 2010; Riegert-Johnson et al. 2002; Sharma et al. 2002; Uehara et al. 2010), and renal abscess (May et al. 2008).

The most important neurologic complications associated with CNS infection include brain abscesses (Adeyemi et al. 2008; Navas et al. 1994) followed by meningitis or meningoencephalitis. Furthermore, cranial epidural and subdural empyemas (Louie et al. 1979; Soto-Hermández et al. 1999) and spinal epidural abscesses (Yung et al. 2000) have been reported. Severe neurologic deficits can result if there is delay in diagnosis or treatment of CNS actinomycosis (Alday et al. 1989).

The most common complications associated with pulmonary actinomycosis include lung abscesses (Nagaoka et al. 2012; Takiguchi et al. 2003), pleural empyemas (Vallet et al. 2004), and necrotizing pneumonia (Costiniuk et al. 2011). Invasion of the chest, soft tissues, and bony structure wall leading to the formation of draining sinus tracts with purulent discharge may occur. Osteomyelitis due to *Actinomyces* infection can involve the bones of the mandible (Bala et al. 2011; Smith et al. 2011), sternum (Panilla et al. 2006), ribs and vertebrae (Honda et al. 2008), and the skull base (Nomura et al. 2011).

Besides its serious nature of organ involvement, other complications include hip prosthesis infection (Wu et al. 2011b; Wüst et al. 2000), septic arthritis (Lequerré et al. 2002), endodontic infection (Xia and Baumgartner 2003), and postoperative endophthalmitis (Scarano et al. 1999). Opportunistic actinomycotic infection has been reported in osteoradionecrosis (Curi et al. 2000) in patients having head and neck cancer.

Actinomyces in Oral Health and Disease

The significance of *Actinomyces* in oral health and disease has been documented (Beighton and Miller 1977; Dent and Marsh 1981; Bowden 1990). *Actinomyces* and *Streptococcus* species dominate among the early colonizing bacteria, both on the teeth and oral mucosal surfaces (Socransky and Manganiello 1971; Gibbons 1989; Hsu et al. 1994; Jenkinson and Lamont 2005). The result of coaggregation and adhesion between *Actinomyces* spp. and *Streptococcus* spp. is the formation of the initial layer of oral biofilm (Palmer et al. 2003). In this layer *Actinomyces* and *Streptococcus* species then offer adhesion sites for additional bacteria, through co-adhesion/coaggregation. *Actinomyces* spp. coaggregate through the type 2 fimbriae (Palmer et al. 2003), while *Streptococcus* spp. have several adhesins involved in coaggregation, including the Ag I/II family, Csh family, and Fap1-including family of adhesins (Elliott et al. 2003; Jakubovics et al. 2005). The coaggregation of different species is highly specific, and each species seems to have specific partners, such as *Streptococcus*/*Actinomyces*, *Veillonella*/*Streptococcus* and *Actinomyces*, and *Prevotella*/*Actinomyces* species (Hughes et al. 1988; Kolenbrander 1988; Nesbitt et al. 1993). Thus, the mature oral biofilm is a multispecies community, with more than 700 different species and phylotypes, the majority belong to the phyla Deferribacteres, Spirochaetes, Fusobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria (Dewhirst et al. 2010; Jenkinson and Lamont 2005; Paster et al. 2001).

Though *Actinomyces* species are part of the normal, resident microbiota of distinct surfaces of the mouth, they contribute to different plaque-involved diseases, e.g., dental caries and periodontal diseases (Syed et al. 1975; Ellen et al. 1985; Bowden 1990; Moore and Moore 1994). The disease results only when there is a shift in the homeostatic balance of the resident microflora due to a change in local environmental conditions (such as pH) which favor the growth of pathogens (Jenkinson and Lamont 2005; Marsh 1994; Marsh and Nyvad 2008). Under such circumstances, it is suggested that acidogenic (acid producing) and aciduric (acid resistant) bacteria other than mutans streptococci (MS), including "low-pH" non-MS and *Veillonella* spp. as well as *Actinomyces naeslundii*, *A. viscosus*, *A. odontolyticus*, and *A. israelii*, are responsible for the initiation of caries (Nyvad and Kilian 1990; van Houte et al. 1994, 1996; Brailsford et al. 1998; Becker and Paster 2002; Becker et al. 2002; Tanner et al. 2002; Aas et al. 2008). Thus, the bacterial

communities associated with root caries are highly diverse and undergo succession during lesion formation, and therefore the caries disease is an outcome of the overall activity of the total plaque microflora and not a specific organism (Loesche 1976).

Dental caries is the localized destruction of the tissues of the tooth by bacterial action. In root caries, enamel or cementum is demineralized by microbial acids, predominantly lactic acid, produced by fermentation of dietary sugars. Evidences of the role of *Actinomyces* species in root surface carious lesion induction were initiated in the 1970s and received some impetus from caries studies on experimental animals where it was shown that strains of *Actinomyces* from humans could cause root surface caries (Socransky et al. 1970; Jordan et al. 1972). *Actinomyces* species possess several attributes that contributes to its success as a cariogenic organism. The expression of cell surface fimbriae is correlated with the ability of these bacteria to adhere to specific receptors on the tooth and mucosal surfaces of the oral cavity (Cisar 1986; Cisar et al. 1997b). Adherence of *Actinomyces* to teeth is mediated by type 1 fimbriae through interactions with salivary proline-rich proteins (PRPs) that coat the enamel surfaces (Cisar et al. 1988; Gibbons et al. 1988; Clark et al. 1989). In contrast, adherence to mucosal epithelial cells depends on the presence of type 2 fimbriae (Brennan et al. 1984, 1986; Sandberg et al. 1986, 1995; Cisar et al. 1995). In addition, *Actinomyces* spp. are known to produce sialidases (Costello et al. 1979; Moncla and Braham 1989), enzymes that enhance fimbriae-mediated adherence by unmasking the fimbrial receptors on mammalian cells (Cisar 1986).

Furthermore, oral *Actinomyces* produce fructosyltransferase (FTF) enzymes which convert sucrose into polymers of D-fructose, known as levans, and these polymers are thought to contribute to the development of dental caries by allowing plaque bacteria to utilize a greater proportion of dietary sucrose over a longer period of time, thus enhancing acid production (Bergeron et al. 2000). Fructans also are effective T-cell-independent antigens, can trigger inflammation, and are mitogenic for B cells (Couthino and Moller 1973; Desaymard and Ivanyl 1976). Thus, it is possible that an immune response to levans may contribute to the inflammation seen in periodontal diseases.

Actinomyces species also can metabolize a variety of sugars, resulting in the production of a number of weak acids, including lactic, succinic, and acetic acids that generate a low-pH environment and enrich for aciduric organism. When the plaque pH drops below about 5, the balance between enamel demineralization and remineralization shifts toward solubility and the caries process is initiated. In contrast, *Actinomyces* species demonstrates urease activity that allows these bacteria to hydrolyze urea to ammonia (Salako and Kleinberg 1989; Morou-Bermudez and Burne 1999). Ammonia production by ureases of oral bacteria in dental plaque causes a considerable increase in plaque pH, inhibiting the emergence of a cariogenic flora and the development of dental caries (Kleinberg 1967; Shu et al. 2007). However, urea metabolism is thought to have detrimental effects as well, enhancing the formation of tartar or calculus (Wong et al. 2002; Wong and Sissons 2007), inducing inflammation at the gingival margin, and enhancing inflammation in periodontal pockets (Burne and Chen 2000). Ureolysis has been found to protect *Actinomyces* against acid damage and allows the organism to use urea as a nitrogen source for growth (Morou-Bermudez and Burne 1999).

Susceptibility of Actinomyces to Antibiotics and Recommended Treatment

Currently there are no CSLS standards published for the reliable testing and reporting of antibiotic susceptibility results for *Actinomyces* species. The use of various enriched broths, undefined inocula, prolonged periods of incubation, and gradations of growth suppression, rather than total growth inhibition, makes it difficult, if not impossible, to compare published results (Blake 1964; Lerner 1974; Schaal and Pape 1980). Despite such extreme variations in assay technique, almost all investigators showed, however, that *Actinomyces* spp. are susceptible to a wide range of beta-lactam antibiotics (Smith et al. 2005; Tanaka-Bandoh et al. 1997). Thus, using the Etest methodology, it was found that all isolates of *A. israelii*, *A. gerencseriae*, *A. turicensis*, *A. funkei*, *A. graevenitzii*, and *A. europaeus* are susceptible to penicillins, cephalosporins, carbapenems, lincomycins, linezolid, macrolides, and tetracyclines (MIC$<$1 mg/L) (Smith et al. 2005). Using the broth microdilution method, poor or intermediate susceptibility to amikacin was observed for *A. israelii*, *A. viscosus*, *A. naeslundii*, and *A. gerencseriae* (Tanaka-Bandoh et al. 1997). In contrast all *Actinomyces* species are resistant to ciprofloxacin, ofloxacin, and metronidazole (Smith et al. 2005; Tanaka-Bandoh et al. 1997; Wade 1989).

Due to the polymicrobial nature of the infection with *Actinomyces*, successful treatment of actinomycosis depends not only on the type of disease and its localization, but also, often more importantly, on the composition of the concomitant flora. Treatment for actinomycosis requires long-term administration of parenteral and oral antibiotics. Optimal duration of antimicrobial therapy should be tailored depending on the severity of illness. However, a longer duration with antimicrobial agents is usually necessary since premature termination of antimicrobial therapy may cause a relapse of actinomycosis. Surgical intervention may be required in selective cases (Berardi 1979; Mabeza and Macfarlane 2003; Hayashi et al. 2010). Penicillin is the drug of choice. Conventional therapy for actinomycosis is high-dose intravenous penicillin at a dosage of 18–24 million units daily for 2–6 weeks, followed by oral penicillin or amoxicillin for a period of 6–12 months (Russo 2005). However, treatment failures and relapses have been documented. This can be attributed to the presence of β-lactamase-producing concomitant flora. Therefore, current treatment schemes include drugs effective against *Actinomyces* species and potential β-lactamase producers such as Staphylococcus aureus, Gram-negative anaerobes, and members of the *Enterobacteriaceae*. A possible scheme for the antibiotic treatment of cervicofacial actinomycosis may consist of amoxicillin plus clavulanic acid or possibly ampicillin plus sulbactam (Smith et al. 2005). The treatment of choice for

abdominal actinomycosis is a combination of amoxicillin and clavulanic acid with metronidazole (or clindamycin) for strict anaerobes plus tobramycin or gentamicin.

In pregnant patients, erythromycin is a safe alternative. In case of patients for whom penicillins allergy is a concern, infections may be treated with ceftriaxone (Skoutelis et al. 1994), doxycycline (Davanos et al. 2008), imipenem (Edelmann et al. 1987; Yew et al. 1994), minocycline (Martin 1985), or erythromycin.

For treatment of lacrimal canaliculitis, a local therapy with penicillin G (100,000 U) or erythromycin, imipenem, or gyrase inhibitor, e.g., norfloxacin or levofloxacin, should be initiated for 1–2 weeks. Caries and periodontitis can usually be treated successfully using the various local measures of conservative and operative dentistry, although administration of suitable antibiotics may facilitate therapy of periodontitis and especially of gingivitis. Recently Comelli et al. (2009) selected a lactic bacterium strain for the preparation of a composition intended for reducing dental plaque and for treating or preventing root caries and other diseases related to *Actinomyces naeslundii* in mammals.

Animal Infections Caused by *Actinomyces* Species

Actinomyces species, which are known to occur commonly on the buccal and nasopharyngeal mucous membranes of several animal species, can cause sporadic pyogranulomatous lesions (Quinn et al. 1994). *A. bovis* is the etiologic agent of actinomycosis, or lumpy jaw, in cattle, horses, goats, sheep, and wildlife species such as elk and pronghorn antelope (Allred and Bradley 1965; Hoefs and Bunch 2001; Vos 2007). The disease is an osteomyelitis that affects the mandible or maxilla. Gross lesions of mandibular osteomyelitis include distortion and proliferation of the jaw bones, damage to teeth, abundant scar tissue, and in some cases fistulas draining through the skin (Bunch et al. 1999). *A. bovis* has also been isolated from nodular abscesses in the lungs of cattle and from infrequent infections in sheep, pigs, dogs, and other mammals, including chronic fistulous withers and chronic poll evil in horses (Kimball and Frank 1945). Furthermore, *A. denticolens* has been reported in a case of mandibular and submandibular abscesses in horses (Albini et al. 2008; Beck et al. 2011).

Actinomyces hyovaginalis is an important cause of disseminated necrotic lung lesions in slaughtered pigs and is believed to be acquired via inhalation (Aalbebaek et al. 2003). Furthermore, it has been associated with rare cases of porcine abortion (Hogg et al. 2012) and lymphadenitis in goat (Schumacher et al. 2009) and is a potential cause of skin abscesses and other sites in small ruminants (Foster et al. 2012).

A. hordeovulneris was isolated from cases of chronic pyogranulomatous pleuritis, pericarditis, peritonitis, visceral abscesses, septic arthritis, and recurrent localized infections in dogs (Buchanan and Scott 1984; Pelle et al. 2000). Infection has been reported in association with penetration of *Hordeum* grass awns.

Actinomyces viscosus is the etiologic agent of canine and feline actinomycosis. Cutaneous actinomycosis is the most common manifestation and is often associated with subcutaneous abscess formation and pyothorax. Infections are usually secondary to perforating injuries caused by bite wounds or foreign bodies. In the most common forms of clinical disease in the dog, the cervicofacial, abdominal, and thoracic regions are involved (Davenport et al. 1975; Donahue and Brightman 1995; Georg et al. 1972). *A. viscosus* has also been isolated from skin pustules and nodules in horses (Specht et al. 1991).

Actinomycosis involving the central nervous system (CNS) is rare in animals. Several cases of *Actinomyces* causing CNS disease have been reported in animals: meningitis in an Arctic fox (Raju et al. 1986), encephalitis associated with hydrocephalus in a dog (Anvik and Lewis 1976), and pyogranulomatous meningoencephalitis in a dog (Couto et al. 2000). In all these cases, however, identification of the organisms could not be made. *A. viscosus* was isolated from the inflammatory tissues of a 3-year-old female domestic cat with paraplegia (Bestetti et al. 1977). *A. naeslundii* has been identified in a case of pyogranulomatous meningoencephalitis and osteomyelitis of the temporal bone in a goat (Hirai et al. 2007).

Furthermore, *Actinomyces* species have been rarely implicated in ocular infections in animals. Sherman et al. (2012) reported on a case of ulcerative keratitis in a dog attributed to *A. bowdenii*. *Actinomyces* sp. has been associated with a case of endophthalmitis in a dog (Barnes and Grahn 2007).

Potential Virulence Factors of *Actinomyces*

Actinomyces species are not virulent or have a low potential to produce diseases, and they require a break in the integrity of the mucous membranes and the presence of devitalized tissue to invade deeper body structures and cause illness. Establishment of infection may require the presence of other bacteria, which participate in the production of infection by elaborating a toxin or enzyme or by inhibiting host defenses. These companion bacteria act as copathogens enhancing the low invasiveness of *Actinomyces* species. After establishment of infection with *Actinomyces*, the host mounts an intense inflammatory response, i.e., suppurative and granulomatous, and fibrosis may develop subsequently. Infection typically spreads contiguously, frequently ignoring tissue planes and invading surrounding tissues or organs. Ultimately, the infection produces draining sinus tracts. Hematogenous dissemination (Apothéloz and Regamey 1996; Colmegna et al. 2003) to distant organs may occur in any stage of the infection, whereas lymphatic dissemination is unusual.

Although the exact mechanism by which *Actinomyces* species exert their pathogenicity has not been totally clarified, there is some evidence that can help explain infections caused by these microorganisms. Several *Actinomyces* species, including *A. naeslundii*, *A. oris*, and *A. viscosus*, have fimbriae which mediate adhesion of these bacteria to dental and mucosal

surfaces and interactions with streptococci as well as other members of the biofilm community (Cisar et al. 1997b; Rickard et al. 2003; Yeung 1999). *Actinomyces* species have two antigenically and functionally distinct types of fimbriae; type 1 fimbriae mediate adhesion of *Actinomyces* to the tooth surface through binding to salivary proline-rich proteins (PRPs) and to statherin (Clark et al. 1989; Gibbons et al. 1988; Wu et al. 2011a), whereas type 2 fimbriae are associated with a lactose-sensitive mechanism (a lectin-like activity) to recognize specific saccharide motifs present in both streptococcal coaggregation receptor polysaccharides (RPS) and host cell surface glycoconjugates (Cisar et al. 1995, 1997a; Mishra et al. 2010; Strömberg et al. 1992). Type 2 fimbrial lectins facilitate phagocytosis by recognizing the lactose-containing receptors on polymorphonuclear leukocytes (Sandberg et al. 1986).

The genetic components for type 1 and 2 fimbrial assembly of *Actinomyces* are arranged in two distinct gene clusters (Donkersloot et al. 1985; Mishra et al. 2007; Yeug and Cisar 1988; Yeung and Ragsdale 1997). Type 1 fimbria of *A. oris*, encoded by the gene cluster *fimQ-fimP-srtC1*, is made of the fimbrial shaft FimP and the tip fimbrillin FimQ, which is the adhesin interacting with PRPs (Wu et al. 2011a). On the other hand, type 2 fimbria, encoded by the gene locus *fimB-fimA-srtC2*, is made of the fimbrial shaft FimA and the tip fimbrillin FimB (Mishra et al. 2007). FimA is essential for coaggregation of *A. oris* with oral streptococci, adherence to red blood cells (RBCs), and biofilm development (Mishra et al. 2010). Assembly of type 1 fimbrial polymers requires sortase SrtC1 (Wu et al. 2011a), whereas type 2 fimbrial assembly involves sortase SrtC2 (Mishra et al. 2010). An *Actinomyces oris* mutant lacking *srtC2* fails to coaggregate with oral streptococci, adhere to RBCs, and form biofilms (Mishra et al. 2010).

In addition, the ability of *Actinomyces* species to produce exopolysaccharides (EPSs) contributes to their survival and the development of persistent infections in the human body. Exopolysaccharides are key components of the matrix in cariogenic oral biofilms and in biofilms formed on abiotic surfaces such as IUD and are recognized virulence factors involved in the pathogenesis of dental caries and pelvic actinomycosis in IUD users (Bowen and Koo 2011). Carrillo et al. (2010) showed that *A. israelii* was able to attach and grow in synthetic intrauterine media and to survive copper toxicity due to its ability to produce EPS and to form biofilms. Yamane et al. (2013) demonstrated the capacity of a strain of *A. oris* to produce EPSs in liquid culture as viscous materials and as meshwork structures around the cell on agar plates and its capability to induce persistent abscess lesions in mice. EPS produced by microorganisms are a complex mixture of biopolymers primarily consisting of polysaccharides, as well as proteins, nucleic acids, lipids, and humic substances. This matrix was considered essential for the existence of the biofilm lifestyle and full expression of virulence by bacterial pathogens (Costerton et al. 1999; Flemming and Wingender 2010). EPS immobilizes biofilm cells and keeps them in close proximity, thus allowing for intense interactions, including cell–cell communication and the formation of synergistic microconsortia (Flemming and Wingender 2010).

Serological Diagnosis of *Actinomyces* Infections

Because of difficulties in obtaining suitable clinical specimens and isolating *Actinomyces* species, there has always been a demand for diagnosing actinomycotic infections by means of serological techniques. Various serological techniques including fluorescent antibody (FA) staining, gel precipitation, enzyme immunoassay, and immunoelectrophoresis have been used for identifying and enumerating *Actinomyces* species. However, few studies have been carried in the past concerning the serological diagnosis of actinomycosis, and the results cannot be regarded as sufficiently satisfactory.

Fluorescent-conjugated antibody (FA) staining provided a rapid method for the identification of various *Actinomyces* species (Slack and Gerenscer 1970, 1976, Gerencser and Slack 1976). In addition, FA permits rapid diagnosis of actinomycosis by direct staining of clinical materials, even after fixation in formalin (Happonen and Viander 1982; Hothci and Schwarz 1972). In a comparative study of fluorescent antibody technique and conventional staining methods for the diagnosis of cervicofacial actinomycosis in formalin-fixed and paraffin-embedded biopsies using antiserum specific against *A. israelii*, a good correlation was found between conventional staining and FA staining in both suspected positive and negative controls (Happonen and Viander 1982).

Holmberg et al. (1975) developed a serological test based on crossed immunoelectrophoresis (CIE) for detecting precipitating antibodies against *A. israelii* and applied it for the detection of humoral antibodies in sera from nine patients with actinomycosis. They found that all sera of patients had antibodies against one or more *A. israelii* antigens in the preparations. In a subsequent study the diagnostic sensitivity of the CIE assay was evaluated by Persson and Holmberg (1984b). The sensitivity of the assay was 83 %, its specificity was 98 %, and its accuracy was 100 % for negative prediction and 45 % for positive prediction. However, the CIE assay could not be relied on for a presumptive diagnosis of actinomycosis as cross-reacting antibodies were detected in serum specimens from some patients, particularly with tuberculosis, nocardiosis, candidiasis, or aspergillosis (Holmberg 1981). Furthermore, although precipitation reactions against *A. israelii* antigens were found in uterine secretions from women with long-term use of IUD, the nature of the precipitating components could not be proved to be immunoglobulins (Persson and Holmberg 1985), and therefore, the method is inconclusive.

Furthermore, the relationship between antibody response to *Actinomyces* antigens and dental caries has been studied (Levine and Movafagh 1984; Levine et al. 1984, 2005). Human serum from many individuals has predominantly IgG precipitating antibodies to two antigens identified in dental plaque and various oral bacteria (Levine and Bush 1981). One antibody (A+) recognizes an antigen from *Actinomyces* spp. (Levine and

Movafagh 1982). The other antibody (S+) is specific for D-alanyl glycerol teichoic acids found in cell walls in many Gram-positive bacteria. The presence of an elevated IgG antibody response to *Actinomyces* antigen (A-Ab) enhances caries protection directly and in association with effective oral hygiene using fluorinated dentifrices (Levine et al. 1984, 2005). Conversely, a low A-Ab response is suggestive of greater caries experience.

Genus *Arcanobacterium*

Arcanobacterium haemolyticum is an opportunistic pathogen that occurs almost exclusively in symptomatic patients, with sporadic finding in healthy individuals. It is believed that humans are the only reservoir of this bacterium. Although *A. haemolyticum* has been most commonly associated with pharyngitis (Funke et al. 1997b), it has been implicated in a wide spectrum of diseases (Linder 1997; Skov et al. 1998; Vargas et al. 2006). These include endocarditis (Alos et al. 1995; Wong et al. 2011), skin and soft tissue infection (Dobinsky et al. 1999; van Loo et al. 2007), urinary tract infection (Ciraj et al. 2006), osteomyelitis (Biswas et al. 2003), peritonitis (Farmer et al. 2007), brain abscess (Vargas et al. 2006), pneumonia (Stacey and Bradlow 1999), pyothorax (Parija et al. 2005), bacteremia (Fernández-suárez et al. 2009), and septic arthritis (Goyal et al. 2005). Infections can be transmitted between humans or from humans to animals.

Human Infections Caused by *Arcanobacterium* Species

A. haemolyticum is almost exclusively a human pathogen, making it somewhat unique within the genus (Jost and Billington 2005). *A. haemolyticum* is a well-recognized cause of pharyngitis, skin, and soft tissue infections (Maclean et al. 1946; Miller et al. 1986). An estimated 0.5–2.5 % of all cases of pharyngitis are caused by *A. haemolyticum* (Mackenzie et al. 1995; Linder 1997). Infection occurs predominantly in adolescents and young adults with maximum incidence in the 15–18-year-old age group (Masckenzie et al. 1995). Clinically, *A. haemolyticum* pharyngitis resembles that caused by *Streptococcus pyogenes*, in that patients complain of sore throat and pharyngeal edema. In addition, about half of the patients complain of fever, swollen lymph glands, nonproductive cough, and purulent secretions from the tonsils. One unique feature of *A. haemolyticum* pharyngitis is that 33–67 % of patients display an erythematous, morbilliform, or scarlatinal rash along with other symptoms (Miller et al. 1986; Carlson et al. 1994c).

An individual with *A. haemolyticum* cutaneous infections displays chronic ulcerations and cellulitis. In addition, bacteremia secondary to skin and soft tissue infections has been reported and in reported cases the source of infection is most likely a skin wound. In some cases, the clinical presentation of soft tissue abscess caused by *A. haemolyticum* mimics a soft tissue tumor in patients without an underlying immunosuppressive condition (van Loo et al. 2007; Dobinsky et al. 1999).

Less commonly, *A. haemolyticum* causes systemic invasive disease and deep-seated infections such as meningitis (Minarik et al. 1997), endocarditis (Alos et al. 1995; Wong et al. 2011), septic arthritis (Goyal et al. 2005), pneumonia (Stacey and Bradlow 1999; Therriault et al. 2008), osteomyelitis (Biswas et al. 2003), and sinusitis (Volante et al. 2008). It has been isolated as a sole cause of infection in a number of cases (Vargas et al. 2006; van Loo et al. 2007; Therriault et al. 2008), but in others it has been isolated in combination with other pathogens (Dobinsky et al. 1999; Younus et al. 2002; Lundblom et al. 2010) such as *Bacteroides* spp., *Staphylococcus aureus*, Group G *Streptococcus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Mycoplasma pneumoniae*, and *Fusobacterium necrophorum*. Invasive disease caused by *A. haemolyticum* shows a special predilection for males and is often seen in two distinct subset of patients: a subset of otherwise healthy adolescents presenting with upper respiratory tract infections (pharyngitis, tonsillitis, sinusitis) and a subset of older, often immunocompromised patients presenting with skin and soft tissue infection (Skov et al. 1998; Tan et al. 2006; Therriault et al. 2008).

The other species are uncommonly isolated from human.

Animal Infections Caused by *Arcanobacterium* Species

A. haemolyticum has been known as an occasional cause of infections in domestic (Hassan et al. 2009; Tyrrell et al. 2002) and farm (Richardson and Smith 1968; Roberts 1969) animals for which human contact is a possible source of infection. The organism has been identified as one of the pathogens responsible for mandibular and maxillary abscesses in pet rabbits (Tyrrell et al. 2002). It has also recently been reported as a cause of necrotizing fasciitis and myositis in cattle (Bancroft-Hunt et al. 2010). *A. hippocoleae* is reported to be associated with vaginitis and placentitis in horse (Hoyles et al. 2002b; Bemis et al. 2008). *A. phocae* was recovered in mixed culture from various tissues and fluids of common seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus*) (Pascual Ramos et al. 1997a). It is a common pathogen of wound infections that is occasionally associated with systemic infections in stranded marine mammals (Johnson et al. 2003). *A. pluranimalium* was recovered from the spleen of a dead harbor porpoise (*Phocoena phocoena*) and lung abscess in a fallow deer (*Dama dama*) (Lawson et al. 2001a) and was frequently isolated from abortion materials derived from ovine (Foster and Hunt 2011). *A. pluranimalium* is thought to be a cause of occasional opportunistic infections in sheep, particularly abortion, in England and Scotland (Duff et al. 2001).

Potential Virulence Factors of *Arcanobacterium*

A. haemolyticum expresses a number of virulence factors that are important for the organism to cause disease. *A. haemolyticum* expresses an unusual phospholipase D (PLD), which is responsible for efficient host cell adhesion by reorganizing lipid rafts (Lucas et al. 2010). PLDs are enzymes which cleave host membrane phospholipids, although the *A. haemolyticum* PLD has restricted substrate specificity, cleaving only sphingomyelin. The activities of PLD promote damage, either directly or via host inflammatory processes, to the epithelial lining, which provides an opportunity for bacterial dissemination from the site of primary infection (throat or skin) to distal sites to cause systemic disease. PLD present in all strains of *A. haemolyticum* and the loss of its production does not affect bacterial invasion (Lucas et al. 2010). This is in contrast to the most closely related PLD of *Corynebacterium pseudotuberculosis* (Cuevas and Songer 1993) that is absolutely required for virulence (McNamara et al. 1994). PLD expressed by intracellular bacteria is cytotoxic to host cells and caused host cell death via necrosis (Soucek and Souckova 1974).

In addition, *A. haemolyticum* secrete arcanolysin (ALN), a cholesterol-dependent cytolysin (CDC), which is most closely related to pyolysin (PLO) from *Trueperella pyogenes*. ALN, like PLD, was present in all strains of *A. haemolyticum*. It has variable hemolytic and cytotoxic activity against mammalian cells from different species with high activity against human, horse, and rabbit cells and lesser activity against cells derived from other species (Jost et al. 2011). This selectivity appears to function at the level of membrane binding and may contribute to the host range of *A. haemolyticum*.

Aside from PLD, *A. haemolyticum* also expresses neuraminidase activity (Mueller 1973) and has been shown to exhibit binding to several human plasma proteins, including fibrinogen and fibronectin (66).

Virulence factors of the other *Arcanobacterium* species have not been studied.

Antigenic Structure

A. haemolyticum induces an antibody response, as demonstrated in sera of patients with acute infection or from convalescents, in patients from whom the organism was cultured (Nyman et al. 1997; Votava et al. 2001). Votava et al. (2001) reported the detection of *A. haemolyticum* PLD-neutralizing antibodies in patients with acute tonsillitis. The antibodies react primarily with four distinct cell wall-associated proteins with estimated molecular masses of 80, 60, 50, and 30 kDa. Of the four proteins, the 80-kDa protein is the dominant antigen (Nyman et al. 1997).

Antigenic properties of PLDs produced in vitro have been used in the serodiagnosis of infections caused by bacteria that produce PLDs in vivo (Cuevas and Songer 1993; McNamara et al. 1995; Skalka et al. 1998; Votava et al. 2001). The sera obtained from spontaneous infection cases react with the homologous PLD of the bacterial species which had caused the infection. The antigenic properties of PLD from *A. haemolyticum* (PLD-A) and from *Corynebacterium pseudotuberculosis* (PLD-C) are similar but not identical (Cuevas and Songer 1993; McNamara et al. 1995). Skalka et al. (1998) used PLD neutralization to examine sera of humans with a spontaneous infection by *A. haemolyticum*. A positive result of neutralization was due to an inhibition of the hemolytic synergism with the equi factor (ef) from *Rhodococcus equi*. They found that the titers of sera neutralizing the homologous PLD were always significantly higher than those neutralizing the heterologous PLD. However, these tests are not yet available as a matter of routine.

Antigens of the other *Arcanobacterium* species have not been studied.

Susceptibility of Arcanobacterium to Antibiotics

A. haemolyticum is susceptible to most classes of antimicrobial drugs, including penicillins, cephalosporins, carbapenems, macrolides, fluoroquinolones, tetracyclines, clindamycin, and vancomycin (Carlson et al. 1994a, 1999; Arikan et al. 1997; Almuzara et al. 2002). General resistance has been reported against trimethoprim/sulfamethoxazole (Carlson et al. 1994a, 1999; Therriault et al. 2008). Although *A. haemolyticum* is susceptible (thus far, universally) to penicillin by in vitro MIC testing, treatment failures have been documented (Nyman et al. 1990; Osterlund 1995; Volante et al. 2008; Bomke et al. 2009). Clinical failures that have been reported were associated with penicillin tolerance (Carlson et al. 1999). Isolates resistant to ciprofloxacin (Vargas et al. 2006), vancomycin (French et al. 1992), macrolides, and quinolones (Carlson et al. 1999) have also been reported.

There are no established guidelines for the treatment of *A. haemolyticum* infections and it is recommended that treatment should be based on in vitro susceptibility profiles of individual strains and on the site of infection (Carlson et al. 1999). Skov et al. (1998) suggest that high doses of parenteral penicillin combined with an aminoglycoside would be preferable to beta-lactam antibiotics in cases of systemic *A. haemolyticum* infection. Theriault et al. (2008) recommend the use of intravenous penicillin or a cephalosporin as first-line pharmacologic management of deep-seated infections caused by this organism, whereas Van der Eerden et al. (2006) found that a macrolide antibiotic was the treatment of choice for a case of pharyngitis caused by *A. haemolyticum*.

Strains of *A. phocae* recovered from marine mammals were 100 % susceptible to amikacin, ampicillin, amoxicillin/clavulanate, cefazolin, ceftiofur, ceftizoxime, chloramphenicol, enrofloxacin, erythromycin, gentamicin, oxacillin, penicillin, rifampin, tetracycline, ticarcillin/clavulanate, and trimethoprim/sulfamethoxazole (Johnson et al. 2003). This suggests that beta-lactam antibiotics should be useful in treating *A. phocae* infections in marine mammals.

Detailed studies on the antibiotic susceptibility of the remaining *Arcanobacterium* species have not been reported.

Genus *Trueperella*

Trueperella are known as opportunistic pathogen of a number of economically important livestock such as domestic animals and pigs, causing a variety of purulent infections involving the skin, joints, and organs (Jost and Billington 2005). Infection is not confined to domestic animals but a number of various animal species including antelopes, bisons, camels, cats, chickens, deer, dogs, elephants, gazelles, horses, macaws, reindeers, turkeys, and wildebeests (Jost and Billington 2005). As a commensal, the source of infections is usually endogenous (Baumann et al. 2001; Dieleman et al. 1989; Lechtenberg et al. 1988; Jonsson et al. 1991; Jost and Billington 2005; Therriault et al. 2008; Volante et al. 2008). Infections can be transmitted from animal to animal, from animals to humans, or even between humans. However, members of different species vary considerably with regard to virulence, types of pathological lesions induced, and host specificity.

Human Infections Caused by *Trueperella* Species

Trueperella pyogenes has not been isolated as a part of the human normal flora (Jost and Billington 2005). It is an infrequent cause of infections in humans, and early reports are plagued by limited details on the microbiological tests performed, raising the possibility of misidentification (Gahrn-Hansen and Frederiksen 1992). Infections are mostly related to patients living in rural areas or had a history of contact with animals (Palmondon et al. 2007; Levy et al. 2009). Severe infections like endocarditis (Palmondon et al. 2007), pneumonia (Hermida et al. 2004), septicemia (Levy et al. 2009), septic arthritis (Lynch et al. 1998), and soft tissue (Kavitha et al. 2010) have been reported.

The role of *Trueperella bernardiae* in human infections has not been clearly established. The organism has been implicated in urinary tract infections (Lepargneur et al. 1998), septic arthritis (Adderson et al. 1998), osteitis (Bemer et al. 2009), and necrotizing fasciitis (Weitzel et al. 2011). The remaining *Trueperella* species appear to occur only in animals.

Animal Infections Caused by *Trueperella* Species

Trueperella pyogenes is an opportunistic pathogen of economically important livestock. As a commensal, the source of *Trueperella pyogenes* infections is often autogenous. Although *T. pyogenes* is capable of acting as a primary pathogen, infection often follows a physical or microbial trauma to the mucous membrane, allowing dissemination of the organism. *T. pyogenes* can cause a variety of suppurative infections involving the skin, joints, testes, and visceral organs. Economically significant disease includes mastitis (Quinn et al. 2002; Unnerstad et al. 2009), endometritis (Williams et al. 2005), liver abscesses (Doré et al. 2007), osteomyelitis (Firth et al. 1987; Bürgi et al. 2001; Martínez et al. 2007), pneumonia (Vogel et al. 2001; Fulton et al. 2009) peritonitis, and pleuritis in cattle, sheep, goats, and swine. *T. pyogenes* was also found to be etiologically involved in pneumonia in deer (Hattel et al. 2004; Tell et al. 2011) and in osteomyelitis in turkeys (Barbour et al. 1991; Brinton et al. 1993) and in chronic otitis externa in cats and canine cystitis (Billington et al. 2002a). It is noteworthy to mention that *T. pyogenes* induces orchitis in experimentally injected ovine (Gouletsou et al. 2004).

Of the remaining *Trueperella* species, *A. bernardiae* has as yet only been isolated from human sources, whereas *T. abortisuis* has been isolated from a sow's placenta after an abortion (Azuma et al. 2009). *T. bialowiezense* and *T. bonasi* were isolated from the prepuce of European bison (*Bison bonasus*) bulls suffering from balanoposthitis (Lehnen et al. 2006).

Potential Virulence Factors of *Trueperella*

A. pyogenes harbors a number of virulence factors that contribute to its pathogenic potential. These factors include pyolysin (PLO), two neuraminidases (NanH and NanP), a collagen-binding protein (CbpA), and fimbriae (Jost and Billington 2005; Pietrocola et al. 2007). Pyolysin (PLO) is a cholesterol-dependent cytotoxin produced by all *T. pyogenes* strains examined to date (Ding and Lämmler 1996; Billington et al. 1997; Jost et al. 1999; Silva et al. 2008) and is detected in culture supernatant as a 55-kDa protein. PLO is cytolytic for a number of cell types including PMNs and macrophages (Jost et al. 1999). It exerts its cytolytic effects through the formation of large pores in eukaryotic cell membranes (Billington et al. 2002a; Jost and Billington 2005). PLO also kills endometrial cells in vitro (Miller 2009) and the tissue damage associated with *T. pyogenes* infection in cows is an important mechanism compromising endometrial health and fertility (Sheldon et al. 2009).

T. pyogenes expresses two cell wall-bound neuraminidases, NanH and NanP. Neuraminidases (sialidases or *N*-acetylneuraminyl hydrolase) remove sialic acid from carbohydrates or glycoproteins. The NanH found in all *T. pyogenes* isolates (Jost et al. 2001), while the NanP is found only in 64.2 % of the examined strains (Jost et al. 2002b). The neuraminidases are important factors in promoting adhesion to host epithelial cells. In addition, the action of neuraminidase can decrease mucus viscosity (Gottschalk 1960), possibly enhancing bacterial colonization of the underlying tissues. Furthermore, neuraminidases impair the host immune response as desialylation increases the susceptibility of mucosal immunoglobulin A (IgA) to bacterial proteases (Frandsen 1994; Reinholdt et al. 1990).

T. pyogenes also expresses a collagen-binding protein, CbpA, which mediates adhesion and promotes colonization of *T. pyogenes* cells to collagen-rich tissues of the host. The mature CbpA protein is 1,124 amino acids long, has a molecular mass of 121.9 kDa, and contains the domain structure typical of microbial surface components recognizing adhesive matrix molecules (MSCRAMM) (Esmay et al. 2003; Pietrocola et al. 2007). CbpA displays 30.9 % identity and 50.4 % similarity to the collagen adhesin (Cna) of *Staphylococcus aureus*. CbpA is present in 48 % of *T. pyogenes* isolates. However, 100 % of turkey osteomyelitis

isolates carry *cbpA* gene, suggesting that CbpA may act as a virulence factor for *T. pyogenes* osteomyelitis (Esmay et al. 2003).

Furthermore, *T. pyogenes* expresses fimbriae, hairlike surface projections which are involved in adhesion to host cells. Four fimbrial biogenesis operons which are required for the expression of fimbriae in *T. pyogenes* are identified (Jost and Billington 2005). One of these operons consists of three genes, *fimB*, *fimA*, and *srtA*, which are similar in genetic organization to those from *Actinomyces naeslundii* type 2. *fimA* encodes a 45.7-kDa protein, FimA, responsible for fimbrial biogenesis, and shows 27 % identity and 41 % similarity to the *A. naeslundii* type 2 fimbrial subunit. fimA and fimB are both carried or neither present in approximately 94 % of examined *T. pyogenes* isolates. In contrast, *srtA* is found in all *T. pyogenes* isolates examined to date, including those lacking fimA and fimB, which suggests that in addition to fimbrial biogenesis, this enzyme may be required for the sorting of other cell surface molecules. Like other bacterial fimbriae, it is assumed that the fimbriae of *T. pyogenes* are involved in adhesion to the host, possibly by binding to fibronectin through FimB. The *T. pyogenes*-specific *fimA* gene is significantly associated with clinical endometritis in cows (Bicalho et al. 2012; Santos et al. 2011).

Antigenic Structure

PLO expressed by *T. pyogenes* is required for bacterial survival in the hosts and, like other thiol-activated cytolysins (TACYs), it is cytotoxic for phagocytic cells (Jost et al. 1999). In addition, PLO is also an important host-protective antigen, as formalin-inactivated, recombinant, His-tagged PLO (HIS-PLO) was shown to be efficacious in mice (Jost et al. 1999). However, the toxicity of PLO limits its usefulness as a vaccine without prior inactivation (Jost et al. 1999). Mutational analysis of the undecapeptide in PLO and other CDCs has identified residues which are critical for cytotoxic activity (Billington et al. 2002b; Korchev et al. 1998; Sekino-Suzuki et al. 1996). Knowledge of the residues critical for toxic activity allowed the design of genetic toxoids, i.e., recombinant toxins with mutations affecting activity, for use as immunoprophylactic agents. Three genetically toxoided, HIS-PLO.F_{497}, HIS-PLO.ΔP_{499}, and HIS-PLO.A_{522}, were found to be nontoxic, and vaccination of the mice with these toxoids protected mice from infection with *T. pyogenes* (Jost et al. 2003). The protective capacity of these vaccines appears to be in the stimulation of a humoral immune response, as passive immunization with anti-PLO goat serum could also prevent *T. pyogenes* infection in the mouse model system (Billington et al. 1997). These results suggest that PLO may be a promising vaccine candidate. However, unlike laboratory mice, healthy domestic animals are naturally colonized with *T. pyogenes* and often have antibodies to the organism and to PLO. Vaccination with whole cell and culture supernatant can increase antibody titers without giving any protection. However, it is uncertain how these neutralizing antibodies in these animals will translate to protection from *T. pyogenes infections*.

Antigens of the other *Trueperella* species have not been studied.

Susceptibility of *Trueperella* to Antibiotics

The antimicrobial susceptibility patterns of *T. pyogenes* vary depending on the host species and the anatomical site from which they were isolated (Cohen et al. 1996; Brooks and Jayarao 2008; Tell et al. 2011). Cohen et al. (1996) observed a difference between susceptible patterns of *T. pyogenes* isolated from bovine udder and bovine uterus to oxytetracycline and trimethoprim–sulfadiazine. They found that most of the isolates that originated from the uterus were resistant to oxytetracycline and trimethoprim–sulfadiazine, whereas udder isolates were sensitive to both drugs. Furthermore, Brooks and Jayarao (2008) found that bovine and porcine isolates of *T. pyogenes* behaved differently to certain antibacterial drugs where more isolates of porcine origin were resistant to oxytetracycline, erythromycin, tilmicosin, and lincomycin as compared to those isolated from cattle.

In a study of antimicrobial resistance in *T. pyogenes* isolated from the uteri of postpartum Holstein dairy cows, Santos et al. (2010) found that there was a broad and variable antimicrobial resistance profile among *T. pyogenes* organisms isolated from bovine uterine fluid. More than 50 % of the total isolates were resistant to amoxicillin, ampicillin, chloramphenicol, florfenicol, oxytetracycline, and penicillin. Malinowski et al. (2011) found that *T. pyogenes* strains isolated from the uteri of cows with metritis/endometritis were susceptible mostly to amoxicillin/clavulanic acid, ceftiofur, and norfloxacin and were resistant or slightly susceptible to oxytetracycline, ampicillin, cloxacillin, rifaximin, and cephapirin. Silva and Lobato (1998) found that in vitro *T. pyogenes* was most sensitive to ampicillin, enrofloxacin, cephalothin, lincomycin, neomycin, penicillin, novobiocin, gentamicin, chloramphenicol, and tetracycline. Sheldon et al. (2004a, b) reported the highest activity of cephalosporins (cefquinome, cephapirin, ceftiofur) and enrofloxacin against *T. pyogenes*.

Although susceptibility standards for human isolates are not available for *T. pyogenes*, Levy et al. (2009) reported the treatment of a case of human sepsis using a combination of ampicillin 6 g/day plus gentamicin 240 mg/day.

Genus *Actinobaculum*

Actinobaculum species are strongly associated with urinary tract infections (UTIs) in swine and human. The spectrum of UTIs primarily ranges from benign cystitis to severe pyelonephritis with urosepsis. *A. suis* is a common cause of cystitis and pyelonephritis in breeding sows in North and South America, Europe, Asia, and Australia. *A. suis* may be transmitted from boars to sows at the time of mating. The source of infections with the human isolates is thought to be endogenous.

Human Infections Caused by *Actinobaculum* Species

Actinobaculum species associated with human infections include *A. massiliense*, *A. schaalii*, and *A. urinale*. It has been reported that they are responsible for numerous urinary tract infections, mainly in elderly patients with underlying urological predispositions (Hall et al. 2003a; Greub and Raoult 2002; Fendukly and Osterman 2005; Reinhard et al. 2005; Bank et al. 2010; Nielsen et al. 2010). A recent study showed that 22 % of 252 urine samples from patients >60 years were positive for *A. schaalii* (Bank et al. 2010). Besides UTI, *A. schaalii* also cause septic complications such as urosepsis (Sturm et al. 2006), bacteremia (Hesstvedt et al. 2006; Gomez et al. 2011), abscess (Tschudin-Sutter et al. 2011), osteomyelitis (Haller et al. 2007), endocarditis (Hoenigl et al. 2010), and Fournier's gangrene (Bempt et al. 2011). It is estimated that *A. schaalii* is associated with an infection—primarily sepsis and abscesses—in 81.5 % of patients (Tschudin-sutter et al. 2011). *A. massiliense* has been reported to cause superficial skin infections (Waghorn 2004).

Animal Infections Caused by *Actinobaculum* Species

A. suis is a well-established veterinary pathogen causing UTI, cystitis, pyelonephritis, and metritis with abortions in sows (Yamini and Slocombe 1988; Walker and MacLachlan 1989; Taylor 2012). In contrast with cystitis caused by other bacteria, infection with *A. suis* resulted in a hematuria and urinary pH values above 8.0 (Liebhold et al. 1995). In acute and severe cases, affected animals die suddenly from acute renal failure.

Potential Virulence Factors of *Actinobaculum*

Larsen et al. (1986) demonstrated that some strains of *A. suis* are heavily fimbriated and adhere to epithelial cells of the porcine bladder where glycoconjugates are specific receptor sites for its attachment. *A. suis* produces the urease enzyme able to cause cleavage of urea into ammonia. The elevated pH, increased from a normal range of 6.0–7.5 to 8.0–9.0, enhances bacterial proliferation and causes an inflammatory reaction of the mucosal surface, inhibiting the growth of competitive microflora and promoting the precipitation of urinary salts and crystals, which in turn increases inflammatory changes in the bladder mucosa and provides a nest for bacterial growth and protection from antibiotics and host defense mechanisms.

Most interesting is the finding that the cell membranes of *Actinobaculum* species are characterized by the presence of choline-containing phosphoglycolipids. These are known as potent virulence factors that participate in the adhesion of pathogenic bacteria to eukaryotic target cells (Rottem 2002). Nevertheless, the exact chemical structure of the choline-containing phosphoglycolipids from *Actinobaculum* is not yet determined and its role in colonization and pathogenesis of *Actinobaculum* species is not examined.

The human pathogen, *A. urinale*, as yet known is the only one which produces the urease enzyme and which may be a functional factor in the pathogenicity of this bacterium.

Antigenic Structure

The 6-deoxyhexoses, rhamnose and 6-deoxytalose, are cell wall constituents of *Actinobaculum* species. Deoxyhexoses are substantial cell wall components of several human pathogens such as *Actinomyces bovis* (MacLennan 1961), *Mycobacterium avium* (MacLennan 1962), and *Aggregatibacter actinomycetemcomitans* (Amano et al. 1989). Because of their presentation on the cell surface, they represent antigenic determinants that contribute to serological specificity (Bishop et al. 1982). However, to date there is no data describing the structure/biological activity of these deoxy sugars and its involvement in the pathogenicity of the genus *Actinobaculum*.

Susceptibility of *Actinobaculum* to Antibiotics

In vitro susceptibility testing showed that all of the type strains of *Actinobaculum* species are susceptible to nearly all β-lactam antibiotics, tetracyclines, vancomycin, linezolid, and clindamycin but are completely resistant to metronidazole (MIC >256 mg/L). All species, except *A. suis* and *A. urinale* (MICs ≥256 mg/L), were highly susceptible to trimethoprim. Although it has been reported that clinical isolates of *Actinobaculum schaalii* are resistant to trimethoprim (Cattoir et al. 2010; Cattoir 2012), MIC determined for the type strain *A. schaalii* CCUG 27420^T indicates that it is susceptible against this antibiotic (MIC <0.002 mg/L). Of the antibiotics tested, fluoroquinolones exhibit variable reactivities. Whereas older fluoroquinolones (i.e., norfloxacin, ciprofloxacin) exhibit weaker antimicrobial effect (MIC 1.5–2 mg/L) against *Actinobaculum* species, newer compounds (i.e., levofloxacin, moxifloxacin) have greater activity (MIC 0.094–0.25 mg/L).

Treatment failure with amoxicillin was reported for patients with chronic UTI due to *A. massiliense* and *A. schaalii* (Greub and Raoult 2002; Reinhard et al. 2005).

Genus *Varibaculum*

Information on the clinical prevalence and pathogenic potential of *Varibaculum cambriense* is inadequate. The organism has been isolated from human abscesses such as brain abscess, postauricular abscess, ischiorectal abscess, submandibular abscess, breast abscesses, cheek abscess, intrauterine contraceptive devices or vaginal swabs, hidradenitis and a fistula, umbilical scar, sebaceous cysts, skin abscess, and hip joint abscess

(Hall et al. 2003c; Chu et al. 2009; A. F. Yassin, unpublished). It was also identified in cultures and tDNA-PCR among the species typically associated with bacterial vaginosis (Verhelst et al. 2005). The organism was always isolated as part of polymicrobial infections associated with other potentially pathogenic bacteria such as *Peptostreptococcus* sp., *Peptoniphilus* sp., *Porphyromonas* sp., *Prevotella* sp., *Propionibacterium* sp., and *Bifidobacterium* sp.

Genus *Mobiluncus*

Mobiluncus species have been associated with several types of infections in humans. They have been isolated, either alone or in concert with other anaerobes, from breast and umbilical abscesses (Glupezynski et al. 1984; Sturn and Sikkenk 1984; Weinbren et al. 1986; Edmiston et al. 1989), blood cultures (Glupezynski et al. 1984; Gomez-Garces et al. 1994; Sahuquillo-Arce et al. 2008), and the chorioamnionic membranes of a placenta at preterm delivery (Hillier et al. 1988). Multivariate analysis of vaginal flora has shown that *Mobiluncus* spp. occur in large numbers in bacterial vaginosis (BV) and may therefore serve as an indicator organism for this clinical syndrome (Spiegel 1995; Hillier et al. 1991). In a study of the prevalence of antibody to *Mobiluncus* among women with and without BV, Moi et al. (1991) found that women with BV had significantly higher titers of IgG antibody than those without BV. Titers of antibody to *M. mulieris* were significantly lower than those to *M. curtisii*. The seroprevalence of IgG antibody to *M. curtisii* was also demonstrated in 75% of a cohort of pregnant females by Schwebke et al. (1996). These results in addition to the ability of *M. curtisii* to escapes phagocytosis more easily as observed in luminol chemiluminescence, indicate that *M. curtisii* is more virulent than *M. mulieris*, and agree with reports of *M. curtisii* found in postoperative and extragenital infections (Moi et al. 1991).

Human Infections Caused by *Mobiluncus* Species

Bacterial Vaginosis (BV)

Bacterial vaginosis (BV) is a polymicrobial, superficial vaginal infection involving a reduction in the amount of hydrogen peroxide-producing lactobacilli and an overgrowth of anaerobic bacteria (Eschenbach 1989). Normally, lactobacilli such as *Lactobacillus crispatus*, *Lactobacillus jensenii*, and *Lactobacillus iners* (Hillier et al. 1993; Antonio et al. 1999; Vásquez et al. 2002; Vallor et al. 2001) constitute 95 % of the bacteria in the vagina. The predominance of lactobacilli helps maintain the ecological balance of the vaginal environment by maintaining a low pH and by preventing overgrowth of other microbes (Aroutcheva et al. 2001). In women with BV, the prevalence of lactobacilli is considerably reduced and the concentration of other bacteria increased by 10^2 to 10^4 (Eschenbach 1989). Microorganisms associated with BV include *Gardnerella vaginalis*, *Bacteroides* sp., *Mobiluncus* species, *Prevotella* species, *Fusobacterium nucleatum*, *Peptostreptococcus* sp., *Ureaplasma urealyticum*, *Mycoplasma hominis*, and a wide variety of other microbes (Krohn et al. 1989). Although most of these organisms are present in small numbers in the normal vagina, *Mobiluncus* is rarely found in the normal vagina and has been considered as a sensitive marker for the diagnosis of BV. *Mobiluncus* has been reported to be found in up to 50 % of women with BV (Spiegel et al. 1983b; Hallén et al. 1987; Thomason et al. 1984b; Hillier et al. 1991; Tohill et al. 2004). The association between *Mobiluncus* spp. and a positive amine odor after KOH preparation ("whiff test") has been reported (Hallén et al. 1987; Pereira et al. 2005; Nyirjesy et al. 2007). Despite an increasing understanding of the microbiology of BV, the etiologic agents remain unknown.

The most common BV symptoms that resulted from the shift in vaginal flora are a malodorous discharge and itching (Amsel et al. 1983). The discharge results in part from degradation of the normal vaginal mucin gel, which is efficiently performed by mucin-degrading enzymes produced by BV-associated bacteria (Olmsted et al. 2003). The odor, usually described as "fishy," is derived from volatilization of the amines produced by the metabolism of anaerobic bacteria. Chen et al. (1979) found methylamine, isobutylamine, putrescine, cadaverine, histamine, tyramine, and phenethylamine in the vaginal discharge of patients with symptoms of nonspecific BV. The profound increase in *Mobiluncus* concentrations is characterized by enhanced production of volatile amines (Cruden and Galask 1988).

Diagnosis of BV Currently the Amsel criteria and the Nugent score (NS) are the most commonly used methods for BV diagnosis. Clinical diagnosis of BV, according to Amsel's clinical criteria, is based on the presence of three of the following four criteria: an elevated (>4.5) vaginal pH, release of a fishy odor on addition of 10 % KOH to the vaginal fluid, an abnormal discharge that is thin and homogenous, and clue cells in the vaginal fluid (Amsel et al. 1983). An increase in pH rapidly releases amines such as trimethylamine (TMA), which are dissolved in the discharge as amino acids when the pH is low. TMA is responsible for the smell of fishy odor and can be detected in vaginal specimens using the sniff test/whiff test by adding one drop of 10–20 % KOH to the discharge. Clue cells are epithelial cells of the vagina whose borders are difficult to see in wet mount microscopic preparation because so many bacteria are found on the surface of the cells. It has been reported that the presence of *Mobiluncus* morphotypes with Gram stain may be associated with higher numbers of clue cells and positive "whiff" tests (Pereira et al. 2005).

Direct Gram stain of vaginal fluids has been used to diagnose BV (Spiegel et al. 1983a; Nugent et al. 1991). For the interpretation of Gram-stained smears, Nugent et al. (1991) proposed a scoring system that is based on the observation of three morphotypes to create a total score of zero to ten. These three morphotypes are large Gram-positive rods (*Lactobacillus*), small Gram-negative or Gram-variable rods (*Bacteroides* or *Gardnerella*), and curved Gram-negative to Gram-variable rods (*Mobiluncus* spp.). The total scores were computed by adding the weighted quantitation (0 to 4+) of the three morphotypes. A score of 0–3 is considered normal flora,

a score of 4–6 is classified as intermediate flora, and a score of 7–10 is consistent with BV. Today, the Nugent scoring is considered as the gold standard for laboratory diagnosis of BV and is employed in many research studies.

Molecular methods have been recently used for the detection and quantification of BV-associated bacteria to improve the diagnosis of BV (Fredricks et al. 2007; Menard et al. 2008, 2010; Obata-Yasuoka et al. 2002; Schwebke and Lawing 2001; Zariffard et al. 2002). Generally, good agreement and high sensitivity and specificity have been reported for PCR-based assay in comparison to standard methods. Schwebke and Lawing (2001) used PCR to characterize *Mobiluncus* spp. in women with BV and a control group of women without BV as determined by Nugent scoring. The authors detected *Mobiluncus* by PCR in 84.5 % of women with BV and in 38 % of women without infection. *M. curtisii* was rarely detected in the latter group, though it was found in 65.3 % of women with bacterial vaginosis. The sensitivity and specificity of Gram stain compared with PCR were 46.9 % and 100 %, respectively. In a multiplex PCR assay for diagnosing BV via quantifying *Mobiluncus mulieris*, *Mobiluncus curtisii*, *Bacteroides fragilis*, and *G. vaginalis* from vaginal swabs in pregnant and nonpregnant women, Obata-Yasuoka et al. (2002) reported 78.4 % and 95.6 % sensitivity and specificity, respectively, for multiplex PCR in comparison to the Nugent score. Fredricks et al. (2007) detected *Mobiluncus curtisii* by PCR in 55.6 % of subjects with BV with a specificity of 93 % and *Mobiluncus mulieris* in 22.2 % of subjects with BV with a specificity of 100 %. PCR assay performance for the *Mobiluncus* species did not improve when the Nugent criteria were used to define BV.

Furthermore, several studies have investigated the utility of quantitative PCR (qPCR) as a diagnostic tool for BV. Sha et al. (2005) demonstrated that qPCR for *Gardnerella vaginalis*, *Mycoplasma hominis*, and lactobacilli significantly correlates with the Nugent Gram stain method to diagnose BV. The authors found that women with BV diagnosed by Nugent score have significantly higher numbers of *G. vaginalis* and *M. hominis* and significantly lower numbers of lactobacillus organisms than women without BV. The sensitivity and specificity of the method were 83 % and 78 %, respectively, in comparison with Nugent score. The specificity and sensitivity of the method for *G. vaginalis* and *M. hominis* and decreasing levels of *Lactobacillus* spp. were significantly associated with BV with a sensitivity of 83 % and 87 % when compared to Nugent score. Furthermore, Menard et al. (2008) found that the molecular quantification of two microorganisms, *Atopobium vaginae* and *G. vaginalis*, had excellent sensitivity (96 %) and specificity (99 %) when compared with the Nugent Gram stain classification of BV. Although the study represents a major step toward a molecular diagnostic test for BV, the generalizability of the results to other populations needs to be established before widespread application of this method is advocated.

Complications Associated with Bacterial Vaginosis Bacterial vaginosis is an important public health problem that is characterized by abnormal changes in the vaginal flora (Koumans and Kendrick 2001). Numerous reports have hightend the association between bacterial vaginosis and serious medical complications in many gynecologic conditions, such as pelvic inflammatory disease (Faro et al. 1993), endometritis (Abner et al. 1998), amniotic fluid infection (Hillier et al. 1988), preterm labor and delivery (Eschenbach et al. 1988; Kurki et al. 1992; Meis et al. 1995), premature rupture of the fetal membranes (Krohn et al. 1993), miscarriage (Hay et al. 1994), and spontaneous abortion (McGregor et al. 1995). Pregnant women with BV are also at increased risk urinary tract infections (Sharami et al. 2007). Moreover, there is now evidence linking bacterial vaginosis to an increased susceptibility to acquisition of other sexually transmitted diseases (STDs), including HIV infection (Hillier 1998; Royce et al. 1999; Taha et al. 1999; Schmid et al. 2000; Sobel, 2000; Cu-Uvin et al. 2001). The enhanced susceptibility to STDs and HIV infection among women with bacterial vaginosis proposed to be related to decreased amounts of H_2O_2-producing lactobacilli in women with bacterial vaginosis (Eschenbach et al. 1989; Hillier and Holmes, 1998a, b). Most women with bacterial vaginosis have a relative decrease in lactobacilli concentration, and some have a complete absence of lactobacilli (Agnew and Hillier 1995; Hillier and Holmes 1998a). H_2O_2 is toxic to many microorganisms at concentrations that are readily achievable in the vaginal fluid and, thus, provides an intrinsic protective mechanism in the vaginal compartment (Eschenbach et al. 1989; Hillier and Holmes, 1998a; Klebanoff and Coombs 1991). The presence of BV also increases the risk of infection with herpes simplex virus type 2 (HSV-2) (Cherpes et al. 2005), acquisition or reactivation of human papillomavirus (HPV) (da Silva et al. 2004) as well as infection with gonorrhea and *Trichomonas vaginalis* and *Chlamydia trachomatis* (Wiesenfeld et al. 2003).

Extragenital *Mobiluncus* Infections

Although *Mobiluncus* species are seen with relative frequency in the genital tract, they are uncommonly isolated from nongenital sites, and reports on their involvement in extravaginal infections in humans are increasingly recognized (Glupezynski et al. 1984; Mayer et al. 1994; Sahuquillo-Arce et al. 2008; Sherlock et al. 2005; Sturm 1989; Weinbren et al. 1986). *Mobiluncus* have been isolated, either alone or in concert with other anaerobes, from several cases of breast and umbilical abscesses (Glupezynski et al. 1984; Sturn and Sikkenk 1984; Sturm 1989; Weinbren et al. 1986; Edmiston et al. 1989), from the chorioamnionic membranes of placenta at preterm delivery (Hillier et al. 1988), and have been found to be associated with septicemia (Glupezynski et al. 1984; Gomez-Garces et al. 1994; Mayer et al. 1994; Hill et al. 1998; Sahuquillo-Arce et al. 2008). *M. mulieris* has been reported as a copathogen with *Haemophilus aphrophilus* in a case of liver abscess (Sherlock et al. 2005). These infections outside of the reproductive tract could indicate that *Mobiluncus* is potentially pathogenic for human.

Potential Virulence Factors of *Mobiluncus*

Mobiluncus species possess several factors that contribute to the impairment of host defense component of the cervicovaginal mucus. *Mobiluncus*, *G. vaginalis*, and other BV-associated organisms produce a host of enzymes such as sialidase, prolidase (proline aminopeptidase), and mucinase (McGregor et al. 1994; Thomason et al. 1988). Sialidases and prolidases are potentially able to degrade several key mucosal protective factors, such as mucins, cytokines, immunoglobulins, antimicrobial molecules, and host cell receptors (Cauci et al. 1998, 2008). The combined action of different hydrolytic enzymes like sialidase and prolidase can dysregulate several crucial host antimicrobial/immune responses, creating a local immunosuppression. In addition, the observation that clue cells (the main hallmark of BV) are positively correlated to microbial enzymes suggests that sialidase and prolidase contribute to exfoliation and detachment of vaginal epithelial cells, which are major effectors of vaginal innate immune responses (Valore et al. 2002, 2006; Wira et al. 2005). Furthermore, *Mobiluncus* may contribute to the pathogenesis of BV infections by direct inhibition of neutrophil function. It is well known that *Mobiluncus* species produce succinic, acetic, and lactic acids as major metabolic by-products. In vitro studies demonstrated that increased succinic acid dramatically impairs neutrophil phagocytic killing, response to chemotactic stimuli, and generation of respiratory bursts required for bacterial killing (Rotstein et al. 1988).

Nevertheless, in a study of the adherence capability, biofilm-forming potential, and cytotoxic activity of *Gardnerella vaginalis* and other BV-associated anaerobes, Patterson et al. (2010) showed that *M. mulieris* and other BV-associated anaerobes were neither able to adhere to ME-180 vaginal epithelial cells nor to display cytotoxic activity under the conditions used. Only *G. vaginalis* and *Peptonophilus* sp. form a dense biofilm, but *M. mulieris* and none of the other BV-associated anaerobes formed biofilms. Together, these findings cast doubt on the role of *Mobiluncus* in the pathogenesis of BV. However, *Mobiluncus* is one important component of the complex bacterial community of BV, a polymicrobial infection in which no one species alone is capable of causing disease, but rather the synergistic effects of the consortium give rise to the pathophysiology (Dowd et al. 2008).

Susceptibility of *Mobiluncus* to Antibiotics and Recommended Treatment

The susceptibility of *Mobiluncus* species to antimicrobial agents have been investigated in several studies (Carlone et al. 1986; Spiegel 1987; Puapermpoonsiri et al. 1997; Gatti 1999; Bahar et al. 2005). All *Mobiluncus* strains were susceptible to amoxicillin, chloramphenicol, clindamycin, penicillin G, amoxicillin/clavulanic, piperacillin/tazobactam, cefotaxime, cefoxitin, cefotetan, erythromycin, imipenem, meropenem, rifampin, tobramycin, and vancomycin. All *M. curtisii* strains and some *M. mulieris* strains have been reported to be resistant to metronidazole. Some strains of *M. curtisii* subsp. *holmesii* were resistant to tetracycline (Gatti 1999). Interestingly, among Gram-positive bacteria, some strains of *M. curtisii* carried one or two of the tetracycline resistance genes, *tetQ* and *tetO*, which encode proteins that protect the bacterial ribosomes from tetracycline both in vivo and in vitro and are often associated with conjugative transposons (Leng et al. 1997).

Recommended treatments for BV in both pregnant and nonpregnant women include application of antimicrobial agents such as metronidazole and clindamycin (Joesoef et al. 1999; Koumans et al. 2002). Either systemic (usually oral) or intravaginal antibiotics can be used to treat BV (Mikamo et al. 1997; Paavonen et al. 2000; Sobel et al. 2001). Although metronidazole is usually effective in treating women with BV leading to resolution of symptoms, high recurrence rates of the disease have been reported (Bradshaw et al. 2006; Hay 1998). Clindamycin reduces the presence of vaginal *Mobiluncus* to a greater extent than metronidazole; this correlates with a higher BV cure rate (Nyirjesy et al. 2007).

Probiotics Treatment

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a beneficial health effect on the host (Senok 2005). Probiotics have been a more recent approach for treatment of bacterial vaginosis. The dominance of lactobacilli in healthy vaginal microbiota and its depletion in BV has given rise to the concept of oral or vaginal instillation of probiotic *Lactobacillus* strains for the management of this condition. Available evidence indicates that certain strains of lactobacilli when administered to patients can colonize the vagina and reduce the risk of BV (Reid et al. 2001). Several studies have shown high cure rate of BV using a combination of metronidazole treatment along with administration of probiotics (Anukam et al. 2006; Martinez et al. 2009). The success of this approach has been attributed to the observation that metronidazole treatment decreases the numbers of pathogenic bacteria while leaving the population of lactobacilli unaffected, thereby allowing the lactobacilli to recolonize the vagina. Studies have been carried out to assess the efficacy of single strain or cocktail of probiotics administered orally or intravaginal in the treatment of BV (Falagas et al. 2007). In addition, the effect of probiotics in conjunction with antibiotic regimen has also been evaluated. Lactobacilli probiotics can be used over a long time without adverse effects, making them an attractive option to antibiotics, particularly in addressing the problem of high recurrence rates.

Application

The main attraction of using members of the family *Actinomycetaceae* centers around their mode of metabolism and the formation of fermentation products which could be of biotechnological importance.

Bioremediation

Actinomyces species are promising candidates for bioremediation of spilled oil. The detection of *Actinomyces* sp. among the bacteria found in soil samples taken from oil-polluted field along a concentration gradient of sulfurous oil and from unpolluted soil treated with dibenzothiophene (DBT)-containing petroleum suggests that they are likely to play a role in hydrocarbon degradation processes in soils (Duarte et al. 2001). Further, the successful isolation of three strains of *A. naturae* (Bowman et al. 2006; Rao et al. 2012) from chlorinated solvent-contaminated groundwater at Petro-Processors of Louisiana (USA) and their ability to grow in the presence of high concentrations of chlorinated (e.g., 1,2-dichloroethane, 1,1,2-trichloroethane, tetrachloroethane) and non-chlorinated solvents (e.g., toluene) has potentially important implications for cleanup at contaminated sites.

Genus I. *Actinomyces* Harz 1877, 133[AL], emend. Georg, Pine and Gerencser 1969, 292[VP].

Ac.ti.no.my'ces. Gr. fem. n. *aktis*, *aktinos*, ray; Gr. masc. n. *mykes*, fungus; M.L. masc. n. *Actinomyces*, ray fungus referring to the radial arrangement of filaments in *Actinomyces bovis* sulfur granules.

Taxonomy, Historical and Current

Cohn (1875) observed a filamentous branched organism in stained preparations of concrements taken from an infected lacrimal duct of a patient. He gave this organism the name "*Streptothrix foersteri*." Thereafter, Bollinger (1877) focused on an organism with branching mycelia in a pathological specimen obtained from the lesions within the jaw bones of cattle and he used the term "actinomycosis" to refer to the disease. In the same year, Harz (1877) named this organism, observed in a pathological specimen submitted to him from Bollinger, the "ray fungus" or *Actinomyces bovis* to refer to the raylike appearance of the organism in the granules that characterize the lesion. No pure culture was obtained. In the following year James Israel (1878), independently of Bollinger and Harz, characterized a similar "fungus" in a human clinical case and he gave an accurate description of the disease, but he, too, failed to cultivate the organism. Later Bujwid (1889) succeeded in isolating and culturing this human pathogen. The cultural characteristics and the anaerobic peculiarity of the human pathogen were described in considerable detail by Wolff and Israel (1891). The name "*Streptothrix israeli*" was applied by Kruse (1896) to the organism isolated by Wolff and Israel. However, the generic name "*Streptothrix*" was not valid as it had been preempted by Corda (1839) for a true fungus designated as "*Streptothrix fuca*." In 1898 Lachner-Sandoval completed a dissertation on the "ray fungi" and applied the name *Actinomyces israelii* Kruse to the isolates of Wolff and Israel. Lachner-Sandoval (1898) was the first to unite *Actinomyces* with the genera *Mycobacterium* and *Corynebacterium* (Lehmann and Neumann 1896) into a family Actinomycetes. This name has latter been altered to *Actinomycetaceae* by Buchanan (1918).

Breed and Conn (1919), who have revised the question of nomenclature very carefully, concluded that *Actinomyces* Harz (1877) is the only valid generic name. Winslow et al. (1920) approved the genus name *Actinomyces* and they designated *A. bovis* as the type species of the genus. This was supported by Lessel (1960) in his extensive review and *A. bovis* has been included in each edition of *Bergey's Manual*, including the eighth edition (Slack 1974). However, a type strain for *A. bovis* has not been proposed. For many years it was believed that *Actinomyces bovis* (Harz 1877), isolated from lumpy jaw of cattle, and *A. israelii* (Kruse 1896) Lachner-Sandoval (Breed et al. 1957), isolated from human actinomycosis, were one single species. However, studies by Erikson (1940, 1949), Thompson (1950), and Pine et al. (1960b) provided morphological and biochemical evidence that there are two agents of actinomycosis: *A. bovis*, usually associated with bovine infection, and *A. israelii*, the common cause of human infections. Division of the agents of actinomycosis into several species has received further support from cell wall analyses by Cummins and Harris (1958), serological studies by King and Meyer (1963), and electron microscopy studies by Overman and Pine (1963). Since type strains for *A. bovis* and *A. israelii* were not designated and because none of the strains on which the original descriptions of these organisms were based upon were extant, Slack and Gerencser (1976) defined strains ATCC 43683[T] and ATCC 12102[T] as the neotype strains of *A. bovis* and A. *israelii*, respectively.

Later, Thompson and Lovestedt (1951) described strains of *Actinomyces* which grew in ambient air on primary isolation. For this group, they proposed the name *Actinomyces naeslundii*. Howell et al. (1959) provided a more precise definition and delineated this group from other *Actinomyces* species. Subsequently, a Gram-positive, catalase-positive, filamentous organism isolated from subgingival plaque in hamsters was described by Howell (1963) and Howell and Jordan (1963). This organism was later named "*Odontomyces viscosus*" (Howell et al. 1965) and then renamed as *Actinomyces viscosus* by Georg et al. (1969) after the generic description of *Actinomyces* was broadened to include catalase-positive organisms.

Rapid progress in molecular biology methods have led to the recognition of several new *Actinomyces* species. Currently, the genus includes 41 species of Gram-positive, mainly facultatively anaerobic or microaerophilic rods with various degrees of branching. *Actinomyces gerencseriae* was named for strains previously classified as *A. israelii* serotype 2 (Johnson et al. 1990). The two species are also distinct in their 16S rRNA sequences (Stackebrandt and Charfreitag 1990). *A. naeslundii* was heterogeneous, as shown by DNA–DNA relatedness data (Johnson et al. 1990), immunological studies (Putnins and Bowden 1993), genomic DNA fingerprinting, and rRNA gene probes (Bowden et al. 1993). There were 3 genetically distinct groups that are sufficiently different to be designated as separate species. Genospecies 1 comprised *A. naeslundii* serotype 1 strains; genospecies 2 included strains previously classified as *A. naeslundii* serotypes 2 and 3, *A. naeslundii* serotype NV, and *A. viscosus* serotype 2 strains. Genospecies 3 comprised *A. naeslundii* serotype WVA 963 (previously serotype 4).

The identification of these genospecies had been problematic because neither 16S rRNA gene sequencing nor biochemical tests discriminate between these three genospecies. However, Henssge et al. (2009) demonstrated that the partial sequences of two housekeeping genes (*atp*A and *met*G) can separate the three *A. naeslundii* genospecies. They proposed the name *A. oris* for *A. naeslundii* genospecies II and the name *A. johnsonii* for *A. naeslundii* genospecies III (genospecies WVA 963). In this classification, *A. naeslundii* genospecies I remains as *A. naeslundii*.

The descriptive part of novel *Actinomyces* species proposals described recently, though useful for identification, appears somewhat monotonous. These have been mainly based on morphological and physiological grounds as well as on a 16S rRNA gene sequence-based phylogeny. Unfortunately, little attention has been paid to investigate the chemotaxonomic markers which are important for delineating taxa. After several revisions some former members of the genus *Actinomyces*, e.g., "*A. bernardiae*," "*A. pyogenes*," and "*A. suis*," have been moved to the closely related genera *Arcanobacterium*, *Actinobaculum*, and *Trueperella* (Lawson et al. 1997; Pascual Ramos et al. 1997a; Yassin et al. 2011). Therefore, the genus *Actinomyces*, in its state, appears as merely a collection of phylogenetic related species, and it is in need of a comprehensive chemotaxonomic study of its members in order to confirm their assignment to the genus.

Molecular Analyses

Similarity values of 16S rRNA gene sequence analyses for members of the genus *Actinomyces* range from 88 % to 99 %, indicating the presence of several phylogenetically defined clusters (*Fig. 4.1a, b*), which are separated by intra-cluster similarity values ranging from 88–90 % to 89–92 %. The idea that the genus *Actinomyces* may not represent a monophyletic taxon has been expressed before by Pascual Ramos et al. (1997b). Because of the lack of phenotypic and chemotaxonomic properties that support a taxonomic dissection of the genus, the genus serves as a phylogenetic dumping ground. Chemotaxonomic properties, demonstrated to correlate well with phylogenetic structure in other actinobacterial genera, are either not available or not exclusive in phylogenic clusters. Although tempting, a purely phylogeny-based dissection is not favored, as novel sequences may blur the present topology of the genus.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that members of the genus *Actinomyces* resolved in two major phylogenetic clusters along with four separate lineages (*Fig. 4.1a, b*). The clusters were designated as cluster I (*Actinomyces bovis* group), comprising 21 species, and cluster II (*Actinomyces meyeri* group) which contains 12 species. Three of the four separate lineages are represented by two species each and are designated according to the species they include: *A. hordeovulneris–A. nasicola* lineage, *A. hongkongensis–A. marimammalium* lineage, and *A. coleocanis–A. europaeus* lineage. The fourth lineage consists of only one species, *A. neuii*, which cluster with the monospecific genus *Varibaculum* and designated *A. neuii–Varibaculum* lineage. These groupings were assigned under the consideration that all members of a particular group or lineage are monophyletic, showing the same topology regardless of the method used: neighbor-joining, maximum-parsimony, or maximum-likelihood, and on the presence of group or lineage-specific signature nucleotides. However, the level at which members of the two groups and the separate lineages are considered taxonomically coherent entities should be decided on the basis of the results of chemotaxonomic investigations.

Description of the Phylogenetic Groups and Lineages

Cluster I (*Actinomyces bovis* Group)

This cluster, including 21 species which share 87.1–98.1 % sequence similarity, can be considered authentic *Actinomyces* species as it contains the type species *A. bovis*. This species is closely related to *A. urogenitalis* (97.3 % similarity) as originally pointed out in the description of this species (Nikolaitchouk et al. 2000) and to *A. weissii* (97 % similarity). Likewise, the similarity values between *A. johnsonii*, *A. naeslundii*, *A. oris*, and *A. viscosus* vary between 96.3 and 98.1 %, indicating that these organisms may be strains of the same species (Stackebrandt and Goebel 1994). However, the results of a DNA–DNA hybridization study (Johnson et al. 1990) and sequence analysis of two housekeeping genes (*atp*A and *met*G) (Henssge et al. 2009) confirmed the identification of these organisms as separate species. The latter four species form a subcluster supported by a bootstrap value of 89 %. The group-specific signature nucleotides (*Table 4.1*) include 146–176 (U-G), 1118–1155 (U-A), 1122–1151 (G-C), and 1311–1326 (U-A).

The distinct phylogenetic position of *A. graevenitzii* sharing less than 93 % 16S rRNA gene sequence similarity with other species of the genus has been verified in several studies (Pascual Ramos et al. 1997b; Nikolaitchouk et al. 2000; Hoyles et al. 2001, 2002a). In all the dendrograms generated, *A. graevenitzii* constituted either the deepest branch or it branched off even deeper, giving the appearance of an isolated phylogenetic lineage within *Actinomyces*. Although *A. graevinitzii* shares with *Actinomyces bovis* group the entire signature nucleotide characteristic of this group, it possesses a set of distinctive signature nucleotides (*Table 4.1*) which is not found elsewhere in the genus. These include 145–177 (U-G), 446–488 (G-C), 450–483 (C-G), 668–738 (U-A), 669–737 (A-U), 835–851 (A-U), and 1308–1329 (U-A). The phylogenetic distinctness clearly indicates that *A. graevinitzii* might represent an individual distant lineage. Further evidence is required to resolve its evolutionary history and taxonomic placement.

Cluster II (*Actinomyces meyeri* Group)

This cluster comprises 12 *Actinomyces* species with an overall 16S rRNA gene sequence similarities ranged from 91.1 % to

97.2 % and a high bootstrap support (83 %). Within this cluster, *A. georgiae*, *A. meyeri*, and *A. odontolyticus* form a subcluster with 88 % bootstrap support (Fig. 4.1b). The three species have very similar host ranges as they all were isolated from human supra- and subgingival plaque. They showed sequence similarity values ranging from 96 % to 97.2 %. The separate species status of the three species has been confirmed by low DNA–DNA hybridization values (Johnson et al. 1990).

A. canis occupied an isolated position at the periphery of cluster II, showing a distant relationship to member of this cluster. The 16S rRNA gene sequence similarity values found between *A. canis* and members of this cluster ranged between 91.3 % and 95.3 %. Its separate phylogenetic placement in a basal position to cluster II organisms (Fig. 4.1b) is in accord with the original description of Hoyles et al. (2000). The most striking feature that distinguishes the 16S rRNA gene sequence of *A. canis* from other members of cluster II is a stretch of base pairs at positions 614–626 (A-U), 615–625 (C-G), and 616–624 (A-U).

A. naturae, isolated from oil-contaminated groundwater (Rao et al. 2012), clustered with the pathogenic species *A. radingae*. They are two remotely related species sharing 91.8 % similarity. Their association was supported by a moderate bootstrap value (76 %). *A. naturae* shared sequence similarities ranging from 91.3 % to 94.7 % whereas *A. radingae* showed 91.1–93.3 % with other members of cluster II. Additional pairs of phylogenetic neighbors are *A. suimastiditis* and *A. hyovaginalis* (96.7 % similarity) and *A. cardiffensis* and *A. turicensis*, (96.5 % similarity). The group-specific signature nucleotides (Table 4.1) include 146–176 (G-Y), 316–337 (U-G), 444–490 (C-G), 450–483 (C-G), 1118–1155 (C-G), 1122–1151 (A-U), and 1311–1326 (G-C).

Individual Lineages

These lineages could be regarded as candidate divisions.

A. coleocanis–A. europaeus Lineage

The two species share a remote relationship of 93.2 % sequence similarity. Although the association of the two species is supported by all tree methods and high bootstrap support (100 %), the branching point of this lineage is not settled, as seen by low bootstrap values and different affiliations of either one or both species in dendrograms previously published in species descriptions (Funke et al. 1997a; Collins et al. 2000; Hoyles et al. 2000, 2002a; Nikolaitchouk et al. 2000). The presence of unique 16S rRNA gene nucleotide signatures at positions 146–176 (U-A), 501–544 (U-A), 502–543 (A-U), and 929–1388 (A-U) highlights the separate phylogenetic position of this lineage.

A. neuii–Varibaculum Lineage

Unlike other *Actinomyces* species, *A. neuii* shares a common node in the phylogenetic tree with the genus *Varibaculum* to form a separate clade (Fig. 4.1a, b). This clade was strongly supported by a 98 % bootstrap score. This clade represents the deepest branching within the family *Actinomycetaceae*. The phylogenetic placement of this lineage sister to the *A. coleocanis–A. europaeus* lineage is poorly supported (61 % bootstrap value). The level of sequence similarity of *A. neuii* and *Varibaculum cambriense* is low (89.8 % sequence similarity), whereas the overall sequence similarity values of *A. neuii* with other *Actinomyces* species ranged from 85.6 % to 90.4 %.

A. hordeovulneris–A. nasicola Lineage

A. hordeovulneris and *A. nasicola* are remotely related species sharing a similarity value of 93.8 %. The phylogenetic placement of *A. hordeovulneris* sister to *A. nasicola* is poorly supported (65 % bootstrap value). The similarity values of *A. hordeovulneris* with other *Actinomyces* species range between 85.8 % and 92.5 % and that of *A. nasicola* with other member of the genus range between 87.5 % and 92.5 %. There are no group-specific signature nucleotides that would support the association of members of this lineage (Table 4.1). The inclusion of new sequences corresponding to new taxa will help to clarify the taxonomic placement and the phylogenetic relationships of the two species.

A. hongkongensis–A. marimammalium Lineage

Phylogenetic analysis indicated that *A. hongkongensis* has a close affinity with *A. marimammalium* and both species form a clade that is moderately supported by bootstrap analysis (79 %). The two species share 93.3 % 16S rRNA gene sequence similarity. In the neighbor-joining tree, this lineage seems to have diverged prior to the main radiation that gave rise to *Mobiluncus*, *A. coleocanis–A. europaeus* lineage, *A. neuii–Varibaculum* lineage, and cluster II of *Actinomyces*. The similarity values of *A. hongkongensis* and *A. marimammalium* with other *Actinomyces* species range between 86.6–91.6 % and 86.8–91.9 %, respectively. The set of signature nucleotides (Table 4.1) support recognition of the isolated lineage: 146–176 (G-U), 590–649 (G-U), and 1244–1293 (C-G).

Phenotypic Analyses

Morphology

Members of the genus *Actinomyces* demonstrate considerable variations in both cellular and colonial morphologies so that morphology becomes insignificant as basic taxonomic criterion in the genus. They may resemble nocardiae, bifidobacteria, corynebacteria, propionibacteria, and even streptococci.

Cellular Morphology

Generally *Actinomyces* cells are Gram-positive, non-acid-fast, non-spore-forming, and (with the exception of *A. naturae*) nonmotile. The cellular morphology may vary from species to species and strain to strain within one species. In *A. bovis* cells appear straight to slightly curved (diphtheroid-shaped) rods (Fig. 4.4); in some species such as *A. bowdenii, A. canis,*

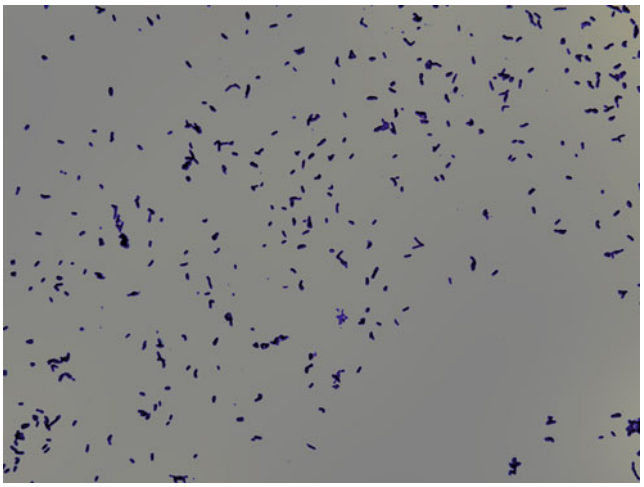

Fig. 4.4
Gram-stained smear from culture of *Actinomyces bovis* ATCC 13683^T on brain heart infusion agar supplemented with 1% Tween 80 showing diphtheroid froms.

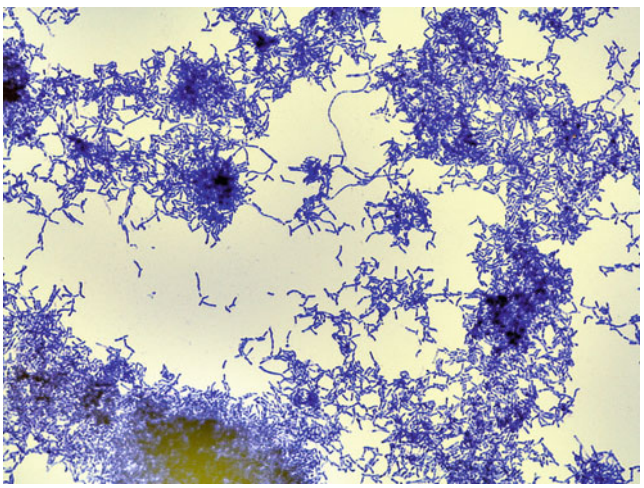

Fig. 4.5
Gram-stained smear from culture of *Actinomyces israelii* ATCC 10048 on CC agar showing branching filamentous forms which stained irregularly given a beaded appearance.

A. catuli, *A. coleocanis*, *A. funkei*, and *A. marimammalium*, curved rods may show branching, which in the case of *A. georgiae* and *A. graevenitzii* have swollen ends. *A. marimammalium*, sometimes with swollen ends in *A. georgiae* and *A. graevenitzii*. In few species, the rods develop into filamentous, beaded, branching forms, e.g., *A. cardiffensis* and *A. dentalis*. Yet other species, e.g., *A. howellii*, *A. radicidentis*, *A. radingae*, *A. nasicola*, *A. neuii*, and *A. vaccimaxillae*, may exhibit coccoid or coccobacillary forms. Moreover, some species are characterized by the production of nonseptate mycelia which are long and exceedingly branched, e.g., *A. israelii* (Fig. 4.5), *A. hordeovulneris*, and *A. viscosus*. Notably, the mycelia may have swollen termini as in *A. hordeovulneris*.

Gross Morphology

For most part, the gross morphology reflects the color, size, and shape of colonies. Variations in gross colonial morphology depend on the species and are affected by the nutrient content of the medium. Most of the *Actinomyces* species colonies appear white, grayish white, or creamy white. However, some species produce red to pinkish colonies. The red pigmentation of mature *Actinomyces odontolyticus* colonies is a well-known distinguishing characteristic of this species. Mature colonies of *A. cardiffensis* are creamy to pinkish, whereas colonies of *A. bowdenii*, *A. europaeus*, *A. funkei*, *A. hominis*, *A. marimammalium*, *A. nasicola*, and *A. radingae* are grayish in appearance. Colonies may be flat or convex, circular, entire edged or irregular, smooth or dry to viscous, shiny, and opaque. Only few species such as *A. europaeus*, *A. georgiae*, *A. howellii*, *A. meyeri*, *A. odontolyticus*, and *A. slackii* produce translucent colonies.

Several species produce pinpoint colonies, e.g., *A. dentalis*, *A. hongkongensis*, *A. marimammalium*, *A. massiliense*, *A. meyeri*, *A. nasicola*, *A. oricola*, and *A. timonensis*. Molar-toothed colonies are rough colonies common in *A. israelii*, *A. gerencseriae*, and *A. hordeovulneris*. Bread crumb-like colonies are prevalent in *A. israelii*, *A. gerencseriae*, *A. hordeovulneris*, *A. dentalis*, and *A. oricola*. Well developed molar-tooth and bread crumb-like colonies are very hard pitting the agar and adhere firmly to the medium. The molar-toothed colonies have tendency to shift to the buttery less adherent bread crumb-like type.

Biochemical Characteristics

Aside from the morphological variations, *Actinomyces* species vary also in the physiological aspects. They are chemoorganotrophs with complex nutritional requirements and a fermentative metabolism. Some species are catalase-positive; others are catalase-negative. Nitrate reduction is variable among species. They are constantly negative for the production of indole, H_2S, and acetoin as well as for blood hemolysis (exception *A. weissii*) and gelatin hydrolysis.

Members of the genus *Actinomyces* vary considerably with respect to their oxygen requirements (Tables 4.4 and 4.5). Strict anaerobic *Actinomyces* species show scant to no growth when incubated in air or in 5 % CO_2 and include *A. bovis*, *A. cardiffensis*, *A. georgiae*, *A. gerencseriae*, *A. hordeovulneris*, *A. hongkongensis*, *A. israelii*, *A. meyeri*, *A. nasicola*, *A. oricola*, *A. ruminicola*, and *A. vaccimaxillae*. Facultative anaerobic or rather capnophilic *Actinomyces* species (all others) showed significantly enhanced growth after 48 h incubation in the presence of 5 % CO_2. It is worth mentioning that some species of the latter group are also able to grow on the surface of suitable agar media after prolonged incubation in air without CO_2. These species could therefore be considered as aerotolerants, e.g., *A. bowdenii*, *A. canis*, *A. catuli*, *A. coleocanis*, *A. europaeus*, *A. funkei*, *A. graevenitzii*, *A. hyovaginalis*, *A. marimammalium*, *A. naturae*, *A. neuii*, *A. odontolyticus*, *A. naeslundii* sensu stricto, *A. oris*,

Table 4.4

Biochemical characteristics of *Actinomyces* species isolated from human

Characteristics	*A. cardiffensis*	*A. europaeus*	*A. funkei*	*A. georgiae*	*A. gerencseriae*	*A. graevenitzii*	*A. hominis*	*A. hongkongensis*	*A. israelii*	*A. meyeri*	*A. massiliensis*	*A. nasicola*	*A. neuii subsp. neuii*	*A.neuii subsp. anitratus*	*A. odontolyticus*	*A. oricola*	*A. radicidentis*	*A. radingae*	*A. turicensis*	*A. timoensis*	*A. urogenitalis*	*A. viscosus*	*A. naeslundii sensu stricto*	*A. oris*	*A. johnsonii*	*A. dentalis*
Cells coccoid or coccobacillary	–	d	–	d	–	–	–	–	–	d	–	+	+	d	(d)	–	+	–	–	–	–	–	–	–	–	–
Microcolonies filamentous	–	–	–	–	+	–	–	–	+	–	–	–	–	–	(d)	–	–	–	–	–	–	d	d	d	d	–
Aerobic growth (without CO_2)	–	+	+	–	–	+	+	–	–	–	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–
Colony color	Pink	Greyish	Grey	White	White	White	White	White	White	White	White	White/ grey	White	White	White	White	White	White	White	White	White	White	White	White	White	White
Acid production from:																										
L-Arabinose	–	–	–	–	–	ND	+	–	+	d	–	–	d	–	d	–	–	d	d	–	d	–	–	–	–	–
Amygdalin	–	–	ND	–	+	–	–	ND	+	d	–	–			–	w	ND	–	–	–	ND	–	ND	ND	–	ND
Cellobiose	–	ND	ND	–	+	–	–	ND	+	–	–	+	d	d	–	+	ND	d	d	–	ND	–	d	d	–	+
D-Fructose	ND	+	ND	–	+	+	+	ND	+	+	+	+	+	+	+	+	ND	d	ND	+	ND	+	+	+	+	+
D-Galactose	ND	+	ND	ND	–	+	+	ND	+	d	+	ND	+	+	ND	ND	ND	d	d	+	ND	+	ND	ND	ND	ND
D-Glucose	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	ND	ND	ND	d	–	+	+	ND	–	d	–	–	+	+	ND	–	ND	ND	ND	w	ND	–	–	ND	ND	ND
Glycogen	–	d	–	+	–	–	+	ND	ND	d	–	–	d	d	d	–	–	+	–	w	–	ND	–	d	+	–
myo-Inositol	ND	ND	ND	–	+	+	+	ND	+	–	–	ND	+	+	–	ND	ND	d	d	+	ND	–	+	+	+	ND
Lactose	–	–	d	–	+	+	–	ND	+	d	+	–	+	+	d	–	+	d	d	+	+	+	d	d	+	w
Maltose	–	+	d	+	+	+	+	ND	+	+	ND	–	+	+	–	ND	+	+	+	+	ND	+	ND	ND	+	+
D-Mannitol	–	–	–	–	+	–	–	ND	d	–	–	–	+	+	–	–	+	d	d	+	d	–	–	–	–	–
Mannose	–	ND	ND	–	+	ND	+	–	+	–	w	–	+	+	–	ND	ND	+	d	w	ND	+	–	–	d	+
Melibiose	–	d	–	–	d	–	–	ND	+	–	–	–	+	+	–	–	+	d	d	+	+	+	ND	ND	+	+
Melezitose	–	d	–	–	d	–	+	ND	–	–	w	–	+	+	–	–	+	d	d	+	+	–	d	d	–	–
Pullulan	–	ND	–	ND	ND	ND	ND	ND	ND	ND	ND	–	ND	ND	ND	–	–	ND	ND	ND	–	ND	ND	ND	ND	–

Table 4.4 (continued)

Characteristics	*A. cardiffensis*	*A. europaeus*	*A. funkei*	*A. georgiae*	*A. gerencseriae*	*A. graevenitzii*	*A. hominis*	*A. hongkongensis*	*A. israelii*	*A. meyeri*	*A. massiliensis*	*A. nasicola*	*A. neuii* subsp. *neuii*	*A. neuii* subsp. *anitratus*	*A. odontolyticus*	*A. oricola*	*A. radicidentis*	*A. radingae*	*A. turicensis*	*A. timoensis*	*A. urogenitalis*	*A. viscosus*	*A. naeslundii* sensu stricto	*A. oris*	*A. johnsonii*	*A. dentalis*
D-Raffinose	–	–	–	–	+	ND	+	–	+	–	–	–	+	+	–	w	+	d	d	+	+	+	–	–	–	+
L-Rhamnose	ND	–	ND	+	–	–	–	ND	–	–	–	ND	ND	–	d	ND	ND	–	–	ND	ND	–	–	–	ND	ND
D-Ribose	d	d	d	+	+	+	+	ND	+	+	w	–	+	+	d	–	+	+	+	w	d	+	–	d	+	–
Salicin	–	–	ND	–	+	–	–	ND	–	–	–	–	d	d	d	+	ND	d	d	+	ND	+	+	d	–	ND
D-Sorbitol	–	–	–	–	–	–	–	ND	–	–	–	–	–	–	–	–	–	–	–	ND	–	–	–	d	d	–
Sucrose	+	d	+	+	+	+	+	ND	+	+	+	–	+	+	d	+	+	+	+	+	+	+	+	+	+	+
Tagatose	–	ND	–	ND	ND	+	ND	ND	ND	ND	–	–	d	d	–	–	–	ND	ND	ND	–	ND	ND	ND	ND	–
Trehalose	–	d	–	+	+	–	ND	–	d	–	+	–	+	+	–	–	+	d	d	+	+	–	d	d	d	+
D-Xylose	–	–	+	+	+	–	–	–	+	+	w	–	+	+	d	–	ND	d	d	+	+	–	d	d	–	–
Hydrolysis of:																										
Esculin	–	d	–	+	+	–	–	–	+	–	ND	–	–	–	d	+	+	–	+	+	+	+	+	+	–	+
Gelatin	–	–	–	d	–	ND	–	ND	–	–	ND	–	–	–	–	–	w	–	–	ND	–	–	–	–	–	–
Hippurate	–	ND	+	ND	–	–	ND	ND	–	ND	ND	–	ND	ND	–	–	–	ND	ND	ND	–	ND	ND	ND	ND	–
Enzyme activities:																										
N-Acetyl-β-glucosaminidase	–	–	d	–	–	+	+	–	–	–	–	+	ND	ND	–	–	–	ND	ND	–	+	–	–	–	–	–
Alkaline phosphatase	–	–	+	ND	–	ND	+	+	–	–	–	–	–	d	–	d	–	–	–	–	d	–	–	–	d	–

Esterase C4	ND	+	–	ND	+	ND	–	ND	–		–	–	ND	ND	–	ND		+	+	–	–	+	ND	ND	ND	ND
Ester lipase C8	ND	+	–	ND	+	–	–	ND	–	ND	ND	–	ND	ND	–	ND	–	+	+	–	–	+	ND	ND	ND	ND
α-Fucosidase	–	ND	–	–	–	–	–	–	–	–	–	–	ND	ND	–	–	–	ND	ND	–	–	+	–	–	–	–
Arginine dihydrolase	+	ND	–	ND	–	ND	ND	+	–	ND	–	d	ND	ND	–	–	–	ND	ND	–	–	–	–	–	–	–
α-Galactosidase	–	+	–	ND	–	–	+	–	–	–	–	–	+	+	–	+	+	ND	ND	–	+	–	+	+	+	+
β-Galactosidase	–	+	d	+	+	+	+	–	+	–	+	+	+	+	–	d	+	ND	ND	–	+	+	+	+	+	w
α-Glucosidase	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	+	ND	ND	–	+	–	+	+	+	+
β-Glucosidase	–	–	–	ND	+	–	–	–	+	–	–	d	–	–	d	+	+	ND	ND	–	+	+	d	d	d	+
β-Glucuronidase	–	–	–	ND	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Leucine arylamidase	+	+	+	ND	ND	+	+	+	–	ND	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
α-Mannosidase	ND	–	–	ND	–	–	+	–	–	–	–	–	+	+	–	–	–	–	–	–	d	–	ND	ND	ND	ND
Pyrazinamidase	–	–	d	ND	+	ND	+	ND	ND	ND	ND	ND	+	+	ND	+	+	+	+		–	ND	ND	ND	ND	ND	–
Valine arylamidase	ND	+	–	ND	+	–	–	ND	–	+	+	+	–	–	–	ND	–	ND	–	–	+	+	–	–	ND	ND
Urease	–	–	–	–	–	–	–	–	–	d	–	–	–	–	–	–	d	–	–	–	–	–	–	–	d	–
Catalase production	–	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	+	–	–	–	–	+	–	d	–	–
Nitrate reduction	d	–	d	d	d	–	+	–	+	–	+	–	+	–	+	–	d	–	–	–	+	–	+	+	+	–
β-Hemolysis on sheep blood agar	–	–	–	–	–	ND	+	–	–	–	ND	ND	+	+	–	–	ND	w	w	ND	ND	–	–	ND	–	ND
CAMP-like reaction	–	–	–	–	–	–	+	–	–	ND	ND	ND	+	+	–	ND	ND	–	–	ND	ND	–	–	ND	ND	ND

Data compiled from Dent and Williams (1984a), Schaal (1986a), Schaal et al. (2006), Hall et al. (2005), Johnson et al. (1990), Henssge et al. (2009), Renvoise et al. (2009)

Symbols: + positive/present, – negative/absent, *d* strain differences, *w* weak, (*d*) some strains weakly positive, *ND* no data available

Table 4.5
Biochemical characteristics of *Actinomyces* species isolated from animals and environment

Characteristics	A. *bovis*	A. *bowdenii*	A. *canis*	A. *catuli*	A. *coleocanis*	A. *denticolens*	A. *hordeovulneris*	A. *howellii*	A. *hyovaginalis*	A. *marimammalium*	A. *slackii*	A. *suimastitidis*	A. *vaccimaxillae*	A. *ruminicola*	A. *weissii*	A. *naturae*
Cells coccoid or coccobacillary	d	–	–	–	–	–	–	–	+	–	+	–	+	–	–	–
Microcolonies filamentous	d	–	–	–	–	d	+		–	–	–	–	–	–	–	–
Aerobic growth (without CO_2)	–	+	+	+	+	d	–	–	+	+	+	–	w	–	+	+
Colony color	White	White	White	White	White	Pink, whi	White	White	White	Grey	White	White	White	White	White	White
Acid production from:																
L-Arabinose	–	–	+	d	–	–	–	–	+	–	–	w	+	ND	ND	ND
Amygdalin	ND	ND	ND	ND	ND	–	ND	–	ND	ND	–	ND	ND	ND	ND	ND
Cellobiose	–	ND	ND	ND	ND	–	+	–	d	ND	–	ND	–	+	ND	ND
D-Fructose	+	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	+	w	ND	ND
D-Galactose	ND	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	ND	ND	ND	–	ND	–	–	–	ND	ND	–	ND	ND	ND	ND	ND
Glycogen	+	–	+	–	+	ND	+	ND	ND	ND	–	ND	–	d	+	–
myo-Inositol	d	ND	ND	ND	ND	d	–	–	d	ND	–	ND	ND	d	ND	ND
Lactose	+	+	+	+	+	+	+	d	d	+	d	–	–	+	+	–
Maltose	+	+	+	+	+	+	+	+	+	+		d	–	ND	+	+
D-Mannitol	–	–	–	–	–	d	–	–	ND	–	–	–	–	d	–	–
Mannose	ND	ND	ND	ND	ND	–	–	d	+	ND	ND	ND	–	ND	ND	+
Melibiose	ND	+	–	d	–	ND	–	d	–	–	ND	+	–	+	ND	–
Melezitose	–	+	–	–	–	–	–		–	–	–	–	–	+	ND	+
Pullulan	ND	–	+	–	+	ND	+	ND	ND	–	–	+	–	ND	ND	–
D-Raffinose	–	+	d	+	–	+	+	+	–	–	+	+	–	ND	ND	–
L-Rhamnose	–	ND	ND	ND	ND	–	–	–	–	ND	–	ND	ND	+	ND	ND
D-Ribose	–	+	+	+	–	d	–	–	ND	–	–	w	+	+	–	ND
Salicin	ND	ND	ND	ND	ND	+	ND	–	+	ND	ND	ND	+	+	ND	ND

D-Sorbitol	–	–	–	–	–	–	–	–	–	–	–	–	–	+	ND	ND
Sucrose	+	+	d	+	–	+	+	+	+	–	d	+	+	+	+	+
Tagatose	ND	–	–	–	–	ND	ND	ND	–	–		–	–	ND	ND	ND
Trehalose	–	+	–	+	–	–	+	d	–	–	d	–	+	+	ND	ND
D-Xylose	–	–	+	+	–	–	+	d	+	–	–	+	+	+	–	ND
Hydrolysis of:																
Esculin	+	+	–	+	–	+	+	+	+	–	–	+	+	d	+	ND
Gelatin	–	–	–	–	–	ND	–	–	–	–	ND	–	–	ND	–	ND
Hippurate	–	–	–	–	–	ND	–	ND	+	–	ND	–	–	–	ND	–
Enzyme activities:																
N-Acetyl-β-glucosaminidase	+	–	+	d	–	–	+	+		+	–	d	–	ND	+	–
Alkaline phosphatase	–	d	–	–	–	–	–	+	+	d	–	d	–	ND	+	–
Esterase C4	–	–	–	d	–	–	–	ND	ND	ND	ND	ND	–	ND	ND	w
Esterase C8	–	–	w	+	–	–	–	ND	ND	ND	ND	ND	–	ND	Nd	–
α-Fucosidase	–	–	+	–	–	–	–	ND	ND	ND	ND	ND	+	ND	ND	–
Arginine dihydrolase	–	–	–	d	–	ND	+	ND	ND	–	ND	–	–	ND	ND	–
α-Galactosidase	–	+	+	d	–	d	+	ND	+	–	+	+	–	ND	ND	w
β-Galactosidase	+	+	+	+	+	+	+	ND	+	+	ND	+	–	ND	+	+
α-Glucosidase	–	d	+	+	+	+	+	ND	ND	–	+	+	d	ND	+	+
β-Glucosidase	+	+	–	+	–	d	+	+	ND	–	+	+	+	ND	+	+
β-Glucuronidase	–	–	–	+	–	–	–	ND	–	–	–	–	–	ND	–	–
Leucine arylamidase	+	+	+	+	–	+	+	ND	+	+	ND	ND	+	ND	ND	+
α-Mannosidase	–	–	–	–	–	–	+	ND	ND	ND	ND	ND	–	ND	+	–
Pyrazinamidase	–	d	w	+	w	ND	–	+	–	–	+	w	+	ND	–	+
Valine arylamidase	w	–	–	–	–	–	+	ND	ND	ND	ND	ND	–	ND	ND	–
Urease	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–
Catalase production	–	+	+	d	–	–	w	+	–	–	+	–	–	–	+	–
Nitrate reduction	–	+	–	+	–	+	–	–	+	–	+	–	–	+	–	–
β-Hemolysis on sheep blood agar	–	–	ND	ND	ND	–	–	ND	–	–	ND	ND	–	ND	w	ND
CAMP-like reaction	–	–	–	ND	–	–	–	ND	–	ND	ND	ND	ND	ND	+	ND

[a]Data compiled from Dent and Williams (1984b), Schaal (1986a), Schaal et al. (2006), An et al. (2006)
Symbols: + positive, present, – negative/absent, *w* weakly positive, *d* strain differences, *ND* available

A. johnsonii, *A. radicidentis*, *A. radingae*, *A. slackii*, *A. turicensis*, *A. urogenitalis*, *A. viscosus* sensu stricto, and *A. weissii*.

The temperature optimum for growth of *Actinomyces* species ranges from 30 °C to 37 °C. Certain members of the genus vary in their response to growth at higher temperatures. Thus, *A. naturae* reported to grow at temperature range of 20–43 °C (optimum 30–37 °C) (Rao et al. 2012). Optimum growth of *A. ruminicola* occurs at 46 °C (An et al. 2006), while strains of *A. viscosus* and *A. naeslundii* may also grow at 45 °C (Holmberg and Nord 1975). In contrast, *A. meyeri* does not grow at all at 45 °C (Cato et al. 1984). Growth of *Actinomyces* species occurred at pH range of 4.5–9.0 (optimum pH 6.5). The pH value of the growth medium continued to decrease to a limiting value of pH 5.0, and colonies harvested continued to grow at the same rate when subcultured to a medium of the same initial pH 7.2.

Apparently, variation in nutrient requirements in *Actinomyces* is minimal, since most species investigated grow well on complex agar media such as Brain Heart Infusion, meat extract, and yeast extract. The nutrient requirements have been investigated for relatively few species. The ability of a strain of *A. israelii* to grow in partially defined media supplemented with various nitrogen compounds has been investigated (Christie and Porteous 1962). They found that L-cysteine and L-tryptophan were essential for growth, whereas glutathione, ammonium sulfate, purine, or pyrimidine bases are not required. For the most part organic nitrogen sources such as casein hydrolysate and peptone are satisfactory sources of nitrogen for *Actinomyces* species.

Some species vary more in response to vitamins and growth factors than to basic elements of nutrition. *A. meyeri* is known to have an absolute requirement for vitamin K_1 for growth (Cato et al. 1984). Addition of 0.02 % Tween 80 to broth media also enhanced growth and carbohydrate fermentation of strains of *A. meyeri* (Cato et al. 1984), *A. georgiae*, *A. gerencseriae*, *A. naeslundii*, *A. odontolyticus*, *A. oris*, and *A. johnsonii* (Johnson et al. 1990). In this context, it is worth mentioning that the growth of almost all *Actinomyces* species is stimulated by the addition of Tween 80 (1 % v/v) to Brain Heart Infusion broth (A. F. Yassin unpublished). Likewise, the addition of serum to the medium enhances growth of *Actinomyces*. Buchanan et al. (1984) reported that the growth of *A. hordeovulneris* is considerably stimulated when the medium is supplemented with 10–20 % (v/v) fetal calf serum. In contrast, addition of 20 % bile inhibits the growth of *A. meyeri* (Cato et al. 1984).

Interestingly, tolerance to organic solvents has been reported for *A. naturae* (Rao et al. 2012). Fermentative growth of *A. naturae* was observed in the presence of near saturation concentrations of perchloroethylene (PCE) and toluene and at concentrations up to at least 24.4 mM and 11.2 mM in the presence of 1,2-dichloroethane (1,2-DCA) and 1,1,2-trichloroethane (1,1,2-TCA), respectively. The ability of *A. naturae* strains to grow in the presence of chlorinated and non-chlorinated solvents has potentially important implications for cleanup at the contaminated sites. Likewise, *A. naturae* grows at NaCl concentrations up to 3.0 % (w/v), while a concentration of 6.5 % NaCl inhibits the growth of *A. meyeri* (Cato et al. 1984).

Furthermore, carbohydrates are the preferred nutrient as they provide immediate carbon and energy sources. Members of the genus *Actinomyces* can utilize a diverse range of carbohydrates that support growth, but the different species may vary in their carbohydrates utilizing abilities (*Table 4.4* and *Table 4.5*). These variations do not correlate in any obvious manner with morphology or recorded pathogenicity and may have little taxonomic significance. In some instances variation in carbohydrates utilization capacity within strains of the same species has been reported (Dent and Williams 1984a, b; Johnson et al. 1990). Glucose is universally utilized by nearly all of the *Actinomyces* species with the exception of *A. hongkongensis* (Woo et al. 2003) and *A. nasicola* (Hall et al. 2003a). The end products of glucose fermentation were acetate, lactate, succinate, and formate.

Actinomyces species exhibit enzymatic activities for the degradation of diverse classes of substrates. The most common enzyme activities present in most species were α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, and leucine arylamidase, while the least common was β-glucuronidase demonstrated only by *A. catuli*. α-Mannosidase activities were demonstrated only by *A. hordeovulneris*, *A. neuii*, and *A. weissii*. None of the *Actinomyces* species are positive for the urease test. However, due to the inconclusiveness of the results, it is difficult to use the enzymatic activity test using the API ZYM test system to differentiate *Actinomyces* species.

Chemotaxonomic Properties

Present members of the genus *Actinomyces* appear to be heterogeneous with regard to their amino acid composition of the cell wall peptidoglycan, cell wall sugars, menaquinone composition, polar lipid profiles, and DNA mol% G+C content (*Table 4.6*). Thus, elucidation of the amino acids sequences of the peptidoglycans across the genus, although many species remain to be investigated, revealed the presence of the main type abbreviated A5 (Schleifer and Seidl 1985). In structural term, the main type A5 includes two subtypes. Peptidoglycan subtype A5α (L-Lys-L-Lys-D-Glu) was found in *A. europaeus* (Funke et al. 1997a). *A. radingae* also exhibits this subtype with L-lysine in position 3 of the tetrapeptide being partially replaced by L-ornithine (L-Lys [L-Orn]-L-Lys-D-Glu) (Wüst et al. 1995). Although *A. bovis* was shown to belong to peptidoglycan type A4, which is characterized by L-lysine in position 3 of the tetrapeptide subunit and a D-asparagine residue forming the interpeptide bridge (L-Lys-D-Asp; Schleifer and Kandler 1972), recent analysis of the peptidoglycan revealed that the peptidoglycan of *A. bovis* (L-Lys-L-Lys-D-Asp) corresponds to subtype A5α (A11.52; according to the nomenclature suggested by Schumann 2011). This is also true for *A. israelii* (www.peptidoglycan-types.info). Subtype A5β (L-Orn-L-Lys-D-Glu) is found in *A. georgiae* (www.peptidoglycan-types.info), *A. gerencseriae*

Table 4.6

Chemotaxonomic characteristics of selected members of the genus *Actinomyces*

Organisms	Chemotaxonomic characteristic					
	Peptidoglycan types	Acyl type	Cell wall sugars	Phospholipid	Menaquinones	Fatty acids
Actinomyces bovis	A5α (L-Lys-L-Lys-D-Asp)	Acetyl	Glucose + mannose + rhamnose + 6-deoxytalose	DPG, PG, PC, PI, PIM	MK-9[a]	C16:0, C16:1ω7c
						C18:1 ω9c, C18:2 ω6,9c
Actinomyces europaeus	A5α (L-Lys-L-Lys-D-Glu)	Acetyl	Galactose	DPG, PC, PI, PIM	MK-9(H4)[a]	C16:0, C18:0,
						C18:1 ω9c
Actinomyces naturae	A5β (L-Orn-L-Lys-D-Glu)		Mannose + rhamnose	DPG, PG, PGL, GL	MK-9(H4)[a]	C14:0, C16:0,
						C18:1 ω9c
Actinomyces turicensis	A5β (L-Orn-L-Lys-D-Glu)	Acetyl	Rhamnose + fucose + 6-deoxytalose	DPG, PC, PI, PIM	MK-9(H4)[a]	C16:0, C16:1ω7c
						C18:1 ω9c, C18:2 ω6,9c

Abbreviations: *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PC* phosphatidylcholine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PGL* phosphoglicolipids, *GL* glycolipids, MK-n(Hx) represents a partially hydrogenated menaquinone with x hydrogen atoms on the side chain containing n isoprene units; S, straight-chain saturated, and *U* monounsaturated

[a]Major component

(www.peptidoglycan-types.info), *A. hyovaginalis* (Collins et al. 1993), *A. naturae* (Rao et al. 2012), and *A. turicensis* (Wüst et al. 1995).

Several cell wall sugars have been detected in *Actinomyces* species. The cell wall of *A. canis*, *A. coleocanis*, *A. europaeus*, *A. israelii*, *A. georgiae*, *A. gerencseriae*, *A. hordeovulneris*, *A. marimammalium*, and *A. neuii* contained only galactose (Buchanan and Scott 1984; A. F. Yassin, unpublished). Dent and Williams (1984) reported that the cell wall of *A. denticolens* contained rhamnose. A variety of cell wall sugars, including glucose, mannose, rhamnose, and 6-deoxytalose, have been detected in the cell wall of *A. bovis* (*Table 4.6*; A. F. Yassin, unpublished). Fucose, in addition to other sugars, is found distributed in the cell wall of *A. meyeri*, *A. turicensis*, and *A. hyovaginalis*. Thus, in term of cell wall sugars composition, members of the genus *Actinomyces* were diverse.

The principal respiratory quinones among *Actinomyces* species are menaquinones with eight, nine, and ten isoprene units. *Actinomyces bovis*, *A. urogenitalis*, and *A. weissii* were found to contain fully unsaturated menaquinones with eight (MK-8) and nine (MK-9) isoprene units, with MK-9 predominating. This data imply that these species are closely related and correlate well with their affiliation to the same phylogenetic cluster I as judged from phylogenetic analysis (*Fig. 4.1b*). Interestingly, the menaquinone pattern of *A. nasicola* deviates from that reported in the original species description by Hall et al. (2003b). Exact mass measurements of the constituent menaquinones extracted from *A. nasicola* using mass spectrometry revealed the presence of tetrahydrogenated menaquinones with eight [MK-8(H_4)] and nine [MK-9(H_4)] isoprene units, with the latter compound being the major component. This result convincingly supports the phylogenetic placement of *A. nasicola* outside the phylogenetic cluster I (*Fig. 4.1b*). Several species, including *A. europaeus*, *A. colecanis*, *A. naturae*, *A. oricola*, and *A. turicensis* contain MK-8(H_4) and MK-9(H_4) as major components.

Polar lipid types have been reported for sparingly few members of the genus *Actinomyces*. Investigation of the polar lipids profile in *A. bovis* revealed the presence of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM), and several unidentified phospholipids (*Table 4.6*; A. F. Yassin, unpublished). It is not surprising to find this pattern, though with variations in the amounts of the different phospholipids, in *A. weissii* (Hijazin et al. 2012a) as both species are members of the same phylogenetic cluster (*Fig. 4.1b*). In contrast, the polar lipids of *A. naturae* were reported to include DPG, PG, phosphoglycolipids (PGL), glycolipids (GL), and an unidentified phospholipid (PL) Rao et al. (2012). From these preliminary data, it seems that there are no universal phospholipids patterns of *Actinomyces* species.

The cellular fatty acids of members of the genus *Actinomyces* consisted of straight-chain saturated and monounsaturated types with $C_{16:0}$, $C_{16:1}$ ω7c, $C_{18:0}$ and $C_{18:1}$ ω9c as the predominating ones. In addition, diunsaturated fatty acid of $C_{18:2}$ ω6,9c was detected in *A. bovis*, *A. cardiffensis*, and *A. weissii*. *A. nasicola* contains an unusual cellular fatty acids profile consisting of a complex mixture of straight-chain saturated, monounsaturated, iso- and anteiso-branched-chain fatty acids (Hall et al. 2003b). *A. massiliense* contains traces of iso-$C_{17:0}$, anteiso-$C_{17:0}$, and anteiso-$C_{17:1}$.

Thus, the genus *Actinomyces* encompasses a chemotaxonomic highly variable group of bacteria and deserves division into separate taxonomic entities. These new entities should be defined using morphological, chemotaxonomic, and molecular data.

Taxonomic Comment

The taxonomy of the genus *Actinomyces*, as currently treated, is largely based on morphology. However, advances in chemical, numerical, and molecular systematic methods have contributed greatly to the circumspection of *Actinomyces*. Therefore, the taxonomic positions of *Actinomyces* species have undergone incisive changes over the past several years and today remain in a state of flux as false names are corrected, existing strains are sorted out into newly recognized genera (e.g., *Actinobaculum* and *Trueperella*), and new species are identified. Nevertheless, the genus *Actinomyces* remains a poorly defined genus.

A wide range of morphological, chemical, and molecular variations are present within members of the genus *Actinomyces*, causing major problems for genus delineation and identification. The morphology of *Actinomyces* species is diverse and varied in some species with the growth stages of the culture. Besides the mycelial state of some species, they can exhibit coryneform morphology with diphtheroidal arrangements (Y, V, T forms) or even coccoid or coccobacillary forms. Considerable variation was recognized also with respect to motility and flagellation. Although all *Actinomyces* species are nonmotile, flagellation and motility was observed in *A. naturae* (Rao et al. 2012). Therefore, retaining *A. naturae* in the genus is not justifiable.

Molecular phylogeny indicates that the genus *Actinomyces* as presently circumscribed is polyphyletic. The phylogeny based on 16S rRNA gene sequences recovers two major clusters and four distinct evolutionary lineages with high bootstrap support. These results strongly support the fact that the genus *Actinomyces* should be dissected into a number of distinct genera. However, this proposal needs to be evaluated critically based on the phenotypic variations among species.

Apart from the phylogenetic evidence, chemotaxonomic data unambiguously indicate that the generic boundaries of *Actinomyces* need further resolution. Although the genus *Actinomyces* has never been subjected to a broad and exhaustive chemotaxonomic study, a wide range of chemical variations or chemosyndromic variations between species have been documented, making the generic chemotaxonomic distinctions challenging. Several chemotypes have been identified in some taxa, e.g., *A. bovis*, *A. europaeus*, *A. naturae*, and *A. turicensis* (Table 4.6). The chemotaxonomic features of *A. bovis* are shared by its phylogenetic closely related species *A. urogenitalis* (97 % sequence similarity) and *A. weissii* (96.9 % sequence similarity). These findings have important implications for the generic delimitation of *Actinomyces*.

In view of the conspicuous chemotaxonomic and phylogenetic variations within the genus *Actinomyces*, it is obvious that the generic boundaries of the genus are in need of further evaluation. Therefore, for correct evaluation of the taxa that are assigned to the genus *Actinomyces*, a proper definition of the genus *Actinomyces* sensu stricto is required. In light of the currently available data on *A. bovis*, the type species of the genus, the following definition of the genus *Actinomyces* sensu stricto is proposed.

Definition of the Genus *Actinomyces* Sensu Stricto

The definition of the genus *Actinomyces* proposed herein, is focused on the close relationship amongst *A. bovis*, *A. urogenitalis* and *A. weissii*. It is supported by 16S rRNA gene sequences and chemotyonomic analyses and provide sound basis for future taxonomic restructuring. Among the criteria for restrictive membership in the genus *Actinomyces* are: Cells are straight or slightly curved rods (with or without swollen ends) which may occur singly or in pairs with diphtheroidal arrangements (Y, V, T forms and palisades). Cells are Gram-reaction-positive, non-acid-fast, non-spore-forming, and nonmotile. Strains grow at 37 °C under anaerobic conditions or in a 5% CO_2 atmosphere. The peptidoglycan is of the type A5α, variation L-Lys–L-Lys–D-Asp. The muramic acid residue of the peptidoglycan is *N*-acetylated. Characteristic whole-cell sugars are glucose, mannose, rhamnose, and 6-deoxytalose. The menaquinones are MK-8 and MK-9, with the latter cs the major component. The phospholipid type is type PIII according to Lechevalier et al. (1977), comprising DPG, PG, PC, PI, and PIM. Long-chain fatty acids are primarily straight-chain saturated and monounsaturated ($C_{16:0}$, $C_{16:1}$ ω7c, $C_{18:0}$, $C_{18:1}$ ω9c) in addition to trace of diunsaturated fatty acid ($C_{18:2}$ 6,9c). The DNA G+C content is 61–63.3 mol%. The type species is *Actinomyces bovis*.

Indeed, it remains to conduct additional studies including all currently described *Actinomyces* species to see whether these characters occur in other species to further elucidate their taxonomic affiliation to the genus. Thereafter, a reevaluation of the existing taxonomy will be necessary to reflect an accurate phylogeny of *Actinomyces*.

Genus II. *Arcanobacterium* Collins, Jones and Schofield, 1983, 438^{VP} (Effective publication: Collins, Jones and Schofield, 1982, 1280), emend. Lehnen, Busse, Frölich, Krasinska, Kämpfer and Speck 2006, 864^{VP}, emend. Yassin, Hupfer, Siering and Schumann 2011, 1272^{VP}.

Ar. ca. no. bac. te' ri. um. L. adj. *arcanus*, secret, hidden, secretive; Gr. neut. dim. n. *bakterion*, a small rod; M. L. neut. n. *Arcanobacterium*, secretive bacterium.

Taxonomy, Historical and Current

The genus *Arcanobacterium* was described by Collins et al. (1982b) to accommodate bacterial strains originally isolated from infected American soldiers and previously named "*Corynebacterium haemolyticum*" (MacLean et al. 1946). However, the species exhibits little similarity to typical corynebacteria, and its placement in the genus *Corynebacterium* was questioned by several workers (Cummins and Harris 1956; Barksdale et al. 1957; Barksdale 1970; Jones 1975; Minnikin et al. 1978; Schofield and Schaal 1981; Collins et al. 1982a). In addition, the relationship of "*C. haemolyticum*" to the species "*C. pyogenes*" (Glage) remained unclear. In a study of cell wall compositions in some Gram-positive bacteria, Cummins and Harris (1956) noted that the cell wall compositions of "*C. pyogenes*" and

"*C. haemolyticum*" were obviously similar to one another but differed both in sugar and amino acid composition from the other corynebacteria, since they contain neither species arabinose nor galactose and lysine is the diamino acid of the peptidoglycan, while DAP is absent. On the other hand, Cummins and Harris (1956) found that rhamnose was present in both organisms, and this, together with the fact that alanine, glutamic acid, and lysine were the major amino acid components, led these authors to suggest that the two organisms were related to the streptococci. This view was upheld by Barksdale et al. (1957) who suggested not only that "*C. haemolyticum*" and "*C. pyogenes*" should be reclassified in the genus *Streptococcus*, but also that "*C. haemolyticum*" was a mutant form of "*C. pyogenes*." In the eighth edition of *Bergey's Manual of Determinative Bacteriology*, both taxa were listed in an addendum to the genus *Corynebacterium* (Cummins et al. 1974), and "*C. haemolyticum*" does not appear in the Approved Lists of Bacterial Names (Skerman et al. 1980).

Later, numerical phenetic (Schofield and Schaal 1981) and chemical (Collins et al. 1982a) studies showed that "*C. haemolyticum*" and "*C. pyogenes*" are two distinct taxa. The discovery of tetrahydrogenated menaquinones with ten isoprene units [MK-10(H4)] as predominant component in "*C. pyogenes*" and tetrahydrogenated menaquinone with nine isoprene units [MK-9(H4)] as predominant component in "*C. haemolyticum*" was not in accord with the inclusion of these taxa in the genus *Streptococcus* or the genus *Corynebacterium* (Collins et al. 1982a). The majority of streptococci completely lack respiratory quinones, although some unsaturated naphthoquinones have been detected in a few group D and group N streptococci (Collins and Jones 1979a, b). The menaquinone patterns of C. *pyogenes* and "*C. haemolyticum*" are also incompatible with the retention of these species in the genus *Corynebacterium*. True corynebacteria generally possess dihydrogenated menaquinones with eight [MK-8(H2)] and nine [MK-9(H2)] isoprene units (Yamada et al. 1976; Collins et al. 1977). Tetrahydrogenated menaquinones with ten and nine isoprene units have, however, been reported in the genera *Actinomyces* (Collins et al. 1977) and *Propionibacterium* (Schwartz 1973; Sone 1974), respectively. In addition, the fatty acids data (Collins et al. 1982a) did not support the views of Barksdale et al. (1957) that "*C. haemolyticum*" and "*C. pyogenes*" should be reclassified in the genus *Streptococcus*. "*C. haemolyticum*" and "*C. pyogenes*" contain major amounts of monounsaturated fatty acids of the oleic acid series (18:1 ω9) (synthesized via an aerobic pathway; Collins et al. 1982a), whereas members of the genus *Streptococcus* possess monounsaturated fatty acids of the *cis*-vaccenic acid series (18:1 ω7) (synthesized via an anaerobic pathway; Kroppenstedt and Kutzner 1978). The presence of lysine in the walls of "*C. haemolyticum*" and "*C. pyogenes*" supports the removal of both species from the genus *Corynebacterium* sensu stricto (Keddie and Cure 1978; Minnikin et al. 1978). The absence of mycolic acids and the presence of predominantly straight-chain and monounsaturated (oleic acid series) fatty acids in "*C. pyogenes*" and "*C. haemolyticum*" is, however, compatible with members of the genus *Actinomyces* (Kroppenstedt and Kutzner 1978). Therefore, Reddy et al. (1982) as well as Collins and Jones (1982) proposed that "*C. pyogenes*" should be reclassified in the genus *Actinomyces* as "*Actinomyces pyogenes*."

The taxonomic position of "*C. haemolyticum*" remained equivocal. The results of lipid analyses did not support the view of Barksdale et al. (1957) that "*C. haemolyticum*" is a mutant of "*C. pyogenes*." Phenotypically, "*C. haemolyticum*" is very similar to *A. bovis* and also contains lysine in the cell wall peptidoglycan. The menaquinone composition of "*C. haemolyticum*" is distinct from that of *A. bovis* (Collins et al. 1977) and resembles that of the propionibacteria (Schwartz 1973; Sone 1974), but the results of cell wall and fatty acid analyses do not support this latter relationship. Therefore, on the basis of phenetic, peptidoglycan, fatty acid, menaquinone, and DNA data (Schleifer and Kandler 1972; Schofield and Schaal 1981; Collins et al. 1982a, b) reclassified "*C. haemolyticum*" in a new genus *Arcanobacterium* as *Arcanobacterium haemolyticum*.

The use of molecular phylogenetic methods of classification has resulted in several species being transferred from other genera to the genus *Arcanobacterium* and new taxa have been assigned to it. Thus, in a phylogenetic analysis based on 16S rRNA gene sequences of the genus *Actinomyces*, Pascual Ramos et al. (1997a) reclassified "*Actinomyces pyogenes*" and "*Actinomyces bernardiae*" in the genus *Arcanobacterium* as "*Arcanobacterium pyogenes*" and "*Arcanobacterium bernardiae*," respectively, and described a new species *Arcanobacterium phocae*. Thereafter, further new species, viz., "*A. pluranimalium*," *A. hippocolea*, "*A. abortisuis*," "*A. bialowiezense*," and "*A. bonsai*," were assigned to the genus. However, the observation that the genus *Arcanobacterium* is not monophyletic and that MK-10(H4) was the principal menaquinone found in "*A. pyogenes*," "*A. abortisuis*," "*A. bialowiezense*," and "*A. bonsai*" is not in accord with the inclusion of these species in the genus *Arcanobacterium*. Therefore, Yassin et al. (2011) proposed that the genus *Arcanobacterium* should be restricted to the species *A. haemolyticum*, *A. phocae*, *A. hippocoleae*, and *A. pluranimalium* and to reclassify the other species in a new genus, *Trueperella*. Currently, the genus *Arcanobacterium* comprises five validly published species, namely, *A. haemolyticum*, *A. hippocoleae*, *A. phocae*, *A. pluranimalium*, and *A. canis*.

Molecular Analysis

Similarity values of 16S rRNA gene sequence analyses for members of the genus *Arcanobacterium* range from 95.1 % to 97.4 %. The maximum-parsimony tree (*Fig. 4.1a*) generated based on sequence analysis of the 16S rRNA gene sequences of *Arcanobacterium* species produced a monophyletic clade within the family *Actinomycetaceae* with poor bootstrap support (63 % bootstrap value). Although only poor bootstrap support exists for the *Arcanobacterium* clade as a whole, the lack of support may be due to the poorly resolved position of *Arcanobacterium hippocoleae*, which is sister to all remaining species in the clade (*Fig. 4.1b*). Two major lineages could be recognized. One lineage includes four species, *A. haemolyticum*, *A. canis*,

A. pluranimalium, and *A. phocae*. The second lineage constitutes a single species, *A. hippocoleae*. The phylogenetic coherence of *A. haemolyticum*, *A. canis*, *A. pluranimalium*, and *A. phocae* to the genus *Arcanobacterium* is supported by a group of signature nucleotides (Table 4.1). This include nucleotides at positions 157–164 (U-G), 440 (U), 443 (C), 446–488 (U-G), 598–642 (U-A), 615–625 (G-C), and 1244–1293 (U-A). *A. hippocoleae* share with these species only signature nucleotides at positions 157–164, 598–642, 615–625, and 1244–1293 (Table 4.7).

From a phylogenetic point of view, the genera *Arcanobacterium* and *Trueperella* are sisters (Fig. 4.1a, b); this relationship is strongly supported (96 % bootstrap value). The position of the genus *Actinobaculum* as the basal of the clade containing the genera *Arcanobacterium* and *Trueperella* is poorly supported (bootstrap value 67 %).

Table 4.7
16S rRNA signature nucleotides that define the genera *Arcanobacterium* and *Trueperella* and their occurrence in the 16S rRNA sequence of *A. hippocoleae*

Position[a]	*Arcanobacterium*	*A. hippocoleae*	*Trueperella*
157–164	U-G	U-G	G-U
440	U	C	C
443	C	U	U
446–488	U-G	A-U	A-U
598–642	U-A	U-A	C-A
615–625	G-C	G-C	G-U
1244–1293	U-A	U-A	R-G,U

[a]*E. coli* position (Brosius et al. 1978)

Phenotypic Analyses

Gross Morphology

Surface colonies of *A. haemolyticum* on blood agar were described to be small after 24 h of incubation at 36 ± 1 °C, becoming larger upon extended incubation (Collins and Cummins 1986). They are circular, discoid, slightly raised, opaque, and nonpigmented with a butyrous consistency, and they show β-hemolysis. *Arcanobacterium phocae* also produces a potent hemolysin, whereas *A. canis* and *A. hippocoleae* are weakly β-hemolytic, and *A. pluranimalium* was reported to be α-hemolytic.

Cellular Morphology

The cellular morphology of the genus *Arcanobacterium* as described by Collins et al. (1982b) for bacteria formerly named "*Corynebacterium haemolyticum*" is as follows: slender, irregular, bacillary forms predominate during the first 18 h of growth; many cells are arranged at an angle to give V formations (Fig. 4.6a). As growth proceeds, cells become granular and segmented so that they resemble small and irregular cocci. Both rods and coccoid cells are Gram-positive, non-acid-fast, and nonmotile; endospores are not formed.

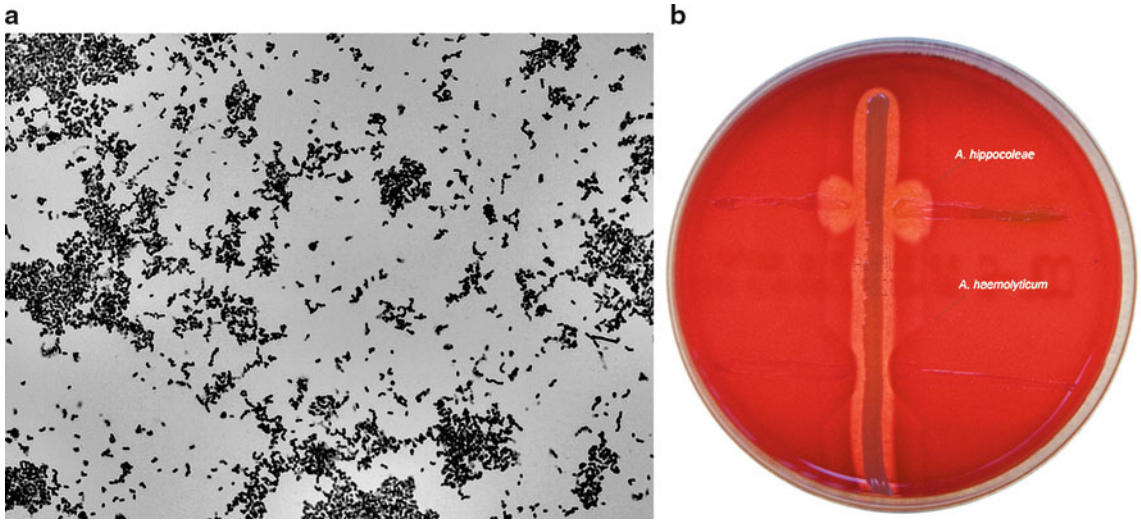

Fig. 4.6
(a) Gram-stained smear from 7-days-old culture of *Arcanobacterium haemolyticum* on Columbia agar with 5% sheep blood showing irregular bacillary forms with V formations. As the growth proceeds cells segmented so that they resembles small irregular cocci. (b) CAMP test showing a positive synergistic result with *Arcanobacterium hippocoleae* DSM15539^T and a positive reverse CAMP test with *Arcanobacterium haemolyticum* DSM20595^T. Staphylococcus aureus ATCC 25923 is streaked down the center of the Columbia agar plate with 5% sheep blood

Table 4.8

Biochemical characteristics of *Arcanobacterium* species

Characteristics	*A. haemolyticum*	*A. hippocoleae*	*A. phocae*	*A. pluranimalium*	*A. canis*
Synergistic CAMP reaction	d	+	+	+	+
Reverse CAMP reaction	+	–	+	–	–
Acid production from:					
L-Arabinose	–	–	–	–	ND
Cellobiose	–	–	–	+	ND
D-Glucose	+	+	+	+	+
Lactose	+	+	+	–	+
Maltose	+	d	+	d	+
D-Mannitol	–	–	d	–	–
Melezitose	ND	–	d	–	ND
D-Raffinose	–	–	–	–	ND
L-Rhamnose	–	–	–	–	ND
D-Ribose	+	–	+	+	+
D-Sorbitol	–	–	–	–	ND
Sucrose	–	–	+	–	+
Trehalose	–	–	d	–	ND
D-Xylose	–	–	d	–	–
Hydrolysis of:					
Esculin	–	w	–	w	–
Gelatin	–	–	–	+	–
Hippurate	ND	+	ND	+	ND
Enzyme activities:					
N-Acetyl-β-glucosaminidase	+	d	–	–	+
Catalase	–	–	d	+	–
Acid phosphatase	+	–	+	ND	ND
Alkaline phosphatase	+	d	+	–	+
Chymotrypsin	–	–	–	ND	ND
Cystine arylamidase	–	–	+	ND	ND
Esterase lipase C4	–	–	+	–	+
Esterase lipase C8	+	–	+	–	ND
α-Fucosidase	–	–	–	–	ND
α-Galactosidase	–	–	+	–	ND
β-Galactosidase	+	+	+	–	+
β-Glucuronidase	–	+	–	+	+
α-Glucosidase	+	+	+	–	+
β-Glucosidase	–	–	–	–	ND
Lipase C14	–	–	–	ND	ND
Leucine arylamidase	+	+	+	+	+
α-Mannosidase	–	–	–	ND	+
Naphthol-AS-BI-phosphohydrolase	–	+	–	ND	ND
Pyrazinamidase	+	–	+	d	–
Trypsin	–	–	d	–	ND
Valine arylamidase	–	–	–	–	ND
Urease	–	–	–	–	–
Nitrate reduction	–	–	–	–	–

Data for *A. hippocoleae* from Hoyles et al. (2002); data for *A. phocae* from Pascual Ramos et al. (1997a); data for *A. pluranimalium* from Lawson et al. (2001a); data for *A. canis* from Hijazin et al. (2012a); data for *A. haemolytocum* from A. F. Yassin (unpublished)

Symbols, + positive/present, – negative/absent, *w* weakly positive, *ND* not determined, *d* differences between strains within one species

Biochemical Characteristics

Arcanobacterium species are facultatively anaerobic bacteria having a fermentative type of carbohydrate metabolism. Their growth is considerably enhanced in an atmosphere of increased CO_2 tension. Growth is sparse on ordinary media but enhanced by blood or serum. The optimum temperature for growth is 37 °C. Organisms will not withstand heating at 60 °C for 15 min. Fermentation end products of carbohydrate metabolism are acetic and lactic acids; the amount of succinic acid produced may vary from species to species and may even be difficult to detect. Catalase activity is usually negative, but some strains of *A. haemolyticum* may show weak catalase production. The physiological properties of *Arcanobacterium* species are summarized in *Table 4.8*. Generally, members of the genus *Arcanobacterium* are characterized by a positive CAMP test (*Fig. 4.6b*), but they are negative for nitrate reduction and urease activity.

Interestingly, two biotypes of *A. haemolyticum* that correlates to the site of isolation have been recognized on the basis of colony morphology, β-hemolysis on horse blood agar, β-glucuronidase activity, and ability to ferment sucrose and/or trehalose identified (Carlson et al. 1994b). One, the smooth type, colonies appear smooth, β-hemolytic, and β-glucuronidase negative and often ferment sucrose and/or trehalose, while the other, the rough type, colonies appear rough, nonhemolytic, and β-glucuronidase positive and do not ferment sucrose and trehalose. Both types ferment glucose and maltose. The smooth biotype is more associated with wound infections, while the rough type is more frequently associated with respiratory tract isolates.

Chemotaxonomic Properties

The primary structure of the peptidoglycan of several *Arcanobacterium* species has been determined (Collins et al. 1982a; Lawson et al. 2001a; Schaal et al. 2006; Yassin et al. 2011). This corresponds to peptidoglycan type A5α according to the nomenclature of Schleifer and Seidl (1985). Thus, *A. haemolyticum*, *A. phocae*, *A. pluranimalium*, and *A. hippocoleae* exhibit variation (L-Lys-L-Lys-D-Glu). The muramic acid residue of the glycan moiety is *N*-acetylated. The principal respiratory quinones are tetrahydrogenated menaquinones with eight MK-8(H4) and nine MK-9(H4) isoprene units, with the latter component being the major one (*Table 4.3*).

Reports on the phospholipids found in *Arcanobacterium* species are controversial. Phosphatidylcholine (PC) has been reported as key diagnostic phospholipid in *A. haemolyticum*, *A. phocae* (Schaal et al. 2006), and in *A. canis* (Hijazin et al. 2012b). However, in a comparative chemotaxonomic study of members of the genus *Arcanobacterium*, phosphatidylcholine was not detected in *A. haemolyticum*, *A. phocae*, *A. pluranimalium*, or *A. hippocoleae* (Yassin et al. 2011). Thus, according to Yassin et al. (2011) the phospholipids found in arcanobacteria include diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylinositol (PI). Nevertheless, in a subsequent emendation of the genus *Arcanobacterium*, Hijazin et al. (2012b) concluded that members of the genus may contain phosphoglycolipid and excluded the presence of PG without referring to the distribution of PC.

The fatty acids profiles are composed of predominantly straight-chain saturated and monounsaturated acids. Major fatty acids are oleic acid ($C_{18:1}\omega9c$) and hexadecanoic acid ($C_{16:0}$). Small amounts of *cis*-vaccenic acid (C18:1ω7c) were detected in *A. haemolyticum*, *A. phocae*, and *A. pluranimalium* (Yassin et al. 2011). Substantial amounts of diunsaturated fatty acids ($C_{18:2}\omega6,9c$) have been detected in all species (Yassin et al. 2011). Methyl-branched-chain (*iso*- and *anteiso*-) were present in minor amounts.

Whole-cell hydrolysates from *A. haemolyticum*, *A. phocae*, *A. pluranimalium*, and *A. hippocoleae* contained rhamnose as diagnostic sugar. Glucose was also detected in these species.

Taxonomic Comments

Arcanobacterium species resemble a variety of other Gram-positive, diphtheroidal, or coccobacillary genera morphologically and/or physiologically. However, recent chemotaxonomic and molecular studies have led to the reevaluation of the genus (Yassin et al. 2011). According to the latter authors the taxonomic circumscription of the genus *Arcanobacterium* established by Collins et al. (1982b) has been modified to emphasize the presence of MK-9(H4) as predominant menaquinone, peptidoglycan type A5α, variation (L-Lys-L-Lys-D-Glu), rhamnose and glucose as diagnostic whole-cell sugars, and phospholipid type PI according to Lechevalier et al. (1977), in addition to a positive CAMP test. These features allowed delimitation of the genus *Arcanobacterium* from the genera *Actinomyces* (as represented by *Actinomyces bovis* the type species of the genus *Actinomyces* sensu stricto), *Actinobaculum*, *Mobiluncus*, *Trueperella*, and *Varibaculum* (*Table 4.3*).

Currently, the genus *Arcanobacterium* comprises five validly published species that form a monophyletic clade in the 16S rRNA gene phylogenetic tree of the family *Actinomycetaceae*. The unresolved placement of *Arcanobacterium hippocoleae* in the phylogenetic tree is due to limited sequence divergence between this species and other members of the genus *Arcanobacterium* (95.1–96.1 % sequence similarity) from one side and members of the genus *Trueperella* (94.1–95.1 % sequence similarity) from the other side. Additionally, there is a potential discrepancy between *A. hippocoleae* and the other species of the genus *Arcanobacterium*, concerning the set of signature nucleotides characteristic for the genus (*Table 4.7*). An obvious explanation for the latter case is that *A. hippocoleae* share with the genus *Trueperella* the set of signature nucleotides at position: 440 (C), 443(U), and 446–488 (A-U). However, the presence of MK-9(H4) as the major menaquinone provides strong evidence that *A. hippocoleae* is specifically related to the genus *Arcanobacterium* and should be retained in the genus.

Besides the chemotaxonomic and phylogenetic evidences, the results of the Christie–Atkins–Munch–Petersen (CAMP) reaction (Christie et al. 1944) are consistent with a distinct phylogenetic group. The CAMP reaction describes the synergistic hemolysis of sheep erythrocytes by the CAMP factor (an extracellular protein) from *Streptococcus agalactiae* and the β-toxin from *Staphylococcus aureus*. All *Arcanobacterium* species are characterized by the production of hemolysis on sheep or horse blood agar and hence display positive CAMP test (*Fig. 4.6b*). This reveals the existence of the gene (*cfa*) that encodes the CAMP factor family protein in the genome of *Arcanobacterium* species, and thus they likely share a common ancestor that already possessed this gene. Although the *cfa* gene has been identified in the only available completed genome sequence of *Arcanobacterium haemolyticum*, the presence of this gene in other species needs to be confirmed.

Definition of the Genus *Arcanobacterium* Sensu Stricto

Cells are slender, irregular, and predominately rod-shaped or arranged at an angle to give V formations during the first 18 h of growth, becoming granular and segmented, resembling small, irregular cocci over time. Both rod-shaped and coccoid cells are Gram-reaction-positive, non-acid-fast, and nonmotile. Endospores are not formed. Strains grow at 37 °C in a 5% CO_2 atmosphere or under anaerobic conditions. Growth is sparse on ordinary media but enhanced on blood- or serum-containing media. The CAMP test is positive with *S. aureus*. The peptidoglycan is of the type A5α, variation L-Lys–L-Lys–D-Glu. The muramic acid residue of the peptidoglycan is *N*-acetylated. The principal menaquinone is MK-9(H_4). Characteristic whole-cell sugars are rhamnose and glucose. The phospholipids comprise DPG and PI but PG and PC may be detected. Mycolic acids are not present. Long-chain fatty acids are primarily straight-chain saturated, monounsaturated, and diunsaturated (C18:2ω6,9c) as well as branched-chain *iso*- and *anteiso*-types. The DNA G+C content is 50–57 mol%. The type species is *Arcanobacterium haemolyticum*.

Identification and Descriptive Characteristics of *Arcanobacterium* Species

The phenotypic characteristics useful for identification of the recognized *Arcanobacterium* species are given in *Table 4.8*.

List of Species of the Genus *Arcanobacterium*

Arcanobacterium haemolyticum (Mac Lean, Liebow, and Rosenberg 1946), Collins, Jones, and Schofield 1983, 438^{VP} (*Corynebacterium haemolyticum* Mac Lean, Liebow, and Rosenberg 1946, 69).

hae. mo. ly' ti. cum. Gr. neut. n. *haema*, blood; Gr. adj. *lyticus*, dissolving, M.L. neut. adj. *haemolyticum*, blood-dissolving, hemolytic.

Surface colonies on blood agar are small (0.75 mm in diameter) after 24 h, becoming large (1.5–2.5 mm in diameter) on extended incubation. Colonies are circular discoid and slightly raised and β-hemolytic. Growth is sparse on ordinary media but is enhanced by blood or serum. Slender, irregular rods predominate during the first 18 h on blood agar; many cells exhibit V forms. Upon extended incubation, organisms become granular and segmented and resemble small irregular cocci. On Loeffler medium, they maintain the slender, irregular, bacillary form but become pleomorphic at 48 h, with numerous club and comma forms. Facultative anaerobi. The optimum temperature for growth is 37 °C. The organism will not withstand heating at 60 °C for 15 min. Acid is produced from glucose, lactose, and some other sugars. Catalase is negative. Extracellular DNase is produced. Gelatin, esculin, and casein are not hydrolyzed. β-Galactosidase and *N*-acetyl-β-glucosaminidase are produced, but β-glucuronidase and α-fucosidase are not. Strain is inhibited by tetracycline (30 pg per disc). The species exhibits the characteristics listed in the definition of the genus *Arcanobacterium* sensu stricto. Other phenotypic features are given in *Table 4.8*.

The mol% G+C of the DNA is 50–52.

Type strain, ATCC 9345 = DSM 20595, isolated from infections among American soldiers.

Arcanobacterium hippocoleae Hoyles, Falsen, Foster, Rogerson and Collins 2002, 619^{VP}.

hip. po. co' le. ae. Gr. masc. and fem. n. *hippos*, horse; Gr. masc. n. koleós, sheath, vagina; M.L. fem. gen. n. *hippocoleae*, of the horse vagina.

Cells are non-branching, irregular-shaped rods which stain Gram-positive, are non-acid-fast, and nonmotile. On Columbia blood agar supplemented with 5% sheep blood, colonies are cirular, entire-edged, convex, shiny, opaque, and gray, surrounded by small zone of beta-hemolysis. Strain is facultatively anaerobic and catalase-negative. Acid is produced from D-glucose and lactose, but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, pullulan, mannitol, melibiose, melezitose, methyl-β-D-glucopyranoside, D-ribose, D-raffinose, sucrose, D-sorbitol, tagatose, trehalose, or D-xylose. Acid may or may not be produced from maltose depending on the test system used. α-Glucosidase, β-galactosidase, β-glucuronidase, leucine arylamidase, and phosphoamidase are detected, but not acid phosphatase, alanine–phenylalanine–proline arylamidase, arginine dihydrolase, chymotrypsin, esterase C-4, ester lipase C8, α-fucosidase, α-galactosidase, β-glucosidase, lipase C14, α-mannosidase, β-mannosidase, pyrrolidonyl arylamidase, pyroglutamic acid arylamidase, pyrazinamidase, trypsin, valine arylamidase, urease, or glycyl-tryptophan arylamidase. *N*-Acetyl-β-glucosaminidase and alkaline phosphatase may or may not be detected depending on the test system used. Esculin (weak reaction) and hippurate are hydrolyzed, but not gelatin. Acetoin is not produced. Nitrate is not reduced to nitrite. The species exhibits the characteristics

listed in the definition of the genus *Arcanobacterium* sensu stricto. Habitat is not known.

The mol% G+C of the DNA has not been reported.

Type strain, CCUG 44697 = CIP 106850, isolated from vaginal discharge from a horse.

Arcanobacterium phocae Pascual Ramos, Foster and Collins 1997, 52^{VP}.

phoc'ae. N.L. gen. n. *phocae*, of *Phoca*, because the organism has been isolated from seals [the genus *Phoca*].

Cells are Gram-stain-positive and non-acid-fast. They are pleomorphic coccbacilli, short rods and dephtheroid. Cells are nonmotile and nonsporeforming. Colonies on blood agar (incubated for 24 h) are white, tiny, circular, low convex, and surrounded by a zone of β-hemolysis which may be two to three times the diameter of the colony. Strain is facultative anaerobic. Growth is not enhanced by increased concentrations of CO_2 (5–10 %). Catalase reaction is variable. Metabolism is strictly fermentative. Acid but not gas is produced from glucose. Acid is produced from glycerol, D-ribose, galactose, D-glucose, D-fructose, *N*-acetylglucosamine, maltose, lactose, sucrose, starch, glycogen, D-turanose, and 5-ketogluconate. Most strains ferment mannose, inositol, trehalose, and melezitose, and a few strains ferment mannitol, D-xylose, D-tagatose, and gluconate. Acid is not produced from erythritol, cellobiose, D-arabinose, L-arabinose, L-xylose, adonitol, β-methyl-D-xyloside, sorbose, rhamnose, dulcitol, D-sorbitol, α-methyl-mannoside, α-methyl-D-glucoside, amygdalin, arbutin, salicin, melibiose, inulin, D-raffinose, xylitol, gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, and 2-ketogluconate. Nitrate reduction is negative. Gelatin, esculin, and urea are not hydrolyzed. Pyrazinamidase, acid phosphatase, alkaline phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, esterase (C4), esterase–lipase (C8), cystine arylamidase, and leucine arylamidase are produced. Production of trypsin is variable. Pyrrolidonyl arylamidase, lipase (C14), valine arylamidase, chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are not produced. The species exhibits the characteristics listed in the definition of the genus *Arcanobacterium* sensu stricto. Other phenotypic features are given in ❯ *Table 4.8*.

Arcanobacterium phocae has been isolated from various tissues and fluids of common seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus*), for which its pathological significance is unclear, but it has been recovered in mixed cultures from pneumonic and septicemic seals.

The mol% G+C of the DNA has not been reported.

Type strain, M1590/94/3 = DSM 10002 = CIP 105740, isolated from the lung of common seal (*Phoca vitulina*). The type strain possesses the characteristics of the species except that it is catalase-positive and produces trypsin. M1590/94/3 ferments inositol, mannitol, mannose, melezitose, trehalose, and D-xylose but does not ferment D-tagatose and gluconate.

Arcanobacterium pluranimalium Lawson, Falsen, Foster, Eriksson, Weiss and Collins 2001, 58^{VP}.

plur. a. ni. ma' li. um. L. comp. adj. *plus*, *pluris*, more, a greater quantity; L. gen. pl. n. *animalium*, from animals; M.L. gen. n. *pluranimalium*, from many animals.

Cells are straight to slightly curved, non-branching, slender rods which stain Gram-positive and are non-acid-fast and nonmotile. Strain is facultative anaerobic and catalase-positive. Acid is produced from D-glucose and D-ribose, but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, pullulan, D-sorbitol, tagatose, mannitol, melibiose, melezitose, lactose, raffinose, sucrose, trehalose, or D-xylose. Maltose is variable. Alanine–phenylalanine–proline arylamidase, pyroglutamic acid arylamidase, and β-glucuronidase are detected, but not arginine dihydrolase, alkaline phosphatase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-galacturonidase, *N*-acetyl-β-glucosaminidase, β-mannosidase, or urease. Pyrazinamidase and glycyl-tryptophan arylamidase may or may not be detected. Esculin (weak reaction), gelatin, and hippurate are hydrolyzed. Acetoin is not produced. Nitrate is not reduced to nitrite. The species exhibits the characteristics listed in the definition of the genus *Arcanobacterium* sensu stricto. Other phenotypic features are given in ❯ *Table 4.8*. Habitat is not known.

The mol% G+C of the DNA is 57.

Type strain, CCUG 42575 = DSM 13483, isolated from a dead harbor porpoise and a dead sallow deer.

Arcanobacterium canis Hijazin, Prenger-Berninghoff, Sammra, Alber, Lämmler, Kämpfer, Glaeser, Busse, Hassan, Abdulmawjood and Zschöck 2012, 2203^{VP}.

can'is. L. gen. n. *canis* of a dog.

Cells are Gram stain-positive, nonmotile, and non-sporeforming rods (1–2 µm long and 0.5 µm wide). Growth occurs on sheep blood agar with a weak zone of hemolysis under microaerobic conditions (the zone of hemolysis is less pronounced under aerobic and anaerobic conditions). CAMP reaction is positive with a strain of *Rhodococcus equi*. Acid is produced from D-glucose, D-ribose, maltose, lactose, sucrose, and glycogen, but not from D-xylose or D-mannitol. Activity of the following enzymes is observed: alkaline phosphatase, β-glucuronidase, β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, DNase, and amylase are positive. Hyaluronidase, pyrazinamidase and pyrrolidonyl arylamidase activities are not detected. Casein, esculin, gelatin and urea are not hydrolyzed. Nitrate is not reduced to nitrite. The menaquinone and polar lipid profiles are typical of the genus. The polyamine pattern contains putrescine, spermine, and spermidine. Cellular fatty acids contain major proportions of $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}\omega9c$, and $C_{18:2}\omega6,9c$/anteiso-$C_{18:0}$. Habitat is not known.

The mol% G+C of the DNA has not been reported.

Type strain, CCUG 61573^{T} = CIP 110339^{T}, isolated from canine otitis externa.

Genus III. *Trueperella* Yassin, Hupfer, Siering and Schumann 2011, 1272^{VP}.

Tru. e. pe. rel 'la. N.L. fem. dim. n. *Trueperella*, named after Hans Georg Trüper, the German microbiologist.

Taxonomy, Historical and Current

The genus *Trueperella* was described by Yassin et al. (2011) to accommodate bacterial species previously classified in the genus *Arcanobacterium*. The description of the genus was based on chemotaxonomy and on phylogenetic data of the 16S rRNA gene sequences. To arrive the current taxonomy, members of the genus had undergone a number of taxonomic changes, such as *Trueperella pyogenes*, the type species of the genus, was proposed for the invalidly named species "*Arcanobacterium pyogenes*" proposed by Pascual Ramos et al. (1997a) for the invalidly named species "*Actinomyces pyogenes*," which in turn was reclassified from the invalidly named species "*Corynebacterium pyogenes*" by Reddy et al. (1982) and Collins and Jones (1982) and *Trueperella bernardiae* named for "*Arcanobacterium bernardiae*" proposed by Pascual Ramos et al. (1997a), which in turn was reclassified from the invalidly named species "*Actinomyces bernardiae*" proposed by Funke et al. (1995) for facultative anaerobic, Gram-positive rods that were referred "CDC fermentative coryneform group 2" by workers at the Centers of Disease Control (CDC).

The taxonomic position of "*Corynebacterium pyogenes*" has been always controversial and its retention within the genus *Corynebacterium* has been questioned by several authors (Cummins and Harris 1956; Barksdale et al. 1957; Barksdale; 1970; Jones 1975; Collins et al. 1982a). Cummins and Harris (1956), on the basis of cell wall sugar and amino acid composition, suggested the inclusion of "*C. pyogenes*" and "*C. haemolyticum*" in the genus *Streptococcus*. This view was supported by Barksdale et al. (1957) who further suggested that "*C. haemolyticum*" was a mutant form of "*C. pyogenes*." In the 8th edition of *Bergey's Manual of Determinative Bacteriology*, "*C. pyogenes*" is retained as an addendum to the genus *Corynebacterium* (Cummins et al. 1974), and it appears in the *Approved Lists of Bacterial Names* (Skerman et al. 1980). Later, numerical taxonomic (Schofield and Schaal 1981) and chemical (Collins et al. 1982a) studies indicated that "*C. pyogenes*" and "*C. haemolyticum*" are distinct taxa. The lipid data are not in accord with the views of Barksdale et al. (1957) and Cummins and Harris (1956) that "*C. pyogenes*" should be reclassified in the genus *Streptococcus*. In addition, the discovery of tetrahydrogenated menaquinones with ten isoprene units [MK-10(H4)] predominated in "*C. pyogenes*" is neither in accord with the inclusion in the genus *Streptococcus* nor the retention of this species in the genus *Corynebacterium*. Moreover, the presence of peptidoglycan based on lysine in "*C. pyogenes*" supports this view since this amino acid is present in the peptidoglycan of *A. bovis* (Schleifer and Kandler 1972). Therefore, because of the close chemical and physiological similarity to *A. bovis*, the type species of the genus *Actinomyces*, Collins and Jones (1982) and Reddy et al. (1982) reclassified "*C. pyogenes*" in the genus *Actinomyces* as "*Actinomyces pyogenes*."

Nonetheless, application of phylogenetic analysis based on 16S rRNA gene sequencing of the genus *Actinomyces* revealed that "*A. pyogenes*" along with "*A. bernardiae*" cluster together with *Arcanobacterium haemolyticum* (Pascual Ramos et al. 1997a). Consequently, "*Actinomyces pyogenes*" and "*Actinomyces bernardiae*" have been reclassified and assigned to the genus *Arcanobacterium* as "*Arcanobacterium pyogenes*" and "*Arcanobacterium bernardiae*," respectively. Finally, further study based on comparative chemotaxonomic and phylogenetic analysis of the genus *Arcanobacterium* indicated that members of the genus *Arcanobacterium* clusters in two groups (Yassin et al. 2011). The presence of MK-10(H4) as predominant menaquinone in conjunction with a set of signature nucleotides in one cluster justified the erection of a new genus, *Trueperella*. Currently, the genus *Trueperella* harbors five species, including *Trueperella pyogenes*, *T. abortisuis*, *T. bernardiae*, *T. bialowiezense*, and *T. bonsai*.

Molecular Analyses

Similarity values of 16S rRNA gene sequence analyses for members of the genus *Trueperella* range from 95.4 % to 98.1 %. Phylogenetic analysis based on 16S rRNA gene sequences revealed a strongly supported monophyletic clade (96 % bootstrap value) sister to the genus *Arcanobacterium* (*Fig. 4.1a*) and together, though weakly supported (67 % bootstrap), appeared as potential sister to the genus *Actinobaculum*. The presence of a set of signature nucleotides (*Table 4.1*) in the 16S rRNA gene sequence of all *Trueperella* species underlines the phylogenetic coherence of this group. This includes nucleotides at 157–164 (G-U), 440 (C), 443 (U), 446–488 (A-U), 598–642 (C-A), and 615–625 (G-U).

Phenotypic Analyses

Gross Morphology

Surface colonies of *Trueperella* species incubated for 48 h under anaerobic conditions on blood agar are nonpigmented, pinpoint, smooth, circular, with entire edge, convex, translucent, and surrounded by a zone of β-hemolysis (Collins and Jones 1982; Funke et al. 1995; Lehnen et al. 2006; Reddy et al. 1982). Colony diameters are 0.2–1.5 mm after 48–72 h of incubation. All *T. pyogenes* strains exhibit β-hemolysis on agar media containing bovine or ovine blood (Pascual Ramos et al. 1997a), although strains of porcine origin are generally more hemolytic (Timoney et al. 1988). Some strains of *T. bernardiae* and *T. abortisuis* either are nonhemolytic or possess a very narrow zone of β-hemolysis.

Cellular Morphology

Gram stains of *Trueperella* strains grown on blood agar show slender, irregular bacillary forms. Cells are 0.2 × 2 μm and occur mostly singly and in pairs (V and T formations and

some palisades). Chains of coccoid forms resembling streptococci may be seen but short, diphtheroid forms normally predominate. All forms are Gram-reaction-positive, but cells from older cultures may be Gram-reaction-variable. Cells are nonmotile and non-acid-fast. Endospores are not formed.

Biochemical Characteristics

Of the different biochemical characteristics evaluated for identification of *Trueperella* species, only four tests yield uniform results. These include fermentation of glucose, lack of catalase, and urease activities and inability to reduce nitrate. The ability to liquify gelatin is at present restricted to *T. pyogenes*. Furthermore, *T. pyogenes* has been found to be positive by the CAMP reaction. The major phenotypic features useful in the separation of *Trueperella* species are listed in ❯ *Table 4.9*.

Trueperella are facultatively anaerobic having a fermentative type of metabolism. Metabolic end products of glucose fermentation include succinate, acetate, and lactate.

Chemotaxonomic Properties

The mode of cross-linkage and the amino acid composition of the tetrapeptide bridge of the peptidoglycan layer of *Trueperella* species have been investigated (Collins et al. 1982a; Funke et al. 1995; Yassin et al. 2011). This corresponds to peptidoglycan type A5α according to the nomenclature of Schleifer and Seidl (1985) (❯ *Table 4.3*). However, two peptidoglycan variations could be identified among individual species: variation L-Lys-L-Ala-L-Lys-D-Glu has been identified in *T. pyogenes*, *T. bernardiae*, and *T. bialowiezensis* and variation L-Lys-L-Lys-D-Glu has been characterized in *T. abortisuis* and *T. bonsai*.

Regardless of the variation encountered in the peptidoglycan, the menaquinone pattern of *Trueperella* species consistently correlate with a cohesive group (❯ *Table 4.3*). All species contain tetrahydrogenated menaquinones with nine, ten, and eleven isoprene units, abbreviated MK-9(H4), MK-10(H4), and MK-11(H4), respectively, with MK-10(H4) being the major component (Collins et al. 1982a; Lehnen et al. 2006; Yassin et al. 2011).

Furthermore, *Trueperella* species possess characteristic phospholipid patterns consisting of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphoglycolipids with unknown chemical structure, and a number of incompletely characterized phospholipids. The presence of phosphoglycolipids is considered an important feature to substantiate the genus (Yassin et al. 2011). Although Lehnen et al. (2006) reported the presence of an unknown aminolipid (AL2) and an unknown aminophospholipid in *T. bialowiezensis* and *T. bonsai* and Azuma et al. (2009) reported the presence of phosphatidylethanolamine (PE) in *T. abortisuis*, the distribution of nitrogen-containing phospholipids is considered absent from the genus (Yassin et al. 2011).

The cellular fatty acids of *Trueperella* species are composed of predominantly straight-chain saturated and monounsaturated acids, in addition to trace amounts of diunsaturated ($C_{18:2}\omega6,9c$), methyl-branched-chain (*anteiso-*, *iso-*) fatty acids. Tetradecanoic acid ($C_{14:0}$), hexadecanoic acid ($C_{16:0}$), and oleic acid ($C_{18:1}\omega9c$) predominated in all *Trueperella* species. Additionally, substantial amounts of hexadecenoic ($C_{16:1}\omega7c$) and octadecanoic ($C_{18:0}$) acids were also detected. Moreover, the cellular fatty acid profiles of *T. bialowiezensis* and *T. bonsai* contain traces of $C_{18:1}\omega7c$.

The cell wall of *Trueperella* species is characterized by the presence of rhamnose and glucose as diagnostic sugars (Cummins and Harris 1956; Yassin et al. 2011). The muramic acid residues of the glycan moiety of the cell wall peptidoglycan are *N*-acetylated. The DNA G+C content of member of the genus range from 56 % to 66 % mol% (Yassin et al. 2011).

Identification and Descriptive Characteristics of *Trueperella* Species

The phenotypic characteristics useful for identification of the recognized *Trueperella* species are given in ❯ *Table 4.9*.

List of Species of the Genus *Trueperella*

Trueperella abortisuis Azuma, Murakami, Ogawa, Okada, Miyazaki, and Makino 2009, 1471^{VP}.

(a.bor.ti.su'is. L. n. *abortus*, *abortus*, an abortion; L. n. *sus*, *suis*, a pig, a sow; N.L. gen. n. *abortisuis*, from an abortion of a sow).

Cells are Gram-stain-positive, diphtheroid-shaped rods and may arranged in V-shaped forms or palisades. Colonies on Columbia blood agar are smooth, entire-edged, translucent, and nonhemolytic. Facultatively anaerobic. The strain is catalase-negative. Indole production is negative. Reduces nitrate. Soluble starch is hydrolyzed, but gelatin is not. Produces acid from fructose, galactose, glucose, cellobiose, lactose, maltose, sucrose, trehalose, melezitose, raffinose, inulin, soluble starch, adonitol, and glycerol. Does not ferment arabinose, ribose, xylose, rhamnose, mannose, sorbose, melibiose, amygdalin, arbutin, salicin, dulcitol, erythritol, mannitol, sorbitol, or inositol. Produces α-galactosidase, β-galactosidase, α-glucosidase, and leucine arylamidase but not urease, β-glucosidase, or pyroglutamic acid arylamidase. Fermentation end products are lactic, succinic, and acetic acids. Whole-cell sugars include rhamnose and ribose. Cell wall peptidoglycan contains glutamic acid, alanine, and lysine in the molar ratio of 1:2:1. Major fatty acids are $C_{14:0}$, $C_{16:1}\omega7$, $C_{16:0}$, and $C_{18:1}\omega9$. The major menaquinone is MK-10(H_4). Polar lipids are phosphatidylethanolamine and two unknown phosphatidylinositol mannosides. The interpeptide bridge within the peptidoglycan has not been reported.

The mol% G+C of the DNA is 63.8.

Table 4.9

Biochemical characteristics of *Trueperella* species

Characteristics	*T. pyogenes*	*T. abortisuis*	*T. bernardiae*	*T. bialowiezensis*	*T. bonasi*
Synergistic CAMP reaction	+	–	–	–	–
Reverse CAMP reaction	–	–	–	–	–
Acid production from:					
L-Arabinose	d	–	–[a]	–	–
Cellobiose	–	ND	–	ND	ND
D-Glucose	+	+	+	+	+
Lactose	+	+	–	–	–
Maltose	+	+	+	–	–
D-Mannitol	d	–	–	–	–
Melezitose	+	+	ND	ND	ND
D-Raffinose	–	+	–[a]	–	–
L-Rhamnose	–	–	ND	ND	ND
D-Ribose	+	–	d	–	–
D-sorbitol	d	–	–	–	–
Sucrose	d	+	–	–	–
Trehalose	+	+	–	–	–
D-xylose	+	–	–	–	–
Hydrolysis of:					
Esculin	–[a]	+[a]	–	–[a]	–[a]
Gelatin	+	–[a]	–	–	–
Hippurate	–	+[a]	–[a]	–[a]	–[a]
Enzyme activities:					
N-Acetyl-β-glucosaminidase	–[a]	–[a]	+[a]	–	–
Catalase	–	–	–	–	–
Acid phosphatase	–	–[a]	–	+[a]	+[a]
Alkaline phosphatase	–[a]	–[a]	–	–	–
Chymotrypsin	–	–[a]	–	–	–
Cystine arylamidase	–	–[a]	–	–	–
Esterase Lipase C4	–	–[a]	–	–[a]	–[a]
Esterase Lipase C8	–	–[a]	–	+	+
α-Fucosidase	–	–[a]	–	–	–
α-Galactosidase	–	+	–	–	–
β-Galactosidase	+[a]	+	–[a]	–	–
β-Glucuronidase	+[a]	+[a]	–	+	+
α-Glucosidase	+[a]	+	+	+	–
β-Glucosidase	–	–	–	–	–
Lipase C14	–	–[a]	–[a]	–	–
Leucine arylamidase	+[a]	+[a]	+	–	w
α-Mannosidase	–	–[a]	–	–	–
Naphthol-AS-BI-phosphohydrolase	–	+[a]	+[a]	+[a]	+[a]
Pyrazinamidase	–[a]	–[a]	+[a]	+[a]	+[a]
Trypsin	–	–[a]	–	–	–
Valine arylamidase	–	–	–[a]	–	–
Urease	–[a]	–	–	–	–
Nitrate reduction	–	–[a]	–	–	–

Data for *T. pyogenes* and *T. bernardiae* are from Pascual Ramos et al. (1997a); data for *T. abortisuis* are from Azuma et al. (2009); data for *T.bialowiezensis* and *T. bonasi* are from Lehnen et al. (2006)

Symbols + positive/present, – negative/absent, *w* weakly positive, *ND* no data available, *d* differences between strains within one species

[a]Denote data from A. F. Yassin

Type strain: Murakami^T (= ATCC BAA-1522^T = DSM 19515^T = JCM 14813^T), isolated from a sow's placenta after an abortion.

Trueperella bernardiae (Funke, Pascual Ramos, Fernández-Garayzábal, Weiss, and Collins 1995), comb. nov. Pascual Ramos, Foster, and Collins 1997, 51^{VP} (*Actinomyces bernardiae* Funke, Pascual Ramos, Fernández-Garayzábal, Weiss, and Collins 1995, 59).

(ber. nar' di.ae. N.L. gen. n. *bernardiae*, of Bernard, named after the contemporary Canadian microbiologist Kathryn A. Bernard for her contributions to the study of asporogenous, Gram-positive rods).

Cells are Gram-positive rods, with coccobacilli predominating. Cells are sometimes arranged in clusters, but primary branching is not observed. Cells are nonmotile and do not form spores. Colonies are circular, smooth, and slightly convex with a glassy appearance. Colony diameters range from 0.2 to 0.5 mm after 48 h of incubation in the presence of 5 % CO_2 on sheep blood agar. Catalase is not produced. Acid is produced from glycerol, ribitol, adonitol, D-glucose, D-fructose, maltose, starch, xylitol, D-arabitol, and 5-ketogluconate. Most strains ferment erythritol, ribose, glycogen, and L-arabitol. Acid is not produced from xylose, mannitol, α-methyl-D-mannoside, amygdalin, arbutin, sucrose, and 2-ketogluconate. Activity is detected for leucine arylamidase and α-glucosidase. No activity is detected for alkaline phosphatase, cystine arylamidase, chymotrypsin, trypsin, acid phosphatase, α-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, and α-fucosidase. Esculin and urea are not hydrolyzed. Nitrate is not reduced. Palmitic and stearic acids are the main straight-chain cellular fatty acids, while oleic acid is the predominant unsaturated fatty acid. The major menaquinone is MK-10(H_4). The interpeptide bridge within the peptidoglycan is L-Lys-L-Ala-L-Lys-D-Glu (type A 5α).

The mol% G+C of the DNA is 63–66.

Type strain, CCUG 33419 = DSM 9152, isolated from human blood.

Trueperella bialowiezense Lehnen, Busse, Frölich, Krasinska, Kämpfer, and Speck 2006, 864^{VP}.

(bi.a.lo.wi.e.zen'se. N.L. neut. adj. *bialowiezense*, pertaining to Bialowieza, Poland, where the type strain was isolated).

Cells are short pleomorphic rods. After 48 h of growth under aerobic conditions on sheep blood agar, colonies are translucent, convex, approx. 0.5 mm in diameter, and surrounded by a narrow zone of β-hemolysis. Cells are nonmotile (motility agar) and facultatively anaerobic and are catalase- and oxidase-negative. Best growth occurs at 37 °C; no growth at 42 °C. No growth is observed on Gassner or MacConkey agar. Nitrate reduction is negative. Esculin and gelatin are not hydrolyzed. Hippurate hydrolysis is positive. Acid is produced from glycerol, D-glucose, and D-fructose. Acid is not produced from adonitol, D-arabitol, L-arabitol, erythritol, glycogen, inulin, D-lactose, maltose, D-mannitol, raffinose, ribose, D-sorbitol, starch, sucrose, trehalose, and D-xylose. Activity is detected for acid phosphatase, esterase–lipase (C8), naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-glucosidase, and pyrazinamidase. No activity is detected for alkaline phosphatase, cystine arylamidase, chymotrypsin, α-galactosidase, *N*-acetyl-β-glucosaminidase, β-glucosidase, α-fucosidase, leucine arylamidase, α-mannosidase, pyrrolidonyl arylamidase, trypsin, and urease. The quinone system contains MK-10(H_4) as the predominant compound. The interpeptide bridge within the peptidoglycan has not been reported.

The mol% G+C of the DNA has not been reported.

Type strain, DSM 17162 = NCTC 13354, isolated from the prepuce of a European bison.

Trueperella bonasi Lehnen, Busse, Frölich, Krasinska, Kämpfer, and Speck 2006, 864^{VP}.

(bo. na' si. L. gen. n. *bonsai*, of the European bison (*Bison bonasus*) from which the type strain was isolated).

Cells are short pleomorphic rods. After 48 h of growth under aerobic conditions on sheep blood agar, colonies are translucent, convex, approx. 0.5 mm in diameter, and surrounded by a narrow zone of β-hemolysis. Cells are nonmotile (motility agar), facultatively anaerobic, and catalase- and oxidase-negative. Best growth occurs at 37 °C; no growth at 42 °C. No growth is observed on Gassner or MacConkey agar. Nitrate reduction is negative. Esculin and gelatin are not hydrolyzed. Hippurate hydrolysis is positive. Acid is produced from D-arabitol, L-arabitol, D-fructose, and trypsin. Acid is weakly produced from adonitol, erythritol, and glycerol. Acid is not produced from erythritol, D-glucose, glycogen, inulin, D-lactose, maltose, D-mannitol, raffinose, ribose, D-sorbitol, starch, sucrose, trehalose, and D-xylose. Activity is detected for acid phosphatase, esterase–lipase (C8), lipase (C14), naphthol-ASBI-phosphohydrolase, β-glucuronidase, pyrazinamidase, and pyrrolidonyl arylamidase. No activity is detected for alkaline phosphatase, cystine arylamidase, chymotrypsin, α-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-fucosidase, leucine arylamidase, α-mannosidase, and urease. The quinone system contains MK-10(H_4) as the predominant compound. The interpeptide bridge within the peptidoglycan has not been reported.

The mol% G+C of the DNA has not been reported.

Type strain, DSM 17163 = NCTC 13355, isolated from the prepuce of a European bison.

Trueperella pyogenes (Glage 1903), Pascual Ramos, Foster and Collins 1997, 51^{VP}. (*Bacillus pyogenes* Glage 1903, 1973; *Corynebacterium pyogenes* (Glage) Eberson 1918, 23; *Actinomyces pyogenes* Reddy, Cornell and Fraga 1982, 427). (Gr. neut. n. *pyon*, pus; Gr. v. *gennaein*, to produce; N. L. adj. *pyogenes*, pus-producing).

Cells are Gram-stain-positive coccobacilli or short rods that may occur singly, in pairs or clusters and may arranged in V-forms or T-forms and palisade. Cells are nonmotile and nonsporeforming. Streptococcal forms in small clumps and short crooked chains are occasionally observed. Cells vary in shape and size (0.2 to 0.9 by 0.3 to 2.5 μm) in different media. Cells from old culture (more than 48 hours growth) stain unevenly and may be Gram-variable. The cell wall ultrastructure is typical of Gram-positive bacteria. The cell walls are 29–30 nm

thick and have a characteristic double-track appearance. Pinpoint, β-hemolytic colonies occur on sheep blood agar after 24 h of incubation. The zones of hemolysis are typically two to three times the diameter of the colony. After 48–72 h, the colonies (diameter 0.5–1.5 mm) are convex, circular, opaque, white, and soft with entire edges. Colonies develop faster and are bigger (diameter 1.5–3.0 mm) on SFM agar plates. Good growth occurs under aerobic and strictly anaerobic conditions. Metabolism is strictly fermentative. Acid but not gas is produced from glucose, fructose, galactose, lactose, cellobiose, trehalose, maltose, melezitose, mannose, glycogen, dextrin, xylose, and starch. The fermentation of adonitol, arabinose, erythritol, glycerol, sucrose, mannitol, and sorbitol varies with the strain. No acid is produced from amygdalin, esculin, melibiose, raffinose, rhamnose, or salicin. Alanine, arginine, aspartate, glycine, and threonine are not fermented. Most strains are catalase-negative, although one strain has been reported to be catalase-positive. Acid clotting and digestion of clots in litmus milk and liquefaction of gelatin are characteristic of all strains. Nitrates are not reduced, and indole is not produced. The optimum growth temperature is 37 °C; the temperature range is 20–40 °C.

Lactic acid is the primary metabolic product in Brain Heart Infusion or tryptose broth with no added HCO_3^-; acetate is a minor product. Glucose is fermented in the presence of CO_2/HCO_3^-, and this reaction yields succinate, acetate, formate, and lactate as major end products. For each 1 mol of CO_2/HCO_3^- fixed, 1 mol of succinate, 1 mol of acetate, and 1 mol of formate are produced. In identical media without added bicarbonate or hemin, lactate is the major product, and smaller amounts of acetate, succinate, and formate are produced. CO_2/HCO_3^- is required for growth. Hemin is highly stimulatory or required for growth. Peptides are required for growth even in the presence of a complete complement of 20 amino acids and $(NH_4)_2SO_4$. Inositol can replace the peptide requirement for growth. Riboflavin and nicotinic acid are required for optimal growth. Adenine and uracil are required for optimal growth of some strains. Characteristic cell wall sugar components are rhamnose and glucose. No mycolic acids are present. Culture filtrates are fatal to mice and rabbits after intravenous injection. The soluble hemolysin produced is active against human, guinea pig, sheep, horse, and rabbit erythrocytes. Both toxic and hemolytic activities of crude cell extracts are neutralized by antitoxin.

This organism is frequently isolated from a wide variety of pyogenic disease conditions in many species of domestic animals and in humans. Presumably, *Arcanobacterium pyogenes* occurs as a commensal organism on the mucous surfaces of warm-blooded animals. The interpeptide bridge within the peptidoglycan is L-Lys-L-Ala-L-Lys-D-Glu (Type A5 α). The principal menaquinones are MK-10(H4).

The mol% G+C of the DNA is 56–58.

Type strain, ATCC 19411 = NCTC 5224.

Genus IV. *Actinobaculum* Lawson, Falsen, Åkervall, Vandamme and Collins 1997, 902^{VP}.

Ac. ti. no. ba'cu. lum. Gr. n. *actis actinos*, ray; L. neut. n. *baculum*, rod, stick; N. L. neut. n. *Actinobaculum* ray stick.

Taxonomy, Historical and Current

The genus *Actinobaculum* was introduced by Lawson et al. (2007) to accommodate bacterial strains previously designated as "*Actinomyces suis*" and also some *Actinomyces*-like organisms originated from human sources. Currently, the genus comprises four validly published species: *Actinobaculum massiliense*, *Actinobaculum schaalii*, *Actinobaculum suis*, and *Actinobaculum urinale*. *A. suis*, the type species of the genus, has a convoluted taxonomic and nomenclatural history. In 1957 Soltys and Spratling proposed the name "*Corynebacterium suis*" for an anaerobic diphtheroid bacterium which was associated with cases of cystitis and pyelonephritis in pigs. The generic assignment was based almost exclusively on the diphtheroid morphology of the organism, a common practice at the time. The name "*Corynebacterium suis*" was not included in the Approved Lists of Bacterial Names (Skerman et al. 1980) and hence has no standing in nomenclature.

In a study by Wegienek and Reddy (1982) to determine the taxonomic status of "*Corynebacterium suis*," they found that "*Corynebacterium suis*" strain Soltys 50052 is anaerobic, has rhamnose and lysine as major cell wall components, and produces acetate, formate, and ethanol as major end products of carbohydrate metabolism. According to *Bergey's Manual of Determinative Bacteriology*, 8th ed., the genus *Corynebacterium* includes aerobic or facultatively anaerobic and non-spore-forming organisms that are characterized by arabinose and galactose as the major cell wall sugar components and *meso*-diaminopimelic acid as the major diamino acid (Rogosa et al. 1974). Furthermore, they produce major amounts of acetate, propionate, and formate and variable amounts of other acids as products of carbohydrate metabolism (Reddy and Kao 1978). Thus, Wegienek and Reddy (1982) concluded that "*Corynebacterium suis*" strain Soltys 50052 did not belong to the genus *Corynebacterium* and proposed, despite differences in cell wall composition and DNA base ratios, that the organism should be included in the genus *Eubacterium*. Their proposal was based on a rather limited number of characteristics such as anaerobiosis and morphology, as well as absence of propionate, lactate, and succinate among the end products of carbohydrate metabolism.

In a subsequent phylogenetic study, based on comparative analysis of the 16S rRNA gene sequences, Ludwig et al. (1992) found that "*Eubacterium suis*" is a close relative of "*Actinomyces pyogenes*" (93.8 % sequence similarity), and they also found that the two organisms share a common cell wall composition (lysine is the diamino acid; rhamnose and traces of mannose are the cell wall sugars). Moreover, according to previous studies (Soltys and Spratling 1957; Schaal and Pulverer 1981; Wegienek and Reddy 1982; Schaal 1986b) both "*Eubacterium suis*" and "*Actinomyces pyogenes*" contain cytochrome *c* and have similar DNA G+C contents (55 and 56–58 mol%, respectively). However, differences in the biochemical characteristics of "*Eubacterium suis*" Soltys and Spratling and the species incertae sedis "*Actinomyces*

suis" Franke 1973 indicate that these two organisms are not identical, and since no type strain of "*Actinomyces suis*" Franke is extant, Ludwig et al. (1992) proposed that "*Eubacterium suis*" should be transferred to the genus *Actinomyces* as "*Actinomyces suis*."

In a systematic phylogenetic study of the genus *Actinomyces*, Pascual et al. (1997) noted that the genus *Actinomyces* comprises a phylogenetically diverse group of organisms and is clearly in urgent need of taxonomic revision. One such organism is *Actinomyces suis* which displays approximately 10–14 % 16S rRNA sequence divergence with the *Actinomyces bovis* cluster of species and exhibit somewhat closer affinity with *Arcanobacterium* species (approximately 8–11 % sequence divergence). Considerations of both sequence divergence and tree topology stimulated Lawson et al. (1997) to reclassify *Actinomyces suis* in a new genus *Actinobaculum* as *Actinobaculum suis*. Interestingly, Lawson et al. (1997) also noted that five unknown bacterial isolates from clinical sources possess a specific affinity with *A. suis*. They confidently cluster with this species (bootstrap value of 100 %) and displayed approximately 6 % sequence divergence. Therefore, they concluded that the five clinical isolates represented a distinct species within the new genus as *Actinobaculum schaalii*.

Molecular Analyses

The molecular phylogeny of *Actinobaculum* based on 16S rRNA gene sequence data shows that the genus resolved as a monophyletic clade supported by 73 % bootstrap (*Fig. 4.1a*). Within this clade two genetically distinguishable lineages could be identified: one lineage (supported by 100% bootstrap value) uniting *A. massiliense* and *A. suis*. Both species sharing 95.2% 16S rRNA gene sequence similarity. The other lineage (not supported by bootstrap value) uniting *A. schaalii* and *A. urinale*. The level of 16S gene sequence similarity between the latter two species is 93.6%. This dichotomy was independently supported by molecular signatures in 16S rRNA genes (*Table 4.10*). The two lineages share 92.1–93.6 % similarity. These data imply that the genus *Actinobaculum* represents a paraphyletic assemblage of taxa and need a thorough revision. However, at present time, the small size of the genus (4 species) troubles this attempt to make an infrageneric classification.

Interestingly, phylogenetic analyses resolved a sister relationship between the genus *Actinobaculum* and the genera *Arcanobacterium* and *Trueperella*. However, this relationship is poorly supported (67 % bootstrap value).

Table 4.10
16S rRNA signature nucleotides that define the two lineages of the genus *Actinobaculum*

Position[a]	Lineage 1		Lineage 2	
	A. massiliense	*A. suis*	*A. schaalii*	*A. urinale*
70–98	A-U	A-U	G-A	G-C
86	U	U	G	G
100	C	C	U	U
140–223	A-U	A-U	C-G	C-G
141–222	A-U	A-U	U-A	U-A
142–221	C-G	C-G	U-A	U-A
144–178	G-C	G-C	U-A	U-G
157–164	G-U	G-U	U-G	U-G
176	U	U	C	C
184	UU	UU	GC	GC
194	U	U	G	G
307	U	U	C	A
457	G	G	A	A
1031	U	U	G	G
1032	U	U	G	G
1035	G	G	U	U
1133	G	G	A	A
1252	U	U	A	A
1283	U	U	C	C
1286	G	G	U	U
1292	C	C	U	U
1424	C	C	U	U
1477	U	U	C	C

[a]*Escherichia coli* position (Brosius et al. 1978).

Phenotypic Analyses

Morphology

Actinobaculum are Gram-positive, non-acid-fast, nonmotile, non-spore-forming straight to slightly curved rods. They grow slowly on blood-containing agar media after anaerobic incubation for 2–3 days. Isolates grew either well or poorly in air with 5 % CO_2 and either poorly or not at all in ambient air. *Actinobaculum suis* produces small, 2–3 mm in diameter, translucent, round, and granular colonies after 2 days growth on Columbia blood agar. After 7 days incubation, colonies flatten and develop a characteristic dry, gray opaque surface with crenated edges, attaining a size of 4–5 mm in diameter after a week of growth (*Fig. 4.7a*). No hemolytic effect is observed around the colonies. The striking colony morphology of *A. suis* easily separates it from other species of the genus. Cells of *A. suis* have coryneform morphology with size varying from 2 to 3 µm in length and from 0.3 to 0.5 µm in width (*Fig. 4.7b*). In contrast to the large, crenate-edged and dry colonies of *A. suis*, colonies of *A. massiliense*, *A. schaalii*, and *A. urinale* are small, <1 mm in diameter, smooth with entire margins, and nonhemolytic (Greub and Raoult 2002; Hall et al. 2003a).

Actinobaculum species are anaerobic bacteria with strict fermentative type of metabolism. Although, acetate has been reported as a basic end product of glucose or maltose catabolism, variations in fermentation end product profiles have been reported.

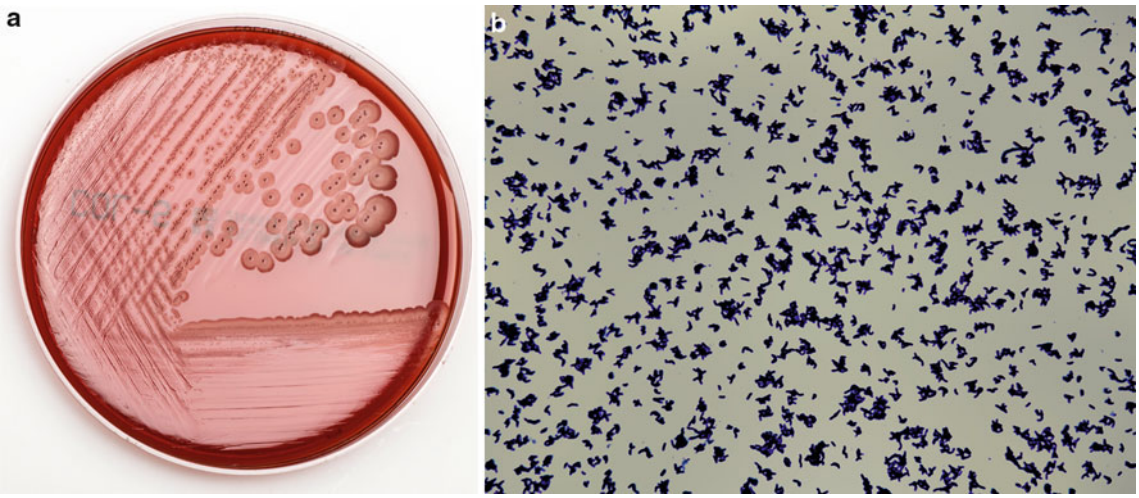

Fig. 4.7
(a) Culture of *Actinobaculum suis* DSM 20639^T on Columbia blood agr with 5% sheep blood The colonies are small, 2-3 mm in diameter, translucent, round and granular. After 7 days incubation, colonies flatten and develop a characteristic dry, gray opaque surface with crenated edges attaining a size of 4-5 mm in diameter. (b) Gram-stained smear from culture of *Actinobaculum suis* DSM20639^T on Columbia agar with 5% sheep blood showing coryneform morphology

A. suis has been reported to produce acetate, ethanol, and formate as main products from maltose fermentation (Wegienek and Reddy 1982) succinate in addition to acetate has been reported to be produced by *A. schaalii* (Lawson et al. 1997), whereas *A. urinale* produces lactate as a major product of glucose catabolism, together with minor amounts of acetate (Hall et al. 2003a). No information is available regarding the fermentation end product profile of *A. massiliense*.

Comprehensive study on the biochemical characteristics of *Actinobaculum* showed that, at present time, three tests yield uniform results for all described species. These include negative reactions for catalase, oxidase, and nitrate reductase. Currently, a positive hippurate hydrolysis test would be useful as a key characteristic of all *Actinobaculum* species. At this time, strains that are both urease and β-glucuronidase positive would be restricted to *A. suis* and *A. urinale*. The general information about the biochemical characteristics of the type strains of *Actinobaculum* species is given in ❯ *Table 4.11*.

Chemotaxonomic Properties

Extensive chemotaxonomic study (Yassin, unpublished) of members of the genus *Actinobaculum* revealed the presence of glucose, rhamnose, and 6-deoxytalose as diagnostic sugars in whole-cell hydrolysates of all species, with the exception of *A. suis* which is characterized by the absence of 6-deoxytalose. The primary structure of the peptidoglycan of the genus *Actinobaculum* belongs to the A5α type. Interestingly, two variations have been identified. Variation (L-Lys-L-Ala-Lys-D-Glu) has been reported in *A. suis* (Ludwig et al. 1992) and variation (L-Lys-L-Lys-D-Glu) has been reported in *A.schaalii* (Lawson et al. 1997). Consistent with the latter variation is the peptidoglycan structure of *A. urinale* (Yassin, unpublished). The muramic acid residue of the glycan moiety of the cell wall peptidoglycan, as far as examined for members of the genus *Actinobaculum*, is *N*-acetylated. The fatty acid profile comprises straight-chain saturated and monounsaturated fatty acids. Respiratory quinones were not detected. The polar lipids pattern contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol dimannoside, and choline-containing phosphoglycolipid. The DNA G+C contents range between 55 and 61.1 mol%.

Taxonomic Comment

Originally, the classification of *A. suis* and *A. schaalii* in a separate genus, *Actinobaculum*, is based exclusively on phylogenetic analysis of the 16S rRNA gene sequences (Lawson et al. 1997). Subsequent assignment of new species to the genus was based on its phylogenetic relationships to *A. suis* and *A. schaalii* and differences in biochemical properties. However, the phylogeny-based classification and the placement of new species in the genus have not been justified yet according to chemical features. Evaluation of the phylogenetic and chemotaxonomic data of the genus *Actinobaculum* does not support the continuous inclusion of *A. suis* in the genus. Comparative analysis of the 16S RNA gene sequences shows that *A. suis* appeared to be moderately related to other *Actinobaculum* species (92.7–95.2 % similarity). Moreover, examination of the aligned 16S rRNA gene sequences demonstrated the presence of a set of signature nucleotides that are uniquely and commonly shared by all *Actinobaculum* species, except *A. suis* (❯ *Table 4.10*).

Furtheremore, notable chemotaxonomic differences (Yassin, unpublished) are found between *A. suis* and other members of

Table 4.11

Biochemical characteristics of *Actinobaculum* species

Characteristics	*A. suis*	*A. schaalii*	*A. massiliense*	*A. urinale*
CAMP reaction	–	w	–	–
Acid production from:				
L-Arabinose	–	d	ND	–
Cellobiose	–	ND	ND	
D-Glucose	–	+	+	+
Lactose	–	–	ND	–
Maltose	+	+	+	d
D-Mannitol	–	–	ND	–
Melezitose	–	–	ND	–
D-Raffinose	–	–	ND	–
L-Rhamnose	–		ND	
D-Ribose	+	+	+	+
D-Sorbitol	–	–	ND	–
Sucrose	–	d	ND	d
Trehalose	–	d	ND	–
D-Xylose	–	+	+	–
Hydrolysis of:				
Esculin	–	–	–	–
Gelatin	–	–	–	–
Hippurate	+	+	+	+
Enzyme activities:				
N-Acetyl-β-glucosaminidase	w	–	–	–
Catalase	–	–	ND	–
Acid phosphatase	+	–	ND	–
Alkaline phosphatase	+	–	ND	–
Chymotrypsin	–	–	ND	–
Cystine arylamidase	–	–	ND	–
Esterase lipase C4	w	–	ND	–
Esterase lipase C8	+	–	ND	–
α-Fucosidase	–	–	ND	–
α-Galactosidase	–	–	ND	–
β-Galactosidase	–	–	–	–
β-Glucuronidase	+	–	ND	+
α-Glucosidase	+	+	+	–
β-Glucosidase	–	–	ND	–
Lipase C14	–	–	ND	–
Leucine arylamidase	+	+	ND	–
α-Mannosidase	+	–	ND	–
Naphthol-AS-BI-phosphohydrolase	+	–	ND	–
Pyrazinamidase	–	d	ND	–
Trypsin	–	–	ND	–
Valine arylamidase	w	–	ND	–
Urease	+	–	–	+
Nitrate reduction	–	–	–	–

Data for *A. suis* from A. F. Yassin; data for *A. schaalii* are from Lawson et al. (1997); data for *A. massiliense* are from Greub and Raoult (2002); data for *A. urinale* are from Hall et al. (2003a)

Symbols + positive/present, – negative/absent, *w* weakly positive, *ND* no data available, *d* differences between strains within one species

the genus. Though *Actinobaculum* species exhibit peptidoglycan type A5α, two distinct variations have been identified. Variation (L-Lys-L-Ala-Lys-D-Glu) has been found in *A. suis*, while *A. schaalii* and *A. urinale* exhibit variation (L-Lys-L-Lys-D-Glu). Besides, analysis of whole-cell hydrolysates revealed that members of the genus are characterized by the presence of glucose, rhamnose, and 6-deoxytalose as diagnostic cell wall sugars. In contrast, 6-deoxytalose is not found in whole-cell hydrolysate of *A. suis*.

Of particular interest is the observation that members of the genus *Actinobaculum* are adapted to different ecological niches. *A. suis* is a well-recognized cause of urinary tract infections and abortion in swine, whereas *A. massiliense*, *A. schaalii*, and *A. urinale* emerged as human pathogens, causing urinary tract infections and systemic diseases. Hence, *A. suis* and all other *Actinobaculum* species represent two different ecotypes. Such adaptations provide a unique opportunity for applying this information for the genus taxonomy. Thus, the genus *Actinobaculum* represents an ecologically diverse clade in the *Actinomycetaceae*.

In this context, the phylogenetic, chemotaxonomic, and ecological evidences argue strongly that *A. massiliense*, *A. schaalii*, and *A. urinale* should at present be excluded from the genus *Actinobaculum* and be elevated to genus rank. However, the decision about whether to retain these species in the genus *Actinobaculum* or to place them in a separate genus depends upon the branching points of other species that will be described and added to this lineage in the future. Such actions to divide *Actinobaculum*, while desirable from the point of view of presenting a more defensible phylogeny, will have considerable epidemiologic repercussions.

Identification and Descriptive Characteristics of *Actinobaculum* Species

The phenotypic characteristics useful for identification of the recognized *Actinobaculum* are given in Table 4.11.

A. massiliense Greub and Raoult 2002, 2025^{VP} mas. si. li. en'se. L. neut. adj. *massiliense*, pertaining to *Massilia*, Latin name of Marseille, where the organism was isolated.

Cells are Gram-positive, non-acid-fast, and straight to slightly curved rods, some of which exhibit branching. Cells are nonmotile. Facultative anaerobe. Catalase- and oxidase-negative. Colonies on sheep blood agar are nonhemolytic, 1 mm in diameter after 72 h of incubation at 37 °C in a 5 % CO_2 atmosphere. Esculin and gelatin are not hydrolyzed. Hippurate hydrolysis is positive. Nitrate is not reduced to nitrite. Acid is produced from glucose, maltose, ribose, xylose, and trehalose, but not from mannose, mannitol, sorbitol, or amidon. Acid production from raffinose is weak. Pyrazinamidase and α-glucosidase activities are positive. Pyrrolidonyl arylamidase, alkaline phosphatase, α-galactosidase, β-glucuronidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, urease, leucine arylamidase, or arginine dihydrolase activity are negative.

The mol% G+C of the DNA has not been reported.

Type strain: CCUG 48898 = CIP 107404 = JCM 15300, isolated from the urine of an 81-year-old woman with cystitis.

Actinobaculum schaalii Lawson, Falsen, Åkervall, Vandamme, and Collins, 1997, 902^{VP} schaalii (N. L. gen. n. schaalii, of Schaal, to honor Klaus P. Schaal, contemporary German microbiologist, for his contributions to actinomycete microbiology).

Cells are straight to slightly curved rods, some of which exhibit branching, nonmotile, non-spore forming, Gram-positive and non-acid-fast. On Columbia blood agar colonies are nonhemolytic, circular, smooth, creamy and with entire edges. A weak CAMP reaction may observed. Facultative anaerobe and catalase-negative. Acetate and succinate are the major end products of glucose fermentation. Acid is produced from glucose, maltose, ribose, and D-xylose. Some strains produce acid from L-arabinose, mannose, starch, sucrose, and trehalose. Acid is not produced from D-arabitol, *N*-acetyl-β-glucosamine, cyclodextrin, glycogen, lactose, mannitol, melezitose, melibiose, methyl-β-D-glucopyranoside, pullulan, raffinose, sorbitol, and D-tagatose. Hippurate is hydrolyzed. Esculin, gelatin, and urea are not hydrolyzed. α-Glucosidase, alanine–phenylalanine–proline arylamidase, and pyroglutamic acid arylamidase are produced. Alkaline phosphatase, arginine dihydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, glycyl-tryptophan arylamidase, and β-mannosidase activities are not detected. Pyrazinamidase and pyrrolidonyl arylamidase activities are shown by some strains. Nitrate is not reduced to nitrite. Acetoin is not produced. The cell wall murein type is type A5a (L-Lys-Lys-D-Glu). The major cellular fatty acids are hexadecanoic, octadecanoic, and *cis*-delta-9-octadecenoic acids.

The mol% G+C of the DNA is 57 mol% G+C.

Type strain, CCUG 27420 = CIP 105739 = DSM 15541 = LMG 1293, isolated from the blood of a 64-year-old male with chronic pyelonephritis.

Actinobaculum suis (Wegienek and Reddy) comb. nov. *Actinobaculum suis* (Eubacterium suis Wegienek and Reddy 1982; "*Corynebacterium suis*" Soltys and Spratling 1957) su'is. L. gen. n. suis, of a hog.

Slender, nonmotile, pleomorphic rods that are 1–3 by 0.5 μm and are arranged singly, in pairs (cells are often at an angle to each other or in palisades), or in small clusters. Cells are Gram-positive, but they are easily decolorized, as culture ages. Not acid fast and nonsporulating; does not survive heating at 80 °C for 10 min. Capsules are not observed by capsule staining; however, a fringelike outer coat external to the cell wall is seen in thin-section electron micrographs. Colonies on blood agar are nonhaemolytic white, circular, granular and have entire to slightly irregular margins after 48 h anaerobic incubation at 37 °C, reaching 0.5–0.3 mm in diameter. Colonies often have slightly raised centers, which gives them a fried-egg appearance. Colonies become flatter reaching 3–5 mm in diamter after 7 days anaerobic incubation at 37 °C. Peptone–yeast extract–starch broth supports excellent growth. The optimal pH is 7–8; no growth occurs at pH 5.0 or less. The optimal temperature is 37 °C; the temperature range for growth is 30–43 °C; and no

growth occurs at 22–23 °C. Cells are anaerobic. Metabolism is strictly fermentative. Maltose, starch, and glycogen are fermented. Acetate, ethanol, and formate are the main end products from maltose fermentation. Adonitol, amygdalin, arabinose, cellobiose, dulcitol, erythritol, esculin, fructose, galactose, glucose, glycerol, inositol, inulin, lactose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, lactate, pyruvate, and threonine are not fermented. Urease activity is positive. Does not produce catalase, indole, hydrogen sulfide, lipase, or lecithinase; ammonia is not produced from peptone. Hippurate is hydrolyzed. Esculin and gelatin are not hydrolyzed. Meat and milk are not digested. Nitrate is not reduced. Activities of *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, esterase lipase (C8), α-glucosidase, β-glucuronidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-mannosidase, pyrrolidonyl arylamidase, and valine arylamidase (weak) are detected. Activities of arginine dihydrolase, esterase (4), α-galactosidase, β-galactosidase, β-glucosidase, pyrazinamidase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, and α-fucosidase are not detected. Major amounts of cytochrome b and minor amounts of cytochrome c are synthesized. Acetoin is not produced. The cell wall sugars are glucose and rhamnose. The cell wall murein type is type A5α (L-Lys-L-Ala-Lys-D-Glu). Exotoxin is not produced.

The G+C content of the DNA is 55 mol%.

Type strain: strain Soltys 50052 = ATCC 331440 = CCUG 19206 = CIP 105361 = DSM 20639 = LMG 18291, isolated from cases of cystitis and pyelonephritis and cases of metritis in pregnant sows.

Actinobaculum urinale Hall, Collins, Hutson, Falsen, Inganäs and Duerden, 2003, 681^{VP}.

u.ri.na'le. N.L. neut. adj. *urinale* pertaining to urine.

Cells are straight to slightly curved Gram-positive rods. Branching is not observed. Cells are non-acid-fast and non-spore-forming. After 48-h anaerobic incubation on Fastidious Anaerobe Agar with 5 % horse blood, colonies are <1 mm in diameter, convex, smooth, entire edged, gray or white, and weakly *β*-hemolytic. Facultative anaerobic and catalase-negative. Lactic acid is the major end product of glucose metabolism, together with minor amounts of acetic acid. Acid is produced from glucose and ribose; acid production from maltose and sucrose is variable and dependent on the test system used. Acid is not formed from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, mannitol, mannose, melibiose, melezitose, methyl-*β*-D-glucopyranoside, pullulan, raffinose, sorbitol, tagatose, trehalose, or D-xylose. Urease is strongly positive. Hippurate is hydrolyzed but gelatin and esculin are not. Arginine arylamidase, *β*-glucuronidase, and proline arylamidase are produced. Alanine–phenylalanine–proline arylamidase, arginine dihydrolase, acid phosphatase, alkaline phosphatase, alanine arylamidase, arginine arylamidase, α-arabinosidase, chymotrypsin, trypsin, cysteine arylamidase, esterase C-4, ester lipase C-8, α-fucosidase, α-galactosidase, *β*-galactosidase, *β*-galactosidase-6-phosphate, α-glucosidase, *β*-glucosidase, glycyl-tryptophan arylamidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase, lipase C-14, α-mannosidase, *β*-mannosidase, *N*-acetyl-*β*-glucosaminidase, phosphoamidase, phenylalanine arylamidase, pyroglutamic acid arylamidase, pyrazinamidase, serine arylamidase, tyrosine arylamidase, and valine arylamidase are not detected. Acetoin is not produced. Indole production is negative. Nitrate is not reduced to nitrite. The cellular fatty acids are of the straight-chain saturated and monounsaturated types, with $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}\omega 9c$ predominating.

The mol% G+C of the DNA has not been reported.

Type strain: CCUG 46093 = CIP 107424 = DSM 15805, isolated from human urine. Habitat is not known.

Genus V. *Varibaculum* Hall, Collins, Lawson, Hutson, Falsen, Inganas, and Duerden, 2003, 627^{VP}.

va.ri.ba'cu.lum. L. adj. *varus*, bent; L. neut. n. *baculum*, small rod; N.L. neut. n. *varibaculum*, small bent rod.

Taxonomy, Historical and Current

The genus *Varibaculum* was introduced by Hall et al. (2003c) to accommodate anaerobic, catalase-negative, Gram-positive diphtheroidal bacteria isolated from human sources. The generic assignment was based exclusively on the biochemical testing, electrophoretic analysis of whole-cell proteins, and phylogenetic analysis of 16S rRNA gene sequences. Currently, the genus comprises only one validly published species: *Varibaculum cambriense*.

Molecular Analyses

Phylogenetic analysis based on 16S rRNA genes conclusively demonstrated that *Varibaculum cambriense* occupies a relatively long and distinct line of descent within the family *Actinomycetaceae* (Hall et al. 2003c). Sequence comparison reveals that *Varibaculum* is equidistantly related to *Actinomyces neuii* (89.9–90.9 % sequence similarity) and *Mobiluncus* species (88.1–88.5 % sequence similarity). The derived phylogenetic tree (Fig. 4.1b) indicates that *Varibaculum cambriense* and *Actinomyces neuii* are sister taxa, a relationship strongly supported by all tree reconstruction methods and 96 % bootstrap. Interestingly, the deduced branching in the phylogenetic tree (Fig. 4.1b) is consistent with the presence of a set of signature nucleotides commonly shared by *Varibaculum cambriense* and *Actinomyces neuii*, but not found in any species of the family *Actinomycetaceae*, and thus provides evidence that they probably descended from a common ancestor. This includes 1239 (U), 1244–1293 (G-C), 1245–1292 (U-G), 1246–1291 (C-G), and 1298 (U). In fact, the placement of *Varibaculum cambriense* and *Actinomyces neuii* as sister group of *Mobiluncus* remains difficult to demonstrate as the 16S rRNA gene-based phylogenetic trees did not resolve the relationships between them with confidence.

Phenotypic Analyses

Morphology

Cells of *Varibaculum cambriense* are Gram-positive, non-acid-fast, nonmotile, non-spore-forming, straight, or curved diphtheroid-shaped rods. The organism grows on blood-containing agar media after anaerobic incubation for 2–3 days. It may grow well or poorly in air containing 5 % CO_2 and either poorly or not at all in ambient air. After anaerobic incubation for 48–72 h on Columbia agar or Fastidious Anaerobe Agar (LabM, Bury, United Kingdom) with horse blood (5 %), colonies are pinpoint, convex, glistening, translucent white or gray, with entire edges, and nonhemolytic.

Varibaculum cambriense is regarded as an anaerobic organism. Like *Actinomyces neuii*, it ferments glucose to a characteristic pattern of metabolic end products consisting of succinic and lactic acid. In contrast to *Actinomyces neuii*, *V. cambriense* is characterized by a negative catalase and synergistic hemolysis (CAMP) test and the inability to produce acid from mannitol.

Chemotaxonomic Properties

The description of the genus *Varibaculum* was chiefly based on phenotypic characteristics derived from biochemical testing and electrophoretic analysis of whole-cell proteins as well as on phylogenetic analysis of 16S rRNA gene sequences. Apart from these phenotypic characteristics and phylogenetic evidence, chemotaxonomic data that provide additional criteria for the delineation of the genus are not available. Comparative chemotaxonomic study (Yassin unpublished) of *Varibaculum cambriense* and *Actinomyces neuii* corroborated the phylogenetic relationships of the two species. Both species have the same chemotaxonomic pattern. This pattern consists of galactose as diagnostic cell wall sugars. The muramic acid residue of the peptidoglycan is *N*-acetylated. The respiratory quinones are [(MK-8(H4)] and nine [MK-9(H4)], with [MK-9(H4)] as the major components. Polar lipids consists of phosphatidylcholine as the characteristic phospholipid, i.e., phospholipid type III sensu Lechevalier et al. (1977), in addition to diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol (PI), and phosphatidylinositol mannosides (PIM). The fatty acid profiles consist of straight-chain saturated, unsaturated, and branched-chain iso- and anteiso-fatty acids. In addition, the G+C contents of the DNA range between 51 and 55 mol%.

Taxonomic Comment

The striking resemblance of the chemotaxonomic profiles of *Varibaculum cambriense* and *Actinomyces neuii* is in complete accordance with the outcome of molecular phylogeny. Thus, it seems likely that *Actinomyces neuii* could be assigned to the genus *Varibaculum* with reasonable confidence. However, in terms of 16S rRNA gene sequence divergence (approximately 10 %), *Varibaculum cambriense* and *Actinomyces neuii* are distantly related and as such, the taxonomic rank of *Actinomyces neuii* is far more problematic. Due to the fact that most generic groupings exhibit a sequence divergence of <6 %, it is obviously evident that *Actinomyces neuii* represents the nucleus of previously unrecognized genus which is closely related to *Varibaculum*.

Identification and Descriptive Characteristics of *Varibaculum* Species

The phenotypic characteristics useful for identification of the genus are previously described by Hall et al. (2003c). Additional descriptive characteristics of the genus are given below (A. F. Yassin unpublished).

Varibaculum

va.ri.ba'cu.lum. L. adj. *varus*, bent; L. neut. n. *baculum*, small rod; N.L. neut. n. *varibaculum*, small bent rod.

Cells consist of short, straight or curved diphtheroid rods which stain Gram-positive and are non-acid fast and nonmotile, nonhemolytic, anaerobic, and catalase-negative. The cell wall contains acetylated muramic acid residues and galactose as diagnostic sugars. The major quinone is menaquinone MK-9(H4). The polar lipids are phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The fatty acid profile comprises straight-chain saturated and unsaturated and branched-chain iso- and anteiso-fatty acids. Lactic and succinic acids are the major end products of glucose metabolism. The G+C content of DNA is 51.7 mol%. The type species is *Varibaculum cambriensis*.

Varibaculum cambriensis Hall, Collins, Lawson, Hutson, Falsen, Inganas, and Duerden, 2003, 627^{VP}

cam. bri. en'sis. L. adj. *cambriensis*, pertaining to cambria, the Latin name of Wales.

Cells consists of short, straight, or curved diphtheroid rods which stain Gram-positive and are non-acid-fast and nonmotile. Colonies after 48 h anaerobic incubation on Fastidious Anaerobic Agar with 5 % horse blood are pinpoint, convex, entire edged, glistening, translucent white, or gray. It is nonhemolytic, anaerobic, and catalase-negative. The end products of glucose metabolism are lactic and succinic acids, together with small amounts of acetic acid. Using conventional biochemical testing, acid is formed from D-glucose, sucrose, and D-ribose; acid may or may not be formed from fructose, mannitol, and xylose. Acid is not produced from amygdalin, L-arabinose, cellobiose, lactose,

mannose, D-raffinose, salicin, or trehalose. It is lecithinase and lipase-negative and indole-negative. Esculin, gelatin, and starch are not hydrolyzed. Using API test systems, acid is produced from D-glucose, and most strains ferment sucrose. Some strains produce acid from maltose, D-ribose, trehalose, and D-xylose. Acid is not produced from D-arabitol, L-arabinose, cyclodextrin, glycogen, mannitol, melibiose, melezitose, methyl-β-D-glucopyranoside, lactose, pullulan, D-raffinose, sorbitol, or tagatose. Hippurate is hydrolyzed by most strains, but esculin and gelatin are not hydrolyzed. Activity for α-glucosidase and leucine arylamidase are detected, and some strains display activity for alanine–phenylalanine–proline arylamidase and β-galactosidase (weak reaction). Activity for acid phosphatase, esterase C-4, ester lipase C8, and phosphoamidase is either weakly positive or negative. No activity is detected for arginine dihydrolase, alkaline phosphatase, chymotrypsin, α-fucosidase, α-galactosidase, β-glucosidase, β-glucuronidase, glycyl-tryptophan arylamidase, lipase C14, α-mannosidase, β-mannosidase, *N*-acetyl-β-glucosaminidase, pyrrolidonyl arylamidase, pyroglutamic acid arylamidase, pyrazinamidase, trypsin, valine arylamidase, or urease. Most strains reduce nitrate to nitrite. Acetoin is not produced.

The mol% G+C of the DNA is 51.7.

Type strain, CCUG 44998 = CIP 107344, isolated from human sources, including breast abscess, brain abscess, cheek abscess, submandibular abscess, postauricular abscess, ischiorectal abscess, and intrauterine contraceptive devices.

Genus VI. *Mobiluncus* Spiegel and Roberts 1984, 180^{VP}, emend. Hoyles, Collins, Falsen, Nikolaitchouk, and McCartney, 2004.

Mo.bi.lun'cus. L. adj. *mobilis* capable of movement, active; L. masc. n. *uncus* hook; N. L. masc. n. *Mobiluncus* a motile curved rod.

Taxonomy, Historical and Current

Krönig (1895) reported on the observation of an anaerobic short-curved bacillus in vaginal fluids. Strains were growing in symbiosis with an anaerobic *Streptococcus*, but only survived one generation. Later, Curtis (1913) was the first to isolate an anaerobic curved bacillus in pure culture from the vagina and cervix of a patient with puerperal infection. The strains grown equally well on blood agar made from human, goat, or sheep blood and colonies appeared as a "dustlike coat," minute, translucent, and nonhemolytic. Curtis assumed that the isolated organism is presumably identical with that early observed by Krönig. Due to the curved cellular morphology of the isolate, Curtis classed it with the vibrios. Prévot (1940) in his manual on classification and identification of anaerobic bacteria used the designation "*Vibrio mulieris*" for the vibrio of Curtis.

In the years following the work of Curtis, no further description of curved rod-shaped bacteria from the female genital tract was published, until Moore (1954) isolated and characterized a number of vibrio-like strains seen in wet preparations of vaginal material from women attending a fertility clinic. These strains required a high atmospheric CO_2 tension as well as anaerobiosis for their primary isolation. Similar or identical serum-requiring strains of "vibrions succinoproducteurs" were characterized by Durieux and Dublanchet (1980). They separated these organisms into two groups on the basis of cellular morphology, fermentation of glucose, and reduction of nitrate.

The taxonomic position of these anaerobic curved rods isolated from the human vagina remained uncertain until Spiegel and Roberts (1984) as well as Hammann et al. (1984) proposed to assign them to two species in the genus *Mobiluncus* and the genus *Falcivibrio*, respectively. The two species were named by Spiegel and Roberts (1984) as *M. mulieris* and *M. curtisii*, the latter being further divided into *M. curtisii* subsp. *curtisii* and *M. curtisii* subsp. *holmesii*, whereas Hammann et al. (1984) designated them as *Falcivibrio grandis* and *Falcivibrio vaginalis*. Although the genus designation *Falcivibrio* appeared first in the literature, it was not validated first, so that *Mobiluncus* has priority. In a subsequent polyphasic taxonomic study of members of the genera *Falcivibrio* and *Mobiluncus*, Hoyles et al. (2004) formally proposed to transfer *F. grandis* and *F. vaginalis* to the genus *Mobiluncus* as *M. mulieris* and *M. curtisii*, respectively. *Mobiluncus* species are etiologically involved in a syndrome called bacterial vaginosis (BV), nonspecific vaginitis, "*Haemophilus vaginalis* vaginitis" (Gardner and Dukes 1955), anaerobic vaginosis (Blackwell et al. 1983), or in German "Aminkolpitis".

Taxonomy

Although the Gram reactions of *Mobiluncus* strains are typically Gram-negative or variable, Spiegel and Roberts (1984) noted that the two species of *Mobiluncus*, *M. curtisii* and *M. mulieris*, exhibit a multilayered cell wall, characteristic for Gram-positive bacteria, but lack an outer membrane. The apparent absence of an outer membrane in the cell walls of the Gram-negative curved rod-shaped organisms led these authors to conclude that they more closely resemble Gram-positive organisms than Gram-negative ones. This conclusion is supported by reports that these organisms are resistant to some antimicrobial agents, e.g., colistin and nalidixic acid, which traditionally inhibit Gram-negative microorganisms, and are susceptible to antimicrobial agents, e.g., penicillin and vancomycin, which inhibit Gram-positive bacteria (Durieux and Dublanchet 1980; Sprott et al. 1983). In addition, hydroxy fatty acids, which are commonly found in Gram-negative cell walls, are absent from these organisms (Skarin et al. 1982). Despite the presence of distinct features of Gram-positive bacteria, Spiegel and Roberts (1984) placed *Mobiluncus* in the family *Bacteroidaceae*, the reason being the obvious phenotypic differences to the *Actinomycetaceae* and *Propionibacteriaceae*. However, a more detailed study on the cell walls of *Mobiluncus* confirmed that their composition was not Gram-negative (Carlone et al. 1986). Furthermore, partial reverse transcriptase sequencing of 16S rRNA gene from *Mobiluncus curtisii* and

Mobiluncus mulieris clearly indicated that the genus *Mobiluncus* is not a member of the *Bacteroidaceae* but belongs to the order *Actinomycetales* (Lassnig et al. 1989). The highest degree of relationship was found with the genus *Actinomyces* which is supported by the presence of common physiological properties.

The subdivision of *M. curtisii* into two subspecies, *M. curtisii* subsp. *curtisii* and *M. curtisii* subsp. *holmesii*, was mainly based on differences in migration patterns in soft agar and the ability to reduce nitrate (Spiegel and Roberts 1984). The analysis of surface antigens of *Mobiluncus* using murine monoclonal antibodies (MAbs) in an enzyme-linked immunosorbent assay and indirect immunofluorescence revealed four MAbs and thus surface components that appeared to be subspecies-specific for *M. curtisii* subsp. *curtisii* (Fohn et al. 1988). One MAb reacted with an epitope shared by *M. curtisii* subsp. *holmesii* and *M. mulieris* but not by *M. curtisii* subsp. *curtisii*. However, neither DNA–DNA homology studies (Spiegel and Roberts 1984) nor comparative analysis of partial 16S rRNA gene sequences supported the division of *M. curtisii* in two subspecies (Tiveljung et al. 1996; Hoyles et al. 2004).

Molecular Analyses

The 16S rRNA gene sequence of *Mobiluncus* showed moderate 16S rRNA sequence similarity with members of the family *Actinomycetaceae* (86.2–91.3 %). The highest 16S rRNA similarity values were observed with *Actinomyces hongkongensis* (90.3–91.3 %), members of the genus *Arcanobacterium* (89.5–91.1 %), members of the genus *Trueperella* (89.4–90.7 %), and the two subspecies of *Actinomyces neuii* (89.4–90 %). A significantly lower 16S rRNA similarity (88.1–88.4 %) was observed with *Varibaculum cambriense*. The 16S rRNA gene signatures characteristics for *Mobiluncus* consisted of nucleotides at 114–313 (C-G), 154–167 (G-U), 166 (C), 408 (A), 411 (G), 441 (U), 560 (U), 591–648 (C-G), 613–627 (A-U), 846 (U), 118–1155 (C-G), 1180 (G), and 1245 (A).

The association of the genus *Mobiluncus* with *Actinomyces* and its related taxa has long been recognized (Lassnig et al. 1989). Phylogenetic analyses based on 16S rRNA gene sequences indicate a monophyletic clade within the *Actinomycetaceae*. Phylogenetic tree reconstructed using the maximum-likelihood method resulted in the placement of the genus *Mobiluncus* as sister to the clade comprising all the other genera of *Actinomycetaceae* (Fig. 4.1a). By contrast, the tree reconstructed using the neighbor-joining method shows that the genus *Mobiluncus* cluster as sister to *A. coleocanis–A. europaeus* lineage and to *A. neuii–Varibaculum cambriense* lineage. In addition, the consensus NJ tree produced by bootstrap analysis of 500 resamplings of the 16S rRNA gene sequences produced a tree with several topological changes and thus did not support this association, although strong bootstrap support (98 %) was shown for the association of *Varibaculum cambriense* and to the two subspecies of *A. neuii* as well as the association of *A. coleocanis* and *A. europaeus* (100 % bootstrap support). Moreover, the clustering of *Mobiluncus* with *Varibaculum cambriense* and the two subspecies of *A. neuii* was broken in the maximum-parsimony tree, albeit the association of *Varibaculum cambriense* and the two subspecies of *A. neuii* as well as the association of *A. coleocanis* and *A. europaeus* remain consistently the same for all tree methods. Thus, the phylogenetic position of *Mobiluncus* within the *Actinomycetaceae* appears to remain unsettled, as shown by the different topologies.

Phenotypic Analyses

Morphology

Mobiluncus cells are typically Gram-variable or Gram-negative curved rods with tapered ends that occur singly or in pairs. The cells are motile by means of multiple subterminal flagella (Spiegel and Roberts 1984; Spiegel 1992). *Mobiluncus curtisii* has one to six flagella per cell with a common origin, while *M. mulieris* possesses one to eight flagella with multiple origins (Hammann et al. 1984; Spiegel 1992). *Mobiluncus curtisii* subsp. *curtisii* was reported to migrate through soft (0.25 %) agar, whereas *M. curtisii* subsp. *holmesii* was not; however, migration through soft agar is also variable in *M. mulieris* (Spiegel and Roberts 1984), while this feature was recently shown not to exactly correspond to *M. curtisii* subsp. *curtisii* (Hoyles et al. 2003). Electron micrographs revealed that *Mobiluncus* species possess a multilayered Gram-positive cell wall lacking an outer membrane. The thinness of the walls was considered to be the reason for the tendency of the curved rod-shaped organisms to stain Gram-negative (Spiegel and Roberts 1984). Pili (fimbriae) were not observed (Hammann et al. 1984). Cells of the two *Mobiluncus* species differ considerably in size: While *M. curtisii* cells are <0.5 µm wide and on average 1.7 µm long rods (Fig. 4.8a), *M. mulieris* cells are significantly longer, measuring <0.5 µm in width but 2.9 µm in mean length (Fig. 4.8b). Colonies on Chocolate agar are cream to light brown in color, convex, smooth, and entire after 5-days anaerobic incubation at 37 °C, reaching 2–4 mm in diameter (Hoyles et al. 2004).

Chemotaxonomic Properties

There are no previous reports on the chemotaxonomy of the genus *Mobiluncus* which can be utilized in the clarification of infrageneric taxonomy of the genus. Sporadic reports in the literature have dealt with fatty acids and whole-cell wall carbohydrates. Carlone et al. (1986) reported on the presence of peptidoglycan based on muramic acid and glucosamine. They also demonstrated that analysis of whole-cell carbohydrates revealed the presence of similar amounts of rhamnose and galactose. Besides, they also reported on the absence of lipopolysaccharide (LPS), 2-keto-3-deoxyoctulosonic acid (KDO), and heptose, characteristic components of Gram-negative bacteria.

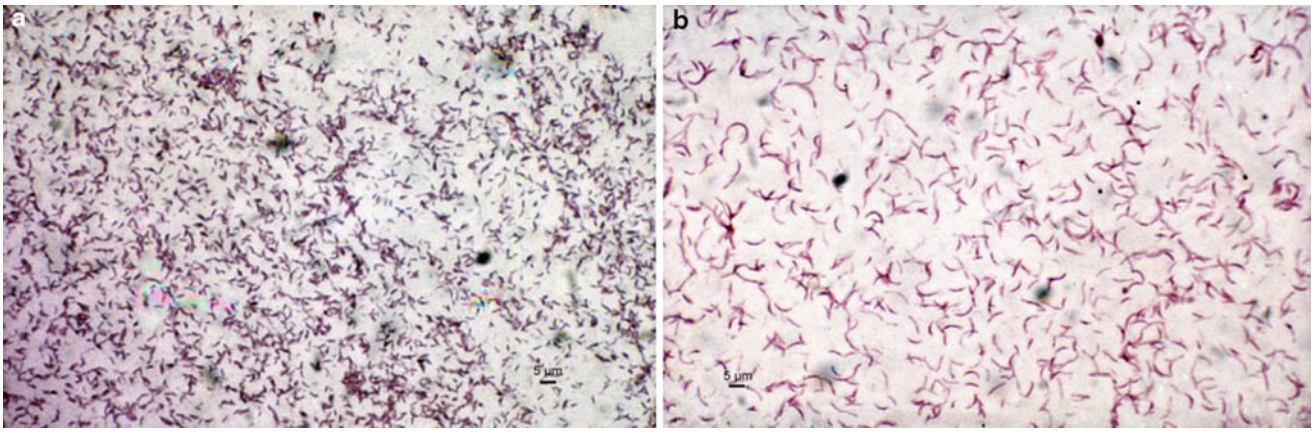

Fig. 4.8
(a) Gram-stained smear from 14-day-old culture of *Mobiluncus curtisii* subsp. *holmesii* on Columbia sheep blood agar incubated under anaerobic condition at 36 °C. Cells measure <0.5 × 1.7 µm. (b) Gram-stained smear from 14-day-old culture of *Mobiluncus mulieris* on Columbia sheep blood agar incubated under anaerobic condition at 36 °C. Cells measure <0.5 × 2.9 µm

Table 4.12
Differential morphological, biochemical and antimicrobial characteristics of *Mobiluncus* species

Characteristic	*Mobiluncus curtisii*		*Mobiluncus mulieris*
	subsp. *curtisii*	subsp. *holmesii*	
Cell length (µm)	1–2	1–2	3–4
Gram reaction	Gram variable	Gram variable	Gram-negative
Growth stimulation by arginine	+	+	–
Reduction of nitrate	–	+	+
Hippurate hydrolysis	+	+	–
Acid production from:			
α-Methyl-D-glucoside	–	–	+
Trehalose	–	–	+
Enzyme activity:			
Arginine dihydrolase	+	+	–
β-galactosidase	+	+	–
Susceptibility to metronidazole	+	+	–

They also assumed that lipoteichoic acids, a cell wall component of Gram-positive bacteria, may be responsible for the intermediate levels of *Limulus* amebocyte lysate (LAL) reactivity (Kessler 1983). The cellular fatty acids profile contained tetradecanoic (14:0), hexadecenoic (16:1ω7c), hexadecanoic (16:0), heptadecanoic (17:0), octadecadienoic (18:2ω6,9c), octadecenoic (18:1ω9c), and octadecanoic (18:0) acids, with 16:0, 18:2, and 18:1 representing more than 50 % of the total fatty acids detected (Carlone et al. 1986). Hydroxylated fatty acids which are commonly found in Gram-negative cell walls are absent from *Mobiluncus* (Skarin et al. 1982; Spiegel and Roberts 1984; Carlone et al. 1986). The G+C content of the DNA of the *Mobiluncus* type strains is 49–52 mol% (Spiegel and Roberts 1984), although values as high as 53.5 ± 0.6 for *M. mulieris* and 55.2 ± 0.6 for *M. curtisii* have been reported (Hammann et al. 1984).

Besides published data, extensive chemotaxonomic investigation of *Mobiluncus* species (Yassin unpublished) indicated that the whole-cell hydrolysates of *Mobiluncus curtisii* subsp. *holmesii*, "*Falcivibrio grandis*," and "*Falcivibrio vaginalis*" were characterized by the presence of galactose and rhamnose as diagnostic sugars (Table 4.3). Analysis of the respiratory quinones of *Mobiluncus curtisii* subsp. *holmesii* revealed the presence of menaquinones with eight (MK-8) and nine (MK-9) isoprene units with MK-9 as the major components (Table 4.3). The polar lipid pattern was phospholipid type V sensu Lechevalier et al. (1977), which is characterized by the presence of *N*-acetylglucosamine in addition to diphosphatidylglycerol and phosphatidylglycerol. The fatty acid profile contained straight-chain saturated and unsaturated fatty acids as well as traces of branched-chain fatty acids of the iso- and anteiso-types (Table 4.3).

Analysis of whole-cell proteins using SDS-PAGE indicated that *Mobiluncus* strains have distinct protein profiles which distinguishes the two species, *M. curtisii* and *M. mulieris* (Baron et al. 1984; Taylor and Owen 1984; Vetere et al. 1987; Schwebke et al. 1990; Drouet et al. 1991; Hoyles et al. 2004). In addition, PAGE-protein analysis allowed defining intraspecies groups association (Drouet et al. 1991; Hoyles et al. 2004). However, this association did not correlate with the biochemical activity of the strains (Drouet et al. 1991). Although, SDS-PAGE analysis of whole-cell proteins is a useful tool for differentiating between the two species of *Mobiluncus* and in intraspecies typing, it is of limited use in subspecies delineation.

Biochemical Characteristics

The genus *Mobiluncus* is divided into two species, *M. curtisii* and *M. mulieris*, on the basis of cell morphology, reaction in Gram's stain, susceptibility to metronidazole, hydrolysis of arginine and hippurate, and β-galactosidase reactions (*Table 4.12*; Spiegel and Roberts 1984; Nord 1984). *Mobiluncus* species are indole, catalase, oxidase, and H_2S negative. *Mobiluncus* strains require extended anaerobic incubation for growth. No growth occurred on media such as CBA incubated in aerobic conditions, or in a candle jar (Vetere et al. 1987). The optimum temperature for multiplication ranges between 33 °C and 37 °C (Spiegel and Roberts 1984; Vetere et al. 1987) and no growth occurs at 20 °C or 42 °C (Holst et al. 1982; Vetere et al. 1987). Scanty growth occurred at pH 4–5 and more enhanced growth occurred at higher pH values above 5, pH >5–8. Furthermore, when grown in peptone–yeast extract–glucose medium (PYG), the metabolic end products of glucose fermentation include succinic, lactic, and acetic acids (Spiegel and Roberts 1984; Vetere et al. 1987).

Phenotypic characterization assays commonly utilized to characterize *Mobiluncus* include carbohydrate fermentations assay. However, the results of individual tests reported in the literature may vary widely, as the performance of such tests are method dependent (Vetere et al. 1987; Spiegel 1992; Hoyles et al. 2004). Therefore, care must be taken when interpreting the results of carbohydrate fermentations assay.

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5 The Genus *Arthrobacter*

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Abstract

The genus *Arthrobacter* is a member of the family *Micrococcaceae* and compared to other genera of the family, it contains the highest number of species. The genus cannot be considered monophyletic because within the clade embracing all *Arthrobacter* species also members of other genera of the family are present. Based on quinone system and peptidoglycan structure, the genus *Arthrobacter* can be subdivided into two major groups. One group is characterized by a quinone system with monosaturated menaquinone [MK-8(H_2 and/or MK-9(H_2) and peptidoglycan type A3α. The second group contains completely unsaturated menaquinones (MK-8, MK-9, and/or MK-10) and peptidoglycan type A4α. Combining chemotaxonomic and 16S rRNA based data, the genus can be subdivided at least into 11 subgroups. The majority of established species has been isolated from soil and sediments, but some were recovered from clinical specimens, as well. Arthrobacters are heterotrophic bacteria that do not require fastidious growth conditions. Most of them are mesophilic with growth optima below 30 °C, but some strains isolated from cold environments (Arctica, Antarctica, glaciers) are psychrotolerant or even psychrophilic. Numerous arthrobacters have been studied that are able to degrade harmful compounds such as 4-chlorophenol, 4-fluorophenol, 4-nitrophenol, or phenanthrene. Some strains have been identified as a source of enzymes including cold-adapted β-galactosidases. Since the genus *Sinomonas* harbors three former *Arthrobacter* species, its species will be dealt with here without separating from *Arthrobacter* species.

Taxonomy: Historical and Current

Short Description of the Genus

Ar.thro.bac'ter. Gr. n. *arthron* a joint; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Arthrobacter* a joint rod. (Modified from *Bergey's Manual*). The description is an emended version of the description given in *Bergey's Manual*, 2nd edition (Busse et al. 2012).

Most species show a marked rod–coccus cycle when grown in complex media. Cells from the stationary-growth phase are composed entirely or largely of coccoid cells that are 0.6–1.0 μm in diameter. Species that are showing only coccoid shape during the growth cycle are known. Major fatty acids are $C_{15:0\ anteiso}$, $C_{15:0\ iso}$, $C_{17:0\ anteiso}$, and $C_{16:0\ iso}$. $C_{16:0}$ may be another major fatty acid. The quinone system contains as the major compound MK-8, MK-9, MK-10, MK-8(H_2), or MK-9(H_2). The diagnostic diamino acid of the peptidoglycan is L-lysine and the peptidoglycan type is A3α or A4α (Schleifer and Kandler 1972). Numerous variations in the composition of the interpeptide bridge do occur. The majority of species analyzed for polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, and a diglycosyldiacylglycerol (either dimannosyldiacylglycerol/monoacyldimannosylmonoacylglycerol, or digalactosyldiacylglycerol). Monogalactosyldiacylglycerol and trimannosyldiacylglycerol may be present, as well. Cell sugars are galactose, glucose, glucosamine, mannose, rhamnose, ribose, and/or xylose. The G+C content of the genomic DNA is 55–72 mol%.

The genus *Arthrobacter* was proposed by Conn and Dimmick (1947) with the type species *Arthrobacter globiforme* and the species *Arthrobacter tumescens* and "*Arthrobacter helvolum*." While the type species was included to the Approved Lists of Bacterial Names (Skerman et al. 1980) as *A. globiformis* and *A. tumescens*, as well, for unknown reasons "*A. helvolum*" was not. Hence, this name has no standing in nomenclature. Actually, after its description "*A. helvolum*" was mentioned only once in the taxonomic literature (Clark 1951). However, it has to be emphasized that "*A. helvolum*" has not been reclassified as *Pseudoclavibacter helvolum* as asserted by Busse et al. (2012).

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In the following *A. tumescens* was first renamed *Pimelobacter tumescens* (Suzuki and Komagata 1983) and subsequently *Terrabacter tumescens* (Collins et al. 1989). Certain other species, primarily classified with the genus *Arthrobacter*, were renamed as well. *Arthrobacter duodecadis* (Lochhead 1958) was assigned to the genus *Tetrasphaera* as *Tetrasphaera duodecadis* (Ishikawa and Yokota 2006); *Arthrobacter flavescens* (Lochhead 1958) was first reclassified as *Aureobacterium flavescens* (Collins et al. 1983) and subsequently as *Microbacterium flavescens* (Takeuchi and Hatano 1998); *Arthrobacter radiotolerans* (Yoshinaka et al. 1973) was reclassified as *Rubrobacter radiotolerans* (Suzuki et al. 1989); *Arthrobacter simplex* (Lochhead 1957) was transferred to the genus *Pimelobacter* as *Pimelobacter simplex* (Suzuki and Komagata 1983); *Arthrobacter variabilis* (Müller 1961) was reclassified as *Corynebacterium variabile* (Collins 1987); and *Arthrobacter atrocyaneus* (Kuhn and Starr 1960), *Arthrobacter albidus*, and *Arthrobacter echigonensis* (Ding et al. 2009) were reclassified in the genus *Sinomonas* as *Sinomonas atrocyanea* (Zhou et al. 2009), *Sinomonas albida*, and *Sinomonas echigonensis*, respectively (Zhou et al. 2012). *Arthrobacter picolinophilus* (Tate and Ensign 1974) was identified as a strain of *Rhodococcus erythropolis* (Koch et al. 1995). *Arthrobacter siderocapsulatus* (Dubinina and Zhdanov 1975) was identified as a strain of *Pseudomonas putida* (Chun et al. 2001). A candidate for reclassfication in another genus is *Arthrobacter viscosus* (Gasdorf et al. 1965) which shares highest 16S rRNA gene sequence similarities with members of the proteobacterial genus *Rhizobium* (Heyrman et al. 2005).

At the time of writing, the genus *Arthrobacter* (except *A. viscous*) embraces 66 species. The genus is a member of the actinobacterial family *Micrococcaceae*. Within the genus, several major branches exist (*Fig. 5.1*) and some of these branches show a closer relationship to other genera of the family than to the type species of the genus, *A. globiformis*. This observation indicates that the genus is not monophyletic.

Heterogeneity within the genus regarding peptidoglycan types (A3α or A4α) and quinone system [menaquinone MK-9 (H_2) or MK-8 and/or MK-9] was already discussed by Stackebrandt et al. (1983). These authors designated *Arthrobacter* species with MK-9 (H_2) and peptidoglycan type A3α the "globiformis" group including *Arthrobacter globiformis, Arthrobacter oxydans, Sinomonas atrocyanea* (formerly *Arthrobacter atrocyaneus*), and *Arthrobacter ureafaciens*. *Arthrobacter* species showing MK-8 and/or MK-9 and peptidoglycan type A4α were placed in the "nicotianae" group including *Arthrobacter nicotianae, Arthrobacter mysorens, Arthrobacter protophormiae, Arthrobacter uratoxydans*, and *Arthrobacter sulfureus*. On the same grounds, species of the "globiformis" group together with *Arthrobacter pascens, Arthrobacter histidinolovorans, Arthrobacter ramosus, Arthrobacter ilicis, Arthrobacter aurescens*, and *Arthrobacter crystallopoietes* were considered to represent the *Arthrobacter globiformis/citreus* group whereas the "nicotianae" group was named *Arthrobacter nicotianae* group (Keddie et al. 1986). Komagata and Suzuki (1987) subdivided *Arthrobacter* species into seven groups based on the composition of the interpeptide bride of the peptidoglycan.

Group I shows Lys-Ser-Thr-Ala found in the species *A. oxydans* and *A. polychromogenes*

Group II shows Lys-Ala-Thr-Ala found in *A. aurescens, A. histidinolovorans, A. ilicis, A. nicotinovorans*, and *A. ureafaciens*

Group III shows Lys-$Ala_{1–4}$ found in *A. crystallopoietes, A. globiformis, A. pascens*, and *A. ramosus*

Group IV shows Lys-Ser-$Ala_{2–3}$ found in *Sinomonas atrocyanea* (formerly *Arthrobacter atrocyaneus*)

Group V shows Lys-Thr-Ala_2 found in *A. citreus*

Group VI shows Lys-Ala-Glu found in *A. nicotianae, A. creatinolyticus, A. uratoxydans*, and *A. protophormiae*

Group VII shows Lys-Glu found in *A. sulfureus*

More recently, the genus *Arthrobacter* was subdivided into 11 groups based on combined data from 16S rRNA gene sequences, quinone system, and peptidoglycan structure (Busse et al. 2012). These groups were named

"*Arthrobacter globiformis* group" (*Arthrobacter sensu stricto*) embraces *Arthrobacter globiformis, Arthrobacter pascens, Arthrobacter humicola* and *Arthrobacter oryzae*.

"*Arthrobacter aurescens* group" embraces *Arthrobacter aurescens, Arthrobacter histidinolovorans, Arthrobacter ilicis, Arthrobacter nicotinovorans, Arthrobacter nitroguajacolicus*, and *Arthrobacter ureafaciens*.

"*Arthrobacter oxydans group*" embraces *Arthrobacter chlorophenolicus, Arthrobacter defluvii, Arthrobacter niigatensis, Arthrobacter oxydans, Arthrobacter phenanthrenivorans, Arthrobacter polychromogenes, Arthrobacter scleromae*, and *Arthrobacter sulfonivorans*. *Arthrobacter equi* (Yassin et al. 2011) can be assigned to "*Arthrobacter oxydans* group" as well because it shares highest 16S rRNA gene sequence similarities with representatives of this group and the amino acid composition of the interpeptide bridge of the peptidoglycan (*Table 5.1*).

"*Arthrobacter protophormiae* group" embraces *Arthrobacter ardleyensis, Arthrobacter arilaitensis, Arthrobacter bergerei, Arthrobacter creatinolyticus Arthrobacter mysorens, Arthrobacter nicotianae, Arthrobacter protophormiae, Arthrobacter rhombi, Arthrobacter soli*, and *Arthrobacter uratoxydans*. Assignment of *A. rhombi* should be carefully reconsidered because this species was reported to contain a menaquinone MK-9(H_2) (Chen et al. 2009) which is in contrast to the common trait of this group containing exclusively unsaturated menaquinones (*Table 5.1*).

"*Arthrobacter sulfureus* group" embraces *Arthrobacter antarcticus, Arthrobacter gangotriensis, Arthrobacter kerguelensis, Arthrobacter psychrophenolicus*, and *Arthrobacter sulfureus*. Another species, *Arthrobacter cryotolerans* (Ganzert et al. 2011) proposed recently, can be considered as another species of this group because it shares its chemotaxonomic traits and phylogenetically, it is the deepest branching species of this group (*Fig. 5.1*).

"*Arthrobacter agilis* group" embraces *Arthrobacter agilis, Arthrobacter flavus, Arthrobacter parietis, Arthrobacter subterraneus, Arthrobacter tecti*, and *Arthrobacter tumbae*.

Fig. 5.1

Phylogenetic reconstruction of the genus *Arthrobacter* and related genera based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, a 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

Table 5.1
Characteristics of *Arthrobacter* groups as defined by Busse et al. (2012)

Group	*Arthrobacter globiformis* group	*Arthrobacter citreus* group	*Arthrobacter psychrolactophilus* group	*Arthrobacter agilis* group	*Arthrobacter pigmenti* group	*Arthrobacter aurescens* group	*Arthrobacter oxydans* group	*Arthrobacter protophormiae* group	*Arthrobacter sulfureus* group	*Arthrobacter albus/ cumminsii* group
Quinone	MK-9 (H_2)	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9(H_2)	MK-9 (H_2)	MK-9 (H_2)[a]	MK-8, MK-9	MK-9, MK-10 or MK-8	MK-8(H_2)
Peptidoglycan[b]	A3α (Lys-Ala_{2-3}) A11.5 or A11.6	A3α (Lys-Thr-Ala_2) A11.27	A3α (Lys-Thr-Ala_{1-3} or Lys-Ala_{1-4}) A11.25, A11.26, A11.27, A11.28 or A11.4, A11.5, A11.6 A11.7	A3α (Lys-Thr-Ala_{2-3}) A11.27 or A11.28	A3α (Lys-Ala-Ser-Ala_3 or Lys-Ala_4) unspecified[c] or A11.7	A3α (Lys-Ala-Thr-Ala) A11.17	A3α (Lys-Ser-Thr-Ala) A11.23	A4α (Lys-Ala-Glu) A11.35	A4α (Lys-Glu) A11.54	A4α (Lys-Ala-Glu or Lys-Ser-Glu) A11.35 or A.11.58

[a]Deviating results were reported for *A. phenanthrenivorans* and *A. scleromae* containing predominantly MK-8 and MK-9(H_2) and MK-8(H_2), respectively (Huang et al. 2005; Kallimanis et al. 2009)
[b]Three digit code according to Schleifer and Kandler (1972) and five digit code as provided by the German Collection of Microorganisms and Cell Cultures (http://www.dsmz.de/catalogues/catalogue-microorganisms/specific-catalogues/peptidoglycans.html#c1252)
[c]The interpeptide bridge Lys-Ala-Ser-Ala_3 is not specified at http://www.dsmz.de/catalogues/catalogue-microorganisms/specific-catalogues/peptidoglycans.html#c1252

"*Arthrobacter citreus* group" embraces *Arthrobacter citreus*, *Arthrobacter gandavensis*, *Arthrobacter koreensis*, and *Arthrobacter luteolus*.

"*Arthrobacter psychrolactophilus* group" embraces *Arthrobacter psychrolactophilus*, *Arthrobacter stackebrandtii*, *Arthrobacter psychrochitiniphilus*, and *Arthrobacter alpinus*. Recently, two proposed species, namely, *Arthrobacter livingstonensis* and *Arthrobacter cryoconiti*, were phylogenetically placed in "*Arthrobacter psychrolactophilus* group" and their peptidoglycan compositions (Ganzert et al. 2011; Margesin et al. 2012) are similar to those of representatives of this group (Table 5.1). Hence, placement of these two species in "*Arthrobacter psychrolactophilus* group" is suggested from these data.

"*Arthrobacter pigmenti* group" embraces *Arthrobacter castelli*, *Arthrobacter monumenti* and *Arthrobacter pigmenti*.

"*Arthrobacter albus/cumminsii* group" embraces *Arthrobacter albus* and *Arthrobacter cumminsii*.

"*Sinomonas* group" embraces *Sinomonas albida* (formerly *Arthrobacter albidus*), *Sinomonas atrocyanea* (formerly *Arthrobacter atrocyaneus*), *Sinomonas echigonensis* (formerly *Arthrobacter echigonensis*), and *Sinomonas flava*.

The major characteristics of these groups are listed in Table 5.1.

However, several species were only tentatively assigned to certain groups. *A. crystallopoietes* was placed in "*Arthrobacter globiformis* group" based on high 16S rRNA gene similarities with *A. globiformis* and a similar peptidoglycan composition. Also *A. ramosus* and *A. methylotrophicus* were tentatively placed in "*Arthrobacter globiformis* group" because the interpeptide bridge in their peptidoglycan contains exclusively alanines. Despite close phylogenetic relationship with *A. methylotrophicus*, *A. alkaliphilus* was placed in "*Arthrobacter oxydans* group" because it shares the group-specific peptidoglycan structure, different from that of *A. methylotrophicus*. *A. russicus* was placed in "*Arthrobacter psychrolactophilus* group" because in many phylogenetic trees, it branches at the root of this group and it shows an interpeptide bridge in the peptidoglycan that is similar to that of a representative of the group, *A. stackebrandtii*.

Arthrobacter halodurans was described in 2009 (Chen et al. 2009), but the name was only validated at the end of 2012. This species was shown to group within the *Arthrobacter* clade next related to *A. rhombi* together comprising a separate line of descent within the genus which is branching at the root of species of "*Arthrobacter protophormiae* group" and "*Arthrobacter sulfureus* group." The predominant quinone of *A. halodurans* is menaquinone MK-9(H_2), the peptidoglycan is of the A4α type (Lys-Ala-Glu) and the polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and an unidentified phospholipid. The peptidoglycan type Lys-Ala-Glu in combination with the quinone system, and the polar lipid profile clearly distinguish *A. halodurans* from members of "*Arthrobacter protophormiae* group" and "*Arthrobacter sulfureus* group" which contain a Lys-Ala-Glu or Lys-Glu type peptidoglycan, exclusively unsaturated menaquinones, and lack phosphatidylinositol. Chen et al. (2009) also reported on the quinone system of *A. rhombi* which was found to have menaquinone MK-9(H_2) predominantly which is in accordance with the close relatedness to *A. halodurans*. However, in the phylogenetic tree shown in Fig. 5.1, *A. halodurans* and *A. rhombi* are clearly separated from each other but both being next related to species of "*Arthrobacter sulfureus* group." These observations indicate that reanalyses of the peptidoglycan structure, the quinone systems, and polar lipid profiles of the two species are desirable in order to confirm these traits which are not in agreement with the traits of "*Arthrobacter sulfureus* group." Confirmation of these data would mean that the definition of "*Arthrobacter sulfureus* group" must be reconsidered and their phylogenetic assignment as well.

The species *A. nasiphocae*, *A. roseus*, *A. sanguinis*, and *A. woluwensis* were not assigned to any *Arthrobacter* group because neither phylogenetic studies nor chemotaxonomic characteristics suggest clear relationships.

Molecular Analyses

Phylogeny

Species of the genus *Arthrobacter* are forming a clade with other members of the family *Micrococcaceae* and they are closely related to the type genus of the family, *Micrococcus*. Phylogenetic trees including *Arthrobacter* species and other representatives of the family indicate that the family is polyphyletic. This suggestion is supported also from heterogeneity in chemotaxonomic traits including peptidoglycan composition and quinone system. Despite high intragroup 16S rRNA gene sequence similarities, only a minority of the *Arthrobacter* groups, as defined by Busse et al. (2012), are forming separate clades which are statistically supported from high bootstrap values. Clades with high statistical support are formed by "*Arthrobacter agilis* group," "*Arthrobacter sulfureus* group," "*Arthrobacter albus/cumminsii* group," "*Arthrobacter citreus* group," and "*Arthrobacter psychrolactophilus* group," which are also sharing ≥97 %, intragroup 16S rRNA sequence similarities. However, also among the representatives of the other *Arthrobacter* groups, relatively high intragroup similarities are found: "*Arthrobacter globiformis* group" (>98.0 %), "*Arthrobacter aurescens* group" (>97.0 %), "*Arthrobacter oxydans* group" (>97.0 %) "*Arthrobacter protophormiae* group" (>96.0 %), "*Arthrobacter pigmenti* group" (>96.0 %), and "*Sinomonas* group" (>97.0 %).

DNA-DNA Hybridizations (DDHs)

The first comprehensive study applying DNA-DNA hybridizations (DDHs) between *Arthrobacter* species was published by Stackebrandt and Fiedler (1979). This DDH study already demonstrated the close relatedness of the two "*Arthrobacter globiformis* group" species, *A. globiformis* DSM 20124^T and *A. pascens* ATCC 13346^T (51 % and 55 % DDH similarity).

In contrast, the two species *A. ramosus* CCM 1646^T and *A. crystallopoietes* DSM 20117^T, which were tentatively assigned to "*Arthrobacter globiformis* group" (Busse et al. 2012) like all other strains included in the study did not show significant DDH similarity (<40 %). The same authors showed that *A. oxydans* DSM 20119^T and *A. polychromogenes* DSM 20136 ("*Arthrobacter oxydans* group") are closely related species exhibiting DDH similarity slightly below the threshold value 70 % for species delineation (61 % and 69 %, respectively). This observation is in agreement with phylogenetic analyses (◉ *Fig. 5.1*) and very high 16S rRNA gene similarity (99.3 %). Furthermore, moderate DDH similarities (44–54 %) indicated that the misclassified strain "*Arthrobacter globiformis* DSM 20125" is a close relative of these two species. No significant DDH similarity (<45 %) was found with other strains included in this study. A close relatedness was also found between the "*Arthrobacter aurescens* group" species *A. aurescens* DSM 20116^T and *A. histidinolovorans* DSM 20115^T (50 % and 55 % DDH similarity, respectively). Both strains show no significant DDH similarities with another member of "*Arthrobacter aurescens* group," namely, *A. ureafaciens* nor with other strains included in this study (<40 % DDH similarity). Moderate DDH similarity (39 % and 48 %, respectively) was observed between the two "*Arthrobacter protophormiae* group" species *A. nicotianae* DSM 20123^T and *A. protophormiae* DSM 20168^T. DDH similarities >70 % with strain DSM 20168^T demonstrated that the misclassified patent strains "*Arthrobacter citreus* ATCC 17775" (US Patent 3,533,914), "*Arthrobacter citreus*" ATCC 21348 (US Patent 3,661,711), and "*Arthrobacter citreus*" ATCC 21040" (US Patent 3,532,600) are strains of the species *A. protophormiae* (formerly *Brevibacterium protophormiae*). No relationship with other strains of the study was suggested from DDH similarities. This study also showed that "*Arthrobacter citreus* ATCC 15170" was misclassified and actually is a strain of the "*Arthrobacter sulfureus* group" species *A. sulfureus* (formerly *Brevibacterium sulfureum*; >70 % DDH similarity). No relatedness with other species of this study was found for the type species of "*Arthrobacter citreus* group" species *A. citreus* and "*Sinomonas* group" species *Sinomonas atrocyanea* (formerly *Arthrobacter atrocyaneus*).

In a following study, Stackebrandt et al. (1983) applied DDH for examination of relationship of several strains with the type strains of the species *A. nicotianae, A. protophormiae,* and *A. sulfureus.* These authors showed that the patent strain (US Patent 3,616,215; British Patent 1,158,199) "*Arthrobacter nucleogenes*" ATCC 21279, *Arthrobacter* sp. AC 250, *Arthrobacter* sp. NCIB 9863, "*Brevibacterium*" sp. AJ 1486, and *Corynebacterium liquefaciens* DSM 20579 are strains of *A. nicotianae,* as demonstrated by DDH similarities >80 % and that *Corynebacterium* sp. ATCC 31021, "*Brevibacterium fuscum*" ATCC 15993, the patent strain *Arthrobacter mysorens* ATCC 31021, "*Corynebacterium uratoxydans*" ATCC 21749^T, and "*Corynebacterium uratoxydans*" ATCC 21752 are closely related to *A. nicotianae* (DDH similarity 40–50 %). Furthermore, a close relatedness of the patent strain (US Patent 3,762,999; US Patent 3,709,786; US Patent 3,560,342; US Patent 3,764,472; US Patent 3,586,606) *Arthrobacter* sp. ATCC 21085 with *A. sulfureus* was suggested from 60 % DDH similarity. No close relationships with other species were identified for *A. citreus* DSM 20133^T, *A. globiformis* DSM 20124^T, *Arthrobacter oxydans* DSM 20119, or *A. ureafaciens* DSM 20126^T subjected to DDH analyses. As a result from this study for "*Corynebacterium uratoxydans*" ATCC 21749^T and "*Corynebacterium uratoxydans*" ATCC 21752, which are sharing 100 % DDH similarity, the species *Arthrobacter uratoxydans* was proposed; for "*Brevibacterium sulfureum*" DSM 20167^T the species *Arthrobacter sulfureus* was proposed and *Brevibacterium protophormiae* DSM 20168^T was reclassified as *Arthrobacter protophormiae.*

In a polyphasic study dealing with the classification of two *Arthrobacter oxydans* strains, DSM 419 and DSM 420^T, including analysis of DDH similarity, Kodama et al. (1992) found that these two strains are misclassified. The two strains show less than 45 % DDH similarity with each other, demonstrating that they are not members of a single species. Strain DSM 419 shows less than 20 % similarity with the type strain of *A. oxydans* and also low levels of DDH similarity (<45 %) with the type strains of *A. aurescens, A. histidinolovorans, A. ilicis,* and *A. citreus* but more than 95 % with the type strain *A. ureafaciens.* From these data, the authors concluded that strain DSM 419 should be classified as *A. ureafaciens.* Strain DSM 420 showed less than 60 % DDH similarity with each of above-mentioned reference species. Based on these results, Kodama et al. (1992) proposed for strain DSM 410^T the novel species *Arthrobacter nicotinovorans.*

Two human isolates from blood designated CF46 and CF39 were identified as strains of *Arthrobacter oxydans* based on >80 % DDH similarity (Wauters et al. 2000).

The distinct position of the *Arthrobacter* isolate A6^T and its status as a novel species was determined by DDH hybridization experiments with the close phylogenetic relatives *A. oxydans* and *Arthrobacter polychromogenes.* DDH similarities below 55 % clearly demonstrated that strain A6^T is representing a novel species for which the name *Arthrobacter chlorophenolicus* was proposed (Westerberg et al. 2000).

The type strain of *Arthrobacter defluvii* was hybridized with a second strain 4C1-b which was isolated in the same study and the type strains of closely related *Arthrobacter* species, as indicated from 16S rRNA gene sequence similarities, namely, *A. chlorophenolicus, A. oxydans, A. scleromae, A. polychromogenes,* and *A. sulfonivorans.* DDH similarity with 4C1-b was 93 % and similarities with the reference strains were <40 %, supporting the description of the novel species (Kim et al. 2008).

Despite high 16S rRNA similarity with the type strain of *A. citreus* (98.7 %), an isolate from an infected surgical wound CF25^T was proposed as *Arthrobacter luteolus* (Wauters et al. 2000) because low DDH similarity (44 %) was observed between the two species.

Storms et al. (2003) reported on the description of the novel species *Arthrobacter gandavensis.* Based on DDH similarity (<90 %), the three strains LMG 21285^T, LMG 21286 and LMG 21287, which had been isolated from mastitic milk of dairy cows and from the uterus of a cow, respectively, were identified as strains of the same species. DDH similarities ≤50 % with the

next phylogenetic relatives *A. citreus* and *A. luteolus* confirmed the status of the novel species, *A. gandavensis*. Studies of the genetic relationship of the novel species *Arthrobacter equi* with its close relatives *A. defluvii* and *A. chlorophenolicus* revealed 32.3 % and 53.9 % DDH similarity, respectively, supporting the description of the novel species (Yassin et al. 2011).

The two isolates CA15-8^T and CA15-9 sharing ≥90 % DDH similarity were identified as strains of the novel species *Arthrobacter koreensis*. The two strains share less than 10 % DDH similarity with the type strain of the next phylogenetic relative *A. luteolus* (Lee et al. 2003).

Three strains GIFU 12498^T, GlFU 12499, and GIFU 12500, which share more than 90 % DDH similarity, were described as strains of *Arthrobacter creatinolyticus* (Hou et al. 1998). *A. creatinolyticus* GIFU 12498^T was shown to exhibit less than 35 % DDH similarity with the type strains of *A. aurescens*, *A. globiformis*, *A. ilicis*, *A. nicotianae*, *A. protophormiae*, *A. sulfureus*, and *A. uratoxidans*.

Arthrobacter gangotriensis Lz1y^T and *Arthrobacter kerguelensis* KGN15^T were described as novel species because they exhibited with each other 50 % and with the type species of the nearest relative *A. sulfureus* 54 % and 12 % DDH similarity, respectively (Gupta et al. 2004).

The proposal of *Arthrobacter psychrochitiniphilus*, which shares 97.8 % 16S rRNA gene sequence similarity with its closest relative *Arthrobacter psychrolactophilus*, was supported by 33.8 % DDH similarity between the type strains of the two species (Wang et al. 2009).

Arthrobacter albus was proposed by Wauters et al. (2000) for human isolates CF43^T and CF44 from blood and urine, respectively, which share 88 % DDH similarity. Though CF43^T shares 99.7 % 16S rRNA gene sequence similarity with the type strain of *Arthrobacter cumminsii*, the DDH similarity between the two isolates and *A. cumminsii* is only 54.5 % and 58.6 %, respectively.

Fourteen isolates from the surfaces of different smear-ripened cheeses could be assigned to the two novel species *Arthrobacter arilaitensis* and *Arthrobacter bergerei* based on results from DDH (Irlinger et al. 2005). Based on DDH similarities >70 % with the type strain Re117^T the isolates Po101, Ep104, Cou102, Gor104, Bres102, Po102, Stp101, and Ma107 could be assigned to the species *A. arilaitensis*. The isolates La101, Sne104, Re127, and Ka101 were identified as strains of *A. bergerei* with which they shared >75 % DDH similarity. The type strains of the two species *A. arilaitensis* and *A. bergerei* showed only <45 % DDH similarity with the type strains of their close phylogenetic neighbors *A. nicotianae*, *A. uratoxydans*, *A. protophormiae*, and *A. sulfureus*.

DDH studies between G2-1^T and its closest phylogenetic relatives *A. ilicis* and *A. aurescens* (<45 % DDH similarity) demonstrated that it is the representative of a novel species for which the name *Arthrobacter nitroguajacolicus* was proposed. Furthermore, DDH similarity of 78.2 % identified a second isolate, designated 8/3, as a strain of *A. nitroguajacolicus* (Kotoucková et al. 2004).

The species *Arthrobacter flavus* was proposed despite 77 % DDH similarity with the type strain of *Arthrobacter agilis* (Reddy et al. 2000) which is clearly above the threshold value of 70 % applied for species distinction. This proposal was supported from significant phenotypic differences.

Arthrobacter humicola and *Arthrobacter oryzae* were described by Kageyama et al. (2008). The type strains of the two species showed approximately 50 % DDH similarity and less than 30 % DDH similarity with the type strains of the closely related species *A. globiformis*, *A. pascens*, and *A. ramosus*. These data unambiguously demonstrated the status of separate species for *A. humicola* and *A. oryzae*.

DDH similarity values <45 % with its closest relatives *A. polychromogenes* and *A. oxydans* supported the description of strain Sphe3^T as the species *Arthrobacter phenanthrenivorans* (Kallimanis et al. 2009).

The species *Arthrobacter alpinus* S6-3^T was described because it shared with its next phylogenetic relatives *A. stackebrandtii* and *S. psychrolactophilus* approximately 11 % and 34 % DDH similarity, respectively (Zhang et al. 2010).

Four *Arthrobacter* species were described by Ding et al. (2009), namely, *Arthrobacter alkaliphilus*, *Arthrobacter niigatensis*, *Sinomonas echigonensis* (formerly *Arthrobacter echigonensis*), and *Sinomonas albida* (formerly *Arthrobacter albidus*) including DDH with the next related reference species. *A. alkaliphilus* LC6^T showed 32.1 % DDH similarity with *A. methylotrophus*. The DDH similarity between *A. niigatensis* LC4^T and *A. chlorophenolicus* was determined to be 32.4 %. The closely related strains *Sinomonas echigonensis* LC10^T and *Sinomonas albida* LC13^T shared 25.3 % with each other and with the next related reference species *Sinomonas atrocyanea* (formerly *Arthrobacter atrocyaneus*) 11.2 % and 9.6 % DDH similarity, respectively. The latter three species recently were reclassified in the novel genus *Sinomonas* as *S. echigonensis*, *S. albida*, and *S. atrocyanea* (Zhou et al. 2009, 2012) with the type species *Sinomonas flava* CW 108^T. *S. flavus* and *S. atrocyanea* share 52.2 % DDH similarity (Zhou et al. 2009). Another species, *Sinomonas soli*, shares 58.3 %, 41.8 %, 21.6 %, and 25.5 % DDH similarity, respectively, with the other four species of the genus, *S. atrocyaneus*, *S. flava*, *S. echigonensis*, and *S. albidus*, respectively (Zhou et al. 2012).

Two strains A1-3^T and A19, which had been isolated from the air in the Russian space laboratory Mir, were classified in a single species, *Arthrobacter russicus* (Li et al. 2004). The two strains exhibit 100 % DDH similarity with each other and only 9.8 % DDH similarity with the next relative *A. polychromogenes*.

In the study describing the six species *Arthrobacter monumenti*, *Arthrobacter pigmenti*, *Arthrobacter castelli*, *Arthrobacter tecti*, *Arthrobacter tumbae*, and *Arthrobacter parietis*, the status of the latter five species was substantiated from the results of DDH values obtained between the type strains of selected novel species and the reference species *A. globiformis*, *A. pascens*, *A. agilis*, and *A. ramosus* (Heyrman et al. 2005). *A. parietis* and *A. tumbae* show approximately 50 % DDH similarity, which is reflecting their close relatedness as shown by 16S rRNA gene sequence analyses but less than 12 % DDH similarity with *A. tecti* and the four reference species. DDH similarity of *A. tecti* with *A. parietis*, *A. tumbae*, and the

four reference species is not higher than 16 %. Among the reference species *A. globiformis* and *A. pascens* show highest DDH similarity with each other (approximately 31 %) but less than 14 % with *A. agilis* and *A. ramosus*. DDH similarity of *A. agilis* and *A. ramosus* is less than 6 % and less than 13 % with the three novel species mentioned above and the other two reference species. In the same study, 26 % DDH similarity between the type strains of *A. pigmenti* and *A. castelli* suggested that the two strains belong to different genospecies.

Most recently, the novel species *Arthrobacter cupressi* was described (Zhang et al. 2012). The levels of DDH similarity of the type strains of *A. cupressi* with those of its close relatives *A. oryzae*, *A. humicola*, *A. alkaliphilus*, *A. niigatensis*, and *A. defluvii* were 36 %, 26 %, 21 %, 20 %, and 20 %, respectively.

Multi-locus Sequence Analysis (MLSA)

Multi-locus sequence analyses (MLSA) have not yet been introduced to the taxonomy of the genus *Arthrobacter*. However, a short stretch (360 bp) of the gene *recA* often sequenced in MLSA studies has been applied to selected *Arthrobacter* species for comparative phylogenetic analyses (van Waasbergen et al. 2000). In agreement with relationships deduced from 16S rRNA sequence data, these analyses revealed a close relatedness between *Arthrobacter globiformis* and *Arthrobacter pascens*; between *Arthrobacter uratoxydans*, *Arthrobacter protophormiae*, *Arthrobacter nicotianae,* and more distantly *Arthrobacter sulfureus*; between *Arthrobacter aurescens*, *Arthrobacter nicotinovorans*, *Arthrobacter histidinolovorans*, and *Arthrobacter ureafaciens*; and between *Arthrobacter oxydans* and *Arthrobacter polychromogenes*. Additional *recA* gene sequences from *Arthrobacter* type strains are accessible from the released genome sequences of *Arthrobacter chlorophenolicus* A6^T (CP001341), *Arthrobacter phenanthrenivorans* Sphe3^T (CP002379), and *Arthrobacter arilaitensis* RE117^T (FQ311875). Sequence similarities with the *recA* gene fragments of type strains of recognized *Arthrobacter* species do not support assignment of *A. chlorophenolicus* to "*Arthrobacter oxydans* group" (Busse et al. 2012) but indicate a position between "*Arthrobacter globiformis* group" and "*Arthrobacter oxydans* group." On the other hand, *recA* similarities suggest placement of. *A. phenanthrenivorans* in "*Arthrobacter oxydans* group." In agreement with the grouping proposed by Busse et al. (2012) *A. arilaitensis* RE117^T is placed in "*Arthrobacter protophormiae* group."

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS has been applied for identification of *Arthrobacter* strains that have been isolated from soil contaminated with heavy metals and aromatic solvents (Vargha et al. 2006), from horseradish rhizosphere soil capable to metabolize biphenyl (Uhlik et al. 2011), and halotolerant, phenanthrene-degrading close relatives of *Arthrobacter crystallopoietes* (Prisyazhnaya et al. 2012).

Vargha et al. (2006) studied 16 *Arthrobacter* isolates and showed that the groupings identified on the basis of MALDI-TOF protein profile corresponded well to phylogenetic relationships. Common to all isolates were two peaks at 4,400 and 5,160 *m/z* whereas all other peaks were group specific or even strain specific.

In a study on classification of biphenyl-degrading isolates from horseradish soil applying MALDI-TOF MS and the MALDI Biotyper 2.0 software (Bruker), seven strains were identified at the species level (*A. arilaitensis, A. aurescens, A. chlorophenolicus, A. ilicis, A. oxydans,* and *A. polychromogenes*, respectively), one strain as a member of the group *A. oxydans/A polychromogenes/A. scleromae* and four strains at genus level (Uhlik et al. 2011). Analyses of the 16S rRNA coding genes of the strains confirmed that they are members of the genus *Arthrobacter*. However, 16S rRNA gene comparisons demonstrated that additional three isolates, of which none were identified by the MALDI Biotyper, are members of the genus *Arthrobacter*, as well.

Seven halotolerant *Arthrobacter* isolates capable to degrade phenanthrene were studied by Prisyazhnaya et al. (2012) which are closely related to *A. crystallopoietes*. These authors evaluated the applicability of MALDI-TOF MS for classification and differentiation and compared the results with those from DDH, 16S rRNA gene sequence phylogeny and genomic fingerprints obtained after BOX-PCR. Isolates and *A. crystallopoietes* share 15 MALDI-TOF peaks of which nine peaks (*m/z* 6,020, 6,704, 7,293, 7,344, 7,524, 7,652, 7,998, 8,516, and 9,472) are only found in this group. Two strains of this group are distinguishable from the other strains by the presence of 15 unique peaks whereas the remaining five isolates are characterized by eight unique peaks. These indicated relationships are also reflected in a dendrogram based on MALDI-TOF profiles in which the seven isolates are forming two groups both next related to *A. crystallopoietes* whereas the type strains of ten other *Arthrobacter* species are significantly more distant. These results are well in agreement with results from DDH, BOX fingerprinting, and phylogeny.

In a study investigating the MALDI-TOF profiles of the majority of established *Arthrobacter* species, several most interesting results were obtained (Peter Schumann; unpublished results) since several MALDI-TOF clusters (*Fig. 5.2*) are composed of species which are also phylogenetically forming a group or are placed in an "*Arthrobacter* group" (*Fig. 5.1*) on the basis of 16S rRNA similarity values and/or chemotaxonomic traits. All species of "*Arthrobacter citreus* group," "*Arthrobacter psychrolactophilus* group," "*Arthrobacter pigmenti* group," "*Arthrobacter albus/cumminsii* group," and "*Arthrobacter aurescens* group," respectively, are also forming separate MALDI-TOF groups. Species of "*Arthrobacter oxydans* group" are forming a MALDI-TOF group as well but *A. globiformis* and *A. pascens* are attached to this group, though their peptidoglycan structure is different and their phylogenetic position is clearly separate. The very close phylogenetic relationship between *A. oryzae* and *A. humicola* is reflected by MALDI-TOF spectra

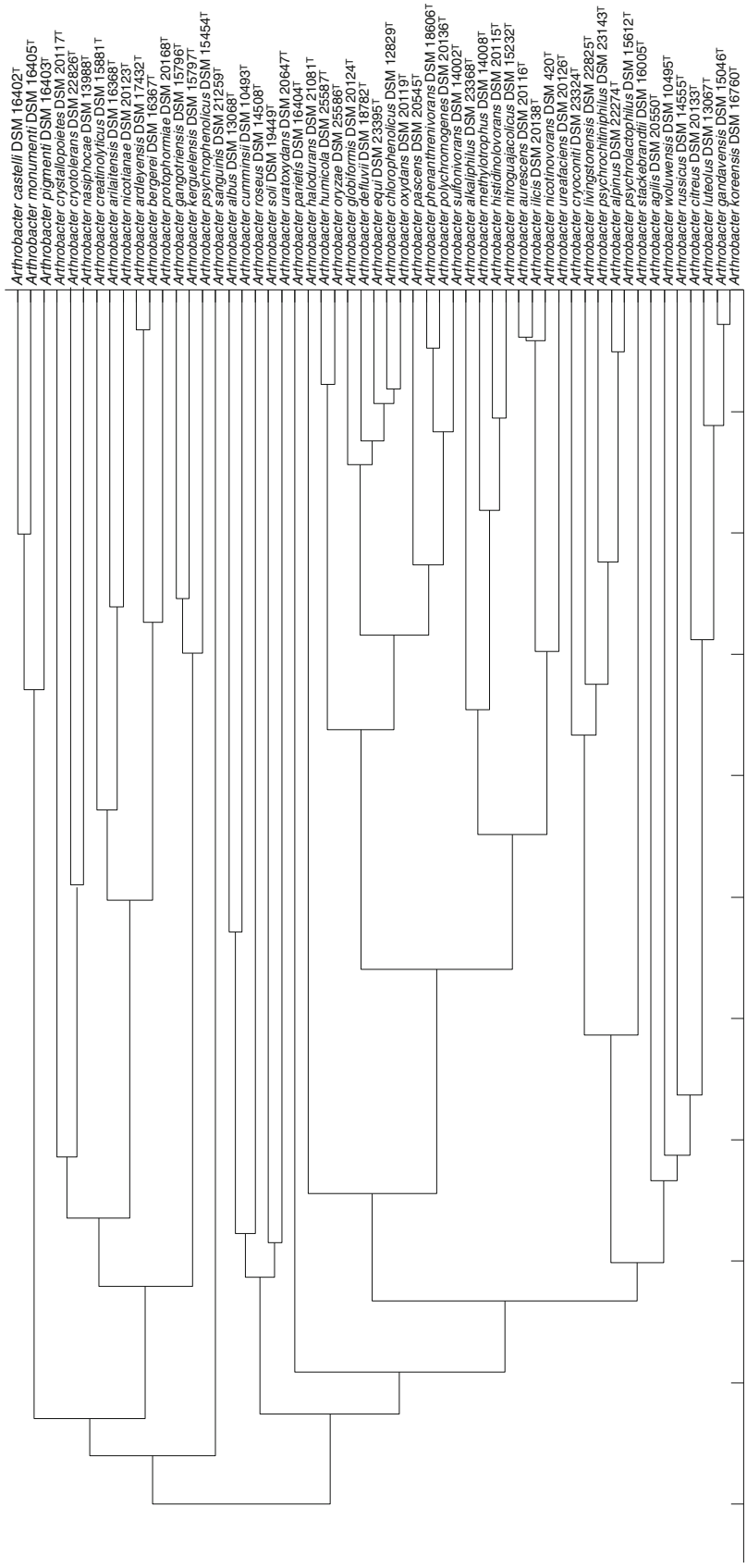

Fig. 5.2
Score oriented dendrogram generated by the Biotyper software (version 3.1, Bruker Daltonics) showing the similarity of MALDI-TOF mass spectra of cell extracts of selected type strains of the genus *Arthrobacter*

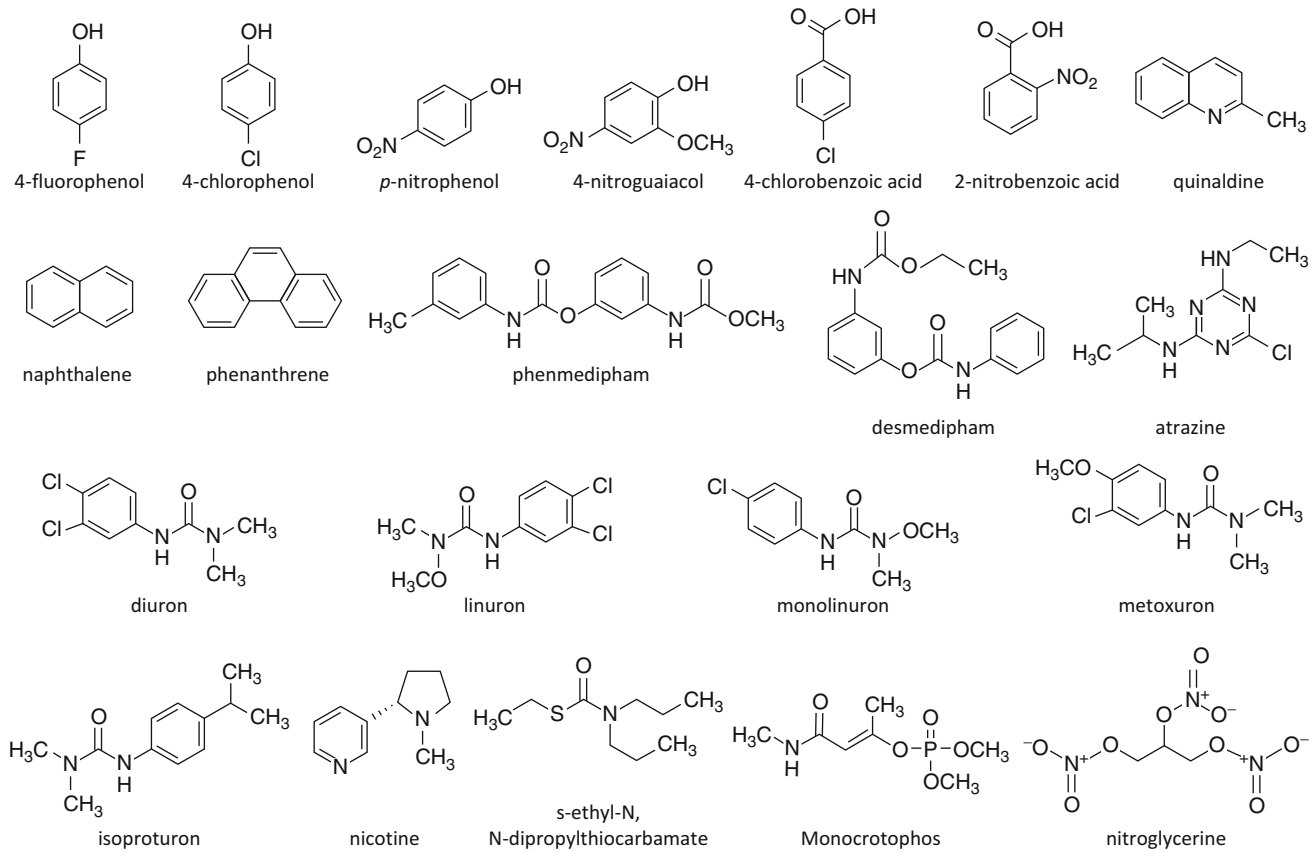

Fig. 5.3
Chemical structures of components attacked by certain *Arthrobacter* strains

as well. Selected species of "*Arthrobacter protophormiae* group" are grouped together by MALDI-TOF profiles except *A. soli* which is clearly separated from this group. Except *A. cryotolerans*, species of "*Arthrobacter sulfureus* group" are also forming a group.

Genomes

So far, nine *Arthrobacter* genomes have been sequenced, including *Arthrobacter globiformis* NBRC 12137^T, *Arthrobacter aurescens* TC1, *Arthrobacter arilaitensis* Re117^T, *Arthrobacter chlorophenolicus* A6^T, *Arthrobacter phenanthrenivorans* Sphe3^T, *Arthrobacter* sp. Rue61a, *Arthrobacter* sp. FB24, *Arthrobacter* sp. strain M2012083, and *Arthrobacter* sp. TB 23. The genomes of *A. chlorophenolicus* A6^T and *A. globiformis* NBRC 12137^T are accessible without associated publications.

The shotgun sequence of the genome of *A. globiformis* NBRC 12137^T (project accession PRJDA71847; accession no. BAEG01000000) comprises 4,954,410 bp, 4,529 coding sequences, three rRNAs, and 50 tRNAs.

The genome of *A. chlorophenolicus* A6^T (project accession PRJNA58969; accession no CP001341) comprises 4,980,870 bp, one chromosome and two plasmids, 4,590 coding sequences, 15 rRNAs, and 88 tRNAs. The G+C content is 66 mol %. The sizes of the chromosome and the two circular plasmids pACHL01 and pACHL02 are 4,395,537 bp, 426,858 bp, and 158,475 bp, respectively.

The 16S rRNA gene sequence of *Arthrobacter* sp. TB23 shares 100 % similarity with the type strain of *Arthrobacter flavus*, and hence, it can be considered to be an authentic member of this species. The shotgun genome sequence of *Arthrobacter* sp. TB 23 (project accession PRJNA170988; accession no ALPM01000000) comprises 3,542,528 bp and the G+C content is 63.3 mol %. The genome contains 3,298 ORFs, and it encodes 46 tRNAs and six rRNA operons. Genes of special interest identified are encoding for type III polyketide synthetase, a non-ribosomal peptide synthetase, and terpene biosynthetic genes (Fondi et al. 2012).

The draft genome of the nicotine-degrading *Arthrobacter* sp. strain M2012083 (accession no AKKK00000000) is 4,629,172 bp long, has a G+C content of 62.0 mol%, and contains 54 genes encoding for tRNAs and 4,312 coding sequences. The genome encodes for 522 proteins involved in carbohydrate metabolism, 59 proteins involved in aromatic compound metabolism, and contains 89 genes involved in stress responses. Genes related to motility are absent (Yao et al. 2012).

Arthrobacter aurescens TC1 was identified (Strong et al. 2002) based on high 16S rRNA sequence similarities with two environmental *A. aurescens* strains, *A. aurescens* TA13 and

strain 51. The complete chromosome of *A. aurescens* TC1 is 4,597,686 bp long and in addition, two circular plasmids are present, pTC1 and pTC2 which are 408,237 bp and 300,725 bp long, respectively (Mongodin et al. 2006). The G+C content is 62.4 mol % which is very close to 61.9 mol % reported for the type strains of *A. aurescens* (Kodama et al. 1992) and *A. nitroguajacolicus* (Kotoučková et al. 2004).

On the chromosome 4,136, open reading frames (ORFs) have been identified of which 2,767 could be assigned to a function. Eighteen ORFs are encoding for rRNA, 54 for tRNA, and one for sRNA; 379 ORFs are assigned to conserved hypothetical proteins, 468 ORFs are encoding for hypothetical proteins, and 522 ORFs are of unknown function.

Eleven genomic islands are present on the chromosome with atypical G+C content. These genomic islands are encoding for 180 genes. In these genomic islands, transposons and related genes, transcriptional regulators, resistance genes, and genes involved in metabolism and transport of substrates are present. On the chromosome 23 sequences are found that are encoding for transposons or insertion sequences. A phage sequence was not detected. Genes coding for flagella synthesis, motility, and chemotaxis are absent. Absence of motility is in agreement with the data published for the type strain of *A. aurescens* by Kodama et al. (1992). The majority of ORFs are associated to the categories energy metabolism (739), encoding for transport and binding proteins (517), related to cell envelope (340) and regulatory functions (332). Additional 186 genes were assigned to cellular processes, 167 genes to protein fate, 141 genes to synthesis of cofactors, prosthetic groups, and carriers, 136 genes to amino acid biosynthesis, 123 genes to protein synthesis, 123 genes to fatty acid and phospholipid metabolism, 117 genes to central intermediary metabolism, 111 genes to DNA metabolism, 89 genes to transcription, 82 genes to purine, pyrimidine, nucleoside, and nucleotides, 54 genes to signal transduction, and 35 genes to mobile and extrachromosomal element function.

The G+C contents of plasmid pTC1 and plasmid pTC2 are 64.6 mol % and 61.3 mol %, respectively. The number of ORFs is 297 and 275, respectively. On plasmid pTC1, a function is assigned to 139 ORFs and on plasmid pTC2 to 275 ORFs; 24 ORFs on pTC1 are encoding for conserved hypothetical proteins and on pTC2 36 ORFs. On pTC1, 104 ORFs are encoding for hypothetical proteins and 30 ORFs are of unknown function. On pTC2 51 ORFs are encoding for hypothetical proteins and 23 ORFs are of unknown function. Except RNA encoding genes on both and protein function related ORFs on pTC2, the two plasmids are harboring at least one gene of each category mentioned above for the genome. Furthermore, a cluster of genes is present on each of the two plasmids encoding for proteins associated with biodegradation of isopropylamine. As already reported earlier (Sajjaphan et al. 2004), the three triazine hydrolase genes *trzN, atzB,* and *atzC* are exclusively located on plasmid pTC1. Also on pTC1, six identical direct tandem repeats of about 16 kb are found. Fourteen ORFs on plasmid pTC1 are encoding for transposons and/or insertion elements. On plasmid pTC2, nine ORFs are encoding for transposons and/or insertion elements.

The chromosomes of *A. aurescens* TC1 and *Arthrobacter* sp. FB24 share 79.1 % similarity at the amino acid level. For a total of 540 genes of *A. aurescens* TC1, no corresponding genes are found on the chromosome of *Arthrobacter* sp. FB24. The two strains share 25 identical proteins of which ten are ribosomal proteins.

Comments on the taxonomy of *Arthrobacter aurescens* TC1: this strain was identified based on highest 16S rRNA gene sequence similarities with *A. aurescens* TA13 (Shimoni et al. 2002; accession number AF467106) and *A. aurescens* strain 51 (Macur et al. 2004; accession number AF388032) but not considering the corresponding sequence of the type strain of the species. Both, strain TA13 and strain 51, share 100 % 16S rRNA sequence similarity with the type strain of *A. nitroguajacolicus* and 99.7 % similarity with the type strain of *A. aurescens*. Hence, it appears to be more likely that strains TA13 and 51 are members of the species *A. nitroguajacolicus* than *A. aurescens*. However, the two environmental isolates TA13 and S1 had been identified at a time when *A. nitroguajacolicus* was not yet described and the best hit for the two 16S rRNA gene sequences actually was that of the type strain of *A. aurescens*. Like the two environmental isolates also, the 16S rRNA gene sequence of *A. aurescens* TC1shows highest similarity with the corresponding sequence of the type strain of *A. nitroguajacolicus* (99.9 %) and that of the type strain of *A. aurescens* (99.7 %). Furthermore, in the *rec*A gene sequence, *A. aurescens* TC1 shows only 91.4 % similarity with the type strain of *A. aurescens* (corresponding sequence of *A. nitroguajacolicus* not available), strongly suggesting that *A. aurescens* TC1has been misclassified. In conclusion from comparison of 16S rRNA and *rec*A gene sequences, it is most likely that *A. aurescens* TC1 is either a strain of the species *A. nitroguajacolicus* or a representative of a so far not described species.

The genome of *Arthrobacter arilaitensis* Re117^T is composed of a circular chromosome and the two plasmids pRE117-1 and pRE117-2 (Monnet et al. 2010). The size of the chromosome is 3,859,257 bp (accession no FQ311875), that of plasmid pRE117-1 is 50,407 bp (accession no FQ311475), and that of plasmid pRE117-2 is 8,528 bp (accession no FQ311476), resulting in total in a genome size of 3,918,192. The size of the chromosome is approximately 0.7–1.3 Mbp smaller than those of the genomes of *Arthrobacter phenanthrenivorans* Sphe3^T, *Arthrobacter globiformis* NBRC 12137^T, *A. aurescens* TC1, *Arthrobacter* sp. FB24, *Arthrobacter chlorophenolicus* A6^T, and *Arthrobacter* sp. strain M2012083 but approximately 0.3 Mbp larger than the chromosome of *Arthrobacter* sp. TB23. The G+C content of the chromosome is 59.2 mol %. The complete genome contains 3,518 genes. The number of rRNA and tRNA genes is 18 and 64, respectively; 3,436 genes are encoding for proteins of which 2,155 are with predicted and 1,281 are without predicted function. In addition, 123 pseudogenes are present in the genome. The organism's genome contains genes that are encoding for proteins associated with repair of DNA lesions, three type I restriction modification systems, a Sec-dependent protein secretion pathway, a twin-arginine translocation pathway, and 356 putative transport proteins. Of the 2,727 gene sequences, 1,210 show highest similarities with corresponding

sequences of *A. aurescens* TC1, *A. chlorophenolicus* A6^T, and *Arthrobacter* sp. FB24. Compared to the latter three strains, the genome of *A. arilaitensis* RE117^T shares with the other three genomes 1,545 coding sequences but 1,246 are unique for *A. arilaitensis* RE117^T. The three reference genomes contain 746 coding sequences which are absent in *A. arilaitensis* RE117^T. The genome encodes for 17 proteins with putative lipase and esterase activity, of which one is a secretory triacylglycerol lipase. This enzyme is considered to contribute significantly to ripening of smear-ripened cheese which is a natural habitat of *A. arilaitensis*. One cluster of genes is associated with the aerobic catabolism of glycerol, including a glycerol uptake protein, a glycerol kinase, and a glycerol-3-phosphate dehydrogenase. Genes encoding enzymes of the β-oxidation pathway are present as well. For five out of 11 putative fatty acid-CoA dehydrogenases and four out of 11 fatty acid-CoA ligases of *A. arilaitensis* RE117^T, no ortholog enzymes are present in the genomes of *A. aurescens* TC1, *Arthrobacter* sp. FB24, and *Arthrobacter chlorophenolicus* A6^T. The genome also contains 30 genes associated with Fe^{3+}/siderophore transporters of which 20 genes do not have orthologs in the genomes of *A. aurescens* TC1, *Arthrobacter* sp. FB24, and *Arthrobacter chlorophenolicus* A6^T. Six putative proteins mediate release of iron from the Fe^{3+}/siderophore complex and five of these proteins are unique compared to *A. aurescens* TC1, *Arthrobacter* sp. FB24, and *Arthrobacter chlorophenolicus* A6^T. The chromosome was found to harbor 4.92 % insertion sequences (109 complete and 35 partial). This is significantly higher than in the chromosomes of the three reference genomes (0.08–0.4 %).

Of 42 ORFs located on plasmid pRE117-1 only 19 genes are encoding for proteins with predicted function. This plasmid contains approximately 10 % insertion sequences. Three sequences are encoding for DNA mobilization proteins, one for a putative secreted peptidase, a single stranded DNA-binding protein, and proteins of unknown function. Five proteins show highest amino acid similarity with proteins encoded from plasmid FB24-2 of *Arthrobacter* sp. FB24. Another five proteins show highest similarity with plasmid proteins of other arthrobacters. Plasmid pRE117-2 harbors 13 gene sequences of which nine are hypothetical proteins whereas two are predicted to encode for mobilization proteins.

The genome of the phenanthrene-metabolizing strain *Arthrobacter phenanthrenivorans* Sphe3^T is composed of a chromosome with 4,250,414 bp (accession no CP002379) and two plasmids with 190,450 bp and 94,456 bp, respectively (Kallimanis et al. 2011). The G+C content of the chromosome is 66 mol % whereas that of the two plasmids is 62 mol %. The genome contains 4,288 putative genes of which 4,212 are encoding for proteins, 76 for RNAs, and 77 for pseudogenes. Four rRNA operons are present. Out of the protein coding genes, 3,167 are assigned to putative function.

The manually annotated genome of *Arthrobacter* sp. FB24 (accession no CP000454), which had been isolated from chromate and xylene enriched soil, is composed of a circular chromosome and the three circular plasmids, p1, p2, and p3 (http://www.genome.jp/kegg-bin/show_organism?org=T00421). The chromosome is 4,698,945 bp long, plasmid p1 is 159,538 bp long (accession no CP000455), plasmid p2 is 115,507 bp long (accession no CP000456), and plasmid p3 is 96,488 bp long (CP000457). The genome encodes for 4,523 protein genes and 69 RNA genes.

The genome of *Arthrobacter* sp. Rue61a comprises a circular chromosome with a length of 4,736,495 bp, the circular plasmid pARUE232 with a length of 231,551 bp, and the linear plasmid pARUE113 with a length of 112,992 bp (Niewerth et al. 2012). The G+C contents are 62.32, 61.58, and 60.88 mol %, respectively. On the genome 4,575 ORFs, six rRNA operons, 53 tRNAs, and nine pseudogenes are found. Out of the total ORFs, 3,382 are assigned to a putative function whereas 1,193 are considered as hypothetical. The genome comprises 303 ORFs that are most similar to genes of phyla other than actinobacteria and 13 putative genomic islands were detected on the chromosome that contains 160 genes. A total of 594 genes are encoding for transporters and binding proteins; 512 of these genes are similar or identical to genes found in the genome of *A. aurescens* TC1. Remaining genes encoding for transporters/binding proteins are found on genomic islands or on plasmid pARUE232. The genome encodes several putative uptake systems for K^+, organic osmoprotectants, and for synthesis of compatible solutes. As indicated by growth experiments, *Arthrobacter* sp. Rue61a encodes proteins that allow utilization of several carbon sources for growth including 4-hydroxybenzoate, protocatechuate, vanillate, hydroxyphenylacetate, homoprotocatechuate, tyrosine, the N-heterocyclic compounds hypoxanthine, xanthine, 1*H*-4-oxoquinaldine, 1*H*-3-hydroxy-4-oxoquinaldine, several sugars, alcohols, organic acids, amines, and amides. Furthermore, genes encoding enzymes are present, allowing the organism to utilize urea, creatinine, and putrescine as sole source of nitrogen and taurine as source of sulfur.

In their study dealing with the complete genome analysis of *Arthrobacter* sp. Rue61a Niewerth et al. (2012) compared the Clusters of Orthologous Groups (COGs) of proteins of the genomes of all available *Arthrobacter* strains. These authors showed that the chromosomes of all *Arthrobacter* strains share only 1,014 homologous genes. Compared to the other genomes, *Arthrobacter globiformis* NBRC 12137^T shows the highest percentage of COG categories related to amino acid transport and metabolism, energy production and conversion, and lowest percentage of the COGs are found related to intracellular trafficking, secretion, and vesicular transport, signal transduction mechanisms, and transcription. In the genome of *A. arilaitensis* Re117^T, the percentage of COGs of the categories replication, recombination, and repair and translation, ribosomal structure, and biogenesis are significantly higher than in the other genomes and category carbohydrate transport and metabolism is the lowest. In the genome of *A. chlorophenolicus*, the percentage of COG category cell motility is approximately sixfold higher than in the other genomes and it also contains the highest percentage of COG category intracellular trafficking, secretion, and vesicular transport. *Arthrobacter* sp. Rue61a shows slightly higher percentages of the COGs carbohydrate transport and metabolism, general function prediction only, lipid transport and metabolism, and transport than the other genomes.

A. phenanthrenivorans Sphe3^T contains the second highest percentage of COG categories replication, recombination, and repair that is still significantly higher than in the remaining five genomes. In the genomes of *A. aurescens* TC1 and *Arthrobacter* FB24, percentages of all COGs are within the average of all genomes.

Bacteriophages

Probably the first report on *Arthrobacter*-lyzing bacteriophages (Conn et al. 1945) was published 2 years earlier than the proposal of the genus *Arthrobacter* (Conn and Dimmick 1947). In this study, Conn et al. (1945) showed that a bacteriophage, which was enriched using a culture labeled "Bact. glob." and showing the typical morphology of "*Bacterium globiforme*", lyzed only cells of the host but not any of some other isolates also regarded as members of *B. globiforme group*. Two bacteriophages of *A. globiformis* were isolated by Gillespie (1960). A bacteriophage reproducing in the type strain of *A. globiformis* was characterized by Einck et al. (1973). This bacteriophage named ΦAG8010 showed a slow adsorption rate to the host cells. On the basis of electron microscopic examination, the bacteriophage was assigned to Bradley's group B (Bradley 1967). It possesses a hexagonal head and an unsheathed tail. Casida and Liu (1974) isolated a virulent bacteriophage named FX-1 which was shown to infect two strains of *A. globiformis,* ATCC 8010^T and ATCC 4,336. These authors found some evidence that the sensitivity of the host cells to the bacteriophage is dependent on the growth phase. Lytic spectra of 11 *Arthrobacter* phage isolates were studied by Germida and Casida (1981) on two strains of *A. globiformis* (ATCC 8010^T and ATCC 4,336), five *Arthrobacter* isolates and several reference strains, of which nowadays only *A. crystallopoietes, A. oxydans*, and *A. ramosus* are still considered as members of the genus. In this study, bacteriophage GAP-15 lyzed only ATCC 8010^T. Bacteriophage GAP-16 lyzed *A. globiformis,* ATCC 8010^T and ATCC 4,336 and bacteriophage GAP-14 lyzed *A. globiformis,* ATCC 8010^T and ATCC 4,336 and *Arthrobacter* isolate SPI-1. This latter isolate was exclusively lyzed by bacteriophage GAP-40. Two bacteriophages, GAP-41 and GAP-42, were shown to cause lysis of the type strain of *A. oxydans*. Two *Arthrobacter* isolates, GSI-5 and GSI-1, were lyzed by bacteriophage GAP-32 and, in addition, *Arthrobacter* isolate GSI-5 was also lyzed by bacteriophage GAP-33. GSI-1 was also lyzed by bacteriophages GAP-30 and GAP-31. *Arthrobacter* isolate GSI-6 was lyzed by two phages, GAP-30 and GAP-34.

Seventeen bacteriophages infecting *Arthrobacter* isolates were recovered from concentrated samples of sewage and river water and nine of which were classified to be distinct (Brown et al. 1978). Based on tail length, these bacteriophages were assigned to Bradley's group B and C, respectively.

A lysogenic bacteriophage hosted by *Arthrobacter polychromogenes* was detected by Schippers-Lammertse et al. (1963) and characterized by Daems (1963). This bacteriophage is composed of a hexagonal head and a long, rigid tail sheath with cross-striations.

Phenotypic Analyses

Arthrobacters are forming colonies of <1–5 mm in diameter depending on medium composition and species examined. Colonies are usually opaque and the majority of arthrobacters are pigmented (light yellow–yellow-orange, creamy, white) or are colorless. Two species, *A. agilis* and *A. roseus*, are red-pink and red-rose pigmented, respectively. *A. polychromogenes* can show different colony pigmentations, depending on the growth medium (colorless, white, grayish, blue or green). Almost all *Arthrobacter* species exhibit a rod–coccus cycle, but *A. agilis* appears always as a coccus and *A. soli* was described to be rod-shaped. The vast majority of arthrobacters are nonmotile, but approximately on third of the species are motile. For some species, peritrichous or lateral flagellae were reported. The majority of species are strictly aerobic, but some are facultatively anaerobic. Catalase is usually produced, whereas oxidase is absent in the vast majority of *Arthrobacter* species. The majority of species are mesophilic, with temperature optima for growth between 20 °C and 30 °C. Usually, growth at 37 °C is slow or even absent. Some species are psychrotolerant, and some may be psychrophilic. The lowest growth temperature reported for *Arthrobacter* species is −6 °C (*A. cryotolerans* and *A. livingstonensis*; Ganzert et al. 2011). Arthrobacters are heterotrophic organisms utilizing a broad range of sugars, amino acids, and organic acids as sole source of carbon and energy. Sugars are utilized oxidatively, but some species are also capable to utilize sugars with production of acid. Some species, e.g., *A. chlorophenolicus, A. defluvii*, and *A. phenanthrenivorans*, are able to degrade toxic compounds such as 4-chlorophenol or phenanthrene.

Fatty acid profiles of arthrobacters are mainly consisting of *iso*- and *anteiso*-methyl branched fatty acids. A typical fatty acid profile of an arthrobacter exhibits the predominant compound *anteiso*-$C_{15:0}$ and usually *iso*-$C_{15:0}$ is the second major compound. Often, relatively high amounts of *iso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$ are present, as well. In few species also significant amounts of the straight-chain fatty acid $C_{16:0}$ were detected. Unfortunately, in the past, fatty acids of arthrobacters were analyzed from biomasses that were not standardized for the physiological age at the point of harvest nor was the same medium composition applied to produce standardized biomasses. Since both, physiological age and medium composition, can influence the fatty acid profile significantly, published data are of little use for species identification or differentiation.

Arthrobacters can be divided into two groups based on differences in the quinone systems. One group contains monosaturated menaquinones whereas the other contains completely unsaturated menaquinones. The majority of established *Arthrobacter* species contain a mono-unsaturated menaquinone with nine isoprenic units in the side chain [MK-9(H_2)]. Three species, *A. scleromae* (Huang et al. 2005), *A. albus,* and *A. cumminsii* (Busse et al. 2012), contain menaquinone MK-8(H_2) and, unique among arthrobacters, *A. phenanthrenivorans* was reported (Kallimanis et al. 2009) to contain a menaquinone system composed of MK-8 and

MK-9(H_2). This quinone system is most surprising. Generally, quinone systems can be considered to be a rather conserved trait and all close relatives of this species comprising "*Arthrobacter oxydans* group" show predominantly menaquinone MK-9(H_2), only. Also unexpected is the observation that the quinone system is composed of a saturated and an unsaturated quinone which differ in the length of the isoprenic side chain. Species with menaquinone MK-9(H_2) were assigned to "globiformis" group (Stackebrandt et al. 1983) or "*A. globiformis/A. citreus group*" (Keddie et al. 1986). The second group contains species which show a quinone system with completely unsaturated isoprenoic units in the side chain with menaquinone MK-8, MK-9, or MK-10 predominating. Members of this group were named by Stackebrandt et al. (1983) the "nicotianae" group and by Keddie et al. (1986) the "*A. nicotianae group*."

The peptidoglycan of all *Arthrobacter* species examined contains lysine as the diagnostic diaminoacid and adjacent peptide side chains are cross-linked via L-lysine of one side chain and D-alanine at position four of the other. The interpeptide bridges contain either mono-carboxylic L-amino acid(s) or a di-carboxylic amino acid. Applying the three digit system of Schleifer and Kandler (1972), these two peptidoglycan types are designated A3α and A4α, respectively. *Arthrobacter* species showing peptidoglycan type A3α were assigned to "globiformis" group (Stackebrandt et al. 1983) or "*A. globiformis/A. citreus group*" (Keddie et al. 1986); species showing peptidoglycan type A4α were assigned to "nicotianae" group or "*A. nicotianae group*." However, large variability is present in the amino acid composition of the interpeptide bridge. On the basis of interpeptide bridge amino acid variability, Komagata and Suzuki (1987) defined seven groups. Group I contains Lys-Ser-Thr-Ala, group II contains Lys-Ala-Thr-Ala, group III contains Lys-$Ala_{1–4}$, group IV contains Lys-Ser-$Ala_{2–3}$, group V contains Lys-Thr-Ala_2, group VI contains Lys-Ala-Glu, and Group VII shows Lys-Glu. By now, two variants of group V have been detected in *Arthrobacter* species. *Arthrobacter agilis* (Koch et al. 1995), *Arthrobacter flavus* (Reddy et al. 2000), *Arthrobacter subterraneus* (Chang et al. 2007), *Arthrobacter tecti, Arthrobacter tumbae* (Heyrman et al. 2005), *Arthrobacter psychrolactophilus* (Loveland-Curtze et al. 1999), and *Arthrobacter alpinus* (Zhang et al. 2010) were shown to exhibit an interpeptide bridge containing of Lys-Thr-Ala_3. Quantitative amino acid analyses of the peptidoglycan of *Arthrobacter psychrochitiniphilus* suggest that the interpeptide bridge is Lys-Thr-Ala (Wang et al. 2009). Furthermore, some so far new amino acid compositions were found in five *Arthrobacter* species. *Arthrobacter castelli* contains Lys-Ala-Ser-Ala_3 in its interpeptide bridge (Heyrman et al. 2005); *Arthrobacter cumminsii* contains Lys-Ser(Gly)-Glu (Funke et al. 1996); *A. nasiphocea* contains Lys-Ala_2-$Gly_{2–3}$-Ala (Gly) (Collins et al. 2002), *Arthrobacter roseus* contains Lys-Gly-Ala_3 (Reddy et al. 2002), and *Arthrobacter woluwensis* contains L-Lys-D-Asp (Funke et al. 1996).

The first report on lipids in an arthrobacter strain was published by Walker and Bastl (1967). These authors showed that in the lipid profile of *Arthrobacter globiformis* strain 616, the glycolipids monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), and dimannosyl diacylglycerol (DMDG) are present. A more complete analysis of the polar lipid profiles of *A. globiformis* strain 616 and the type strains of *Arthrobacter crystallopoietes* and *Arthrobacter pascens* was reported by Shaw and Stead (1971). They confirmed the presence of the three glycolipids MGDG, DGDG, and DMDG in *A. globiformis* strain 616 and detected the same glycolipids in *A. crystallopoietes* and *A. pascens*. Evidence for the presence of trimannosyl diacylglycerol in the three strains was provided, as well and presence of tetramannosyl diacylglycerol was supposed. Furthermore the phospholipids phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and phosphatidylinositol (PI) were detected in all three strains. Kostiw et al. (1972) were only able to confirm the presence of the glycolipids MGDG and DGDG and the phospholipid PG, DPG, and PI in *A. crystallopoietes*.

In the early1980s, the polar lipid profiles of additional *Arthrobacter* strains were analyzed by 2-dimensional thin layer chromatography (Collins et al. 1981, 1982; Collins and Kroppenstedt 1983) and an overall similarity in the polar lipid profiles was shown. All strains analyzed showed the presence of DPG, PG, and one or more unidentified glycolipids. Except *Arthrobacter nicotianae, Arthrobacter protophormiae,* and *Arthrobacter sulfureus,* all strains also exhibited the presence of PI. Furthermore, it was shown that the chromatographic motility of the unidentified major glycolipid of *A. sulfureus* was clearly different from those of all *Arthrobacter* strains studied. In conclusion from these studies, Keddie et al. (1986) supposed that one major glycolipid of all *Arthrobacter* species is dimannosyl diacylglycerol except *A. sulfureus* which contains the major glycolipid digalactosyl diacylglycerol.

The majority of *Arthrobacter* species, which were also analyzed for their polar lipids, were reported to contain DPG, PG, PI, and one or more unidentified glycolipids. However, reservations have to be expressed concerning polar lipid profiles reported for several *Arthrobacter* species. These reservations are based on the conserved character of polar lipid profiles indicated by the fact that closely related species usually do not show significant differences in their polar lipid profiles. The species *A. phenanthrenivorans* (Kallimanis et al. 2009), *A. antarcticus* (Pindi et al. 2010), *A. flavus* (Reddy et al. 2000), and *A. roseus* (Reddy et al. 2002) were reported to contain phosphatidylethanolamine (PE) and to lack any glycolipid. Within the family *Micrococcaceae*, the ability to synthesize PE appears to be absent whereas common to all *Micrococcaceae* species is the presence of at least one glycolipid. In the case of *A. roseus,* this reservation could be confirmed by 2D-TLC which demonstrated that this species does not contain PE but several glycolipids, of which one shows the chromatographic motility of DMDG (Busse, unpublished results). Also absence of any glycolipid in the polar lipid profiles of *A. tecti, A. tumbae* (Heyrman et al. 2005), and *A. russicus* (Li et al. 2004) is not in accordance with characteristics of their close relatives and re-analysis of the polar lipids would be desirable. A polar lipid profile composed exclusively of PG was reported for *A. livingstonensis* and *A. cryotolerans* (Ganzert et al. 2011). However, in addition to PG, the polar lipid profile of *A. livingstonensis* also contains

DPG, PI, and four unidentified glycolipids and *A. cryotolerans* contains in addition to PG also DPG and an unidentified glycolipid (Kai Mangelsdorf, pers. communication). This additional information concerning the polar lipid profile of *A. livingstonensis* and *A. cryotolerans* is well in accordance with the polar lipid profiles that were reported for related species.

Recently, the chemical structures of the major glycolipids of the type strains of *A. globiformis* and *Arthrobacter scleromae* were analyzed applying more sophisticated methods including matrix-assisted laser desorption/deionization Fourier transform ion cyclotron resonance mass spectrometry (MALDI FT ICR-MS) and 1D and 2D nuclear magnetic resonance (NMR) spectroscopy (Paściak et al. 2010). These studies demonstrated that in both strains, the major glycolipids are acyldimannosyl monoacylglycerol (ADMMG) and monogalactosyl diacylglycerol (MGDG). The presence of ADMMG was also detected in two other representatives of the *Micrococcaceae,* the type strains of *Sinomonas atrocyanea* (formerly *Arthrobacter atrocyaneus*) and *Rothia mucilaginosa* (Niepel et al. 1997; Paściak et al. 2004) whereas analyses of the chemical structure of the major glycolipid of the type strain of *Micrococcus luteus* revealed the presence of a dimannosyl diacylglycerol (Pakkiri et al. 2004).

Isolation, Enrichment, and Maintenance Procedures

The majority of *Arthrobacter* type strains were isolated, applying standard procedures regardless of their source of isolation. Only few of them were recovered applying enrichment procedures, especially those that were shown to degrade certain compounds, e.g., 4-chlorophenol.

Arthrobacter chlorophenolicus was recovered from aridic halplustoll, sandy loam (pH 7.5, 68.6 % sand, 16.1 % silt, 15.3 % clay) after enrichment with 4-chlorophenol (4-CP) (Westerberg et al. 2000). Two grams of soil were added to 300 ml GM medium (l^{-1}: 2.1 g K_2HPO_4, 0.4 g KH_2PO_4,0.5 g NH_4NO_3, 0.2 g $MgSO_4 \times 7H_2O$, 0.023 g $CaCl_2 \times 2H_2O$, 0.002 g $FeCl_2 \times 6\ H_2O$) containing 50 ppm 4-CP. When 30–50 % of 4-CP had disappeared, new 4-CP was added and this procedure was repeated over several times, increasing the concentration up to 350 ppm. Bacteria able to degrade 4-CP were isolated from the enrichment culture on GM agar plates containing 300 ppm 4-CP and 0.3 % yeast extract hardened at a slant with a top layer of GM agar, creating a diffusion gradient of 4-CP. 4-CP resistant bacteria were isolated by cross-streaking against the 4-CP gradient on the agar plates and purified by repeated streaking on GM agar containing 200 ppm 4-CP.

Also the 4-CP degrader *Arthrobacter defluvii* was isolated after enrichment with 4-CP (Kim et al. 2008). A sewage sample supplemented with 50 ppm 4-CP was applied to enrich 4-CP degraders and, after incubation, samples from a dilution series were plated on R2A agar and incubated for 14 days. Creamy white colonies were then selected for further characterization.

Arthrobacter nitroguajacolicus was isolated after enrichment in mineral medium supplemented with 0. 1 mM 4-nitrophenol (Kotoučková et al. 2004). The minimal medium consisted of (l^{-1}): $MgSO_4 \times 7H_2O$, 0.00025 g $FeCl_2 \times 6\ H_2O$, $(NH4)_2SO_4$, 0.42 g K_2HPO_4, 0.17 g KH_2PO_4, 0.88 g $Na_2HPO_4 \times 12\ H_2O$. After incubation on a rotary shaker at 28 °C, an aliquot of the enrichment culture (10 ml) was transferred to 100 ml minimal medium containing 4-nitroguaiacol. After decolonization of the medium, indicating degradation of 4-nitroguaiacol, samples were spread on solid minimal medium supplemented with 4-nitroguaiacol and incubated until appearance of single colonies.

Arthrobacter phenanthrenivorans was isolated from a creosote-polluted soil after enrichment with phenanthrene; 100 ml minimal medium (Sambrook et al. 1989) was supplemented with 0.01 % phenanthrene as the sole source of carbon and energy and incubated for 7 days at 30 °C. After serial dilutions on solid medium, colonies able to grow in the presence of phenanthrene were selected, out of which one was characterized in detail.

Arthrobacter sulfonivorans and *Arthrobacter methylotrophus* were isolated after enrichment with dimethylsulfone as the sole source of carbon and energy (Borodina et al. 2000; 2002). Enrichment was carried out in minimal medium which was composed of (l^{-1}) 7.9 g $Na_2HPO_4 \times 2\ H_2O$, 1.5 g K_2HPO_4, 0.1 g $MgSO_4$, 0.8 g NH_4Cl, and 10 ml trace element solution (Tuovinen and Kelly 1973) and supplemented with 10 mM dimethylsulfone. The medium (100 ml) was inoculated with 10 g soil, and incubation was carried out at 10–15 °C. After serial incubation in fresh medium, samples were spread on agar supplemented with saturated aqueous phenol red in order to detect acid production as a result of the oxidation of dimethylsulfone. Positive colonies were transferred to agar medium and incubated at 25 °C.

Arthrobacter psychrochitiniphilus was isolated from fresh penguin guano samples after enrichment with colloidal chitin (Wang et al. 2009). Agar solidified M9 minimal medium [(l^{-1}) 12.8 g $Na_2HPO_4 \times 7\ H_2O$, 3.0 g K_2HPO_4, 0.5 g NaCl, 0.1 g, 1.0 g NH_4Cl] was inoculated with dilutions of the guano samples. Agar plates were incubated for 7–28 day at 10 °C and colonies were subcultivated for purification.

Arthrobacter creatinolyticus strains were isolated from urine with unusually low creatinine concentrations and due to their ability to hydrolyze creatinine (Hou et al. 1998).

Arthrobacters are not fastidious organisms and since, for long-term conservation, standard procedures can be applied including freezing of cell suspensions in glycerol solution at −80 °C or lyophilization.

Ecology

Arthrobacters are considered to be ubiquitous as they could be found in soil, water, and air. Type strains of the genus were isolated from soil including forest soil, humus soil, rockery soil, paddy soil, soil of the root system, a filtration substrate made from volcanic rock and alpine soil, from wastewater sediments, Antarctic lake sediments and Antarctic sea sediments, from deep surface water, sewage and sea water, from cyanobacterial mats from the Antarctica, from human skins and human clinical

sources, from animals sources like a cattle with mammary and uterine infections, the nose of the common seal, the fly *Protophormia terraenovae*, organs of Greenland halibut, the guano of Adelie penguins, chicken feces and poultry litter, from plant, e.g., cured tobacco leaves, from surfaces of cheese and biofilms, and from the air in the Russian space station Mir.

Arthrobacter strains are commonly detected among cultured strains of microbial communities of terrestrial subsurfaces and are often among the predominant members. Crocker et al. (2000) isolated *Arthrobacter* strains from unconsolidated subsurface sediments. *Arthrobacter*-like strains accounted for 24 % of the 169 isolates obtained. However, the relative proportion of *Arthrobacter*-like isolates varies from one lithology to another. Twenty-nine percent of the 91 strains isolated from lacusterine sediment samples were closely related to *Arthrobacter*, whereas only one out of 47 isolates from the upper paleosol samples was identified *Arthrobacter*-like.

Arthrobacter has also been detected in several other subsurface environments including saturated Atlantic coastal plain sediments (20 % of 187 heterotrophic isolates; Balkwill and Boone 1997; Reeves et al. 1995), deep mine gallery clays (Boivin-Jahns et al. 1995), a karstic aquifer (Rusterholtz and Mallory 1994), a basalt aquifer (Zheng and Kellog 1994), unwelded volcanic tuffs (21 % of 119 isolates; Haldeman et al. 1993), and root domains of mature sugar beet (12 % of 556 isolates; Lilley et al. 1996). Lee and Kim (2003) observed *Arthrobacter* strains to be the dominant population of the biofilm from a Seoul water distribution system at operating day 7.

Members of the genus *Arthrobacter* are usually regarded as obligate aerobes. In order to survive periods of oxygen limitations due to changes in oxygen tension in the upper layer of soil, some *Arthrobacter* species have developed alternative, oxygen-independent growth strategies. For instance, under anaerobic conditions, *Arthrobacter nicotinae* reduces nitrate to ammonia, while *Arthrobacter globiformis* uses nitrate as terminal electron acceptor and is able to carry out mixed acid fermentation (Eschbach et al. 2003).

Members of the genus *Arthrobacter* utilize a wide variety of organic compounds, xenobiotica, and other harmful substances (see applications), and hence, they play a significant role in the transformation of organic matter in natural environment. Certain *Arthrobacter* strains are able to tolerate high concentrations of heavy metals like cadmium and copper or even carry out detoxification by accumulation of the heavy metals in their outer layer which may support degradation of harmful compounds in metal-contaminated soils (Roane and Pepper 2000; Konstantinidis et al. 2003).

Pathogenicity: Clinical Relevance

Arthrobacter spp. are widely distributed in the environment, especially in soil where they are considered to constitute a major part of the community but recently certain species have been recognized as opportunistic pathogens.

Due to difficulties in identifying *Arthrobacter* strains by conventional biochemical assays, the first *Arthrobacter* strains from clinical specimen were identified by using 16S rRNA gene sequence analyses (Funke et al. 1996). After this initial finding, several other studies have been published regarding the appearance of *Arthrobacter* spp. in clinical specimens (Park et al. 2012; Mages et al. 2008; Kim et al. 2007; Huang et al. 2005; Bernasconi et al. 2004; Wauters et al. 2000; Hou et al. 1998; Hsu et al. 1998). Among the 66 established *Arthrobacter* species, six were isolated from clinical sources, namely, *Arthrobacter albus* (Wauters et al. 2000), *Arthrobacter creatinolyticus* (Hou et al. 1998), *Arthrobacter cumminsii* (Funke et al. 1996), *Arthrobacter luteolus* (Wauters et al. 2000), *Arthrobacter woluwensis* (Funke et al. 1996), and *Arthrobacter sanguinis* (Mages et al. 2008). In addition, some strains of *Arthrobacter oxydans* were isolated from blood (Wauters et al. 2000). *Arthrobacter scleromae* (Huang et al. 2005) was isolated from swollen scleromata of a dermatosis patient, *A. protophormiae* from urine, *A. aurescens* from wound swab, and *A. oryzae* from neck abscess (Mages et al. 2008). According to the study from Mages et al. (2008), the most frequently encountered arthrobacter was *Arthrobacter cumminsii*, isolated from urine, wound swab, blood culture, tracheal secretion, cervix, and otitis externa. This investigation is in accordance with the previously published data (Funke et al. 1998; Wauters et al. 2000). Furthermore, *A. oxydans* represented more than 20 % of the clinical strains isolated from wound swab, nasal swab, vaginal swab, eye, blood culture, and lung swab at autopsy. In the study of Wauters et al. (2000), two out of five clinical *Arthrobacter* strains were identified as members of the species *A. oxydans*. The third most frequently found *Arthrobacter* species was *A. aurescens* isolated from wound swab and urine which has not been reported before being isolated from human clinical specimens.

Arthrobacter spp. were identified to be the cause of human diseases, e.g., bacteremia, postoperative endophthalmitis, Whipple's disease-like syndrome, and phlebitis (Bernasconi et al. 2004; Shin et al. 2006; Park et al. 2012). *Arthrobacter cumminsii* might be the bacterial agent responsible for selected cases of urinary tract infections (Mages et al. 2008).

Antibiotica Sensitivity

Only few data are available concerning susceptibility of *Arthrobacter* toward antibiotics. Funke et al. (1996) tested 24 *Arthrobacter* strains, including the type strains of *A. globiformis*, *Sinomonas atrocyanea* (formerly *A. atrocyaneus*), *A. aurescens*, *A. crystallopoietes*, *A. histidinolovorans*, *A. nicotinovorans*, *A. oxydans*, *A. pascens*, *A. ramosus*, *A. ureafaciens*, *A. nicotianae*, *A. protophormiae*, *A. uratoxydans*, *A. cumminsii*, and *A. woluwensis* for susceptibility to the antibiotics amoxicillin-clavulanic acid, ampicillin, ceftriaxone, cefuroxime, cefalothin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, penicillin, rifampin, teichoplanin, tetracycline, and vancomycin. Only the glycopeptide antibiotic teichoplanin suppressed growth of all *Arthrobacter* strains

included in this study. Except *A. woluwensis,* all investigated strains were susceptible to amoxicillin-clavulanic acid, ampicillin, cefalothin, erythromycin, penicillin, and vancomycin. Only *Arthrobacter woluwensis* type strain showed a multidrug resistant pattern, being only susceptible to teichoplanin and moderately susceptible to tetracycline. Mages et al. (2005) investigated the susceptibility patterns of 38 *Arthrobacter* strains from cinical sources. Nearly all strains were susceptible to β-lactam antibiotics, doxycycline, gentamicin, linezolid, rifampin, and vancomycin. In contrast to the result of Funke et al. (1996), strains of *Arthrobacter woluwensis* exhibited no multidrug resistance, indicating that this trait is not characteristic for this species.

Application

Members of the genus *Arthrobacter* are abundant and ubiquitous in soil and are capable of degrading a wide variety of harmful synthetic organic compounds including aliphatic, aromatic, and polycyclic aromatic compounds (*Fig. 5.3*). Hence, they are playing an important role in the bioremediation of soils contaminated with pesticides, herbicides, and insecticides.

The enormous degradation potential of *Arthrobacter* strains seems to be based on the adoption to new environments and numerous biotic and abiotic stresses in the course of evolution. These adoptions might be the result of gene recruitment, gene modifications, and/or horizontal transfer. This notion is strengthened by the fact that degradation genes of many xenobiotics are often plasmid encoded.

Interaction between different degradable compounds can significantly influence their decomposition rates. *Arthrobacter* species MTCC 1553 efficiently degrades phenol, *o*-cresol, and *p*-cresol. In mixtures of phenol and *p*-cresol, degradation of phenol is strongly inhibited rather than *p*-cresol degradation. On the other hand, in a mixture of phenol and *o*-cresol, degradation of phenol is slightly increased whereas degradation of *o*-cresol is unaffected (Kar et al. 1997).

Sinomonas atrocyanea (formerly *Arthrobacter atrocyaneus*) MCM B-425 shows the capability of 93 % removal of the extremely harmful, organophosphorus pesticide Monocrotophos (MCP) from a synthetic medium containing 1,000 mg l^{-1} of the pesticide with the end products phosphates, ammonia, and carbon dioxide (Bhadbhade et al. 2002).

Arthrobacter oxydans strain P52 degrades the phenylcarbamate herbicides phenmedipham and desmedipham co-metabolically (Pohlenz et al. 1992). The degradative enzyme, the phenylcarbamate hydrolase, is a monomer with a molecular weight of 55,000 and, most likely, the corresponding gene is plasmid encoded.

Arthrobacter strain TE1 utilizes s-ethyl-*N*,*N*-dipropylthiocarbamate as a sole source of carbon (Tam et al. 1987). The enzyme is encoded on a 50.5 mDa plasmid. Hayatsu et al. (1999) isolated *Arthrobacter* sp. strain RC100 from carbaryl-treated soil which is capable of utilizing carbaryl (1-naphthyl-*N*-methylcarbamate) as a sole carbon source. The carbaryl degradation pathway of the strain is encoded by two distinct large plasmids. The initial reaction from carbaryl (1-naphthyl-*N*-methylcarbamate) to 1-naphthol is encoded by plasmid pRC1 and the following reactions to salicylaldehyde, salicylic acid, and gentisic acid are encoded by plasmid pRC2. Final reactions leading to the end products CO_2 and H_2O are encoded on the chromosome. *Arthrobacter* sp. GLP-1 and *Sinomonas atrocyaneus* ATCCC 13752 have been shown to utilize the herbicide glyphosate as a sole source of phosphorus and nitrogen (Pipke and Amrhein 1988a, b). However, in contrast to *Arthrobacter* sp. strain GLP-1, *S. atrocyaneus* could only use a rather limited number of organophosphonates as sole source of phosphorus, including aminomethylphosphonic acid, 1-amino-butylphosphonic acid, and glyphosate.

Cullington and Walker (1999) studied an isolate, designated D47, for degradation capabilities of phenylurea herbicides. This isolate was obtained after enrichment of a soil sample with diuron. The latter authors demonstrated that strain D47 was able to utilize the phenylurea herbicides linuron, diuron, monolinuron, metoxuron, and isoproturon as sole source of carbon whereas the derivatives 3-(3,4-dichlorophenyl)-1-methylurea and 3-(3,4-dichlorophenyl)-urea were not. In the following study, Turnbull et al. (2001a) identified strain D47 as a member of the genus *Arthrobacter* and despite the closer relationship to *Arthrobacter citreus* and *Arthrobacter polychromogenes*, the strain was named *Arthrobacter globiformis* D47. In this study, it was also demonstrated that *Arthrobacter globiformis* D47 metabolizes diuron by hydrolysis of the urea side chain at the carbonyl group with accumulation of 3,4-dichloroaniline and loss of carbon dioxide (Turnbull et al. 2001a). In cell-free extracts, it was shown that the degradative activity is optimal at a temperature between 15 °C and 30 °C and a pH between 6.5 and 8.0 whereas salt concentrations >50 mM reduce the activity. Turnbull et al. (2001b) showed that diuron degradative genes are located on a 47 kb plasmid. The diuron degradative enzyme consists of 456 amino acids as predicted from the cloned gene sequence and named phenylurea hydrolase (*puh* gene). Another strain, *Arthrobacter* sp. N2 (CIP 105365), which had been isolated after enrichment with diuron from long-term herbicide treated soil, was shown to metabolize the diuron to 3,4-dichloroaniline (Wideham et al. 2002). Degradation of diuron was shown to be more efficient in the presence of alternative carbon and nitrogen sources. *Arthrobacter* sp. N2 is also able to biotransform the phenylurea herbicides isoproturon and chlorotoluron, resulting in the formation of the corresponding substituted anilines isopropylaniline and 3-chloro-4-methylaniline, respectively (Tixier et al. 2002). Complete diuron degradation was achieved by a mixed culture, consisting of *Arthrobacter* sp. N4 (close to aforementioned N2) and *Delftia acidovorans* W34 (Bazot et al. 2007). Total mineralization of diuron was observed with free cells and with alginate fixed cells.

Rousseaux et al. (2001) isolated three *Arthrobacter crystallopoietes*–related strains from three geographically different soils in France that are capable of atrazine degradation. These strains are not able to mineralize the atrazine ring as

indicated from the accumulated intermediate compound cyanuric acid. The three strains harbor the genes *atz*B and *atzC* both of which sharing high gene sequence similarity with the corresponding genes of the well-known atrazine degrader *Pseudomonas* sp. ADP. These genes are encoding enzymes catalyzing degradation of hydroxyatrazine to *N*-isopropylammelide and cyanuric acid, respectively. In addition, evidence for presence of a gene similar to *atz*A was provided that shows only low sequence similarity with the corresponding gene of *Pseudomonas* sp. ADP. On the other hand, *atz*D found in *Pseudomonas* sp. strain ADP is absent in the three *A. crystallopoietes* similar strains. In a following study, Rousseaux et al. (2002) reported the presence of a gene homologous to the triazine hydrolase *trz*N gene in the three *A. crystallopoietes*-related strains.

Arthrobacter aurescens strain TC1 uses diverse s-triazine ring compounds as the sole source of nitrogen, carbon, and energy, including the herbicides atrazine, ametryn, atratone, cyanazine, prometryn, and simazine. Moreover, atrazine substrate analogs containing fluorine, mercaptan, and a cyano group in place of the chlorine substituent are also used as growth substrates. *A. aurescens* strain TC1 also metabolizes compounds containing chlorine plus N-ethyl, N-propyl, N-butyl, N-isobutyl, or N-t-butyl substituents on the s-triazine ring. Atrazine is metabolized to alkylamines and cyanuric acid, consuming up to 3 mg atrazine per liter (Strong et al. 2002). Strain TC1 contains the genes *atzB*, *atzC*, and *trzN* but not *atz*A. Sajjaphan et al. (2004) showed that *Arthrobacter aurescens* strain TC1 contains two large plasmids pAA1 and pAA2, and suggested that the genes *trzN*, *atzB* and *atzC* are located on plasmid pAA1.

Cai et al. (2003) isolated *Arthrobacter* sp. strain AD1 from industrial waste water from a herbicide production facility in china. On the basis of high 16S rRNA sequence similarity (>99.8 %), *Arthrobacter* sp. strain AD1 was identified as a close relative of *Arthrobacter ureafaciens*. *Arthrobacter* sp. strain AD1 degrades atrazine very efficiently. It utilizes sucrose and sodium citrate as carbon sources and atrazine as a sole nitrogen source. Bioremediation experiments using soil experimentally contaminated with up to 1 mg/g of atrazine resulted in 96 % of atrazine removal by strain AD1 at 30 °C within 4 weeks. In a control experiment, only 83 % of atrazine was removed by *Pseudomonas* sp. strain ADP.

Chlorinated benzoic acids (CBAs) are known to be intermediates in the metabolism of herbicides and chlorinated biphenyls (Abramowicz 1995; Komancová et al. 2003; Pettigrew et al. 1990). Hydrolytic dehalogenation of 4-CBA has been described for *Arthrobacter* TM-1 which can use this component as sole source of energy and carbon (Marks et al. 1984). In the initial step, 4-CBA is dehalogenated to 4-hydroxybenzoate and further metabolized to protocatechuate. In cell extracts, 4-fluoro- and 4-bromobezoate are dehalogenated as well.

Schmitz et al. (1992) reported 4-CBA dehalogenation in cell extracts and intact cells of *Arthrobacter* sp. strains TM1 and SU. Cell-free extracts of strain TM1 also dehalogenated 3- and 2-CBA but with less efficiency. However, 3-CBA was only dehalogenated by intact cells if 4-CBA was added as a co-substrate whereas intact cells did not metabolize 2-CBA under any condition. In strains TM1 and SU, 4-CBA is hydrolytically dehalogenated to *p*-hydroxybenzoate, requiring ATP and CoA, suggesting involvement of 4-CBA-CoA ester. As also reported for *A. globiformis* KZT1 (Zaitsev et al. 1991), the dehalogenese genes of *Arthrobacter* sp. strain SU are encoded on a plasmid (pASU) and organized in an operon containing three open reading frames (ORFs). Two of these genes exhibit high sequence similarity to corresponding genes of *Pseudomonas* sp. strain CBS3 encoding for 4-CBA-CoA-ligase (*fchA* gene) and 4-CBA-CoA-delagogenase (*fcbB* gene), whereas no significant sequence similarity was found for the third ORF (*fcbC* gene) supposed to encode for a thioesterase. In substrate activity experiments with the purified FcbC protein of strain SU, Zhuang et al. (2003) identified the enzyme as a 4-hydroxybenzoate-CoA thioesterase. Since no plasmid could be detected in strain TM1, obviously, genes mediating dehalogenation of 4-CBA are encoded on the chromosome.

Evidence was provided that also in other 4-CBA-degrading *Arthrobacter* isolates, the corresponding dehalogenation gene cluster is either located on the chromosome or a plasmid. Yi et al. (2000) isolated three *Arthrobacter* strains from upland and rice-field soils in South Korea which are capable to utilize 4-CBA. *Arthrobacter* sp. strain HR15 (devoid of any plasmid) was able to degrade 4-CBA, 4-bromobenzoic acid, and benzoic acid; *A. protophormiae* strain HR17 (carrying one plasmid) utilized 4-CBA and benzoic acid; *A. globiformis* strain HR19 (devoid of any plasmid) utilized 4-CBA, 4-iodobenzoic acid and 4-bromobenzoic acid.

The type strain of *Arthrobacter chlorophenolicus* A6^T was isolated from a soil suspension after enrichment with 4-chlorophenol (4-CP). During adaptation (165 day) to 4-CP, the ability of strain A6^T to degrade 4-CP and the rate of degradation increased from 50 to 350 ppm 4-CP and 8.0 to 33.2 ppm d^{-1} Strain A6^T utilizes 4-CP as sole source of carbon and energy and removed 4-CP completely from the medium. It can also grow on phenol, *p*-bromophenol, *p*-nitrophenol, and *p*-fluorophenol. Strain A6^T retains 4-CP-degradation ability even when serially cultivated on non-selective medium. Since no plasmid could be detected in A6^T, it is assumed that the degradative genes are chromosomally encoded (Westerberg et al. 2000).

Successful bioremediation of soil contaminated with high concentrations of 4-chlorophenol (175µg l^1 dry weight) with the 4-CP adapted strain *Arthrobacter chlorophenolicus* A6 was described by Elväng et al. (2001). The 4-CP was completely degraded in soil to background level after incubation for 7 days. Therefore, this strain is promising for bioremediation of field sites contaminated with high concentrations of 4-CP. Jernberg and Jansson (2002) applied a variant of *Arthrobacter chlorophenolicus* A6, chromosomically tagged with the firefly luciferase gene *luc* (strain A6L), for monitoring changes in the population of strain A6L corresponding to biodegrading of 4-CP and members of the indigenous microbiota and was found to have a significantly higher abundance is soils contaminated with 4-CP.

Another taxonomically sufficiently characterized 4-CP degrader, *Arthrobacter defluvii*, was described by Kim et al. (2008). The two isolates assigned to the species *A. defluvii* were

recovered from 4-CP stimulated sewage in Korea. The isolates removed 100 ppm 4-CP completely from the medium and were resistant at up to 200 ppm 4-CP.

A new pathway of 4-chlorophenol (4-CP) degradation was found in the isolate *Arthrobacter ureafaciens* strain CPR706 (Bae et al. 1996). Strain CPR706 degrades 4-CP via a hydroquinone pathway, in which the chloro-substituent is eliminated in the first step and hydroquinone is produced as a transient intermediate. Compared to other 4-CP-degrading bacteria carrying out hydroxylation of 4-CP to form chlorocatechol, this strain exhibited much higher substrate tolerance and degradation rate. In addition to 4-CP, strain CPR706 also degrades other para-substituted phenols such as 4-nitro, 4-bromo, 4-iodo, and 4-fluorophenol) via the same pathway.

A 4-fluorophenol degrading *Arthrobacter* sp. strain IF1 was isolated by Ferreira et al. (2008) after enrichment from soil samples that are contaminated with halogenated aliphatic compounds. The isolate IF1 showed highest 16S rRNA gene sequence similarity with the type strain of *Arthrobacter nitroguajacolicus*. The strain grows on catechol, hydroquinone, hydroxyquinol, benzoate, phenol, 4-fluorocinnamic acid, and 4-nitrophenol as substrates but not with 2-fluorophenol, 3-fluorophenol, 4-chlorophenol, 4-bromophenol, 4-iodophenol, fluoroacetate, trifluoroacetate, fluoroacetamide, trifluoroethanol, or on 2-bromoethanol. In batch culture, it is able to mineralize 5 mM 4-fluorophenol and to use it as the sole source of carbon and energy. It is suggested that strain IF1 degrades 4-fluorophenol, starting with removal of fluorine, yielding benzoquinone by a monooxygenase which is subsequently reduced to hydroquinone and in the following step, the hydroquinone is hydroxylated to hydroxyquinol which is then converted to maleylacetate and subsequently to 3-oxoadipate.

Ferreira et al. (2009) showed that *Arthrobacter* sp. strain IF1 harbors two gene clusters (clusters A and B). Cluster A contains four ORFs encoding for putative proteins associated with 4-fluorophenol degradation, *fpdA1, fpdD, fpdE,* and *fdpT1*. Cluster B contains the ORFs *fpdA2, fpdB, fpdC, fpdR, fpdT2,* and *fdpX*. Interestingly, in both gene clusters, ORFs are found encoding for a monooxygenase (*fpdA1* and *fdpA2*). At the deduced amino acid level, the two enzymes are sharing 98.9 % sequence similarity. The two sequences share highest similarity with a 4-nitrophenol monooxygenase of *Arthrobacter* sp. strain JS443 and the hydrolase component of 4-chlorophenol monooxygenase of *Arthrobacter chlorophenolicus* A6. Open reading frames *fpdD* and *fpdE* (cluster A) show some sequence similarity with a maleylacetate reductase of *Arthrobacter* sp. strain JS443 which is involved in degradation of *p*-nitrophenol and to α/β-hydrolase fold family enzyme, respectively. The putative reductase (*fpdB* ORF) is only present in cluster B. The deduced protein shares highest amino acid sequence similarities with the reductase component of the monooxygenase systems of *Arthrobacter* sp. strain JS443 (92 %) and *Arthrobacter chlorophenolicus* A6 (86 %) that reduce FAD to $FADH_2$ (flavine reductase). ORF *fpdC* encodes a putative protein with a high similarity to hydroxyquinol dioxygenase which is involved in 4-chlorophenol degradation in *Arthrobacter chlorophenolicus* A6. ORF *fpdX* is assumed to encode a putative periplasmic binding protein. In ORF *fpdR*, a nucleotide-binding domain and a helix-turn-helix (HTH) motif are present that are similar to putative regulator genes encoded in *p*-nitrophenol and 4-chlorophenol degradation gene clusters found in *Arthrobacter* sp. strain JS443 and *Arthrobacter chlorophenolicus* A6. So far, ORF *fpdT2* is of unknown function. It is located at a similar position like a corresponding ORF in the *p*-nitrophenol gene cluster in *Arthrobacter* sp. strain JS443.

The isolate *Arthrobacter protophormiae* RKJ100 was recovered from a soil sample after enrichment with *p-nitrophenol* (Chauhan and Jain 2000). RKJ100 was shown to utilize *o*-nitrobenzoate as the sole source of nitrogen, carbon, and energy. The degradation of *o*-nitrobenzoate is an oxygen-insensitive reductive catabolic pathway and yields *o*-hydroxylaminobenzoate and anthranilic acid as the terminal aromatic intermediate. Experimental data suggested that the corresponding genes are located on a 65 kb plasmid.

A *p*-nitrophenol-degrading isolate, *Arthrobacter* strain JS443, was described by Jain et al. (1994). The 16S rRNA gene sequence of strain JS443 (accession no: ef078488; Perry and Zylstra 2007) shares highest similarities (>99 %) with the type strains of *Arthrobacter ureafaciens* and *Arthrobacter nicotinovorans,* placing it in the "*Arthrobacter aurescens* group." Mainly on the basis of growth experiments, Jain et al. (1994) supposed that strain JS443 transforms *p*-nitrophenol to 1,2,4-benzenetriol, via 4-nitrocatechol and 4-nitroresorcinol and, after ring fission to maleylacetic acid, degradation follows the β-ketoadipate pathway.

A deviating catabolic pathway was proposed by Perry and Zylstra (2007) for degradation of *p*-nitrophenol by *Arthrobacter* strain JS443. These authors identified in strain JS443 a gene cluster designated *rpd*. The gene cluster harbors the genes *npdA1, npdA2 npdB, npdC, npdR, npd X,* and *orf6*. Based on cloned genes, sequencing, and comparison with genes of *Arthrobacter chlorophenolicus* encoding enzymes of the 4-chlorophenol degradation pathway, *npdA1* was identified to encode a putative NADH-dependent flavin reductase and *npdA2* a putative *p*-nitrophenol monooxygenase belonging to the two-component flavin-diffusible monooxygenase family. Gene *npdB* encodes a hydroxyquinol 1,2-dioxygenase, gene *npdC* a putative maleylacetate reductase, and *npdR* is assumed to be a regulatory gene. No function was assigned to the genes *npdX* and *orf6*. On the basis of genes putatively identified in JS443 and degradation experiments, Perry and Zylstra (2007) proposed that in the first reaction, the NADH-dependent flavin reductase catalyzes the release of nitrite and transformation of *p-nitrophenol* to 2-hydroxy-1,4-benzoquinone via 1,4-benzoquinone which is then reduced by an unidentified reductase to 1,2,4-hydroxyquinol. The hydroxyquinol 1,2-dioxygenase catalyzes cleavage of 1,2,4-hydroxyquinol to maleylacetate reduced to β-ketoadipate. As indicated by the appearance of 4-nitrocatechol in the reactions in a sideway, an unknown oxygenase may convert *p*-nitrophenol to 4-nitrocatechol and NADH-dependent flavin reductase catalyzes the release of nitrite and formation of 2-hydroxy-1,4-benzoquinone.

Arthrobacter strain JS443 has been successfully applied for development of a whole cell amperometric biosensor for *p*-nitrophenol (Lei et al. 2003). The biosensor measures the oxidation current of the intermediates of *p*-nitrophenol degradation, namely, 4-nitrocatechol and 1,2,4-hydroxyquinol (1,2,4-benzenetriol). Under optimized conditions, the biosensor measured as low as 5 nM *p*-nitrophenol.

Three *Arthrobacter* strains were isolated from the microbial community in soil surrounding an outdoor coal storage pile which were capable of growth with naphthalene as sole carbon source at low pH values (Dore et al. 2003). Phylogenetically, one strain was placed in the vicinity of "*Arthrobacter aurescens* group" (isolate 5), whereas the other two strains clustered next to "*Arthrobacter psychrolactophilus* group" (isolates 4 and 13). They showed a range of acid and heavy-metal tolerances (pH 3–7, mercury tolerance: 6–20µg/ml). None of the genes related to naphthalene degradation were found in the three isolates: neither *nahAc, nahAd, phnAc, nahH, xylE,* nor *GST.* However, evidence was provided that these *Arthrobacter* isolates do not mineralize naphthalene completely.

A phenanthrene degrading strain, *Arthrobacter* sp. P1-1, was isolated from a PAH contaminated soil collected from Hilo, Hawaii (USA), after enrichment. The strain was identified as a member of the genus *Arthrobacter* on the basis of highest sequence similarity with another strain, *Arthrobacter* sp. BS20 (Seo et al. 2006). A pre-grown culture (optical density 0.05 at 540 nm) of *Arthrobacter* sp. P1-1 completely degrades 40 mg l^{-1} of phenanthrene in a minimal medium shake culture at 28 °C within 7 days, suggesting that it utilizes this compound as a sole source of carbon and energy. Metabolic analyses suggest that the degradation starts with dioxygenation on 1,2-, 3,4-, and 9,10-C of phenanthrene. Intermediate degradation products indicated that phenanthrene-1,2- and −3,4-diols undergo *meta*-cleavage, but to a limited extent also *ortho*-cleavage may occur. Naphthalene-1,2-diol is then formed via 1-[(*E*)-2-carboxyvinyl]-2-naphthoic acid, naphthalene-1,2-dicarboxylic acid, and 1-hydroxy-2-naphthoic acid. Naphthalene-1,2-diol is then further degraded through the phthalic acid and salicylic acid pathways.

Even higher phenanthrene concentrations are degraded by *Arthrobacter phenanthrenivorans* Sphe3^T (Kallimanis et al. 2007). This strain catabolizes 90 % of 400 mg/l phenanthrene in a minimal medium within 4 days. Vandera et al. (2012) identified in strain Sphe3 two gene loci for 1-hydroxy-2 naphthoic acid dioxygenases which are associated with phenanthrene utilization. The *diox1* gene is located on a plasmid and the *diox2* gene on the chromosome. At the amino acid level, the two enzymes share 93 % sequence homology but only 85 % with the corresponding protein of a *Nocardioides* strain. Both genes are induced in the presence of phenanthrene in the medium, but only minimal expression occurs in the presence of glucose or glucose + phenanthrene. The *diox2* gene is twofold higher upregulated than the *diox1* gene. The occurrence of two genes encoding 1-hydroxy-2 naphthoic acid dioxygenases in Sphe3 renders the strain more advantageous for PAH biodegradation and hence more adjustable to polluted environments. These duplicates may provide an advantage for microbial adaption to environment changes.

Promising results of phenanthrene degradation in soils after inoculation with a phenanthrene-degrading *Arthrobacter* strain were obtained by Schwartz and Scow (1999) and Schwartz et al. (2000). These authors carried out experiments with *Arthrobacter* strain RP17, which had been isolated from soil after enrichment with phenanthrene as a sole carbon source in Forbes soil, which contained a microbial community unable to rapidly degrade phenanthrene. After inoculation with a culture of the nonindigenous *Arthrobacter* strain RP17, a significant decrease of phenanthrene in the soil could be measured, suggesting phenanthrene degradation.

Quinaldine is utilized by *Arthrobacter nitroguajacolicus* Ru61a^T as a sole source of carbon and energy via the anthranilate pathway (Parschat et al. 2003, 2007). Genes for quinaldine degradation are encoded on the linear catabolic plasmid pAL1 (Overhage et al. 2005), and they are organized in two operons. Evidence was provided that quinaldine is converted to anthranilate via 1*H*-4-oxoquinaldine, 1*H*-3-hydroxy-4-oxoquinaldine, and *N*-acetylanthranilic acid involving the enzymes quinaldine 4-oxidase (Qox), 1*H*-4-oxoquinaldine 3-monooxygenase (Moq), 1*H*-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase (Hod), and *N*-acetylanthranilate amide hydrolase. It is hypothesized that anthranilate is then degraded by two different routes. Detection of the intermediate catechol and the ability of a pAL1-deficient mutant of Ru61a^T to grow on anthranilate and catechol as carbon sources strongly suggest that degradation proceeds via the β-ketoadipate pathway. However, genes encoding for the catechol-generating enzymes anthranilate 1,2-dioxygenase and a catechol dioxygenase were not detected on the plasmid but might be encoded by the chromosome. Presence of a second route for anthranilate degradation is assumed by relatively high amino acid sequence similarities of proteins deduced from OFRs abd encoded on plasmid pAL1. These proteins indicate degradation of anthranilate via 2-aminobenzoyl-CoA and 2-amino-5-oxo-cyclohex-1-ene-carbonyl-CoA.

Arthrobacter nicotinovorans (Kodama et al. 1992) has the ability to use the tobacco alkaloid nicotine as its sole carbon and energy source (Decker et al. 1961; Eberwein et al. 1961; Gherna et al. 1965; Hamm and Decker 1978). Corresponding genes are encoded by plasmid pAO1, including a heterotrimeric 6-hydroxypseudooxynicotine dehydrogenase and 2,6-dihydroxypyridine 3-hydroxylase (Baitsch et al. 2001). Gabor and Brandsch (2003) identified on the plasmid the genes for a nicotine dehydrogenase, a 6-hydroxy-L-nicotine oxidase, a ketone dehydrogenase, and an L-nicotine uptake system.

Arthrobacter ureafaciens SP1b-5 isolated from soil of a wash water soakway at a disused nitroglycerin manufacturing plant in Somerset West, South Africa, after enrichment is capable to utilize nitroglycerin as its sole nitrogen source (Marshall and White 2001). Strain SP1b-5 converts nitroglycerin (glycerol trinitrate) to 1,2-glyceroldinitrate and 1,3-glyceroldinitrate in a ratio of approximately 2–1, but mononitrates were not detected during the course of the experiment. Detection of transient intermediates and simultaneous adaptation studies

with potential intermediates indicated that the degradation pathway involves the conversion of nitroglycerin to glycerol via 1,2-dinitroglycerin and 1-mononitroglycerin, with concomitant release of nitrite. Glycerol then serves as the source of carbon and energy. Another strain of *Arthrobacter*, strain JBH1, was isolated after selective enrichment from a nitroglycerin contaminated soil at a facility that formerly manufactured explosives after 8 months. Partial 16S rRNA gene sequence similarity identified strain JBH1 as a close relative of *Arthrobacter pascens*. Strain JBH1 degraded 1,2-dinitritroglycerol, 1,3-dinitroglycerol, and 1-mononitroglycerol, respectively, as sole source of carbon but not 2-mononitroglycerol (Husserl et al. 2010). Enzyme assays with crude extracts from strain JBH1 indicated that the conversion of nitroglycerin to 1,2 dinitroglycerin is NADPH dependent with concomitant release of nitrite, whereas the transformation of 1-mononitroglycerin is ATP dependent and neither nitrate nor nitrite is released (Husserl et al. 2012). Most likely, 1-mononitroglycerin is then phosphorylated at carbon 3 and the nitrite is released by subsequent reactions.

Arthrobacter sp. strain G1 has been isolated after enrichment with 4-fluorocinnamic acid and 4-fluorobenzoic acid from a site in the Netherlands which was contaminated with chlorobenzene and halogenated aliphatic compounds (Hasan et al. 2011). *Arthrobacter* sp. strain G1, a close relative of *Arthrobacter nitroguajacolicus*, is able to degrade 4-fluorocinnamic acid to 4-fluorobenzoic acid and uses the two-carbon side chain for growth. Further degradation does only occur in co-culture with *Ralstonia* sp. strain H1.

Arthrobacter strain D9 plays a special role in degradation of the herbicide 2,4-dichlorophenoxyacetic acid in co-culture with *Alcaligenes eutrophus* JMP134 in a cadmium/2,4-dichlorophenoxyacetic acid–polluted soil (Roane et al. 2001). *Arthrobacter* strain D9 is cadmium resistant but is not able to degrade 2,4-dichlorophenoxyacetic acid, whereas *Alcaligenes eutrophus* JMP134 is cadmium sensitive and able to degrade 2,4-dichlorophenoxyacetic acid. In a laboratory soil microcosm, co-inoculated with the two strains and co-contaminated with both, cadmium and 2,4-dichlorophenoxyacetic acid, 500 $\mu g\ l^{-1}$ of the herbicide could not be detected after 6 weeks of incubation. In broth co-culture, the herbicide was almost undetectable after 5 days of incubation. These results indicate that strain D9 carries out detoxification of the environment from accessible cadmium. Accumulation of cadmium in the outer layer suggests that the metal is bound to the exopolysaccharide which is produced by strains D9.

However, when arthrobacters are applied to remediate xenobiotic compounds from soils, the ability of certain *Arthrobacter* strains to produce antibiotics and antagonistic effects on other members of the community should be considered. Kamigiri et al. (1996) described *Arthrobacter* strain YL-02729S which was recovered from soil collected from West Kalimantan (Indonesia). This strain is producing a quinolone antibiotic with activity against Gram-positive bacteria including multiple resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Seven *Arthrobacter* isolates collected at different geographical Arctic sites (copepod, surface water, sea ice, deep sea, and melt water) were described by Wietz et al. (2012). These *Artrhobacter* strains shared almost identical 16S rRNA gene sequences and are identical or almost identical to that of *Arthrobacter russicus*. The seven isolates were shown to produce arthrobacillins A, B, and C under different culture conditions. Also other unidentified compounds were detected which may contribute to the antagonistic action of these strains, as well.

Formation of Added-Value Products

Microorganisms play an important role in ripening of the surface of smear cheeses and other cheeses, as well. Species of the genus *Arthrobacter* are major components in the smear microflora of surface-ripened chesses and also of mold surface-ripened cheeses, such as Brie and Camembert. They belong to the group of principal secondary microorganisms contributing to cheese ripening. The most important function of these microorganisms is the production of extracellular enzymes and deacidification of the cheese (Sousa et al. 2001). In a study on analysis of the microbial flora of the smears of Austrian Tilsit cheeses and three varieties of soft cheeses among the members of the microbial smear flora in addition to species of the genera *Brevibacterium* and *Corynebacterium*, also strains of the species *Sinomonas atrocyanea* (formerly *Arthrobacter atrocyaneus*), *Arthrobacter citreus*, *Arthrobacter globiformis*, *Arthrobacter nicotianae*, *Arthrobacter protophormiae*, *Arthrobacter uratoxydans*, and *Arthrobacter* spp. were identified (Eliskases-Lechner and Ginzinger 1995).

Arthrobacter nicotianae is a typical species present in the bacterial smear of surface ripened cheeses. *Arthrobacter nicotianae* 9458 produces two extracellular serine proteinases, P1 and P2 (Smacchi et al. 1999a). Another proteinase of *A. nicotianae* 9458 was identified as an extracellular proline iminopeptidase (Smacchi et al. 1999b). These enzymes are well active at conditions applied during cheese ripening and may contribute to proteolysis of the cheese during ripening. *A. nicotianae* 9458 also produces an extracellular esterase (Smacchi et al. 2000). This enzyme shows highest activity on β-naphthyl butyrate at pH 7.0 and 30 °C and as well may play an important role in cheese ripening.

Results from Bockelmann and Hoppe-Seyler (2001) showed that the red-brown or orange pigments are most likely due to the yellow pigmented *Arthrobacter* sp. in the surface flora. The successful use of a defined five strain starter culture consisting of *Debaryomyces hansenii*, *Brevibacterium linens*, *Arthrobacter nicotianae*, *Corynebacterium ammoniagenes*, and *Staphylococcus sciuri* for Tilsit cheese ripening was demonstrated and *Arthrobacter* sp. is involved in the flavor and color development.

Smit et al. (2004) analyzed *Arthrobacter* sp. used in dairy fermentations such as cheese making for their potential to convert leucine into flavor components, most notable 3-methylbutanal. Flavor formation in cheese and other fermented dairy products results mainly from catabolism of milk proteins, sugar,

and lipids. It was demonstrated that *Arthrobacter nicotianae* strains are involved in the formation of very diverse flavors in dairy products.

Arthrobacter aurescens TA13 is capable of utilizing *t*-anethole, anisic acid, anisaldehyde, and anisic alcohol as the sole carbon source. These findings provide new routes for environmental friendly production processes of valuable aromatic chemicals via bioconversion of phenylpropenoides. Valuable aroma compounds are produced as intermediates in the degradation pathways of such phenylpropenoides. *t*-Anethole is the major component of several essential oils, including star anise, anise seed oil, and sweet fennel. Hence, microorganisms capable of utilizing *t*-anethole may be applied to produce intermediates as 4-methoxylated aromatic flavor and fragrance compounds, including anisic alcohol, anis aldehyde, or anisic acid. Shimoni et al. (2002) developed a biotransformation process for valuable aromatic chemicals, indicating that a high yield transformation of various phenylpropenoides such as eugenol, estragol, and safrole into valuable aromatic compounds will be possible.

Arthrobacter sp. DS7 was isolated from a heavy-oil contaminated soil (D'Addario 1996) as a desulfurizer. *Arthrobacter* sp. DS7 is able to cleave C-S bonds from organosulfur hetercyclic compounds such as thiobenzothiophene (Serbolisca et al. 1999). The desulfurization operon was found to be located in a large plasmid that also bears the genes conferring cadmium and arsenic resistance. The desulfurization capacity of the strain depends on the activity of the 4-S pathway enzymes already described for other bacteria. The sequence of the *sox*-operon is completely identical to that of *Rhodococcus* sp IGTS38. Since there is a considerable interest in lowering sulfur levels in petroleum distillates, *Arthrobacter* sp. DS7 may be applied for this purpose because it appears to be already adapted to this special environment.

Enzymes

Arthrobacter ilicis isolated from the marine sponge *Spirastrella* sp. produces extracellular serine type acetylcholine esterase. The maximum enzyme activity was found at 45 °C and pH 8.0 (Mohapatra and Bapuji 1998). The microbial production of this enzyme was also shown for *Arthrobacter globiformis* (Shmeleva et al. 1989).

Arthrobacter psychrolactophilus was described to produce an extracellular α-amylase (Loveland-Curtze et al. 1999; Trimbur et al. 1994). This amylase is thermally labile, calcium dependent, and has an optimum activity temperature of 50 °C for a synthetic substrate, a property associated with mesophilic enzymes rather than with psychrophilic enzymes. The relatively high temperature optimum was assumed likely to be induced by the synthetic substrate applied for the experiments and does not accurately reflect the catalytic efficiency at low temperatures. The amylase possesses a raw starch-binding domain which could make it useful for processes where uncooked starch granules need to be hydrolyzed at ambient temperatures. In crude concentrations, the amylase initially hydrolyzes raw starch at 30 °C at about the same rate as an equal number of units of barely α-amylase, but it loses most of its activity after only a few hours (Smith and Zahnley 2005).

A *β*-fructofuranosidase with wide range of acceptor specificities has been isolated from *Arthrobacter globiformis* IFO 3062 which is applicable for the production of heterooligosaccharides (Win et al. 2004). The enzyme has a molecular mass of 60 kDa and its activity for sucrose is 101 U mg^{-1}. The enzyme exhibits maximum activity at pH 6.8 and still 90 % activity is found at pH between 5.5 and 9.5. Temperature optimum is 37 °C and >90 % activity remains at 40° C. By fructosyl transfer to cellobiose and cellotriose, the enzyme catalyzes the formation of nonreducing oligosaccharides, such as *O*-*β*-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl- (1→2)-α, *β*-D-fructofuranoside and *O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→2)-α, *β*-D-fructofuranoside, respectively. The two oligosaccharides resist heating at 100 °C for 30 min even at pH 2.0.

The psychrotrophic *Arthrobacter* strain B7 was isolated from Pennsylvania farmlands (Loveland-Curtze et al. 1999). Strain B7 produces three different cold-active-β-galactosidase isozymes when grown with lactose as the sole carbon source. The sizes of the three different β-galactosidase isozymes are 111kDa, 71kDa, and 52 kDa, respectively. Due to high homology with the *lacZ* from *E. coli*, the gene encoding the largest β-galactosidase was designated *lacZ*, as well. The protein sequence deduced from the nucleotide sequence contains the conserved acid–base and nucleophilic sites involved in catalysis and typically found in *lacZ* enzymes. The enzyme exhibits a temperature optimum about 20 °C below that of the *E. coli* β-galactosidase (Trimbur et al. 1994). The gene encoding the medium-sized isozyme lacks significant homology with the *lacZ* site but shows homology with isozymes from the thermophile *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) and the mesophile *Bacillus circulans*. The enzyme shows homology to the acid–base sites of the *lacZ* family but not with the nucleophilic region. It was assigned to a new β-galactosidase family designated *lacG* which corresponds to glycosyl hydrolase family 42 (Henrissat and Bairouch 1993). The temperature optimum is between 45 °C and 50 °C (Gutshall et al. 1995). The smallest β-galactosidase exhibits homology with the lysosomal acid β-galactosidases from human and mice and with an enzyme of *Xanthomonas manihotis* (Gutshall et al. 1997) and in agreement with this observation, phylogenetic analysis of the amino acid sequence led to the unexpected clustering within the eukaryotic clade. The enzyme hydrolyzes substrates with either a β-2,4 or a β-1,3 linkage with an temperature optimum similar to the temperature optimum of the *E. coli lacZ* β-galactosidase. Meanwhile strain B7 has been described as the type strain of *Arthrobacter psychrolactophilus* (Loveland et al. 1994).

Arthrobacter strain SB was isolated from Antarctic Dry Valley soil. On the basis of 16S rRNA gene analysis, strain SB was identified as a close relative of *Arthrobacter sulfonivorans*. The strain was tested positive for β-galactosidase activity. The corresponding gene (*bgaS*) shows highest sequence similarities with *lacZ*-like genes of *Arthrobacter* sp. C2-2 (71 %) and *Arthrobacter psychrolactophilus* B7 (66 %). These results suggest that the

β-galactosidase of strain SB belongs to the *lacZ* family β-galactosidases. The enzyme is cold active with an optimal activity at 18 °C, retaining 50 % activity at 0 °C. It is heat labile as it can be inactivated by incubation for 10 min at 37 °C (Coker et al. 2003).

Arthrobacter psychrolactophilus F2 is able to hydrolyze lactose at a temperature below 5 °C. Only one type of cold-active β-galactosidase could be identified in this strain (Nakagawa et al. 2003). This β-galactosidase exhibits a high activity at 0 °C, its temperature and pH optima are 10 °C and 8.0, respectively. It is inactivated rapidly at 45 °C within 5 min (Nakagawa et al. 2006).

Arthrobacter sp. 32c was isolated from Antarctic soil due to its ability to hydrolyze the chromogenic lactose analog X-Gal. Analysis of its 16S rRNA gene sequence identified strain 32c as a close relative of *Arthrobacter oxydans* and *Arthrobacter polychromogenes* (Hildebrandt et al. 2009), placing it in the "*Arthrobacter oxydans* group" (Busse et al. 2012). The β-D-galactosidase gene encodes a protein consisting of 694 amino acids and a deduced mass of 76 kDa. The β-D-galactosidase gene sequence shares highest similarities with those of *Arthrobacter* sp. FB24 (77.1 %) and *Arthrobacter aurescens* TC1 (71.8 %). However, comparison with newly accessible genome sequences identifies *Arthrobacter phenanthrenivorans* Sphe3 (82.5 %) and *Arthrobacter chlorophenolicus* A6 (82.3 %) harboring highest similar sequences. The enzyme shows highest activity at pH 6.5 and 50 °C, approximately 60 % activity is retained at 25 °C and 15 % at 0 °C.

A strain isolated from a soil sample near the Great Wall Station in Antarctica and designated *Arthrobacter* ON14 was described recently (Xu et al. 2011). Phylogenetically, strain ON14 is closely related to *Arthrobacter psychrochitiniphilus* and *A. psychrolactophilus,* suggesting its placement in the "*Arthrobacter psychrolactophilus* group" (Busse et al. 2012). It was shown to contain two genes encoding different β-galactosidases which were designated *galA* and *galB*. The *galA* gene is highly expressed at low temperature (4 °C) and repressed at high temperature (28 °C). The corresponding enzyme GalA shows highest amino acid homology (97.3 %) to BglA of *Arthrobacter psychrolactophilus* strain F2. The maximum activity is at 15 °C and pH 8.0. The *galB* gene is repressed at low temperature and induced at high temperature. Its maximum activity is at 37 °C. The GalB isozyme shows highest amino acid homology (42.4 %) with the 71 kDa β-galactosidase isozyme of *A. psychrolactophilus* B7, and hence, it is assigned to the *lacG* family.

The Antarctic *Arthrobacter* sp. D10 produces two extracellular alkaline phosphatases, designated D10A and D10B. Enzyme D10A shows a pH optimum between 7 and 11 and a maximum at pH 9.5. Enzyme D10B shows calcium-dependent maximum activity at pH 9 and a minimum at pH 10. Both enzymes are heat labile (de Prada et al. 1996).

A D-threonine aldolase was purified and characterized from *Arthrobacter* sp. DK-38. The enzyme catalyzes the cleavage of D-threonine into glycine and acetaldehyde and the reaction is reversible. The molecular mass of the enzyme is approximately 51 kDa and requires pyridoxal 5′-phosphate and divalent cations such as Co^{2+}, Ni^{2+}, Mn^{2+}, or Mg^{2+} for its activity. Both D-threonine and D-allothreonine act as substrates for the enzyme. The aldolase also cleaves other D-β-hydroxy-α-amino acids (Kataoka et al. 1997).

Arthrobacter nicotianae 9458 produces three extracellular proteases, two serine proteinases (P1 and P2), and a proline iminopeptidase (Smacchi et al. 1999a, b). The molecular masses of the two serine enzymes are approximately 54 kDa and 71 kDa, respectively. They differ in respect of temperature optimum which is 55–60 °C and 37 °C, respectively. Both enzymes tolerate NaCl concentrations higher than 5 %, show optimum activity at pH 9.0–9.5, and are still active at pH 6.0. The extracellular proline iminopeptidase shows a temperature optimum of 37 °C and a pH optimum of 8. It is completely inactivated by heating at 80 °C. However, a very high acitivity of the enzyme is observed at 10 °C, pH 7.0, and 5.0 % NaCl concentration; approximately 60 % of the activity is retained at 13 °C, pH 6.5, 3.75 % NaCl, and 13 °C, pH 6.5, 6.25 % NaCl concentration, respectively. *A. nicotianae* 9458 also produces an extracellular esterase (Smacchi et al. 2000). This enzyme shows highest activity on β-naphthyl butyrate at pH 7.0 and 30 °C.

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6 The Family *Beutenbergiaceae*

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Abstract
The family *Beutenbergiaceae* is a member of the order *Micrococcales* and comprises the monospecific genera *Beutenbergia*, *Miniimonas*, *Salana*, and *Serinibacter*. Cells of members of the family are irregular rods and cocci and may exhibit a rod–coccus life cycle. All the species are Gram-stain-positive, catalase-positive, non-motile, non-acid fast, and do not contain mycolic acids. Oxidase activity is variable, and endospores are not formed. Members of the family are defined by a wide range of chemotaxonomic properties. Cross-linkage of the peptidoglycan is of the A type with an L-serine residue at position 1 of the peptide subunit (with the exception of the genus *Beutenbergia*); the diagnostic diamino acids are either L-lysine (*Beutenbergia* and *Serinibacter*) or L-ornithine (*Salana* and *Miniimonas*). The interpeptide bridge contains L-glutamic acid. The predominant menaquinone is MK-8(H_4). The cellular fatty acid profiles consist of iso- and anteiso-branched fatty acids. Saturated straight-chain fatty acids may be present as major components. The G + C content of the genomic DNA ranges between 71 mol% and 75 mol%. The phylogenetic neighbors are the families *Bogoriellaceae*, *Ruaniaceae*, *Jonesiaceae*, and *Actinomycetaceae*.

Taxonomy: Historical and Current

Short Description of the Family

The family description is mainly based on its phylogenetic position and the pattern of its 16S rRNA gene sequence signatures.

Beutenbergiaceae: Zhi, Li, and Stackebrandt 2009, 597^{VP} Emend Hamada, Iino, Tamura, Iwami, Harayama, and Suzuki 2009, 2813^{VP} Emend Ue, Matsuo, Kasai, and Yokota 2011, 125^{VP}

Beu′ten.ber.gi.a.ce′a.e. N.L. fem. n. *Beutenbergia* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Beutenbergiaceae* the family of *Beutenbergia*.

The pattern of 16S rRNA gene sequence signatures consists of nucleotide positions 144:178 (C–G), 280 (U), 293:304 (G–U), 668:738 (A–U), 1003:1038 (G–U), 1027:1034 (U–A), and 1414:1486 (U–A) (Hamada et al. 2009).

The family is a member of the order *Micrococcales* (Busse 2012) within the class *Actinobacteria* (Stackebrandt et al. 1997) and contains the type genus *Beutenbergia* (Groth et al. 1999) and the genera *Miniimonas* (Ue et al. 2011), *Salana* (von Wintzingerode et al. 2001), and *Serinibacter* (Hamada et al. 2009).

Phylogenetic Structure of the Family

A phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of the members of the family *Beutenbergiaceae* is shown in ❯ *Fig. 6.1*. The phylogenetic tree indicates that the members of the family form a monophyletic and reliable cluster within the order *Micrococcales*. The family is moderately related to the families *Bogoriellaceae*, *Ruaniaceae*, *Jonesiaceae*, and *Actinomycetaceae* according to a RaxML 16S rRNA gene tree of the All-Species Living Tree Project (Yarza et al. 2008).

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_172,

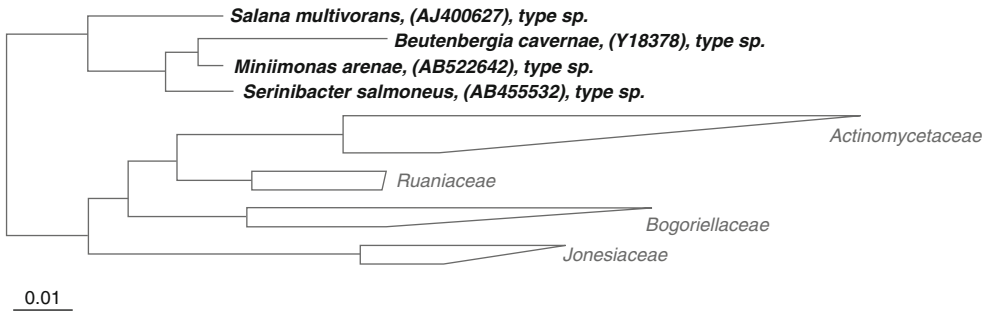

Fig. 6.1
A maximum likelihood phylogenetic tree derived from the 16S rRNA gene sequences of the members of the family *Beutenbergiaceae* and its taxonomic neighbors. The tree was constructed using the RAxML algorithm (Stamatakis 2006)

The family *Beutenbergiaceae* was established for the genera *Beutenbergia*, *Salana*, and *Georgenia* (Altenburger et al. 2002) by Zhi et al. (2009) on the basis of phylogenetic position and the presence of a unique set of 16S rRNA gene sequence signature nucleotides. Later, the genus *Serinibacter* was proposed by Hamada et al. (2009) as a new genus of the family *Beutenbergiaceae* with the emendation of the families *Beutenbergiaceae* and *Bogoriellaceae*. They reevaluated these families based on phylogenetic position and a set of signature nucleotides and concluded that the genus *Georgenia* should be transferred from the family *Beutenbergiaceae* to the family *Bogoriellaceae*. Based on their study, the family *Beutenbergiaceae* was amended to accommodate the genera *Beutenbergia*, *Salana*, and *Serinibacter*, whereas the genus *Georgenia* was placed in the family *Bogoriellaceae*. Recently, the genus *Miniimonas* was described by Ue et al. (2011). They confirmed the results of Hamada et al. (2009) and placed the genus *Miniimonas* in the family *Beutenbergiaceae*. In addition, the family description was emended to change the nucleotide pair at position 131:231 to A–G and not C–G as per Zhi et al. (2009). This time, the family *Beutenbergiaceae* is defined on the basis of a unique set of 16S rRNA gene signature nucleotides, i.e., nucleotides at positions 144:178 (C–G), 280 (U), 293:304 (G–U), 668–738 (A–U), 1003:1038 (G–U), 1027:1034 (U–A), and 1414:1486 (U–A) (Hamada et al. 2009).

Molecular Analyses

MALDI-TOF (matrix-assisted laser-desorption/ionization time-of-flight) mass spectrometric analysis of the members of the family *Beutenbergiaceae* and their phylogenetic neighbors shows a wide range of spectra similarities (*Fig. 6.2*). The type strains of the family *Beutenbergiaceae* form a coherent cluster; however, all the strains have major differences in their spectra when compared with each other (Hamada unpublished). The only study to include the ribotyping method was that of von Wintzingerode et al. (2001). The three isolates of *Salana multivorans* (Se-3111^T, Se13111, and Se1311A) and their closest phylogenetic neighbor *Beutenbergia cavernae* DSM 12333^T were ribotyped using the restriction enzyme *Pvu*II. The similarity values between the ribotype patterns of the three isolates ranged from 65 % to greater than 95 % as compared with less than 20 % similarity to the ribotype pattern of *Beutenbergia cavernae* DSM 12333^T. Thus, it was concluded that the novel isolates were genotypically very similar and represent a single species. DNA–DNA hybridization, riboprinting, and multilocus sequence analysis have not been performed for any members of the family *Beutenbergiaceae*.

Genome Analyses

Beutenbergia cavernae HKI 0122^T is the only strain of the family *Beutenbergiaceae* for which the complete genome sequence has been released (INSDC ID: CP001618) (Land et al. 2009). The genome of the strain is 4,669,183 base pairs long and comprises a circular chromosome with 73.12 % G + C content. This G + C content is slightly higher than that determined originally by HPLC (71 mol%; Groth et al. 1999). The sequences of the two 16S rRNA gene copies in the genome of strain HKI 0122^T are identical but differ by 4 nucleotides from the previously published 16S rRNA gene sequence determined from DSM 12333^T (Y18378). Of the 4,278 genes predicted, 4,225 were protein-coding genes and 53 were RNA genes. Twenty-eight pseudogenes were also identified. The majority of the genes (74.3 %) were assigned a putative function. The percentage of genes assigned to clusters of orthologous groups (COGs) was 72.67 %. The remaining genes were annotated as hypothetical proteins. The classification of the genes into COG functional categories showed that a higher number of genes are involved in carbohydrate transport and metabolism (546 genes; 12.9 %), followed by involvement in transcription (384 genes; 9.1 %), amino acid transport and metabolism (264 genes; 6.3 %), inorganic ion transport and metabolism (183 genes; 4.3 %), and translation, ribosomal structure, and biogenesis (169 genes; 4.0 %). Sixty-two genes (1.5 %) were found to code for secondary metabolite biosynthesis, transport, and catabolism. The details are provided in Land et al. (2009).

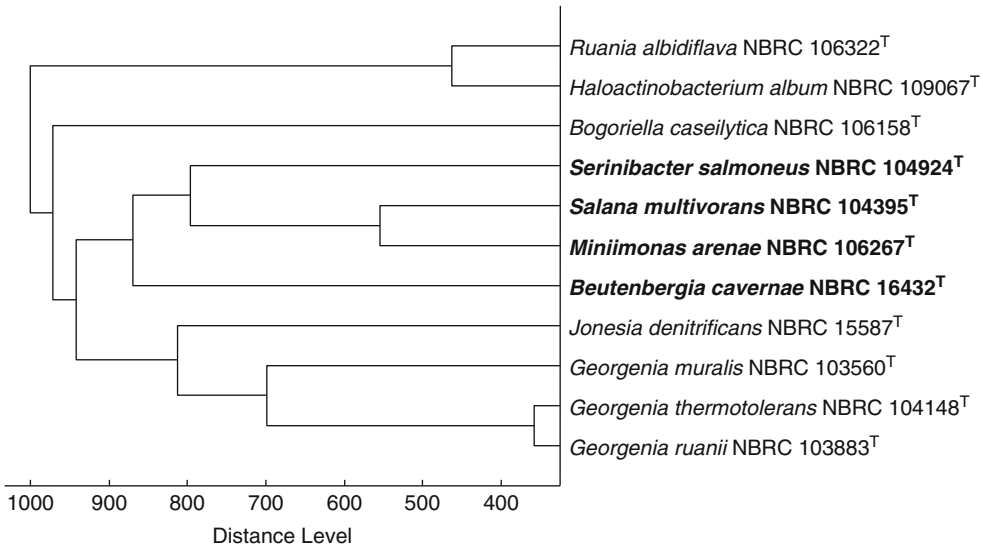

Fig. 6.2
A dendrogram generated by the BioTyper (version 2.0; Bruker Daltonics) software showing the similarity of MALDI-TOF mass spectra of cell extracts from type strains of the family *Beutenbergiaceae* and some related taxa

Phenotypic Analyses

The genera belonging to the family *Beutenbergiaceae* are mainly characterized by differences in chemotaxonomic features such as peptidoglycan type, polar lipids, and cellular fatty acids. The major characteristics of the genera *Beutenbergia*, *Miniimonas*, *Salana*, and *Serinibacter* are indicated in Table 6.1. Specifically, members show diversity in terms of their peptidoglycan structures. Cross-linkage of the peptidoglycans is of the A type and the diagnostic diamino acids are either L-lysine (*Beutenbergia* and *Serinibacter*) or L-ornithine (*Salana* and *Miniimonas*). Furthermore, it has been reported that L-serine occupies position 1 of the peptide subunit of the members of the genera *Salana* and *Serinibacter* (von Wintzingerode et al. 2001; Hamada et al. 2009). Detailed peptidoglycan analyses of the members of the family revealed that the peptidoglycan structure of the genus *Miniimonas* is identical to that of the genus *Salana* (Table 6.2). Specifically, it was concluded that the peptidoglycan of the genus *Miniimonas* was of the A4β type with an L-serine residue at position 1 of the peptide subunit (Hamada unpublished).

Beutenbergia: Groth, Schumann, Schuetze, Augsten, Kramer, and Stackebrandt 1999, 1738^{VP}

Beu.ten.ber′gi.a. N.L. fem. n. *Beutenbergia* referring to Beutenberg, the geographical location of the institute in which the soil sample was studied.

Cells are irregular rods, and cocci occur singly, in pairs, short chains, or clusters and exhibit a rod–coccus growth cycle. Gram-stain-positive, non-acid fast, non-motile, no formation of spores. Aerobic to microaerophilic. Oxidase-negative, catalase-positive. The peptidoglycan type is A4α with an L-Lys–L-Glu interpeptide bridge. The acyl type is acetyl. The major menaquinone is MK-8(H_4). The polar lipids are phosphatidylinositol, diphosphatidylglycerol, and three unknown phospholipids. The major fatty acids are iso-$C_{15:0}$ and anteiso-$C_{15:0}$.

The type strain of *Beutenbergia cavernae* HKI 0122^T was isolated from a soil sample collected in a cleft between the rocks of the Reed Flute Cave near Guilin, Guangxi, China. It shows the following additional properties. Cell sizes in rods vary from 1.0 to 3.1 μm in length. The diameters of the cocci are 0.7–1.0 μm. Colonies are 0.7–1.8 mm in diameter, cream to bright yellow colored, circular, convex. Acids are produced from L-arabinose, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, inulin, maltose, D-mannose, D-raffinose, L-rhamnose, D-ribose, salicin, sucrose, starch, trehalose, and D-xylose. There is no acid production from D-glucitol, lactose, or D-mannitol. Acetate, aconitate, benzoate, citrate, formate, malate, succinate, and DL-tartrate are not utilized. Nitrate is reduced to nitrite and H_2S is produced, whereas indole is not produced. Methyl red and Voges–Proskauer reactions are negative. Casein, aesculin, gelatin, and potato starch are decomposed; adenine, hippurate, hypoxanthine, xanthine, Tween 80, and tyrosine are not decomposed. Urease activity is negative. NaCl in combination with R medium is well tolerated up to a concentration of 4 % (w/v). Good growth occurs at 28 °C, growth is weak at 37 °C, and no growth occurs at 42 °C. Cells are susceptible to ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), neomycin (30 μg), oxytetracycline (30 μg), penicillin G (2 IU), and rifampin (2 μg). Susceptibility to polymyxin B (300 IU) is weakly expressed, and there is no susceptibility to ciprofloxacin (5 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), nitrofuran (300 μg), oxacillin (5 μg), streptomycin (10 μg), and sulfonamide. Based on the API ZYM enzyme assay, strain HKI 0122^T is positive for

Table 6.1
Phenotypic characteristics of the genera *Beutenbergia*, *Miniimonas*, *Salana*, and *Serinibacter*

Characteristic	*Beutenbergia*	*Miniimonas*	*Salana*	*Serinibacter*
Cell morphology	Irregular rods and cocci, rod–coccus cycle	Rods to cocci	Rods, coccoid, club-like	Irregular rods
Oxygen relationship	Aerobic to microaerobic	Facultatively anaerobic	Facultatively anaerobic	Facultatively anaerobic
Peptidoglycan type	A4α	A4β	A4β	A4α
Predominant menaquinone	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
Major fatty acids	iso-$C_{15:0}$, anteiso-$C_{15:0}$	anteiso-$C_{15:0}$, $C_{16:0}$	anteiso-$C_{15:0}$, $C_{14:0}$	anteiso-$C_{15:0}$, $C_{16:0}$
Polar lipids	DPG, PI, 3 PL	PG, DPG, PI, 1 PL	DPG, PG, PLs	PE, PG
DNA G + C content (mol%)	71	74	75	71

Data are from Groth et al. (1999), von Wintzingerode et al. (2001), Hamada et al. (2009), and Ue et al. (2011)
Abbreviations: *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PL* unidentified phospholipid

Table 6.2
Comparison of the peptidoglycan structures of the genera *Beutenbergia*, *Miniimonas*, *Salana*, and *Serinibacter*

Characteristic	*Beutenbergia*	*Miniimonas*	*Salana*	*Serinibacter*
Peptidoglycan type	A4α	A4β	A4β	A4α
Components of peptidoglycan[a]				
Alanine	2.0	0.9	0.9	1.0
Glutamic acid	2.0	2.0	2.0	2.0
Lysine	0.9	–	–	0.9
Ornithine	–	0.8	0.6	–
Serine	–	0.9	1.2	0.8
Enantiomer	D-Ala, L-Ala, D-Glu, L-Glu, L-Lys	D-Ala, D-Glu, L-Glu, L-Orn, L-Ser	D-Ala, D-Glu, L-Glu, L-Orn, L-Ser	D-Ala, D-Glu, L-Glu, L-Lys, L-Ser
Cell-wall diamino acid	L-Lys	L-Orn	L-Orn	L-Lys
Interpeptide bridge	L-Lys–L-Glu	L-Orn–L-Glu	L-Orn–L-Glu	L-Lys–L-Glu
Amino acid at position 1 of peptide subunit	L-Ala	L-Ser	L-Ser	L-Ser

[a]Figure means molar ratio of components

phosphatase alkaline, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, phosphatase acid, naphthol-AS-BI-phosphohydrolase, α-galactosidase, *p*-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase, and negative for lipase (C14), valine arylamidase, trypsin, chymotrypsin, β-glucuronidase, and β-glucosidase. The DNA G + C content of the type strain is 71 mol%.

Miniimonas: Ue, Matsuo, Kasai, and Yokota 2011, 125[VP]

Mi.ni.i.mo′nas. L. adj. *minius* cinnabar-red, vermilion; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Miniimonas* vermilion monad, referring to the cell mass color.

Gram-stain-positive, non-motile, coccoid- to rod-shaped, oxidase-negative, and catalase-positive. Growth occurs under both aerobic and anaerobic conditions. The peptidoglycan is of the A4β type with an L-Orn–L-Glu interpeptide bridge and an L-Ser residue at position 1 of the peptide subunit. The acyl type of muramic acid is acetyl. The major menaquinone is MK-8(H_4). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and one unknown phospholipid. The fatty acid pattern is of the iso- and anteiso-branched and straight-chain saturated type. The major fatty acids are anteiso-$C_{15:0}$ and $C_{16:0}$. The whole-cell sugars are galactose, xylose, and ribose. Mycolic acids are absent.

The type strain of *Miniimonas arenae* YM18-15^T was isolated from sea sand in Teguma fishing harbor in Nagasaki, Japan. It shows the following additional properties. Rod-shaped cells are 0.6–3.7 μm in length. Cells exhibit a rod–coccus cycle.

The diameters of the cocci are 1.0–1.7 μm. Growth occurs weakly at 25–30 °C. The pH range for growth is 5–11, with optimum growth at pH 7–7.5. Cells grow in the absence of NaCl, but tolerate up to 5 % NaCl (w/v). When grown aerobically for 7 days on LB agar or IL8 medium agar, the type strain forms a vermilion-colored cell mass and does not form spores. Cells form circular and smooth colonies that are 1–4 mm in diameter after 7 days at 30 °C on LB agar. Nitrate is reduced to nitrite. H_2S is not produced. Hydrolysis of starch is negative. The type strain forms vermilion-colored colonies. Based on API ZYM tests, cells are positive for esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, and β-glucosidase, but negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Cells are weakly positive for esterase (C4) and acid phosphatase. Cells utilize the following substrates as sole carbon sources: L-arabinose, D-mannose, D-xylose, D-glucose, D-fructose, L-rhamnose, and maltose monohydrate. The strain does not utilize D-mannitol. The API 20A test for the anaerobic formation of acid is positive for glucose, sucrose, maltose, D-xylose, L-arabinose, D-mannose, raffinose, L-rhamnose, and trehalose; weakly positive for cellobiose; and negative for D-mannitol, lactose, salicin, glycerol, melezitose, and D-sorbitol. Based on API 20A tests, cells are anaerobically positive for the hydrolysis of aesculin, but negative for the hydrolysis of gelatin and for the production of indole, arginine dihydrolase, and urease. The DNA G + C content of the type strain is 74.2 mol%.

Salana: von Wintzingerode, Göbel, Siddiqui, Rösick, Schumann, Frühling, Rohde, Pukall, and Stackebrandt 2001, 1659[VP]

Sa.la′na. N.L. fem. n. *Salana* referring to the German river Saale, the source of the bioreactor culture.

Cells form irregular rods and cocci and sometimes club-like forms. They are Gram-stain-positive and facultatively anaerobic bacteria. A variety of organic electron donors may be utilized. The peptidoglycan is of the A4β type with an L-Orn–L-Glu interpeptide bridge and an L-Ser residue at position 1 of the peptide subunit. The major menaquinone is MK-8(H_4). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and several unidentified phospholipids in minor amounts. The fatty acid pattern is of the iso- and anteiso-branched and straight-chain saturated type. The major cellular fatty acids are anteiso-$C_{15:0}$ and $C_{14:0}$.

The type strain of *Salana multivorans* Se-3111^T was isolated from an anaerobic dechlorinating consortium enriched from river sediment. It shows the following additional properties. Cells are 0.4–0.7 μm wide and 1.2–3.1 μm long. Growth occurs under both aerobic and anaerobic conditions. Under aerobic conditions, optimal growth occurs at pH 7.5 and 30 °C or 37 °C. Under anaerobic conditions, cells reduce selenate to elemental selenium. Gelatin and aesculin are not hydrolyzed. Indole is not formed and urease is negative. According to API ZYM reactions, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and valine arylamidase are positive, whereas trypsin, chymotrypsin, α-fucosidase, and β-glucuronidase are negative. Acid is formed under aerobic conditions (according to API 50CH) from L-arabinose, galactose, D-glucose, D-fructose, D-mannose, maltose, melibiose, sucrose, trehalose, D-raffinose, D-turanose, and D-lyxose, but not formed from erythritol, L-xylose, adonitol, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl *a*-dglucoside, *N*-acetylglucosamine, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, and 5-ketogluconate. Acid production from amygdalin, D-arabinose, gentiobiose, glycerol, inulin, methyl β-D-xyloside, ribose, xylitol, and D-xylose is variable. Anaerobically, acid is produced from arabinose, cellobiose, glucose, glycerol, lactose, maltose, mannitol, mannose, melezitose, raffinose, rhamnose, salicin, sorbitol, trehalose, and xylose. The DNA G + C content of the type strain is 75 mol%.

Serinibacter Hamada, Iino, Tamura, Iwami, Harayama, and Suzuki 2009, 2813[VP]

Se.ri.ni.bac′ter. N.L. n. *serinum* serine; N.L. masc. n. *bacter* rod; N.L. masc. n. *Serinibacter* a rod with serine in the cell wall.

Cells are irregular, rod-shaped, Gram-stain-positive, and non-endospore-forming. Growth occurs under both aerobic and anaerobic conditions. The cell-wall peptidoglycan is of the A4α type with an L-Lys–L-Glu interpeptide bridge and an L-Ser residue at position 1 of the peptide subunit. The acyl type of muramic acid is *N*-acetyl. The major menaquinone is MK-8(H_4), and the major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The fatty acid profile consists of iso- and anteiso- branched and straight-chain fatty acids. The major cellular fatty acids are anteiso-$C_{15:0}$ and $C_{16:0}$. The major cell-wall sugar is galactose. Mycolic acids are absent.

The type strain of *Serinibacter salmoneus* Kis4-28^T was isolated from the intestinal tract of sillago fish (*Sillago japonica*) collected from Kyonan beach on the coast of Tokyo Bay, Japan. It shows the following additional properties. Cells are 0.4–0.5 × 1.0–2.0 μm and non-motile. Colonies are circular, smooth, and yellowish orange, but cells grown in liquid culture are yellowish pink. Catalase-positive and oxidase-negative. The Voges–Proskauer test is positive, while the methyl red test is negative. Growth occurs at the NaCl concentrations of 0–10 % (w/v) but not at 15 % NaCl, and the optimal NaCl concentration range for growth is 0–5 %. The temperature range for growth is 10–37 °C, and the optimal temperature for growth is 28 °C. The pH range for growth is 6.0–9.0, and the optimal pH is 7.0. Acid is produced from *N*-acetylglucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, glycogen, lactose, maltose, D-mannose, methyl α-D-glucopyranoside, methyl

β-D-xylopyranoside, starch, sucrose, trehalose, and D-xylose. Esterase lipase (C8), leucine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, pyrazinamidase, and pyrrolidonyl arylamidase are present, whereas alkaline phosphatase, lipase (C4), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine- and ornithine decarboxylases, and tryptophan- and phenylalanine deaminases are absent. H_2S and indole are not produced. Aesculin is hydrolyzed, while gelatin, urea, and DNA are not hydrolyzed. Nitrate is not reduced. The DNA G + C content of the type strain is 70.7 mol%.

Isolation, Enrichment, and Maintenance Procedures

Beutenbergia cavernae HKI 0122^T (Groth et al. 1999) was isolated from a soil sample collected from a cleft between the rocks of the Reed Flute Cave near Guilin, Guangxi, China. One gram of soil sample was suspended in 10 ml sterile phosphate buffer (pH 7.2) and thoroughly mixed by shaking. Soil particles were allowed to sediment, and then the liquid phase was serially diluted and spread onto casein mineral medium (Altenburger et al. 1996) containing 0.6 g/l K_2HPO_4, 0.5 g/l $Na_2HPO_4 \cdot 2H_2O$, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.1 g $MgCl_2 \cdot 7H_2O$, 0.2 g/l KNO_3, 0.01 g/l $FeCl_3 \cdot 6H_2O$, 0.8 g/l casein, and 0.4 g/l yeast extract (pH 7.0). Another strain, HKI 0132, was isolated from the same sample on PY-BHI agar (Yokota et al. 1993) containing 10.0 g/l peptone, 2.0 g/l yeast extract, 2.0 g/l Bacto brain heart infusion (Difco), 2.0 g/l NaCl, and 2.0 g/l glucose (pH 7.0).

Miniimonas arenae YM18-15^T (Ue et al. 2011) was isolated from sea sand from the Teguma fishing harbor in Nagasaki Prefecture, Japan. Isolation was performed at 25 °C for 30 days on H medium (Ue et al. 2011).

Salana multivorans Se-3111^T (von Wintzingerode et al. 2001) was isolated from an anaerobic bioreactor. Samples were taken from a fluidized bed reactor inoculated with an anaerobic, trichlorobenzene-dechlorinating consortium enriched from sediment of the River Saale near Jena, Germany (Selent 1999). Within the fluidized bed reactor, the dechlorinating consortium was immobilized on polyurethane foam cubes. For enrichment of anaerobic, selenate-reducing bacteria, the foam cubes were removed from the bioreactor using sterile forceps and transferred to reduced RAMM medium (Shelton and Tiedje 1984). After a 7-day incubation at 30 °C under anaerobic conditions (GasPak anaerobic jars, Anaerogen), enrichment cultures were set up by transferring 1 foam cube each to an Erlenmeyer flask with Se medium (Macy et al. 1989) containing 2.2 g/l NaCl, 0.3 g/l KCl, 0.3 g/l NH_4Cl, 0.2 g/l KH_2PO_4, 0.15 g/l $CaCl_2 \cdot 2H_2O$, 0.4 g/l $MgCl_2 \cdot 6H_2O$, 0.6 g/l $NaHCO_3$, 3.78 g/l Na_2SeO_4, 3.78 g/l potassium acetate, 10 ml/l trace-metal solution SL8, 10 ml/l vitamin solution, and 800 µl/l methanol. After 5 days of anaerobic incubation in anaerobic jars (CO_2, N_2, and H_2 atmosphere), a red precipitate was visible and served as an inoculum for subcultures in modified Se medium (Se medium supplemented with 1.2 g/l NaCl, 1–4 g/l yeast extract, 0.3 g Na_2SO_4, and 2.02 g/l KNO_3). Pure cultures were obtained by repeated streaking on agar plates containing the above medium.

Serinibacter salmoneus Kis4-28^T (Hamada et al. 2009) was isolated from the intestinal tract of a fish species (Japanese sillago; *Sillago japonica*) collected from Kyonan Beach on the coast of Tokyo Bay, Chiba Prefecture, Japan. The sillago was dissected, and an intestinal tract sample of approximately 1 g was used for the isolation of bacteria. Suspensions of the intestinal tract in 10 ml saline were serially diluted and spread onto LYPm agar (Iino et al. 2007) containing 10 g/l α-lactose, 20 g/l NaCl, 10 g/l yeast extract, 5 g/l polypeptone, 0.025 g/l Tween 80, 5 ml/l salt solution, and 15 g/l agar (pH 6.0). The salt solution contained 40 g/l $MgSO_4 \cdot 7H_2O$, 2.0 g/l $MnSO_4 \cdot 4H_2O$, 2.0 g/l $FeSO_4 \cdot 7H_2O$, and 2.0 g/l NaCl. The inoculated plates were cultivated at room temperature (approximately 20–25 °C) in a sealed nylon bag with an O_2-absorbing and CO_2-generating agent (Anaero-Pack, Mitsubishi Gas Chemical) for at least 1 month. Visible colonies on the agar plates were picked up and then transferred to fresh LYPm agar in aerobic conditions. Pure cultures were obtained by repeated streaking on LYPm agar.

Members of the family *Beutenbergiaceae* grow readily in complex liquid or on solidified media, i.e., Bacto nutrient agar, Bacto tryptic soy agar, and NBRC medium 802 containing 10 g/l polypeptone, 2.0 g/l yeast extract, and 1.0 g/l $MgSO_4 \cdot 7H_2O$ (pH 7.0). Members of the family do not require special procedures for maintenance and preservation. Cultures of the members can be maintained by serial transfers onto appropriate solid media. Growth on agar slants can be kept at 4 °C for about 1 month. Medium-term preservation is in 12–15 % (v/v) glycerol suspensions at −80 °C. Long-term preservation of liquid cultures supplemented with 12–15 % (v/v) glycerol or 7 % (v/v) dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−150 °C). Freeze-drying and L-drying methods can also be applied for long-term storage.

Ecology

Members of the family *Beutenbergiaceae* were isolated from cave soil, river sediment, sea sand, and the intestinal tract of a fish. However, ecological information is insufficient because most species are defined only by a type strain. Further studies are required in order to elucidate the ecological niches and the functions of the members of the family. The NCBI taxonomy browser lists several unnamed strains and clones affiliated to the family *Beutenbergiaceae*, e.g., *Beutenbergia* sp. 91196 (accession number AY996854), an uncultured *Beutenbergiaceae* bacterium clone 741-8-06 from endosphere of hybrid poplar (Ulrich et al. 2008) (AM489678), and two uncultured *Salana* sp. clones OT44-13 and F5OHPNU07INO5B from an organic household waste in anaerobic reactor (Cardinali-Rezende et al. 2009) (FJ982876) and a tailing pond (Ramos-Padrón et al. 2011) (HQ091860), respectively.

Pathogenicity and Clinical Relevance

Pathogenicity has not been reported in any strains belonging to the family *Beutenbergiaceae*. *Beutenbergia cavernae* cells are susceptible to ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), neomycin (30 μg), oxytetracycline (30 μg), penicillin G (2 IU), and rifampin (2 μg). The cells demonstrate weak susceptibility to polymyxin B (300 IU), and are resistant to ciproflaxin (5 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), nitrofuran (300 μg), oxacillin (5 μg), streptomycin (10 μg), and sulfonamide. No information on antibiotic sensitivity and resistance is available for *Miniimonas arenae*, *Salana multivorans*, and *Serinibacter salmoneus*.

Application

It has been reported that strains of *Salana multivorans* are capable of selenate reduction (von Wintzingerode et al. 2001).

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7 The Family *Brevibacteriaceae*

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Abstract
Brevibacterium constitutes a ubiquitous range of species for which only the halotolerant sulfur aroma production has been utilized in the dairy food industry. Recently, however, a wide range of new isolates from different environments including soil, sediment, and seawater has been assigned to this genus. Since these isolates show quite diverse metabolic properties, they pave the way to new applications such as metal processing or bioremediation. Despite its long-time industrial use, especially as inoculants in the cheese-making industry, the genomic era for this genus has just started, with the first genomes of *B. aurantiacum* ATCC9174 (an industrial strain), *B. massiliense* DSM23039 (a pathogenic strain), and, finally, *Brevibacterium* sp. JC43 (a human gut strain) being released recently. Other genome sequences of *Brevibacteriaceae* are on their way. This includes the genome sequences from several strains of the industrially used *B. linens* (ATCC9172 and OC2 strains) and the genital pathogen *Brevibacterium mcbrellneri*. Genomic data should open investigation of the valuable but not yet fully deciphered metabolic properties of species and, on the other side, allow a better understanding of the specialization of distinct species within this genus as pathogens. Genetic manipulation of members of this genus is also not yet fully developed and tools remain to be derived from the genomic information. The recently obtained but still limited genomic data should be screened carefully with special focus on genetic elements such as genomic islands and plasmids. In addition, the investigation of mechanism underlying lateral gene transfer will also give novel insights and potentially applicable genetic tools for this genus.

Taxonomy, Historical and Current State

In 1953, Breed first established the genus *Brevibacterium* with *B. linens* as the type species. The genus' name comes from the Latin *brevis*, short, and the Greek βακτερια, rod. Classically, the genus has presented taxonomists with difficulties because of its close morphologic resemblance with other genera including *Corynebacterium*, *Arthrobacter*, and *Rhodococcus*. Recognized as a genus in the seventh edition of the *Bergey's Manual of Determinative Bacteriology*, it was listed as *incertae sedis* in its 8th edition. Numerical taxonomy has demonstrated that *Brevibacterium* indeed constitutes a distinct genus. Although remaining in continuous reclassification on the basis of 16S rRNA gene sequencing and DNA/DNA hybridization, *Brevibacterium* today remains the unique genus of the *Brevibacteriaceae* family located in the order *Micrococcales*, class *Actinobacteria*. The genus shows, however, an extreme heterogeneity in physiological, biochemical, and chemical features, resulting today in the delineation of 28 *Brevibacterium* species which were isolated from habitats as diverse as dairy products, poultry skin, insects, soil, mural paintings, wall, clinical samples, the human microbiome, brown algae, and salt-lake, marine, or beach sediments (*Table 7.1*). Historically, *B. linens* and *B. iodinum* were the first species described within this genus (Collins et al. 1980), followed by *B. casei* (Collins et al. 1983), *B. epidermidis* (Collins et al. 1983), *B. mcbrellneri* (McBride et al. 1993), *B. otitidis* (Pascual et al. 1996), *B. avium* (Pascual and Collins 1999), *B. paucivorans* (Wauters et al. 2001), *B. luteolum* (Wauters et al. 2003), *B. sanguinis* (Wauters et al. 2004), *B. celere* (Ivanova et al. 2004), *B. permense* (Gavrish et al. 2004), *B. picturae* (Heyrman et al. 2004), *B. samyangense* (Lee 2006), *B. ravenspurgense* (Mages et al. 2008), *B. album* (Tang et al. 2008), *B. marinum* (Lee 2008), *B. oceani* (Bhadra et al. 2008), *B. massiliense* (Roux and Raoult 2009), *B. sandarakinum* (Kämpfer et al. 2010), *B. salitolerans* (Guan et al. 2010),

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Table 7.1

Phenotypic characteristics of the 28 species of *Brevibacterium* (Data are from the original descriptions)

Species	Type strain number	Origin	Colony pigmentation	Colony morphology	Temperature			NaCl tolerance %
					<12 °C	20 °C	37 °C	
B. album	DSM18261	Saline soil	White	Smooth, circular	–		+	15
B. ammoniilyticum	JCM 17537	Sludge of a wastewater	Cream	Circular, convex	–	+	+	11
B. antiquum	VKM Ac-2118	Permafrost sediments	Orange	Smooth	+		–	18
B. aurantiacum	DSM20426	Cheese	Orange	Smooth	+	+	–	–
B. avium	DSM15880	Skin poultry	Cream grey	Smooth	+	+	+	nd
B. casei	DSM 20657	Fermented milk	Cream grey	Smooth	–	+	+	15
B. celere	DSM 15453	Brown algae	Cream yellow	Smooth	+	+	+	15
B. daeguense	JCM 17458	Sludge of a wastewater	Pale yellow	Circular	+	+	+	5
B. epidermidis	DSM 20660	Human skin	Cream yellow	Smooth	–	+	+	15
B. iodinum	DSM 20626	Milk	Cream with purple spot	Smooth	–	+	+	12
B. linens	DSM 20425	Cheese	Orange	Smooth	–	+	w	15
B. lutescens	DSM15022	Peritoneal fluid	Cream yellow	Smooth	–	+	+	10
B. marinum	DSM 18964	Seawater	Yellow	Smooth	+	+	nd	+
B. massilense	DSM23039	Human ankle discharge	Cream	Smooth, circular	–	–	+	10
B. mcbrellneri	ATCC49030	Infected genital hair	Cream grey	Dry	–	–	+	nd
B. oceani	BBH7	Deep-sea sediment	Orange	Sticky	+	nd	–	12
B. otitidis	ATCC700348	Infected ear	Cream yellow	Smooth	nd	–	nd	nd
B. paucivorans	DSM13657	Human blood	Cream grey	Smooth	–	–	+	nd
B. permense	VKM Ac-2280	Permafrost sediments	Orange	Smooth	–	+	+	18
B. picturae	DSM 16132	Damaged mural painting	White	Smooth, circular	nd	+	v	15
B. pityocampae	DSM21720	Caterpillar	Yellow	Circular	nd	nd	+	10
B. ravenspurgense	DSM21258	Wound swab	nd	Slightly convex	nd	nd	nd	nd
B. salitolerans	TRM 415	Salt-lake sediment	White yellow	Smooth, circular	–	+	+	18
B. samyangense	DSM19451	Beach sediment	Cream	Smooth	nd	+	nd	nd
B. sandarakinum	DSM22082	Indoor wall	Orange	nd	+	+	–	10
B. sanguinis	DSM15677	Human blood	Cream grey	Sticky or smooth	nd	+	+	10
B. siliguriense	DSM 23676	River water	White	Circular, convex	–	+	+	15
B. yomogidense	DSM 24850	Poultry manure	Pale yellow	Circular, smooth convex	+	+	+	17

+ positive, *nd* no data, *w* weakly positive, *v* variable

B. pityocampae (Kati et al. 2010), *B. siliguriense* (Kumar et al. 2011), *B. ammoniilyticum* (Kim et al. 2012), *B. daeguense* (Cui et al. 2013), and *B. yomogidense* (Tonouchi et al. 2013). In addition to the description of novel species, other resulted from the reclassification of heterogeneous ones. This was the case for the species *B. linens* which showed heterogeneity in DNA/DNA hybridization properties. In 2004, Gavrish and collaborators split this species into three new species: *B. linens*, *B. antiquum*, and *B. aurantiacum* (Gavrish et al. 2004). Moreover, our own work suggests that the cheese strain of economic interest, CNRZ918, should be reclassified as *B. antiquum* (Forquin et al. 2009). The list of *Brevibacteriaceae* is not extensive, and

novel species have to be defined from novel metagenomic approaches as shown for the gut microbiome for instance. Indeed a *Brevibacterium* sp. JC43 was lately isolated from the cultivable microbiome of human feces (Lagier et al. 2012) that is not yet classified within known *Brevibacterium* species although it presents a 16S sequence close to the one from the recently described *B. yomogidense*. New species of *Brevibacteriaceae* are thus shortly expected. Alternatively, some species were recently removed from the *Brevibacteriaceae* such as *Brevibacterium stationis* which was first described in 1944 as "*Achromobacter stationis*" (ZoBell and Upham 1944) and later assigned to the genus *Brevibacterium* by Breed (Breed 1953). This species was recently reclassified as *Corynebacterium stationis* comb. nov. (Bernard et al. 2010). Classification of *Brevibacterium* remains ambiguous, and a number of strains described as *Brevibacterium* in the literature or public generalist or dedicated databases have to be reclassified. The phylogenetic relationships of the 28 described species of *Brevibacterium* at the time of publication, built from 16S rRNA gene sequences, are shown in *Fig. 7.1*.

Molecular Analyses

The classification and typing of *Brevibacterium* have been done using DNA/DNA hybridization (Fiedler et al. 1981; Stackebrandt and Fiedler 1979), 16S rRNA gene sequence typing (Gavrish et al. 2004; Stackebrandt et al. 1997), or spectroscopic methods such as FT-IR (Oberreuter et al. 2002), DNA restriction enzyme analysis (ARDRA), pulsed-field gel electrophoresis (PFGE), and ribotyping (Hoppe-Seyler et al. 2007; Lima and Correia 2000). Nowadays, the identification is based mainly on classical molecular methods such as 16S DNA sequencing coupled with phenotypic characterization. Recently, a study developed and used both Multilocus Sequence Typing (MLST) and Comparative Genomic Hybridization approaches (CGH) to study the genomic variability of cheese-related *Brevibacterium* strains (Forquin et al. 2009). This study showed that MLST can be efficiently used to identify and type *Brevibacterium* species in a single step. CGH appears also interesting for screening purposes of specific metabolic routes but its use remains restricted to the analysis of the diversity of *B. aurantiacum* isolates (Forquin et al. 2009). Indeed, such tools were up to now developed from the only completed *B. aurantiacum* ATCC9174 genomic sequence. Specific tools should however soon be derived from the increasing number of released genomic sequences of *Brevibacteriaceae* genomes.

Until recently, a few data are available regarding *Brevibacterium* sp. genomes, plasmids, and even gene sequences. Released public information is briefly resumed in *Table 7.2*. Most available *Brevibacterium* sequence data corresponds to 16S rDNA genes. Regarding plasmids, data still remain scarce. Some *Brevibacteriaceae* have been reported as not harboring plasmids while several distinct plasmids, generally circular and of smaller sizes, were identified in others (Nardi et al. 2005). In addition, a large, linear plasmid (which is typically a characteristic of *Streptomyces*, *Arthrobacter*, *Nocardia*, and *Rhodococcus*) of 436 kb has recently been reported in an environmental *Brevibacterium* sp. isolated in Argentina (Dib et al. 2010). Historically, a first multicopy plasmid of a size of 7.75 kb, designated pBL100, has been isolated from *B. linens* DSM 20158 (Sandoval et al. 1985). Further, a plasmid designated pBL33 (7.3 kb) has been isolated from *B. aurantiacum* ATCC 9174 which, interestingly, showed a restriction map very similar to that of the pBL100 plasmid. It also appeared identical to plasmids previously isolated by Kato et al. (1989) from a range of *Brevibacteriaceae*. Small plasmids with similar restriction profiles were further confirmed in six strains of *B. linens* (Holtz et al. 1992) suggesting that small, putatively conjugative plasmids with potentially wider host ranges within the genus may exist in *Brevibacteriaceae*. It has been indicated in older studies that some of these plasmids encoded extracellular proteases. This has to be confirmed by modern sequence data analysis such as the one from the *B. linens* OC2 genome. Indeed, plasmid is likely to be harbored by the currently sequenced *B. linens* OC2 genome (our unpublished data), but this has to be confirmed after gap closure. Systematic genome sequencing in the genus is thus aimed at increasing the knowledge and the characterization of *Brevibacteriaceae* plasmids for which maintenance, replication, and transfer mechanisms are not yet fully understood. Some findings on plasmid biology were indeed published for strains which were nowadays reclassified as *Rhodococcus* sp. or *Corynebacterium* sp. and no systematic plasmid screening has been performed until recently. In addition, plasmids classically used for the genetic manipulation of *Corynebacterium* sp. were derived from strains like "*B. lactofermentum*" or "*B. flavum*" that are all nowadays reclassified as *C. glutamicum*. The lack of tools necessary to perform genetic modifications in this species, for long, hindered the systematic genetic investigation of *Brevibacteriaceae* metabolism (Nardi et al. 2005). Thus, traditionally, selection under pressure rather than genetic manipulation has been used for industrial strain improvement but also for evident reasons as food safety and public acceptance, explaining the lack of interest for the development of genetic engineering tools. Difficulties for genetic manipulation may also have arisen from the lack of knowledge on DNA methylation or CRISPR-like defense mechanisms of *Brevibacteriaceae* against foreign DNA (Roux et al. 2012).

Nowadays, several sequencing projects of *Brevibacterium* genomes have started (*Table 7.2*). One *B. casei* S18 *Brevibacterium* genome project is listed on the NCBI website and other projects are on their way including the genome sequence of *B.marinum*, *B. casei*, *B epidermidis*, *B. picturae*, *B. permense*, *B. antiquum*, and *B linens* (T. Vallaeys, M.P. Forquin-Gomez, F. Irlinger; J Kalinowski, unpublished information).

Three draft genomes are available on the NCBI website. The draft genome of *B. mcbrellneri* consists of 96 contigs, has a total length of 2.56 Mb with a G+C content of 58 %, and 2,490 genes are predicted that encode 2,432 proteins. The draft genome of *B. massiliense* DSM23039 has just been released (Roux et al. 2012). It contains 7 scaffolds and 27 contigs (>1,500 bp) and a total length of 2.36 Mb. Its G+C content was 62.3 %.

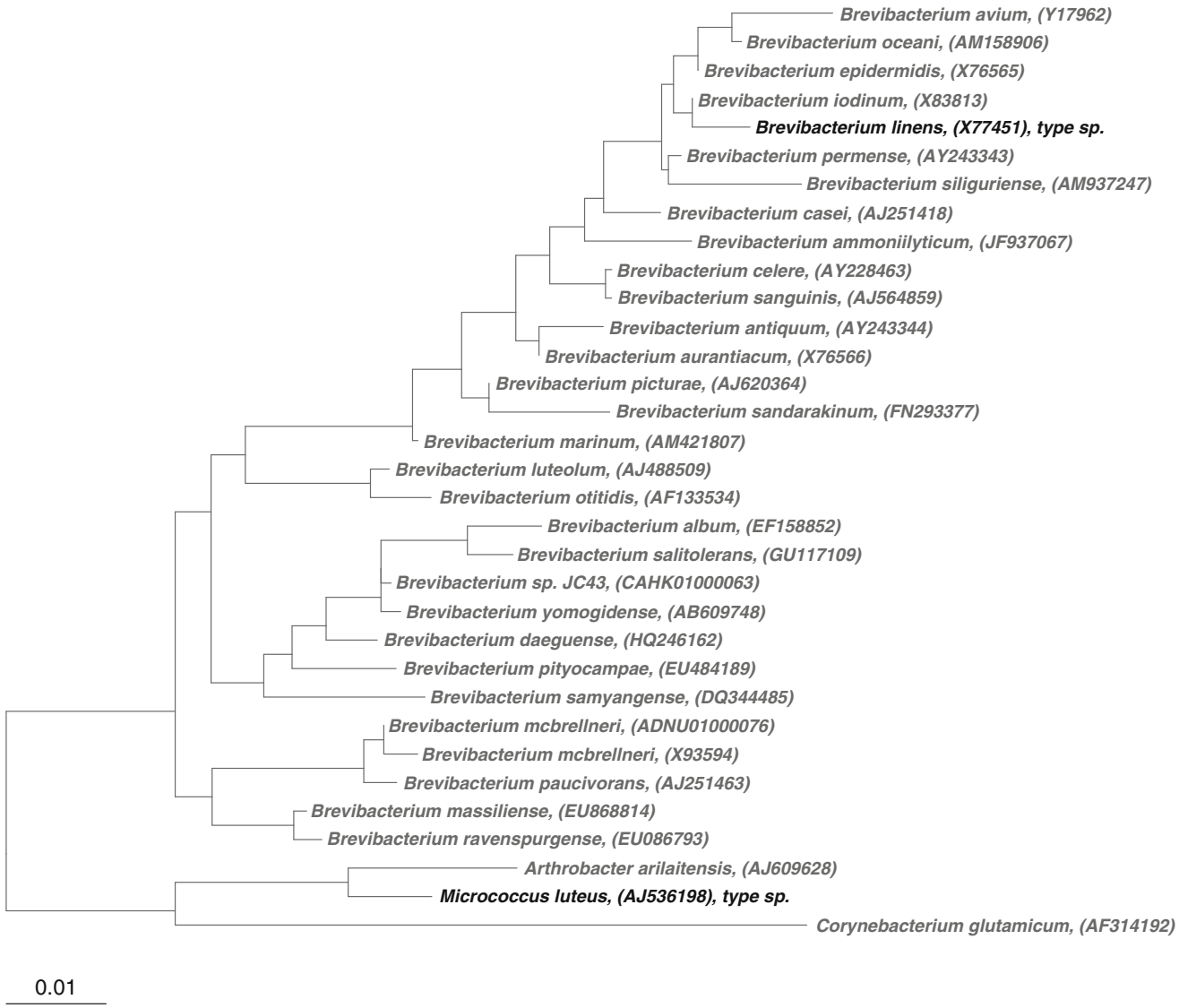

Fig. 7.1

Phylogenetic reconstruction of the family *Brevibacteriaceae* 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

The genome contains 43 tRNA genes and encodes 2,154 putative proteins. The last draft genome sequence available belongs to *Brevibacterium* sp. JC43 (accession number CAHK01000000). This *Brevibacterium* was isolated from the cultivable microbiome of humans, and this strain is apparently related to *B. yomogidense*. The genome of *B. aurantiacum* ATCC9174 has been completely determined (Forquin-Gomez et al., unpublished). The genome consists of a circular chromosome of 4.4 Mb and one plasmid of 7.3 kb. The entire genome with a G+C content of 62.3 % contains 4,104 predicted protein-coding genes (4,097 in the chromosome and 7 on the plasmid) and 48 tRNA genes. Four rDNA operons were identified on the chromosome, organized in the order 16S-23S-5S. The annotation was performed and protein functions according to the COG (Clusters of Orthologous Groups) classification have been assigned. The categories "unknown function" and "general function prediction only" represent 38 % of the genes in the genome. The analysis showed that the highest number of predicted functions (8 %) belongs to the category "amino acid metabolism" and 7 % of genes are involved in transcription. The category carbohydrate metabolism represents only 4 % of the protein-coding genes in the genome. Similar data

Table 7.2
Sequence information on *Brevibacteriaceae*

Species	Accession number 16S rRNA gene	Genome sequencing	Genome GC content %	Plasmid
B. album	EF158852	nd	70.7	nd
B. ammoniilyticum	JF937067	nd	70.7	nd
B. antiquum	AY243344	CNRZ918: Project	60.1–64.3	nd
B. aurantiacum	X76566	ATCC 9174: Complete[b]	62.3	pL33
		DSM20426: Draft[b]		
B. avium	Y17962	nd	nd	nd
B. casei	AM411119	S 18: Project	66.2–67.2	nd
		DSM20657: Draft[b]		
B. celere	AY228463	nd	61.4	nd
B. daeguense	HQ246162	nd	66.4	nd
B. epidermidis	X76565	DSM20660: Draft[b]	63.5	nd
B. iodinum	X83813	nd	63	nd
B. linens	AF426135[a]	DSM20425: Draft[b]	62.5	pL33 and pL100
		OC2: Draft[b]		pRBL1: U39878
				pLIM: AY004211
B. lutescens	AJ488509	nd	68.8	nd
B. marinum	AM421807	Starting project	71.4	nd
B. massilense	EU868814	Draft CAJD00000000	62.3	nd
B. mcbrellneri	X93594	Draft ADNU01000001-96	63.1	nd
B. oceani	AM158906	nd	59.8–60.2	nd
B. otitidis	AF133534	nd	nd	nd
B. paucivorans	AJ251463	nd	55.8	nd
B. permense	AY243343	nd	60.1–64.3	nd
B. picturae	AJ620364	DSM16132: Draft[b]	63.3	nd
B. pityocampae	EU484189	nd	69.8	nd
B. ravenspurgense	EU086793	nd	nd	nd
B. salitolerans	GU117109	Starting project	69.14	nd
B. samyangense	DQ344485	nd	70.7	nd
B. sandarakinum	FN293377	nd	70	nd
B. sanguinis	AJ564859	nd	69.9	nd
B. siliguriense	AM937247	nd	64.6	nd
B. yomogidense	AB609748	nd	67.4	nd

nd no data
[a]Accession number 16S DNA of the type strain
[b]Unpublished

were obtained for *B. linens* OC2, this draft genome has been assembled in 4 scaffolds which G+C content is 62.3 % (unpublished data).

Phenotypic Analyses

Brevibacterium are gram-positive coryneform bacteria and strictly aerobic; nonmotile, except for *B. album* (Tang et al. 2008) and *B. iodinum* (Collins et al. 1980); non-spore forming; catalase positive; and frequently halotolerant bacteria (5 % NaCl is a standard but up to 20 % NaCl is tolerated by given environmental isolates). Their optimal growth temperatures range from 20 °C to 30 °C for the environmental or food-derived strains and up to 37 °C for the human isolates (Table 7.1). However, given *Brevibacteriaceae* were reported to grow at temperatures below 12 °C (Table 7.1). *Brevibacterium* strains grow from pH 5.8 on with an optimum at pH 7. *Brevibacterium* sp. usually alkalinizes its growth medium (up to pH 9.5). Interestingly, in complex media, *Brevibacterium* exhibits a typical distinct rod-coccus cycle. In exponential phase, the cells are rod-shaped, becoming

coccoid-shaped in the stationary phase (3–7 days old cultures). Cell length is thus variable but remains within the range of 0.6–1 μM. The typical arrangement of cells seen under the microscope is V-shaped with cells adherent at one side with an angle resulting from an unequal rupture of the cell walls after cell division. The cell-wall peptidoglycan of *Brevibacterium* is based on cross-linked meso-diaminopimelic acid (Schleifer and Kandler 1972), and complex teichoic acids assembling neutral sugars, amino sugars, and sugar alcohols (Anderton and Wilkinson 1980; Fiedler et al. 1981). Cellular fatty acids are typically branched and long chained, belonging to the anteiso- and iso-methyl types, with 12-methyltetradecanoic and 14-methylhexadecanoic acids predominating (Bousfield et al. 1983; Collins et al. 1980, 1983). Although some strains synthesize phosphatidylinositol (Collins et al. 1980, 1983), diphosphatidylglycerol, phosphatidylglycerol, and dimannosyl-diacylglycerol constitute major polar lipids. Menaquinones constitute the sole respiratory quinones detected in the cellular membrane of *Brevibacteriaceae* with preponderant dihydrogenated menaquinones carrying eight isoprene units (MK- 8[II-H2]) (Collins et al. 1980, 1983).

Production of volatile sulfur compounds (VSCs) such as methanethiol, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), or *S*-methyl thioesters are among the major characteristics of *Brevibacterium*, especially for strains of industrial use. A number of *Brevibacteriaceae* typically convert methionine into methanethiol via a unique one-step reaction using methionine γ-lyase. This compound is then oxidized into VSCs. The VSCs of *B. linens* and *B. aurantiacum* have been well described (Bonnarme et al. 2000, 2001; Dias and Weimer 1998a, b; Forquin et al. 2011; Weimer et al. 1999). Interestingly, VSC production is not limited to strains of industrial importance, and *B. mcbrellneri* (McBride et al. 1993), *B. sanguinis* (Wauters et al. 2004), *B. epidermidis*, *B. casei*, *B. marinum*, *B. celere*, and *B. picturae* (Heyrman et al. 2004) also produce some VSCs (Forquin-Gomez, personal observations). *B. antiquum* and *B. aurantiacum* are also able to produce *S*-methyl thioesters using short-chain fatty acids or branched-chain amino acids as precursors (Sourabié et al. 2012). VSCs production by *B. casei* and *B. epidermidis* is also suspected to be responsible for unpleasant body odors (Jones and Keddie 1986).

A number of *Brevibacteriaceae* are producing pigments, constituting another phenotypic characteristic of the genus. The color of mature colonies varies from clear white beige or grey (*B. epidermidis*, *B. casei*) to orange (*B. linens*) and cream with purple dots (*B. iodinum*) (*Table 7.1*). This typical dark red coloration is due to the secretion of purple crystals of a derivative of the phenazine derivative iodine by *B. iodinum*, which gave the name to the species. Orange colors result from carotenoids, the production of which has been reported to be enhanced by light. Novel metabolic characteristics reported in lately described isolates of environmental *Brevibacteriaceae* include heavy metal metabolism and degradation of long-chain aliphatics (crude oil) and aromatic carbon for which pathways remain mostly undeciphered (Ng et al. 2010; Verma et al. 2013). Such characteristics pave the way to the setting up of new culture media for the selective isolation of this for long-neglected group of environmental *Brevibacteriaceae*.

Isolation, Enrichment, and Maintenance Procedures

Brevibacterium globally comprises easy cultivable species. Its diversity of habitats, originally thought to be restricted to dairy products, has long been masked by the lack of attempts to isolate it from other sources. *Brevibacteriaceae* are indeed grown easily on most peptone-yeast extract agars as well as on brain-heart infusion agars under aerobic conditions as long as salt is added (up to 4 % NaCl is suitable). They are relatively fast growers and can be isolated after incubation at ambient temperatures (mostly 20–25 °C) for 3–5 days. Higher temperatures select for those species living on the human skin and for animal isolates while temperatures of 10–20 °C are preferable for the isolation and culture of soil-inhabiting and marine strains. *Brevibacterium* species are mainly aerobic but growth in microaerophilic conditions, and even weak growth in anaerobic conditions, has been reported (Roux and Raoult 2009). No selective media are available and apart from microscopic characteristics such as the so-called coryneform morphology, 16S sequencing is the method of choice for their identification. Extended metabolic analysis resulting from genome sequences may however soon fill the gap by elaboration of more selective or at least enriching growth media.

Ecology

Brevibacteriaceae are typically halotolerant bacteria and are predominantly found in habitats with increased salt concentrations, including marine environments and salted foods (*Table 7.1*). Indeed, several species are related to saltwater. *B. oceani* has been isolated from deep-sea sediments, *B. samyangense* from beach sediments, *B. salitolerans* from salt-lake sediment, *B. marinum* from seawater, *B. siliguriense* from river water, and *B. celere* from brown algae. *Brevibacterium* species were also recently isolated from lagunal samples and salines in southern France and Tunisia (Abbes and Vallaeys, unpublished data). Similarly, other strains were isolated from saline terrestrial environments: *B. album* was isolated from a saline soil, but *B. antiquum* and *B. permense* were isolated from permafrost soils. However, a *B. antiquum* relative was also isolated from cheese (Forquin et al. 2009). Salted foods constitute another preferential habitat for *Brevibacteriaceae*: *B. aurantiacum and B. linens* are major components of the microflora of surface-ripened cheese such as Munster, Limburger, Tilsiter, and Romadour (Brennan et al. 2002; Feurer et al. 2004a, b; Mounier et al. 2005, 2009), however, sharing the smear niche with other coryneforms such as *Arthrobacter*, lactic acid bacteria, and yeasts (Feurer et al. 2004a, b). Interestingly, a strain of *B. linens* T4 has been found to represent over

96 % of the assigned cloned 16S rDNA sequences obtained from a library constructed from total DNA extracted from the curd of a farm house-produced *Fourme de Montbrison* suggesting either increased competitiveness or antibacterial and antifungal activity for this strain (Vallaeys unpublished data). Similarly, *B. casei* has been isolated from cheese curd and cheddar cheese but also from milk. Interestingly, some strains of this species were isolated from human clinical specimens. Further, *Brevibacterium* appears as a ubiquitous species colonizing a wide range of natural but also human-shaped habitats. Two species were indeed isolated form indoor environments: *B. sandarakinum* from a wall and *B. picturae* from a damaged mural painting at the Saint-Catherine chapel of Castle Herberstein in Austria. An extended diversity of habitats is further suggested by recent studies: *B. iodinum* was originally isolated from milk and *B. avium* was isolated from poultry skin, *B. yomogidense* from poultry manure. *B. ammoniilyticum* and *B. daeguense* have been isolated from the sludge of a wastewater treatment plant. *B. luteolum* has been isolated from environmental and human clinical samples. Nowadays, more and more species are isolated from humans, *Brevibacterium epidermidis* forms part of the resident human skin microflora, and *B. sanguinis*, *B. mcbrellneri*, *B. ravenspurgense*, *B. paucivorans*, *B. otitidis*, and *B. massiliense* were also isolated from humans, suggesting that given *Brevibacterium* species could present an infective potential.

Pathogenicity, Clinical Relevance

Until relatively recently, *Brevibacteriaceae* were not associated with human infections. However, it seems that *Brevibacteriaceae* have recently emerged as background opportunistic pathogens, affecting immunocompromised patients. Of the nine known species of *Brevibacterium* isolated from human clinical samples, *B. casei* is the most frequently reported species from clinical specimens. Indeed, several human infections involving *B. casei* have been reported (Brazzola et al. 2000; Cannon et al. 2005; Gruner et al. 1994; Janda et al. 2003; Kumar et al. 2011; Ulrich et al. 2006). Cases involving *B. epidermidis* (Manetos et al. 2011) and *B. otitidis* (Dass et al. 2002; Ulrich et al. 2006; Wauters et al. 2000) were also published. Moreover, *B. mcbrellneri* was isolated from genital hair of patients infected with white piedra in association with *Trichosporon beigelii* (McBride et al. 1993). *B. luteolum* (Wauters et al. 2003), *B. paucivorans* (Wauters et al. 2001), and *B. sanguinis* (Wauters et al. 2004) were also associated to human infections. Isolates belonging to the *Brevibacterium* genus have been also implicated in corneal ulcers (Ghosheh et al. 2007) but also bacteremia (Ulrich et al. 2006). Implications of *Brevibacterium* in pericardial infections (Cannon et al. 2005), endocarditis (Dass et al. 2002), peritonitis (Antonoiu et al. 1997; Wauters et al. 2000), osteomyelitis (Neumeister et al. 1993), and even brain abscess (Kumar et al. 2011) were reported. Recently, *B. massiliense* has been isolated from a human ankle discharge. However, for long *Brevibacterium* infections were almost exclusively described in immunocompromised patients. Only three patients with *Brevibacterium* bacteremia were not immunocompromised in a classical sense, but still suffered from severe diseases. One can thus consider that given members of the *Brevibacteriaceae* constitute opportunistic pathogens rather than pathogens of clinical relevance.

General Metabolism

General metabolic characteristics of the genus are detailed in *Table 7.3*. Historically, the main metabolic studies in *Brevibacterium* sp. were related to cheese production. *Brevibacterium* strains are indeed known to produce volatile sulfur compounds that are key aromas of cheese flavor (Dias and Weimer 1998a, b; Forquin et al. 2011; Sourabié et al. 2012, 2011; Weimer et al. 1999). Industrial *Brevibacterium* species, mainly related to *B. linens* and *B. aurantiacum* but also *B. antiquum* (Forquin et al. 2009), convert methionine into methanethiol by means of methionine γ-lyase (Dias and Weimer 1998a; Forquin et al. 2011), which then is converted to dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and *S*-methylthioesters (Sourabié et al. 2012). The expression of genes encoding methionine γ-lyase and a methionine low-affinity transporter is induced in the presence of methionine, in combination with a significant increase in production of volatile sulfur compounds (Forquin et al. 2011).

The *Brevibacteriaceae* can produce different types of proteinases or peptidases which are extracellular, intracellular, or cell wall associated (see Rattray and Fox 1999 for a review). Various studies have shown the ability of proteinases from *Brevibacteriaceae* to hydrolyze milk caseins (Coskun and Sienkiewicz 1999; Frings et al. 1993; Rattray et al. 1995, 1996) (*Table 7.3*). Extracellular proteinolytic activity has been detected in 15 strains of *Brevibacterium* (Foissy 1974), and six extracellular proteases have been purified and characterized, showing high variability in optimal temperatures and pH (Rattray and Fox 1999). *The Brevibacteriaceae* additionally produce several intracellular proteinases (Fernández et al. 2000; Rattray and Fox 1997b) and cell-wall-associated proteinases, whose activities are relatively low when compared to those of the extracellular enzymes (Rattray and Fox 1999). Interestingly, the genome analysis of *B. aurantiacum* ATCC 9147 shows the presence of 17 aminopeptidases, 10 carboxypeptidases, 7 endopeptidases, and 4 serine proteases. (Forquin et al. Unpublished Data) suggesting the major role of amino acids in *Brevibacterium* life environment.

Brevibacterium also produces lipases. The lipolytic activity has been demonstrated to be mainly cell associated, with a maximal activity at 37 °C and alkaline pH (Adamitsch and Hampel 2000; Adamitsch et al. 2003). However, lipase activity appears to be distributed heterogeneously in the genus and even within the species. Early studies performed on *B. linens* showed, indeed, an extracellular lipase activity in selected isolates, while others appeared to be restricted to the intracellular compartment.

Table 7.3

Phenotypic characteristics of the 28 species of *Brevibacterium*. Species*: 1 B. album*, 2 *B. ammoniilyticum*, *3 B. antiquum*, 4 *B. aurantiacum*, 5 *B. avium*, 6 *B. casei*, 7 *B. celere*, 8 *B. daeguense*, 9 *B. epidermidis*, 10 *B. iodinum*, 11 *B.* linens, 12 *B. lutescens*, 13 *B. marinum*, 14 *B. massiliense,; 15 B. mcbrellneri*, 16 *B. oceani*, *17 B. otitidis*, 18 *B. paucivorans*, *19 B. permense*, *20 B. picturae*, 21 *B. pityocampae*, *22 B. ravenspurgense*, *23 B. salitolerans*, *24 B. samyangense*, *25 B. sandarakinum*, *26 B. sanguinis*, *27 B. siliguriense*, *and 28 B. yomogidense* (Data from the original descriptions)

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Enzymes activities																												
Acid phosphatase	nd	+	–	+	+	+	+	nd	–	–	–	–	–	–	nd	–	nd	–	w	+	nd	–	nd	–	nd	+	nd	+
Alkaline phosphatase	+	+	–	+	+	+	+	nd	+	+	–	–	+	–	nd	–	nd	–	+	+	nd	w	+	+	nd	+	nd	+
Cystine arylamidase	+	+	–	+	+	–	w	nd	w	w	–	nd	–	–	nd	w	nd	–	+	+	nd	nd	nd	w	nd	w	nd	nd
α-Galactosidase	–	+	–	–	–	–	–	nd	–	–	–	nd	–	–	nd	–	nd	–	–	–	nd	–	nd	–	nd	–	nd	–
β-Galactosidase	–	+	–	+	–	–	–	–	–	–	–	nd	–	–	nd	–	nd	–	–	–	–	–	–	+	nd	–	+	–
α-Glucosidase	–	+	–	–	–	+	+	–	–	–	–	nd	–	–	nd	–	–	–	–	+	nd	–	–	+	nd	+	–	–
β-Glucosidase	–	–	–	–	–	–	–	nd	–	–	–	nd	+	–	nd	–	nd	–	–	–	nd	–	+	–	nd	–	nd	–
α-Glucuronidase	–	–	–	+	+	–	–	nd	–	–	–	nd	–	–	nd	–	nd	–	–	–	nd	–	nd	–	nd	–	nd	–
Leucine arylamidase	+	–	–	–	+	+	+	nd	–	+	–	+	–	+	nd	+	nd	+	+	+	nd	+	nd	+	nd	+	+	+
α-Mannosidase	nd	w	–	–	–	–		nd	–	–	–	nd	–	–	nd	–	nd	–	–	–	nd		nd	–	nd	–	–	–
Protease (gelatin hydrolysis)	+	+	+	+	+	+	+	–	+	+	+	+	+	–	nd	+	nd	w	+	+	–	–	nd	–	nd	–	+	+
Protease (casein hydrolysis)	nd	nd	nd	+	nd	nd	nd	nd		nd	+	+	nd	nd	nd		nd	–	nd	nd	nd	nd	+	nd	nd	+	nd	+
Pyrazinamidase	+	–	nd	–	+	–	–	nd	+	+	+	–	+	w	nd	+	nd	–	+	–	nd	+	+	+	nd	–	+	+
Pyrrolidonyl arylamidase	+	+	nd	–	+	+	+	nd	–	–	–	nd	+	–	nd	–	nd	–	–	+	nd	–	+	+	nd	+	–	+
Urease	–	–	+	+	+	–	+	–	–	–	–	–	–	–	nd	–	nd	–	w	–	nd	–	–	–	nd	+	nd	–
Valine arylamidase	+	+	nd	–	–	–	–	nd	–	–	–		–	nd	nd	–	nd	–	–	–	nd	–	nd	w	nd	–	+	+
Esterase lipase C8	+	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	+	w	w	nd		nd	w	nd	nd	nd	+	nd	+	nd	+	nd	+
Utilization																												
Glucose	–	+	nd	+	–	+	–	–	–	–	–	–	–	nd	nd	–	nd	–	–	–	–	nd	–	w	nd	–	nd	–
D-Lactose	–	–	nd	+	–	–	–	nd	–	–	–	–	–	nd	nd	–	nd	–	–	–	nd	nd	–	–	nd	–	–	–
Maltose	–	+	nd	–	–	–	–	nd	–	–	–	–	–	nd	nd	–	nd	–	–	–	nd	nd	–	w	nd	+	nd	–
Ribose	+	+	nd	–	–	–	–	nd	–	–	w	–	–	nd	–	–	nd	–	–	–	nd	nd	+	–	nd	–	–	–
Saccharose	–	–	nd	+	–	–	–	nd	–	–	–	–	–	nd	nd	–	nd	–	–	–	–	nd	–	w	nd	–	–	–
Xylose	–	–	nd	–	–	+	–	nd	–	–	–	–	–	nd	nd	–	nd	–	–	–	nd	nd	–	–	nd	–	–	–
Mannitol	–	+	–	–	–	–	–	nd	–	–	–	–	–	nd	nd	–	nd	+	–	–	–	nd	–	–	nd	–	+	nd
Glycogen	–	+	nd	–	–	–	–	nd	–	–	–	–	–	nd	nd	–	nd	nd	–	–	nd	nd	–	–	nd	–	nd	–

Assimilation																												
4-Hydroxybenzoate	nd	w	nd	–	+	–	+	–	+	+	w	nd	–	nd	nd	+	nd	nd	–	+	–	nd	nd	nd	nd	+	nd	nd
D,L-3-Hydroxybutyrate	nd	+	nd	+	–	+	+	+	+	–	w	nd	+	nd	nd	+	nd	nd	–	+	+	nd	nd	nd	+	+	nd	nd
α-Ketogluconate	–	–	nd	–	–	w	–	–	–	–	+	nd	–	nd	nd	–	nd	nd	nd	–	–	nd	nd	nd	nd	+	nd	nd
Acetate	–	+	nd	+	–	+	+	–	+	+	–	nd	+	nd	nd	+	nd	nd	–	+	+	nd	nd	+	w	+	nd	+
Citrate	nd	+	nd	+	w	+	+	–	+	+	–	nd	+	nd	nd	+	nd	nd	–	+	–	nd	+	nd	+	+	+	+
Itaconate	nd	–	nd	–	–	–	–	–	–	–	–	nd	–	nd	nd	+	nd	nd	–	–	–	nd	nd	nd	nd	–	nd	nd
D,L-Lactate	–	+	nd	+	+	+	+	w	+	–	–	nd	+	nd	nd	+	nd	nd	–	+	+	nd	+	+	nd	+	nd	+
Malonate	+	+	nd	–	–	+	w		+	–	–	nd	+	nd	nd	+	nd	nd	+	–	+	nd	nd		nd	+	nd	nd
Propionate	–		nd	–	–	+	+	–	+	+	–	nd	+	nd	nd	–	nd	nd	–	+	–	nd	+	+	+	+	nd	+
Suberate	nd	+	nd	–	–	+	w	+	+	+	–	nd	+	nd	nd	+	nd	nd	–	–	+	nd		nd	nd	+	nd	nd
n-Valerate	nd	+	nd	–	–	+	+	+	+	+	–	nd	+	nd	nd	+	nd	nd	–	+	nd	nd	nd	nd	nd	+	nd	nd
L-Fucose	+	+	nd	+	–	+	+	–	+	–	–	–	+	–	nd	–	nd	–	–	+	+	–	+	+	+	+	nd	nd
D-Glucose	–	+	nd	+	+	–	+	–	+	+	–	–	w	nd	nd	w	nd	–	–	+	+	–	–	nd	nd	+	nd	+
D-Maltose	–	w	nd	w	–	–	+	–	–	–	–	–	–	nd	nd	–	nd	–	–	+	–	nd	–	nd	nd	+	nd	nd
L-Rhamnose	nd	+	–	–	–	+	w	–	w	w	–	–	w	nd	nd	+	nd	–	–	–	–	nd	–	+	–	w	nd	–
D-Ribose	+	+	nd	–	–	–	–	–	–	+	–	–	–	w	nd	–	nd	–	–	–	–	–	–	+	+	–	nd	+
D-Sucrose	–	–	nd		–	–	+	–	–	+	–	–	–	nd	nd	–	nd	–	–	+	w	–	–	+	–	+	nd	–
myo-Inositol	+	–	–	–	–	+	+	nd	+	+	–	nd	–	–	nd	–	nd	nd	–	+	nd	nd	–	+	–	+	nd	–
D-Mannitol	+	–	–	+	+	–	–	–	+	w	+	nd	w	nd	nd	–	nd	nd	–	–	nd	–	–	+	nd	–	nd	–
L-Alanine	–	+	nd	–	–	w	+	–	+	+	–	nd	+	–	nd	+	nd	nd	–	+	+	nd	+	+	–	+	nd	nd
L-Histidine	nd	w	nd	–	–	–	+	–	+	+	–	nd	+	nd	nd	+	nd	nd	–	+	+	nd	nd	nd	+	+	nd	nd
L-Proline	nd	+	nd	–	+	+	+	–	+	+	–	nd	+	nd	nd	–	nd	nd	–	+	+	nd	nd	nd	nd	+	nd	nd
L-Serine	–	+	nd	+	+	w	+	–	+	+	–	nd	+	–	nd	w	nd	nd	–	+	–	nd	+	+	nd	+	nd	nd
N-Acetyl-D-glucosamine	+	+	nd	–	+	+	+	w	+	+	–	nd	–	–	nd	–	nd	nd	–	+	+	–	–	nd	–	+	nd	nd
Salicin	+	–	+	–	–	+	–	nd	–	–	–	nd	–	–	nd	–	nd	nd	–	–	+	nd	–	+	–	–	–	–
Glycogen	–	+	nd	+	+	w	–	w	–	–	w	nd	–	–	nd	–	nd	nd	–	–	–	nd	+	nd	nd	w	nd	nd
Nitrate reduction	+	+	–	+	+	–	–	+	+	+	+	–	–	–	nd	–	–	–	–	–	+	–	+	–	nd	–	+	+
Methanthiol production	nd	+	+	+	nd	+	nd	nd	+	nd	+	+	+	nd	+	nd	nd	+	–	+		nd	nd		nd	+	nd	nd

+ positive, *nd* no data, *w* weakly positive, *v* variable

Esterase activity is also found in *Brevibacteriaceae*. Intracellular esterase activity was reported early using α-naphthyl acetate, β − naphthyl butyrate, or tributyrin, *o*-nitrophenyl, and *p*-nitrophenyl derivatives of acetic and butyric acids as substrates (Foissy 1974). One esterase was purified and characterized. Its pH and temperature optima were 7.5 and 35 °C, respectively (Rattray and Fox 1997a).

Catabolism of aromatic compounds is another typical metabolic characteristic of the given members of the genus playing a major role in the formation of flavors and their precursors, especially through the metabolism of aromatic amino acids that were suspected to be actively transported through the membrane by three high affinity permeases in *B. linens*. Phenol and indole are indeed both found at elevated concentration in red smear cheeses. Transamination was then early suspected to constitute the first step in the utilization of aromatic amino acids that could be used as sole nitrogen sources in *B. linens* (Rattray and Fox 1999) and aromatic amino acid aminotransferase activity was detected. Aromatic ring cleavage is then carried out by dioxygenases, and the involvement of a 3,4-dihydroxyphenylacetate 2,3-dioxygenase was early proposed although catechol 2,3-dioxygenases are principal enzymes involved in the breakdown of non-substituted aromatics (Topp et al. 1997). These early data have thus to be confirmed by modern genomic approaches.

Some *Brevibacteriaceae* are also characterized by the fact that they produce a typical orange to red pigmentation. Pigments involved in the orange color were identified as aromatic carotenoids: the isorenieratene, 3-hydroxy-isorenieratene, and, finally, 3,3′-dihydroxyisorenieratene. The genes encoding the synthesis pathway of these pigments were identified in *B. aurantiacum* DSM20426 and are part of the *crt* cluster (Krubasik and Sandmann 2000). The formation of these compounds from isopentenyl pyrophosphate (IPP) occurs by the successive action of IPP isomerase, Idi; a geranylgeranyl pyrophosphate (GGPP) synthase, CrtE; a phytoene synthase, CrtB; β-carotene desaturase, CrtU; and finally a cytochrome P450 (Dufossé and de Echanove 2005; Krubasik and Sandmann 2000).

Brevibacterium has been reported to produce a variety of bacteriocins and antimicrobial substances. Three of the compounds have been characterized in detail. Some strains of *B. linens* produce a bacteriocin, Linocin M18, active against species of the actinobacterial genera *Arthrobacter*, *Corynebacterium*, and *Micrococcus* as well as to those of Firmicutes such as *Bacillus* and *Listeria* (Valdes-Stauber and Scherer 1994). The Linencin A is only active against some other *B. linens* strains but not against other species of the genera *Brevibacterium*, *Corynebacterium*, and *Micrococcus* (Kato et al. 1991). Finally, *B. linens* strain OC2 produced a bacteriocin designated Linenscin OC2, which is different from the two other bacteriocins Linecin A and Linocin M18 (Maisnier-Patin and Richard 1995). Linenscin OC2 inhibits growth of gram-negative bacteria by altering outer membrane permeability and gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) (Boucabeille et al. 1998). More genome sequencing projects to be carried out in the future are expected to further enlighten typical and strain-specific metabolic characteristics of strains from the genus *Brevibacterium*.

Applications

Brevibacterium has for long been recognized for its major industrial potential in dairy production, particularly in the maturation of surface-ripened cheeses, including Munster, Cheddar, and Camembert. *Brevibacteriaceae* produce multiple flavor compounds that include compounds derived from lipolysis, fatty acids, and volatile sulfur compounds (Dias and Weimer 1998a, b; Ganesan et al. 2004; Ummadi and Weimer 1996, 2001; Weimer et al. 1999). In *Brevibacteriaceae*, the proteolytic and lipolytic abilities are also used to accelerate cheese ripening (Weimer et al. 1997). *Brevibacterium* species are also thought to produce specific antibacterial molecules to control growth of food pathogens (Boucabeille et al. 1998; Maisnier-Patin et al. 1992; Motta and Brandelli 2002), as well as production of orange pigmentation (Guyomarc'h et al. 2000; Krubasik and Sandmann 2000). All these characteristics contribute to give *Brevibacterium* a major role in the elaboration of the organoleptic properties of cheese. These properties brought industrials and scientists to screen for *Brevibacteriaceae* strains, investigate their sulfur metabolism, protease production, pathways involved in pigment production, and finally, their genomes. Bacteriocin production but also, potentially, antibiotic production constitute also alternatives, especially, in the latter case if given *Brevibacteriaceae* appear as potential carriers of large, linear, (Makarova et al. 2006) Streptomycete-like plasmids (Dib et al. 2010). More recently, this somehow restricted range of applications has extended. Some indigenous *Brevibacterium* strains of heavy metal-contaminated Kuwaiti soils showed, along with indigenous *Agrobacterium* and *Corynebacterium* strains, a high tolerance to heavy metal pollution and hydrocarbon pollutions (Ali et al. 2012). Even further, *Brevibacterium linens* strains were shown able to grow using *n*-alkanes as carbon substrates. An obvious application for these metabolisms is to be used to clean up crude oil contamination. Whether the presence of some contaminants like sodium arsenate or cadmium sulfate facilitates this metabolic pathway is yet unclear but the direct crude oil consumption rate seemed promising. Such data underline the potential of given indigenous *Brevibacteriaceae* to constitute an interesting source of organisms for bioremediation of mixed organic–inorganic pollutions. This also includes *B. casei* that has been used to produce gold and silver nanoparticles (Kalishwaralal et al. 2010) and to detoxify hexavalent chromium (Ng et al. 2010; Verma and Singh 2013) as well as pentachlorophenol (Verma and Singh 2013). Bioremediation may thus very soon emerge as a novel application for a range of environmental *Brevibacteriaceae* that have been generally neglected up to now.

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8 The Order *Catenulisporales*

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Abstract
The suborder *Catenulisporineae* (Cavaletti et al. 2006) has recently been elevated to the order level (Ludwig et al. 2012) in the course of an adjustment of higher ranks within *Actinobacteria* (Stackebrandt et al. 1997). This order forms an independent lineage within class I *Actinobacteria*, phylum Actinobacteria. The order comprises the two families *Catenulisporaceae* (Busti et al. 2006a) and *Actinospicaceae* (Cavaletti et al. 2006) and two genera, *Catenulispora* and *Actinospica* which harbor five and two species, respectively. The two families share moderate 16S rRNA gene sequence relatedness of 93.1 % similarity among each other and less than 92.5 % similarity with any other, mainly mycelium-forming members of class I. Strains of both genera, including the type strains of *C. acidiphila, A. robiniae and A. acidiphila*, possess genes coding for non-ribosomal peptide synthases and polyketidsynthases I and II, indicative of antimicrobial activity. Isolated mainly from forest soil, paddy fields and rhizophere and at least *Catenulispora* strains appear globally distributed.

Taxonomy, Historical and Current

Short Description of the Families

Despription of the Order *Catenulispora*les

The order contains the families *Catenulisporaceae* and *Actinospicaceae*. The pattern of 16S rRNA gene signatures consists of nt 127: 234 (G–C), 138: 225 (U–A), 139: 224 (C–G), 140: 223 (C–G), 141: 222 (A–U), 157: 164 (G–C), 449 (C), 589: 650 (C–G), 602: 636 (R–U), 603: 635 (A–U), 694 (G) and 1251 (G) (Busti et al. 2006a). The type genus of the order is *Catenulispora*.

Description of the Family *Catenulisporaceae*

Ca.te.nu.li.spo.ra'ce.ae. N.L. fem. n. *Catenulispora* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Catenulisporaceae* the *Catenulispora* family. The pattern of 16S rRNA gene signatures consists of nt 127: 234 (G–C), 129: 232 (U–A), 449 (C), 580: 761 (U–A), 586: 755 (U–A), 591: 648 (C–G), 824: 876 (A–U), 825: 875 (A–U), 834: 852 (G–U), 838: 848 (U–G), 952: 1229 (U–A), 999: 1041 (U–U) and 1000: 1040 (U–U). The type genus is *Catenulispora* (Busti et al. 2006a).

Description of the Family *Actinospicaceae*

(Ac.ti.no.spi.ca'ce.ae. N.L. fem. n. *Actinospica* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Actinospicaceae* the *Actinospica* family).

The pattern of 16S rRNA gene signatures consists of nt 127: 234 (G–C), 129: 232 (C–G), 344 (G), 449 (C), 450: 483 (C–G), 560 (U), 576 (G), 590: 649 (C–G), 591: 648 (U–R), 859 (G), 952: 1229 (C–G), 1122: 1151 (G–C), 1123: 1150 (U–G), 1124: 1149 (A–U). An insert of seven to nine extra nucleotides is present between positions 1134 and 1140. The type genus is *Actinospica* (Cavaletti et al. 2006).

Despite their remote relatedness members of the two families share several common morphological and chemotaxonomic features. With respect to fatty acid, polar lipid and menaquinone composition members of the two genera are similar (*Table 8.1*), though differences exist in the quantitative

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Table 8.1

Cultural and chemotaxonomic properties differentiating type strains of *Catenulispora* (Data from the original species descriptions)

Characteristics	*C. acidiphila* ID139908^T	*C. rubra* Aac-30^T	*C. subtropica* TT 99-48^T	*C. yoronensis* TT NO2-20^T	*C. graminis* BR-34^T
Aerial mycelium	Straight to slightly flexuous	Hook-like or lexuous	nr	nr	nr
Surface of cylindrical arthrospores	Rugose	Smooth	nr	nr	nr
Reverse color on ISP 2	Dark brown	Red	Greyish reddish-orange[a]	Yellowish brown[a]	Ivory
Temperature range °C (optimum)	11–37 (22–28)	(20–30)	10–37 (25–30)	10–37 (25–30)	nr
pH range (optimum)	4.3–6.8 (6.0)	4.0–60 (5.0)	5–8 (6–7)	5–7 (6–7)	nr
Diagnostic amino acids in peptidoglycan (type A3γ)	LL-Dpm-Gly	LL-Dpm, Gly	LL-Dpm, Gly	LL-Dpm, Gly	LL-Dpm, Gly
Whole-cell sugar pattern	Ara, Xyl, Rib, Rha, Glu	Rib,Man, Ara, Glu	Man, Ara,Gal	Man, Ara,Gal	Ara, Xyl
Menaquinone	MK-9(H_6)	MK-9(H_6)	MK-9(H_8)	MK-9(H_8)	MK-9(H_6)
	MK-9(H_4)	MK-9(H_8)	MK-9(H_6)	MK-9(H_6)	MK-9(H_8)
	MK-9(H_8)[b]	MK-9(H_4)[b]	MK-9(H_4)[b]	MK-9(H_4)[b]	MK-9(H_4)[b]
			MK9(H_{10})[b]	MK9(H_10)[b]	
Polar lipids	PG, DPG, PI, PIM, 2 unknown PL	PG, PI	DPG	DPG	PIM,PG,PS, 3 unknown PL
Major fatty acids for all	i-$C_{16:0}$, ai-$C_{17:0}$	i-$C_{16:0}$, ai-$C_{17:0}$	i-$C_{16:0}$, ai-$C_{17:0}$	i-$C_{16:0}$, ai-$C_{17:0}$	i-$C_{16:0}$
Mol% G+C of DNA	71.5	69.1	70–71	69.0	72.8

Abbreviations: Whole cell sugars: *Ara* arabinose, *Xyl* xylose, *Rib* ribose, *Rha* rhamnose, *Glu* glucose, *Man* mannose, *Gal* galactose
Polar lipids: *PG* phosphatidylglycerol, *DGP* diphosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PS* phosphoserine
nr not reported
[a]Color of colonies
[b]Minor compounds

composition, even among species of the same genus. The fatty acid composition given for the various *Catenulispora* species description is somewhat confusing and may be due to differences in growth conditions and methods used for generating their profiles and interpretation. Most authors find iso-C16:0 and anteiso-C17:0 fatty acids (Busti et al. 2006a; Tamura et al. 2007, 2008), though Lee et al. (2011) in their description for *C. graminis* could not verify the high amounts of antesio-C17:0. Morphologically strains are defined by a non-fragmentary vegetative mycelium and the formation of aerial mycelium. Members of both genera form straight to slightly flexuous hyphae which are arranged in tufts in members of *Actinospica*. In aged aerial mycelium hyphae septate into chains of cylindrical arthrospores. Of the properties traditionally used to separate actinobacterial genera it is mainly the composition of the amino acid composition of the peptidoglycan in which members of the two genera differ (*Tables 8.1* and *8.3*). *Actinospica* strains possess hydroxyl-diaminopimelic acid (Dpm) and traces of meso-Dpm (Dpm) (peptidoglycan type A1γ, see www.peptidoglycan-types.info). As in members of *Actinoplanes* and *Micromonospora* (Schleifer and Kandler 1972), glycine has replaced L-alanin bound to muramic acid. Strains of *Catenulispora*, on the other hand, possess meso-Dpm and glycine forms the interpeptide bridge; this type (A3γ) is found for example in members of *Streptomycetaceae, Nocardioides, Luteococcus, Marmoricola and in Propionibacterium.*

Phylogenetic Structure of the Family and Its Genera

According to the original descriptions of the genera, the two families are moderately related (93.1 % 16S rRNA gene sequence similarity) and share less than 92.5 % similarity with *Sporychthya polymorpha, Cryptosporangium arvum* (Busti et al. 2006a; Cavaletti et al. 2006). These two genera are not included in the phylogenetic dendrogram of Lee et al. (2011), though *Micromonospora, Nakamurella* and other genera defined by mycelium forming organisms are also found as nearest neighbors.

The type strains of two species of *Actinospica*, *A. robiniae* and *A. acidophila* are closely related, sharing 97.5 % sequence similarity. Of the five species of *Catenulispora C. rubra* Aac-30^T and *C. acidophila* ID139908^T are highly related (99.4 %). The similarities between *C. subtropica* 99-48^T, *C. yoronensis* TT N02-20^T,

Table 8.2

Metabolic properties differentiating type strains of *Catenulispora* (Data from the original species descriptions)

	C. acidiphila ID139908^T	*C. rubra* Aac-30^T	*C. subtropica* TT 99-48^T	*C. yoronensis* TT NO2-20^T 6	*C. graminis* BR-34^T
Characteristic					
Nitrate reduction	–	–	+	–	nd
β-glucuronidase	–	+	–	–	–
β-galactosidase	+	w	+	–	+
α-fucosidase	–	–	+	+	–
Trypsin	–	+	+	–	–
Catalase	+	–	+	+	–
Gelatin hydrolysis	+	–	+	–	+
Starch hydrolysis					+
Esculin hydrolysis	+	+	+	+	–
Utilization of					
D-fructose	+	+	–	–	+
D-galactose	+	–	+	–	+
D-mannitol	–	+	–	–	+
Gluconate	+	–	+	+	–
Glycerol	–	+	+	–	–
L-arabinose	–	+	+	–	–
Glycogen	–	–	–	–	+
Methyl-α-D-glucopyranoside	–	+	–	–	–
Methyl-β-D-xylopyranoside	–	–	w	–	–
N-acetylglucosamin	w	+	+	–	–
Salicin	+	w	+	–	–
Sucrose	–	+	–	w	–
D-ribose	–	–	–	–	+
D-xylose	+	+	+	+	–

Abbreviations: +positive, –negative, *w* weak, *nd* not determined

and *C. acidiphila* ID139908^T and *C. rubra* Aac-30^T ranged between 97.4 and 98.7 % (Tamura et al. 2008). *C. graminis* BR-34^T (its sequence has not yet been included in the LTP database) appears as most unrelated species of the genus, sharing less than 97.4 % with any other type strain (*Fig. 8.1*).

Molecular Analyses

DNA reassociation studies using the fluorimetric method of Ezaki et al. (1989) have been performed on a few *Catenulispora* strains to confirm either the strain affiliation to the same species (Tamura et al. 2008) or to verify the species status. In any pair wise comparison similarity values were lower than 50 % (Tamura et al. 2008; Lee et al. 2011).

The level of phylogenetic distinctness of 29 *Catenulispora* and 34 *Actinospica* isolates was assessed by capillary electrophoresis of PCR amplified ITS spacer regions (Busti et al. 2006b). Twelve strains with unique patterns were identified which were further analyzed by 16S rRNA gene sequence analysis and screened for the presence of non-ribosomal peptide synthases and polyketidsynthases (PKS) I and II, Primers used for the amplification of the latter genes and modifications of the PCR protocol of Courtois et al. (2003) as well as phylogenetic analysis of translated gene fragment sequences are indicated by Busti et al. (2006b).

The genome sequences of *Catenulispora acidophila* DSM 44928^T has been generated in the frame of the GEBA (*Genomic **E**ncyclopedia of **B**acteria and **A**rchaea*) Project (Copeland et al. 2009). The genome is 10.6 Mb and comprises one circular chromosome. The DNA G+C content is 69.8 mol% GC content, hence 1.7 mol% lower than reported in the original description of the species. 9,056 genes coding for proteins were identified, 66 genes code for RNAs, and 142 pseudogenes were found. Of the identified genes 68.2 % and 31.8 % of the identified genes were assigned with a putative function and annotated as hypothetical proteins, respectively. Of the 5,707 genes associated with the general COG functional categories, 265 genes are associated to

Table 8.3

Physiological and chemotaxonomic properties characterising type strains of *Actinospica* (Cavaletti et al. 2006). Both strains are Gram-positive, aerobic and catalase positive. Nitrate reduction, tyrosin reaction and gelatin liquefication negative. Casein hydrolysis weak. H_2S is produced. Do not tolerate 100 μg lysozyme ml^{-1}

	Actinospica robiniae GE134769^T	*Actinospica acidiphila* GE134766^T
Temperature range °C (optimum)	17–33 (22–28)	17–33 (28)
pH range (optimum)	4.8–6.2 (5.5)	4.2–6.0 (5.0)
1 % NaCl (w/v) tolerated	–	+
Starch hydrolysis	–	+
Peptidoglycan diamino acids (type A1γ)	Hydroxy-Dpm, traces of meso-Dpm	Hydroxy-Dpm, traces of meso-Dpm
Whole-cell sugar pattern	Man, Rha, Gal	Man, Rha, Ara, Xyl
Major manaquinone	MK-9(H6)	MK-9(H4)
	MK-9(H8)	MK-9(H6)
	MK9(H4)[a]	MK-9(H8)
Polar lipids	PI,DPG, PE, methyl-PE	PI, DPG, PE, methyl-PE
Major fatty acids	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{15:0}$	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{15:0}$, i-C17: 1ω9c
Mol% G+C of DNA	70.8	69.2

For abbreviations see footnote of Tables 8.1 and 8.2
[a]Trace

secondary metabolites biosynthesis, transport and catabolism. Among the easily recognizable clusters within the genome are those for the biosynthesis of leinamycin-, thiopeptide- and actinorhodin-related compounds (S.D., unpublished). The latter is probably dedicated to the synthesis of the isochromanequinone GTRI-BB described by Busti et al. (2006b). The genome sequence corroborates the finding of Busti et al. (2006b) on the antibiotic producing ability of the taxon; *Streptosporangium roseum* DSM 43021^T, a known antibiotic-producing strain with a similar genome size contains 315 of such genes (Nolan et al. 2010). On the other hand, only 50 such genes have been reported to be present in the, albeit lower, 4.1 Mb genome of *Cellulomonas flavigena* DSM 20109^T, a rod-shaped actinobacterium for which no such potential has been described (Abt et al. 2010). The genome size has been determined for two additional strains of *Catenulispora* and one *Actinospica* strain, using pulsed-field electrophoresis of *Ase*I, and *Dra*I restriction fragments. The genome size estimated from the fragment sizes obtained from the two enzymes ranged between 8.3–8.6 and 8.3–9.9 Mb for *Catenulispora* isolates (Neo1 and Neo2, respectively) and 8.3–8.8 for *Actinospica* strain Gamma 3 (Busti et al. 2006b).

Phenotypic Analyses

The main phenotypic properties distinguishing *Catenulispora* and *Actinospica* as well as the type strains of the five *Catenulispora* species are indicated in Tables 8.1 and 8.3, respectively. Cultural characteristics for all members of the families were tested on ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7 agar plates (Shirling and Gottlieb 1966), acidified to pH 4.5–5.5. Type strains of both genera differ from each other in the combination of characteristics determined for each medium. Detailed information is available in the original descriptions. *Actinospica* type strains did not exhibit enhanced growth in the presence of carbon sources added to CMM supplemented (Busti et al. 2006a) ISP9 medium and ISP4 medium (omitting starch and pH adjusted to 5.0–5.5); carbon utilization can therefore not be determined for these type strains, nor compared to the reactions available for *Catenulispora* type strains.

Catenulispora Busti et al. 2006, 1745^{VL}, emend Tamura et al. 2008

Ca.te.nu.li.spo'ra. L. fem. n. catenula small chain; Gr. fem. n. spora seed; N.L. fem. n. *Catenulispora* a thin chain of spores).

The emendation of the genus was based on four species and not all of the morphological properties have been determined for all strains. These are Gram-positive, non-acid-fast, and aerobic. The vegetative mycelium does not fragment and the branching aerial hyphae start to septate in chains of cylindrical arthrospores during aging. Motile elements are not produced. Most strains are acidophilic but one type strain tolerates growth at pH 8.0. The peptidoglycan contains LL-A_2pm with glycine as the interpeptide bridge. Iso-$C_{16:0}$ and anteiso-$C_{17:0}$ are present as major cellular fatty acids in most strains. Whole cell sugar composition and polar lipid composition are variable. Menaquinones MK-9(H6) and MK-9(H8) are predominant in most strains. The G+C content of the DNA is 69–72 mol%. The type species is *Catenulispora* acidiphila.

Differentiating metabolic reactions are indicated in Table 8.2. In contrast to *Actinospica* strains most *Catenulispora* strains are metabolically active as they are able to utilize a wide range of carbohydrates and are enzymatically active. All strains hydrolyse starch and do not utilize methyl β-xylopyranoside (Lee et al. 2011). As not all strains have been tested by the same commercial kits we refrain from listing addition positive and negative reactions and refer the reader to the original species descriptions. As judged from the data indicated in Tables 8.1 and 8.2, *C. graminis* appears to be more diverse than the other four type strains, supporting the deepest branching 16S rRNA lineage within the genus.

Actinospica Cavaletti et al. 2006, 1751^{VL}

Ac.ti.no.spi'ca. Gr. n. actinos a ray; L. fem. n. spica tuft; N.L. fem. n. *Actinospica* an actinomycete with tufts of aerial hyphae.

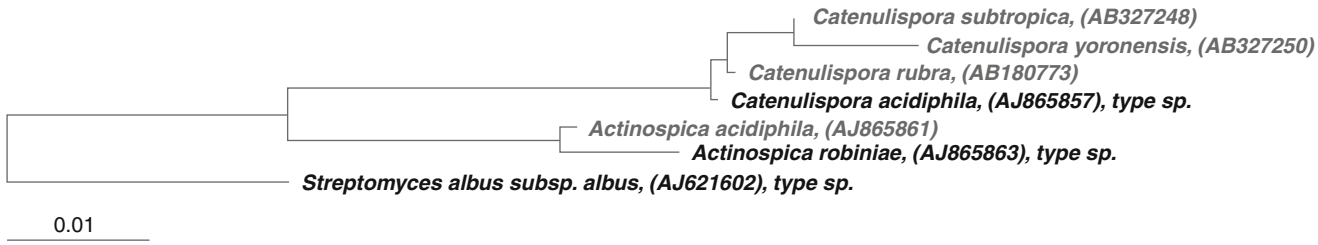

Fig. 8.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of *Catenulisporales*. present in the LTP_106 (Yarza et al. 2010). Representative sequences from close relative genera were used to stabilize the tree topology. In addition, a 40 % maximum frequency filter was applied to remove hypervariable positions from the alignment. *Scale bar* indicates estimated sequence divergence

In addition to properties indicated in *Table 8.2*, the following traits were recorded (Cavaletti et al. 2006): Gram-positive, aerobic, mesophilic, acidophilic and catalase positive. Non-fragmenting vegatative hyphae. Tufts of straight to slightly flexuous hyphae originating from very short sporophorous branching in few sporogenous hyphae. Hyphae form chains of cylindrical arthrospores in aging cultures. Motile elements are not produced. Growth is not supported at pH values higher than 6.2. Grow better on acidic yeast extract–malt extract agar (ISP2) and acidic oatmeal agar (ISP3)

The two type strains share moderate 16S rRNA gene sequence similarity but they are very similar otherwise (*Table 8.3*). As each species is defined by a single strain only even these differences may be strain-, rather than species—specific, such as differences in tolerance towards 1 % NaCl (w/v), starch hydrolysis, size of cylindrical spores and cultural appearance on different ISP media.

Isolation, Enrichment and Maintenance Procedures

The isolation procedure described for members of the two genera varied widely. While *C. graminis* BR34^{T} was isolated on acidified (pH5.5) ISP medium (Lee et al. 2011), *C. subtropica* TT99-48^{T} and *C. yoroensis* TT N02-20^{T} were isolated by using the yeast extract/SDS method (Hayakawa and Nonomura 1989) on humic acid/vitamin (HV) agar (Hayakawa and Nonomura 1987), containing per litre 20 mg nalidixic acid and 50 mg cycloheximide (Tamura et al. 2008). The cultivation medium for *C. rubra* Aac-^{30T} consisted of equal parts of a glucose/yeast extract agar medium pH.5.0 and a mineral salt broth, pH about 4.0 (Tamura et al. 2007). *C. acidiphila* ID 39908^{T} (Busti et al. 2006a), and *A. robiniae* GE134769T and *A. acidiphila* GE 134766*T* (Cavaletti et al. 2006) were isolated from a dried soil sample which was resuspended in a 18.2 mM citric acid, 164 mM Na_2HPO_4 buffer (pH7). Serial dilutions were spread onto a soil extract, gellan gum, $CaCl_2$ medium, supplemented with traces of a vitamin solution. The precise protocol is given by Busti et al. (2006a). Colonies of the latter isolates were picked after 8 weeks of growth.

Strains can be maintained on ISP 2 agar or in GYM *Streptomyces* medium (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium65.pdf). Medium-term preservation is done at −86 °C in the presence of 20 % (v/v) glycerol, while freeze-drying or storage under N_2 vapor is recommended for long term preservation (DSMZ information).

Ecology

Habitat

Acidic forest soil appears to be the main habitat though strains have been isolated from other soil samples and a rice paddy field as well. The functional role and possible interactions with other organisms is unknown. Busti et al. (2006b) noted, citing a more general observation by Sait et al. (2002), that members of the two genera have not been reported before in the decades long search for antibiotic-producing mycelium-forming actinomycetes (Williams et al. 1971; Khan and Williams 1975; Kim et al. 2003, 2004), especially as these organisms can be isolated on standard ISP 2 medium. On the other hand, within a short period of 5 years two genera with seven species has been added to the actinobacteria proper. In addition to the described type strains several other undescribed isolates cluster with either *Catenulispora* or *Actinospica* species. Within *Catenulispora* there are the six unnamed Neo isolates from acidic soils from Italy and Nicaragua which were included in their study on antibiotic-producing ability (Busti et al. 2006b). Of these, Neo 3 became later the type strain of *C. acidiphila*. Similarly highly related (>99 %) are the so called "Ellin" strains from soil (for Dairy Research Institute, Ellinbank, Victoria, Australia (38°14.55′S, 145°56.11′E) (Joseph et al. 2003) and other unidentified isolates, e.g., strain 12202 [AY639903]; and IMER-B1-10 [FJ796419] from a nonspecified habitats as well as several strains from Cliff soil: CR3-AC11 [FM998837], CR3-AC10 [FM998836], or CR3-AC7T [FM998835], tentatively named "*Catenulispora cavernae*." Using a 16S rRNA gene based PCR protocol for the specific detection of members of *Catenulispora*, Busti et al. (2006b) were able to detect signals in 33 % of those 100 different soil samples from Europe, Africa and America which had a pH of 5.5 or lower.

Clustering adjacent to the two type strains of *Actinospica* are more than 20 isolates from acidic soils (Tamura and Sakane

unpublished) as well as the so called Gamma strains included in the study by Busti et al. (2006b). The phylogenetic position of some of these isolates from both genera is shown by Cavaletti et al. (2006). It is noteworthy that there are hardly any records of environmental clone sequences falling into the phylogenetic radiation of the members of the two genera. Clone ncd2045h10c1 (JF176612) from an international skin consortium (Kong et al. unpublished) and clone mat161 (JN590556) of white lupin cluster roots (Weisskopf et al. 2011) are the only two records found after BLAST search. A 16S rRNA gene fragment sequence found in a clone library from DNA retrieved from acidic grasslands was moderately similar to the homologous gene sequence of *Actinospica acidiphila* (Jenkins et al. 2009). As a result of their study of community dynamics on plots of long-termed managed grassland undergone different fertilizing regimes, these authors also stress the prevalence of members of *Streptomyces*, *Acidimicrobium* and *Actinospica* in acidic soils thriving in.

Pathogenicity, Clinical Relevance

All members of the families are categorized as risk group 1 organisms by the DSMZ-internal risk assessment.

The antimicrobial activities of some *Catenulispora* (Neo) and *Actinospica* (Gamma) strains were evaluated by Busti et al. (2006b). Few bioactive metabolites have been reported from *Actinospica* and *Catenulispora* strains. Busti et al. (2006b) reported that strain Neo3 (the *C. acidiphila* type strain) produced a compound with anti-staphylococcal activity that showed UV–VIS maxima and a 629 $[M-H]^-$ *m/z* signal identical to those of isochromanequinone GTRI-BB (Yeo et al. 2002), although no further characterization was reported. As mentioned above, the *C. acidiphila* genome contains a gene cluster highly related to those involved into isochromanequinone biosynthesis. After prediction from genome analysis, *C. acidiphila* was demonstrated to produce the class III lantipeptide catenulipeptin, a 27-amino acid peptide containing two labionin bridges (Wang and van der Donk 2012). Catenulipeptin was apparently devoid of antimicrobial activity, but was able to stimulate aerial mycelium formation in surfactin-treated *Streptomyces coelicolor* (Wang and van der Donk 2012). Busti et al. (2006b) also reported that *Actinospica* strain Gamma22, an isolate with 16S highly related to that of *A. robiniae*, produced an unidentified compound with antimicrobial activities. Recent work has shows that this strain produces the new metabolite 6-hydroxychrolactomycin, along with minor amounts of chrolactomycin (Iorio et al. in preparation).

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9 The Family *Cellulomonadaceae*

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Abstract
Cellulomonadaceae, a family within the order *Actinomycetales*, embraces the genera *Cellulomonas, Oerskovia, Paraoerskovia* (including *Koreibacter*), *Actinotalea*, and *Tropheryma*. Irrespective of algorithms applied to the set of 16S rRNA gene sequences of type strains, *Tropheryma whipplei* and *Actinotalea fermentans* branch deeply and have *Cellulomonas bogoriensis* as their phylogenetic neighbor. Based upon the fragmentary phenotypic data on *Tropheryma whipplei* and comparative analysis of full genome sequences of some members of the suborder *Micrococcineae*, the position of the genus *Tropheryma* must be considered tentative. Members of the family are defined by a wide range of morphological and chemotaxonomic properties, such as polar lipids, fatty acids, amino acids of peptidoglycan, and whole cell sugars which are used for the delination of genera and species. Members of the family are mainly found in soil, but they have been isolated from patient material and the marine environment as well. Many species are described for their ability to decompose not only plant-derived macromolecules such as cellulose, starch, and xanthan but also chitin, DNA, and gelatine. Some strains of the genera *Cellulomonas* and *Oerskovia* are opportunistic pathogens. This contribution is a modified and updated version of previous family descriptions (Stackebrandt E, Schumann P, Prauser H (2006) The family *Cellulomonadaceae*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes, 3rd edn. Springer, New York, pp 983–1001; Stackebrandt E, Schumann P (2012) *Cellulomonadaceae*. In: Whitman WB, Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Garrity G, Ludwig W, Suzuki K-I (eds) Bergey's manual of systematic bacteriology, vol 6, 2nd edn. Springer, New York, p 699).

Taxonomy: Historical and Current

Short Description of the Family

Phylogenetically a member of the order *Micrococcales* (Busse 2012), phylum Actinobacteria. The family contains the type genus *Cellulomonas* (Bergey et al. 1923; emended by Clark 1953; Stackebrandt et al. 1982), *Oerskovia* (Prauser et al. 1970; emended by Lechevalier 1972), *Actinotalea* (Yi et al. 2007), *Paraoerskovia* (Khan et al. 2009), and *Koreibacter* (Lee and Lee 2010). *Tropheryma* (La Scola et al. 2001) is included in this family in several communications and databases (e.g., http://www.bacterio.cict.fr/), but its membership to the family is doubtful (see comment below). Gram-positive but many of the older cells stain Gram-negative. Morphological forms vary from slender irregular rods of varying length, which may show primary branching, short filaments and coccoid forms may occur in late exponential phase cultures; or vegetative mycelia with the oldest parts of the mycelium fragmenting into sections of different size and more or less irregular shape; fragmentation may continue to yield rod-like cells of coryneform appearance and arrangement. Aerial mycelium is not formed. Motile by means of one or several polar or subpolar flagella, or peritrichous flagella; some species are nonmotile. Aerobic to facultatively anaerobic, producing acid from a variety of carbohydrates. Cross-linkage of the peptidoglycan is by the A type; diagnostic diamino acids

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

	Cellulomonas[a]	*Actinotalea*[d]	*Oerskovia*[b]	*Paraoerskovia*[c,f]	*Tropheryma*[e]
Morphology	Straight, curved or coccoid rods, Filaments, fragmenting into diphtheroid or coccoid forms	Straight or curved rods	Extensively branching vegetative hyphae which break up into motile or non-motile rod-like elements	Rods	Short rods when cultivated on human embryonic lung cell monolayers. When extracellular, has a tendency to aggregate in masses of bacteria embedded in an extracellular matrix, with the appearance of long, rope-like structures
Gram-stain	Positive	Negative	Positive	Positive	Negative
Motility	+ or –	–	+ or –	–	–
Metabolism	Aerobic, anaerobic growth in stab culture possible	Aerobic and anaerobic	Aerobic to facultative anaerobic	Facultative anaerobic	ND
Peptidoglcan type	A4β	44β	A4α	A4α	ND
Diagnostic peptidoglycan amino acids	L-Orn-D-Asp, L-Orn-D-Glu	L-Orn-D-Asp	L-Lys-L-Thr-D-Glu, L-Lys-L-Thr-D-Asp	L-Lys-LSer-D-Glu	ND
Major polar lipids	Different combinations of DPG, PG, PI, PIM, PE	nd	PG, DPG, PI	PG, DGP, PI, PIM	ND
Major fatty acids	$C_{15:0\ ante}$, $C_{15:0\ iso}$, $C_{16:0}$, $C_{17:0\ ante}$, $C_{15:0}$, $C_{15:0}$	$C_{15:0\ ante}$	$C_{15:0\ ante}$	$C_{15:0\ ante}$, $C_{17:0\ ante}$, $C_{16:0}$, $C_{18:0}$	ND
Menaquinone	MK-9(H_4)	MK10(H_4)	MK-9(H_4)	MK-9(H_4)	ND
G+C content	68.5–76.0	75.8	71	71	59.5

[a]Stackebrandt and Schumann (2012)
[b]Stackebrandt et al. (2002)
[c]Khan et al. (2009)
[d]Lee and Lee (2010)
[e]La Scola et al. (2001)
[f]Schumann et al. (2013)
For Abbreviations see *Table 9.2*.

are either ornithine or lysine. Interpeptide bridges contain either aspartic acid or glutamic acid; L-Thr in combination with a dicarboxylic amino acid occurs in one genus. 12-Methyltetradecanoic (anteiso(ai)-$C_{15:0}$) is the prominent fatty acid; $C_{16:0}$, i-$C_{15:0}$, $C_{14:0}$, and ai-$C_{17:0}$ may also occur. Menaquinone MK-9(H4) is the predominant isoprenoid quinone (*Table 9.1*). The set of 16S rRNA gene sequence signature nucleotides is comprised of position 120 (A), 131:231 (A–G), 196 (C), 342–347 (C–G), 444–490 (A–U), 580–761 (C–G), 602–636 (C–G), 670–736 (A–U), 822–878 (U–C), 823–877 (A–C), 826–874 (U–G), 827 (G), 843 (C), 950–1231 (U–A), 1047–1210 (G–C), 1109 (C), 1145 (G), 1309–1328 (G–C), 1361 (G), and 1383 (C).

If analyzed, polar lipids are diphosphatidylglycerol and phosphatidylglycerol besides unidentified lipids, phospholipids, and phosphoglycolipids. Mycolic acids, diagnostic whole cell sugars, and teichoic acids are absent. G+C values of DNA range between 68 and 76 mol%. Usually found in soil, compost, and cellulose-enriched environments such as decaying plant materials, bark, wood, sugar fields, rumen, and activated sludge; occasionally isolated from various clinical specimens. Members of two genera have been isolated from marine environment.

Comment on *Tropheryma*: The members of the subcommittee on the taxonomy of the suborder *Micrococcineae* did not consider the genus *Tropheryma* La Scola et al. 2001 a member of the family *Cellulomonadaceae* because of insufficient arguments for this classification (Schumann et al. 2009). These authors preferred to list this genus under genera incertae sedis. In the NCBI taxonomic browser (http://www.ncbi.nlm.nih.gov/ Taxonomy/Browser), this genus is listed under unclassified *Micrococcineae*. In the recent description of *Cellulomonadaceae* (Stackebrandt and Schumann 2012), neither *Tropheryma* nor *Demequina* was originally considered to be members of this family; both genera were included by the editors of *Bergey's Manual of Systematic Bacteriology*, vol 2 (Whitman et al. 2012). In this communication, *Tropheryma* will be dealt with only in the context of phenotypic and genomic comparison with members of *Cellulomonadaceae* (see also below).

Phylogenetic Structure of the Family and Its Genera

According to the phylogenetic branching of actinobacterial type strains in the RaxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010), the family is moderately related to the families *Promicromonosporaceae*, *Sanguibacteraceae*, and *Rarobacteraceae*. A phylogenetically broad group containing *Micrococcaceae*, *Actinomycetaceae*, and several other less species-rich families appear as a sister clade of these four families, while a clade embracing *Microbacteriaceae* and *Bifidobacteriaceae* is branching slighly deeper.

16S rRNA cataloguing (Stackebrandt et al. 1980a; Stackebrandt and Woese 1981) indicated *Cellulomonas* species to be related to members of *Arthrobacter*, *Micrococcus*, and related taxa. This relationship was supported by 5S rRNA analysis performed on *Cellulomonas biazotea* and a variety of coryneform bacteria (Park et al. 1987) and later by almost complete sequences of 16S rRNA gene sequences, characteristic indels in protein sequences analyzed from completely sequenced genomes (Gao and Gupta 2005), and recently from the fully sequenced genome of *Cellulomonas flavigena* (Abt et al. 2010).

Based upon phylogenetic evidence, the genera *Cellulomonas* and *Oerskovia* were united into the genus *Cellulomonas* (Stackebrandt et al. 1982). When the high correlation between phylogenetic clustering and chemotaxonomic distinctness within the actinobacteria became apparent, the phenotypic properties of cellulomonads and *Oerskovia*e were reevaluted. As a consequence, the generic status of *Oerskovia* was reconfirmed, *Cellulomonas cellulans* was excluded from *Cellulomonas* and reclassified as *Cellulosimicrobium cellulans*, while *Promicromonospora enterophila* was reclassified as *Oerskovia enterophila* (Stackebrandt et al. 2002).

Cellulomonas, *Oerskovia*, *Promicromonospora*, and *Jonesia* were members in the original description of the family *Cellulomonadaceae* (Stackebrandt and Prauser 1991, 480^{VL}, effective publication Stackebrandt and Prauser 1991, 263, emend. Stackebrandt et al. 1997, 484, emend. Stackebrandt and Schumann 2000, 1284). With the availability of sequences of several newly described type strains, the phylogenetic branching pattern of the family was refined (Rainey et al. 1995). Consequently, the genera *Promicromonospora* and *Jonesia* were excluded in the description of a new hierarchic structure of the *Actinobacteria* classis nov. (Stackebrandt et al. 1997) and the families *Promicromonosporaceae* (Rainey, Ward-Rainey and Stackebrandt) and *Jonesiaceae* (Stackebrandt, Rainey and Ward-Rainey), respectively, were described for the two genera. The emended family *Cellulomonadaceae* (Stackebrandt et al. 1997), described on the basis of phylogenetic position and signature nucleotides of 16S rRNA gene sequences, was affiliated to the suborder *Micrococcineae*, order *Actinomycetales* (Stackebrandt et al. 1997). The family then contained the genera *Cellulomonas*, *Oerskovia*, and *Rarobacter* (Yamamoto et al. 1988). Shortly afterward, several new genera were described as new members of the suborder *Micrococcineae* (Groth et al. 1997a, b, 1999; Martin et al. 1997); the addition of new 16S rRNA gene sequences to the database of actinomycete species changed the phylogenetic position of a few taxa while stabilizing other taxa. New families were described, i. e., *Rarobacteraceae*, *Bogoriellaceae*, *Dermacoccaceae*, and *Sanguibacteraceae* (Stackebrandt and Schumann 2000). 16S rRNA gene sequence signatures given in these family descriptions were adjusted according to novel sequences included in recent descriptions of *Cellulomonas* (Rivas et al. 2004; An et al. 2005; Brown et al. 2005; Jones et al. 2005; Kang et al. 2007) and *Oerskovia* species (Stackebrandt et al. 2002). A full update and revision of the signature-based hierarchic structure (Stackebrandt et al. 1997) of *Actinobacteria* was published by Zhi et al (2009) who included the genera *Actinotalea* (Yi et al. 2007), *Tropheryma* (La Scola et al. 2001), and *Demequina* (Yi et al. 2007) into *Cellulomonadaceae*. The latter genus was excluded from this family as a family of its own, *Demequinaceae* (Ue et al. 2011), on the basis of chemotaxonomic differences and the pattern of 16S rRNA signatures. The highly related genera *Paraoerskovia* and *Koreibacter* were described in 2009 (Khan et al. 2009) and 2010 (Lee and Lee 2010), respecively, but only *Paraoerskovia* has been formally included into *Cellulomonadaceae*. In the phylogenetic dendrogram given in the original description of *Koreibacter* (Lee and Lee 2010), the type strain K. algae DSW-2^T branched between the genera *Sanguibacter* and *Demequina*. The type strain of *Paraoerskovia* marina CTT-37^T, published 1 year earlier, was not included in the analysis of *Koreibacter algae*, hence excluding detection of a possible high phylogenetic relatedness. Blast analysis of the 16S rRNA gene sequences for the type strains of *K. algae* (FM995611) and *P. marina* (AB445007) resulted in a similarity of 100 %. The agreement in chemotaxonomic properties (peptidoglycan type A4α L-Lys-L-Ser-L-Glu, major menaquinone MK-9(H4), major fatty acids anteiso (ai)-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:0}$, $C_{18:0}$), and the DNA-DNA similarity of 94 % led to the reclassification of the type strain DSW-2^T of *K. algae* as a strain of *P. marina* (Schumann et al. 2013).

The genus *Oerskovia* with its four species forms a monophyletic cluster. In the maximum likelihood (◉ *Fig. 9.1*) and parsimony (not shown) trees, 15 of the 18 *Cellulomonas* species form a coherent cluster, while *C. bogoriensis*, *C. hominis*, and *C. denverensis* cluster outside the *Cellulomonas*/*Oerskovia*/*Paraoerskovia* group. The species *Cellulomonas phragmiteti*, described recently (Rusznyák et al. 2011), will be added in the next update of the LTP tree. Its nearest phylogenetic neighbor is *C. flavigena*.

Molecular Analyses

DNA-DNA Hybridization Studies

Almost all descriptions of *Cellulomonas* and *Oerskovia* species include results of DNA-DNA hybridization (DDH) studies and a few species only were found to be closely related (>40 % similarity), e.g., *Cellulomonas biazotea* and *C. fimi*, and a cluster comprising *C. uda*, *C. flavigena*, *C. persica*, and

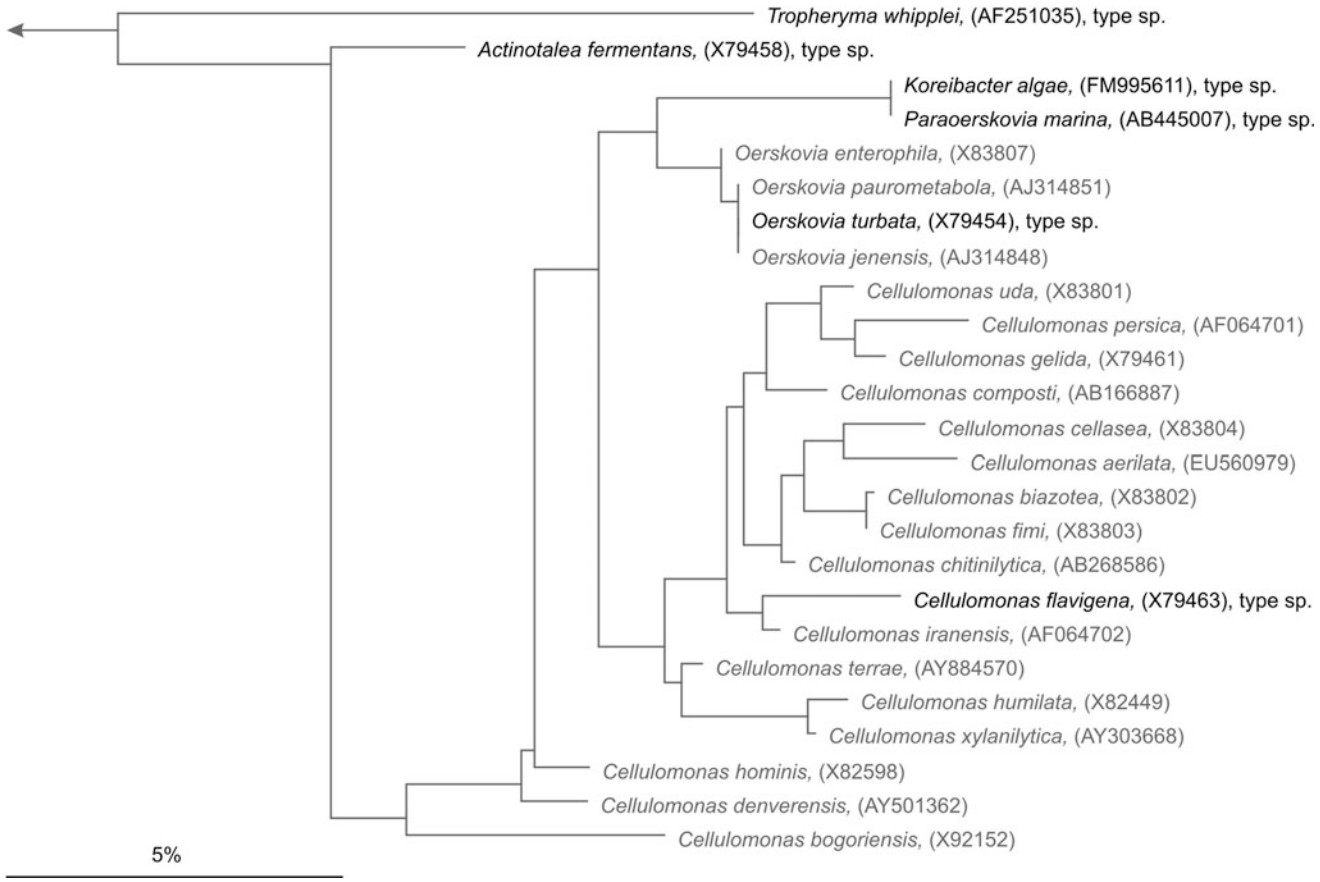

Fig. 9.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the family *Cellulomonadaceae* present in the LTP_106 (Yarza et al. 2010). The tree was reconstructed by using a subset of sequences List of type strains used for dendrogram construction: *Actinotalea fermentans* DSM 3133^T, *Cellulomonas aerilata* 5420S-23^T, *C. biazotea* DSM 20112^T, *C. bogoriensis* 69B4^T, *C. cellasea* DSM 20118^T, *C. chitinilytica* X.bu-b^T, *C.composti* TR7-06^T, *C.* denverensis W6929^T, *C. fimi* DSM 20113^T, *C. flavigena* NCIMB 8073^T, *C. gelida* NCIMB 8076^T, *C. hominis* DMMZ CE40^T, *C. humilata* NCTC 25174^T, *C. iranensis* O^T, *C. persica* I^T, *C. terrae* DB5^T, C. uda DSM 20107^T, *C. xylanilytica* XIL 11^T, *Koreibacter algae* DSW-2^T, *Oerskovia enterophila* DSM 43852^T, *Oerskovia jenensis* DSM 46000^T, *Oerskovia paurometabola* DSM 14281^T, *Oerskovia turbata* NCIMB 10587^T, *Paraoerskovia marina* CTT-37^T, *Tropheryma whipplei* Twist-Marseille^T. *Cellulomonas carbonis* T26^T, *C. soli* Kc1^T and *C. oligotrophica* Kc5^T are not included because of their recent descriptions (see genus description)

C. iranensis (Stackebrandt and Kandler 1979; Elberson et al. 2000). Others were more distantly related such as those reported for the type strains of *C. xyl*anilytica and *C. humilata* (37 %; An et al. 2005), between *C. cellasea* (<35 %) and some other *Cellulomonas* strains, between *C. denverensis* and *C. hominis* (24–33 %, Brown et al. 2005), between *C. aerilata* and related strains (16–28 %, Lee et al. 2008), between *C. chitinilytica* and other cellulomonads (7–23 %, Yoon et al. 2008) as well as between *C. soli* and *C. oligotrophica* and 13 type strains of *Cellulomonas* (<35 %, Hatayama et al. 2012). Moderate DDH relatedness of 37–40 % between type strains of *Actinotalea* (then [*Cellulomonas*] *fermentans* and *C. uda*, Bagnara et al. 1985), however, is no proof for intrageneric membership as shown by the later exclusion of *C. fermentans* from *Cellulomonas* and its reclassification as *Actinotalea fermentans* on the basis of chemotaxonomic properties. Discrimination of the type strains of the four *Oerskovia* species by DDH revealed similarity values below 70 %, indicative of members of the separate genomospecies. DDH values are moderately high with values ranging between 55 % and 75 % similarity. *Oerskovia paurometabola* and *O. jenensis* are close neighbours, as strains of these species share between 64% and 75 % DNA similarity (Stackebrandt et al. 2002).

The description of *Paraoerskovia* gen. nov. was substantiated by a moderate DDH value determined for the type specis *Paraoerskovia marina* and *Oerskovia enterophila* JCM 7350T (10–12 %). The necessity of the transfer of *Koreibacter* algae DSW-2^T into the species *Paraoerskovia marina* has been indicated by a DNA-DNA similarity of 94 %.

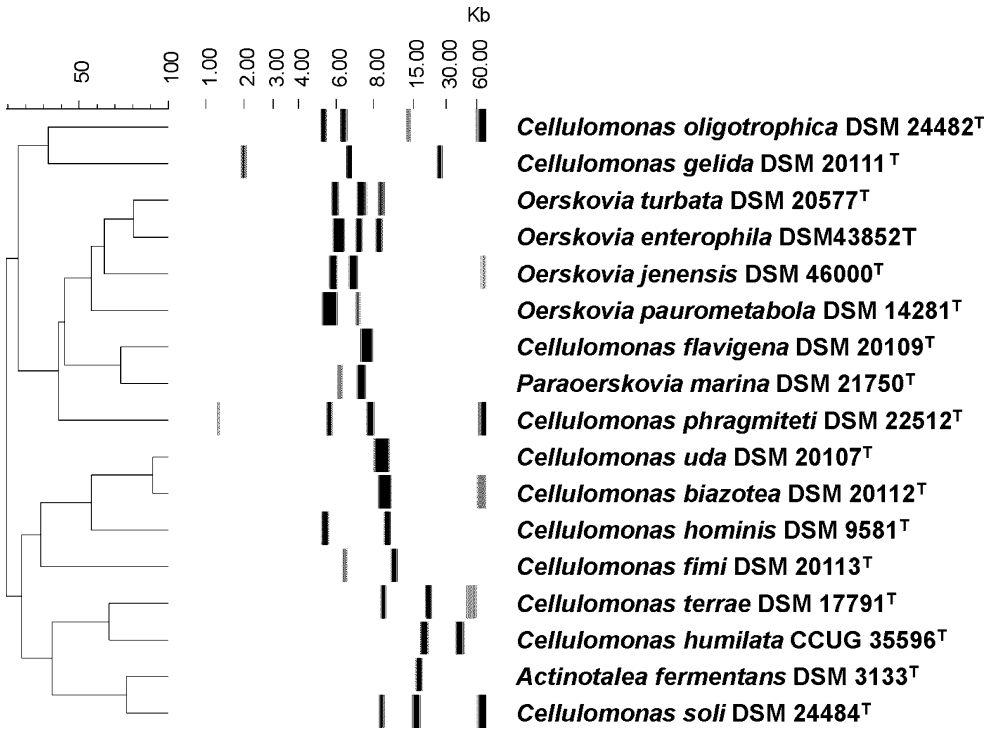

Fig. 9.2

RiboPrint patterns of those type strains of the family *Cellulomonadaceae* species which are cut by *Pst*I. The dendrogram has been generated with the BioNumerics software (Applied Math, Kortrijk, Belgium)

Riboprinting and Ribotyping

The presence of restriction sites for different nucleases and the presence of conserved regions next to more highly variable ITS regions and flanking regions have made the rrn operons an ideal target for species characterization. While the automated robot RiboPrint® system works with entire rrn operons and flanking DNA regions, the ribotype approach usually works with PCR amplified 16S rRNA genes which are then subjected to restrictions and one-dimensional gel electrophoresis. Because the RiboPrint system targets the entire rrn operons at the genome level, its discrimination is superior.

RiboPrint pattern of strains of members of *Cellulomonadaceae* are routinely generated and used in the authentication process of the DSMZ collection. However, as the standard restriction enzyme *Eco*RI does not cut at all and as the cleavage sites of enzymes *Pvu*II and *Pst*I are not present in all members of the family, two subsets need to be generated. *Figure 9.2* shows the *Pst*I patterns of the *Oerskovia* and some *Cellulomonas* species (Stackebrandt et al. 2002), as well as those of the type strains of *Paraoerskovia* marina and *Actinotalea fermentans*. *Figure 9.3* displays the second set of strains in which the patterns were generated with *Pvu*II (*Paraoerskovia marina* is cut by both enzymes *Pvu*II and *Pst*I). The highest RiboPrint similarity of over 90 % is seen between the type strains of *C. uda* and *C. biazotea* (*Fig. 9.2*). This relationship is neither supported by an exclusively high 16S rRNA gene sequence similarity nor for DDH similarities between *C. uda* and *C. biazotea* for which these data are available. These results reinforce the notion that RiboPrint analyses are not indicative of intrageneric relationships.

Riboprinting and ribotyping of members of *Cellulomonadaceae* have been generated in taxonomic studies (Stackebrandt et al. 2002), in a molecular epidemiology study of *Oerskovia turbata* causing endocarditis and *Cellulomonas hominis* (McNeil et al. 2004) and on a single *Oerskovia* strain isolated from paper mill pulps (Suihko and Skyttä 2009).

MALDI-TOF

A recent introduction into the molecular tool box is the MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometric approach, a cheaper and faster way of strain characterization than the generation of riboprints. This method compares the mass spectra recorded in the range of 2.000–20.000 m/z of biomolecules (mainly ribosomal proteins) extracted from the microbial cells by a simple sample preparation (Tóth et al. 2008). These mass spectrometric fingerprints are used for the reliable species identification of bacteria, yeasts, and fungi by comparison with reference databases. Dendrograms based on the similarity of MALDI-TOF mass spectra provide an insight into the phylogenetic relationship of species in good agreement with results obtained by sequence comparison of housekeeping genes (Schumann, unpublished).

The MALDI-TOF analyses of type strains of *Cellulomonadaceae*, also routinely performed in the DSMZ collection for

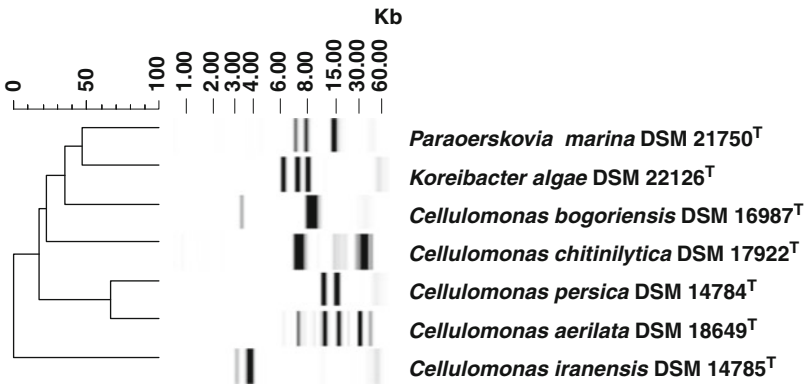

Fig. 9.3

RiboPrint patterns of those type strains of the family *Cellulomonadaceae* species which are cut by *Pvu*II. The dendrogram has been generated with the BioNumerics software (Applied Math, Kortrijk, Belgium)

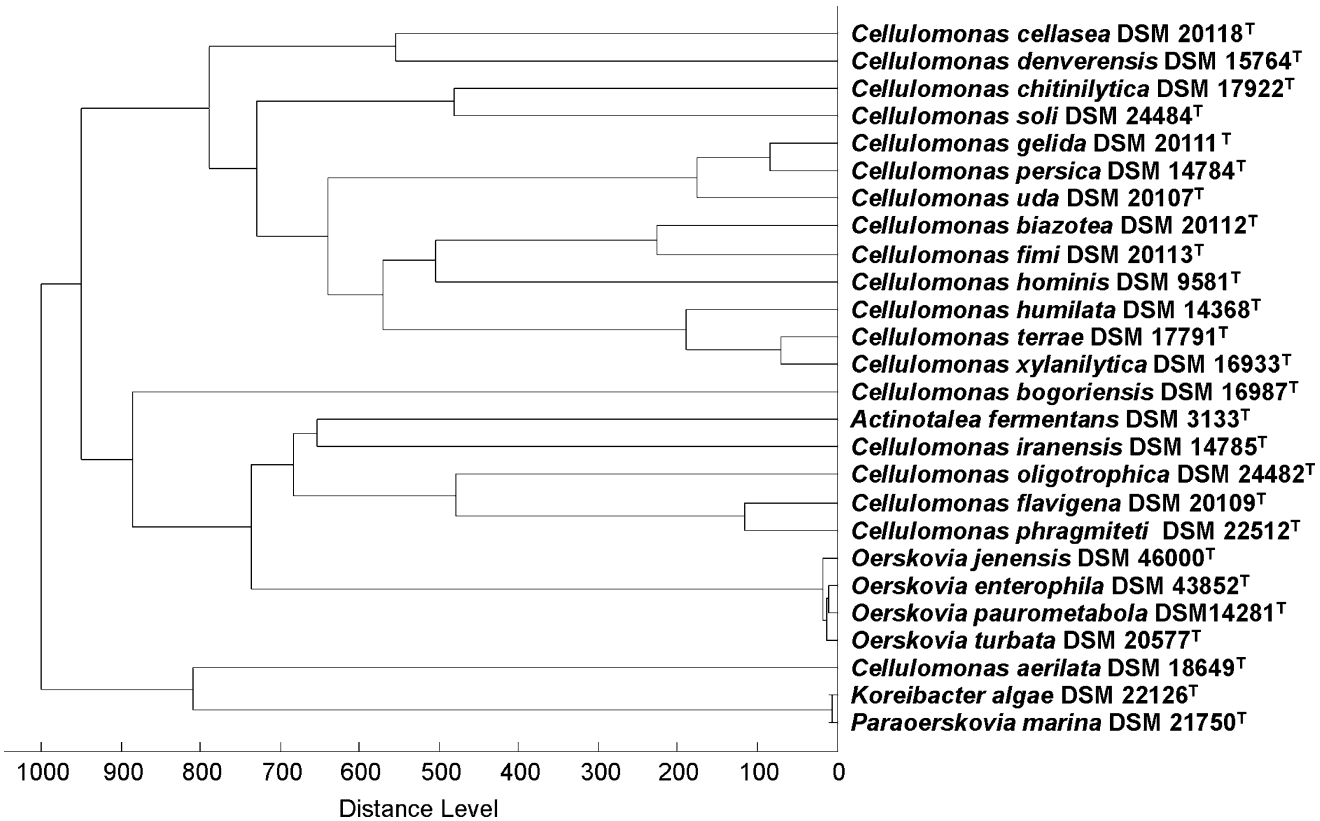

Fig. 9.4

Score oriented dendrogram generated by the BioTyper software (version 2.0, Bruker Daltonics) showing the similarity of MALDI-TOF mass spectra of cell extracts of selected type strains of the family *Cellulomonadaceae*

authentication purposes, display a wide range of spectral similarities. Similar to the 16S rRNA gene sequence, dendrogram type strains of *Oerskovia* as well as *Paraoerskovia marina* and *Koreibacter algae* (see below) are highly related (Fig. 9.4). *Cellulomonas* strains do not form a coherent cluster, but the strains branching outside the main cluster are different to those in the 16S rRNA gene tree. However, several clusters of *Cellulomonas* species agree in the 16S rRNA gene sequence and MALDI-TOF MS–based dendrograms: *C. gelida*/*C. persica*/*C. uda*, *C. fimi*/*C. biazotea*, *C. flavigena*/*C. phragmiteti*, *C. xylanilytica*/*C. terrae*/*C.*

humilata. The MALDI-TOF approach has been mainly used for functional studies such as on carbohydrate-binding modules of xylanase from *Cellulomonas fimi* (Boraston et al. 2001; Boraston et al. 2003) and on studies of mannose from *C. fimi* (Hekmat et al. 2010).

Genome Comparison

The complete genome sequences of two species branching within the 16S rRNA gene tree of *Cellulomonadaceae* have been released. The genome of the type strain of *Cellulomonas flavigena* DSM 20109T (Abt et al. 2010).(GOLD ID Gc01326) is 4,123,179 bp long, contains 3.788 genes, including six copies for the 16S rRNA gene, and the mol% G+C of DNA is 74.3 %. The latter value falls into the range of 72.7–74.8 mol% determined for the species (*Table 9.2*) by HPLC and thermal denaturation. Annotation of the 3.735 genes coding for proteins gene sequences revealed the presence of several proteins related to the synthesis of carotenoids (similar to those found in *Beutenbergia cavernae*, *Leifsonia xyli* subsp. *xyli*, and *Sanguibacter keddii*). The highest number of genes associated with general COG functional categories is found for carbohydrate transport and metabolism, followed by transcription (272 and 270, respectively) and amino acid transport and metabolism (209). In accordance with the species description of *C. flavigena* is the absence of genes related to flagellum structure and flagellum biogenesis (though eight genes are listed to be responsible for cell motility), as well as the presence of several genes involved in the degradation of xylane and cellulose. The presence of a chitinase points toward the ability of *C. flavigena* to degrade chitin though this property has never been reported.

The second species is *Tropheryma whipplei* with the genomes of two strains (strain Twist and TW08/27) fully sequenced. These two strains are highly related as their ANI (average nucleotide identity) value is 99.3 %, thus above the 95 % threshold value determined to separate different species (Konstantinidis and Tiedje 2005) and the presence of about 900 genes shared at the nucleotide level and 773 genes share at the amino acid level (AAI) (Konstantinidis, personal communication). These two genomes are, however, not closely related to the genomes of *C. flavigena*, *Brevibacterium linens and Beutenbergia cavernae*, as indicated by the very low number of less than 10 shared genes at the nucleotide level and about 500 shared genes at the aminoacid level. The ANI values are as low as about 83 % and unreliable because of the low number of shared genes. At the amino acid level, *Tropheryma* strains shows 48 % AAI to either of the other three genomes compared, indicating that *T. whipplei* is not closer related to *Cellulomonas flavigena* than it is to the type strains of *Beutenbergia cavernae* or *Brevibacterium* linens. As mentioned above, the membership of *Tropheryma* to the family is a moot point, a notion that is confirmed by the genome analyses.

Phages

The set of phages proposed to be specific of the genus *Oerskovia* (Stackebrandt and Prauser 1991; Prauser 1984, 1986; Prauser and Falta 1968) are phages O2 (DSM 49109) (host *O. turbata* DSM 43878), O3 (DSM 49138) (host *O. jenensis* DSM 46000 T), and O6 (DSM 49111) (host *O. turbata* DSM 20577 T). The hosts for phages O5 (DSM 49112) and O13 (DSM 49139) described originally as *Cellulomonas cellulans* (DSM 43881 and DSM 46215, respectively) have been reclassified as *Cellulosimicrobium cellulans*.

Phenotypic Analyses

The main features of members of *Cellulomonadaceae* are listed in *Tables 9.1–9.3*.

Cellulomonas Bergey et al. 1923, 154, emend. mut. char. Clark 1952, 504^{AL}.

Cellu.lo.mo'nas. M.L. n. cellulosa cellulose; Gr. n. monas a unit, monad; M.L. fem. n. *Cellulomonas* cellulose monad.

Young cultures of *Cellulomonas* consist of slender, irregular rods ranging between ~0.4 and 0.8 μm in diameter. The rods vary considerably in length and may appear as short filaments in late exponential phase cultures (24 h) when examined by the methods described by Cure and Keddie (1973). They may also show primary branching. As growth proceeds, the rods become shorter and V formations become more obvious. One-week old cultures or older cultures are usually composed mainly of short rods but a proportion of the cells may be coccoid. When placed on fresh solid medium, growth of coccoid cells occurs by elongation from one or sometimes two parts of the cell to give rods which appear club-shaped or jointed. However, they do not show the marked rod-coccus cycle characteristic of *Arthrobacter* and *Brevibacterium*. Only *Cellulomonas humilata* forms a mycelium with true branching that fragments into diphtheroid and coccoid elements (Collins and Pascual 2000).

Moderate growth occurs in air at 30 °C on meat extract, peptone agar or yeast-extract, peptone-based agar media at near neutral pH (Keddie and Jones 1981). Colonies on such media are opaque, usually convex, ~1–3 min in diameter, and usually yellow, but sometimes white. *C. humilata* forms branched, filamentous microcolonies (Gledhill and Casida 1969). Some strains grow at 10 °C, some at 5 °C, and the maximum temperatures are in the range 36–43 °C (cited in Stackebrandt and Keddie 1986). If tested, strains do not survive heating at 63 °C for 30 min (Keddie et al. 1966). All strains grow best aerobically and most give markedly reduced growth under anaerobic conditions (Keddie 1974; Keddie and Cure 1977). *C. soli* and *C. oligotrophica* can grow anaerobically on basal medium agar without Fe(III)-nitrilotriacetic acid (Hatayama et al. 2012). *C. humilata* is microaerophilic (Gledhill and Casida 1969). Glucose, maltose, and sucrose are fermented by all species, while a few species fermented one or two of the carbohydrates

Table 9.2

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

Characteristic	*C. flavigena*[a,b]	*C. aerilata*	*C. biazotea*[a,b]	*C. bogoriensis*[c]	*C. cellasea*[a,b]	*C. chitinilytica*[d]
Morphology	Straight or curved rods	Short rods or coccoid	Straight or curved rods	Straight and rod-shaped	Straight or curved rods	Rods
Mycelium	–	–	–	–	–	–
Motility	+[a,b]	+	+[a]	+	–[a]	–
Catalase	+	+	+	+	+	+
Nitrate reduction	+	–	+	–	+	+
Urease	–	+	–	–	–	
Utilization of						
Acetate	+	–	+	nd	+	+
Dextrin	+	nd	–	nd	–	
Gluconate	+	–	–	–	–	+
Lactose	–	nd	+	–	–	+
Lactate	–	–	+	–	+	–
Mannitol	–	+	–	–	+	+
Mannose	nd	–	+	+	+	+
Raffinose	–	nd	+	–	–	+
Rhamnose	–	–	+	w	–	–
Ribose	+	–	–	–	–	–
Xylose	+	nd	+	+	+	+
Hydrolysis of						
Aesculin	+	+	+	+	+	nd
DNase	–	–	–	+	–	–
Gelatin	+	+	+	+	–	+
Cellulose	+	+	+	+	+	w
Casein	nd	–	nd	+	nd	–
Starch	+	+	nd	+	nd	+
Peptidoglycan composition[d]	L-Orn←Asp	L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Asp	L-Orn←D-Glu	L-Orn←D-Glu
Predominant cellular fatty acids[m]	ai-$C_{15:0}$	ai-$C_{15:0}$, $C_{16:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, $C_{16:0}$	ai-$C_{15:0}$, $C_{16:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:0}$	ai-$C_{15:0\ ante}$, ai-$C_{17:0\ ante}$
Cell-wall sugars[n]	$GlcNH_2$, Rha, Man, Rib	Gal, Glu, xyl	$GlcNH_2$, Rha, Gal, 6dTal	ND	Rha, Man, 6dTal	Gal, Rib, Xyl, Rha
Major polar lipids	DPG, PI, unidentified phosphoglycolipids	DPG, PG	nd	PG	nd	DPG, PG
DNA G+C content (mol%)	72.7–74.8	74	71.5–75.6	71.5	75	73.6
Characteristic	***C. denverensis*[e]**	***C. composti*[f]**	***C. fimi*[a,b,e]**	***C. gelida*[a,b]**	***C. hominis*[b,e]**	***C. humilata*[g]**
Morphology	Short rods	Rods	Straight or curved rods	Straight or curved rods	Regular short rods	Filaments, fragmenting into diphtheroid or coccoid forms
Mycelium	–	–	–	–	–	+
Motility	+	–	+[a]	+a	+	–
Catalase	+	–	+	+	+	–
Nitrate reduction	+	+	+	–	+	–
Urease	–	–	–	–	–	–

Table 9.2 (continued)

Characteristic	*C. denverensis*[e]	*C. composti*[f]	*C. fimi*[a,b,e]	*C. gelida*[a,b]	*C. hominis*[b,e]	*C. humilata*[g]
Growth on						
Acetate	nd	nd	–	+	nd	–
Dextrin	nd	nd	w	–	+	+
Gluconate	nd	–	–	–	+	+
Lactose	nd	w	+	v	+	+
Lactate	nd	nd	+	–	nd	–
Mannitol	nd	–	–	nd	–	+
Mannose	nd	+	+	nd	nd	+
Raffinose	–		–	–	+	w
Rhamnose	nd	–	+	–	+	+
Ribose	nd	w	–	–	–	w
Xylose	v	+	+	+	+	w
Hydrolysis of						
Aesculin	+	+	+	nd	+	+
DNA	nd	+	–	+	+	nd
Gelatin	–	w	+	+	+	w
Cellulose	nd	+	+	+	–	w
Casein	–	nd	nd	nd	nd	+
Starch	nd	+	nd	+	ND	+
Peptidoglycan composition[m]	nd	L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Glu
Predominant cellular fatty acids[n]	i-$C_{15:0}$, ai-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, $C_{16:0}$, $C_{14:0}$, $C_{18:0}$,	$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, $C_{15:0}$	ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:0}$
Cell-wall sugars[o]	Man, Rha, Rib	Man, Glu	$GlcNH_2$,Rha, Fuc, Glc	$GlcNH_2$, Glc	Man, Fuc, Rham	Rha, Fuc, Glc
Major polar lipids[p]	nd	DGP, PI, PE	nd	nd	nd	nd
DNA G+C content (mol%)	68.5	73.7	71.0–72.0	72.4–74.4	76	73
Characteristic	***C. iranensis***[h]	***C. persica***[h]	***C. phragmitetis***[i]	***C. terrae***[j]	***C. uda***[a,b]	***C. xylanilytica***[g,j]
Morphology	Straight or curved rods	Straight or curved rods	Straight rods	Straight rods	Straight or curved rods	Curved rods or coccoid
Mycelium	–	–	–	–	–	–
Motility	+	+	+	–	–	–
Catalase	nd	nd	+	–	+	+
Nitrate reduction	+	+	–	+	+	+
Urease	+	+	+	–	–	–
Growth on						
Acetate	+	+	v	–	+	–
Dextrin	+	+	v	v	+	nd
Gluconate	–	–	nd	–	–	–
Lactose	–	–	–	+	+	+
Lactate	–	–	nd	–	–	–
Mannitol	nd	nd	+	–	–	–
Mannose	+	+	+	+	nd	+
Raffinose	–	–	–	–	–	nd
Rhamnose	nd	nd	–	w	–	+
Ribose	–	–	v	–	–	–

Table 9.2 (continued)

Characteristic	*C. iranensis*[h]	*C. persica*[h]	*C. phragmitetis*[i]	*C. terrae*[j]	*C. uda*[a,b]	*C. xylanilytica*[g,j]
Xylose	nd	nd	+	+	+	+
Hydrolysis of						
Aesculin	nd	ND	+	+	+	
DNA	+	+	nd	+	+	nd
Gelatin	W	w	+	nd	+	w
Cellulose	+	+	–	+	+	+
Casein	nd	nd	+	–	nd	–
Starch	+	+	+	+	+	+
Peptidoglycan composition[m]	L-Orn←D-Asp	L-Orn←D-Asp	L-Orn←D-Asp	L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Glu
Predominant cellular fatty acids[n]	ND	ND	ai-$C_{15:0}$, $C_{16:0}$, ai-$C_{15:1}$	ai-$C_{15:0}$, i-$C_{15:0}$, $C_{16:0}$	ND	ai-$C_{15:0}$, i-$C_{15:0}$, $C_{18:0}$
Cell-wall sugars[o]	$GlcNH_2$, Rha, (Man)	$GlcNH_2$, Rha, (Man)	nd	Rha, Gal, Glc	$GlcNH_2$, Man	Rha, Man, Fuc
Major polar lipids[p]	nd	nd	nd	DPG, PG, PIM, PE	nd	DGP, PI, PIM, PE
DNA G+C content (mol%)	nd	nd	74.8	73.9	72	73
Characteristic	***C. carbonis***[k]	***C. soli***[l]	***C. oligotrophica***[l]			
Morphology	Rods	Slender irregular rods	Slender irregular rods			
Mycelium		–	–			
Motility	+	+	+			
Catalase	+	+	+			
Nitrate reduction	+	+	+			
Urease	–	–	–			
Growth on						
Acetate	–	–	+			
Dextrin	nd	nd	nd			
Gluconate	+	+ (acid production)	–			
Lactose	+	nd	nd			
Lactate	nd	–	–			
Mannitol	–	–	+			
Mannose	+	+	+			
Raffinose	+	+	–			
Rhamnose	–	–	–			
Ribose	–	–	–			
Xylose	+	+	+			
Hydrolysis of						
Aesculin	+	nd	nd			
DNA	–	nd	nd			
Gelatin	+	w	+			
Cellulose	+	+	+			
Casein	–	–	w			

Table 9.2 (continued)

Characteristic	*C. carbonis*[k]	*C. soli*[l]	*C. oligotrophica*[l]			
Starch	+	–	+			
Peptidoglycan composition[m]	L-Orn←D-Glu	L-Orn←D-Glu	L-Orn←D-Glu			
Predominant cellular fatty acids[n]	ai-$C_{15:0}$, $C_{16:0}$, $C_{14:0}$, ai-$C_{15:1}$	ai-$C_{15:0}$	ai-$C_{15:0}$, $C_{16:0}$, $C_{15:0}$, ai-$C_{15:1}$			
Cell-wall sugars[o]	Rha, Gal, Xyl, Ino	Rha, Gal, man	Rha, Glc			
Major polar lipids[p]	DPG, PG, PIM, PI	DPG, PIM	DPG, PIM			
DNA G+C content (mol%)	74.4	73.6	75.8			

Data taken from
[a]Stackebrandt and Kandler 1979; Stackebrandt et al. 1982; Stackebrandt and Prauser 1991
[b]Funke et al. 1995
[c]Jones et al. 2005
[d]Yoon et al. (2008)
[e]Brown et al. 2005
[f]Kang et al. 2007
[g]Rivas et al. 2004
[h]Elberson et al. 2000
[i]Rusznyák et al. (2011)
[j]An et al. 2005
[k]Shi et al. (2012)
[l]Hatayama et al. (2012)
[m]Asp, aspartic acid; Glu, glutamic acid; Orn, ornithine
[n]A number before a colon indicates the number of carbons; the number after the colon is the number of double bonds; iso indicates a methyl branch at the iso position; anteiso indicates a methyl branch at the anteiso position
[o]*6dTal* 6-Deoxytalose, *Fuc* fucose, *Gal* galactose, *Glc* glucose, *Man* mannose, *Rha* rhamnose, *Xyl* xylose, *Ino* inositol
[p]*PG* phosphatidylglycerol, *DGP* diphosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PE* phosphatidylethanolamine
Symbols and abbreviations: + positive, – negative, *w* weakly positive, nd not determined, *v* variable

mannitol, xylose, dextrin, ß-methyl-xyloside, rhamnose, and gluconat. The main products of glucose dissimilation in resting cell suspensions of several type strains are acetic acid, L-lactic acid, formic acid, succinic acid, ethanol, and CO_2. End products of aerobic glucose dissimilation are mainly CO_2 and either acetate or acetate and L-lactate (Stackebrandt and Kandler 1979, 1980a, b). Lactic acid is the main endproduct of sugar dissimilation by *C. humilata* (Gledhill and Casida 1969) while end products were not determined for strains described in the past years. The major route for glucose dissimilation is the Embden-Meyerhof-Parnas pathway; a small amount of glucose is metabolized via the hexose monophosphate pathway (Stackebrandt and Kandler 1974; 1980b). This finding is supported by determination of respective key enzyme activities of these pathways in *C. flavigena* (Kim 1987) and C. uda (Marschoun et al. 1987). Under anaerobic conditions, resting cells produce mainly CO_2, acetate, lactate, and ethanol as well as smaller amounts of succinate and formate. As shown with *C. flavigena*, gluconate is catabolized via the Entner-Doudoroff (ED) pathway and HMP shunt (Kim 1987).

Biotin and thiamine are the only exogenous organic growth factors required by the type strains of *C. flavigena*, *C. biazotea*, *C. cellasea*, *C. gelida*, and *C. uda* (Keddie et al. 1966); when provided with these vitamins, growth occurs in suitable mineral media with glucose as carbon + energy source and an ammonium salt (or nitrate for most strains) as nitrogen source (Owens and Keddie 1969).

Cellulomonas species have been extensively characterized with respect to chemotaxonomic properties. The diagnostic amino acid in position 3 of the peptide subunit of the peptidoglycan is ornithine with the interpeptide bridge containing either D-aspartic acid (*C. flavigena*, *C. phragmiteti*, *C. iranensis*, *C. persica*) or D-glutamic acid (all other species) (*Table 9.2*). Rhamnose is the major diagnostic cell wall sugar in most strains. Menaquinone MK-9(H4) is the predominant isoprenoid quinone (Collins and Jones 1981; Collins and Pascual 2000). 12-Methyltetradecanoic (ai-$C_{15:0}$) and hexadecanoic ($C_{16:0}$) acids are the dominant components of fatty acid patterns of cellulomonads, other branched-chain (e.g., i-$C_{15:0}$, ai-$C_{17:0}$) and straight-chain (e.g., $C_{14:0}$, $C_{15:0}$) fatty acids occur in lower amounts (Funke et al. 1995; no data are available on cellular fatty acids of *C. iranensis*, *C. persica*, and *C. humilata*). Phosphatidylglycerol (Lechevalier et al. 1981), diphosphatidylglycerol, and phosphoglycolipid are the major polar lipids (Minnikin et al. 1979). No data are available on isoprenoid quinones and polar lipids of *C. iranensis*, *C. persica*, *C. humilata*, and *C. hominis*.

Chemotaxis toward cellobiose and hemicellulose hydrolysis products, e.g., cellotriose, D-glucose, xylobiose, and D-xylose as well as other sugars, has been observed in *C. gelida* ATCC 486 (Hsing and Canale-Parola 1992). Two types of separately regulated cellobiose receptors (Cb1 and Cb2) were described, allowing the motile organism to migrate toward

Table 9.3
Differentiating phenotypic properties of *Oerskovia* strains

Characteristics	*O. turbata* DSM 20577^T, DSM 43878	*O. jenensis* DSM 46001^T, DSM 46000, DSM 46097	*O. enterophila* DSM 43852^T	*O. paurometabola* DSM 14281^T
Utilization of (BIOLOG panel)				
Mannan	+	+	+	–
α–acetyl mannosamine	+	+	+	–
Amygdalin	+	+	+	–
Arbutin	+	+	+	–
Cellobiose	+	+	+	–
D-fructose	+	+	+	–
D-L-fucose	–	–	+	–
D-galactose	+	+	+	–
D-galacturonic acid	–	–	+	–
Gentobiose	+	+	+	–
m-inositol	–	–	+	–
α-D-lactose	+	–	+	–
D-melibiose	+	+	+	–
α-methyl D-galactoside	+	+	+	–
Sedoheptulosan	–	–	+	–
Stachyose	–	–	+	–
Acetic acid	+	+	+	–
α-hydroxybutyric acid	–	–	+	–
γ- hydroxybutyric acid	–	–	+	–
lactamide	–	–	+	–
D-lactic acid methylester	+	+	+	–
L-lactic acid	+	+	+	–
D-malic acid	–	–	+	–
L-asparagine	–	–	+	–
Fructose-6-phosphate	–	–	+	–
API "Coryne"				
Pyrazinamidase	–	–	+	–
ß-galactosidase	+	w	+	–
Urease	–	v	+	+
Gelatine hydrolysis	+	–	–	–
Glycogen fermentation	v	+	+	+

w weak reaction, *v* variable

plant containing cellulose and hemicellulose by swimming up concentration gradients of cellobiose and other sugars. While one receptor Cb1 was inducible and bound to cellobiose and xylobiose, receptor Cb2 was synthesized constitutively and bound to cellobiose, cellotriose, xylobiose, and D-glucose. In a following paper, the authors (Hsing and Canale-Parola 1996) described that L-methionine is required for normal cell motility and chemotaxis and that S-adenosylmethionin is involved in sugar chemotaxis. Methylation occurred posttranslationally, increased upon addition of sugar

attractants and decreased after removal of the stimulating sugars. The cellulolytic enzymes are controlled by catabolic repression, as activity against carboxymethyl cellulose is only low during growth on glucose or cellobiose (Stoppok et al. 1982; Choi et al. 1978)

All cellulomonads are able to grow under aerobic and microaerophilic conditions and *C. uda* ATCC 21399 (Dermoun et al. 1988) grows under strictly anaerobic conditions as well. Glucose uptake of whole cell suspension of an unidentified *C. fimi* isolate was two-fold higher under aerobic condition than under N_2 or H_2; cellobiose negatively affected glucose uptake (Khanna 1993).

The three *Cellulomonas* species described recently, not included in ❯ *Fig. 9.1*, show the following 16S rRNA gene sequence relatedness to other species: *C. carbonis* T26^T is moderately related to *C. bogoriensis* DSM 69B4^T (69.4 % similarity) in trees obtained with various algorithms, forming a sister clade to the other *Cellulomonas* type strains (Shi et al. 2012). *C. soli* Kc1^T and *C. oligotrophica* Kc5^T, sharing 97.8 % sequence similarity between each other, display highest similarities with *C. terrae* DB5^T (98.1–98.4 %) (Hatayama et al. 2012).

Oerskovia Prauser et al. 1970, 534^{AL} emend Stackebrandt et al. 2002.

Oers.ko'vi.a M.L. dim. *-ia* ending; M.L. fem.n. *Oerskovia* in honor of J. Øerskov who first described this organism.

*Oerskovia*e are typical nocardioforms but may show a coryneform appearance depending on the particular strain, the age of the culture, and the external growth conditions. In general, they are characterized by extensively branching vegetative hyphae (ca. 0.5 µm in diameter) which grow on the surface of the agar or penetrate into it. The substrate hyphae fragment into bacillary and coccoid or spore-like elements, which can be motile by differently arranged flagella, i.e., subpolar tufts of one to three flagella (Higgins et al. 1967) and monotrichous flagella (for short cellular elements) and peritrichous flagella (for longer cells) (Sukapure et al. 1970). All elements, motile and nonmotile, resulting from fragmentation, may give rise to new mycelia independent of their size. No aerial mycelium is formed. The growth appears bacteroid in smears. Colonies are lemon-yellow to whitish. Their consistency is smooth and the surface is glistening with a tendency to dull. Edges show mycelial or at least hyphal character, resembling those of other nocardioforms.

The most straightforward placement of an unidentified strain into the genus *Oerskovia* is a combination of 16S rRNA gene sequence analysis and determination of the peptidoglycan type A4α. Teichoic acids are lacking (Evtushenko et al. 1984b). The cytochromes belong to the a, b, and c types (Seidl et al. 1980).

While *Oerskovia enterophila* DSM 43852^T utilizes a broad range of BIOLOG compounds (76 of 95 substrates), *O. paurometabola* DSM 14281 reacts mostly negative toward the BIOLOG substrate panel (28 of 95) (❯ *Table 9.3*). *O. turbata* and *O. jenensis* are metabolically similar (Stackebrandt et al. 2002). Differentiating phenotypic properties are displayed in ❯ *Table 9.2*. All *Oerskovia* strains were positive in the following reactions API "coryne" reactions: oxidase, catalase, nitrate reduction, pyrazimidase, α-glucosidase, *N*-acetyl-ß-glucosidase, ß-glucosidase, and fermentation of glucose, ribose, xylose, maltose, and saccharose. All strains were negative in the following reactions: pyrrolidonyl arylamidase, ß-glucuronidase, and fermentation of mannitol and lactose.

All strains utilized the following Biolog GP substrates: ß-cyclodextrin, dextrin, glycogen, Tween 40, *N*-acetyl glucosamine, D-gluconic acid, α-D-glucose, maltose, maltotriose, mannose, ribose, salicin, sucrose, trehalose, turanose, D-xylose, methyl-pyruvate, glycerol, adenosine, 2-deoxy adenosine, inosine, thymidine, uridine, and adenosine-5′-monophosphate. The following substrates were not utilized by any *Oerskovia* strain: inuline, D-arabitol, melizitose, *p*-hydroxyphenyl acetic acid, α-keto glutaric acid, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, glucose-1-phosphate, and glucose-6-phosphate.

All *Oerskovia* strains were positive in the following reactions API "coryne" reactions: oxidase, catalase, nitrate reduction, pyrazimidase, α-glucosidase, *N*-acetyl-ß-glucosidase, ß-glucosidase, and fermentation of glucose, ribose, xylose, maltose, and saccharose. All strains were negative in the following reactions: pyrrolidonyl arylamidase, ß-glucuronidase, and fermentation of mannitol and lactose.

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Actinotalea, Paraoerskovia, and *Koreibacter* are monospecific genera. Some of their salient properties have been covered unter "short description of genera" and in ❯ *Table 9.1*.

Paraoerskovia Khan et al. 2009, 2094^{AL}. emend. Schumann et al. 2013.

Pa.ra.oer.sko'via. Gr. prep. *para* beside; N.L. fem.n. *Oerskovia* a bacterial genus name; N.L. fem. n. *Paraoerskovia* beside or close to *Oerskovia*.

Koreibacter alga is a later heterotypic synonym of *Paraoerskovia marina* and the unification required the emendation of *Paraoeroskovia. Paraoerskovia marina* (Khan et al. 2009) is catalase-positive and oxidase-negative. Cells are 0.4–0.6-µm wide and 1.0–1.6-µm long. Colonies on HSMA or ISP2 plates after 3–5 days of incubation at 28 °C are creamy yellow-coloured colonies, 1–2 mm in diameter. Carotenoid-type pigments are present. Growth occurs at 10–35 °C (optimum at 28 °C) and pH 6.0–10.0 (optimum pH 7.0–8.0). Growth occurs at 0–8 % NaCl (w/v).

According to the API 50CH system, it is positive for acid production from starch, glycogen, cellobiose, sucrose, trehalose, gentiobiose, maltose, L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, D-xylose, esculin ferric citrate, glycerol, turanose (type strain) and negative for 33 other tests of the

panel. Positive for the degradation of CM-cellulose and starch and negative for the degradation of casein, cellulose, chitin, and gelatin. In the API ZYM system, positive for leucine arylamidase, α- and β-glucosidase, lipase *N*-acetyl-ß glucosaminidase, and esterase (type strain) and negative for all other enzymes included in the test panel. For the type strain, menaquinones MK-9 and MK-9(H2) are detected in addition to the major menaquinone MK-9(H4). Traces of xylose occur in addition to the major cell-wall sugar galactose.

Actinotalea Yi et al. 2007, 155^{AL}.

Ac.ti.no.tal'e.a. Gr. n. actis, actinis ray; L. fem. n. talea a slender staff, rod, stick; N.L. fem. n. *Actinotalea* ray stick.

Actinotalea fermentans (Bagnara et al. 1985) was isolated as *Cellulomonas fermentans* from a methanogenic enrichment culture from a municipal dumping ground of unrecorded origin in France. The type strain M^T exhibits a coryneform polymorphism, 0.2–0.5 by 0.6–1.7 µm. Colonies are white with an undulate margin, 2–3 mm in diameter. A zone of cellulose digestion around colonies is observed in cellulose agar. Anaerobic, but shows growth under aerobic conditions without any apparent benefit. Growth on arabinose, cellulose, fructose, galactose, glucose, glycogen, lactose, maltose, mannose, mannitol, raffinose, starch, sucrose, trehalose, xylose, and xylan but not on asparagine, arbutine, glycerol, inulin, melibiose, melezitose, pectin, rhamnose, ribose, pyruvate, salicin, sorbitol, and urea. Fermentation products are acetic acid, formic acid, ethanol, occasionally L-(+)-lactic acid, and, in smaller amounts, carbon dioxide and succinic acid. No growth under aerobic conditions on butanol, butyrate, citrate, ethanol, gluconate, lactate, propanol, propionate, or succinate but grows slowly on acetate. Yeast extract (0.05 %) and casamino acids (0.2 %) support only slight growth. Growth factors other than biotin and thiamine together are required and can be provided by 0.05 % yeast extract. Gelatin is liquefied, nitrate reduction positive, NH_3 is produced from peptone. Neither acetyl methyl carbinol nor indole is produced. Catalase- and urease-negative. Optimum temperature between 30 °C and 37 °C. No growth at 50 °C. Optimum pH around 7.4. Growth rate decreases substantially when pH is under 7 and over 8. Cell wall sugars are glucose, rhamnose, and ribose.

Isolation, Enrichment, and Maintenance Procedures

Members of the family *Cellulomonadaceae* grow on a wide range of media as do many other soil bacteria. The main problem in their isolation is the exclusion of organisms that cover large areas of the isolation plates, e.g., swarming bacilli, pseudomonads, and hyphal fungi, as well as the suppression of the numerous streptomycetes, which may be confused at first sight with young stages on the isolation plates and which may possibly antagonize the *Oerskoviae*. Cultures may be enriched in a mineral-based medium containing a low (0.05-0.1 %) concentration of yeast extract to provide the necessary organic growth factors, and filter paper as cellulose source (Stackebrandt and Keddie 1986). This is followed by plating on a similar solid medium but containing cellulose in dispersed form. Cellulolytic bacteria produce colonies surrounded by zones of clearing. Direct plating on cellulose agar, or R2A medium (Reasoner and Geldreich 1985) without previous enrichment, may also be used. The methods are not selective for *Cellulomonas* and isolates must be screened for those with a coryneform morphology, followed by molecular characterization.

Suspensions or macerates of the material being examined, soil, compost, etc., may be streaked directly onto the surface of cellulose agar (Stewart and Leatherwood 1976) of the following composition (g/100 ml of distilled water): $NaNO_3$, 0.1; K_2HPO_4, 0.1; KCl, 0.05; $MgSO_4$, 0.05; yeast extract, Difco, 0.05; agar, 1.7; ball-milled filter paper, 0.1; glucose 0.1; pH 7.0. To prepare the ball-milled filter paper, a 3 % (w/v) aqueous suspension of filter paper (Whatman No. 1) is ball-milled for 3 days. Other suitable sources of dispersed cellulose may be used, e.g., microcrystalline cellulose (Avicel, FNIL) at a concentration of 0.1 % (w/v) (Kaufmann et al. 1976), Avicel, Solka floc, CF11 cellulose, carboxymethyl cellulose, or phosphoric acid-treated cellulose (Kauri and Kushner 1985). In other similar versions of the medium, the glucose is omitted. The plates are incubated at 30 °C for 5–7 days; colonies showing zones of clearing are replated on the same medium until pure cultures are obtained. Yellow to yellowish isolates which show a coryneform morphology in combination with cellulolytic activity are presumptive members of the genus *Cellulomonas*.

Cellulomonas enrichments may be prepared by a method similar to that described by Han and Srinivasan (1968). A liquid version of the cellulose agar described above is used but with the glucose omitted and with strips of filter paper replacing the finely divided cellulose. Other mineral bases may be used, e.g., that of Han and Srinivasan (1968); Owens and Keddie (1969); Bagnara et al. 1985; Malekzadeh et al. (1993). Moderate growth occurs on meat extract, peptone agar, or media based on yeast extract or peptone at around neutral pH. Growth-promoting factors in yeast extract are, in part, thiamine and biotin. These factors can be supplemented by adding a few drops of a sterile commercially available multivitamin solution (e.g., Multibionta, Merck). Other strains grow on Trypticase soy agar (TSA) or nutrient agar at 30 °C. Xylanic strains may be isolated on XED medium (xylan, 0.7 %; yeast extract, 0.3 %; agar, 2.5 %) (Rivas et al. 2004). *C. phragmiteti* has been isolated and cultivated on Kings B medium adjusted to pH 9.0 (Rusnyak et al. 2011) while *C. aerilata* was enriched and maintained on R2A agar (Lee et al. 2008).

C. soli and *C. oligotrophica* from soil were enriched anaerobically on basal medium agar (Lovley et al. 1984), supplemented with Fe(III)-nitrilotriacetic acid (Roden and Lovley 1993), but are able to grow aerobically on TSA and R2A agar. The same media support growth of *C. carbonis*, isolated from a coal mine (Shi et al. 2012), which was enriched on a chemically defined medium (Weeger et al. 1999).

Strains from clinical specimen were cultured on Columbia agar or Trypticase soy agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) with 5 % sheep blood at 37 °C in

a 5 % CO_2 atmosphere (Funke et al. 1995). *C. humilata* can be cultivated on brain heart infusion agar at 30 °C (http://www.atcc.org/) but does not grow well at 37 °C, in chemically defined media or in those lacking organic nitrogen and in anaerobic conditions (Collins and Pascual 2000).

Alkaliphilic cellulomonads may be enriched at 37 °C on an alkaline casein medium containing the following (g l^{-1}): glucose (10), Difco peptone (5), Difco yeast extract (5), K_2HPO_4 (1), $MgSO_4.7H_2O$ (0·2), NaCl (40), Na_2CO_3 (10), casein (20), and agar (20) (Jones et al. 2005). Cultivation is achieved in glucose alkaline medium consisting of two parts. Solution A contained the following, dissolved in 800-ml distilled water and sterilized: glucose (10 g), Difco peptone (5 g), Difco yeast extract (5 g), K_2HPO_4 (1 g), and $MgSO_4.7H_2O$ (0·2 g). Solution B contained 40 g NaCl and 10 g Na_2CO_3 dissolved in 200-ml distilled water and sterilized. The two solutions were then mixed. Solid medium was prepared by adding agar (2 %, w/v) to solution A before sterilization (Duckworth et al. 1996).

Procedures and media used for the isolation of members of the genus *Promicromonospora* (Stackebrandt and Prauser 1991) can also be recommended for *Oerskovia*e. Tapwater agar (1.5 % crude agar in tap water; Lechevalier and Lechevalier 1989) may also be used to isolate *Oerskovia*e.

Paraoerskovia marina, isolated from marine sediment, was enriched on half strength marine agar (Khan et al. 2009), while a second strain was isolated from seaweed, enriched on starch-casein sea water medium (for composition see Lee and Lee 2010).

Actinotalea fermentans is the only member of the family *Cellulomonadaceae* that is able to grow under strict anaerobic conditions: The basal medium used had the following composition: K_2HPO_4, 2.21 g; KH_2PO_4, 1.50 g; $FeS0_4$ solution (0.5 % [wt/vol]), 0.25 ml; yeast extract, 5 g; and distilled water to bring the final volume to 1 l. The pH was adjusted to 7.4 with 8 N NaOH.

For isolation purposes, the basal medium was supplemented with 4.0 % (w/vol) ball-milled MN300 cellulose and 2.0 % agar and used by the Hungate roll-tube technique (Hungate 1969).

For anaerobic cultures in liquid medium, 10 ml of sterile cysteine-hydrochloride solution (5.0 %) and 10 ml of sterile sodium bicarbonate solution (8.0 %) per liter were added after autoclaving. The anaerobic culture technique used was that of Hungate (1950) as modified by Bryant (1972).

When carbon sources were soluble carbohydrates, they were autoclaved separately (30 min, 110 °C) and added just before inoculation. For culture maintenance, the basal medium was supplemented with 0.5 % MN300 cellulose and dispensed in 10-ml volumes into Hungate tubes under an O_2-free argon atmosphere. All cultures were incubated at 30 °C without agitation.

For short-term preservation, stab cultures in semisolid medium should remain viable for several months at room temperature. Serial transfers on nutrient agar at 4-week intervals followed by maintenance at 4 °C are recommended for medium-term storage as is maintenance of cells as 20 % (w/v) lycerol suspensions in an appropriate medium at −20 °C and at −80 °C. Long-term preservation methods include freeze-drying in skim milk and maintenance in liquid nitrogen at −196 °C.

Ecology

Habitat

The main habitat of cellulomonads appears to be the soil, including decayed wood, cellulose-containing material, compost, and municipal waste from which the original cultures were isolated (Kellerman et al. 1913; Bergey et al. 1923; Kauri and Kushner 1985; Stackebrandt and Keddie 1986). This applies also to *Cellulomonas* species described or reclassified recently: *C. persica* and *C. iranensis* were isolated from forest soils (Elberson et al. 2000), *C. terrae* (An et al. 2005) and *C. humilata* (basonym *Actinomyces humiferus*) originated from organically rich soils (Collins and Pascual 2000), *C. soli* and *C. oligotrophica* (Hatayama et al. 2012) were isolated from wet to most Japanese soils, while *C. carbonis* was found in subsurface soil of a chinese coal mine Shi et al. 2012. *C. xylanilytica* was isolated from a decayed elm tree (Rivas et al. 2004). Cattle-farm compost has been the source of *C. chitinilytica* (Yoon et al. 2008), and *C. composti* (Kang et al. 2007) while *C. aerilata* was found in an air sample (Lee et al. 2008). Two species have been isolated from alkaline environment, i.e., *C. bogoriensis* (Jones et al. 2005) from the litteral zone of Lake Bogoria and *C. phragmiteti* (Rusznyák et al. 2011) from reed growing in a soda pond in Hungary. Reports on the occurrence of cellulomonads in natural environments are rare and they almost never are found as a dominating population. Emphasis placed on the cellulolytic activity of these organisms has resulted in the successful isolation of *Cellulomonas* strains from activated sludge (Ramasamy et al. 1981), and cellulose-enriched environments such as bark and wood (Deschamps 1982; Przybyl 1979), coffee beans (Silva et al. 2000), soils enriched on flax or sisal fibres (Lednicka et al. 2000) and sugar fields (de Leon and Joson 1980). A strain of *C. flavigena* has been isolated from the great spruce bark beetle, *Dendroctonus micans* (Yaman et al. 2010), and culture and culture-independent studies demonstrated the presence of cellulomonads in the gut of the turpentine beetle, *Dendroctonus valens LeConte* (Morales-Jiménez et al. 2009). Cellulolytic culturable bacteria closely related to strains of the genus *Cellulomonas* as revealed by 16S rDNA sequence comparison have been isolated from refuse of a landfill (Pourcher et al. 2001), from an agricultural encatchment (Ulrich and Wirth 1999), and they were found among other endophytic bacteria on growing shoot tips of banana (Thomas and Soly 2009) and among populations associated with rice seeds (Cottyn et al. 2001). A high number of uncharacterised *Cellulomonas* strains have been isolated from mature coffee beans of *Coffea arabica* in Brazil (Silva et al. 2000). Under dry conditions, cellulomonads dominated the population of Gram-positive organism. Their role, however, has not been elucidated.

Cellulomonads were found among a halophilic oil-utilizing community in the Arabian Gulf cost (Al-Awadhi et al. 2007) and as hydrocarbonoclastic bacteria in mangrove sediments in Brazil (Brito et al. 2006). They were part of the dominating cultrable population in a 2,347-year-old permafrost soil from Spitsbergen (Hansen et al. 2007).

C. hominis and *C. denverensis* were the first representatives of the genus which were isolated from human clinical samples: *C. hominis* from cerebrospinal fluid (Funke et al. 1995) and *C. denverensis* and *C. hominis* from blood, cerebrospinal fluid, homograft valve, lip wound, and pilonidal cyst (Brown et al. 2005). Recently, Ohtaki et al. (2009) reported *C. denverensis* as the cause of an acute cholecystitis.

*Oerskovia*e were randomly and rarely isolated from various types of soils from different geographical regions, composts, decaying plant materials, and occasionally from various clinical specimens (Cruickshank et al. 1979; Reller et al. 1975; Sottnek et al. 1977). *Oerskovia enterophila* (*Promicromonospora enterophila*) constitutes the major part of the actinomycete microflora of the intestines and feces of litter-inhabiting millipedes, e.g., *Chromatoiulus projectus* (Dzingov et al. 1982; Jáger et al. 1983; Szabó et al. 1983, 1986). The species occurs also in the feces of the cave-inhabiting blind isopode *Mesoniscus graniger* (Bodnar et al. 1989). A large homogenous population of facultatively anaerobic *Oerskovia*-type nocardioforms was also found in the gut contents of adult specimens of the common earthworm *Lumbricus polyphemus* (Ravasz et al. 1987; Szabó et al. 1986). None of these *Oerskovia*e could be isolated from the surrounding feeding habitats of the animals. Moreover, the inability of the gut and feces nocardioforms to survive in the natural soil and litter habitat could be demonstrated (Márialigeti et al. 1985). However, among 311 culturable cellulolytic bacterial isolates from soil of an agricultural encatchment, 13 strains were found to show 99.6 % 16S rRNA gene similarity to *Oerskovia enterophila* (Ulrich and Wirth 1999), classified as "*Paramicromonospora enterophila*" by the authors. Similarly, the name *Oerskovia xanthineolytica* is frequently used even in the recent literature while this organism has been reclassified as *Cellulolomicrobium cellulans* (Schumann et al. 2001). *Oerkovia turbata* has frequently been associated with endocarditis (see below).

The habitat of members of the monospecific genera and of those species for which only a single strain, the type strain, is available must be considered tentative.

As pointed out by Abt et al. (2010) for *Cellulomonas flavigena*, a survey of metagenome libraries do not, until 2010, provide information about the presence of close relatives in any of the environments tested. Also, 16S rRNA based studies did hardly point toward the presence of cellulomonads in soil environments such as grassland soils (Felske et al. 1998), Amazonan soil Borneman and Triplett (1997), or arid soil (Kuske et al. 1997). One of the rare reports demonstrating the presence of four clone sequences related to *Cellulomonas* spp. comes from the analysis of grass pasture in the UK (McCaig et al. 1999). Apparently, cellulomonads and *Oerskovia*e are not dominating in soil habitats (though they have been isolated therefrom) but are more affiliated to the gut environment of invertebrates like earthworms and termites and in close contact with decaying plant material.

Pathogenicity: Clinical Relevance

Strains of *Cellulomonas denverensis* and *Cellulomonas hominis*, rare human pathogens, were isolated from blood and cerebrospinal fluid, and some other specimen (Brown et al. 2005; Funke et al. 1995).

Oerskovia turbata, Cellulomonas strains described until 1995, and *C. hominis* are susceptible to tetracycline and vancomycin and, except of *Oerskovia turbata*, also subsceptible *to* rifampicin (Funke et al. 1995). Rifampicin and vancomycin were also suitable antimicrobial agents for *C. denverensis* (Brown et al. 2005). Additionally, clarithromycin, clindamycin, imipenem, and minocycline were considered active against *C. hominis* and *C. denverensis* strains (Brown et al. 2005). Two isolates from blood cultures from patients at the Gaziantep University Hospital (south-east Turkey) that were identified as members of the genus *Cellulomonas* were susceptible to teicoplanin, rifampicin, vancomycin, gentamicin and ampicillin-sulbactam (Balci et al. 2002). The environmental type strain of *C. bogoriensis* (Jones et al. 2005) is susceptible to ampicillin (25 μg), chloramphenicol (25 μg), erythromycin (5 μg), fusidic acid (10 μg), methicillin (10 μg), novobiocin (5 μg), streptomycin (10 μg), tetracycline (25 μg), sulphafurazole (100 μg), oleandomycin (5 μg), polymyxin (300 IU), rifampicin (2 μg), vancomycin (30 μg) and bacitracin (10 IU) and resistant to gentamicin (10 μg), nitrofurantoin (50 μg), nalidixic acid (30 μg), sulphamethoxazole (50 μg), trimethoprim (2·5 μg), penicillin G (1 IU), neomycin (30 μg) and kanamycin (30 μg) (Jones et al. 2005). A similar sensitivity spectrum was determined for the type strain of *C. carbonis* (Shi et al. 2012). *C. terrae* is resistant to ampicillin (30 μg), tetracycline (15 μg), streptomycin (15 μg) and kanamycin (2 mg) (An et al. 2005). *Paraoerskovia marina* is sensitive to vancomycin (50 μg), and resistant to bacitracin (10 μg), gentamycin (30 μg), kanamycin (30 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), nystatin (100 IU) and streptomycin (10 μg) (Khan et al. 2009). Other members of *Cellulomonadaceae* have not yet been tested for antibiotic resistance and susceptibility.

C. flavigena ATCC 482, *C. gelida* ATCC 488, *C. uda* ATCC 491, *C. fimi* ATCC 15724 and *Cellulomonas* sp. ATCC 21399 were subjected to toxicity test by injecting cell extracts as well as viable cells in fertile chicken eggs and some rodents, respectively. Neither were cell extracts lethal to the embryos, nor did the viable cells cause generalized or local infections in rats, mice or rabbits. The sexual maturity, fertility and organs of adult rats as well as the vitality of their progeny were not affected by feeding 10 % protein from the test strains (Dey and Fields 1995).

C. hominis was isolated from cerebrospinal fluids of a male and a female patient (Funke et al. 1995). Although no patients'

records were available for the evaluation of the clinical significance of the isolates, this was the first report on members of the genus *Cellulomonas* that were isolated from clinical specimens. Three additional clinical isolates from cerebrospinal fluid (strain W7335), pilonidal cyst (strain W7336) and a lip wound (strain W7387) were identified as *C. hominis* mainly on the basis of DNA-DNA hybridization (Brown et al. 2005). Two isolates from a patient with endocarditis from Denver (W6929^T from blood and W6124 from a homograft valve) and a blood isolate (strain W6117) from a patient from Ohio differed in their ability to fermentation of sorbitol and low DNA-DNA similarity values sufficiently from *C. hominis* to be classified in a new species, *C. denverensis* (Brown et al. 2005). Although it is difficult to differentiate between true infection and contamination and to identify *Cellulomonas* strains unambiguously by phenotype-based commercial diagnostic test systems, the role of members of the genus *Cellulomonas* as potential pathogens in clinical cases especially of immunocompromised patients must be taken into consideration. A recent review on infective endocarditis and osteomyelitis by cellulomonads has been published by Lai et al. (2009).

When reading the literature care must be taken in the interpretation of strains reported to be either *Oerskovia* or *Cellulosimicrobium* species. According to Betancourt Castellanos et al. (2011) there are four *Oerskovia* cases of peritoneal infection published, caused by *O. xanthineolytica* or *O. turbata*. The authors then conclude on the basis of published literature that these two species are currently known as *Cellulosimicrobium cellulans* (Rowlinson et al. 2006) and *Cellulosimicrobium funkei* (Brown et al. 2006), respectively. The latter authors however did not reclassify *O. turbata* as *Cellulosimicrobium funkei* but only a set of strains formerly assigned to *O. turbata*. Indeed, the majority of cases are assigned to *Cellulosimicrobium* [*xanthineolytica*] *cellulans*.

The pathogenic potential of *Oerskoviae* was first described by Reller et al. (1975). Strains identified as *O. turbata* were frequently isolated from the blood taken from a patient suffering from endocarditis after homograft replacement of the aortic valve. The source of *O. turbata* remained speculative although contamination of harvested heart valves with *O. turbata* have been reported (Reller et al. 1975). The occurrence of *Oerskoviae* in various clinical sources was documented (Sottnek et al. 1977) when a large number of motile, Gram-positive, non-sporeforming, yellow-pigmented organisms could be allocated to *O. turbata* and to the former *O. xanthineolytica* (now *Cellulosimicrobium cellulans*). Nine of 31 clinical isolates, collected by the Bacteriology Division, Centers for Disease Control (CDC) over a period of 20 years, were identified as *O. turbata*. The source of the *O. turbata* isolates was heart tissues, heart valves, blood, and tissues. Since no satisfactory case history for any of the isolates was available, their clinical significance could not be elucidated.

Vancomycin-sensitive *Oerskovia* strains have been frequently isolated from the commensal bowel flora in humans, where they may cause opportunistic infections. The *van*A gene, coding for vancomycin and teicoplanin resistance has been sequenced from the clinical isolate *Oerskovia* sp. strain 892 (Power et al. 1995). The sequence of the plasmid-born gene was found to be highly similar to that of *Arcanobacterium haemolyticum* and *Enterococcus faecium*. Sialidase (neuraminidase) activity has been detected in culture collection strains of *O. turbata* and strain isolated from a liver abscess (Müller 1995). Some of the non-type strains have been reclassified as the new species *O. jenensis* (Stackebrandt et al. 2002).

Application

Waste Treatment and Removal

The ability of cellulomonads to attack cellulose and wheat straw under microaerobic or even anaerobic conditions has been used in mixed cultures to provide nitrogen-fixing strains of *Bacillus macerans* and *Azospirillum brasilense* with energy-yielding products (Halsall and Gibson 1985, 1986; Halsall and Goodchild 1986). Good nitrogen-fixing rates have been reported for the pair *Azospirillum brasilense* ATCC 29145 and *Cellulomonas* sp. strain CS117. The latter is a mutant strain selected for its increased production of cellulase and reduced sensitivity to inhibition or repression by accumulated cellobiose and glucose (Haggatt et al. 1978).

Of the large variety of hydrolytic starch, xylan, and cellulose-degrading enzymes, detected in *Cellulomonadaceae*, the cellulases are the most salient ones. In a comparative study *C. biazotea* produced the highest filter-paper cellulase and endo-glucanase activities, followed by *C. flavigena*, *C. cellasea*, and *C. fimi* (Rajoka and Malik 1997). However, most of the molecular work on cellulase and xylanase genes was determined with *C. fimi*.

Like other cellulose- and hemicellulose-degrading organisms, *Cellulomonas* strains have been considered potential candidates for waste disposal (Ramasamy et al. 1981; Dunlap and Callihan 1974), and composting flax and sisal fibres (Lednicka et al. 2000), bagasse (Richard and Peiris 1981), pith, leaves of sugar canes (Diaz and Guirola 1983; Richard and Peiris 1981; Rajoka and Malik 1986), dried palm oil mill effluent (Agamuthu and Tan 1985), shredded newspapers (Rapp et al. 1984), or even as producers of chemicals from low-cost substrates. Mutants of *C. flavigena*, showing elevated xylanase and carboxymethyl cellulase acitivity, were able to use a larger portion of sugar cane bagasse than their wild type strains (Ponce-Noyola and de la Torre 1995; Mayorga-Reyes and Ponce-Noyola 1998). The isolation of cellulomonads from a landfill of domestic refuse, e.g., *Actinotalea fermentans, C. hominis*, *C. biazotea*/ *C. fimi* and *C. flavigena*/*C. uda* (the latter two pairs of organisms were indistinguishable by numerical analysis, Pourcher et al. 2001) or strains isolated from cattle compost indicate their potential to degrade solid cellulolytic waste. *Cellulomonas* sp. strain DOT 21, isolated from

a domestic refuse (Bichet-Hébé et al. 1999), has been used to estimate paper degradation by reduction of a whiting fluorescent agent added to white paper prior to the degradation process.

Bioremediation

The bioremediating abilities have been investigated in a few cases. *C. flavigena* strain HR5, was isolated from agricultural soil in South Korea, contaminated with 4-chlorobenzoate. This plasmid-bearing strain was also able to utilize well 4-bromobenzoic acid, benzoic acid and less well 4-iodobenzoic acid, but not 3-chlorobenzoic acid and 2,4-dichlorophenoxyacetic acid (Yi et al. 2000). A bench scale experiment with *Cellulomonas* sp. strain ES6 resulted in the reduction of Cr(VI) and Fe(III) without continuous nutrient supply in the subsurface thus offering a viable and economical alternative technology for in situ remediation of Cr(VI)-contaminated groundwater through formation of permeable reactive biobarriers (Viamajala et al. 2008).

Formation of Added-Value Products

Cellulomonas strains ATCC 482, ATCC 488, ATCC 491, ATCC 15392 and ATCC 21399 were found to contain high protein and essential amino acid values (Dey 1976) and different strains have been used for single-cell protein production from a variety of waste products, such as sugar cane bagasse (Han et al. 1971; Rodríguez et al. 1993) and rice straw (Han et al. 1971), hemstock (Jedar et al. 1987) and ground corn and stalks (Fields et al. 1991).

Cellulolases from *Cellulomonas* sp. YJ5 has been used to hydrolyse suspensions of *Chlorella* with favorable results concerning yield of soluble proteins, peptides, Fe(2+) chelating ability, antioxidation capacity and reducing power (Yin et al. 2010).

Mixed cultures consisting of *Cellulomonas* sp. ATCC 21399, *Desulfovibrio vulgaris* strain J.J., and *Methanosarcina barkeri* 227 were highly efficient in converting xylan to methane via hydrolysis and acidogenesis (strain ATCC 21399), acetogenesis (strain J.J.), and methanogenesis (strain 227) (Guyot 1986). The same *Cellulomonas* strain has also been used in mixed cultures with *Rhodopseudomonas capsulata* to photoevolve molecular hydrogen by the nitrogenase system of the phototrophic strain with cellulose as the sole carbon source (Odom and Wall 1983).

The ability of *Actinotalea fermentans* to degrade cellulose and to produce acetate has been used by Bayer et al. (2009) to construct a consortium consisting of this cellulomonad and a recombinant strain of *Saccharomyces cerevisiae* to turn raw cellulolytic material into methyl halide which can be easily converted to fuel. This inexpensive process works at low temperature using unprocessed switchgrass, corn stover, sugar cane bagasse and poplar as feed for *A. fermentans*. *S. cerevisiae* with its cloned methyl halide transferase gene from turtleweed transfers the acetate into methyl halide which can be converted into gasoline, benzene, toluene, xylene, ethylene, propylene, methanol or dimethyl ether.

Enzymes

The earlier literature has been summarized by Stackebrandt and Prauser (1991). Different kinds of cellulose preparations and derivatives had been tested, including amorphous (Dermoun and Belaich 1985), swollen, phosphoric-acid-treated (Kauri and Kushner 1985), microcrystalline (Vladut-Talor et al. 1986; Dermoun and Belaich 1988; Poulsen and Petersen 1988), and carboxymethyl cellulose (CMC). As reported by Kauri and Kushner (1985), degradation of cellulose does not depend on cell-to-fiber contact, but cellulases from three *Cellulomonas* strains were active even when they were physically separated from the fibers. Microcrystalline cellulose (Avicel pH 101, Cellulose MN300, Whatman cc41) appears to be less efficiently attacked than amorphous cellulose (phosphoric-acid-treated Whatman cc41 cellulose) (Dermoun and Belaich 1985). Under anaerobic conditions, celluloses with varying degrees of crystallinity were hydrolized by *C. uda* ATCC 21399 with the same efficiency as by aerobically grown cells though the growth yield was reduced significantly (Dermoun et al. 1988). Optimal liquefaction of CMC gels occurred in a synthetic medium at 40 °C at pH of 7.0–7.5 (Thayer et al. 1984).

A wide range of carbohydrate-binding modules, carbohydrate esterases and glycoside hydrolases have been studied in detail at the molecular level. Several enzymes have been identified to be involved in the hydrolysis of xylane (endo-1,4-xylanases [Mayorga-Reyes et al. 2002; Horcasitas et al. 1998]), cellulose (endo-1,4-glucanases [Gutiérrez-Nava et al. 2003] ß-glucosidase, cellobiohydrolases), chitine (exo-*N*,*N*'-diacetylchitobiohydrolase [Chen et al. 1997] chitinase) and mannan (ß-mannanase). Most of the species studied were *C. flavigena* and *C. fimi*. The earlier molecular data on these enzymes were compiled by Stackebrandt et al. (2002) and the reader is asked to read the chapter of Bayer (Bayer et al. 2000) and his contribution in this edition.

As indicated above, the annotation of the recently fully sequenced genome of *C. flavigena* DSM 20109^T revealed that 9.6 % of encoded proteins are classified into the COG category "carbohydrate transport and metabolism"; among these are genes coding for xylan degrading enzymes; 14 genes coding for putative endo-1,4-β-xylanases belonging to glycoside hydrolase family 10, and five genes encoding β-xylosidases. Also identified were two genes coding for endo-1,4-β-glucanases (Cfla_0016, Cfla_1897), three genes encoding 1,4-β-cellobiohydrolases (Cfla_1896, Cfla_2912, Cfla_2913) and three genes coding β-glucosidases (Cfla_1129, Cfla_3027, Cfla_2913).

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10 The Families *Conexibacteraceae*, *Patulibacteraceae* and *Solirubrobacteraceae*

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Abstract
Three families will be here discussed, *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacteraceae*, which are members of the order *Solirubrobacterales* together in this chapter. Members of this order are Gram-positive, mesophilic, and the peptidoglycan contains meso-diaminopimelic acid (*meso*-Dpm) as diagnostic diamino acid. The pattern of 16S rRNA signatures consist of nucleotides at positions 63 : 104 (G-C), 70 : 98 (G-C), 370 : 391 (C-G), 580 : 776 (U-A), 657 : 749 (U-A), 670 : 736 (A-U), 681 : 709 (U-A), 941 : 1342 (A-U), 953 : 1228 (G-C), 954 : 1226 (G-C), 1051 : 1207 (G-C), 1118 : 1155 (U-A) and 1311 : 1326 (A-U). The three families of the order *Solirubrobacterales* are monogeneric: The family *Conexibacteraceae* contains two species, *Conexibacter woesei* and *C. arvalis* (Monciardini et al., Int J Syst Evol Microbiol 53:569–576, 2003; Seki et al., Int J Syst Evol Microbiol 53:2400–2404, 2012); the family *Patulibacteraceae* contains three species, *Pautulibacter minatonensis*, *P. americanus*, and *P. ginsengiterrae* (Kim et al., Int J Syst Evol Microbiol 62:563–568, 2012; Takahashi et al., Int J Syst Evol Microbiol 56:401–406, 2006; Reddy et al., Int J Syst Evol Microbiol 59:87–94, 2009) and the family *Solirubrobacteraceae* comprises three species, *Solirubrobacter pauli*, *S. soli* and *S. ginsenosidimutans* (An et al., Int J Syst Bacteriol 61:2606–2609, 2011; Kim et al., Int J Syst Evol Microbiol 57:1453–1455, 2007; Singleton et al., Int J Syst Evol Microbiol 53:485–490, 2003). These organisms are phylogenetic neighbors of the families *Gaiellaceae*, *Rubrobacteraceae*, and *Thermoleophilaceae* (Albuquerque et al., Syst Appl Microbiol 34:595–599, 2011; Reddy and Garcia-Pichel, Int J Syst Evol Microbiol 59:87–94, 2009).

Taxonomy: Historical and Current

Short Description of the Families and Genera

Conexibacteraceae Stackebrandt 2005; Emend. Zhi et al. 2009

Conexibacteraceae (Co.ne.xi.bac.te.ra.ce'a.e. N.L. masc. n. *Conexibacter*, type genus of the family; suff. -aceae, ending to denote a family; N.L. fem. pl. n. *Conexibacteraceae*, the *Conexibacter* family).

The members of the family *Conexibacteraceae* of the order *Solirubrobacterales* stain Gram-positive. Form rod-shaped cells and endospores are not formed. These organisms are strictly aerobic and chemoorganotrophic. The peptidoglycan contains *meso*-Dpm as diagnostic diamino acid. The peptidoglycan type is A1γ' (based on *meso*-A_2pm, directly cross-linkage). Unsaturated straight chain and iso-fatty acids are present. The major respiratory lipoquinone is MK-7 or derivatives. The pattern of 16S rRNA signatures consist of nucleotides at positions 52 : 359 (U-A), 127 : 234 (G-C), 139 : 224 (G-C), 144 : 178 (U-A), 145 : 177 (U-A), 291 : 309 (U-A), 293 : 304 (G-C), 377 : 386 (C-G), 408 : 434 (A-U), 418 : 425 (U-A), 590 : 649 (U-A), 600 : 638 (U-G), 823 : 877 (G-C), 906 (A), 955 : 1225 (U-A), 999 : 1041 (G-U), 1115 : 1185 (C-G) and 1410 : 1490 (U-A). The type and only genus of this family is *Conexibacter* (Monciardini et al. 2003; Reddy and Garcia-Pichel 2009; Seki et al. 2012; Zhi et al. 2009).

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_200,

Patulibacteraceae Takahashi et al. 2006; Emend. Zhi et al. 2009; Emend. Kim et al. 2012

Patulibacteraceae (Pa.tu.li.bac.te.ra.ce'a.e. N.L. masc. n. *Patulibacter* type genus of the family; suff. –aceae, ending to denote a family; N.L. fem. pl. n. *Patulibacteraceae*, the *Patulibacter* family).

A family of the order *Solirubrobacterales*. Cells are Gram-positive and form rod-shaped cells. Endospores are not formed. Strictly aerobic and chemoorganotrophic. The peptidoglycan contains *meso*-Dpm as diagnostic diamino acid. The peptidoglycan type is A1γ'. Unsaturated straight chain and anteiso-fatty acids are present. The major respiratory quinone is MK-7 or derivatives. The pattern of 16S rRNA gene sequence signature nucleotides of members of the family consists of 52 : 359 (C-G), 98 (A), 127 : 234 (G-C), 139 : 224 (G-C), 144–178 (C-G), 291 : 309 (U-A), 293 : 304 (G-C), 377 : 386 (C-G), 408 : 434 (G-C), 502 : 543 (G-C), 590 : 649 (U-A), 600 : 638 (U-G), 823 : 877 (A-U), 906 (A), 955 : 1225 (U-A), 999 : 1041 (U-A), 1115 : 1185 (C-G), 1354 : 1368 (U-G) and 1410 : 1490 (A-U). The type and only genus of this family is *Patulibacter* (Kim et al. 2012; Reddy and Garcia-Pichel 2009; Takahashi et al. 2006; Zhi et al. 2009).

Solirubrobacteraceae Stackebrandt 2005; Emend. Zhi et al. 2009

Solirubrobacteraceae (So.li.ru.bro.bac.te.ra.ce'a.e. N.L. masc. n. *Solirubrobacter*, type genus of the family; suff. -*aceae*, ending to denote a family; N.L. fem. pl. n. *Solirubrobacteraceae*, the *Solirubrobacter* family).

A family of the order *Solirubrobacterales*. Cells are Gram-positive and form rod-shaped cells. Endospores are not formed. Strictly aerobic and chemoorganotrophic. The peptidoglycan contains *meso*-Dpm as diagnostic diamino acid. The peptidoglycan type is A1γ'. Unsaturated straight chain and iso-fatty acids are present. The major respiratory quinone is MK-7 or derivatives. The pattern of 16S rRNA signatures consists of nucleotides at positions 52 : 359 (C-G), 127 : 234 (G-C), 139 : 224 (A-U), 144 : 178 (C-G), 145 : 177 (C-G), 293 : 304 (G-C), 408 : 434 (G-C), 590 : 649 (C-G), 600 : 638 (C-G), 823 : 877 (G-C), 906 (A), 955 : 1225 (U-A), 999 : 1041 (U-A), 1115 : 1185 (C-G) and 1410 : 1490 (U-A). The family comprises the type and only genus *Solirubrobacter* (An et al. 2011; Kim et al. 2007; Reddy and Garcia-Pichel 2009; Singleton et al. 2003; Stackebrandt 2004; Zhi et al. 2009).

Phylogenetic Structure of the Family and Its Genera

The order *Solirubrobacterales* comprises three monogeneric families, namely, *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacteraceae* which form a monophyletic clade at a level of about 92–93 % 16S rRNA gene sequence similarity (*Fig. 10.1*). They are most closely related to the species of the genus *Thermoleophylum* (family *Thermoleophylaceae*) with sequence similarity of about 89–90 %. The three validly named species of the genus *Solirubrobacter* have 16S rRNA gene sequence similarities varying from 96.4 % between

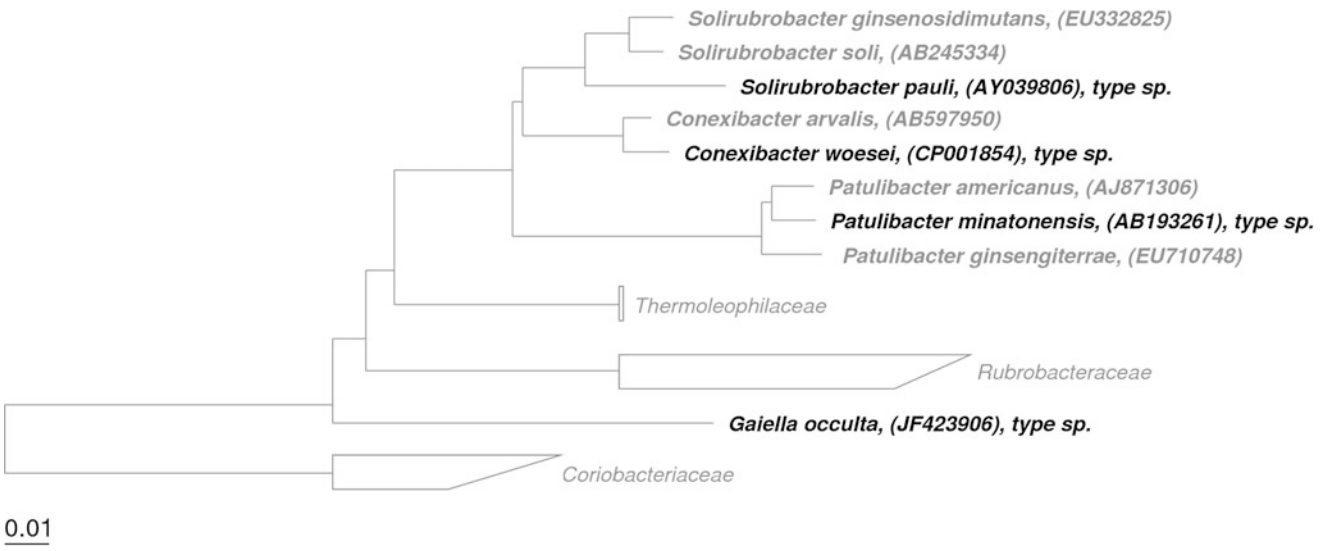

Fig. 10.1

Phylogenetic reconstruction of the families *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacteraceae* 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

S. ginsenosidimutans BXN5-15^T (EU332825) and *S. pauli* B33D1^T (AY039806) and 98.4 % between *S. soli* Gsoil 355^T (AB245334) and *S. pauli* B33D1^T (AY039806).

The species *Conexibacter arvalis* KV-962^T (AB597950) and the type species *C. woesei* ID131577^T (CP001854) share 98.6 % 16S rRNA sequence similarity, while the species of the genus *Patulibacter*, namely, *P. americanus* CP177-2^T (AJ871306) and *P. ginsengiterrae* P4-5^T (EU710748), share 98.6 % and 98.5 % 16S rRNA gene sequence similarity with the type strain of the genus, *P. minatonensis* KV-614^T (AB193261), respectively.

The close relationship in phylogeny deduced from 16S rRNA gene sequence analysis is confirmed by similar chemotaxonomic characteristics, namely, the respiratory quinones, peptidoglycan type, and fatty acid composition, indicating that the *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacteraceae* are closely related (see Chap. 19 on the Family *Gaiellaceae*).

Phenotypic Analyses

The main features of the families *Conexibacteraceae*, *Patulibacteraceae*, *and Solirubrobacteraceae* are listed in Table 10.1. The main features of *Conexibacter woesei* and *Conexibacter arvalis* are listed in Table 10.2. The main features of *Patulibacter minatonensis*, *Patulibacter americanus*, and *Patulibacter ginsengiterrae* are listed in Table 10.3. The main

Table 10.1

Phenotypic and chemotaxonomic characteristics of the families *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacteraceae*

	Conexibacteraceae[1,2,3,4]	*Patulibacteraceae*[1,2,5,6]	*Solirubrobacteraceae*[1,2,7,8,9]
Morphology	Rods	Rods	Rods
Gram-stain	Positive	Positive	Positive
Pigmentation	Whitish cream/White to ivory	Pale yellow/Pink	Non-pigmented/Yellowish/Pink
Motility	+	+	–
Temperature for growth (°C)			
Range	5–46	5–37	15–38
Optimum	28–37	24–27	25–30
pH for growth			
Range	5.0–10.0	5.0–9.0	6.0–nd
Optimum	7.0–9.0	7.0	6.5–7.0
NaCl for growth (%)			
Range	0–4	0–3	0–1
Metabolism	Aerobic	Aerobic	Aerobic
Nitrate reduction	+	–	–
Presence of			
Oxidase	Variable	Variable	Variable
Catalase	+	+	+
Peptidoglycan type	A1γ′	A1γ′	A1γ′
Diagnostic peptidoglycan amino acids[a]	*meso*-Dpm	*meso*-Dpm	*meso*-Dpm
Presence of mycolic acids	–	–	nd
Major fatty acids	$C_{18:1}\omega9c$, $C_{17:1}\omega6c$, iso-$C_{16:0}$	$C_{18:1}\omega9c$, anteiso-$C_{15:0}$	$C_{18:1}\omega9c$, iso-$C_{16:0}$
Polar lipids[b]	PI, PL	PI, DPG, PG, PGL, PL(s), GL(s), AL(s), UL (s)	PL(s)
Major respiratory lipoquinone[c]	MK-7(H_4)	MK-7(H_2)/DMK-7	MK-7(H_4)
G+C content (mol%)	71.0–75.0	72.0–75.0	70.6–71.8

Symbols: + positive, – negative, *nd* not determined

[1]Albuquerque et al. 2011; [2]Reddy and Garcia-Pichel 2009; [3]Monciardini et al. 2003; [4]Seki et al. 2012; [5]Kim et al. 2012; [6]Takahashi et al. 2006; [7]An et al. 2011; [8]Kim et al. 2007; [9]Singleton et al. 2003

[a]*meso*-Dpm, *meso*-diaminopimelic acid

[b]DGP, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PGL, unknown phosphoglycolipid; AL(s), unknown aminolipid(s); GL(s), unknown glycolipid(s); PL(s), unknown phospholipid(s); UL(s), unknown lipid(s)

[c]MK, menaquinone; DMK, demethylmenaquinone

Table 10.2

Phenotypic and chemotaxonomic properties of members of the genus *Conexibacter*. All of the organisms are catalase positive and reduce nitrate. All of the strains hydrolyze gelatin and utilize D-trehalose. All of the organisms are urease and arginine dihydrolase negative, and do not produce indole. None of the strains utilize methyl-αD-mannopyranoside, methyl-αD-glucopyranoside and D-cellobiose. In Api ZYM tests, all organisms are positive for esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and negative for α-chymotrypsin, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. All organisms are sensitive to amikacin, gentamicin, chloramphenicol, tetracycline, vancomycin, polymyxin B and resistant to aztreonam, ceftazidime, ciprofloxacin, norfloxacin, oxacillin

Characteristic	*C. woesei*[a] ID131577^T	*C. arvalis*[b] KV-962^T
Cell size (μm)	0.6–0.7 × 0.9–1.2	0.8–1.0 × 1.6–2.3
Pigmentation	White to cream	White to ivory
Colony morphology	Smooth, mucoid to sticky	nd
Motility	Motile by long peritrichous flagella	Motile by long flagella
Temperature for growth (°C)		
Range	nd	5–46
Optimum	28–37	28–38
pH for growth		
Range	6.0–8.0	5.0–10.0
Optimum	7.0–7.5	7.0–9.0
NaCl for growth		
Range	0–1	0–4
Presence of		
Oxidase	+	−
β-Galactosidase	nd	−
Enzymes (Api Zym)		
Alkaline phosphatase	−	+
Lipase (C14)	−	+
Valine arylamidase	−	+
Cystine arylamidase	−	+
Trypsin	−	+
α-Galactosidase	−	+
Hydrolysis of		
Esculin	+	−
Assimilation of (using API 50 CH)		
Glycerol	w	+
Erythritol	w	−
D-Arabinose	w	−
L-Arabinose	nd	−
D-Ribose	w	−
D-Xylose	w	−
L-Xylose	−	w
D-Adonitol	nd	−
Methyl-βD-xylopyranoside	nd	−
D-Galactose	nd	−
D-Glucose	w	+
D-Fructose	nd	−
D-Mannose	w	+
L-Sorbose	−	w
L-Rhamnose	w	+
Dulcitol	−	w

Table 10.2 (continued)

Characteristic	*C. woesei*[a] ID131577^T	*C. arvalis*[b] KV-962^T
Inositol	–	+
D-Mannitol	nd	–
D-Sorbitol	nd	–
N-Acetylglucosamine	w	–
Amygdalin	nd	+
Arbutin	–	w
Esculin ferric citrate	+	–
Salicin	–	w
D-Maltose	nd	–
D-Lactose	nd	–
D-Melibiose	nd	–
D-Sucrose	nd	–
Inulin	nd	–
D-Melezitose	nd	–
D-Raffinose	nd	–
Starch	nd	–
Glycogen	nd	–
Xylitol	nd	–
Gentiobiose	nd	–
D-Turanose	–	+
D-Lyxose	–	w
D-Tagatose	w	w
D-Fucose	nd	+
L-Fucose	w	w
D-Arabitol	–	w
L-Arabitol	–	w
Potassium gluconate	nd	–
Potassium 2-ketogluconate	nd	–
Potassium 5-ketogluconate	+	w
Biolog GP2 microplate		
Glycerol	+	nd
L-Arabinose	+	nd
D-Ribose	+	nd
D-Xylose	+	nd
Acetic acid	+	nd
α-Ketovaleric acid	+	nd
Propionic acid	+	nd
Pyruvic acid	+	nd
Biolog GN2 microplate		
Methylpyruvate	+	nd
β-Hydroxybutyric acid	+	nd
α-Ketoglutaric acid	+	nd
α-Ketovaleric acid	+	nd
Sensitivity to		
Nitrofurantoin	+	nd
Novobiocin	+	nd
Teicoplanin	+	nd

Table 10.2 (continued)

Characteristic	*C. woesei*[a] ID131577^T	*C. arvalis*[b] KV-962^T
Ampicillin	–	+
Erythromycin	+	–
Clindamycin	–	nd
Kanamycin	–	nd
Methicillin	–	nd
Rifampicin	–	nd
Imipenem	nd	–
Nalidixic acid	nd	–
Streptomycin	–	nd
Trimethoprim	–	nd
Tobramycin	–	+
G+C content (mol%)	71.0	74.0

For symbols see *Table 10.1*
w, weakly positive
[a]Monciardini et al. 2003
[b]Seki et al. 2012

features of *Solirubrobacter pauli*, *Solirubrobacter soli*, *and Solirubrobacter ginsenosidimutans* are listed in *Table 10.4*. All organisms of the three families are strictly aerobic, chemoorganotrophic, and mesophilic with an optimum growth pH between about 6.5 and 7.5, except for *Conexibacter arvalis*, which is reported to have an optimum pH for growth of 7.0–9.0. The peptidoglycan is of the A1γ' type with *meso*-Dpm; the predominant respiratory lipoquinone is menaquinone 7 (MK-7 or derivates of MK-7). The predominant fatty acids of the families *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacteraceae* are unsaturated straight chain, and iso- and anteiso-branched chain fatty acids. The G+C content of the DNA is around 71–75 %.

Conexibacter Monciardini et al. 2003

Conexibacter (Co.nex.i.bac'ter. L. part. adj. *conexus* bound, tied; N.L. masc. n. *bacter* from Gr. N. *baktron* rod; N.L. masc. n. *Conexibacter* a rod that is bound).

Conexibacter forms rod-shaped cells. Motile by long flagella. Mesophilic. Catalase positive and oxidase variable. The Biolog GP2 and GN2 microplates were used to assess the carbon metabolism of the species *Conexibacter woesei*, while the API 50 CH system was used to assess the single carbon source assimilation of the species *Conexibacter arvalis*. Some assimilations of *C. woesei* were also performed by Seki et al. (2012). *C. woesei* and *C. arvalis* were negative for glucose fermentation. The optimum pH for growth varies from about 7.0 to 7.5 in *C. woesei* and between pH 7.0 and 9.0 in *C. arvalis*. Mycolic acids are absent. The major isoprenoid respiratory lipoquinone is a fully saturated menaquinone 7, tetrahydrogenated menaquinone 7 [MK-7(H_4)]. The polar lipid pattern consists of phosphatidylinositol (PI) and an unknown phospholipid (PL). The major fatty acids are $C_{18:1}\omega9c$, $C_{17:1}\omega6c$ and iso-$C_{16:0}$. The G+C content of the DNA is in the range 71–75 mol%. The type species is *Conexibacter woesei*. The type strain ID131577^T (=DSM 14684^T =JCM 11494^T) was isolated from a temperate forest soil in Gerenzano, Italy (Monciardini et al. 2003). *Conexibacter arvalis* is the second species of this genus; the type strain is KV-962^T (=DSM 23288^T =NBRC 106558^T), isolated from soil collected from a field in Japan. Strain KV-963, isolated from the same source, is a reference strain of *C. arvalis* (Seki et al. 2012).

Patulibacter Takahashi et al. 2006; Emend. Reddy and Garcia-Pichel 2009; Emend. Kim et al. 2012

Patulibacter (Pa.tu.li.bac'ter. L. adj. *patulus*, spreading; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Patulibacter* bacterium with spreading growth).

Patulibacter forms rod-shaped cells; motility is variable. Mesophilic. Catalase positive and oxidase variable. The Biolog GP2 microplates were used to assess the carbon metabolism of the species *Patulibacter minatonensis*, the classical single carbon and energy source assimilations were used to assess the carbon source assimilation of the species *Patulibacter americanus*, and the API 50 CH and the API 20 NE systems were used to assess the carbon source assimilations of the species *Patulibacter ginsengiterrae* making comparisons of assimilation patterns impossible. Acid production was only examined in *P. ginsengiterrae* using the API 50 CH, being negative for all carbohydrates. *P. minatonensis* was reported to be negative for the fermentation of glucose, which was the only sugar examined.

Table 10.3

Phenotypic and chemotaxonomic characteristics of members of the genus *Patulibacter*. All of the organisms are catalase positive, and hydrolyze tween 20 and tween 80. All of the strains are DNAse, β-galactosidase, lysine decarboxylase, phenylalanine deaminase, methyl red, voges-proskauer negative, do not reduce nitrate, and do not produce indole and H_2S. None of the strains hydrolyze gelatin, esculin, and starch. All organisms are sensitive to polymyxin B, vancomycin, gentamicin and resistant to aztreonam

Characteristic	*P. minatonensis*[a,c] KV-614^T	*P. americanus*[b,c] CP177-2^T	*P. ginsengiterrae*[c] P4-5^T
Cell size (μm)	0.6–0.7 × 1.2–1.5	nd	0.4–0.6 × 0.8–1.0
Pigmentation	Pale yellow	Pink	Creamy white
Colony morphology	Flat and nearly transparent	Convex, entire, smooth and slightly mucoid	Circular and semi-translucent
Motility	Motile by long flagella	Variable (when present occurs through jerking cellular motions)	Motile by a single polar flagellum
Temperature for growth (°C)			
Range	16–28	10–30	5–37
Optimum	24–27	25	25
pH for growth			
Range	6.0–8.0	5.0–9.0	5.5–9.0
Optimum	nd	7.0	7.0
NaCl for growth (%)			
Range	0–1	0–2	0–3
Growth on Simmon's citrate	nd	–	nd
Presence of			
Oxidase	–[a],w[c]	–	+
Urease	–	–	+
Lecithinase	nd	nd	+
Arginine dihydrolase	–	–	+
Ornithine decarboxylase	–	–	–
Enzymes (Api Zym)			
Alkaline phosphatase	+	nd	–
Esterase (C 4)	+	nd	+
Esterase lipase (C 8)	+	nd	+
Lipase (C14)	+	nd	+
Leucine arylamidase	+	nd	+
Valine arylamidase	–	nd	–
Cystine arylamidase	–	nd	+
Trypsin	–	nd	–
α-Chymotrypsin	–	nd	–
Acid phosphatase	+	nd	+
Naphthol-AS-BI-phosphohydrolase	+	nd	+
α-Galactosidase	–	nd	–
β-Galactosidase	–	nd	–
β-Glucuronidase	–	nd	–
α-Glucosidase	–	nd	–
β-Glucosidase	–	nd	–
N-Acetyl-β-glucosaminidase	–	nd	–
α-Mannosidase	–	nd	–
α-Fucosidase	–	nd	–

Table 10.3 (continued)

Characteristic	*P. minatonensis*[a,c] KV-614^T	*P. americanus*[b,c] CP177-2^T	*P. ginsengiterrae*[c] P4-5^T
Hydrolysis of			
Casein	nd	–	–
Cellulose	nd	–	+
Fermentation of glucose	–	nd	nd
Assimilation of			
D-Glucose	nd	+	+
D-Laevulose	nd	+	nd
Melibiose	nd	+	+
Sucrose	nd	+	+
Raffinose	nd	–	–
L-Sorbose	nd	–	nd
Trehalose	nd	–	nd
Arabinose	nd	–	nd
L-Arabinose	nd	nd	+
L-Fucose	nd	nd	+
Rhamnose	nd	–	nd
L-Rhamnose	nd	nd	+
Trehalose	nd	–	+
Cellobiose	nd	–	–
Galactose	nd	–	–
Maltose	nd	+	+
Mannose	nd	–	+
Gentiobiose	nd	nd	–
Melezitose	nd	nd	–
Ribose	nd	–	–
Xylose	nd	+	+
D-Fructose	nd	–	–
Lactose	nd	–	–
Turanose	nd	nd	–
Dextran	nd	–	nd
Inulin	nd	+	nd
Amygdalin	nd	nd	–
Salicin	nd	nd	+
myo-Inositol	nd	–	+
Mannitol	nd	–	+
Sorbitol	nd	+	+
Glycerol	nd	–	+
Adonitol	nd	–	nd
Dulcitol	nd	–	nd
L-Arabitol	nd	nd	–
Xylitol	nd	nd	–
Pyruvate	nd	–	nd
Succinate	nd	–	nd
Acetate	nd	–	+
Citrate	nd	–	+
Gluconate	nd	nd	+
Malate	nd	nd	+

Table 10.3 (continued)

Characteristic	*P. minatonensis*[a,c] KV-614^T	*P. americanus*[b,c] CP177-2^T	*P. ginsengiterrae*[c] P4-5^T
Malonate	nd	nd	–
Propionate	nd	nd	+
L-Aspartic acid	nd	–	nd
L-Glutamic acid	nd	–	nd
Lactic acid	nd	–	+
Nicotinic acid	nd	–	nd
Tartaric acid	nd	–	nd
Fumaric acid	nd	–	nd
Oxalate	nd	–	nd
N-Acetylglucosamine	nd	nd	–
Ethanolamine	nd	–	nd
L-Alanine	nd	–	+
L-Cysteine	nd	–	nd
L-Glycine	nd	–	nd
L-Glutamine	nd	–	nd
L-Histidine	nd	–	nd
L-Leucine	nd	–	nd
L-Lysine	nd	–	nd
L-Phenylalanine	nd	–	nd
L-Tryptophan	nd	–	nd
L-Tyrosine	nd	–	nd
Adenine	nd	–	nd
Cytosine	nd	–	nd
Guanine	nd	–	nd
Thymidine	nd	–	nd
Arginine	nd	+	nd
Serine	nd	–	+
L-Histidine	nd	nd	–
L-Asparagine	nd	–	nd
L-Isoleucine	nd	–	nd
L-Methionine	nd	–	nd
L-Proline	nd	–	+
L-Threonine	nd	–	nd
L-Valine	nd	–	nd
Phenanthrene	nd	–	nd
Indole	nd	–	nd
Biolog GP2 microplate			
Dextrin	+	nd	nd
Inulin	+	nd	nd
Mannan	+	nd	nd
N-Acetyl-D-mannosamine	+	nd	nd
L-Arabinose	+	nd	nd
D-Arabitol	+	nd	nd
Cellobiose	+	nd	nd
D-Fructose	+	nd	nd
L-Fucose	+	nd	nd
D-Galactose	+	nd	nd

Table 10.3 (continued)

Characteristic	*P. minatonensis*[a,c] KV-614^T	*P. americanus*[b,c] CP177-2^T	*P. ginsengiterrae*[c] P4-5^T
D-Galacturic acid	+	nd	nd
α-D-Glucose	+	nd	nd
myo-Inositol	+	nd	nd
Maltose	+	nd	nd
Maltotriose	+	nd	nd
D-Mannitol	+	nd	nd
D-Mannose	+	nd	nd
D-Melezitose	+	nd	nd
Methyl α-D-galactoside	+	nd	nd
Methyl β-D-glucoside	+	nd	nd
Methyl α-D-glucoside	+	nd	nd
Methyl α-D-mannoside	+	nd	nd
D-Psicose	+	nd	nd
L-Rhamnose	+	nd	nd
Salicin	+	nd	nd
Sedoheptulosan	+	nd	nd
Turanose	+	nd	nd
α-Hydroxybutyric acid	+	nd	nd
Lactamide	+	nd	nd
D-Lactic acid methyl ester	+	nd	nd
L-Lactic acid	+	nd	nd
L-Serine	+	nd	nd
2,3-Butanediol	+	nd	nd
Adenosine	+	nd	nd
Inosine	+	nd	nd
Uridine	+	nd	nd
Adenosine 5′-monophosphate	+	nd	nd
Fructose 6-phosphate	+	nd	nd
Glucose 1-phosphate	+	nd	nd
Glucose 6-phosphate	+	nd	nd
DL-α-Glycerol phosphate	+	nd	nd
D-Ribose	+	nd	nd
D-Tagatose	+	nd	nd
D-Xylose	+	nd	nd
Acetic acid	+	nd	nd
Propionic acid	+	nd	nd
D-Alanine	+	nd	nd
Putrescine	+	nd	nd
Maltose	+	nd	nd
Potassium gluconate	+	nd	nd
Adipic acid	+	nd	nd
L-Malic acid	+	nd	nd
Acid production from carbohydrates using API 50 CH	nd	nd	–

Table 10.3 (continued)

Characteristic	*P. minatonensis*[a,c] KV-614^T	*P. americanus*[b,c] CP177-2^T	*P. ginsengiterrae*[c] P4-5^T
Sensitivity to			
Bacitracin	nd	+	–
Ceftriaxone	nd	+	+
Doxycycline	nd	+	+
Novobiocin	nd	+	–
Rifampicin	nd	+	+
Streptomycin	nd	+	+
Tetracycline	+	+	–
Amikacin	+	nd	+
Chloramphenicol	+	+	–
Erythromycin	+	–	–
Ciprofloxacin	+	nd	nd
Tobramycin	+	nd	+
Kanamycin	+	nd	+
Ampicillin	+	nd	+
Imipenem	+	nd	+
Cephalothin	nd	+	+
Ceftazidime	–	nd	–
Nalidixic acid	–	nd	–
Norfloxacin	–	nd	–
Oxacillin	–	nd	–
Azithromycin	nd	–	nd
Carbenicillin	nd	–	+
Ciprofloxacin	nd	–	–
Colistin	nd	–	–
Ethambutol	nd	–	nd
Nitrofurantoin	nd	–	nd
Penicillin	nd	–	nd
Sulfathiazole	nd	–	nd
Sulfisoxazole	nd	+	nd
Trimethoprim	nd	–	nd
Major polar lipids	DPG, PG, 2 GL(s)	DPG, PG	DPG, PG
Major respiratory lipoquinone	DMK-7	MK-7(H_2)	DMK-7
G+C content (mol%)	72.0[a]	72.0[b]	74.6
	72.3[c]	71.9[c]	

For symbols, see *Tables 10.1* and *10.2*
[a]Takahashi et al. 2006
[b]Reddy and Garcia-Pichel 2009
[c]Kim et al. 2012

Cell-wall peptidoglycan contains *meso*-diaminopimelic acid as diagnostic diamino acid along with alanine and glutamic acid. Major fatty acids are $C_{18:1}\omega9c$ and anteiso-$C_{15:0}$. Mycolic acids are absent. The predominant isoprenoid respiratory lipoquinone is a demethylmenaquinone 7 (DMK-7) or is a fully saturated menaquinone 7 [MK-7(H_2)]. Major polar lipids include phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG). The DNA G+C content is in the range 72–75 mol%. The type species is *Patulibacter minatonensis*. The type strain, KV-614^T (=NRRL B-24346^T=JCM 12834^T =NBRC 100761^T), was isolated from soil (Takahashi et al. 2006). *Patulibacter americanus* is other species and the type strain is CP177-2^T (=ATCC BAA-1038^T =DSM 16766^T), isolated from biological soil crusts from the Colorado Plateau, USA. Strain CP153-3 (=ATCC BAA-1037), isolated from the same source, is a reference strain of *P. americanus* (Reddy and Garcia-Pichel 2009). Another species

Table 10.4

Phenotypic and chemotaxonomic characteristics of members of the genus *Solirubrobacter*. All of the organisms are catalase positive. All of the strains are urease and arginine dihydrolase negative, do not reduce nitrate, and do not produce indole and H_2S. None of the strains utilize citrate and malate. In Api ZYM tests, all organisms are positive for esterase (C4), esterase lipase (C8), leucine arylamidase, α-glucosidase, β-glucosidase and negative for lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-fucosidase

Characteristic	*S. pauli*[a,c,d] B33D1[T]	*S. soli*[b,c] Gsoil 355[T]	*S. ginsenosidimutans*[c] BXN5-15[T]
Cell size (μm)	0.7 × 1.4	1.0 × 3.0	0.4–0.6 × 1.8–2.5
Pigmentation	Pink	Non-pigmented	Yellowish
Colony morphology	Round and convex	nd	Smooth, circular, and convex
Temperature for growth (°C)			
Range	19–38	15–35	15–37
Optimum	28–30	25–30	30
pH for growth			
Range	6.0–7.5	nd	nd
Optimum	6.5	nd	7.0
NaCl for growth (%)			
Range	0	0–1	0
Presence of			
Oxidase	–	w	+
Enzymes (Api Zym)			
Alkaline phosphatase	nd	+	nd
Valine arylamidase	+	+	w
Cystine arylamidase	–	+[b],–[c]	–
Acid phosphatase	nd	+	nd
Naphthol-AS-BI-phosphohydrolase	+	–	–
β-Galactosidase	–	+[b],–[c]	+
N-Acetyl-β-glucosaminidase	–	+	–
α-Mannosidase	+	–	–
Hydrolysis of			
Gelatin	–	+	–
Assimilation of			
Fructose	+	nd	nd
Galactose	+	nd	nd
Glucose	+[a],w[c]	+	–
Lactose	+	nd	nd
Mannose	+	+	–
Sucrose	+	+	nd
Xylose	+	nd	nd
Arabinose	w	+	+
L-Arabinose	nd	+	nd
L-Fucose	nd	+	nd
Maltose	–	+	–
D-Melibiose	nd	+	nd
L-Rhamnose	nd	+	nd
D-Ribose	nd	+	nd
Cellobiose	–	nd	nd

Table 10.4 (continued)

Characteristic	*S. pauli*[a,c,d] B33D1^T	*S. soli*[b,c] Gsoil 355^T	*S. ginsenosidimutans*[c] BXN5-15^T
Casaminoacids	+	nd	nd
Pyruvate	+	nd	nd
Acetate	+	–	nd
Adipate	–	+	–
Gluconate	+	+[b],w[c]	+
2-Ketogluconate	nd	–	nd
3-Hydroxybenzoate	nd	–	nd
3-Hydroxybutyrate	nd	–	nd
4-Hydroxybenzoate	nd	–	nd
5-Ketogluconate	nd	–	nd
Caprate	nd	–	–
Itaconate	nd	–	nd
Lactate	nd	–	nd
Succinate	–	nd	nd
Malonate	nd	–	nd
Phenyl acetate	nd	–	–
Propionate	nd	–	nd
Suberate	nd	–	nd
n-Valarate	nd	–	nd
Sorbitol	+	–	nd
D-Mannitol	–	–[b],w[c]	w
Glycerol	+	nd	nd
myo-Inositol	nd	+	nd
L-Alanine	+	–	nd
Arginine	+	nd	nd
Lysine	+	nd	nd
L-Histidine	nd	–	nd
Proline	nd	+	nd
N-Acetyl-D-glucosamine	–	+	–
Salicin	nd	+	nd
Glycogen	nd	+	nd
Chlorogenic acid	+	nd	nd
Methanol	–	nd	nd
Ethanol	–	nd	nd
1-Propanol	–	nd	nd
2-Propanol	–	nd	nd
Butanol	–	nd	nd
Isobutyl alcohol	–	nd	nd
Isoamyl alcohol	–	nd	nd
Anthranilic acid	–	nd	nd
Benzoic acid	–	nd	nd
Catechol	–	nd	nd
Protocatechuic acid	–	nd	nd
p-Coumaric acid	–	nd	nd
Gentisic acid	–	nd	nd
Ferulic acid	–	nd	nd

Table 10.4 (continued)

Characteristic	*S. pauli*[a,c,d] B33D1^T	*S. soli*[b,c] Gsoil 355^T	*S. ginsenosidimutans*[c] BXN5-15^T
p-Hydroxybenzoic acid	–	nd	nd
Syringic acid	–	nd	nd
Vanillic acid	–	nd	nd
Major polar lipids	PL(s)[d]	nd	nd
Major respiratory lipoquinone	nd	MK-7(H_4)	MK-7(H_4)
G+C content (mol%)	71.8	71.5	70.6

For symbols, see *Tables 10.1* and *10.2*
[a]Singleton et al. 2003
[b]Kim et al. 2007
[c]An et al. 2011
[d]Albuquerque et al. 2011

is *Patulibacter ginsengiterrae* and the type strain is P4-5^T (=KCTC 19427^T =CECT 7603^T), isolated from soil of a ginseng field in Republic of Korea (Kim et al. 2012).

Solirubrobacter Singleton et al. 2003

Solirubrobacter (So.li.ru.bro.bac'ter. L. n. *solum*, soil; N.L. n. *Rubrobacter*, a bacterial genus; N.L. masc. n. *Solirubrobacter*, a *Rubrobacter*-like bacterium from soil).

Solirubrobacter forms rod-shaped cells and nonmotile. Mesophilic. Catalase positive and oxidase variable. One species *Solirubrobacter pauli* grows on common sugars, polyols, organic acids, and amino acids as sole carbon sources. The API 20 NE and the API ID32 GN systems were used to assess the carbon metabolism of the species *Solirubrobacter soli* and *Solirubrobacter ginsenosidimutans*. None of the strains produce acid from glucose. Major fatty acids are $C_{18:1}\omega9c$ and iso-$C_{16:0}$. The major respiratory isoprenoid lipoquinone is a fully saturated menaquinone 7 [MK-7(H_4)]. The DNA G+C content is approximately 71 mol%. The type species is *Solirubrobacter pauli*. The type strain is B33D1^T (=ATCC BAA-492^T =DSM 14954^T) and was isolated from a burrow of the epigeic earthworm *Lumbricus rubellus* in an agricultural soil (Singleton et al. 2003). The genus *Solirubrobacter* also includes two additional species: *Solirubrobacter soli*, the type strain is Gsoil 355^T (=KCTC 12628^T =LMG 23485^T) that was isolated from soil from a ginseng field in Daejeon, South Korea (Kim et al. 2007); *Solirubrobacter ginsenosidimutans*, the type strain is BXN5-15^T (=KACC 20671^T =LMG 24459^T) that was isolated from soil of a ginseng field of Baekdu Mountain, China (An et al. 2011).

Isolation, Enrichment, and Maintenance Procedures

Conexibacter woesei was isolated from a temperate forest soil in Gerenzano, Italy, by Monciardini et al. (2003). Soil was plated on half-strength humic acid vitamins agar (HV/2 medium) (Hayakawa and Nonomura 1987) following dilution in water. Single colonies were transferred on Internacional Streptomyces Project medium 3 (ISP3, Oatmeal agar: 20 g oatmeal, 18 g agar, 1 L deionized water) (Küster 1959; Shirling and Gottlieb 1966). Colonies were serially transferred on HV/2, ISP3, and Todd–Hewitt agar (THA, Difco) plates until a pure culture was obtained. The strain was also cultured on brain heart infusion agar (BHI, Difco), Luria-Bertani (LB, Difco), tryptic soy agar (TSA, Oxoid), R2A agar (Difco), and Internacional Streptomyces Project medium 2 (ISP2, Yeast extract-malt extract agar: 4 g bacto yeast extract, 10 g bacto malt extract, 4 g bacto dextrose, 20 g agar, 1 L deionized water) (Pridham et al. 1956–57; Shirling and Gottlieb 1966). For liquid cultures, Todd–Hewitt medium, BHI, tryptone soy broth (TSB, Oxoid), and R2A medium were used.

Conexibacter arvalis was isolated from a soil sample collected from a field in Saitama, Japan, by Seki et al. (2012). Soil was plated on 1/5-strength nutrient agar (1/5 NA, Difco) with 0.002 % benlate (Dupon) and incubated at 27 °C for 21 days. The strain was cultured on 1/5 NA, NA, ISP medium 5 (DAIGO; Nihon Pharmaceutical), brain heart infusion agar (BHI, Difco), 1/5 strength brain heart infusion agar (1/5 BHI), R2A agar (Difco), 1/5 strength R2A agar (1/5 R2A agar), tryptic soy agar (TSA, Difco), Todd–Hewitt agar (THA, Difco), 1/5 strength Todd–Hewitt agar (1/5 THA), and GPM agar [1 % (w/v) glucose, 0.5 % (w/v) peptone, 0.5 % (w/v) meat extract, 0.3 % (w/v) NaCl, 1.2 % (w/v) agar, pH 7.0] at 27 °C for 14 days.

Patulibacter minatonensis was isolated from a soil sample collected at Minato-Ku, Tokyo, Japan, (Takahashi et al. 2006) using an agar medium supplemented with superoxide dismutase (SOD) following the method of Takahashi et al. (2003). GPM agar medium, consisting of 1 % (w/v) D-glucose, 0.5 % (w/v) peptone, 0.5 % (w/v) meat extract, 0.3 % (w/v) NaCl, and 1.2 % (w/v) agar supplemented with 30 U ml^{-1} *Escherichia coli* SOD (Sigma), was used for strain isolation. The strain was cultured on 1/5 strength nutrient agar (1/5 NA, Difco), ISP3 medium (Küster 1959; Shirling and Gottlieb 1966), heart infusion agar, R2A agar, and Todd–Hewitt agar (THA, Difco) and Yeast

extract/glucose agar [YD agar: 1 % (w/v) yeast extract, 1 % (w/v) glucose and 1.2 % (w/v) agar] for 7 days at 27 °C. Trypticase soy broth (TSB, BBL) was used for liquid culture.

Patulibacter americanus was isolated from a biological soil crusts (BSC) collected from the Colorado Plateau (Reddy and Garcia-Pichel 2005; Reddy and Garcia-Pichel 2009). The medium used for isolation was BG11-PGY [10 % (v/v) BG11 mineral medium, 0.25 % (w/v) peptone, 0.25 % (w/v) yeast extract, 0.25 % (w/v) glucose, 1.5 % (w/v) agar]. The composition of BG11 base was: 1.5 g $NaNO_3$, 40 mg $K_2HPO_4.3H_2O$, 75 mg $MgSO_4.7H_2O$, 36 mg $CaCl_2.2H_2O$, 6 mg citric acid, 6 mg ferric ammonium citrate, 1 mg EDTA (disodium magnesium), 20 mg Na_2CO_3, 1 ml trace metal solution in 1 L Milli-Q water, pH 7.4 [the composition of the trace metal solution is as given in Rippka et al. (1979)]. Initially, 0.5 g of crust sample was suspended in Ringer's solution (9 g NaCl, 0.042 g KCl, 0.025 g CaCl2, and 100 mL deionized water) (Reddy et al. 2006) and vortexed for 30 min. The suspension was allowed to settle and then 100 mL supernatant was plated on BG11-PGY and incubated at room temperature for 15 days. Pink-colored colonies were isolated, purified on 1/10-strength BG11-PGY (1/10 BG11-PGY) by streaking, and maintained on the same medium.

Patulibacter ginsengiterrae was isolated from soil of a ginseng field located in Geumsan Country, Republic of Korea, by Kim et al. (2012). The soil sample was diluted serially in sterile deionized water, and samples of each serial dilution were spread on 1/10 strength nutrient agar (1/10 NA, Difco) and incubated at 25 °C for 4 weeks. One colony was selected and purified by subculturing on the same medium. The strain was routinely cultured on trypticase soy agar (TSA, Difco) or in trypticase soy broth (TSB, Difco) at 25 °C for 3 days.

Solirubrobacter pauli was isolated from a burrow of the epigeic earthworm *Lumbricus rubellus* in an agricultural soil (Furlong et al. 2002), on a plate composed of 50 % nutrient broth medium (NB, Difco) at 23 °C. Isolates were allowed to grow for 2 weeks before colonies were picked. One pink colony was selected from a dilution series and maintained on the same medium. The strain was routinely cultured on NB at 30 °C.

Solirubrobacter soli was isolated by direct plating of serially diluted soil sample from a ginseng field in Daejeon, South Korea, on R2A agar (Difco) by Kim et al. (2007). Single colonies from these plates were transferred to new plates and incubated for 5 days at 30 °C. The strain was routinely cultured on R2A agar at 30 °C.

Solirubrobacter ginsenosidimutans was isolated from soil of a ginseng field of Baekdu Mountain in China (An et al. 2011). The soil sample was suspended in 50 mM phosphate buffer (pH 7.0) and spread on modified xylan-nutrient agar (0.02 g tryptone, 0.02 g yeast extract, 0.02 g malt extract, 0.02 g beef extract, 0.02 g casaminoacid, 0.02 g soytone, 1 g xylan, 0.1 g sodium pyruvate, 0.3 g K_2HPO_4, 0.05 g $MgSO_4$, 0.05 g $CaCl_2$, 15 g agar, and 1 L deionized water, pH 7.0) plates after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for 1 month. The strain was routinely cultured on R2A agar (Difco) at 25 °C.

Members of this family do not require special procedures for maintenance and long-term storage. Generally, strains are maintained on the isolation medium at 4 °C for a few days and can be stored frozen at −70 °C in medium containing 15 % glycerol without loss of viability. Long-term preservation is by lyophilization.

Ecology

Habitat

All species of the genera *Conexibacter*, *Patulibacter*, and *Solirubrobacter* originate from soil samples, indicating that the members of the three genera inhabit soils with generally low temperatures and neutral pH. Since none of the species seem to tolerate or necessitate high salt media for growth, it is unlikely that these organisms inhabit saline environments.

Environmental 16S rRNA gene sequences closely related to the species of the genera *Conexibacter*, *Patulibacter* and *Solirubrobacter* corroborate the hypothesis that most of the members of the order *Solirubrobacteriales* inhabit soil habitats. Sequences with 98 % similarity or higher to the species of *Solirubrobacter*, namely, JF176876, JF176927, HM845968, and HM845830, were recovered from human skin. Other clone sequences such as JX133394, HQ119173, and HQ397109 were encountered in soil. The same habitat seems to be true of the species of *Conexibacter* which are mostly recovered from soil (JF806520, EU223949). Clone sequences or strains closely related to *Patulibacter* spp. were detected in the intestine of an earthworm (FJ542906) and an endophytic habitat (JQ660040).

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11 The Family *Coriobacteriaceae*

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Abstract
Coriobacteriaceae is a family within the order *Coriobacteriales* (phylum Actinobacteria), which includes 30 species belonging to 14 genera: *Adlercreutzia*, *Asaccharobacter*, *Atopobium*, *Collinsella*, *Coriobacterium* (type genus), *Cryptobacterium*, *Denitrobacterium*, *Eggerthella*, *Enterorhabdus*, *Gordonibacter*, *Olsenella*, *Paraeggerthella*, *Parvibacter*, and *Slackia*. These bacteria are normal dwellers of mammalian body habitats such as the oral cavity, the gastrointestinal tract, and the genital tract. In the gut, *Coriobacteriaceae* carry out functions of importance such as the conversion of bile salts and steroids as well as the activation of dietary polyphenols. However, they can also be considered as pathobionts, because their occurrence has been associated with a range of pathologies such as bacteremia, periodontitis, and vaginosis. *Coriobacteriaceae* are usually nonmotile, nonspore-forming, nonhemolytic, and strictly anaerobic bacteria that grow as small rods; stain Gram-positive; are negative for oxidase, urease, and indole production; and are characterized by a high G+C content of DNA (around 60 mol%). Many species are asaccharolytic and possess a variety of aminopeptidases. Typical cellular fatty acids are $C_{18:1}$w9c as well as saturated fatty acids ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$) and derivatives thereof. The production of menaquinone-6 homologues of vitamin K_2 seems also to be an attribute of the family. Taking into account the aforementioned metabolic functions of *Coriobacteriaceae*, their clinical relevance and the fact that an increasing number of novel species have been described very recently, this bacterial family will surely gain an increasing attention in the field of host/bacteria interactions in the near future.

Taxonomy, Historical and Current

The proposal to create the family *Coriobacteriaceae* (Co.ri.o.bac.te.ri.a'ce.ae. M.L. neut. n. *Coriobacterium* type genus of the family; *-aceae* ending to denote a family; M.L. fern. pl. n. *Coriobacteriaceae* the *Coriobacterium* family) was first published in 1997 by Stackebrandt et al. who reported a novel hierarchic classification of the phylum Actinobacteria according to 16S ribosomal RNA (rRNA) gene-based phylogeny (Stackebrandt et al. 1997). The type genus of the family, *Coriobacterium*, includes only one species, *Coriobacterium glomerans*, originally cultured from the intestine of a red soldier bug (Haas and König 1988).

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_343,

Only five of the current members of the family were isolated before the advent of molecular phylogeny in the mid-1980s. All of them have been subjected to amended description: *Atopobium minutum* (formerly *Bacteroides minutum*, *Eubacterium minutum*, or *Lactobacillus minutus*) (Collins and Wallbanks 1992), *Atopobium parvulum* (formerly *Peptostreptococcus parvulus* or *Streptococcus parvulus*) (Collins and Wallbanks 1992), *Collinsella aerofaciens* (formerly *Bacteroides aerofaciens*, *Eubacterium aerofaciens*, or *Pseudobacterium aerofaciens*) (Kageyama et al. 1999a), *Eggerthella lenta* (formerly *Bacteroides lentus*, *Eubacterium lentum*, or *Pseudobacterium lentum*) (Wade et al. 1999), and *Slackia heliotrinireducens* (formerly *Peptococcus heliotrinreducans* or *Peptostreptococcus heliotrinreducens*) (Wade et al. 1999). The main phenotypic traits still used nowadays for the identification of most family members are as follows: Gram-positive staining; nonmotile (with the exception of *Gordonibacter pamelaeae*); nonspore-forming; nonhemolytic; mesophilic (typically with a relatively narrow range of growth temperatures around the optimum of 37 °C); usually neutrophilic and acidotolerant; strictly anaerobic, albeit some members reported to be aerotolerant (*Eggerthella lenta*, *Enterorhabdus*, and *Parvibacter* spp.) and others microaerophiles (*Olsenella* spp.) or facultative anaerobes (*Atopobium vaginae*); grow as small rods or coccobacilli that mostly occur as single cells, pairs, or chains (e.g., *Adlercreutzia equolifaciens*, *Collinsella aerofaciens*, *Collinsella tanakaei*, *Coriobacterium glomerans*, *Eggerthella* spp., *Olsenella umbonata*, *Paraeggerthella hongkongensis*); grow usually to low optical density in liquid medium (with the exception of *Atopobium*, *Collinsella*, and *Olsenella* spp.); enhanced growth in the presence of arginine (e.g., *Cryptobacterium*, *Eggerthella*, *Gordonibacter*, and *Slackia* spp.) or Tween 80 (e.g., *Atopobium* and *Olsenella* spp.); positive for arginine dihydrolase and a variety of aminopeptidases; and negative for indole production, oxidase, and urease. Many species are asaccharolytic or convert a very limited number of sugars, e.g., *Adlercreutzia equolifaciens*, *Asaccharobacter celatus*, *Eggerthella* spp., *Enterorhabdus* spp., *Paraeggerthella hongkongensis*, *Parvibacter caecicola*, and all *Slackia* species.

Researchers who isolated strains of *Coriobacteriaceae* in the early days focused mainly on the description of isolates from feces, wounds, abscesses, and gingival crevices, which drew attention to the pathogenic potential of these bacteria. To date, however, nearly all species within the *Coriobacteriaceae* are known as commensal members of mammalian microbiota. The last 5 years have seen a bloom in the number of newly described bacteria belonging to the family: 11 of the 30 known species with a standing name in nomenclature have been described since 2008 (Maruo et al. 2008; Minamida et al. 2008; Clavel et al. 2009, 2010, 2013; Matthies et al. 2009; Würdemann et al. 2009; Jin et al. 2010; Nagai et al. 2010; Kraatz et al. 2011). In light of these novel descriptions, chemotaxonomic features have emerged as important parameters for reliable taxonomic classification of isolates. Most members of the *Coriobacteriaceae* contain a high proportion of saturated cellular fatty acids (e.g., $C_{14:0}$, $C_{16:0}$, or $C_{18:0}$ and dimethyl acetal thereof) and/or $C_{18:1}$w9c. The major menaquinones hitherto reported are menaquinone-6 (MK-6) (e.g., in *Eggerthella lenta*, *Gordonibacter pamelaeae*, and *Paraeggerthella hongkongensis*), monomethylmenaquinone-6 (MMK-6) (e.g., in *Eggerthella sinensis*, *Enterorhabdus* spp., and *Parvibacter caecicola*), and dimethylmenaquinone-6 (DMMK-6) (e.g., in *Adlercreutzia equolifaciens*, *Eggerthella* spp., and *Enterorhabdus caecimuris*). The latter group of quinones seems to be unique to the *Coriobacteriaceae* (Würdemann et al. 2009). So far analyzed peptidoglycan structures are of type A4*a*, A4*b*, as well as A4*g* or A1*g* based on the presence of LL- or *meso*-diaminopimelic acid, respectively. In all species examined for the presence of polar lipids, phosphatidylglycerol and diphosphatidylglycerol as well as up to four glycolipids and three phospholipids were detected.

Molecular Analyses

Phylogenetic Structure of the Family and Its Genera

A 16S rRNA gene sequence-based phylogenetic tree of the 30 members of the family is shown in ❯ *Fig. 11.1*. The trees were reconstructed by using a subset of sequences representative of most closely related genera to stabilize the tree topology.

The first phylogenetic description of the family *Coriobacteriaceae* was published by Stackebrandt et al. (1997). Due to newly described species within the phylum Actinobacteria and the availability of their 16S rRNA gene sequences, an emended description of the family was recently published based on the analysis of 2,642 actinobacterial sequences with >1,300 unambiguous nucleotides (between position 100 and 1,400) (Zhi et al. 2009). The authors reported that the order *Coriobacteriales* (and thus *Coriobacteriaceae*, the sole family within this order) constitutes one of the deepest branches within the phylum Actinobacteria together with the lineages of the order *Rubrobacterales* (e.g., *Thermoleophilaceae*, *Conexibacteraceae*) and *Acidimicrobiales*. The pattern of 16S rRNA signatures of *Coriobacteriaceae* consists of nucleotides at positions 242 : 284 (C–G), 291 : 309 (C–G), 316 : 337 (U–G), 819 (A), 952 : 1229 (U–A), and 1115: 1185 (C–G). Before the first description of the family by Stackebrandt et al. (1997), 16S rRNA-based phylogeny had already played an important role for the sake of emended description of several misclassified member species, including *Lactobacillus minutus*, *Lactobacillus rimae*, and *Streptococcus parvulus* (Collins and Wallbanks 1992). The genus *Atopobium* has then served, together with *Coriobacterium*, as a phylogenetic core of the *Coriobacteriaceae* and has been used to demonstrate that the inclusion of a broad range of physiologically diverse bacteria and thoughtful selection of out-groups are essential prerequisites for drawing proper phylogenetic conclusion (Rainey et al. 1994; Stackebrandt and Ludwig 1994). Thereafter, 16S rRNA gene-based phylogenetic evidence has largely contributed to the reclassification of additional members of the *Coriobacteriaceae*, e.g., *Atopobium fossor*, *Collinsella aerofaciens*, *Eggerthella lenta*, *Slackia exigua*, and *Slackia heliotrinireducens* (Kageyama et al. 1999a, b; Wade et al. 1999).

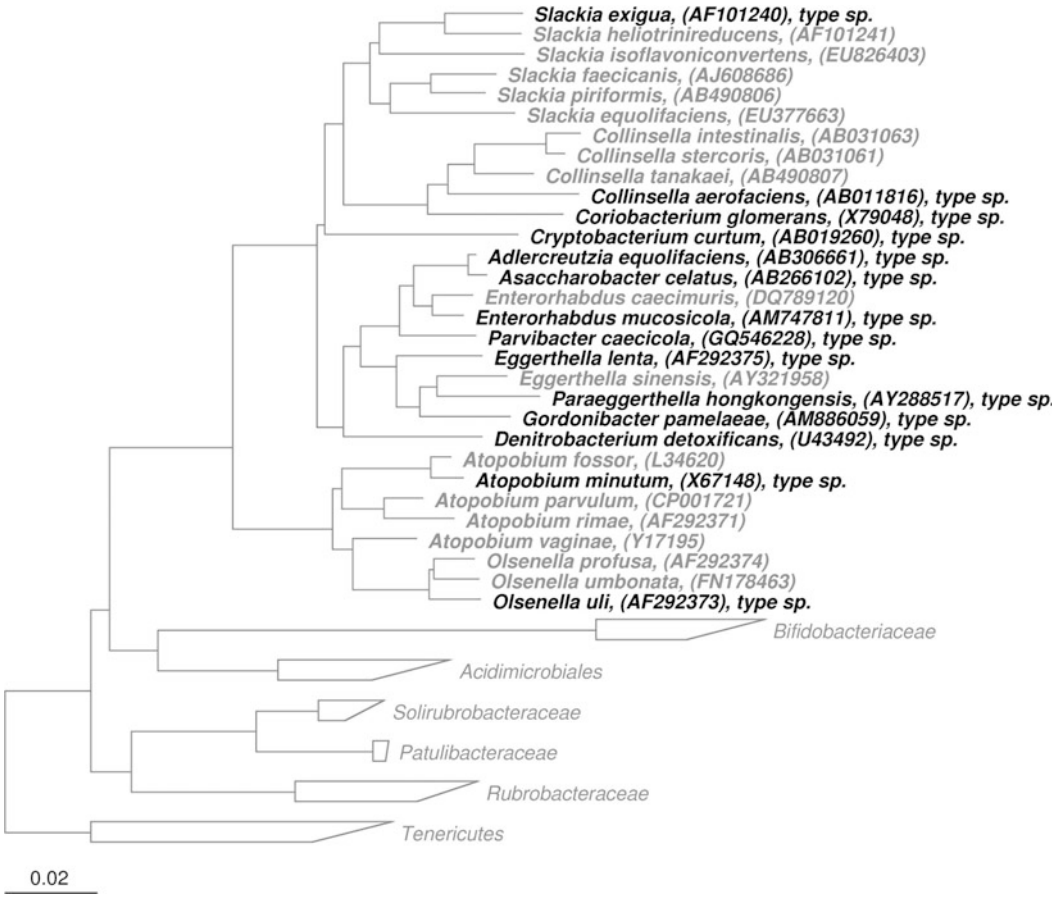

Fig. 11.1

Phylogenetic reconstruction of the family *Coriobacteriaceae* 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 amongst 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

According to information retrieved from the Ribosomal Database Project, 749 isolates that relate to the *Coriobacteriaceae* family have been described (full and partial 16S rDNA length), most of them from the *Atopobium* genus (337) and unclassified *Coriobacteriaceae* (117), followed by *Olsenella* (94), *Collinsella* (63), *Cryptobacterium* (39), *Eggerthella* (25), *Slackia* (22), *Paraeggerthella* (20), *Coriobacterium* (10), *Adlercreutzia* (9), *Gordonibacter* (6), *Denitrobacterium* (4), *Enterorhabdus* (2), and *Asaccharobacter* (1). Even though the number of 16S rRNA operons varies greatly between species (from 1 operon to 7 in *Collinsella aerofaciens*), their average number is low (2.4) since most of the sequenced strains have only one to two 16S rRNA operons.

DNA-Based Analysis and Genome Comparison

With the exception of *Atopobium* spp. and *Cryptobacterium curtum*, family members are characterized by a high G+C content of DNA (approximately 60 mol% and above). All DNA-DNA relatedness values available in the literature for members of the *Coriobacteriaceae* are given in *Table 11.1*.

Table 11.2 gathers most relevant information on genome sequencing projects focused on members of the *Coriobacteriaceae*. Representative genomes are available for 24 species belonging to 8 of the *Coriobacteriaceae* genera: *Atopobium* ($n = 6$ genomes), including *Atopobium parvulum*, *Atopobium rimae*, and *Atopobium vaginae* (3 strains); *Collinsella* ($n = 4$ genomes), including *Collinsella aerofaciens*, *Collinsella intestinalis*, *Collinsella stercoris*, and *Collinsella tanakaei*; *Coriobacterium glomerans* ($n = 1$ genome); *Cryptobacterium curtum* ($n = 1$ genome); *Eggerthella lenta* ($n = 4$ genomes from 3 strains); *Gordonibacter pamelaeae* ($n = 1$ genome); *Olsenella* ($n = 2$ genomes), including *Olsenella uli* and *Olsenella* sp. oral taxon 809; *Slackia* ($n = 4$ genomes), including *Slackia exigua*, *Slackia heliotrinireducens*, and *Slackia piriformis* (2 strains); and unclassified *Coriobacteriaceae* ($n = 1$ genome; *Coriobacteriaceae* bacterium JC110).

Table 11.1
DNA-DNA homology between species of *Coriobacteriaceae*

Strain 1	Strain 2	%	Reference
Atopobium minutum VPI 9428^T	*Atopobium parvulum* VPI 0546^T	<16	Olsen et al. (1991)
Atopobium minutum VPI 9428^T	*Atopobium rimae* VPI D140H-11A^T	<11	Olsen et al. (1991)
Atopobium parvulum VPI 0546^T	*Atopobium rimae* VPI D140H-11A^T	16	Olsen et al. (1991)
Atopobium minutum VPI 9428^T	*Olsenella uli* VPI D76D-27C^T	4	Olsen et al. (1991)
Atopobium parvulum VPI 0546^T	*Olsenella uli* VPI D76D-27C^T	5	Olsen et al. (1991)
Atopobium rimae VPI D140H-11A^T	*Olsenella uli* VPI D76D-27C^T	8	Olsen et al. (1991)
Collinsella aerofaciens JCM 10188^T	*Collinsella intestinalis* RCA56-68^T	8	Kageyama and Benno (2000)
Collinsella aerofaciens JCM 10188^T	*Collinsella stercoris* RCA 55-54^T	8	Kageyama and Benno (2000)
Collinsella intestinalis RCA56–68^T	*Collinsella stercoris* RCA 55-54^T	<25	Kageyama and Benno (2000)
Cryptobacterium curtum 12–3^T	*Eggerthella lenta* ATCC 25559^T	<5	Nakazawa et al. (1999), Nakazawa and Hoshino (2004)
Cryptobacterium curtum 12–3^T	*Slackia exigua* ATCC 700122^T	4	Nakazawa and Hoshino (2004)
Cryptobacterium curtum 12–3^T	*Slackia heliotrinireducens* ATCC 29202^T	5	Nakazawa and Hoshino (2004)
Eggerthella lenta ATCC 25559^T	*Slackia exigua* ATCC 700122^T	<11	Poco et al. (1996), Nakazawa and Hoshino (2004)
Eggerthella lenta ATCC 25559^T	*Slackia heliotrinireducens* ATCC 29202^T	10	Nakazawa and Hoshino (2004)
Enterorhabdus caecimuris B7^T	*Enterorhabdus mucosicola* Mt1-B8^T	28	Clavel et al. (2010)
Olsenella profusa CCUG 45371^T	*Olsenella uli* CCUG 31166^T	33	Kraatz et al. (2011)
Olsenella profusa CCUG 45371^T	*Olsenella umbonata* lac31^T	50	Kraatz et al. (2011)
Olsenella uli CCUG 31166^T	*Olsenella umbonata* lac31^T	47	Kraatz et al. (2011)
Slackia exigua ATCC 700122^T	*Slackia heliotrinireducens* ATCC 29202^T	33	Nakazawa and Hoshino (2004)
Slackia isoflavoniconvertens HE8^T	*Slackia exigua* CCUG 44588^T	18	Matthies et al. (2009)
Slackia isoflavoniconvertens HE8^T	*Slackia faecicanis* DSM 17537^T	29	Matthies et al. (2009)
Slackia isoflavoniconvertens HE8^T	*Slackia heliotrinireducens* DSM 20476^T	22	Matthies et al. (2009)

A complete genome is available for eight of the sequenced organisms, whereas the others are whole genome shotgun under completion. Fourteen of the 24 sequenced species are human isolates; four of them were isolated from diseased patients (caries, periodontitis, or bacteremia). Genome size ranges from 1,418,601 (*Atopobium vaginae* DSM 15829^T) to 3,632,260 bp (*Eggerthella lenta* DSM 2243^T). No plasmids have been described. One chromosome has been described for each of the sequenced strains. The number of genes is lowest in the *Atopobium* genus and highest in *Eggerthella lenta* DSM 2243^T. On average, 73.2 % of genes can be assigned to Clusters of Orthologous Groups (COGs). This ranges from 66.5 % in *Collinsella* spp. (min. 60.0 % in *Collinsella stercoris*) to 80.7 % in *Coriobacterium glomerans* PW2. In *Eggerthella* sp. YY7918, Yokoyama et al. reported an incomplete carbohydrate metabolic pathway in KEGG, supporting the observation that members of this genus are known to be asaccharolytic (Yokoyama et al. 2011). Several phage-related genes have been described in the genomes of all members of the family (*Table 11.3*). The highest number of phage-related genes is observed in the genome of *Atopobium rimae* ATCC 49626 ($n = 20$) and *Collinsella stercoris* DSM 13279 ($n = 16$). However, no phages have been described to lyse or infect strains of the *Coriobacteriaceae*.

The complete genomes of six sequenced strains were compared to the biggest genome of the family, i.e., *Eggerthella lenta* DSM 2243^T, using RAST (Aziz et al. 2008) (*Fig. 11.2*). Genes were annotated to proteins and results were computed using BLASTP (uni- and bidirectionally) to compare every protein in the reference genome (*Eggerthella lenta*) to every protein in the comparison genomes. Out of the 3,308 total proteins in *Eggerthella lenta*, 115 proteins were shared with the six other sequenced strains at a threshold of 60 % similarity. A major part of these genes were related to ribosomal proteins. Proteins that were not directly related to ribosomal proteins ($n = 73$) belonged to several COGs family, but originated mainly from the family J (translation, ribosomal structure, and biogenesis), L (DNA replication, recombination, and repair), O (posttranslational modification, protein turnover, chaperones), and R (general function prediction only). As expected, the genome of *Eggerthella* sp. YY7918 was the most closely related to that of *Eggerthella lenta*, followed by *Slackia heliotrinireducens*.

Phenotypic Analyses

Unless otherwise stated, all so far described species are Gram-positive, nonspore-forming, nonmotile, strictly anaerobic small rods or coccobacilli (*Fig.11.3*) that are negative for oxidase, urease, hemolysis, and indole production. The main

Table 11.2

***Coriobacteriaceae* family members for which the genome is completely or partially sequenced. Bacteria are listed according to their genome size. Data were extracted from the PATRIC resource (Gillespie et al. 2011). *Abbreviations*: *WGS* whole genome shotgun, *CDS* coding sequences**

Genome name	NCBI taxon Id	Genome status	Type strain	Publication (PMID)	GenBank accession	Genome length	GC content	RAST CDS
Atopobium vaginae DSM 15829	525256	WGS	Yes	Unpublished	ADNA00000000	1,418,601	42.7	1,214
Atopobium vaginae DSM 15829	525256	WGS	Yes	Unpublished	ACGK00000000	1,435,317	42.7	1,197
Atopobium vaginae PB189-T1-4	866774	WGS	No	Unpublished	AEDQ00000000	1,448,900		1,282
Atopobium parvulum DSM 20469	521095	Complete	Yes	21304653	CP001721	1,543,805	45.7	1,329
Cryptobacterium curtum DSM 15641	469378	Complete	Yes	21304644	CP001682	1,617,804	50.9	1,351
Atopobium rimae ATCC 49626	553184	WGS	No	Unpublished	ACFE00000000	1,626,291	49.3	1,480
Collinsella intestinalis DSM 13280	521003	WGS	Yes	Unpublished	ABXH00000000	1,809,497	62.5	1,537
Olsenella uli DSM 7084	633147	Complete	Yes	21304694	CP002106	2,051,896		1,805
Slackia sp. CM382	1111137	WGS	No	Unpublished	ALNO01	2,051,910	-	1,803
Slackia exigua ATCC 700122	649764	WGS	No	Unpublished	ACUX00000000	2,096,289	62.1	1,813
Slackia piriformis YIT 12062	742818	WGS	Yes	Unpublished	ADMD01	2,100,457	-	1,967
Coriobacterium glomerans PW2	700015	Complete	No	Unpublished	CP002628	2,115,681	60	1,936
Olsenella sp. oral taxon 809 str. F0356	661087	WGS	No	Unpublished	ACVE01	2,159,805	-	1,905
Coriobacteriaceae bacterium JC110	1034345	WGS	No	Unpublished	CAEM01	2,354,438	62.1	1,973
Atopobium sp. ICM58	1105030	WGS	No	Unpublished	ALIY01	2,390,495	-	1,968
Collinsella aerofaciens ATCC 25986	411903	WGS	Yes	Unpublished	AAVN00000000	2,439,869	60.5	2,110
Collinsella stercoris DSM 13279	445975	WGS	Yes	Unpublished	ABXJ00000000	2,475,429	63.2	1,805
Collinsella tanakaei YIT 12063	742742	WGS	Yes	Unpublished	ADLS01	2,482,197	–	2,190
Eggerthella sp. YY7918	502558	Complete	No	21914883	AP012211	3,123,671		2,715
Slackia heliotrinireducens DSM 20476	471855	Complete	Yes	Unpublished	CP001684	3,165,038	60.2	2,824
Eggerthella sp. HGA1	910311	WGS	No	Unpublished	AEXR00000000	3,362,931		3,021
Eggerthella sp. 1_3_56FAA	665943	WGS	No	Unpublished	ACWN00000000	3,453,272		3,045
Gordonibacter pamelaeae 7–10–1-b	657308	Complete	No	Unpublished	FP929047	3,608,022		3,083
Eggerthella lenta DSM 2243	479437	Complete	Yes	21304654	CP001726	3,632,260	64.2	3,212

discriminative features of *Coriobacteriaceae* at the genus level are listed in *Table 11.4*. Many species possess a range of amino-peptidases likely to be important for amino acid release from the environment, N cycling processes, ammonia production, and which are useful selective parameters for the classification of *Coriobacteriaceae*. Thus, information on arginine dihydrolase and amino acid arylamidases is summarized at the species level in *Table 11.5*.

Adlercreutzia Maruo et al. (2008)

Ad.ler.creut'zi.a. N.L. fem. n. *Adlercreutzia* named after H. Adlercreutz (Emeritus Professor, University of Helsinki, Finland), for his contributions to research on the effects of phytoestrogens on human health.

The genus is represented only by the type species *Adlercreutzia equolifaciens* (e.quo.li.fa'ci.ens. N.L. n. *equol-olis*

Table 11.3
Listing of phage-related genes in sequenced genomes of *Coriobacteriaceae*

Gene product name (sample locus tag)	*Atopobium vaginae* DSM 15829	*Atopobium vaginae* PB189-T1-4	*Atopobium rimae* ATCC 49626	*Atopobium parvulum* IPP 1246, DSM 20469	*Collinsella aerofaciens* ATCC 25986	*Collinsella stercoris* DSM 13279	*Collinsella intestinalis* DSM 13280	*Collinsella tanakaei* YIT 12063	*Cryptobacterium curtum* 12-3, DSM 15641	*Eggerthella* sp. HGA1	*Eggerthella* sp. 1_3_56FAA	*Eggerthella lenta* VPI 0255, DSM 2243	*Eggerthella* sp. YY7918	*Gordonibacter pamelaeae* 7-10-1-bT, DSM 19378	*Slackia heliotrinireducens* RHS1, DSM 20476	*Slackiaexigua* ATCC 700122	*Olsenella uli* VPI, DSM 7084	*Coriobacterium glomerans* PW2, DSM 20642
Conserved hypothetical phage AbiD protein (HMPREF0091_0188)	1																	
Holin, phage phi LC3 family (Apar_0594)			1	1	1	1			1									
Hypothetical membrane protein with similarity to phage infection protein (ATORI0001_0646)			1															
Integrase/recombinase, phage integrase family protein (HMPREF9404_4029)										1								
Lambda family phage portal protein (HMPREF9452_00397)								1										
Phage capsid family (GPA_22850)														1				
Phage DNA replication protein (predicted replicative helicase loader) (COLAER_00687)					1									1	1			
Phage family integrase (ATORI0001_0862)			1															
Phage integrase (HMPREF1023_00378)											4					2		
Phage integrase family protein (ATORI0001_1210)			1															
Phage integrase family (COLAER_00798)					2	3												
Phage integrase, N-terminal SAM domain protein (HMPREF9248_0962)		2								1								
Phage major capsid protein, HK97 family (Shel_08230)															1			
Phage major tail protein, -1 family (ATORI0001_0997)			1															
Phage major tail protein, phi13 family (COLSTE_02157)						1												
Phage minor capsid protein 2 (COLSTE_01119)						1												
Phage minor structural protein (Elen_2638)												1			1			
Phage minor structural protein, N-terminal region (COLSTE_01099)						2												
Phage *N*-6-adenine-methyltransferase (Elen_2611)											1	1						
Phage portal protein (ATORI0001_1181)			1															

Phage portal protein, HK97 family (Shel_08250)															1			
Phage portal protein, SPP1 family (ATORI0001_1007)			1															
Phage portal protein, SPP1 Gp6-like (COLAER_00274)					1									1				
Phage prohead protease, HK97 family (COLSTE_02163)	2					1									1			
Phage protein (ATORI0001_1002)			5															
Phage putative head morphogenesis protein, SPP1 gp7 family (COLAER_00268)					1													
Phage putative tail component, N-terminal domain (COLSTE_01100)						2												
Phage recombination protein Bet (Ccur_02960)					1	1			1									
Phage-related protein (ATORI0001_1186)			1															
Phage scaffold protein (ATORI0001_1003)			1															
Phage shock protein (HMPREF0762_01151)															1			
Phage shock protein A (IM30), suppresses sigma54-dependent transcription (GPA_11810)													2	1				
Phage shock protein C, PspC (Elen_0248)								1										
Phage SPO1 DNA polymerase-related protein (Corgl_0200)				1								1					1	1
Phage structural protein (ATORI0001_0992)			1															
Phage tail component (Elen_2637)											1							
Phage tail protein I (HMPREF1023_00258)										1								
Phage tail tape measure protein, family, core region (ATORI0001_1171)			1															
Phage tail tape measure protein, TP901 family (Ccur_02660)						1			1			1						
Phage tape measure protein (Apar_0588)				1														
Phage terminase-like protein, large subunit (GPA_22790)						1		1					1	1				
Phage terminase, large subunit, PBSX family (ATORI0001_1521)			1		1	1			1			1						
Phage terminase (COLINT_00860)							1											
Phage uncharacterized protein, XkdX family (COLAER_01621)					1													
Phage-associated deoxyribonuclease (HMPREF0762_01369)															1			
Phage-associated protein (EGYY_10090)												1						
Phage-related protein (COLINT_00124)						1	2											
Prophage LambdaCh01, site-specific recombinase, phage integrase family (HMPREF0762_01054)															1			
Putative phage head-tail adaptor (HMPREF9248_0591)		1																
Putative phage integrase (HMPREF0762_01085)															1			
Putative phage major tail protein (ATORI0001_1174)			1															
Putative phage *N*-6-adenine-methyltransferase (HMPREF9404_4960)										1								
Putative phage repressor (Corgl_1278)																		1
Putative phage terminase, large subunit (ATORI0001_1183)			1															
Site-specific recombinase, phage integrase family (HMPREF9248_0052)		1	1							2					1			
Total phage-related genes	3	4	20	3	9	16	3	3	4	5	6	6	1	6	7	7	1	2

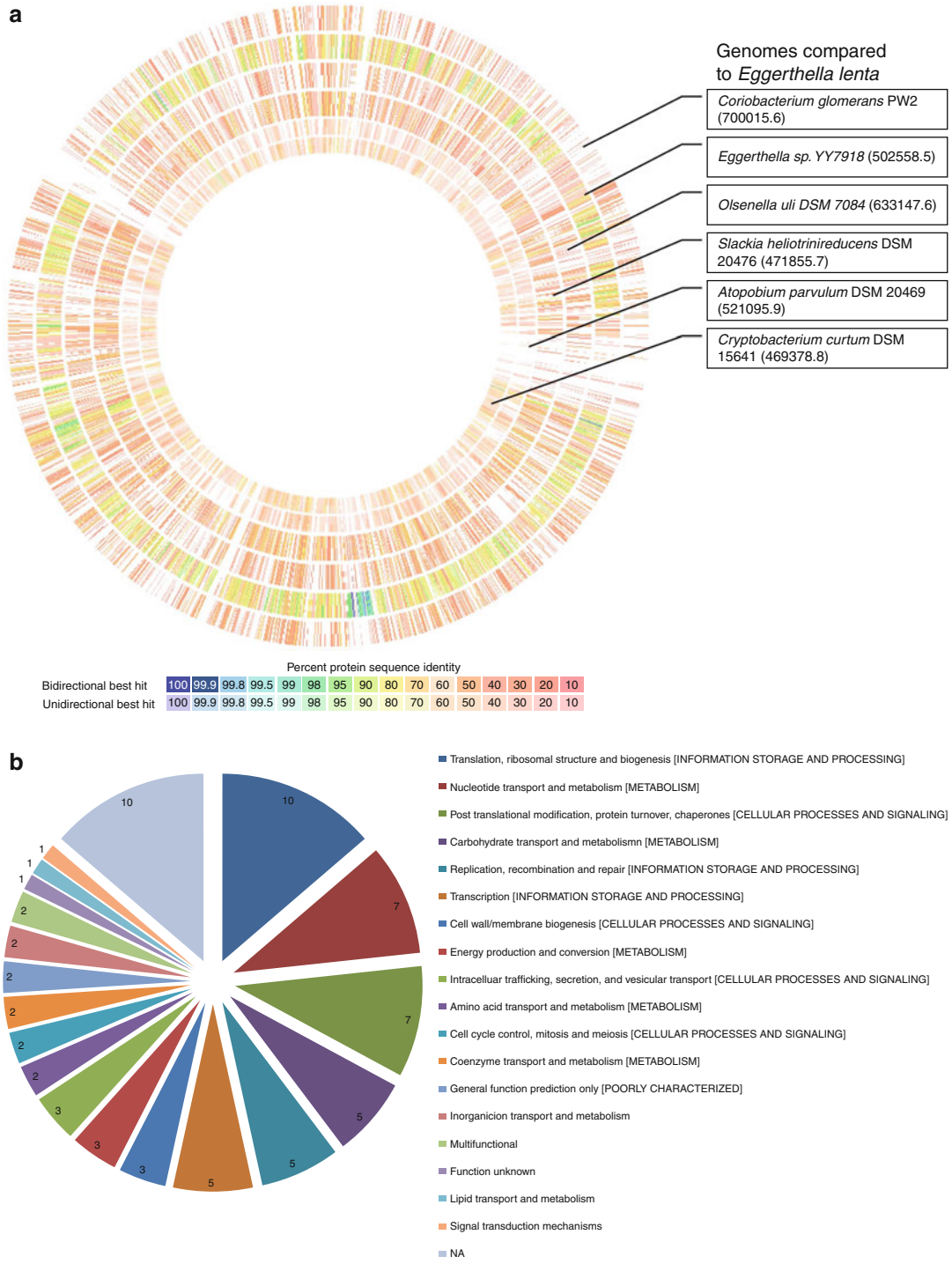

Fig. 11.2

Comparative genome analysis of *Coriobacteriaceae*. (a) Circular map of proteins encoded by the different compared genomes with percent similarity to the reference genome *Eggerthella lenta* (the order of proteins refers to the order of the contigs/genes in the reference genome). The amino acid identity of the query genomes relative to the reference is color coded on a logarithmic scale following the visible spectrum. (b) Functional category distribution of non-ribosomal proteins ($n = 73$) shared by the seven *Coriobacteriaceae* genomes

equol; L. part. adj. *faciens* making; N.L. part. adj. *equolifaciens* equol-producing). Cells are coccobacilli (0.6–0.7 × 1.5–2.7 μm) arranged in chains. Colonies on blood agar are 1–2 mm in diameter, grey to off-white grey, circular, entire, slightly convex, and smooth. The species does not grow in 20 % bile. It is asaccharolytic and positive for arginine dihydrolase as well as arginine and leucine arylamidase. No metabolic end product is detected in peptone-yeast extract medium

supplemented with glucose. Growth is stimulated by arginine. Nitrate is not reduced. *Adlercreutzia equolifaciens* converts the isoflavone daidzein into equol. Its cell wall contains A1*g*-type peptidoglycan with an (L-Ala)–D-Glu–m-Dpm peptide subunit. The diamino acid in the peptidoglycan is *meso*-diaminopimelic acid. The principal respiratory quinone is DMMK-6 (70–96 %). MMK-6 is a minor component (1–29 %). The major cellular fatty acid is $C_{18:1}$*cis*9. The G+C content of DNA is 64–67 mol% (64 mol% for the type strain). The description is based on the study of four strains: FJC-A10, FJC-B9, FJC-B20, and FJC-D53. The type strain is FJC-B9^T (= JCM 14793^T = DSM 19450^T = CCUG 54925^T).

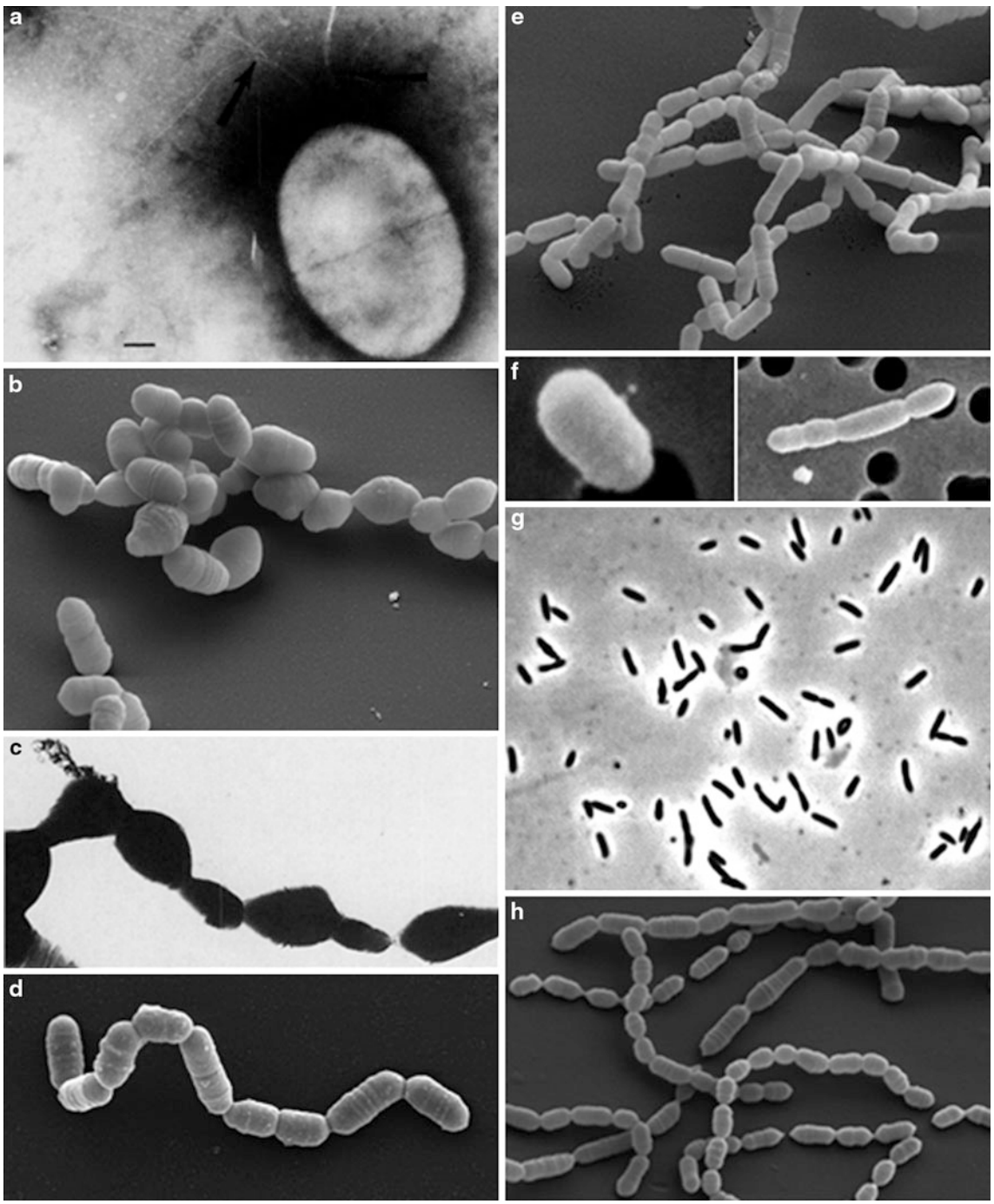

Fig. 11.3 (Continued)

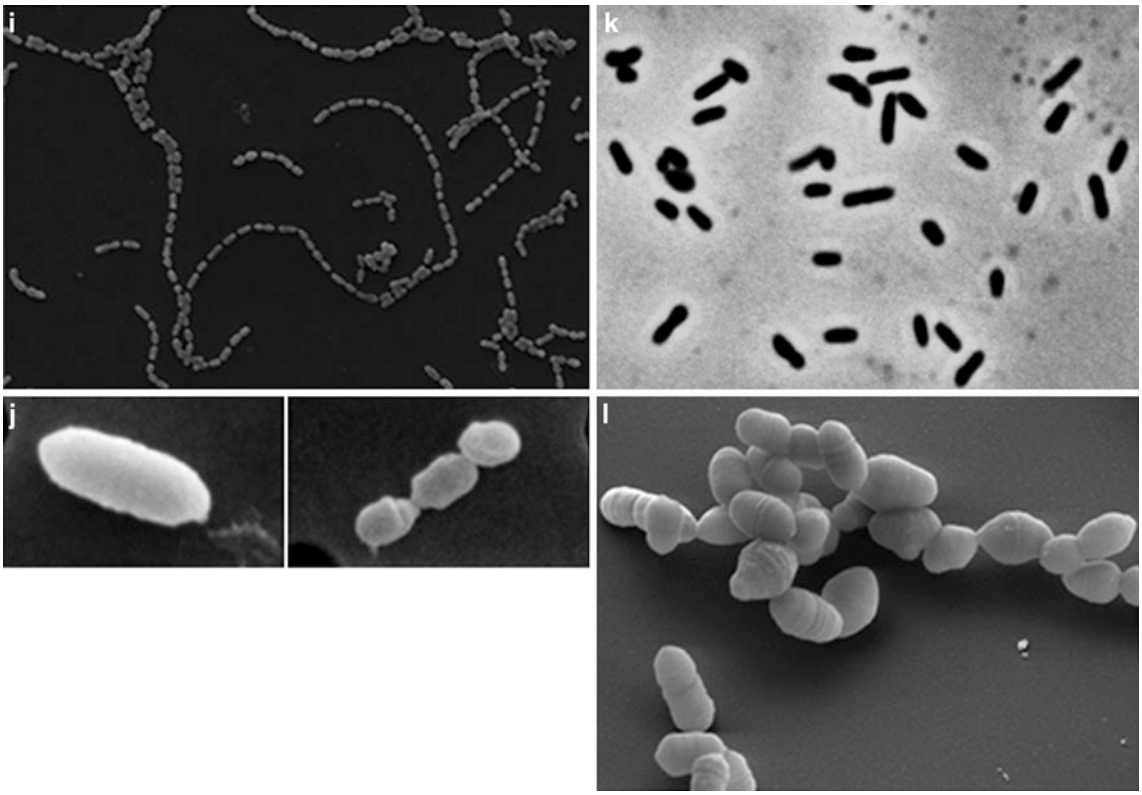

Fig. 11.3
Cell morphology of members of the *Coriobacteriaceae*. (a) *Atopobium fossor* (Bailey and Love 1986), (b) *Atopobium parvulum* (Copeland et al. 2009), (c) *Coriobacterium glomerans* (Haas and König 1988), (d) *Cryptobacterium curtum* (Mavrommatis et al. 2009), (e) *Eggerthella lenta* (Saunders et al. 2009), (f) *Eggerthella sinensis* (Lau et al. 2004b), (g) *Enterorhabdus mucosicola* (Clavel et al. 2009), (h) *Olsenella uli* (Goker et al. 2010), (i) *Olsenella umbonata* (Kraatz et al. 2011), (j) *Paraeggerthella hongkongensis* (Lau et al. 2004a), (k) *Parvibacter caecicola* (Clavel et al. 2013), (l) *Slackia heliotrinireducens* (Pukall et al. 2009)

Asaccharobacter Minamida et al. (2008)

A.sac.cha.ro.bac'ter. Gr. pref. *a-* not; Gr.n.*saccharon* sugar; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Asaccharobacter* rod that does not digest sugar.

The genus is represented only by the type species *Asaccharobacter celatus* (ce.la'tus. L. masc. adj. *celatus* conceal, hide, keep secret). This species is phylogenetically closely related to *Adlercreutzia equolifaciens* FJC-B9^T and strain Julong 732 (>99 % similarity) based on partial 16S rRNA gene sequence analysis. DNA-DNA hybridization analysis of these three isolates has not been performed so far. In contrast to *Adlercreutzia equolifaciens*, *Asaccharobacter celatus* can grow in 20 % bile, is negative for leucine arylamidase, and is characterized by the presence of a dominant lipoquinone that is not MK, MMK, DMMK, ubiquinone, or rhodoquinone. Cells are rod-shaped (0.45 × 2.3–2.7 μm). Colonies are smooth, clear, and colorless on GAM agar, reaching 1 mm in diameter after 2 days at 37 °C. Growth is enhanced in the presence of arginine, but not Tween 80. The species does not reduce nitrate, is asaccharolytic, and produces trace amounts of organic acids (lactic, acetic, and succinic acid) in medium containing peptone, yeast extract, and glucose. It is capable of converting the isoflavone daidzein to equol. Cells do not produce acid from/show negative test results in the API 50 CH system with the following substrates: glycerol, glucose, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl *b*-D-xyloside, galactose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl *a*-D-mannoside, methyl *a*-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, and 5-ketogluconate. Cells show strong naphthol-AS-BI-phosphohydrolase activity, medium acid phosphatase activity, and weak alkaline phosphatase and esterase (C4) activities. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid, alanine, and glutamic acid. The predominant cellular fatty acid is $C_{18:1}$*cis*9. The G+C content of DNA is 63 mol%. The type strain is do03^T (= JCM 14811^T = DSM 18785^T = AHU 1763^T).

Table 11.4

Comparison of selected characteristics of genera within the family *Coriobacteriaceae*

Characteristic	*Adlercreutzia*	*Asaccharobacter*	*Atopobium*	*Collinsella*	*Coriobacterium*
Growth requirement	Strictly anaerobic	Strictly anaerobic	Strictly or facultative anaerobic	Strictly anaerobic	Strictly anaerobic
Motility	–	–	–	–	–
Growth stimulated by arginine	+	+	–	ND	ND
Growth stimulated by Tween 80	–	–	+	+	ND
Nitrate reduction	–	–	–	–	–
Catalase	–	–	–	–	–
Esculin hydrolysis	ND	–	v	v	ND
Asaccharolytic	+	+	v	–	–
Lactate production	–	trace	+	trace	+
Main CFA	$C_{18:1}$w9c	$C_{18:1}$w9c	$C_{18:1}$w9c FAME/DMA	$C_{18:1}$w9c; $C_{18:1}$w9c DMA	ND
% saturated CFA (major sCFA)	67 ($C_{18:0}$ DMA)	20 ($C_{18:0}$)	14–16 ($C_{10:0}$ FAME; $C_{16:0}$ DMA)	3–30 ($C_{16:0}$ DMA; $C_{18:0}$ DMA)	ND
Major respiratory quinone	DMMK-6	Unidentified	ND	Not detected	ND
G+C mol%	64–67	63	39–46	60–64	60–61
Characteristic	***Cryptobacterium***	***Denitrobacterium***	***Eggerthella***	***Enterorhabdus***	***Gordonibacter***
Growth requirement	Strictly anaerobic	Strictly anaerobic	Strictly anaerobic	Strictly anaerobic	Strictly anaerobic
Motility	–	–	–	–	+
Growth stimulated by arginine	+	ND	+	ND	+
Growth stimulated by Tween 80	–	ND	–	ND	ND
Nitrate reduction	–	+	v	–	–
Catalase	–	–	+	–	+
Esculin hydrolysis	–	ND	–	v	ND
Asaccharolytic	+	+	+	+	–
Lactate production	–	ND	trace	ND	ND
Main CFA	ND	$C_{14:0}$ FAME; $C_{16:0}$ DMA	$C_{16:0}$ DMA	$C_{16:0}$	ai-$C_{15:0}$; $C_{16:0}$ DMA
% saturated CFA (major sCFA)	ND	87 ($C_{14:0}$ FAME; $C_{16:0}$ DMA)	61–76 ($C_{16:0}$ DMA)	70–71 ($C_{16:0}$)	89 (ai-$C_{15:0}$; $C_{16:0}$ DMA)
Major respiratory quinone	ND	ND	MK-6; MMK-6; DMMK-6	MMK-6	MK-6
G+C mol%	50–51	56–60	62–66	64–65	66

Table 11.4 (continued)

Characteristic	*Olsenella*	*Paraeggerthella*	*Parvibacter*	*Slackia*
Growth requirement	Microaerophilic or strictly anaerobic	Strictly anaerobic	Strictly anaerobic	Strictly anaerobic
Motility	−	−	−	−
Growth stimulated by arginine	−	ND	ND	+
Growth stimulated by Tween 80	+	ND	ND	−
Nitrate reduction	−	−	−	v
Catalase	−	+	−	−
Esculin hydrolysis	v	ND	ND	−
Asaccharolytic	−	+	+	+
Lactate production	+	ND	ND	−
Main CFA	$C_{14:0}$; $C_{18:0}$; $C_{18:1}$w9c; $C_{18:1}$w9c DMA	$C_{18:1}$w9c	$C_{16:0}$	$C_{18:1}$w9c; $C_{18:1}$w9c DMA
% saturated CFA (major sCFA)	54–100 ($C_{14:0}$; $C_{18:0}$)	49 ($C_{16:0}$ DMA)	75 ($C_{16:0}$)	16–42 ($C_{14:0}$; $C_{16:0}$DMA; $C_{18:0}$DMA)
Major respiratory quinone	ND	MK-6	MMK-6	ND or not detected
G+C mol%	63–64	61–62	63	58–64

Symbols and abbreviations: + positive, − negative, *ai* anteiso, *DMA* dimethyl acetal, *FAME* fatty acid methyl ester, *MK* menaquinone, *MMK* methylmenaquinone, *DMMK* dimethylmenaquinone, *ND* not determined, *v* variable depending on species, *CFA* cellular fatty acids

Table 11.5
Detection of aminopeptidase activity in *Coriobacteriaceae* species

Aminopeptidase	*Adlercreutzia equolifaciens*^{T}	*Asaccharobacter celatus*^{T}	*Atopobium minutum*^{T}	*Atopobium parvulum*	*Atopobium rimae*
Arginine dihydrolase	+	ND	v	−	−
Alanine arylamidase	ND	ND	v	+	−
Arginine arylamidase	+	ND	+	+	−
Cystine arylamidase	ND	−	ND	ND	ND
Glycine arylamidase	ND	ND	V	+	−
Histidine arylamidase	ND	ND	V	−	−
Leucine arylamidase	+	−	V	+	−
Leucyl glycine arylamidase	ND	ND	+	ND	ND
Lysine arylamidase	ND	ND	ND	ND	ND
Phenylalanine arylamidase	ND	ND	−	ND	ND
Proline arylamidase	ND	ND	v	−	−
Serine arylamidase	ND	ND	−	−	−
Tyrosine arylamidase	ND	ND	−	+	−
Valine arylamidase	ND	−	ND	ND	ND

Aminopeptidase	*Atopobium vaginae*	*Collinsella aerofaciens*^{T}	*Collinsella intestinalis*	*Collinsella stercoris*	*Collinsella tanakaei*
Arginine dihydrolase	+	+	+	+	+
Alanine arylamidase	−	−	−	+	−
Arginine arylamidase	+	+	+	+	+
Cystine arylamidase	ND	−	−	−	−
Glycine arylamidase	+	+	+	+	+
Histidine arylamidase	+	+	+	+	+
Leucine arylamidase	+	+	+	+	+

Table 11.5 (continued)

Aminopeptidase	*Atopobium vaginae*	*Collinsella aerofaciens*^{T}	*Collinsella intestinalis*	*Collinsella stercoris*	*Collinsella tanakaei*
Leucyl glycine arylamidase	ND	+	+	+	+
Lysine arylamidase	ND	ND	ND	ND	ND
Phenylalanine arylamidase	ND	–	–	–	–
Proline arylamidase	+	+	–	–	+
Serine arylamidase	+	–	–	+	–
Tyrosine arylamidase	–	–	–	+	–
Valine arylamidase	ND	–	–	–	–
Aminopeptidase	***Cryptobacterium curtum*^{T}**	***Eggerthella lenta*^{T}**	***Eggerthella sinensis***	***Enterorhabdus caecimuris***	***Enterorhabdus mucosicola*^{T}**
Arginine dihydrolase	+	+	+	+	+
Alanine arylamidase	ND	–	–	–	+
Arginine arylamidase	ND	v	+	–	–
Cystine arylamidase	ND	ND	ND	ND	ND
Glycine arylamidase	ND	–	–	–	+
Histidine arylamidase	ND	–	–	–	+
Leucine arylamidase	ND	–	–	–	+
Leucyl glycine arylamidase	ND	–	–	–	–
Lysine arylamidase	ND	ND	+	–	+
Phenylalanine arylamidase	ND	–	–	–	+
Proline arylamidase	ND	–	–	–	+
Serine arylamidase	ND	–	–	–	+
Tyrosine arylamidase	ND	–	–	–	+
Valine arylamidase	ND	ND	ND	ND	ND
Aminopeptidase	***Gordonibacter pamelaeae*^{T}**	***Olsenella profusa***	***Olsenella uli*^{T}**	***Olsenella umbonata***	***Paraeggerthella hongkongensis*^{T}**
Arginine dihydrolase	+	+	+	+	+
Alanine arylamidase	–	+	+	+	v
Arginine arylamidase	v	+	+	+	v
Cystine arylamidase	ND	+	+	+	ND
Glycine arylamidase	–	+	+	+	–
Histidine arylamidase	–	+	+	+	–
Leucine arylamidase	–	+	+	+	v
Leucyl glycine arylamidase	ND	+	+	+	–
Lysine arylamidase	ND	ND	ND	ND	v
Phenylalanine arylamidase	–	+	+	+	–
Proline arylamidase	–	+	+	+	–
Serine arylamidase	–	+	+	+	–
Tyrosine arylamidase	–	+	+	+	–
Valine arylamidase	ND	+	+	+	ND

Table 11.5 (continued)

Aminopeptidase	*Parvibacter caecicola*^{T}	*Slackia equolifaciens*	*Slackia exigua*^{T}	*Slackia faecicanis*	*Slackia heliotrinireducens*
Arginine dihydrolase	–	+	+	+	+
Alanine arylamidase	+	+	+	–	+
Arginine arylamidase	–	–	+	–	–
Cystine arylamidase	ND	ND	+	–	v
Glycine arylamidase	+	+	+	–	+
Histidine arylamidase	–	+	+	–	+
Leucine arylamidase	+	+	+	–	+
Leucyl glycine arylamidase	–	v	–	–	–
Lysine arylamidase	ND	ND	ND	ND	ND
Phenylalanine arylamidase	+	+	+	–	+
Proline arylamidase	+	+	+	–	+
Serine arylamidase	+	+	+	v	+
Tyrosine arylamidase	+	+	+	–	+
Valine arylamidase	ND	ND	+	–	+

Aminopeptidase	*Slackia isoflavoniconvertens*	*Slackia piriformis*
Arginine dihydrolase	+	+
Alanine arylamidase	–	+
Arginine arylamidase	–	–
Cystine arylamidase	–	+
Glycine arylamidase	–	+
Histidine arylamidase	–	+
Leucine arylamidase	–	+
Leucyl glycine arylamidase	–	–
Lysine arylamidase	ND	ND
Phenylalanine arylamidase	–	+
Proline arylamidase	–	+
Serine arylamidase	–	+
Tyrosine arylamidase	–	+
Valine arylamidase	–	+

Atopobium fossor, *Coriobacterium glomerans*, and *Denitrobacterium detoxificans* were not included in the table since, to the best of our knowledge, no information is available in the literature on any of the listed enzymes for these species

Symbols and abbreviations: + positive, – negative, *v* variable depending on strains, *ND* not determined

Atopobium Collins and Wallbanks (1992)

A.to.po'bi.um. Gr. adj. *atopos* having no place, strange; Gr. neu. part. used as noun; *bion* living thing; N.L. neu. n. *Atopobium* strange living thing.

The genus name *Atopobium* was initially proposed in 1992 following the pioneering 16S rRNA-based phylogenetic analysis of 40 lactic acid bacteria by Collins and Wallbanks. The genus was created to accommodate the species formerly classified as follows: (a) *Lactobacillus minutus* (synonyms: *Bacteroides minutum*, *Eubacterium minutum*) → *Atopobium minutum* comb. nov. (mi.nu'tum. L. neut. adj. *minutum* little, small); (b) *Lactobacillus rimae* → *Atopobium rimae* comb. nov. (L. gen. n. *rimae* of a fissure, here pertaining to the gingival crevice); and (c) *Streptococcus parvulus* (synonym: *Peptostreptococcus parvulus*) → *Atopobium parvulum* comb. nov. (L. neut. dim. adj. *parvulum* very small) (Collins and Wallbanks 1992). The genus also includes *Atopobium vaginae* (va.gi'nae. L. n. *vagina* vagina; L. gen. n. *vaginae* of the vagina) (Rodriguez Jovita et al. 1999) and *Atopobium fossor* (fos'sor. L.n. *fossor*, a digger, delver), originally described as [*Eubacterium fossor*] (Bailey and Love 1986; Kageyama et al. 1999b). The type species of the genus is *Atopobium minutum*. Of note, Olsen et al. published already in 1991 an amended description of [*Lactobacillus minutus*] and [*Streptococcus parvulus*] (Olsen et al. 1991), which were originally described in 1937 (Hauduroy et al. 1937; Weinberg et al. 1937). The transfer of [*Peptostreptococcus parvulus*] to the genus *Streptococcus* was published by Cato in 1983 (Cato 1983).

Table 11.6
Phenotypic features of *Atopobium* spp.

Characteristic	*Atopobium fossor*	*Atopobium minutum*^{T}	*Atopobium parvulum*	*Atopobium rimae*	*Atopobium vaginae*
Growth atmosphere	Strictly anaerobic	Strictly anaerobic	Strictly anaerobic	Strictly anaerobic	Facultative anaerobic
Esculin hydrolysis	–	–	+	v	–
b-Galactosidase	ND	–	+	–	–
Pyroglutamic acid arylamidase	ND	v	+	+	–
Growth in 6.5 % NaCl	ND	v (4/11)	v (6/67)	–	ND
G+C content of DNA (mol%)	43–46	44	39	45	44
Type strain	ATCC 43386 = CIP 106638 = JCM 9981 = NCTC 11919 = VPB 2127	VPI 9428 = ATCC 33267 = CCUG 31167 = DSM 20586 = JCM 1118 = LMG 9439 = NCIMB 702751 (NCFB 2751)	IPP 1246 = ATCC 33793 = CCUG 32760 = CIP 102970 = DSM 20469 = JCM 10300 = VPI 0546	VPI D140H-11A = ATCC 49626 = CCUG 31168 = DSM 7090 = IFO (now NBRC) 15546 = JCM 10299 = LMG 11476	ATCC BAA-55 = CCUG 38953 = CIP 106431

Symbols: *v* variable (number of positive strains/total number of strains tested)

The growth of *Atopobium* spp. is usually stimulated by the presence of Tween 80. Cells consist of short rods, often with central swellings, or small cocci that may appear to be elliptical. Cells occur singly, in pairs, or short chains. The major fermentation products from glucose are lactic acid, together with acetic and formic acid; trace amounts of succinic acid may also be formed. H_2 is not produced. Gelatin is not liquefied; meat is not digested. These bacteria are usually strictly anaerobic, but *Atopobium vaginae* can also grow under aerobic conditions (5 % CO_2). The G+C content of DNA is 35–46 mol%. Discriminative features of *Atopobium* spp. are shown in *Table 11.6*.

Collinsella Kageyama et al. (1999c), Emend. Kageyama and Benno (2000)

Col.lin.sel'la. M.L. fem. dim. ending *-ella*, M.L. fem. n. *Collinsella* named to honor Matthew D. Collins, a contemporary English microbiologist, for his outstanding contribution to microbial taxonomy and phylogeny.

The genus *Collinsella* was created in 1999 to accommodate [*Eubacterium*] *aerofaciens* (ae.ro.fa'ci.ens. Gr. n. *aer* gas; L. v. *facere* to make, to produce; M.L. part. adj. *aerofaciens* gas-producing), which had been previously published as *Bacteroides aerofaciens* (Eggerth 1935). The proposal to create *Collinsella* gen. nov. was based on 16S rRNA gene sequence analysis showing that three strains of [*Eubacterium*] *aerofaciens* (JCM 10188^T, JCM 7790, and JCM 7791) formed a cluster closest to *Atopobium* spp. and *Coriobacterium glomerans*. The three strains were also characterized by higher G+C content of DNA (60–61 vs. 45–47 mol%) when compared with *Eubacterium* sensu stricto (*Eubacterium limosum*, *Eubacterium barkeri*, *Eubacterium callanderi*). The genus currently comprises four species: *Collinsella intestinalis* (in'test.in.alis. N. L. adj. *intestinalis* pertaining to the intestine) (Kageyama and Benno 2000), *Collinsella stercoris* (ster'co.ris. L. n. *stercus* feces; L. gen. n. *stercoris* of feces, referring to the source of the isolate) (Kageyama and Benno 2000), *Collinsella tanakaei* (ta.na.ka'e.i. N.L. masc. gen. n. *tanakaei* of Tanaka, to honor Ryuichiro Tanaka, a Japanese microbiologist, for his contribution to increased knowledge about human intestinal microbiota and probiotics) (Nagai et al. 2010), and the type species *Collinsella aerofaciens*. *Collinsella* spp. occur in chains of rod-shaped cells (0.5–1.0 × 1–3 μm). Fermentation products of glucose are H_2, ethanol, formate, and lactate. All strains are positive for naphthol-AS-BI-phosphohydrolase, acid from glucose and D-mannose. They are negative for *a*-arabinosidase, *a*-fucosidase, *a*-galactosidase, *a*-mannosidase, chymotrypsin, esterase (C4), esterase lipase (C8), glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, lipase (C14), pyroglutamic acid arylamidase and acid from L-arabinose, glycerol, D-mannitol, melezitose, raffinose, L-rhamnose, D-sorbitol, and D-xylose. It has been reported that the growth of *Collinsella* is stimulated by Tween 80 (Dewhirst et al. 2001; Maruo et al. 2008), but this characteristic is absent from the single description of all *Collinsella* species (Kageyama et al. 1999a; Kageyama and Benno 2000; Nagai et al. 2010). Cells of *Collinsella tanakaei* are resistant to 20 % bile (no data available for the other species). The cell wall contains a A4-type peptidoglycan. Respiratory quinones are not detected. The G+C content of DNA is

Table 11.7
Phenotypic features of *Collinsella* spp.

Characteristic	*Collinsella aerofaciens*^{T}	*Collinsella intestinalis*	*Collinsella stercoris*	*Collinsella tanakaei*
Acid produced from				
Cellobiose	–	+	+	+
Lactose	+	–	+	+
Maltose	+	–	+	+
Acid phosphatase	–	+	+	+
Alkaline phosphatase	–	+	+	+
b-Galactosidase	+	–	+	–
a-Glucosidase	+	–	–	–
b-Glucosidase	–	v	+	+
b-Glucuronidase	–	–	–	+
N-Acetyl *b*-glucosaminidase	–	+	+	–
6-phospho-*b*-galactosidase	–	+	–	–
Esculin hydrolysis	–	v	–	+
Peptidoglycan type	A4*b* [(L-Ala)-D-Glu-L-Orn-D-Asp]	A4*a* [(L-Ala)-D-Glu-L-Lys-D-Glu]	A4*b* [(L-Ala)-D-Glu-L-Orn-D-Asp]	ND
% saturated CFA	31	3	3	18
Type strain	VPI 1003 = ATCC 25986 = CCUG 28087 = DSM 3979 = JCM10188 = NCTC 11838	RCA56-68 = CCUG45296 = CIP 106914 JCM 10643 = DSM 13280	RCA55-54 = CCUG45295 = CIP 106913 = DSM 13279 = JCM 10641	YIT 12063 = DSM 22478 = JCM 16071

CFA cellular fatty acids

60–64 mol%. All strains were isolated from human feces. Discriminative features of the *Collinsella* spp. are shown in *Table 11.7*.

Coriobacterium Haas and König (1988)

Co.ri.o.bac.ter'i.um. Gr. fem. n. *koris* bug; Gr. neut. n. *bakterion* a small rod; M.L. neut. n. *Coriobacterium* rodlet associated with bugs.

The genus is represented only by the type species *Coriobacterium glomerans* (glo'me.rans. L. part. adj. *glomerans* agglomerating; the cells form flocculent, wooly sediments with a clear supernatant in fluid media). Cells grow as long chains (>150 μm) of pear-shaped to irregularly shaped rods (0.44–1.80 μm long). Spherical involution forms are common. The filamentous cell chains are attached to the epithelia in the intestine of bugs. The organisms grow on Columbia blood agar, supplemented Schaedler agar (BBL), and TPY agar at 25 and 30 °C. When grown in TPY medium, the fermentation products of glucose (−7.8 μmol/mL) are acetic acid (7.5 μmol/mL), L-lactic acid (6.5 μmol/mL), ethanol (6.1 μmol/mL), CO_2, and H_2. D-Lactic acid, formic acid, volatile short-chain alcohols, or other volatile fatty acids are not formed. The cells ferment glucose, L-arabinose, D-xylose, D-ribose, mannose, sucrose, maltose, cellobiose, mannitol, and salicin. Lactose, melibiose, raffinose, inulin, starch, and inositol are not fermented. The cells have an electron-dense Gram-positive 40-nm-wide cell wall. The peptidoglycan belongs to the Lys-Asp type. The G+C content of the DNA is 60–61 mol%. The type strain is PW2^T (= DSM 20642^T = ATCC 49209^T = JCM 10262^T). The species was originally reported to occur in the third bulbous midgut portion of all stages of the red soldier bug (*Pyrrhocoris apterus*), except the eggs. However, recent in situ hybridization experiments and sterilization of eggs revealed that vertical transmission of *Coriobacterium glomerans* occurs via the egg surface (Kaltenpoth et al. 2009).

Cryptobacterium Nakazawa et al. (1999)

Crypt.o.bac.te'ri.um. Gr. n. *kryptos* hidden; Gr. n. *bakterion* a small rod; M.L. neut. n. *Cryptobacterium* a hidden rod-shaped bacterium.

The genus is represented only by the type species *Cryptobacterium curtum* (cur'tum. L. neut. adj. *curtum*

shortened, a shortened cell of this organism). Cells are asaccharolytic short rods. On BHI-blood agar, minute, circular, convex, and translucent colonies less than 1 mm in diameter are formed, even after prolonged incubation. Growth in broth media is poor with or without carbohydrates. Starch is not hydrolyzed and no liquefaction of gelatin occurs. Ammonia is produced from arginine (Uematsu et al. 2006). Adonitol, amygdalin, arabinose, cellobiose, erythritol, fructose, galactose, glucose, glycogen, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose, and xylose are not utilized. No metabolic end product is detected in peptone-yeast extract medium supplemented with glucose. Maruo et al. (2008) reported that growth is stimulated by arginine but not Tween 80, yet this statement is not found in the original description by Nakazawa et al., and no amended description has been proposed. The G+C content of DNA is 50–51 mol%. The type strain is 12-3^T (= ATCC 700863^T = DSM 15641^T).

Denitrobacterium Anderson et al. (2000)

De.nit.ro.bac.te'ri.um. L. pref. *de* from; L. n. *nitro* nitro-compound; Gr. neut. dim.n. *bakterion* a small rod; M.L. neut. n. *Denitrobacterium* nitro-compound-reducing rod.

The genus is represented only by the type species *Denitrobacterium detoxificans* (de.tox.if'i.cans. L. pref. *de* from; L. n. *toxicum* poison; L. neut. n. *detoxificans* poison reducer). Cells are chemoorganotrophic and rod-shaped (0.5–1.0 × 1.0–1.5 μm); bulbous ends may be present. The species grows equally well at 32, 37, and 39 °C. Growth occurs in media containing clarified rumen fluid, peptone, and a suitable electron acceptor, including nitrate, 3-nitropropanol, 2-nitropropanol, 3-nitropropionate, nitroethanol, nitroethane, 1-nitropropane, 2-nitrobutane, DMSO, trimethylamine oxide, hydrogen, formate, or (DL)-lactate. H_2S is not produced, and gelatin is not hydrolyzed. Little if any acid is produced during growth in medium with hydrogen or formate as electron donor. Acetate is the major product after growth on lactate; D-lactate is used more readily than L-lactate. The G+C content of DNA ranges from 56 to 60 mol % (thermal denaturation method). A *c*-type cytochrome was found in the type strain NPOH1^T (= ATCC 700546^T = CCUG 56741^T), isolated from a population of ruminal microbes enriched for enhanced metabolism of 3-nitropropanol, the toxic aglycone of miserotoxin (3-nitro-1-propyl-b- d-glucopyranoside) (Anderson et al. 1996). Strain NPOH1^T differs from other strains of the species (NPOH2 = ATCC 700547; NPOH3 = ATCC 700548; and MAJ1 = ATCC 700549) in that it has the ability to reduce nitrate.

Eggerthella Wade et al. (1999), Emend. Maruo et al. (2008), Emend. Würdemann et al. (2009)

Eg.ger.thel'la. L. dim. ending *-ella*; M.L. fem. n. *Eggerthella* named after Arnold H. Eggerth, an American microbiologist who was the first person to report the isolation of [*Eubacterium lentum*] from human feces in 1935 (Eggerth 1935).

The genus *Eggerthella* comprises two species: *Eggerthella sinensis* (M.L. gen. n. *sinae* of China; N.L. fem. adj. *sinensis* pertaining to China, the country where the bacterium was discovered) (Lau et al. 2004b) and the type species *Eggerthella lenta* (len'ta. L. fem. adj. *lenta* slow). *Eggerthella lenta* was originally referred to as *Eubacterium lentum* (Moore et al. 1971; Holdeman et al. 1977). Other synonyms of this species include [*Bacteroides lentus*] and [*Pseudobacterium lentum*]. The proposal to create the name *Eggerthella lenta* was first published in 1999 by Wade et al. on the basis of 16S rRNA gene-based phylogenetic evidence, which showed that [*Eubacterium lentum*], [*Eubacterium exiguum*], and [*Peptostreptococcus heliotrinreducens*] formed a coherent cluster closely related to *Atopobium* spp. and *Coriobacterium glomerans* but only distantly related to *Eubacterium limosum*, the type species of the genus *Eubacterium* (Wade et al. 1999). Kageyama et al. also published a similar study in 1999 and proposed to create the name *Eggerthella* gen. nov. to accommodate [*Eubacterium lentum*] (Kageyama et al. 1999c). However, the work by Wade et al. has priority. Kageyama et al. reported as well that the cell wall of *Eggerthella lenta* contains type A3 peptidoglycan, yet this information cannot be found in the original work by Schleifer and Kandler to which Kageyama et al. referred (Schleifer and Kandler 1972). In their amended description of the genus *Eggerthella*, Maruo et al. stated that the cell wall contains A4*g*-type peptidoglycan with an (L-Ala)–D-Glu–m-Dpm–D-Glu peptide subunit and an inter-peptide bridge that consists only of D-Glu (Maruo et al. 2008). In 2009, Saunders et al. published the genome sequence of the type strain of *Eggerthella lenta* and stated that its cell wall contains A1*g*-type peptidoglycan (Saunders et al. 2009). The latest description with standing in nomenclature is the one by Maruo et al. 2008. The major respiratory quinones are MK-6 (dominant in *Eggerthella lenta*) and MMK-6 (dominant in *Eggerthela sinensis*). DMMK-6 is also detected in *Eggerthella sinensis*. Polar lipids consist of two phospholipids, phosphatidylglycerol and diphosphatidylglycerol, and four glycolipids. The main cellular fatty acid is $C_{16:0}$ DMA. The proportion of saturated cellular fatty acids is 61–76 %. Growth is stimulated by arginine (Sperry and Wilkins 1976a). Cells are usually arranged in chains. They are catalase- and arginine dihydrolase-positive. Colonies on blood agar are as follows: 0.25–1.0 mm, circular, entire, slightly raised, smooth, grey, and translucent to semiopaque (*Eggerthella lenta*) and greyish white, 0.5 mm in diameter after 48 h at 37 °C (*Eggerthella sinensis*). *Eggerthella lenta* reduces nitrate and has been found to produce ammonia from arginine and to contain cytochromes *a*, *b*, and *c* and a carbon monoxide-binding pigment (Sperry and Wilkins 1976b). The G+C content of DNA is 61–64 mol% (*Eggerthella lenta*) and 65–66 mol% (*Eggerthella sinensis*). The type strain of *Eggerthella lenta* is DSM 2243^T (= ATCC 25559^T = CCUG 17323A^T = CIP 106637^T = JCM 9979^T = NCAIM B.01418^T = NCTC 11813^T). The type strain of *Eggerthella sinensis* is HKU14^T (= DSM 16107^T = JCM 14551^T = LMG 22123^T). Discriminative features of *Eggerthella* spp. are shown in Table 11.8.

Table 11.8
Phenotypic features of *Eggerthella* spp.

Characteristic	*Eggerthella lenta*^{T}	*Eggerthella sinensis*
Nitrate reduction	+	–
Major respiratory quinone	MK-6 (64 %)	MMK-6 (60 %)
Bile resistance	+	ND
Lysine arylamidase	ND	+

Abbreviations: *MK* menaquinone, *MMK* methylmenaquinone

Table 11.9
Phenotypic features of *Enterorhabdus* spp

Characteristic	*Enterorhabdus caecimuris*	*Enterorhabdus mucosicola*^{T}
Diamino pimelic acid	*meso*	LL
Respiratory quinone	MMK-6 (60 %); DMMK-6 (40 %)	MMK-6 (100 %)
Glucose in whole-cell sugars	+	–
Polar lipids	DPG, PG, 2 GL, 1PL, 1 L	DPG, PG, 4 GL, 3 PL
Aminopeptidases	–	+
Glutamic acid decarboxylase	+	–
Equol production	–	+

Abbreviations: *DPG* diphosphatidylglycerol, *GL* glycolipids, *L* unidentified lipid, *MMK* methylmenaquinone, *PG* phosphatidylglycerol, *PL* phospholipids

Enterorhabdus Clavel et al. (2009), Emend. Clavel et al. (2010)

En.te.ro.rhab'dus. Gr. n. *enteron* intestine; Gr. fem. n. *rhabdos* a rod; N.L. fem. n. *Enterorhabdus* a rod isolated from the intestine.

The genus *Enterorhabdus* comprises two species: *Enterorhabdus caecimuris* (ca.e.ci.mu'ris. L. n. *caecum* caecum; L. n. mus *muris* mouse; N.L. gen. n. *caecimuris* of the caecum of a mouse) and the type species *Enterorhabdus mucosicola* (mu.co.si'co.la. N.L. n. *mucosa* mucosa from L. adj. *mucosus -a -um* mucous; L. suff. *-cola* (from L. n. *incola*) inhabitant, dweller; N.L. n. *mucosicola* inhabitant of the intestinal mucosa). These species are mesophilic, aerotolerant anaerobes that grow as single short rods (0.5 × 2.0 μm) that do not produce glycosidases. Cultures in the stationary phase of growth in anoxic Wilkins-Chalgren-Anaerobe broth are characterized by stable pH (6.9–7.1) and a typically low turbidity (<0.5 McFarland standard). They grow well in the temperature range 30–40 °C. No growth occurs in the presence of 0.5 % (w/v) bile salts. *Enterorhabdus caecimuris* grows in the presence of 2 % (w/v) NaCl. Both species form pinpoint colonies on blood agar. The major cellular fatty acids are $C_{14:0}$, $C_{16:0}$, and $C_{16:0}$ DMA. Whole-cell sugars include galactose and ribose. The most dominant respiratory quinone is MMK-6. The G+C content is 64.2–64.5 mol%. The major polar lipids are diphosphatidylglycerol and two glycolipids. The type strain of *Enterorhabdus mucosicola* is Mt1B8^T(= DSM 19490^T = CCUG54980^T). The type strain of *Enterorhabdus caecimuris* is B7^T (= DSM 21839^T, =CCUG 56815^T). Discriminative features of *Enterorhabdus* spp. are shown in Table 11.9.

Gordonibacter Würdemann et al. (2009)

Gor.do'ni.bac'ter. N.L. masc. n. *Gordon* named after Jeffrey I. Gordon, MD, the Dr Robert J. Glaser Distinguished University Professor and Director of the Center for Genome Sciences at Washington University School of Medicine, St. Louis, MO, USA; N.L. masc. n. *bacter* a rod; N.L. masc.n. *Gordonibacter* a rod named after Jeffrey I. Gordon.

The genus is represented only by the type species *Gordonibacter pamelaeae* (pa.me'la.eae. N.L. fem. n. *pamelaeae* named after Dr Pamela Lee Oxley (née Fredericks), biochemist, environmentalist, teacher, mentor, and mother). Cells are catalase-positive coccobacilli (0.5–0.6 × 0.8–1.2 μm) with a conical cell apex. They are motile and characterized by the presence of a subpolarly inserted flagella when grown in BHI medium. Of note, one clinical isolate identified as *Gordonibacter pamelaeae* on the basis of 16S rRNA gene sequencing and phenotypic analysis was reported to be nonmotile (Woo et al. 2010). Growth is generally slow on BHI and Schaedler anaerobic media (Oxoid) supplemented with 5 % defibrinated horse blood, with pale-white, semitranslucent colonies forming after 48–72 h at 37 °C. Growth is enhanced by 1 % (w/v) arginine-HCl. Arabinose, glucose, mannose, raffinose, trehalose, xylose, L-methionine, L-phenylalanine, L-valine, L-valine plus L-aspartic acid, dextrin, and D-glucose 6-phosphate are not metabolized. Nitrate is not reduced. Only weak conversion of pyruvic acid and pyruvic acid methyl ester is observed. All other organic substrates included in the Biolog AN MicroPlate are not metabolized. Cellular fatty acids consist mainly (approximately 90 %) of saturated fatty acids (predominantly C_{15} and C_{16}). The major respiratory lipoquinone present is MK-6; MMK-6 is a minor component. The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and four glycolipids. The G+C content of DNA is 66 mol%. The type strain is 7–10-1-b^T(= DSM 19378^T = CCUG55131^T).

Olsenella Dewhirst et al. (2001)

Ol.sen.el'la. L. fem. dim. ending *-ella*, N.L. fem. n. *Olsenella* of Olsen, named to honor Ingar Olsen, a contemporary Norwegian microbiologist, who first described *Lactobacillus uli*.

The genus currently comprises three species: (a) *Olsenella profusa* (pro.fus'a. L. adj. *profusus* profuse, referring to the good growth of the organism), (b) *Olsenella umbonata* (um.bo.na'ta. N.L. fem. adj. *umbonata* bossed, umbonate (from L. masc. n.

Table 11.10
Phenotypic features of *Olsenella* spp.

Characteristic	*Olsenella profusa*	*Olsenella uli*^{T}	*Olsenella umbonata*
Cell morphology	Single, pairs, or chains	Single, pairs, or chains	Short to very long serpentine chains
Acid produced from			
Mannitol	+	–	–
Lactose	+	v	–
Arabinose	+	–	–
Cellobiose	+	–	–
Raffinose	+	–	–
Alkaline phosphatase	+	–	–
b-Galactosidase	+	–	–
a-Glucosidase	+	–	+
b-Glucosidase	+	+	–
N-Acetyl-*b*-glucosaminidase	+	–	–
6-phospho-*b*-galactosidase	+	–	–
Growth stimulation by Tween 80	slight	+	+
Esculin hydrolysis	+	v	–
% saturated CFA (main)	93–97 (ai-$C_{14:0}$)	54–87 ($C_{18:0}$)	85–100 ($C_{14:0}$; $C_{18:0}$)
Type strain	D315A-29 = CCUG 45371 = CIP 106885 = DSM 13989 = JCM 14553	VPI D76D-27C = ATCC 49627 = CCUG 31166 = DSM 7084 = JCM 12494 = LMG 11480 = VPI D76D-27C	lac31 = CCUG 58604 = DSM 22620 = JCM 16156

Abbreviations: ai, anteiso, *CFA* cellular fatty acids

umbo, *umbonis* a shield boss), referring to the umbonate elevations of outgrown colonies on solid culture media) (Kraatz et al. 2011), and (c) the type species *Olsenella uli* (u'li.Gr. n. *oulon* the gum; N.L. gen. n. *uli* of the gum). Cells are microaerotolerantly (moderately obligately) anaerobic (less than 5 % O_2, v/v). They grow as small, elliptical rods that occur singly, in pairs, or short to very long serpentine chains. Convert a variety of sugars. Lactic acid is the major product from glucose. Minor products are formic and acetic acid. Able to grow on mucin from porcine stomach. All strains are negative for urease, *a*-galactosidase, *a*-arabinosidase, *b*-glucuronidase, *a*-mannosidase, *a*-fucosidase, raffinose fermentation, acidification of glycerol and melezitose, trypsin, *a*-chymotrypsin, reduction of nitrate, pyroglutamic acid arylamidase, glutamic acid decarboxylase, and glutamyl glutamic acid arylamidase. All strains are positive for mannose fermentation, acidification of glucose, and gelatin hydrolysis. Growth is stimulated by Tween 80 but not arginine. The cellular fatty acids consist mainly of saturated fatty acids. The G+C content of DNA is 63–64 mol%. Original values reported for [*Lactobacillus*] *uli* were $C_{18:1}$*cis*9 (major cellular fatty acid) and 53 mol% (G+C content of DNA) (Olsen et al. 1991). Göker et al. recently reported that *Olsenella uli* is characterized by the presence of a A4*b*-type peptidoglycan based on L-Orn-D-Asp (Goker et al. 2010). *Olsenella profusa* was previously designated *Eubacterium* group D52 (Holdeman et al. 1977). The description of *Olsenella umbonata* refers to the analysis of four strains (A2, lac 15, lac 16, and lac31^T). All lac strains were isolated from pig jejunal mucosa (Kraatz and Taras 2008), whereas strain A2 was isolated from sheep rumen as part of a study focusing on ammonia-producing bacteria (Eschenlauer et al. 2002). *Olsenella umbonata* was found to produce ammonium from peptone under anaerobic and unreduced microaerobic conditions (ca. 12 and 9 mmol/l, respectively). Growth of this species is positive in 20 % bile but absent in 6.5 % NaCl. Strain A2 (=CCUG 58212 = DSM 22619 = JCM 16157), which had been informally named [*Olsenella* (*Atopobium*) *oviles*] (Dewhirst et al. 2001; Eschenlauer et al. 2002), can be differentiated from the type strain lac31^T by a negative result for acidification of trehalose in the API 20 A strip. Discriminative features of *Olsenella* spp. are shown in *Table 11.10*.

Paraeggerthella Würdemann et al. (2009)

Pa'ra.eg.ger.thel'la. L. prep. *para* beside; N.L. fem. n. *Eggerthella* a bacterial genus name; N.L. fem. n. *Paraeggerthella* beside *Eggerthella*, named in recognition of the close relationship to the genus *Eggerthella*.

The genus is represented only by the type species *Paraeggerthella hongkongensis* (N.L. fem. adj. *hongkongensis*, pertaining to Hong Kong, the city where the bacterium was discovered). This species had been previously described as [*Eggerthella*] *hongkongensis* (Lau et al. 2004b), for which an emended description was published by Maruo et al. (2008). The type strain is HKU10^T (= DSM 16106^T = CCUG 49250^T), isolated in 1998 from the blood of a 30-year-old male patient suffering from alcoholic cirrhosis, portal hypertension, and epilepsy and diagnosed with perianal abscess (Lau et al. 2004a). Additional strains (HKU11, HKU12, HKU13) were isolated from blood cultures of a patient with an infected rectal tumor, a liver abscess, and acute appendicitis, respectively. These additional strains were not further studied in amended descriptions. The rationale for reclassifying [*Eggerthella*] *hongkongensis* into the novel genus *Paraeggerthella* was based on several major differences observed between strain HKU10^T and *Eggerthella* species: (a) 16S rRNA gene similarity values <95 %, (b) a lower amount of saturated cellular fatty acids (45 vs. 61–63 %), (c) the presence of $C_{18:1}$ w9c instead of $C_{16:0}$ DMA as major cellular fatty acid, (d) different polar lipid profiles (three instead of four glycolipids), and (e) the ability of *Paraeggerthella hongkongensis* to metabolize 3-methyl-D-glucose, palatinose, L-rhamnose, L-methionine, L-valine, L-valine plus L -aspartic acid, and uridine 5′-monophosphate. Physiological testing using Rapid ID32A and API 20A revealed just one positive reaction, for arginine dihydrolase. Lau et al. reported a positive reaction for *b*-glucosidase, which was not confirmed by Würdemann and colleagues. Results obtained with Biolog AN MicroPlates indicated that urocanic acid and L-threonine are metabolized. Weak conversion of rhamnose is observed. The other organic substrates included in the Biolog AN MicroPlate are not metabolized. No significant conversion of the flavonoids quercetin, rutin, genistein, and phloretin is observed. Cells are catalase-positive coccobacilli arranged in chains. They grow on blood agar as greyish white colonies of 0.5 mm in diameter after 48 h at 37 °C. The cell wall contains the A4*g*-type peptidoglycan. According to Würdemann et al., the major respiratory lipoquinone is MK-6 (68 %); MMK-6 is a minor component (32 %). Maruo et al. found that the principal respiratory quinone is MMK-6 and that minor menaquinones are MK-6 and DMMK-6. This discrepancy is likely due to growth conditions and technical issues, e.g., the fact that DMMK-6 can be difficult to detect using HPLC. Polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, and three glycolipids (GL1, GL2, andGL4). The G+C content of DNA of strain HKU10^T is 61–62 mol%.

Parvibacter Clavel et al. (2013)

Par.vi.bac'ter. L. adj. *parvus* small; N.L. masc. n. *bacter* rod; N.L. masc. n. *Parvibacter* small rod.

The genus is represented only by the type species *Parvibacter caecicola* (ca.e.ci'co.la. N.L. n. *caecum* blind pouch, caecum; L. suff. *-cola* (from L. n. *incola*), dweller, inhabitant; N.L. n. *caecicola* caecum dweller). Cells are aerotolerant small rods (0.5 × 1.5 μm) that grow only under strictly anoxic conditions in the temperature range from 25 to 37 °C. After 48 h at 37 °C on Wilkins-Chalgren-Anaerobe agar under anoxic conditions, colonies are circular, entire, pinpoint, and grey. Positive for proline, phenylalanine, leucine, tyrosine, alanine, glycine, and serine arylamidase. Negative for urease activity, arginine dihydrolase, *a*- and *b*-galactosidase, *a*- and *b*-glucosidase, *a*-arabinosidase, *b*-glucuronidase, *b*-*N*-acetylglucosamine, mannose and raffinose fermentation, glutamic acid decarboxylase, *a*-fucosidase, nitrate reduction, indole production, and alkaline phosphatase as well as arginine, leucyl glycine, pyroglutamic acid, histidine, and glutamyl glutamic acid arylamidase. The major cellular fatty acids are $C_{16:0}$ (26 %) and i-$C_{15:0}$ (11 %). Galactose, glucose, and ribose are detected as whole-cell sugars. The principal respiratory quinone is MMK-6. The diamino acid in the peptidoglycan is *meso*-diaminopimelic acid. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, three phospholipids, four glycolipids, and one unidentified lipid. The G+C content of DNA is 62.5 %. The type strain is NR06^T (= DSM 22242^T = CCUG 57646^T).

Slackia Wade et al. (1999), Emend. Nagai (2010)

Slack'ia. M.L. fem. n. named to honor Geoffrey Slack, distinguished British microbiologist and dental researcher.

The rationale for creating the genus name *Slackia* was to accommodate [*Eubacterium exiguum*] (Poco et al. 1996) and [*Peptococcus heliotrinreducans*] (Lanigan 1976) on the basis of 16S rRNA phylogenetic evidence showing that these two species formed a distinct cluster within the *Coriobacteriaceae*. The genus *Slackia* currently comprises six species: (a) *Slackia equolifaciens* (e.quo.li.fa'ci.ens. N.L. n. *equol-olis* equol; L. part. adj. *faciens* making; N.L. part. adj. *equolifaciens* equol-producing) (Jin et al. 2010), (b) *Slackia faecicanis* (fae.ci.ca'nis. L. n. *faex, faecis* feces; L. gen. n. *canis* dog; N.L. gen. n. *faecicanis* from dog feces) (Lawson et al. 2005), (c) *Slackia heliotrinireducens* (he.li.o.trin.i.re.duc. ens. M.L. n. *heliotrinum* derived from heliotrine, a pyrrolizidine alkaloid; L.adj. *reducans* reducing M.L. adj. *heliotrinireducens* referring to the ability to bring about oxidative cleavage of the heliotrine molecule), (d) *Slackia isoflavoniconvertens* (i.so.fla.vo.ni.con.ver'tens. N.L. neut. n. *isoflavonum* isoflavone; L. part. adj. *convertens* converting; *isoflavoniconvertens* isoflavone-converting) (Matthies et al. 2009), (e) *Slackia piriformis* (pi.ri.for'mis. L. n. *pirum* pear; L. adj.

Table 11.11
Phenotypic features of *Slackia* spp.

Characteristic	*Slackia equolifaciens*	*Slackia exigua*^{T}	*Slackia faecicanis*	*Slackia heliotrinireducens*	*Slackia isoflavoniconvertens*	*Slackia piriformis*
Nitrate reduction	–	–	v	+	–	–
Bile resistance	ND	–	w[a]	–	ND	w[a]
% saturated CFA (main)	42 ($C_{14:0}$)	22–35 ($C_{14:0}$; $C_{16:0}$ DMA)	18–30 ($C_{14:0}$; $C_{18:0}$ DMA)	16 (i-$C_{14:0}$)	ND	26 ($C_{18:0}$ DMA)
G+C content of DNA (mol%)	61	60–64	61	61	58.5	58
Colony morphology (agar medium)	1–2 mm, translucent grey (GAM[b] + 0.5 % arginine-HCl)	<1 mm, circular, convex, translucent (BHI-blood)	1–2 mm, translucent to grey, uneven surface, irregular edges (anaerobic blood)	1–2 mm, effuse, entire edge, colorless, transparent (tryptone-yeast-mineral salts)	1 mm, smooth, translucent (Columbia blood)	0.1–1.0 mm, translucent to beige, circular, uneven surface, irregular edges (GAM[b])
Type strain	DZE (=CCUG 58231 = JCM 16059)	S-7 = ATCC 700122 = CIP 105133 = JCM 11022 = CCUG 44588	5WC12 = CCUG 48399 = CIP 108281 = JCM 14555 = DSM 17537	RHS1 = ATCC 29202 = CCUG 47954 = DSM 20476 = JCM 14554 = NCTC 11029	HE8 = CCUG57679 = DSM 22006 = JCM 16137	YIT 12062 = DSM 22477 = JCM 16070

Abbreviations: *BHI* brain-heart infusion, *DMA* dimethyl acetal, *i* iso, *ND* not determined, *w* weak
[a]*w* weak, cells grew on medium containing 2 % oxgall, but the number of colonies was decreased compared with control medium without oxgall (5 % and 50 % cfu for *Slackia faecicanis* and *Slackia piriformis*, respectively)
[b]General anaerobic medium, Nissui Pharmaceutical, Tokyo, Japan

suffix-*formis*-like, in the shape of; N.L. fem. adj. *piriformis* pear-shaped, referring to the cell shape) (Nagai et al. 2010), and (f) the type species *Slackia exigua* (ex.i.gu'a. L. adj. *exigua* scanty, small, referring to the scanty or poor growth of this organism). Cells are cocci, coccobacilli, or short bacilli, the growth of which is stimulated by 0.5 % arginine. Sugars are not fermented. Positive for naphthol-AS-BI-phosphohydrolase but negative for alkaline phosphatase, *a*-arabinosidase, *N*-acetyl-*b*-glucosaminidase, chymotrypsin, *a*-fucosidase, *a*-galactosidase, *b*-galactosidase, *a*-glucosidase, *b*-glucosidase, *b*-glucuronidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, lipase (C14), *a*-mannosidase, 6-phospho-*b*-galactosidase, pyroglutamic acid arylamidase, trypsin, urease, and esculin hydrolysis. The main cellular fatty acids are $C_{18:1}$w9c and $C_{18:1}$w9c DMA. Respiratory quinones have not been detected in *Slackia piriformis*, *Slackia exigua*, *Slackia heliotrinireducens*, and *Slackia faecicanis* (*Slackia equolifaciens* and *Slackia isoflavoniconvertens* have not been analyzed). *Slackia heliotrinireducens* was isolated for its ability to reductively cleave hepatotoxic pyrrolizidines found in forages. It also contains a *c*-type cytochrome. This species was originally published as *Peptococcus heliotrinreducans* (Lanigan 1976), before its transfer to the genus *Peptostreptococcus* as *Peptostreptococcus heliotrinreducens* in 1986 on the basis of its high G+C content of DNA and the presence of various aminopeptidases (Ezaki and Yabuuchi 1986). Discriminative features of *Slackia* spp. are shown in *Table 11.11*.

Isolation, Enrichment, and Maintenance Procedures

It is striking that all members of the *Coriobacteriaceae* have been so far isolated only from body habitats of mammals and insects, which hints at evolutionary driving forces that made these bacteria best suited for efficient colonization and survival in such environments. The first cultivable representatives of the family, i.e., *Collinsella aerofaciens* and *Eggerthella lenta*, were recovered from human feces (Eggerth 1935). All strains of so far described species have been isolated by chance using either nonselective rich media or selective media and isolation procedures targeting specific metabolic functions or bacterial populations, e.g., conversion of isoflavones (*Asaccharobacter celatus*, *Slackia equolifaciens*, *Slackia isoflavoniconvertens*), mucosa-associated bacteria

(*Gordonibacter pamelaeae*, *Enterorhabdus mucosicola*, *Olsenella umbonata*), reduction of nitro-compounds (*Denitrobacterium detoxificans*), or ammonia production (*Olsenella umbonata*, *Slackia heliotrinireducens*). For this reason, and due as well to the metabolic versatility of the 30 species of the family, there is to date no selective medium available for exhaustive enrichment of *Coriobacteriaceae*. The efficacy of blood, arginine, or Tween 80 to stimulate growth as well as the resistance towards bile and antibiotics has hitherto not been tested for all species. Moreover, while strictly anoxic culture techniques are suited for cultivation of most species, *Olsenella* spp. grow under microaerophilic conditions and *Atopobium vaginae* is a facultative anaerobe. The isolation and maintenance conditions reported for the 30 species of the family are summarized in Table 11.12.

Ecology

The family *Coriobacteriaceae* includes a large majority of strictly anaerobic strains with fastidious growth requirements. They frequently coexist with a number of other microorganisms in complex ecosystems. As a result, the ecology of this family (as in the sense of the occurrence and functions of its members) was poorly studied until the emergence (and affordability) of culture-independent techniques such as polymerase chain reaction (PCR), sequencing of 16S rRNA genes as well as metabolomics and system biology approaches (Woo et al. 2008; Claus et al. 2011). It is now becoming clear that these previously understudied bacterial species carry out important physiological functions within their hosts.

Habitat and Occurrence

At the time of writing, the family accommodates 14 genera, 13 of which originate from the gastrointestinal tract of mammals (human, mouse, rat, dog, cow, and sheep). *Coriobacterium glomerans*, the type species of the family, has been so far retrieved only from the gut of insects (Haas and König 1988; Kaltenpoth et al. 2009). The diversity and composition of the human intestinal microbiota varies greatly between individuals (Qin et al. 2010). Nevertheless, *Coriobacteriaceae* can be considered as prevalent and dominant dwellers of the human intestine (and by extension of the mammalian intestine in general). Dominant means that certain species can be found at cell densities above 10^8 cells per gram intestinal content. Still, actinobacteria, and thus *Coriobacteriaceae*, represent usually a minor fraction of gut bacterial diversity (<2–5 % of total 16S rRNA gene sequences) when compared with members of the phyla Bacteroidetes and Firmicutes.

Culture-independent studies have demonstrated that the genus *Collinsella* is the most abundant human gut taxon of the family (Kageyama et al. 2000). The species *Collinsella aerofaciens* seems to be a member of the core human gut microbiome, i.e., "a set of bacterial molecular species that are altogether dominant and prevalent within the fecal microbiota of healthy humans" (Tap et al. 2009; Qin et al. 2010). Based on the use of specific 16S rRNA-targeted oligonucleotide probes for fluorescence in situ hybridization, Harmsen et al. found that the *Collinsella* and *Atopobium* phylogenetic groups were part of the dominant microbiota in 26 of 33 adult subjects, with cell counts $>10^9$ cell/g dry feces (Harmsen et al. 2000). In another similar study, mean proportions of the *Atopobium* group were >3 % of dominant bacteria in 39 postmenopausal women (Clavel et al. 2005). Thus, *Atopobium* spp. also seem to be predominant in human feces. However, it is important to note that the specificity of 16S probes warrants detection of relatively broad phylogenetic groups rather than specific species (e.g., the *Atopobium* probe S-*-Ato-0291-a-A-17 targets also other *Coriobacteriaceae*). In one study based on the use of quantitative PCR, *Slackia* spp. were detected in 16 of 40 fecal samples from healthy Japanese adults at a mean population density of $\log_{10}$ 6.4 ± 2.4 cell/g wet weight (Tsuji et al. 2010). PCR-based assays have been used as well to assess the occurrence of *Eggerthella lenta* in human feces, revealing that this species is detected in 30–40 % of tested samples (Schwiertz et al. 2000; Kageyama and Benno 2001). In fact, part of the aforementioned molecular data confirmed the pioneering culture-based work by W. E. C. Moore, S. M. Finegold, and L. V. Holdeman, who readily isolated a number of strains of [*Eubacterium*] *aerofaciens* and [*Eubacterium lentum*] from feces of healthy human adults. These isolates were usually recovered from 50 % of analyzed subjects at mean densities of $>10^9$ cfu/g dry weight (Moore and Holdeman 1974; Finegold et al. 1983). Some *Coriobacteriaceae* have also been detected in sewage samples using massively parallel pyrosequencing of hypervariable regions in microbial rRNA genes (McLellan et al. 2010). The genus *Collinsella* was detected at 0.27 % and 1.07 % total sequences in sewage and human fecal samples, respectively, but not in surface water. The presence of fecal microbial taxa in sewage water appears to be the consequence of human fecal pollution of the wastewater treatment plants rather than such environmental samples being the natural habitat of *Coriobacteriaceae*.

Regarding more recently described taxa within the family, *Enterorhabdus* spp. have been repeatedly found in high-throughput 16S rRNA gene sequence datasets from the mouse, human, and bovine intestinal tract (Benson et al. 2010; Werner et al. 2011; Hristov et al. 2012; Martinez et al. 2012). This speaks in favor of a widespread occurrence in various gut ecosystems, yet most likely at lower population densities. With respect to specific niches occupied by *Coriobacteriaceae* in the gut, it is worth noting that some members may be well suited for colonization of mucosal surfaces, as suggested by (a) the isolation of strains from mucosal samples or using selective culture media containing mucin (*Enterorhabdus mucosicola*, *Gordonibacter pamelaeae*, *Olsenella umbonata*), (b) the symbiotic relationship they may have with their hosts (*Coriobacterium glomerans*), and (c) their detection in mucosal samples using molecular-based techniques (*Atopobium* and *Collinsella* spp.) (Collado and Sanz 2007a, b; Nadal et al. 2007; Lyra et al. 2012).

Table 11.12

Origin, isolation, and growth conditions of type strains of *Coriobacteriaceae*

	Adlercreutzia equolifaciens	*Asaccharobacter celatus*	*Atopobium fossor*	*Atopobium minutum*	*Atopobium parvulum*
Publication	2008	2008	1986	1937[a]	1937[a]
Sample type	Feces of a 25-year-old healthy woman	Caecal content (frozen glycerol stock) of a male Sprague–Dawley rat[b]	Pharyngeal tonsillar surface of normal horses	Human oral cavity	Human oral cavity
Agar medium	BL[c] + 5 % horse blood	GAM[c] + 2 g Fujiflavone P10[d]	Sheep blood (5 %) + vitamin K-hemin + formate-fumarate[e]	Nonselective D4[f]	Nonselective D4[f]
Incubation	3 d, 37 °C	2 d, 37 °C	NR (d), 37 °C	5 d, NR (t°C)	5 d, NR (t°C)
Atmosphere	Anaerobic[g]	$N_2/H_2/CO_2$ (85:5:10)	$N_2/H_2/CO_2$ (80:10:10)	$N_2/H_2/CO_2$ (85:3:12)	$N_2/H_2/CO_2$ (85:3:12)
Additional maintenance media	GAM[c] + 0.5 % arginine, pH 7.0	GAM[c]	Tryptose agar[h]	/	/
References	Maruo et al. (2008)	Minamida et al. (2008)	Bailey and Love (1986)	Moore et al. (1982), Moore et al. (1983)	Moore et al. (1982), Moore et al. (1983)
	Atopobium rimae	*Atopobium vaginae*	*Collinsella aerofaciens*	*Collinsella intestinalis*	*Collinsella stercoris*
Publication	1991	1999	1935	2000	2000
Sample type	Human gingival crevice	Human vagina	Human feces	Human feces	Human feces
Medium	NR	NR	Beef-heart infusion agar[i]	EG agar[j]	EG agar[j]
Incubation	NR	NR	5–6 d, NR (t°C)	2 d, 37 °C	2 d, 37 °C
Atmosphere	NR	NR	Anaerobic[g] As for Adlercreutzia	100 % CO_2	100 % CO_2
Additional maintenance media	Reduced and unreduced PYG (DSMZ medium 104)	Columbia CNA (Difco) + 5 % horse blood; 37 °C; 5 % CO_2 in air	Liver infusion agar	/	/
References	Olsen et al. (1991)	Rodriguez Jovita et al. (1999)	Eggerth (1935)	Kageyama and Benno (2000)	Kageyama and Benno (2000)
	Collinsella tanakaei	*Coriobacterium glomerans*	*Cryptobacterium curtum*	*Denitrobacterium detoxificans*	*Eggerthella lenta*
Publication	2010	1988	1999	1996	1935
Sample type	Human feces	Intestinal tract of a red soldier bug (*Pyrrhocoris apterus*)	Human periodontal pocket	Rumen content, cow #1 reared at NADC[k] and fed an alfalfa/corn (9:1) diet	Human feces
Medium	GAM[c] + 1 % (w/v) NaCl + fosfomycin (60 μg/mL)	Blood agar (BD)	NR	Enrichment in medium A[l]	Beef-heart infusion agar[i]
Incubation	3 d, 37 °C	10–20 d, 25–30 °C	NR	24 h of consecutive batch cultures; 39 °C	5–6 d, NR (t°C)

Table 11.12 (continued)

	Collinsella tanakaei	*Coriobacterium glomerans*	*Cryptobacterium curtum*	*Denitrobacterium detoxificans*	*Eggerthella lenta*
Atmosphere	$N_2/H_2/CO_2$ (88:7:5)	N_2/CO_2 (80:20)	NR	H_2/CO_2 (50:50)	Anaerobic (Eggerth 1935)
Additional maintenance media	GAM[c]	TPY medium (11) + Na2S + cysteine-HCl (each 0.45 g/l)	BHI-blood agar; 3 d, 37 °C; $N_2/H_2/CO_2$ (80:10:10)	Medium B and C[m]	Liver infusion agar
References	Nagai et al. (2010)	Haas and König (1988)	Sato et al. (1998), Nakazawa et al. (1999)	Anderson et al. (1996), Anderson et al. (2000)	Eggerth (1935)
	Eggerthella sinensis	*Enterorhabdus caecimuris*	*Enterorhabdus mucosicola*	*Gordonibacter pamelaeae*	*Olsenella profusa*
Publication	2004	2010	2009	2009	2001
Sample type	Blood of a 59-year-old female patient with acute proctitis and a history of cervical carcinoma	Caecum of a C3H/HeJBir mouse	Ileal mucosa of a 12-week-old female heterozygous $TNF^{deltaARE}$ C57BL/6 mouse with ileitis	Sigmoid region of the colon of a 33-year-old male patient suffering from active Crohn's disease[n]	Human subgingival plaque in adults with periodontitis
Medium	BACTEC 9240 blood culture system (Becton Dickinson, Sparks, MD, USA)	ATCC medium 602E	Mucin-containing medium[o]	Schaedler basal agar (Oxoid) with 5 % defibrinated horse blood	NR
Incubation	NR	3 d, 37 °C	9 d, 37 °C	37 °C	NR
Atmosphere	Anaerobic[g]	$N_2/H_2/CO_2$ (90:5:5)	AnaeroGen catalyzer (Oxoid)	$N_2/H_2/CO_2$ (80:10:10)	NR
Additional maintenance media	Blood agar	BHI (BD 211059) +2 g/l each yeast extract and glucose + 0.05 % (w/v) cysteine; 100 % N_2	BHI (BD 211059) + 2 g/l each yeast extract and glucose + 0.05 % (w/v) cysteine; 100 % N_2	Pre-reduced BHI + 1 % (w/v) arginine-HCl	Fastidious anaerobe agar (LabM) with 5 % horse blood
References	Lau et al. (2004a), Lau et al. (2004b)	Duck et al. (2007)	Clavel et al. (2009)	Würdemann et al. (2009)	Holdeman et al. (1977), Dewhirst et al. (2001)
	Olsenella uli	*Olsenella umbonata*	*Paraeggerthella hongkongensis*	*Parvibacter caecicola*	*Slackia equolifaciens*
Publication	1991	2011	2004	2013	2010
Sample type	Human gingival crevice	Jejunal mucosa of a healthy 62-day-old pig	Blood of a 30-year-old male patient[p]	Caecal content of a 25-week-old male heterozygous $TNF^{deltaARE}$ C57BL/6 mouse with ileitis	Human fecal enrichment in GAM broth + 0.1 mM daidzein
Medium	NR	LAB selective medium with porcine gastric mucin (type III; Sigma)[q]	BACTEC 9240 blood culture system (Becton Dickinson, Sparks, MD, USA)	WCA +1 % (v/v) autoclaved rumen fluid, 0.05 % (w/v) cysteine and 0.02 % DTT	GAM[c] agar
Incubation	NR	7–14 d, 37 °C	NR	6 d, 37 °C	3 d, 37 °C
Atmosphere	NR	Anaerocult A (Merck)	Anaerobic[g]	$N_2/H_2/CO_2$ (85:5:10)	100 % CO_2

Table 11.12 (continued)

	Olsenella uli	*Olsenella umbonata*	*Paraeggerthella hongkongensis*	*Parvibacter caecicola*	*Slackia equolifaciens*
Additional maintenance media	Reduced and unreduced PYG (DSMZ medium 104)	Reduced and unreduced PYG (DSMZ medium 104)	Blood agar	WCA with cysteine and DTT; 100 % N_2	GAM[c] + 0.5 % arginine-HCl
References	Olsen et al. (1991)	Eschenlauer et al. (2002), Kraatz and Taras (2008)	Lau et al. (2004a), Lau et al. (2004b)	Clavel et al. (2013)	Jin et al. (2010)

	Slackia exigua	*Slackia faecicanis*	*Slackia heliotrinireducens*	*Slackia isoflavoniconvertens*	*Slackia piriformis*
Publication	1996	2005	1976	2009	2010
Sample type	Human deciduous teeth with endodontic lesions	Feces of a healthy male Labrador dog	Sheep rumen	Feces of a healthy 37-year-old woman	Human feces
Medium	BHI-blood agar	*Bacteroides* agar[r]	Rich medium with rumen fluid and heliotrine (2 mg/mL)	BHI + 100 μM daidzein + 10 μg/mL tetracyclin	GAM + 6 % Bacto oxgall (Difco)
Incubation	7 d, 37 °C	2 d, 37 °C	7d, 38 °C	Enrichment by limiting dilution; cycles of 37 °C, 72 h	3 d, 37 °C
Atmosphere	$N_2/H_2/CO_2$ (80:10:10)	$N_2/H_2/CO_2$ (80:10:10)	CO_2	CO_2/H_2(80:20)	$N_2/H_2/CO_2$ (88:7:5)
Additional maintenance media	/	Chocolate or blood agar	/	BHI or Columbia agar	GAM
References	Sato et al. (1993), Poco et al. (1996)	Lawson et al. (2005)	Lanigan (1976)	Matthies et al. (2009)	Nagai et al. (2010)

Abbreviations: *BHI* brain-heart infusion, *d* days, *GAM* general anaerobic medium, *NR* not reported, *PYG* peptone-yeast-glucose

[a]The isolation procedure in the table refers to the work by Moore et al. 1982, 1983

[b]SLC Japan, Tokyo; the rat was fed an AIN-93G casein diet for 3 weeks

[c]Nissui Pharmaceutical, Tokyo, Japan

[d]Fujicco, Kobe, Japan

[e]Smibert and Holdeman (1976), Holdeman et al. (1977)

[g]Details on gas phase were not provided

[f]Per L, 37 g brain-heart infusion, 5 g yeast extract, 5 mL 6 % (w/v) ammonium formate solution, 0.5 g cysteine-HCl, 5 mg hemin, 2.5 mg resazurin, 1 mg vitamin K_1, 4 % rabbit blood, pH 7.0

[h]Per L, 5 g NaCl, 15 g agar, 20 g tryptose, 2.5 g tryptone, 1 g yeast extract, 1 g of glucose, pH 7.4–7.6

[i]1.5 % agar, 1 % Parke Davis peptone, 0.4 % Na_2HPO_4. $12H_20$, 5 % blood, 0.15 % glucose, pH 7.6

[j]Per L: 3 g beef extract, 5 g yeast extract, 10 g peptone, 1.5 g glucose, 0.5 g L-cysteine. HCl, 0.2 g L-cystine, 4 g Na_2HPO_4, 0.5 g soluble starch, 0.5 g Tween 80, 0.5 g silicone, 15 g agar, 5 % horse blood, pH 7.7

[k]The National Animal Disease Center in Ames (IA, USA)

[l]Contained Na_2CO_3, resazurin, L-cysteine-HCl, and vitamins at concentrations that were the same as in the complete medium of Bryant and Robinson (Bryant and Robinson 1961). Also contained (in 1 L) 800 mg phytone peptone, 5 μg lipoic acid, 2 μg vitamin B_{12}, 40 % (v/v) clarified rumen fluid, and the same minerals as in the non-rumen fluid medium of Dawson et al. (Dawson et al. 1980). Supplemented for enrichment with milk vetch or alfafa forage + 4.2 mM nitropropanol

[m]Same as medium A with 8 and 0 % rumen fluid, respectively

[n]Treated with azathioprine, mutaflor and cortisone

[o]Per L, 5 g mucin (Sigma M1778), 0.5 % (v/v) ethanol, 500 mg L-cysteine, 1 mg yeast extract, 20 mg folic acid, 20 mg vitamin B_{12}, 50 mmol $NaHCO_3$, 10 mmol sodium acetate, 5 mmol Na_2HPO_4, 5 mmol NaCl, 3 mmol KH_2PO_4, 1 mmol $CaCl_2$, 1 mmol $MgCl_2$, 10 mmol $FeCl_3$, 1 % (w/v) agar, pH 7.7

[p]Suffered from alcoholic cirrhosis, portal hypertension, and epilepsy and diagnosed with perianal abscess

[q]Per L, 10.0 g mucin, 0.01 g peptone, 0.01 g yeast extract, 0.01 g glucose, 0.3 g NaCl, 0.1 g $CaCl_2$, 6.0 g KH_2PO_4, 5 mL Rogosa's salt solution, 1 mL modified (lacking elements already included in Rogosa's salt solution) Pfennig's SL8 trace element solution, 0.2 mL vitamin solution, 0.5 mg resazurin, 4–7.5 g agar, pH 5 (Kraatz and Taras 2008)

[r]Holdeman et al. (1977)

A number of species of the family have also been isolated from mammalian body habitats other than the gut. However, their prevalence in these other environments has not been investigated, apart from *Atopobium* spp. in the mouth and vagina (Zhou et al. 2004; Ravel et al. 2011; Belda-Ferre et al. 2012; Liu et al. 2012; Santiago et al. 2012). The other body origins of *Coriobacteriaceae* include:

(a) The blood: *Atopobium rimae* (Angelakis et al. 2009), *Eggerthella sinensis* and *Paraeggerthella hongkongensis* (Lau et al. 2004b), and *Gordonibacter pamelaeae* (Woo et al. 2010).
(b) The perineum region and vagina: *Atopobium minutum* (Hauduroy et al. 1937; Collins and Wallbanks 1992) and *Atopobium vaginae* (Rodriguez Jovita et al. 1999). The latter species is usually found in biofilms adherent to the vaginal mucosa rather than in the vaginal fluid (Verhelst et al. 2004; Swidsinski et al. 2005; Polatti 2012).
(c) The oral cavity and respiratory tract: *Atopobium fossor* (Bailey and Love 1986; Kageyama et al. 1999b), *Atopobium parvulum* and *Atopobium rimae* (Weinberg et al. 1937; Olsen et al. 1991; Collins and Wallbanks 1992), *Cryptobacterium curtum* (Nakazawa et al. 1999), *Olsenella profusa* and *Olsenella uli* (Dewhirst et al. 2001), and *Slackia exigua* (Poco et al. 1996; Wade et al. 1999). Using 16S rRNA gene sequencing, Dewhirst et al. identified *Olsenella uli* and *Olsenella profusa* from subgingival plaques in patients with severe periodontal disease, suggesting that, similarly to other *Coriobacteriaceae* in the gut and vaginal mucosa, *Olsenella uli* and *Olsenella profusa* favor an adherent mode of growth. However, in sheep rumen, *Olsenella umbonata* was isolated from ruminal fluid, indicating variability in the mode of growth of this genus (Kraatz et al. 2011).

Metabolic Activities

Conversion of Cholesterol-Derived Host Metabolites

The potential of *Coriobacteriaceae* to modulate host metabolism in vivo has been recently brought to light by reports of significant correlations between their occurrence and altered metabolic parameters, including (a) higher intestinal cholesterol absorption and higher levels of plasma non-high-density lipoprotein (non-HDL) cholesterol in hamsters (Martinez et al. 2009, 2012) and (b) energy metabolism via decreased glycogenesis and enhanced triglycerides synthesis as well as hepatic detoxification pathways (higher 2*b*- and 6*b*-hydroxylase activity) in mice (Claus et al. 2011). Moreover, a recent metagenomic analysis of fecal samples from approximately 350 human subjects indicated that the prevalence of *Eggerthella lenta* is linked to type-2 diabetes (Qin et al. 2012). However, these data are descriptive and there is yet no direct proof of molecular mechanisms underlying the impact of *Coriobacteriaceae* on host metabolism. In other words, research on bacteria/host interactions with respect to *Coriobacteriaceae* is in its infancy.

The best studied metabolic functions of *Coriobacteriaceae* are the dehydrogenation and dehydroxylation of cholesterol-derived host factors (Ridlon et al. 2006). The type and various strains of *Eggerthella lenta* and *Collinsella aerofaciens* possess hydroxysteroid dehydrogenases (HSDH), which are responsible for stereospecific oxidation and epimerization (change from *a* to *b* configuration or vice versa) of bile acids, thereby generating stable oxo-bile acid intermediates. Hitherto detected dehydrogenases include both 3*a*- and 12*a*-HSDH in *Eggerthella lenta* and 7*b*-HSDH in *Collinsella aerofaciens* (Eyssen and Verhulst 1984; Ridlon et al. 2006). This hints at metabolic chains between *Coriobacteriaceae* and other bacteria, since the combined activity of two position-specific, stereochemically distinct HSDH (e.g., 3*a* and 3*b*) is required for epimerization of bile salts (Ridlon et al. 2006).

Although early work reported that *Eubacterium* spp., especially strain VPI 12708, were also capable of dehydroxylating free primary bile acids (cholic and chenodeoxycholic acid) into secondary bile acids (deoxycholic and lithocholic acid) (White et al. 1988; Takamine and Imamura 1995), deeper taxonomic assignment revealed that these bacteria actually belong to the genus *Clostridium* (Kitahara et al. 2000). There is to date no report on bile acid dehydroxylase activity in *Eggerthella lenta* or other *Coriobacteriaceae*. One paper referred to 7*a*-dehydroxylation by one isolate related to [*Eubacterium lentum*] without standing in nomenclature (Hirano and Masuda 1982). Bacterial dehydroxylation renders bile acids more hydrophobic, thereby favoring passive reabsorption in the proximal colon (enterohepatic circulation). However, secondary bile salts may also contribute to the pathogenesis of cholesterol gallstones and colon cancer (Ridlon et al. 2006). Altered bile acid metabolism has also been associated with chronic intestinal inflammation (Gnewuch et al. 2009; Devkota et al. 2012; Duboc et al. 2012).

Transformation of bile salts by HSDH and dehydroxylases is believed to serve as an energy source for the bacteria and reduce the levels of bile acids with antimicrobial activities (Ridlon et al. 2006). Several *Coriobacteriaceae* are reported to be bile resistant, e.g., *Asaccharobacter celatus*, *Eggerthella lenta*, *Olsenella umbonata*, and *Slackia piriformis*. Additionally, the favorable generation of oxo-bile acids by *a*-HSDH at higher redox potentials such as those encountered at mucosal surfaces may be one additional reason for the colonization of these areas by some *Coriobacteriaceae* (Ridlon et al. 2006). Finally, *Eggerthella lenta* is also able to dehydroxylate corticoids such as deoxycorticosterone to form progesterone via 21-dehydroxylase activity (Bokkenheuser et al. 1977). This species also carries a corticoid-converting 16*a*-dehydroxylase (Bokkenheuser et al. 1980) and a 3*a*-HSDH (Bokkenheuser et al. 1979). Strikingly, despite the apparent implication for the host of this bacterial rearrangement of hormonal networks in the gut, related functional studies in experimental animal models have not yet been performed.

Polyphenol Metabolism

One of the most peculiar enzymatic properties of *Coriobacteriaceae* is the conversion of food polyphenols, especially the activation of the isoflavone daidzein to the bioactive metabolite equol (Clavel and Mapesa 2013). Isoflavones are dietary phytoestrogens that are abundant in soybean and soy-derived products. They share structural similarities with steroid hormones such as 17-*b*-estradiol and thus have low binding affinity to estrogen receptors (Kuiper et al. 1998; Kostelac et al. 2003). Equol is known to be the most potent isoflavone metabolite, e.g., it has stronger affinity to estrogen receptors than its substrate (Clavel and Mapesa 2013). The biological properties of equol have been given attention since the 1930s when reproductive failures started to affect sheep grazing on clover containing high amounts of isoflavones and later in the 1980s in captive cheetahs fed a soy-based diet (Setchell et al. 1987; Messina 2010). Since then, equol has been associated with protective effects against cardiovascular diseases, bone disorders, prostate and breast cancer, and other hormone-related conditions, even though gold-standard randomized control trials are urgently needed to substantiate results (Clavel and Mapesa 2013). In humans, only 30–50 % of individuals are able to produce equol from daidzein, possibly due to the absence of specific equol-producing bacteria in the rest of the population (Xu et al. 1995; Rowland et al. 2000).

Evidence of intestinal microbial equol production dates back from the early 1980s (Axelson and Setchell 1981). However, it was only in 2005 that the first equol-producing isolate, strain Julong 732, was cultured from human feces (Wang et al. 2005). To date, only ten bacterial strains capable of producing equol from daidzein have been isolated from intestinal samples of pigs, rodents, and humans. Nearly all of them ($n = 9$) fall into the family *Coriobacteriaceae* based on 16S rRNA gene sequence analysis. These strains include five type strains, which have been fully described and assigned valid names (human isolates are marked with stars in the following list): (1) *Adlercreutzia equolifaciens** FJC-B9^T (=DSM 19450^T) (GenBank accession AB306661) (Maruo et al. 2008), (2) *Asaccharobacter celatus* do03^T (=DSM 18785^T) (AB266102) (Minamida et al. 2006, 2008), (3) *Enterorhabdus mucosicola* Mt1B8^T (=DSM 19490^T) (AM747811) (Matthies et al. 2008; Clavel et al. 2009), (4) strain Julong 732* (AY310748) (Wang et al. 2005), (5) '*Eggerthella*' sp. YY7918* (AB379693) (Yokoyama and Suzuki 2008), (6) *Slackia equolifaciens** DZE^T (=CCUG 58231^T) (EU377663) (Jin et al. 2010), (7) *Slackia isoflavoniconvertens** HE8^T (=DSM 22006^T) (EU826403) (Matthies et al. 2009), (8) '*Slackia*' sp. NATTS* (AB505075) (Tsuji et al. 2010), and (9) strain D1 (DQ904563) (Yu et al. 2008). Of note, *Adlercreutzia equolifaciens*, *Asaccharobacter celatus*, and strain Julong 732 share >99 % similarity based on 16S rRNA-based phylogeny (Maruo et al. 2008). One additional equol-producing isolate, strain D2, seems not to belong to the *Coriobacteriaceae* based on 16S rRNA gene sequencing (DQ904564) (Yu et al. 2008). Interestingly, the production of equol from daidzein by '*Slackia*' sp. NATTS was found to be two to fourfold higher after addition of 1 g/L autoclaved adonitol, arabinose, galactose, lactitol, inositol, melezitose, ribose, sorbitol, sorbose, trehalose, or xylose to the culture medium(Tsuji et al. 2010). Conversely, the addition of fructooligosaccharides, galactooligosaccharides, inulin, lactose, raffinose, or sucrose inhibited equol production. This may fit with the observation that resistant polysaccharides do not enhance equol production in vivo (Larkin et al. 2007; Mathey et al. 2007).

In addition to isoflavones, dietary lignans are phytoestrogens that can also be activated by *Coriobacteriaceae*. Conversion of plant lignans (pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, and corresponding glycosides) by gut bacteria involves two to five different reactions (deglycosylation, reduction, demethylation, dehydroxylation, and dehydrogenation) to form the enterolignans enterodiol and enterolactone (Clavel et al. 2006). Enterolignans were actually thought to be new steroid hormones after their first detection in urine samples from female primates and human adults (Setchell et al. 1980; Stitch et al. 1980). Their bacterial origin was highlighted shortly thereafter (Setchell et al. 1981; Borriello et al. 1985). Several strains of *Eggerthella lenta* were found to reduce and dehydroxylate plant lignans and intermediate metabolites thereof (Clavel 2006). Thus, beyond the metabolism of host-derived bile acids and steroid hormones, the species *Eggerthella lenta* is also involved in metabolic chains leading to the production of bioactive molecules from plant substrates in the gut. Recently, this species was also found to reductively cleave the heterocyclic C-ring of the flavanols epicatechin and catechin (Kutschera et al. 2011). Most importantly, the successful isolation and cultivation of phytoestrogen-converting strains open ways to assess the effects of bacterial metabolites on host health in detail using, for instance, gnotobiotic approaches (i.e., colonization of germfree animals with specific strains of interest) (Woting et al. 2010; Becker et al. 2011; Mabrok et al. 2012).

Pathogenicity, Clinical Relevance

As seen above, members of the *Coriobacteriaceae* carry out functions of importance for their hosts. However, several members of the genera *Atopobium*, *Eggerthella*, *Gordonibacter*, *Olsenella*, and *Paraeggerthella* have been also implicated in the development of various clinical pathologies including abscesses, intestinal diseases and tumors, periodontitis, vaginosis, and bacteremia. *Coriobacteriaceae* can thus be considered as pathobionts, i.e., potentially pathogenic commensal species of host body microbiota (Chow et al. 2011). However, one can say that nearly all published studies on *Coriobacteriaceae* refer to descriptive work, for instance, the enumeration of bacteria in diseased versus healthy tissues/subjects or the isolation of bacteria from clinical specimens. Hence, fundamental knowledge on how and when *Coriobacteriaceae* start to be detrimental to their hosts is lacking. The antimicrobial susceptibility profile of some family members has been well defined in various studies and is summarized in *Table 11.13*.

Table 11.13
Antimicrobial susceptibility profiles of *Coriobacteriaceae*

Antibiotic class	Antibiotic	*Atopobium parvulum*	*Atopobium rimae*	*Atopobium vaginae*	*Collinsella aerofaciens*	*Eggerthella lenta*	*Eggerthella sinensis*	*Enterorhabdus caecimuris*	*Enterorhabdus mucosicola*	*Olsenella uli*	*Paraeggerthella hongkongensis*	*Parvibacter cecicola*	*Slackia exigua*
Penicillins	Amoxicillin					1							
	Ampicillin	0.125	0.023	<0.016–0.94	≤0.03–1	0.5–2							0.094–0.19
	Oxacillin							36	4.667			6	
	Penicillin		0.064	0.008–0.25	≤0.03–2	1–4	0.5			≤0.03–1	0.25–2		0.064–0.125
	Piperacillin					1–16							
Tetracyclines	Doxycycline			0.19–0.75									
	Minocycline	0.25											
	Tetracycline				0.06–8	6		0.12	0.115	0.125–32		0.069	
	Tigecycline					0.12–25					0.06–0.25		
Macrolides	Azithromycin		<0.016	<0.016–0.32	≤0.03–0.25								
	Clarithromycin	<0.004						<0.016	<0.016			< 0.016	
	Erythromycin		<0.016		≤0.03–0.25	3		<0.016	0.048			< 0.016	0.016–0.023
Aminoglycosides	Kanamycin			8–16									
	Tobramycin							4.333	2.667			0.6	
Quinolones	Ciprofloxacin		0.06	0.023–0.25	≤0.5–2			0.305	>32	≤0.5– > 8		0.061	
	Levofloxacin	0.25–0.5			≤0.06–2	0.5				0.25–8			
	Moxifloxacin			0.06–1		0.25– > 32					0.25–4		
	Nalidixic acid			>256									
	Nemofloxacin					0.25– > 32					0.5–2		
	Nifuratel			0.125–1									
	Trovafloxacin			<0.015–2									
Polypeptides	Bacitracin			1–4									
	Colistin			>1,024					>256			> 256	
Lincosamides	Clindamycin	1		<0.016–2	≤0.03–0.25	<0.06– > 32		0.105	<0.016	≤0.03– > 32	4	< 0.016	0.016–0.023

Oxazolidinones	Linezolid			0.016–0.25		0.5–4					1		
Sulfonamide	Trimethoprim/ sulfamethoxazole												>32
Carbapenems	Doripenem					0.25					0.25		
	Ertapenem												0.016–2
	Imipenem	0.25	0.032	<0.015–0.5	≤0.03–0.25	0.06–8					0.5–1		
	Meropenem			<0.015–0.5		0.5					0.5		
Cephems	Cefalexin				0.25 –8					0.25– > 32			
	Cefepime			1									
	Cefmetazole	2				16–32					8–16		
	Cefotaxime					0.5– > 256	0.25	>32	1.25		>32	4.167	
	Cefotetan					8–128							
	Cefoxitin			2–3									0.5–3
	Ceftriaxone	0.5		0.25–2		1– > 128							0.002–0.25
	Cefuroxime			0.016–0.25									
	Flomoxef					8–16					8–16		
B-Lactam/B-lactamase inhibitor combinations	Amoxicillin/ clavulanic acid		0.012		≤0.015–0.25	1–4				≤0.015–2			0.094–0.25
	Ampicillin-sulbactam			0.032–0.25		4					2–4		
	Piperacillin-tazobactam				≤0.03–16	32–64					16–64		
Glycopeptides	Vancomycin	2	1.25	1–4	0.25–2	0.5–2	3	1.5	1.333		0.75–2	0.585	0.032–0.5
	Teicoplanin					0.06–0.5							
Lipopeptides	Daptomycin				0.06– > 32	0.25–0.5					0.25		
Macrocyclic antibiotics	Fidaxomicin					0.015–0.25					0.12–0.25		
Nitroimidazoles	Metronidazole	2		0.12– >256	≤0.03–2	0.25–2	0.25	0.016	0.034	0.125–>32	0.25–4	0.069	0.064–0.38
Phenicols	Chloramphenicol					8–16							

Table 11.13 (continued)

Antibiotic class	Antibiotic	*Atopobium parvulum*	*Atopobium rimae*	*Atopobium vaginae*	*Collinsella aerofaciens*	*Eggerthella lenta*	*Eggerthella sinensis*	*Enterorhabdus caecimuris*	*Enterorhabdus mucosicola*	*Olsenella uli*	*Paraeggerthella hongkongensis*	*Parvibacter cecicola*	*Slackia exigua*
Rifamycin	Rifampicin			<0.002									
Streptogramins	Quinupristin/ dalfopristin				0.06–8	0.25–2							
References		a, b	c	d, e, f, g, h, i	j, k	a, l, m, n, o, p	q	r	s	k	q, m	t	u

The shaded boxes show antimicrobial resistance according to the 2012 CLSI MIC breakpoints for anaerobes (M100-S22)

References (when more than one strain was analyzed, the number of strains is shown within brackets after the corresponding reference):

[a]Tanaka et al. (2006)

[b]Hirokawa et al. (2008)

[c]Angelakis et al. (2009)

[d]Knoester et al. (2011)

[e]Salimnia et al. (2008)

[f]Polatti (2012)

[g]Ferris et al. (2004) (3)

[h]De Backer et al. (2006) (9)

[i]Chan et al. (2012)

[j]Goldstein et al. (2003) (9)

[k]Merriam et al. (2006) (7)

[l]Liderot et al. (2010)

[m]Lee et al. (2012) (8)

[n]Mosca et al. (1998) (29)

[o]Sneath et al. (1986) (12)

[p]Credito and Appelbaum (2004) (10)

[q]Lau et al. (2004b)

[r]Clavel et al. (2010)

[s]Clavel et al. (2009)

[t]Clavel et al. (2013)

[u]Kim et al. (2010) (6)

Bacteremia

Five of the 14 genera of the family include species which have been already isolated from blood samples of human patients: *Atopobium*, *Eggerthella*, *Gordonibacter*, *Olsenella*, and *Paraeggerthella*. There seems to be a consensus about the reservoir of infection as being the natural habitats of *Coriobacteriaceae*, i.e., the mouth and the gastrointestinal or genital tract, or acutely infected organs (Lau et al. 2004a; Salimnia et al. 2008; Angelakis et al. 2009; Woo et al. 2010; Thota et al. 2011).

The best documented cases of *Coriobacteriaceae*-driven bacteremia relate to *Eggerthella* spp. and closely related species. In Hong Kong, between 1998 and 2001, *Eggerthella lenta* was associated with five of 16 clinically relevant cases of bacteremia, whereas five additional cases were associated with the presence of its relatives *Eggerthella sinensis* and *Paraeggerthella hongkongensis* (Lau et al. 2004a, b). Lee et al. very recently published 10 additional cases of bacteremia due to *Eggerthella lenta* and *Paraeggerthella hongkongensis* in Taiwanese subjects hospitalized between 2001 and 2010 (Lee et al. 2012). Landais et al. also reported two cases of bacteremia in France that were associated with the presence of *Eggerthella lenta* based on 16S rRNA gene sequencing of isolates (the authors erroneously cited the genus name as '*Eggerthela*') (Landais et al. 2007). In this study, patient 1 was admitted to the hospital with fecal peritonitis related to intestinal perforation, whereas patient 2 had acute appendicitis. They received imipenem (1.5 g/day for 3 weeks) and amoxicillin/clavulanic acid (3 g/day), respectively, with favorable outcomes. Two additional clinically relevant strains of *Eggerthella lenta* have been reported, including one strain identified on the basis of the VITEK system after isolation from the blood of a 21-year-old African-American woman diagnosed with Crohn's disease who developed bacteremia after ileocaecal resection (Chan and Mercer 2008; Thota et al. 2011). This case of *Eggerthella lenta* bacteremia was successfully treated with a combination of meropenem, metronidazole, and vancomycin. Finally, one case of polymicrobial bloodstream infection with *Eggerthella lenta* and *Desulfovibrio desulfuricans* was reported in Sweden in one 86-year-old woman who was successfully treated with cefuroxime and amoxicillin (Liderot et al. 2010). Of note, a rather broad range of diseases may underlie translocation of *Eggerthella* spp. from the gut to the blood stream, since patients positive for these species in blood cultures were hospitalized for a variety of reasons (pelvic inflammatory disease, infected bed sore, perianal abscess, infected rectal tumor, liver abscess, acute appendicitis, and proctitis) and suffered from a variety of chronic diseases (lung, cervical and colon cancer, alcoholic cirrhosis, diabetes, cardiovascular disorders, recurrent pyogenic cholangitis) (Lau et al. 2004a; Landais et al. 2007). Finally, *Eggerthella lenta* was also isolated from (a) the pus of a hepatic abscess from a 42-year-old patient who was treated favorably with a course of metronidazole (1.5 g/day) (Landais et al. 2007) and (b) bone biopsy samples of the spine in one 82-year-old Chinese women with spondylodiscitis who was treated with trimethoprim/sulfamethoxazole and metronidazole (Bok and Ng 2009).

The genus *Atopobium* also gained attention following the isolation of strains from clinical samples (Olsen et al. 1991; Kumar et al. 2005). *Atopobium rimae*, together with *Streptococcus gordonii*, was recently associated with a case of septic shock in a 77-year-old woman in France, from whom two isolates were recovered from blood cultures on two separate occasions during hospitalization for pneumonia (Angelakis et al. 2009). Treatment of the patient with intravenous amoxicillin-clavulanate (2 g/200 mg) led to full recovery within 7 days. Beforehand, *Atopobium rimae* had been already identified in blood samples from a 47-year-old man with liver cirrhosis, who was treated with success using metronidazole and imipenem (Chung et al. 2007). Another *Atopobium* species phylogenetically closely related to *Atopobium rimae* (98 % 16S rRNA gene sequence identity) has also been associated with bacteremia (Salimnia et al. 2008). This species, provisionally named "*Atopobium detroiti*", was isolated from the blood of a 38-year-old paraplegic male patient hospitalized for presumed sepsis and characterized by a necrotic decubitus ulcer of the hip and poor oral hygiene after physical examination. Finally, the species *Atopobium vaginae* has also been identified in the context of intrauterine infection leading to fetal death and maternal bacteremia in a 40-year-old woman undergoing transcervical chorionic villus sampling (Knoester et al. 2011). Unlike *Eggerthella lenta*, *Atopobium vaginae* has been associated with metronidazole resistance (Ferris et al. 2004; De Backer et al. 2006; Knoester et al. 2011), and successful treatment of *Atopobium vaginae* bacteremia usually involves a course of *b*-lactam antibiotics alone or in combination with *b*-lactamase inhibitors or clindamycin (Knoester et al. 2011; Chan et al. 2012).

Less frequently reported cases of *Coriobacteriaceae*-driven bacteremia relate to bacteria other than *Eggerthella* and *Atopobium*. One isolate identified as *Gordonibacter pamelaeae* based on 16S rRNA gene sequencing and phenotypic description was recently recovered from the blood of an 82-year-old Chinese man diagnosed to have rectosigmoid carcinoma with lung metastasis (Woo et al. 2010). In contrast to the type strain of the species, this isolate was found to be nonmotile and positive for arginine arylamidase. The patient was successfully treated with a course of intravenous amoxicillin-clavulanate for 9 days. Finally, one case of bacteremia associated with a strain of *Olsenella uli* obtained from the blood of one 43-year-old male subject suffering from acute cholangitis has been reported (Lau et al. 2004a).

In summary, when compared with bacteremia due to usual suspects such as *Bacteroides fragilis*, enterobacteria, enterococci, or staphylococci, cases of *Coriobacteriaceae*-driven bacteremia seem to be relatively rare, but are very often clinically relevant. More research effort is needed to identify environmental factors and molecular mechanisms that favor initial colonization and survival of *Coriobacteriaceae* in the blood. Of note, only three genera within the family are positive for catalase activity: *Eggerthella*, *Gordonibacter*, and *Paraeggerthella*. All three have been associated with cases of bacteremia. The presence of catalase may help these organisms coping with oxidative stress during infection.

Gastrointestinal Pathologies

Although there are an increasing number of studies investigating the gut microbiota in colorectal cancer (CRC), the exact contribution of bacteria to molecular mechanisms underlying disease remains unclear. Intestinal bacteria are proposed to play a role in CRC via two main mechanisms: (1) the production of metabolites such as hydrogen sulfide or ammonia, which can have detrimental effects on host cell functions (Blaut and Clavel 2007), and (2) the alteration of innate immune mechanisms (Rakoff-Nahoum and Medzhitov 2007).

The role of a variety of bacteria such as enterotoxigenic *Bacteroides fragilis*, *Enterococcus faecalis*, *Fusobacterium* spp., *Prevotella* spp., and *Streptococcus bovis* in CRC has already been discussed (Wu et al. 2009; Al-Jashamy et al. 2010; Sobhani et al. 2011; Kostic et al. 2012). *Coriobacteriaceae* have gained attention in this field very recently. The occurrence of *Collinsella*, *Eggerthella*, *Olsenella*, and *Slackia* spp. was significantly higher on tumor site versus adjacent nonmalignant tissue in six Dutch patients who underwent resection for primary colon adenocarcinoma (Marchesi et al. 2011). Other recent studies on bacterial diversity in CRC patients found an increased prevalence of 16S rRNA gene sequences classified as *Actinobacteria*, including *Collinsella* spp., in feces from CRC versus healthy control subjects (Chen et al. 2012; Wang et al. 2012). In the study by Chen et al., the prevalence of sequences assigned to the *Coriobacteriaceae* was 1.19 % in CRC patients versus 0.74 % in healthy individuals. Still, these data refer only to the density of bacterial populations, and there is no indication that *Coriobacteriaceae* have overall positive or negative effects on tumorigenesis. *Coriobacteriaceae* have recently been referred to as "passenger" bacteria in CRC, in contrast to "driver" bacteria such as *Bacteroides fragilis* which seem to be involved in the initiation of disease (Tjalsma et al. 2012). Passenger bacteria are proposed to be best suited for colonization of disturbed microenvironments in the vicinity of tumors. In that context, the effect of local production of equol by *Slackia* spp. that colonize tumor sites in the gut may be worth investigating considering the biological properties of this bacterial product (Magee et al. 2006; Choi 2009). The effects of ammonia production by, for instance, *Olsenella* spp. or *Eggerthella lenta* may be worth investigating too (Eschenlauer et al. 2002; Kraatz et al. 2011).

Apart from cancer, the role of *Coriobacteriaceae* in other pathologies associated with gastrointestinal dysfunctions is ill defined. Isolates of *Eggerthella lenta* identified on the basis of fermentation and biochemical reactions were recovered in 44 % of 41 appendix tissue samples from children with suspected acute appendicitis (Rautio et al. 2000). Moreover, although clinical case reports and targeted isolation procedures hint at the relevance of *Coriobacteriaceae* in inflammatory bowel diseases, there is to date no corresponding quantitative or functional data available (Clavel et al. 2009, 2013; Würdemann et al. 2009; Joossens et al. 2011; Thota et al. 2011). Finally, there is an increasing body of evidence pointing at the involvement of gut bacteria in host energy balance and metabolic disorders (Backhed et al. 2004; Qin et al. 2012). *Coriobacteriaceae* have been detected in the feces of 14 overweight and obese human volunteers with no history of gastrointestinal disease (Walker et al. 2011). As previously reported in healthy individuals (Harmsen et al. 2000; Kageyama et al. 2000; Tap et al. 2009), *Collinsella aerofaciens* was amongst the most abundant taxonomic units (3.7 % of 16S rRNA clones) after *Faecalibacterium prausnitzii* (8.0 %), *Eubacterium rectale* (4.4 %), and *Clostridium clostridioforme* (3.8 %) in the fecal sample from six of the 14 volunteers. The proportion of *Collinsella aerofaciens* was significantly reduced to 0.6 % after consumption of a protein-rich, fat, and carbohydrate-reduced weight-loss diet. In another study, the number of 16S rRNA gene sequences assigned to *Coriobacteriaceae* in the feces of three obese subjects was found to be higher than in lean controls and in subjects after gastric bypass-induced weight loss (Zhang et al. 2009). Based on these descriptive findings on the dominance of *Coriobacteriaceae* in the gut and considering their metabolic potential with regard to hepatic functions and lipid homeostasis (see metabolic activities), their role in the regulation of host metabolic disorders is worth investigating in more details.

Allergy

Commensal gut microbial communities are known to influence host immune responses beyond the gut. For instance, they have been implicated in the regulation of molecular mechanisms underlying allergies (Hormannsperger et al. 2012). A molecular study comparing the fecal microbiota in <12-month-old infants with cow's milk protein allergy versus nonallergic infants ($n = 46$ each) found higher median counts of the *Atopobium* group in allergic infants (0.6 vs. 0.0 % of total bacteria) (Thompson-Chagoyan et al. 2011). Of note, in a former study, the *Atopobium* and *Collinsella* group represented a substantial proportion of the gut microbiota in the feces of formula-fed infants when compared with breast-fed infants (>17 vs. 0.5 % of total bacteria; $n = 6$ each) (Harmsen et al. 2000). Breast-feeding is proposed to have protective effects on the development of atopic disorders, although more data are needed to reach consensus in results (Mimouni Bloch et al. 2002; Batchelor et al. 2010; Brew et al. 2011).

Dental Caries and Abscess

In the human oral cavity, *Coriobacteriaceae*, including *Atopobium parvulum*, *Atopobium rimae*, and *Olsenella profusa*, have been detected during the final phase of caries extension in dental pulp with established and advanced infection (Nadkarni et al. 2010). The spatial distribution of these bacteria suggested an intricate association with members of the *Bacteroidetes* in tightly concentrated biomass, even though underlying reasons were unclear. Identification of bacterial pathogens by 16S rRNA gene-targeted PCR in the oral cavity of 21 patients suffering from primary or persistent endodontic infections revealed that some *Coriobacteriaceae* were amongst the most prevalent phylotypes: *Olsenella uli*,

Olsenella profusa, and *Atopobium parvulum* were identified in 33, 9.5, and 5 % of cases of infection, respectively (Siqueira and Rocas 2005). *Olsenella uli* was also identified in persistent endodontic infections in this study. This species was also found to be one of the most prevalent species in root canals from 139 teeth with apical periodontitis (Dewhirst et al. 2001; Chavez de Paz et al. 2004). A number of additional papers reported the detection of *Coriobacteriaceae*, especially *Atopobium* and *Olsenella* spp., in oral clinical samples (Kumar et al. 2003, 2005; Aas et al. 2008; Preza et al. 2008; Subramanian and Mickel 2009; Lima et al. 2011). In one additional study, high-throughput sequencing of 16S rRNA genes allowed the identification of *Coriobacteriaceae* in the oral cavity, infected root canal, and periapical abscess of eight patients (Hsiao et al. 2012). The genus *Atopobium* was mostly found in root canal samples, whereas the genus *Collinsella* was significantly overrepresented in abscess samples. Other *Coriobacteriaceae*, including *Olsenella*, *Slackia*, *Cryptobacterium*, and *Eggerthella* were seldom identified in oral cavity samples.

Bacterial Vaginosis

Bacterial vaginosis is a frequently reported polymicrobial infection in which the commensal microbiota usually dominated by lactobacilli is replaced by obligate anaerobes (Danielsson et al. 2011). The type strain of *Atopobium vaginae* was isolated from the vagina of a healthy woman (Rodriguez Jovita et al. 1999). The pathogenic potential of this species was highlighted in 2003 by a case of tubo-ovarian abscess following transvaginal oocyte recovery (Geissdorfer et al. 2003). Clinical isolates have also been recovered in the context of uterine endometritis (Yamagishi et al. 2011) and intrauterine infection (Knoester et al. 2011). Thanks to molecular techniques, this bacterium has been frequently detected in vaginal infections and is thought to be involved in 55–95 % of cases and responsible for therapeutic failures (Ferris et al. 2004; Verhelst et al. 2004; Polatti 2012). A recent evaluation of the microbiota in vaginal swabs from 220 women using pyrosequencing of 16S rRNA gene amplicons showed that women with vaginosis are characterized by diverse heterogeneous communities with a high prevalence of *Atopobium vaginae* and *Eggerthella* species (Srinivasan et al. 2012).

Atopobium vaginae is commonly identified alongside *Gardnerella vaginalis* in clinical samples, and their association appears to provide a reliable diagnosis (Lamont et al. 2011; Srinivasan et al. 2012). Fluorescence in situ hybridization analysis of vaginal biopsies provided further evidence of the strong co-occurrence of these species, which accounts for more than 90 % of the biofilm mass on vaginal epithelial surfaces (Swidsinski et al. 2005). The biofilm-forming properties of *Atopobium vaginae* and *Gardnerella vaginalis* contribute to the recalcitrance of infection by conferring a protective environment against both antibacterial therapies and immune responses. A 5-day treatment of polymicrobial *Gardnerella*, *Atopobium*, and *Lactobacillus* spp. biofilm using 400 mg/day moxifloxacin in women with bacterial vaginosis showed a significant decrease in *Atopobium* and *Gardnerella* coupled to an increase in lactobacilli in biofilms (Swidsinski et al. 2011). However, despite short-term clinical efficacy, moxifloxacin (similarly to metronidazole and clindamycin) fails to prevent the recurrence of vaginosis (Swidsinski et al. 2011; Bradshaw et al. 2012).

The antibiotic susceptibility profile of *Atopobium vaginae* reveals resistance to the antibiotics nalidixic acid and colistin with MIC values higher than 256 and 1,024 μg/mL, respectively, while metronidazole resistance was reported for a number of strains (Ferris et al. 2004; De Backer et al. 2006; Polatti 2012). Also, *Atopobium vaginae* was found to be susceptible to a range of antibiotics including clindamycin, the antibiotic of choice for bacterial vaginosis, as well as ampicillin, ampicillin-sulbactam, azithromycin, ceftriaxone, ciprofloxacin, imipenem, linezolid, meropenem, moxifloxacin, penicillin, rifampicin, and trovafloxacin (Ferris et al. 2004; De Backer et al. 2006). It was recently suggested that the nitrofuran derivative, nifuratel, provides an alternative therapy for bacterial vaginosis involving the common pathogens *Atopobium vaginae* and *Gardnerella vaginalis*, without affecting the commensal microbiota of the vagina (Togni et al. 2011; Polatti 2012).

Application

Due to the recent description of a substantial number of *Coriobacteriaceae* species and to the even more recent reports that highlight some of their physiologically and clinically relevant functions, the use of these bacteria for application purposes has been very limited so far, but is at favorable odds for the near future.

The ability of *Coriobacteriaceae* to convert dietary isoflavones into the bioactive product equol is of particular interest for potential nutraceutical or pharmaceutical applications. The observation that two thirds of the human population cannot produce equol has spurred considerable interest on applied microbiological approaches aimed at triggering equol production in non-equol producers, along with the hypothesis that people hosting equol-producing *Coriobacteriaceae* are more likely to benefit from potentially beneficial health effects of soyfood and isoflavone intake. However, most attempts fell short of their target. First, there is to date no official nutritional recommendation on the benefit of dietary soy isoflavones on human health and state-of-the-art intervention trials are needed (Clavel and Mapesa 2013). Second, a number of animal and human studies examined the use of probiotic strains to boost equol production but failed to establish clear evidence (Larkin et al. 2007; Clavel and Mapesa 2013). Finally, the intake of *Coriobacteriaceae* themselves as probiotic strains in human subjects is for obvious safety issues not sound. However, the use of already isolated and characterized *Coriobacteriaceae* can be of great value in several ways: (1) for gathering functional evidence that equol is indeed directly linked to beneficial health effects using gnotobiotic mouse models of diseases colonized with, for instance, equol-producing or steroid-dehydroxylating versus

non-active strains (Woting et al. 2010; Becker et al. 2011), (2) for studying the production and effects of so far unknown isoflavone products such as 5-hydroxy-equol (Matthies et al. 2008), and (3) for large-scale affordable production of equol, for instance, for the sake of intervention trials that require large quantity of pure material. With respect to the latter point, the enantiospecificity of equol production is noteworthy. Gut bacteria are known to produce exclusively the *S*-enantiomer of equol, which seems to be more biologically active than its counterpart *R*-equol (Setchell et al. 2005; Wang et al. 2005, 2007; Shinkaruk et al. 2010). Patents related to the bacterial or synthetic production of enantiomeric equol and to the isolation of involved bacterial enzymes have already been registered (Setchell et al., US2009/7528267, Shimada et al., US2010/0330627; Isono et al., US2011/0189134; Tsuji et al., US2011/0318309). *Coriobacteriaceae*-based applications for the sake of metabolite production are also valid with respect to secondary bile acids, as recently studied using a 7*b*-HSDH from *Collinsella aerofaciens* (Braun et al. 2012).

The aforementioned use of *Coriobacteriaceae* in gnotobiotic mouse models can actually be extended to the study of host metabolic functions. Such experiments would help deciphering, for instance, the health implication of *Eggerthella*-encoded bile acid and steroid dehydroxylases as well as the role of these bacteria on hepatic functions, e.g., lipid metabolism and detoxification pathways (Ridlon et al. 2006; Claus et al. 2011; Martinez et al. 2012). In addition, one member of the *Coriobacteriaceae* isolated from the bovine rumen, *Denitrobacterium detoxificans*, is capable of metabolizing the nitrotoxins 3-nitro-1-propanol and 3-nitro-1-propionate found in forages, thereby providing potential industrial application for clearance of nitrocompounds from environmental samples or enhancement of tolerance towards environmental toxins in cattle (Anderson et al. 2000, 2005). *Slackia heliotrinireducens* may also be of interest for the reduction of pyrrolizidine alkaloid poisoning in cattle (Hovermale and Craig 2002).

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12 The Family *Corynebacteriaceae*

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Abstract
The family *Corynebacteriaceae* is composed of the type genus *Corynebacterium* with almost 90 species and the monospecific genus *Turicella*. The status of *Turicella* as a genus is supported by phenotypic characteristics. Both taxa form a distinct clade in the order *Corynebacteriales* which is clearly separated from the related families *Dietziaceae* and *Tsukamurellaceae*. Most *Corynebacterium* species contain mycolic acids with 22–36 carbons, though few species and *Turicella otitidis* lack this component. The peptidoglycan is of the A1γ type with directly cross-linked *meso*-diaminopimelic acid. Arabinose and galactose are the major cell-wall sugars. Fatty acid profiles are rich in saturated and unsaturated components and may contain tuberculostearic acid. The quinone systems reported for *Corynebacterium* species consist exclusively of menaquinones, with major amounts of MK-8(H_2), MK-9(H_2), or a mixture of both. *Turicella otitidis* contains completely unsaturated menaquinones (MK-10, MK-11). A significant variability in the G+C content of genomic DNA of *Corynebacterium* species has been reported, ranging from ∼46 mol% (*Corynebacterium kutscheri*) to 74 mol% (*Corynebacterium auris*). Members of the family *Corynebacteriaceae* are found in diverse environments. Some species are used in industrial applications and food production, whereas other species are serious pathogens of humans or domestic animals. Many of the medically relevant species can be recovered as commensals or contaminants from a variety of clinical specimens. Some *Corynebacterium* species usually associated with animals have also been documented to cause human infections by zoonotic transmission. The closely related organisms *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, and *Corynebacterium pseudotuberculosis* are the only species which may produce potent exotoxins, i.e., diphtheria toxin and phospholipase D which both play a significant role in pathogenicity.

Taxonomy, Historical and Current

Short Description of the Family

Family *Corynebacteriaceae* Lehmann and Neumann 1907[AL] emend. Stackebrandt, Rainey and Ward-Rainey 1997, 485 emend. Zhi, Li and Stackebrandt 2009, 593

Co.ry.ne.bac.te.ri.a.ce'a.e. N.L. neut. n. *Corynebacterium* type genus of the family; suff. −*aceae* ending to denote a family; N.L. fem. pl. n. *Corynebacteriaceae* the *Corynebacterium* family.

The family *Corynebacteriaceae* (Lehmann and Neumann 1907; Stackebrandt et al. 1997; Zhi et al. 2009) belongs to the phylum Actinobacteria, class *Actinobacteria*, and order *Corynebacteriales* (Goodfellow 2012; Stackebrandt et al. 1997; Goodfellow and Jones 2012). The family encompasses the type genus *Corynebacterium* (Lehmann and Neumann 1896; Bernard et al. 2010) and the monospecific genus *Turicella* (Funke, et al. 1994). The cell-wall peptidoglycan is of type A1γ with directly cross-linked *meso*-diaminopimelic acid, and the major cell-wall sugars are arabinose and galactose (also referred to as wall chemotype IV). The glycan type of the cell walls

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contains acetyl residues. The major fatty acids are $C_{16:0}$ and $C_{18:1}$ ω9*c*; tuberculostearic acid (10-methyl $C_{18:0}$) may be present. Most *Corynebacterium* species contain major amounts of menaquinones with either eight or nine isoprenoic units in the side chain, one of which is saturated [MK-8(H_2), MK-9(H_2), or a mixture of both]. Completely unsaturated menaquinones (MK-10, MK-11) are found in *Turicella otitidis*. *Corynebacterium* species typically contain short-chain mycolic acids with a length of 22–36 carbon atoms (corynemycolic acids), but some species and *Turicella otitidis* lack mycolic acids entirely. The G+C content of the genomic DNA varies from ~46 to 74 (mol%). Specific 16S rRNA signature nucleotides for this family have been described (Zhi et al. 2009). The specific pattern of 16S rRNA signatures consists of nucleotides or nucleotide pairs at positions 250 (U), 316:337 (U–G), 418:425 (C–G), 586:755 (U–G), 599:639 (C–G), 662:743 (U–G), 987:1218 (G–C), and 1059:1198 (U–A).

Phylogenetic Structure of the Family and Its Genera

According to recent improvements in the taxonomy of actinomycetes, the family *Corynebacteriaceae* belongs to the phylum Actinobacteria, class *Actinobacteria*, and order *Corynebacteriales* (Goodfellow 2012; Stackebrandt et al. 1997; Goodfellow and Jones 2012). *The order Corynebacteriales includes* aerobic or facultatively anaerobic, Gram-stain-positive, catalase-positive actinomycetes. Arabinose and galactose are the major cell-wall sugars. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid and is of type A1γ (wall chemotype IV). Fatty acid profiles are rich in saturated and unsaturated components and usually contain tuberculostearic acid. Members of the order *Corynebacteriales* typically contain mycolic acids. Until recently, wall chemotype IV actinomycetes that contain mycolic acids were assigned to genera in the suborder *Corynebacterineae* (Stackebrandt et al. 1997; Zhi et al. 2009) which was recasted into the order *Corynebacteriales* in 2012 (Goodfellow and Jones 2012). In the revised taxonomy of the actinobacteria, the taxonomic ranks of subclasses and suborders are eliminated. The phylum Actinobacteria is now divided into six classes, and the class *Actinobacteria* now contains a total of 15 orders (Goodfellow and Jones 2012). Families and genera classified in the order *Corynebacteriales* can be distinguished from one another and from corresponding taxa in the phylum Actinobacteria by 16S rRNA similarity values and by taxon-specific 16S rRNA nucleotide sequences (Zhi et al. 2009). The pattern of 16S rRNA signatures for the order *Corynebacteriales* consists of nucleotides at positions 127:134 (G–Y), 564 (C), 672:734 (U–G), 833:835 (U–G), 952:1229 (U–A), and 986:1219 (U–A). Moreover, analyses of protein sequences from sequenced *Corynebacteriales* genomes have identified conserved signature proteins and conserved signature indels which are highly specific for this order (Gao and Gupta 2012). A two-amino-acid insert in a conserved region of a macrolide transporter ATP-binding protein is specifically present in all of the *Corynebacteriales* proteins, but not in the orthologous counterparts of other actinobacteria Likewise, a one-amino-acid deletion is uniquely present in a conserved region of the enzyme α-ketoglutarate decarboxylase in all *Corynebacteriales* protein sequences (Gao and Gupta 2012).

Members of the order *Corynebacteriales* are found in diverse environments, notably in the soil ecosystem, and some species are serious pathogens of humans and domestic animals (Goodfellow and Jones 2012). The order *Corynebacteriales* comprises the families *Corynebacteriaceae*, *Dietziaceae*, *Gordoniaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Segniliparaceae*, and *Tsukamurellaceae* and includes the recently described taxa *Millisia*, *Skermania*, *Smaragdicoccus*, *Tomitella*, and *Williamsia* (Goodfellow and Jones 2012). The genera classified in the families *Gordoniaceae*, *Mycobacteriaceae*, *Nocardiaceae*, and *Tsukamurellaceae* contain *N*-glycolated muramic acid and a phospholipid pattern which includes the taxonomically significant nitrogenous phospholipid phosphatidylethanolamine (phospholipid type II). They can be distinguished on the basis of the menaquinone composition and the overall chain length of their mycolic acids (Goodfellow and Jones 2012). Genera assigned to the families *Corynebacteriaceae* and *Dietziaceae* can be distinguished from the other mycolic acid-containing genera as they contain *N*-acetyl residues in the glycan moiety of the peptidoglycan. Recent analyses of genome sequences identified molecular signatures for the main clades of the phylum Actinobacteria (Gao and Gupta 2012). The detected conserved signature proteins and conserved signature indels provide useful markers for an understanding of the phylogeny of actinobacteria and the relationship of a number of clades in the order *Corynebacteriales*. A molecular signature based on the absence of 12 conserved signature proteins supports the deep branching of a clade of *Corynebacterium* species and *Dietzia* within the order *Corynebacteriales* (Gao and Gupta 2012).

The family *Corynebacteriaceae* is currently composed of the genus *Corynebacterium* with 89 species and the monospecific genus *Turicella* (Bernard and Funke 2012; Aravena-Román et al. 2012; Zhou et al. 2012; Frischmann et al. 2012; Wu et al. 2011; Shin et al. 2011a). Numerous phylogenetic studies based on 16S rRNA or *rpoB* gene sequences indicated that the genus *Turicella* forms a deeply branching lineage within the clade comprising the species of the genus *Corynebacterium* (Pascual et al. 1995; Ruimy et al. 1995; Riegel et al. 1995a, 1997a; Funke et al. 1995a, 1997a, 1998a; Collins et al. 1998; Fernández-Garayzábal et al. 1998; Wattiau et al. 2000; Brennan et al. 2001; Renaud et al. 2001; Goyache et al. 2003a; Khamis et al. 2004, 2005). This phylogenetic placement of the species *Turicella otitidis* among *Corynebacterium* species creates a non-monophyletic genus *Corynebacterium* (❯ *Fig. 12.1*), indicating that the current taxonomy needs to be revised (Pascual et al. 1995; Busse 2012). However, the status of *Turicella* as a genus was supported by the lack of mycolic acids and by the menaquinone pattern (MK-10, MK-11) (Funke et al. 1994). The latter feature excludes *Turicella otitidis* from the genus *Corynebacterium,* in which MK-9(H_2) or MK-8(H_2) or both are the major menaquinones (Collins et al. 1977; Funke et al. 1994), though significant

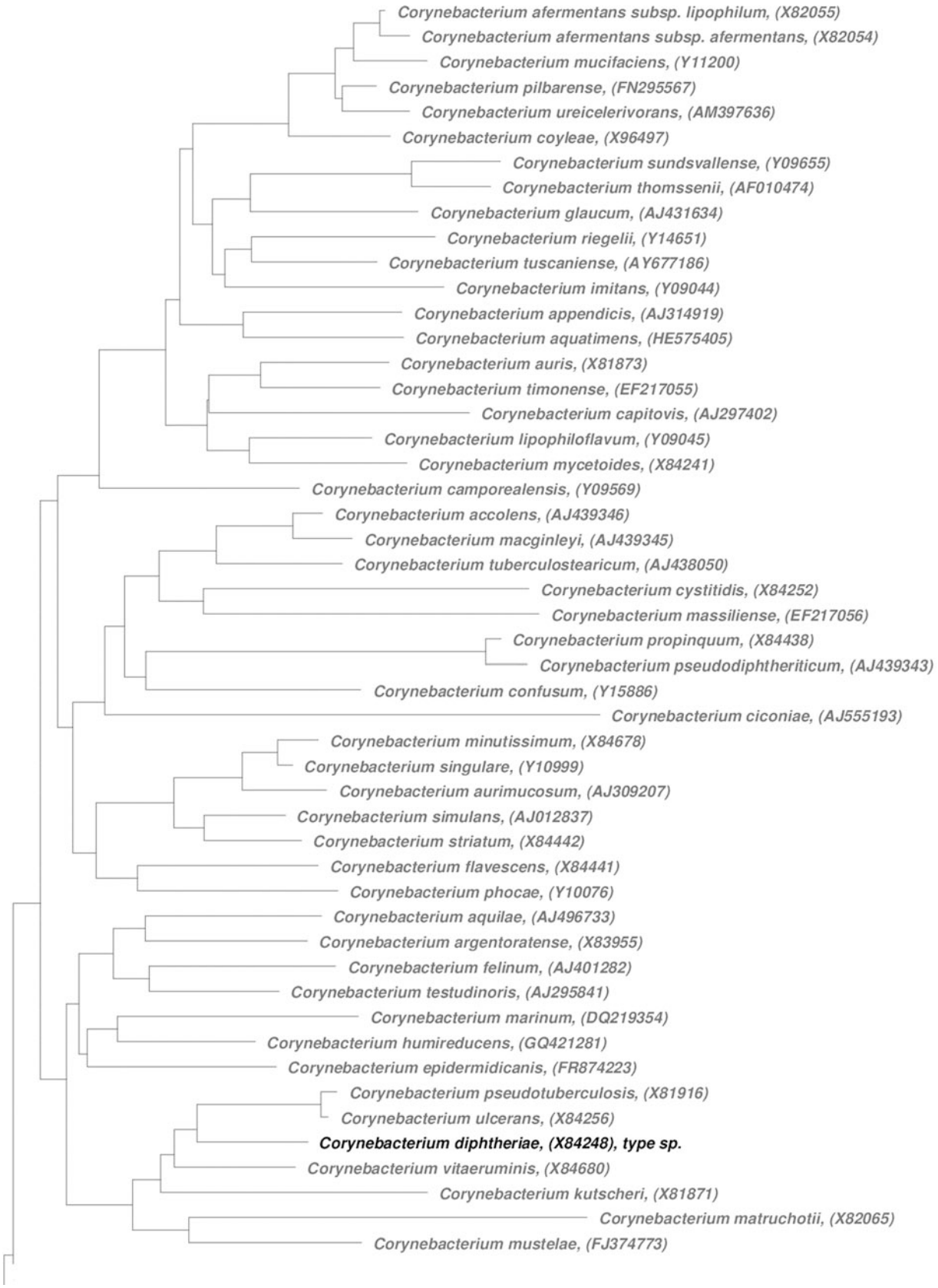

Fig. 12.1 (continued)

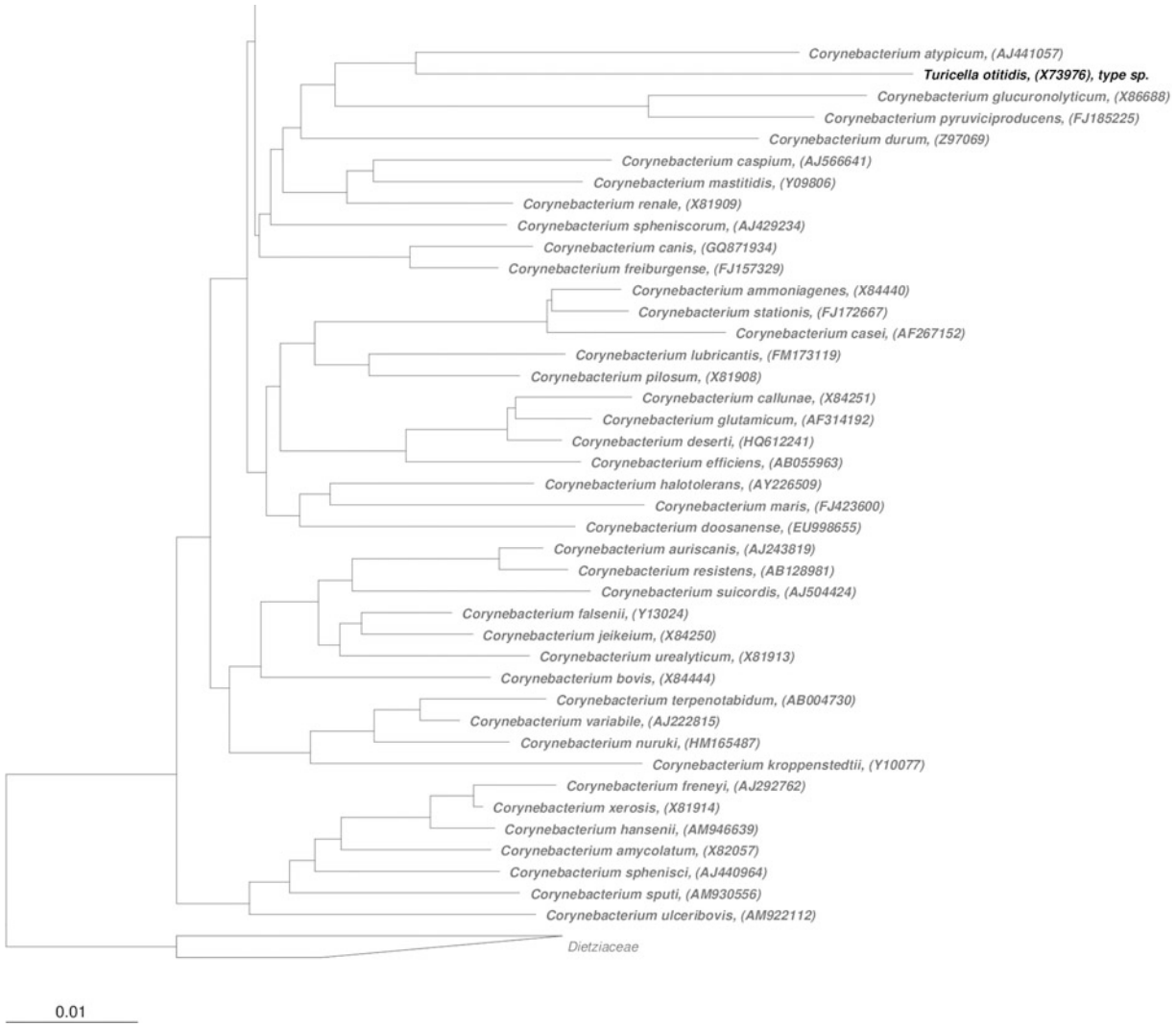

Fig. 12.1

Phylogenetic reconstruction of the family *Corynebacteriaceae* 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 database (Yarza et al. 2010). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Each branch of the tree contains species name and sequence accession number. Taxa of the family *Dietziaceae* were used as outliers. Scale bar indicates estimated sequence divergence

amounts of MK-7(H_2) may also be present (*Corynebacterium lubricantis*) (Kämpfer et al. 2009). *Corynebacterium glaucum* contains MK-7(H_2), MK-8(H_2), and MK-9(H_2) as respiratory menaquinones, with MK-8(H_2) as the major component (Yassin et al. 2003). Small amounts of MK-10(H_2) were found in *Corynebacterium bovis* (Collins et al. 1977) and *Corynebacterium thomssenii* (Zimmermann et al. 1998). Most *Corynebacterium* species contain mycolic acids as a component of the cell envelope, with the exception of a *Corynebacterium afermentans*-like organism isolated from middle ear fluid (Simonet et al. 1993), *Corynebacterium amycolatum* (Collins et al. 1988a), *Corynebacterium atypicum* (Hall et al. 2003), *Corynebacterium caspium* (Collins et al. 2004), *Corynebacterium ciconiae* (Fernández-Garayzábal et al. 2004), and *Corynebacterium kroppenstedtii* (Collins et al. 1998) (*Table 12.1*). These species form distinct phyletic lines within the phylogenetic tree of the family *Corynebacteriaceae* (*Fig. 12.1*). The lack of mycolic acids in *Corynebacterium kroppenstedtii* is obviously caused by gene loss, comprising at least a condensate gene cluster and a mycolate reductase gene which both contribute to the biosynthesis of mycolic acids in corynebacteria (Tauch et al. 2008a; Gande et al. 2004; Lea-Smith et al. 2007). The length of

Table 12.1

Characteristics of *Corynebacterium* species and *Turicella otitidis*

Corynebacterium species and *Turicella otitidis*	G+C content of genomic DNA[a]	Metabolic process	Lipophilism	Mycolic acids[b]	TBSA[c]	Nitrate reduction[d]	Urease[d]	Oxidase[d]	Acid production[d]			Other features
									Glucose	Maltose	Sucrose	
C. accolens	53.9–63.2	F	+	+ (22–36)	n.a.	+	–	–	+	–	v	Satellite growth in the vicinity of *Staphylococcus aureus*
C. afermentans subsp. *afermentans*	66–67	O	–	+ (30–36)	n.a.	–	–	–	–	–	–	–
C. afermentans subsp. *lipophilum*	68	O	+	+ (30–36)	n.a.	–	–	–	–	–	–	–
C. ammoniagenes	53.7–55.8	F	–	+ (32–36)	+	+	+	n.a.	+	–	–	Growth occurs at 10–37 ˚C, but not at 45 ˚C
C. amycolatum	61	F	–	–	n.a.	v	v	–	+	v	v	Some strains grow at 40 ˚C; multidrug resistance observed
C. appendicis	65.8	F	+	+	+	–	+	n.a.	(+)	(+)	–	–
C. aquatimens	60.8	F	+	+ (28–36)	–	–	–	–	+	–	–	Cells grow very slow under aerobic conditions
C. aquilae	n.a.	F	–	+ (30–36)	n.a.	–	–	–	+	–	–	–
C. argentoratense	60–61	F	–	+ (26–36)	n.a.	–	–	–	+	–	–	–
C. atypicum	n.a.	F	–	–	–	–	–	n.a.	+	+	+	–
C. aurimucosum	63.7 (60.6)	F	–	+	+	–	–	(+)	+	+	+	Black-pigmented variants detected
C. auris	68–74	O	–	+	–	–	–	n.a.	–	–	–	Colonies slightly adhere to agar
C. auriscanis	61	O	–	+ (28–34)	–	–	–	n.a.	+	–	–	–
C. bovis	67.8–69.7 (72.55)	F	+	+ (22–36)	+	–	–	(+)	+	–	–	MK-10(H_2) detected
C. callunae	51.2	F	–	+	n.a.	–	+	n.a.	+	+	+	–
C. camporealensis	n.a.	F	–	+	n.a.	–	–	–	+	–	–	–
C. canis	n.a.	F	–	+	–	+	–	n.a.	+	+	+	Colonies adhere to agar; filamentous rods observed (>15 mm in length)
C. capitovis	n.a.	F	–	+ (32–36)	–	–	–	n.a.	+	–	–	Colonies are lemon-pigmented
C. casei	51 (55.3)	F	–	+ (22–36)	n.a.	+	–	–	+	–	–	–
C. caspium	n.a.	F	–	–	–	–	+	–	+	–	–	Growth occurs at 22–42 ˚C
C. cicionae	n.a.	F	–	–	n.a.	–	–	n.a.	+	+	–	–
C. confusum	n.a.	F	–	+	+	+	–	n.a.	(+)	–	–	–
C. coyleae	62–64	F	–	+	n.a.	–	–	n.a.	(+)	–	–	–

Table 12.1 (continued)

Corynebacterium species and *Turicella otitidis*	G+C content of genomic DNA[a]	Metabolic process	Lipophilism	Mycolic acids[b]	TBSA[c]	Nitrate reduction[d]	Urease[d]	Oxidase[d]	Acid production[d]			Other features
									Glucose	Maltose	Sucrose	
C. cystitidis	52.6–53.9	F	–	+	n.a.	–	+	–	+	+	–	–
C. deserti	61.7	O	–	+	+	–	+	–	n.a.	n.a.	n.a.	–
C. diphtheriae	52–55 (53.48)	F	–/+ biovar *intermedius*	+	n.a.	+/– biovar *belfanti*	–	n.a.	+	+	–	Multidrug resistance observed (biovar *mitis*); adhesive pili described
C. doosanense	53.5	F	n.a.	+ (22–32)	n.a.	+	–	+	+	–	–	–
C. durum	55	F	–	+ (26–36)	n.a.	+	(v)	n.a.	+	+	+	Colonies strongly adhere to agar
C. efficiens	59–60.2 (63.4)	F	–	+	–	+	v	–	+	+	n.a.	Growth occurs at 45 ˚C
C. epidermidicanis	n.a.	F	–	n.a.	–	–	–	–	+	+	–	–
C. falsenii	n.a.	F	–	+	n.a.	–	(+)	n.a.	(+)	v	–	Urea hydrolysis is positive, but delayed
C. felinum	n.a.	F	–	+ (32–36)	–	–	–	n.a.	+	+	–	–
C. flavescens	58.3	F	–	n.a.	n.a.	–	–	n.a.	+	–	–	–
C. freiburgense	n.a.	F	–	+	n.a.	+	–	n.a.	+	+	+	Colonies strongly adhere to blood agar; older colonies exhibited a “spoke-wheel”macroscopic morphology
C. freneyi	n.a.	F	–	+	n.a.	v	–	n.a.	+	+	+	Growth occurs at 20–42 ˚C
C. glaucum	64.3	F	–	+	–	–	–	n.a.	+	–	+	MK-7(H_2) detected
C. glucuronolyticum	52–58	F	–	+	n.a.	v	v	n.a.	+	v	+	Strong β-glucuronidase activity
C. glutamicum	55–55.7 (53.8)	F	–	+ (30–36)	n.a.	+	+	n.a.	+	+	+	Able to produce glutamic acid
C. halotolerans	63	O	n.a.	+ (32–36)	n.a.	+	–	–	+	–	–	Optimum growth at 28 ˚C and 10 % KCl
C. hansenii	n.a.	F	–	n.a.	n.a.	–	–	n.a.	+	+	+	Growth occurs at 20 ˚C
C. humireducens	59	F	–	n.a.	n.a.	–	–	–	+	–	–	Growth occurs from 4–45 ˚C; optimum growth at 37 ˚C, 8 % NaCl and pH 9.0
C. imitans	62	F	–	+	n.a.	–	–	n.a.	+	+	(+)	–
C. jeikeium	58–61 (61.4)	O	+	+ (32–36)	+	–	–	n.a.	+	v	–	No growth anaerobically; multidrug resistance observed
C. kroppenstedtii	62 (57.5)	F	+	–	+	–	–	n.a.	+	v	+	Growth occurs in 10 % NaCl and at 42 ˚C

C. kutscheri	~46	F	−	n.a.	n.a.	+	+	n.a.	+	+	+	−
C. lipophiloflavum	65	O	(+)	+	n.a.	−	(+)	n.a.	−	−	−	−
C. lubricantis	n.a.	O	n.a.	n.a.	+	−	−	−	+	−	−	MK-7(H_2) detected
C. macginleyi	58	F	+	+ (26–36)	n.a.	+	−	−	+	−	+	−
C. marinum	65	F	−	n.a.	n.a.	+	−	−	+	+	+	Growth occurs from 4 ˚C to 37 ˚C; optimum growth at 30–32 ˚C and 1 % NaCl
C. maris	66.6	O	n.a.	+ (30–36)	+	−	−	+	−	−	−	Growth occurs at 0.5–4.0 % salinity
C. massiliense	n.a.	O	n.a.	n.a.	+	−	−	−	−	−	−	Temperature range for growth is 30–44 ˚C; optimum growth at, 37 ˚C
C. mastitidis	n.a.	O	+	+	−	−	v	−	−	−	−	−
C. matruchotii	55–58	F	−	+	−	+	−	n.a.	+	+	+	Nonseptate and septate filaments observed
C. minutissimum	56–58	F	−	+	+	−	−	n.a.	+	+	v	−
C. mucifaciens	63–65	O	−	+	+	−	−	n.a.	+	−	v	Mucoid colonies observed
C. mustelae	n.a.	F	−	+	−	−	−	n.a.	+	+	+	Colonies strongly adhere to agar
C. mycetoides	59	O	−	+ (30–36)	+	−	−	n.a.	+	−	−	−
C. nuruki	73.6 (69.5)	O	−	+	+	−	+	+	+	n.a.	n.a.	Growth occurs from 10 ˚C to 45 ˚C in the presence of 0–10 % NaCl and at pH 6.0–9.0; optimum growth at 37 ˚C, 1 % NaCl, and pH 8
C. phocae	58	F	−	+ (30–34)	−	−	v	n.a.	+	+	v	Urea hydrolysis is variable
C. pilbarense	n.a.	F	−	+	−	−	−	−	+	−	+	−
C. pilosum	57.9–60.9	F	−	+	+	+	+	−	+	+	−	Densely piliated cells observed
C. propinquum	57–59	O	−	+ (30–36)	n.a.	+	−	−	−	−	−	−
C. pseudodiphtheriticum	54.9–56.8	O	−	+	n.a.	+	+	n.a.	−	−	−	−
C. pseudotuberculosis	51.8–52.5 (52.2)	F	−	+	−	− biovar ovis; + biovar *equi*	+	n.a.	+	+	v	−
C. pyruviciproducens	62	F	+	+ (22–36)	−	−	−	n.a.	+	+	+	Able to produce pyruvic acid
C. renale	53–58	F	−	+ (30–36)	−	−	+	n.a.	+	v	−	−
C. resistens	54.6 (57.1)	F	+	n.a.	n.a.	−	−	−	+	−	−	Multidrug resistance observed
C. riegelii	n.a.	F	−	+	−	−	+	n.a.	−	(+)	−	Strong urease activity
C. simulans	n.a.	F	−	+ (22–36)	n.a.	+	−	n.a.	+	−	+	−
C. singulare	62	F	−	+ (26–36)	n.a.	−	+	−	+	+	+	−
C. sphenisci	n.a.	F	−	+ (22–32)	−	+	−	−	+	+	−	Mycolic acids are produced, but in small amounts
C. spheniscorum	n.a.	F	−	+ (32–36)	−	−	−	n.a.	+	+	−	−
C. sputi	n.a.	F	+	+	+	−	+	−	+	−	−	−

Table 12.1 (continued)

Corynebacterium species and *Turicella otitidis*	G+C content of genomic DNA[a]	Metabolic process	Lipophilism	Mycolic acids[b]	TBSA[c]	Nitrate reduction[d]	Urease[d]	Oxidase[d]	Acid production[d]			Other features
									Glucose	Maltose	Sucrose	
C. stationis	53.9	F	−	+	+	+	+	−	(+)	−	−	−
C. striatum	57.6	F	−	+	−	v	−	n.a.	+	−	v	Brown-pigmented variants detected; multidrug resistance observed
C. suicordis	n.a.	F	−	+ (28–36)	n.a.	−	+	−	−	−	−	−
C. sundsvallense	64	F	−	+	−	−	+	−	+	+	+	Some cells display bulges or knobs at their ends
C. terpenotabidum	67.5	O	−	+	+	−	+	−	−	n.a.	n.a.	Able to degrade squalene
C. testudinoris	n.a.	F	−	+ (30–36)	n.a.	+	−	−	+	+	+	−
C. thomssenii	n.a.	F	−	+ (32–36)	n.a.	−	+	n.a.	+	+	+	Strong *N*-acetyl-β-glucosaminidase activity; MK-10(H_2) detected
C. timonense	n.a.	F	−	n.a.	−	−	−	−	+	−	−	Temperature range for growth is 25–50 °C; optimum growth at 37 °C
C. tuberculostearicum	n.a.	F	+	+	+	v	−	−	+	v	v	−
C. tuscaniense	n.a.	O	−	+ (26–36)	n.a.	−	−	n.a.	+	+	−	−
C. ulcerans	53 (53.4)	F	−	+ (26–36)	n.a.	−	+	n.a.	+	+	−	−
C. ulceribovis	n.a.	F	−	+	−	−	−	−	+	−	−	Large colonies observed (approx. 2–4 mm in diameter)
C. urealyticum	61–62 (64.4)	O	+	+ (26–36)	+	−	+	−	−	−	−	Strong urease activity; multidrug resistance observed
C. ureicelerivorans	n.a.	F	+	+	+	−	+	−	+	−	−	Strong urease activity
C. variabile	65 (67.15)	O	−	+ (30–36)	+	v	+	−	+	−	−	Optimum growth at 25–30 °C
C. vitaeruminis	64.8	F	−	n.a.	−	+	+	n.a.	+	+	+	−
C. xerosis	67.3	F	−	+	−	v	−	n.a.	+	+	+	Multidrug resistance observed
T. otitidis	65–72 (71.35)	O	−	−	+	−	−	−	−	−	−	MK-10, MK-11 detected

Abbreviations: *F* fermentative, *O* oxidative, + feature present, − feature absent, *n.a.* data not available

[a]The G+C content of the genomic DNA is given in mol%. Data were generated with different methodologies. Data in parenthesis are from genome sequences

[b]Data in parenthesis indicate the number of carbons

[c]The presence of varying amounts (including trace amounts) of tuberculostearic acid is listed. Abbreviation: *TBSA* tuberculostearic acid

[d]Data in parenthesis indicate weak or delayed reaction. Abbreviation: *v* variable

mycolic acids found in *Corynebacterium* species mainly varies between 22 and 36 carbon atoms (Collins et al. 1982; Athalye et al. 1984) (Table 12.1), though ranges from 20 to 38 carbon atoms were also described (Butler et al. 1986; de Briel et al. 1992; Embley and Stackebrandt 1994). These so-called corynemycolic acids represent the simplest forms of the mycolate family, since they possess the shortest chain length (Daffé 2005). The composition of the corynemycolic acids from a *Corynebacterium* species might depend on the growth conditions, as fatty acids from lipid-rich media may be incorporated into both parts of the mycolic acid molecule (Daffé 2005). Lipophilic *Corynebacterium* species grown on Tween 80 exhibit a high content of unsaturated mycolic acids, presumably due to the incorporation of oleic acid from the detergent (Chevalier et al. 1988). Genome sequencing projects revealed that lipophilism of *Corynebacterium* species is also caused by gene loss, comprising the fatty acid synthase gene (Tauch et al. 2005, 2008a, b; Schröder et al. 2012a, b). The evolutionary event of gene loss occurred independently in distinct phyletic lines within the phylogenetic tree of the family *Corynebacteriaceae* (Fig. 12.1) and probably includes not only the hitherto examined species *Corynebacterium bovis* (Funke et al. 1997b), *Corynebacterium jeikeium* (Jackman et al. 1987), *Corynebacterium kroppenstedtii* (Collins et al. 1998), *Corynebacterium resistens* (Otsuka et al. 2005), and *Corynebacterium urealyticum* (Pitcher et al. 1992) but also the other known lipophilic species *Corynebacterium accolens* (Neubauer et al. 1991), *Corynebacterium afermentans* subsp. *lipophilum* (Riegel et al. 1993a), *Corynebacterium appendicis* (Yassin et al. 2002a), *Corynebacterium aquatimens* (Aravena-Román et al. 2012), *Corynebacterium lipophiloflavum* (Funke et al. 1997c), *Corynebacterium macginleyi* (Riegel et al. 1995b), *Corynebacterium mastitidis* (Fernández-Garayzábal et al. 1997), *Corynebacterium pyruviciproducens* (Tong et al. 2010), *Corynebacterium sputi* (Yassin and Siering 2008), *Corynebacterium tuberculostearicum* (Feurer et al. 2004), and *Corynebacterium ureicelerivorans* (Yassin 2007). *Corynebacterium diphtheriae* biotype *intermedius* is also regarded as a lipophilic bacterium (Funke et al. 1997b) (Table 12.1). *Corynebacterium lipophiloflavum* was described as slightly lipophilic (Funke et al. 1997c), although no fatty acid synthase gene is detectable in the draft genome sequence (Pagani et al. 2012). The family *Corynebacteriaceae* includes organisms that possess a cell-wall peptidoglycan of type A1γ with the characteristic *meso*-diaminopimelic acid. Major fatty acids are $C_{16:0}$ and $C_{18:1}$ ω9*c* (Bernard et al. 1991). Some species contain varying amounts of tuberculostearic acid (10-methyl $C_{18:0}$) (Table 12.1) which was detected in *Corynebacterium ammoniagenes* (Collins 1987a), *Corynebacterium appendicis* (Yassin et al. 2002a), *Corynebacterium aurimucosum* (Yassin et al. 2002b), *Corynebacterium bovis* (Funke et al. 1997b), *Corynebacterium confusum* (Funke et al. 1998a), *Corynebacterium deserti* (Zhou et al. 2012), *Corynebacterium jeikeium* (Jackman et al. 1987), *Corynebacterium kroppenstedtii* (Collins et al. 1998), *Corynebacterium lubricantis* (Kämpfer et al. 2009), *Corynebacterium maris* (Ben-Dov et al. 2009), *Corynebacterium massiliense* (Merhej et al. 2009), *Corynebacterium minutissimum* (Collins and Jones 1983a), *Corynebacterium mucifaciens* (Funke et al. 1997d), *Corynebacterium mycetoides* (Bernard et al. 1991), *Corynebacterium nuruki* (Shin et al. 2011a), *Corynebacterium pilosum* (Bernard et al. 1991), *Corynebacterium sputi* (Yassin and Siering 2008), *Corynebacterium stationis* (Bernard et al. 2010), *Corynebacterium terpenotabidum* (Takeuchi et al. 1999), *Corynebacterium tuberculostearicum* (Feurer et al. 2004), *Corynebacterium urealyticum* (Pitcher et al. 1992), *Corynebacterium ureicelerivorans* (Yassin 2007), *Corynebacterium variabile* (Collins 1987a), and *Turicella otitidis* (Funke et al. 1994). A remarkable variability in the G+C content of the genomic DNA of *Corynebacterium* species has been detected (Table 12.1), as the lowest value was reported for *Corynebacterium kutscheri* (~46 mol%) and the highest for *Corynebacterium auris* (74 mol%). Besides these two exceptions, the genomic G+C content of *Corynebacterium* species varies in principle between 51 and 68 mol% (Table 12.1). The genomic G+C content of *Corynebacterium accolens* isolates varies between 53.9 and 63.2 mol%, indicating that the characterized collection of strains contains various genomospecies (Neubauer et al. 1991).

The phylogenetic relatedness among *Corynebacterium* species is demonstrated by the 16S rRNA gene sequence-based tree of the family *Corynebacteriaceae* (Fig. 12.1). Molecular typing by 16S rRNA gene sequencing clearly distinguishes among most species of the genus *Corynebacterium,* as these can be separated by significant sequence variance. It is now recognized that a 16S rRNA gene sequence similarity range above 98.7–99 % should be mandatory for establishing the genomic uniqueness of a novel isolate (Stackebrandt and Ebers 2006). Several genomically distinct species within the genus *Corynebacterium* exhibit comparable or even higher levels of relatedness: *Corynebacterium aurimucosum* and *Corynebacterium minutissimum* (98.9 %) (Yassin et al. 2002b), *Corynebacterium coyleae* and *Corynebacterium afermentans* subsp. *afermentans* (98.2 %) (Funke et al. 1997a), *Corynebacterium deserti* and *Corynebacterium glutamicum* (98.4 %) (Zhou et al. 2012), *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* (98.5 %) and *Corynebacterium pseudotuberculosis* (98.5 %) (Pascual et al. 1995), *Corynebacterium hansenii* and both *Corynebacterium freneyi* (99.0 %) and *Corynebacterium xerosis* (98.5 %) (Renaud et al. 2007), *Corynebacterium macginleyi* and *Corynebacterium accolens* (98.7 %) (Riegel et al. 1995b), *Corynebacterium mucifaciens* and both subspecies of *Corynebacterium afermentans* (98.5 %) (Funke et al. 1997d), *Corynebacterium propinquum* and *Corynebacterium pseudodiphtheriticum* (99.2 %) (Pascual et al. 1995), *Corynebacterium singulare* and *Corynebacterium minutissimum* (99.1–99.6 %) (Riegel et al. 1997a), *Corynebacterium sundsvallense* and *Corynebacterium thomssenii* (98.8 %) (Bernard 2012), and *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* (99.8 %) (Riegel et al. 1995c). Analysis of the levels of DNA relatedness revealed four genomospecies among *Corynebacterium jeikeium* isolates (Riegel et al. 1994). Although the respective *Corynebacterium jeikeium* strains showed genomic diversity at the species level, biochemical characteristics allowed their classification in this single taxon.

Molecular Analyses

16S rRNA Gene Sequence Analysis and Molecular Typing

Molecular typing by 16S rRNA gene sequencing is recommended as a starting point for characterization of *Corynebacterium* species and is in principle an efficient means to deduce phylogenetic relationships (Bernard 2012). However, the reliable identification of some *Corynebacterium* species from clinical specimens remains challenging (Coyle et al. 1993). Several strains of *Corynebacterium minutissimum*, *Corynebacterium striatum*, and *Corynebacterium xerosis* have been misidentified in the routine clinical laboratory and belong to the species *Corynebacterium amycolatum* (Funke et al. 1996; Wauters et al. 1996; Zinkernagel et al. 1996). A chemotaxonomic study on 25 clinical isolates originally identified as *Corynebacterium xerosis* revealed two different groups of strains, one of which unambiguously belonging to the species *Corynebacterium amycolatum* (Funke et al. 1996). Heterogeneity was also observed in a collection of 48 clinical strains that were tentatively identified as *Corynebacterium minutissimum* on the basis of standard biochemical reactions, and it was explained by misidentified *Corynebacterium amycolatum* strains (Zinkernagel et al. 1996). This observation indicates that the clinical microbiologist must be careful not to misidentify isolates of *Corynebacterium amycolatum* as *Corynebacterium minutissimum*. Detailed data on the biochemical and molecular identification of *Corynebacterium tuberculostearicum* isolated from clinical specimens from 18 hospitalized patients demonstrated that routine biochemical tests do not provide reliable identification of this species (Hinic et al. 2012). It was therefore proposed that *Corynebacterium tuberculostearicum* is often misidentified and its clinical relevance is underestimated. The use of matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry was evaluated and shown to represent a helpful tool for the reliable identification of *Corynebacterium tuberculostearicum* isolates (Hinic et al. 2012). Analysis of the diversity within reference strains of *Corynebacterium matruchotii* by whole-cell fatty acid profiles, DNA-DNA dot blot hybridization, sequencing of two hypervariable regions of the 16S rRNA gene, and the pyrrolidonyl arylamidase test indicated that two reference strains, ATCC 33449 and ATCC 33822, are members of the species *Corynebacterium durum* (Rassoulian Barrett et al. 2001).

Four *Corynebacterium* species were reclassified within the genus and assigned to other species, including *Corynebacterium mooreparkense* (Brennan et al. 2001), *Corynebacterium nigricans* (Shukla et al. 2003a), *Corynebacterium seminale* (Riegel et al. 1995a), and *Corynebacterium lilium* (Liebl et al. 1991). According to additional data based on 16S rRNA gene sequencing and DNA-DNA hybridizations, *Corynebacterium mooreparkense* turned out to be a later heterotypic synonym of *Corynebacterium variabile* (Gelsomino et al. 2005). All *Corynebacterium mooreparkense* strains had a 16S rRNA gene sequence similarity of at least 99.5 % with *Corynebacterium variabile* and the DNA-DNA relatedness was 95 %. Likewise, the sequencing of the 16S rRNA gene and DNA-DNA hybridization analysis revealed that *Corynebacterium nigricans* can be reclassified as a charcoal-black-pigmented variant of *Corynebacterium aurimucosum* (Daneshvar et al. 2004). The type strains of *Corynebacterium seminale* (Riegel et al. 1995a) and *Corynebacterium glucuronolyticum* (Funke et al. 1995b) and other clinical isolates were shown by DNA-DNA hybridization and sequencing of the 16S rRNA gene to be related at the species level (Devriese et al. 2000). All strains were classified as *Corynebacterium glucuronolyticum*, because this species name has nomenclatural priority over *Corynebacterium seminale* (Devriese et al. 2000). *Corynebacterium lilium* and two glutamic acid-producing strains of *Corynebacterium ammoniagenes*, ATCC 13745 and ATCC 13746, were transferred to the species *Corynebacterium glutamicum* (Liebl et al. 1991; Oberreuter et al. 2002), whereas three environmental isolates that had tentatively been named *Corynebacterium thermoaminogenes* in a Japanese patent application were classified as the new species *Corynebacterium efficiens* (Fudou et al. 2002).

All *Corynebacterium* species previously associated with plants and attributed to diseases in plants have been reassigned. Therefore, the genus *Corynebacterium* is currently devoid of a species assigned as plant pathogenic. *Corynebacterium* species reclassified in other genera include the following: *Corynebacterium betae, Corynebacterium flaccumfaciens, Corynebacterium ilicis*, *Corynebacterium oortii*, and *Corynebacterium poinsettiae* in the genus *Curtobacterium*, as *Curtobacterium flaccumfaciens* (Collins and Jones 1983b; Young et al. 2004); *Corynebacterium beticola* in the genus *Erwinia*, as *Erwinia herbicola*, and later to *Pantoea agglomerans* (Collins and Jones 1982); *Corynebacterium equi* and *Corynebacterium hoagii* in the genus *Rhodococcus*, as *Rhodococcus equi* (Goodfellow and Alderson 1977); *Corynebacterium fascians* in the genus *Rhodococcus*, as *Rhodococcus fascians* (Goodfellow 1984); *Corynebacterium insidiosum*, *Corynebacterium michiganense*, *Corynebacterium nebraskense*, and *Corynebacterium sepedonicum* in the genus *Clavibacter*, as subspecies of *Clavibacter michiganensis* (Davis et al. 1984); *Corynebacterium iranicum*, *Corynebacterium rathayi*, and *Corynebacterium tritici* in the genus *Rathayibacter*, as *Rathayibacter iranicus*, *Rathayibacter rathayi*, and *Rathayibacter tritici*, respectively (Zgurskaya et al. 1993); *Corynebacterium paurometabolum* in the genus *Tsukamurella*, as *Tsukamurella paurometabola* (Collins et al. 1988b); and *Corynebacterium pyogenes* in the genus *Trueperella*, as *Trueperella pyogenes* (Ramos et al. 1997; Yassin et al. 2011).

Since the 16S rRNA gene sequences of *Corynebacterium* species show very little polymorphism, accurate molecular identification is only possible by sequencing the complete 16S rRNA gene (Khamis et al. 2004). *Corynebacterium* species which cannot be distinguished by 16S rRNA gene sequences can be further characterized by sequencing of other target genes, for instance the *rpoB* gene (Khamis et al. 2004). It was proposed that two *Corynebacterium* isolates belong to the same species if they show $\geq$95 % *rpoB* sequence similarity (Khamis et al. 2005). Higher proportions of corynebacterial isolates were

positively identified by partial *rpoB* gene sequence determination than by classification based on 16S rRNA gene sequences. However, in some ambiguous cases, *rpoB* gene sequencing should be used in conjunction with other tests for definitive species identification (Khamis et al. 2005). A number of other molecular techniques have been applied to separate closely related *Corynebacterium* species. Amplified rDNA restriction analysis (ARDRA) with the enzymes AluI, CfoI, and RsaI and the combination of the ARDRA patterns obtained after restriction with the three enzymes enabled the differentiation between the following species: *Corynebacterium accolens*, *Corynebacterium afermentans* subsp. *afermentans*, *Corynebacterium afermentans* subsp. *lipophilum*, *Corynebacterium amycolatum*, *Corynebacterium cystitidis*, *Corynebacterium diphtheriae*, *Corynebacterium jeikeium*, *Corynebacterium macginleyi*, *Corynebacterium minutissimum*, *Corynebacterium pilosum*, *Corynebacterium pseudotuberculosis*, *Corynebacterium renale*, *Corynebacterium striatum*, *Corynebacterium urealyticum*, and *Corynebacterium xerosis* (Vaneechoutte et al. 1995). Moreover, the value of rRNA gene RFLP analysis (ribotyping) as a molecular tool for the identification of *Corynebacterium* and *Turicella* species was evaluated, using the enzymes BstEII, SmaI, and SphI (Björkroth et al. 1999). BstEII clustering of many species followed known phylogenetic lineages, although the information provided by all three enzymes was considered essential for the reliable linking of strains of unknown identity with defined species (Björkroth et al. 1999). Ribotyping studies on a collection of *Corynebacterium urealyticum* isolates from humans and animals with HindIII revealed twelve different ribotypes. Most human isolates were found to be multidrug resistant and clustered into ribotypes named 8, 9, and 10, whereas strains from animals were significantly less antibiotic resistant and assigned to ribotypes 5 and 6 (Nieto et al. 2000). Ribotyping of *Corynebacterium pseudotuberculosis* with ApaI revealed differences between, but not within, the two biotypes *ovis* and *equi* derived from various types of lesions and different geographical locations (Sutherland et al. 1996; Costa et al. 1998). Ribotyping of *Corynebacterium ulcerans* from a female diphtheria patient and a chronic labial ulcer of her dog revealed that both isolates correspond to a single strain (Lartigue et al. 2005). Likewise, ribotyping of brown-pigmented *Corynebacterium striatum* strains provided evidence of person-to-person transmission in intensive care units (Leonard et al. 1994). The combination of ribotyping and pulsed-field gel electrophoresis (PFGE) demonstrated that *Corynebacterium imitans* strains from closely related persons were of clonal origin (Funke et al. 1997e). On the other hand, ribotyping was used to distinguish two toxigenic *Corynebacterium ulcerans* isolates from pharyngeal swabs of two patients from the same hospital which could not be distinguished by PFGE (Komiya et al. 2010). PFGE was used to characterize 48 *Corynebacterium striatum* isolates recovered from clinical specimens from long-term hospitalized patients with underlying diseases (Otsuka et al. 2006). The PFGE profiles revealed 14 distinct patterns with 20 subtypes, of which three types with four subtypes were related to a nosocomial outbreak of *Corynebacterium striatum*. Moreover, PFGE analysis confirmed that a single multidrug-resistant clone of *Corynebacterium striatum* is emerging as a pathogen in Italy (Campanile et al. 2009). Molecular genotyping of *Corynebacterium pseudotuberculosis* isolates from the United Kingdom by PFGE showed their epidemiological relatedness to an outbreak strain of caseous lymphadenitis in goats (Connor et al. 2000). Likewise, a clonally expanding epidemic of *Corynebacterium pseudotuberculosis* infections in horses was observed in Utah by molecular typing methods (Foley et al. 2004). Further genotyping studies of multinational ovine and caprine *Corynebacterium pseudotuberculosis* isolates using PFGE displayed a remarkable homogeneity among the investigated strains from Australia, Canada, Ireland, Northern Ireland, and The Netherlands (Connor et al. 2007). Based on these results, it would appear that the genome of *Corynebacterium pseudotuberculosis* is highly conserved, irrespective of the country of strain origin (Connor et al. 2007). Analysis of the length polymorphisms of the 16S–23S rDNA intergenic spacer by PCR-mediated amplification was used to reveal heterogeneity among strains of *Corynebacterium* species otherwise thought to be monophyletic (Aubel et al. 1997). Strains assigned to *Corynebacterium amycolatum*, *Corynebacterium jeikeium*, *Corynebacterium minutissimum*, and *Corynebacterium striatum* did not produce identical PCR profiles, suggesting that genomic heterogeneity occurs in these four species. RFLP analysis of the 16S–23S rRNA gene spacer using the enzyme CfoI allows differentiation between *Corynebacterium freneyi* and *Corynebacterium xerosis* (Funke and Frodl 2008). A randomly amplified polymorphic DNA (RAPD) method was applied to detect an association between *Corynebacterium striatum* from the bloodstream of a patient and that from the patient's central venous catheter (Chen et al. 2012). A molecular typing by RAPD analysis was also performed to demonstrate the patient-to-patient spread of a single strain of *Corynebacterium striatum* in a surgical intensive care unit. The same strain was isolated from surfaces and air sampled in the direct vicinity of infected patients and from the hands of personnel attending to infected patients (Brandenburg et al. 1996). RAPD and ribotyping were combined to prove that cutaneous sites and blood culture isolates of *Corynebacterium striatum* from the same patient were identical (Martín et al. 2003). RAPD was also combined with the amplification of DNA fragments surrounding rare restriction sites (ADSRRS fingerprinting) to distinguish *Corynebacterium pseudotuberculosis* strains isolated from goats (Stefańska et al. 2008). The value of discrimination was higher for ADSRRS fingerprinting, indicating that this rapid technique may be applied for epidemiological studies of intraspecific relatedness of *Corynebacterium pseudotuberculosis* isolates. Ophthalmic clinical isolates of the species *Corynebacterium macginleyi* were characterized by multilocus sequence typing (MLST) analysis of seven housekeeping genes: *adk*, *dnaA*, *fumC*, *gltA*, *gyrB*, *icd*, and *purA*. The MLST analysis grouped the majority of *Corynebacterium macginleyi* isolates into a single lineage. RAPD analysis supported this finding and indicated that a particular lineage of *Corynebacterium macginleyi* was dominant on the human ocular surface (Eguchi et al. 2008). A molecular method based

on the use of different primers for amplification of the cell division gene *divIVA* by conventional or real-time PCR was developed to identify *Corynebacterium amycolatum* (Letek et al. 2006). This technique allows to distinguish *Corynebacterium amycolatum* from the closely related *Corynebacterium minutissimum*, *Corynebacterium striatum*, and *Corynebacterium xerosis*, without the requirement of further molecular analysis (Letek et al. 2006). The differentiation of *Corynebacterium amycolatum*, *Corynebacterium minutissimum*, *Corynebacterium striatum,* and related species was also evaluated by pyrolysis-gas–liquid chromatography with atomic emission detection. This phenotypic method, which analyzes the whole chemical composition of bacteria, clearly separates *Corynebacterium amycolatum* from other species (Voisin et al. 2002). Real-time PCR is a rapid tool to confirm the presence of the diphtheria toxin gene *tox* in an isolate or specimen, although some toxigenic *Corynebacterium ulcerans* strains show atypical results in a real-time PCR for *tox*, revealing potential for false-negative real-time PCR results (Cassiday et al. 2008). The rapid detection and molecular differentiation of toxigenic *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* strains is possible by LightCycler PCR. Since the *tox* genes of these species differ from each other in DNA and amino acid sequences, both *tox* genes can be covered by real-time PCR methods. A LightCycler PCR assay reliably recognized *tox* genes from *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* and differentiated these target genes by fluorescence resonance energy transfer (FRET) hybridization probe melting curve analysis (Sing et al. 2011). A combined PCR assay for the *tox* and *rpoB* genes was established for the direct identification of the diphtheria toxin gene of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* and the molecular discrimination of both species, irrespective of their toxigenic status (Mancini et al. 2012). A PCR-based assay to detect the *dtxR* gene associated with *Corynebacterium diphtheriae* strains has been used as a method to screen for this organism (Pimenta et al. 2008).

Identification of *Corynebacterium* species was also evaluated by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. The identification of 92 clinical isolates was compared with the classification using *rpoB* or 16S rRNA gene sequencing. Eighty isolates were correctly identified to the species level, with the exception of *Corynebacterium aurimucosum* being misidentified as the closely related *Corynebacterium minutissimum* (Alatoom et al. 2012). Another study used a bacterial collection including 40 *Corynebacterium diphtheriae,* 13 *Corynebacterium pseudotuberculosis,* 19 *Corynebacterium ulcerans,* and 270 other *Corynebacterium* isolates for evaluating the identification of *Corynebacterium* species by MALDI-TOF mass spectrometry (Farfour et al. 2012). This new method provides also a helpful tool for the reliable identification of *Corynebacterium tuberculostearicum* that is often misidentified in clinical samples (Hinic et al. 2012). Moreover, 52 multiresistant *Corynebacterium striatum* strains from different Spanish hospitals were characterized by MALDI-TOF mass spectrometry, revealing different clonal populations within the clinical samples (Gomila et al. 2012). The identification of the potentially toxigenic species *Corynebacterium diphtheriae, Corynebacterium ulcerans,* and *Corynebacterium pseudotuberculosis* is essential for rapid diagnosis and treatment of diphtheria and diphtheria-like diseases. MALDI-TOF mass spectrometry was performed in comparison with classical microbiological and molecular methods on 116 *Corynebacterium* strains. All 90 potentially toxigenic *Corynebacterium* strains collected by the German National Consiliary Laboratory on Diphtheria were correctly identified by MALDI-TOF mass spectrometry (Konrad et al. 2010).

A broad spectrum of molecular typing schemes was applied to *Corynebacterium diphtheriae* isolates, in order to establish their epidemiological relatedness and genetic divergence and to analyze their circulation in the human population (De Zoysa et al. 1995; Popovic et al. 1996; Marston et al. 2001; Damian et al. 2002; Zasada et al. 2010). Four typing methods, including ribotyping, pulsed-field gel electrophoresis (PFGE), random amplification of polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) were compared for their use in the characterization of *Corynebacterium diphtheriae* strains (De Zoysa et al. 2008). This study revealed that ribotyping is highly discriminatory and reproducible and is probably the method of choice for typing *Corynebacterium diphtheriae*. PFGE and AFLP were less discriminatory than ribotyping and RAPD. An assessment of the transcontinental spread of the organism showed that several genotypes of *Corynebacterium diphtheriae* circulated on different continents and that each outbreak was caused by a distinct clone. The ribotypes seen in Europe appeared to be distinct from those seen elsewhere, and certain ribotypes appeared to be unique to particular countries (De Zoysa et al. 2008). A multilocus sequence typing (MLST) scheme has been developed for *Corynebacterium diphtheriae* surveillance and is based on data derived from sequencing of seven housekeeping genes: *atpA*, *dnaE*, *dnaK*, *fusA*, *leuA*, *odhA*, and *rpoB* (Bolt et al. 2010). The sequence data are stored and curated in databases and used to assign allelic numbers to a sequence type (Jolley et al. 2004; Dallman et al. 2008). MLST was applied to analyze 150 *Corynebacterium diphtheriae* isolates from 18 countries and encompassing a period of 50 years. Strain discrimination was in accordance with ribotyping data, and clonal complexes associated with disease outbreaks were clearly identified by MLST. Therefore, the MLST scheme provides a valuable tool for monitoring and characterizing *Corynebacterium diphtheriae* strains (Bolt et al. 2010). Moreover, a macroarray-based spoligotyping method was evaluated for *Corynebacterium diphtheriae* strain typing (Mokrousov et al. 2005, 2007, 2009). The spoligotyping method is based on simultaneous reverse-hybridization analysis of two clustered regularly interspaced short palindromic repeats (CRISPR) loci present in the genome of *Corynebacterium diphtheriae*. The method was evaluated with 154 clinical strains of *Corynebacterium diphtheriae* previously identified as belonging to the ribotypes Sankt-Peterburg and Rossija. The 154 strains were subdivided into 34 spoligotypes, comprising 14 unique strains and 20 types shared by 2–46 strains (Mokrousov et al. 2005). Therefore, spoligotyping allows efficient discrimination

within an epidemic clonal group of *Corynebacterium diphtheriae* and is applicable to phylogenetic reconstruction and epidemiological investigations (Mokrousov et al. 2005, 2009; Trost et al. 2012). Another genome-based method is comparative genomic hybridization, thereby detecting the heterogeneity in the distribution of pathogenicity islands in the genomes of *Corynebacterium diphtheriae* isolates (Iwaki et al. 2010). The future use of multiple genome sequences can give a phylogenomic overview of the genus *Corynebacterium* by using a core gene set as the basis of multilocus sequence typing (Adékambi et al. 2011) or by generating alignments for genomic regions that are characteristic or unique for a specified subgroup of species (Ågren et al. 2012).

Genome Analysis

A new generation of DNA sequencing approaches, collectively called next-generation DNA sequencing technologies, has provided unprecedented opportunities for high-throughput genome research (Mardis 2008; Shendure and Ji 2008). The application of these DNA sequencing technologies has provided detailed insights into the genome architecture and gene content of *Corynebacterium* species. The first next-generation DNA sequencing studies have focused on the genomes of corynebacterial pathogens because of their importance in human and animal disease. Complete genome sequences are meanwhile available for a black-pigmented *Corynebacterium aurimucosum* isolate (Trost et al. 2010a), multiple toxigenic and non-toxigenic strains of *Corynebacterium diphtheriae* (Cerdeño-Tarrága et al. 2003; Trost et al. 2012), *Corynebacterium jeikeium* (Tauch et al. 2005), *Corynebacterium kroppenstedtii* (Tauch et al. 2008a), multiple strains of the *Corynebacterium pseudotuberculosis* biovars *ovis* and *equi* (Trost et al. 2010b; Cerdeira et al. 2011a, b; Ruiz et al. 2011; Silva et al. 2011, 2012; Hassan et al. 2012; Lopes et al. 2012; Pethick et al. 2012a, b; Ramos et al. 2012), *Corynebacterium resistens* (Schröder et al. 2012a), *Corynebacterium ulcerans* from human and animal sources (Trost et al. 2011; Sekizuka et al. 2012), and *Corynebacterium urealyticum* (Tauch et al. 2008b). Complete genome sequences from environmental corynebacteria include *Corynebacterium efficiens* (Nishio, et al. 2003), *Corynebacterium glutamicum* (Kalinowski et al. 2003; Ikeda and Nakagawa 2003; Yukawa et al. 2007), and *Corynebacterium variabile* (Schröder et al. 2011). The genome of *Turicella otitidis* was sequenced to a high-quality draft status (Brinkrolf et al. 2012). The Genomes OnLine Database GOLD lists additional corynebacterial species that are targeted for genome sequencing or whose genomes have already been sequenced and annotated to a permanent high-quality draft status (Pagani et al. 2012). The latter section includes the additional species: *Corynebacterium accolens*, *Corynebacterium ammoniagenes*, *Corynebacterium amycolatum*, *Corynebacterium glucuronolyticum*, *Corynebacterium lipophiloflavum*, *Corynebacterium matruchotii*, *Corynebacterium striatum*, and *Corynebacterium tuberculostearicum*. The draft genome sequencing projects of *Corynebacterium bovis* (Schröder et al. 2012b), *Corynebacterium casei* (Monnet et al. 2012), *Corynebacterium nuruki* (Shin et al. 2011b), and *Turicella otitidis* (Brinkrolf et al. 2012) were published briefly as genome announcements.

The corynebacterial genome consists in principle of a single, circular chromosome within a size range from 2.28 Mb (*Corynebacterium pseudotuberculosis*) to 3.43 Mb (*Corynebacterium variabile*), although plasmids were described in several species. The chromosomes of corynebacteria from human and animal sources are generally smaller (2.28–2.96 Mb) than those from environmental species (3.11–3.43 Mb), and approximately 2,000–3,000 protein-coding genes were predicted by bioinformatic methods (Pagani et al. 2012). The number of *rrn* operons varies from three copies (e.g., *Corynebacterium jeikeium*) to six copies (e.g., *Corynebacterium variabile*) per chromosome. The G+C content of the genomic DNA varies in a wide range between the species (*Table 12.1*), as it can been expected when considering the taxonomic description of *Corynebacterium* species (Funke et al. 1997b). However, some genome sequences of type strains revealed a significantly lower or higher G+C content of the genomic DNA than that deduced earlier from experimental taxonomic studies, for instance *Corynebacterium kroppenstedtii* (Tauch et al. 2008a), *Corynebacterium nuruki* (Shin et al. 2011b), and *Corynebacterium resistens* (Schröder et al. 2012a) (*Table 12.1*). The G+C content of the genomic DNA of *Turicella otitidis* is 71.35 mol% (Brinkrolf et al. 2012) which is in the range of 65–72 mol% published previously (Funke et al. 1994). The draft genome sequence of *Turicella otitidis* has a size of 2.08 Mb (Brinkrolf et al. 2012). The sequenced corynebacteria revealed a high degree of synteny of orthologous genes over the entire length of the chromosomes. A remarkable difference in the overall synteny of genes was only observed in the chromosomes of *Corynebacterium jeikeium* (Tauch et al. 2005), *Corynebacterium resistens* (Schröder et al. 2012a), *Corynebacterium urealyticum* (Tauch et al. 2008b), and *Corynebacterium variabile* (Schröder et al. 2011). These genomes are characterized by three similar inversions which provided evidence for a common evolutionary scenario for these species. A new order of orthologous coding regions was also detected in the chromosome of *Corynebacterium kroppenstedtii* indicating the occurrence of genomic inversions during the evolution of this mycolic acid-free species (Tauch et al. 2008a). In principle, corynebacteria have an exceptionally stable genome structure with respect to the order of orthologous genes and have apparently maintained an ancestral genome organization (Ventura et al. 2007; Tauch 2008). A survey for recombinational repair systems among corynebacterial genomes suggested that the absence of the *recBCD* genes is responsible for the suppression of genome shuffling in these species (Nakamura et al. 2003). Nevertheless, mechanisms of horizontal gene transfer played an important role in the evolution of corynebacterial genomes and in intra-species genome plasticity, as it is exemplified by the presence of genomic islands that can cause the acquisition of blocks of genes, thereby producing evolution in quantum leaps (Schmidt and Hensel 2004). During a pan-genomic study of the

species *Corynebacterium diphtheriae*, 57 genomic islands were detected among the 13 sequenced strains (Trost et al. 2012). Comparative content analysis of the detected genomic islands revealed that some genomic regions are strain specific, whereas others are completely or partially conserved in more than one strain. The detected genomic islands were classified as pathogenicity islands, resistance islands, phage islands, or metabolic islands according to their predicted gene content (Trost et al. 2012). The gene content of genomic islands was also deduced from the genome sequences of *Corynebacterium efficiens* and *Corynebacterium glutamicum*, revealing the presence of metabolic islands and phage islands in these environmental species (Zhang and Zhang 2005; Tauch 2008).

Prophages

Prophages harboring the *tox* gene for diphtheria toxin were identified in the genome sequence of five *Corynebacterium diphtheriae* strains (Cerdeño-Tarrága et al. 2003; Trost et al. 2012). Two almost identical copies of corynephage ω^{tox+} were detected in the chromosome of *Corynebacterium diphtheriae* PW8 (Trost et al. 2012), as deduced previously from restriction endonuclease maps of phage DNA (Rappuoli et al. 1983a). The non-tandem copies of the prophage are separated by a 2-kb gene region that is flanked by two copies of a $tRNA^{Arg}$ gene in all *Corynebacterium diphtheriae* genomes (Trost et al. 2012). Nucleotide sequence comparisons revealed that the ω^{tox+} phage of *Corynebacterium diphtheriae* PW8 is homologous to corynephage β^{tox+} which has been integrated into the chromosome of the avirulent strain *Corynebacterium diphtheriae* C7 (Freeman 1951). This observation confirms an early study demonstrating by restriction mapping that the two phages differ in only three genomic regions (Rappuoli et al. 1983a). A different tox^+ phage was detected in the genome of *Coynebacterium diphtheriae* 31A, although all *tox* genes sequenced during the pan-genomic study showed a perfect nucleotide sequence identity (Trost et al. 2012). It has been proposed that the diphtheria toxin gene was acquired by corynephage β due to the terminal location of *tox* in the genome of the prophage and the significantly decreased G+C content of this gene region (Cerdeño-Tarrága et al. 2003). The detection of an identical *tox* gene in the prophage of *Corynebacterium diphtheriae* 31A indicates that the acquisition of *tox* had occurred independently in different corynephages or that gene shuffling is a common mechanism in corynephages. Interestingly, another type of tox^+ corynephage was recently identified in the chromosome of *Corynebacterium ulcerans* 0102 (Sekizuka et al. 2012). This observation suggests that tox^+ corynephages can have different genome architectures and that different phages contribute to the spread of the *tox* gene in the human population.

Prophages or phage islands were also identified in other corynebacterial genomes due to the lack of homology of the respective chromosomal region to the genomes of other *Corynebacterium* species and variations of the local G+C content of the genomic DNA. Five putative prophages were detected in *Corynebacterium ulcerans* (Trost et al. 2011). The prophage-like regions ΦCULC22I and ΦCULC809I have a size of about 42 kb and are characterized by highly similar genetic maps. The annotation of the *Corynebacterium aurimucosum* chromosome revealed the presence of two putative prophages, ΦCauriI and ΦCauriII (Trost et al. 2010a). Both prophage regions contain at their 3′ ends genes encoding λ repressor-like transcription regulators and phage-related integrases. Almost all genes of the prophage regions are encoded on the leading strand of the left replichore of the *Corynebacterium aurimucosum* chromosome. The size difference between both prophage genomes suggests that at least ΦCauriI is incomplete and a defective remnant of a formerly active corynephage. Two breakpoints of synteny between the chromosomes of *Corynebacterium resistens* and *Corynebacterium jeikeium* are caused by the presence of genes related to two prophages, named ΦCRES I and ΦCRES II (Schröder et al. 2012a). The genomic segment assigned to ΦCRES I has a size of about 58.7 kb and comprises 51 genes, whereas the ΦCRES II region has a size of about 40.2 kb and comprises 44 genes. Both putative prophage genomes share not only a very similar set of protein-coding regions but also a highly similar order of these genes, suggesting that the respective phages are genetically related. A phage island present in the *Corynebacterium variabile* chromosome has a size of about 48.3 kb and comprises 60 genes (Schröder et al. 2011). Most genes (39 out of 60) identified in the phage island encode hypothetical proteins of unknown function, whereas others encode enzymes involved in phage DNA replication, recombination, and repair. The structural proteins encoded by the phage island revealed similarities to the tail proteins of *Rhodococcus* phage ReqiPine5 and to the capsid structure of *Listeria* phage A006 (Schröder et al. 2011).

Another structural feature of some corynebacterial genomes is arrays of so-called clustered regularly interspaced short palindromic repeats (CRISPRs) (Mokrousov et al. 2007; Trost et al. 2010a, 2012; Schröder et al. 2012a). These arrays are composed of direct repeats that are separated by non-repetitive, similar-sized spacers. Together with a repertoire of associated *cas* genes, CRISPR arrays have been suggested to confer resistance to bacteriophages by RNA interference-like mechanisms, with a specificity that is determined by sequence similarities between the spacers and foreign DNA sequences (Barrangou et al. 2007; Terns and Terns 2011). Three different types of CRISPR arrays were detected in the genomes of sequenced *Corynebacterium diphtheriae* strains (Trost et al. 2012). These CRISPR arrays vary by the nucleotide sequence of the direct repeats, the number of spacer sequences, and the set of associated *cas* genes. *Corynebacterium diphtheriae* CRISPR type I is composed of three associated *cas* genes, and the number of spacers ranged from 1 to 28. CRISPR type II contains eight *cas* genes, and the number of repeats in these arrays ranged from 4 to 26, whereas the number of repeats in CRISPR type III ranged from 12 to 42, with eight flanking *cas* genes. Combinations of CRISPR type I and CRISPR type II were detected in three *Corynebacterium diphtheriae* genomes. The comparison of the spacer sequences revealed

that only 48 out of the 219 spacers are shared by two or three *Corynebacterium diphtheriae* strains, demonstrating that CRISPR arrays provide a solid basis to discriminate between different isolates (Trost et al. 2012). A CRISPR-based method to determine the phylogenetic relationship of *Corynebacterium diphtheriae* isolates is spoligotyping (Mokrousov et al. 2007, 2009). Targets for spoligotyping are the variable spacer regions between the conserved direct repeats of CRISPR arrays, as variations in the number or nucleotide sequence of spacers may provide patterns for the differentiation between subtypes of bacterial isolates (Grissa et al. 2008). An array of 98 CRISPRs and seven *cas* genes was found in the *Corynebacterium aurimucosum* chromosome (Trost et al. 2010a), and a DNA region with 73 CRISPRs and eight *cas* genes was annotated in the chromosome of *Corynebacterium resistens* (Schröder et al. 2012a). CRISPR arrays might be present also in other corynebacterial genome sequences. They can be identified in silico by means of the CRISPR Finder tool (Grissa et al. 2007).

Plasmids

Several plasmids were detected in *Corynebacterium* species and completely sequenced. Plasmid pNG2 (15.1 kb) from *Corynebacterium diphtheriae* is a prototype of a new plasmid family which is widely distributed in corynebacteria (Tauch et al. 2003a). This plasmid is characterized by a novel type of replication initiator protein and an associated 22-bp box element. Plasmid pNG2 and derivatives were initially detected in *Corynebacterium diphtheriae* strains and mediated erythromycin resistance (Schiller et al. 1980). R-plasmids were also found in *Corynebacterium xerosis* strains (Kono et al. 1983), although at least one host strain (M82B) was later reclassified as *Corynebacterium striatum* (Tauch et al. 2000a). The R-plasmid of M82B, pTP10 (51.4 kb), represents a mosaic structure of DNA elements associated with plasmid maintenance functions or multidrug resistance. The complete DNA sequence of pTP10 provides genetic information regarding the mechanisms of resistance against 16 antimicrobial agents that belong to six structural classes (Tauch et al. 2000a). A screening of 62 clinical isolates of *Corynebacterium jeikeium* revealed that 17 strains possessed small plasmids ranging in size from 7.6 to 14.9 kb. The plasmids formed four genetic groups according to DNA restriction analysis, and the complete DNA sequence of a representative from each group (pK43, pK64, pCJ84, and pB85766) was determined (Tauch et al. 2004). Plasmid pK64 contains the *ypkK* gene which encodes corynicin JK, a member of a new family of antimicrobial proteins (bacteriocins) with modular structure in Gram-positive bacteria (Swe et al. 2007). Corynicin JK is active against *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* (Swe et al. 2007). Plasmid pKW4 (14.3 kb) from *Corynebacterium jeikeium* was also associated with the production of a bacteriocin-like substance with a narrow killing spectrum, although the genetic determinant of this feature remained unknown (Kerry-Williams and Noble 1984; Tauch et al. 2005). The multidrug resistance of *Corynebacterium resistens* was mainly attributed to the presence of plasmid pJA144188 (28.3 kb) which harbors several antibiotic resistance genes, including a *tet*(W) tetracycline-minocycline resistance region which was detected for the first time in corynebacteria (Schröder et al. 2012a). Likewise, the presence of plasmid pET44827 (29 kb) in *Corynebacterium aurimucosum* explained the black pigmentation of the host strain, as it harbors five genes that might be responsible for the synthesis of a 3,3′-bipyridyl-like pigment. According to the annotation of pET44827 genes, it is most likely that the black pigment of *Corynebacterium aurimucosum* ATCC 700975 is synthesized by the concerted action of a non-ribosomal peptide synthetase and an unknown condensase function (Trost et al. 2010a). The small cryptic plasmid p1B146 (4,2 kb) was identified in a *Corynebacterium tuberculostearicum* strain derived from healthy human skin (Wieteska et al. 2011), whereas the cryptic plasmids pCR1−pCR4 (1.4–5.7 kb) were found in a single strain of *Corynebacterium renale* (Nath and Deb 1995; Srivastava et al. 2010).

Plasmids were also detected during a systematic survey of *Corynebacterium glutamicum* strains (Tauch et al. 2003b; Tauch 2005). They range in size from 2.4 kb (pXZ10142) to 95 kb (pGX1906). Sequenced plasmids were classified into four plasmid families according to the type of replication and the amino acid sequence similarities of the replication initiator proteins. The cryptic plasmids pEC2 (23.7 kb) and pEC3 (48.7 kb) are part of the *Corynebacterium efficiens* genome (Nishio et al. 2003). The small cryptic plasmid pCC1 (4.1 kb) was isolated from *Corynebacterium callunae* (Venkova-Canova et al. 2004), and plasmid pBY503 (15 kb) was detected in *Corynebacterium stationis* (Kurusu et al. 1991). Most *Corynebacterium glutamicum* plasmids are also cryptic, but pAG1, pCG4, pTET, and pXZ10145 were associated with antibiotic resistances (Tauch et al. 2003b; Tauch 2005).

Class 1 integrons were detected on plasmids pCG4 (*aadA2* gene cassette) and pTET3 (*aadA9* gene cassette) from *Corynebacterium glutamicum* (Nešvera et al. 1998; Tauch et al. 2002, 2003b) and on plasmid pJA144188 from *Corynebacterium resistens* (Schröder et al. 2012a). The class 1 integron of pJA144188 is characterized by small deletions in the 5′ and 3′ conserved segments and by a gene cassette array that comprises the rare *aacA1*:*gcuG* tandem gene cassette and the *aadA1a* gene cassette (Schröder et al. 2012a). Another class 1 integron with *dfrA16* and *qacH* gene cassettes is present in the genome of a *Corynebacterium diphtheriae* biovar *mitis* strain (Barraud et al. 2011).

Proteome Analysis

Comprehensive proteome maps are available for *Corynebacterium glutamicum* (Hermann et al. 2001; Hansmeier et al. 2006a), *Corynebacterium efficiens* (Hansmeier et al. 2006a), and *Corynebacterium jeikeium* (Hansmeier et al. 2007), including the cytosolic, cell surface, and extracellular proteome fractions. The extracellular and cell surface proteome of *Corynebacterium diphtheriae* was also mapped (Hansmeier et al. 2006b).

Phenotypic Analyses

Genus I. *Corynebacterium* Lehmann and Neumann 1896, 350[AL] emend. Bernard, Wiebe, Burdz, Reimer, Ng, Singh, Schindle and Pacheco 2010, 877

Co.ry.ne.bac.te'ri.um. Gr. n. *coryne* a club; L. neut. n. *bacterium* a rod, and in biology a bacterium (so called because the first ones observed were rod-shaped); N.L. neut. n. *Corynebacterium* a club bacterium.

Cells are Gram-positive, nonmotile, nonspore-forming, straight to slightly curved rods with tapered ends. Rods are usually short or of medium length. Club-shaped forms may be observed; sometimes ellipsoidal, ovoid, or rarely "whip handles" (*Corynebacterium matruchotii*) or thinner rods with bulges (*Corynebacterium sundsvallense*) are observed. Some cells stain unevenly. No species has aerial mycelium. Snapping division produces angular and palisade arrangements of cells. Metachromatic (polyphosphate) granules may be observed for some species. All species are catalase positive. All species are oxidase negative, with few exceptions (Table 12.1). Many species are facultatively anaerobic and some are (strictly) aerobic. Chemoorganotrophs. Some species are lipophilic or lipid auxotroph (Table 12.1). Many species produce acid from glucose and some other sugars (Table 12.1). Several species alkalinize citrate as the sole carbon source. The cell-wall peptidoglycan is based on *meso*-diaminopimelic acid (A1γ type). The glycan type of the cell walls contains acetyl residues. Major cell-wall sugars are arabinose and galactose (arabinogalactan). Short-chain mycolic acids with 22–36 carbon atoms in length may be present, but some species lack corynemycolates entirely (Table 12.1). Long-chained cellular fatty acids are of the straight-chain saturated and monounsaturated types, with significant amounts of palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and oleic acid ($C_{18:1}$ w9*c*) as major components. Small or moderate amounts of tuberculostearic acid (10-methyl $C_{18:0}$) and other cellular fatty acids may also be present (Bernard et al. 1991). Branched-chain or hydroxylated fatty acids are absent or found only in trace amounts. Fermentation products may include small volumes of acetic, succinic, and lactic acids, but production of propionic acid is species specific (Bernard et al. 2002). The major respiratory menaquinones are MK-8(H_2) and/or MK-9(H_2). MK-7(H_2) has been detected in *Corynebacterium glaucum* and *Corynebacterium lubricantis*, and small amounts of MK-10(H_2) have been found in *Corynebacterium thomssenii*. Phospholipids include phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol dimannosides, and other glycolipids (Yagüe et al. 2003). Phosphatidylethanolamine is absent, with the exception of *Corynebacterium bovis* and *Corynebacterium urealyticum*. The DNA G+C content varies between ~46 and 74 mol%. Type species is *Corynebacterium diphtheriae* (Kruse 1886) Lehmann and Neumann 1896, 350[AL].

Genus II. *Turicella* Funke, Stubbs, Altwegg, Carlotti, and Collins 1994, 272[VP]

Tu.ri.cel'la. *Turicum* proper name Turicum; L. fem. dim. suff. *−ella*; N.L. fem. dim. n. *Turicella* pertaining to Turicum, the Latin name of Zurich, Switzerland, where the first isolates were collected.

Cells are Gram-positive, nonmotile, nonspore-forming diphtheroids that occur as single cells or are arranged in V-shaped forms or palisades. Colonies are circular, convex, and creamy in color and range from 1.0 to 2.0 mm in diameter after 48 h of incubation at 37 °C on sheep blood agar. Catalase positive and oxidase negative. Metabolism is respiratory. The cell wall contains *meso*-diaminopimelic acid, arabinose, and galactose. Mycolic acids are not present. The principal menaquinones are MK-10 and MK-11. The straight-chain saturated fatty acids are mainly palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids. Oleic acid ($C_{18:1}$ w9*c*) is the predominant unsaturated fatty acid. Tuberculostearic acid (10-methyl $C_{18:0}$) is produced. The DNA G+C content varies between 65 and 72 mol%. Type species is *Turicella otitidis* Funke, Stubbs, Altwegg, Carlotti, and Collins 1994, 272[VP].

Turicella otitidis can be cultivated on Columbia agar base supplemented with 5 % sheep blood in a 5 % CO_2 atm, trypticase soy agar with 5 % sheep blood, chocolate agar supplemented with pyridoxal in a 5 % CO_2 atm, cooked-meat broth, and brain-heart infusion broth supplemented with 2 % yeast extract (Bernard and Funke 2012). Acid is not produced from D-glucose, glycogen, lactose, maltose, D-mannitol, ribose, sucrose, and D-xylose, or on triple sugar iron agar. Nitrate is not reduced. Indole is not produced. Urea and esculin are not hydrolyzed. Growth is visible in the presence of 6.5 % NaCl. Acid phosphatase, alkaline phosphatase, and leucine arylamidase are produced (Funke et al. 1994). The detection of completely unsaturated menaquinones (MK-10, MK-11) and the lack of mycolic acids distinguishes *Turicella otitidis* from *Corynebacterium* species. The non-fermentative metabolism is another characteristic distinguishing *Turicella otitidis* from the majority of *Corynebacterium* species (Table 12.1). The lipoarabinomannan of *Turicella otitidis* has a novel truncated structure which has been determined to consist of a mannosyl phosphatidylinositol anchor unit carrying an (α 1→6)-linked mannan core and substituted with terminal-arabinosyl branches (Gilleron et al. 2005).

Metabolic Pathways and Metabolism

The knowledge of metabolic pathways and the metabolism of *Corynebacterium* species and *Turicella otitidis* is very limited. Standard biochemical testing can be performed using commercially available manual identification test strips (Bernard 2012). *Corynebacterium* species and coryneforms exhibit a variety of metabolic processes, i.e., they can be fermentative, oxidative, assimulative, or nonreactive to most or all substrates on a species-specific basis (Bernard 2012). The metabolism of

Corynebacterium glutamicum has been extensively studied because of its relevance in engineering high-performance strains for the production of amino acids and other valuable products (Eggeling and Bott 2005; Yukawa and Inui 2013). These metabolic analyses were based on the annotated genome sequence and were additionally facilitated by the availability of transcriptomic and proteomic techniques. In the view of systems biology (Vertès et al. 2012), comprehensive genome-scale metabolic networks of *Corynebacterium glutamicum* were reconstructed in silico (Kjeldsen and Nielsen 2009; Shinfuku et al. 2009). The transcriptional regulatory repertoire of *Corynebacterium glutamicum* was also used for the in silico reconstruction of a gene-regulatory network (Schröder and Tauch 2010), and both networks were combined in an integrated version containing genes of pathways involved in lysine and glutamate production (Brinkrolf et al. 2010). A genome-scale cellular network of *Corynebacterium glutamicum* was also reconstructed by integrating data of the genome-wide metabolic and gene-regulatory subnetworks. This integrated cellular network is composed of 1,384 reactions, 1,276 metabolites, 88 transcriptional regulators, and 999 pairs of regulatory relationships. The transcriptional regulatory subnetwork was arranged into five hierarchical layers (Schröder and Tauch 2010), and the metabolic subnetwork presented a clear bow-tie structure (Jiang et al. 2012). In silico reconstructions of metabolic networks in *Corynebacterium* species can be performed almost automatically with the software tool CARMEN (Schneider et al. 2010). This tool supports the visualization of metabolic networks based on pathway information from the KEGG database or from user-defined templates and supports the interpretation of genome data in a functional context. The in silico reconstruction of pathways involved in the central metabolism of *Corynebacterium aurimucosum* (Trost et al. 2010a) and in the central carbohydrate and amino acid metabolism of *Corynebacterium resistens* (Schröder et al. 2012a) helped to deduce the lifestyle of both bacteria from their genome sequences. Metabolic features of *Corynebacterium variabile* relevant for the lifestyle on the surface of smear-ripened cheeses and during cheese ripening were deduced from the reconstruction of pathways involved in the central carbohydrate metabolism, in the biosynthesis of vitamins and cofactors, and in amino acid metabolism (Schröder et al. 2011). This analysis provided insights into the iron metabolism and the capacity of *Corynebacterium variabile* to perform lipolysis and proteolysis, thereby revealing a strong adaption of this bacterium to the iron-depleted and lipid-rich cheese surface habitat (Schröder et al. 2011).

Isolation, Enrichment and Maintenance Procedures

No selective media or enrichment procedures are known that are specifically suited for this group of organisms (Liebl 2005). However, special selective agars exist for *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* from respiratory specimens (Efstratiou and George 1999). Growth of *Corynebacterium* species and *Turicella otitidis* is generally achieved using a temperature range from 30 °C to 37 °C, particularly in air plus 5 % CO_2. Most species originally obtained from clinical specimens are routinely cultured on complex media, such as Columbia agar, brain-heart infusion agar, trypticase soy agar, or chocolate agar (*Corynebacterium aquatimens*), supplemented with 5 % horse or sheep blood where appropriate. Fastidious *Corynebacterium* species can be missed by routine culturing, because the organism does not grow after standard overnight incubation. Therefore, incubation may last longer than 24 h, at least in the case of *Corynebacterium urealyticum* (Soriano and Tauch 2008). A selective medium for the isolation of *Corynebacterium urealyticum* from urine and skin samples of hospitalized patients has been described (Zapardiel et al. 1998). Specific media have been designed and applied for the isolation of environmental corynebacteria from activated sludge (*Corynebacterium doosanense*) (Lee et al. 2009), the anode of a wastewater-fed microbial fuel cell (*Corynebacterium humireducens*) (Wu et al. 2011), saline soil (*Corynebacterium halotolerans*) (Chen et al. 2004), and coastal sediment (*Corynebacterium marinum*) (Du et al. 2010).

Growth of some *Corynebacterium* species is enhanced by the addition of 5 % horse or sheep blood to the synthetic medium or by using mixtures of lipids (e.g., 1 % Tween 80) as media supplements (Funke et al. 1997b). These species are generally referred to as lipophilic or lipid-requiring corynebacteria, since they grow poorly on standard laboratory media but show enhanced growth on sheep blood or brain-heart infusion broth supplemented with 0.1–1 % Tween 80 (Riegel et al. 1995b; Funke et al. 1997b). Recent genome sequencing projects revealed that the absence of fatty acid synthase genes apparently results in a strict nutritional requirement for exogenous fatty acids in the lipophilic species *Corynebacterium bovis* (Schröder et al. 2012b), *Corynebacterium jeikeium* (Tauch et al. 2005), *Corynebacterium kroppenstedtii* (Tauch et al. 2008a), *Corynebacterium resistens* (Schröder et al. 2012a), and *Corynebacterium urealyticum* (Tauch et al. 2008b). *Corynebacterium jeikeium* has a cellular fatty acid composition with the majority of these compounds being of the straight-chain, monounsaturated types (Yagüe et al. 2003), and it is worth mentioning that the chemical composition of Tween 80 almost exactly meets this requirement of *C. jeikeium*. Therefore, the term lipid auxotroph is also appropriate for the description of lipophilic corynebacteria lacking a fatty acid synthase gene (Tauch et al. 2005). However, it remains to be elucidated whether this genetic explanation is also applicable for the phenotypic description of other lipophilic *Corynebacterium* species (*Table 12.1*).

Members of the genus *Corynebacterium* may require additional supplements (amino acids, vitamins, purins, or pyrimidines) in synthetic culture media to grow, in particular when cultivated in minimal media. The knowledge of metabolic pathways deduced from complete genome sequences may help

to design minimal media for a species of interest (Brune et al. 2011). However, systematic growth assays with *Corynebacterium resistens* revealed a cysteine auxotrophy (Schröder et al. 2012c), despite the presence of all genes of the cysteine biosynthesis pathway (Schröder et al. 2012a). Most, if not all, *Corynebacterium* species are auxotrophic for the vitamin biotin due to the lack of a varying number of genes involved in the biotin biosynthesis pathway (Brune et al. 2012).

Cells of *Corynebacterium* species and *Turicella otitidis* do not require special protocols and procedures for storage. Short-term storage can be performed at 4 °C or room temperature on enriched media such as Columbia agar or brain-heart agar supplemented with 5 % horse or sheep blood where appropriate. Medium-term storage of *Corynebacterium* cells and *Turicella otitidis* can be done by using commercially available cryosystems to prepare cells for storage at −80 °C. Long-term preservation may be performed by standard lyophilization procedures.

Ecology

Currently, about 50 *Corynebacterium* species have been described that were initially isolated from humans or human clinical samples. Only *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, and *Corynebacterium pseudotuberculosis* can be regarded as toxigenic species, as they may produce potent exotoxins, i.e., diphtheria toxin or phospholipase D (Barksdale et al. 1981; Groman et al. 1984; Wong and Groman 1984; Holmes 2000). Other species of medical origin may be assigned opportunistic pathogens, as they rarely cause human infections and are in principle part of the human microbiome (Lewis et al. 2012; The Human Microbiome Project Consortium 2012a). However, there have been numerous case reports claiming an association of *Corynebacterium* species with disease. This increased detection of *Corynebacterium* species is amongst others caused by the growing number of immunocompromised patients whose diagnosis and treatment have become ever more intensive and invasive, resulting in better growth conditions for microbes (Funke et al. 1997b). Many of the medically relevant *Corynebacterium* species can also be recovered as commensals or contaminants from a variety of clinical specimens (Bernard 2012). Species associated with humans in addition to *Corynebacterium diphtheriae* include the following: *Corynebacterium accolens* from various clinical materials isolated from both patients and healthy individuals, a breast abscess, a case of endocarditis of native aortic and mitral valves, and the anterior nares (Neubauer et al. 1991; Claeys et al. 1996; Ang and Brown 2007; The Human Microbiome Project Consortium 2012b); *Corynebacterium afermentans* from blood cultures and multiple abscesses in brain, lung, and liver (Riegel et al. 1993a; Dykhuizen et al. 1995; Minkin and Shapiro 2004); *Corynebacterium ammoniagenes* from infant feces and floor dust samples (Collins 1987b; Täubel et al. 2009); *Corynebacterium amycolatum* from human skin, clinical specimens, and female patients with mastitis (Collins et al. 1988a; Lagrou et al. 1998; Paviour et al. 2002); *Corynebacterium appendicis* from an abdominal swab of a patient with appendicitis accompanied with abscess formation (Yassin et al. 2002a); *Corynebacterium aquatimens* from blood cultures of a patient with bacteremia (Aravena-Román et al. 2012); *Corynebacterium argentoratense* from human throat specimens (Riegel et al. 1995d); *Corynebacterium atypicum* from an unknown human clinical source (Hall et al. 2003); *Corynebacterium aurimucosum* from various clinical sources and in samples from the female urogenital tract (Yassin et al. 2002b; Daneshvar et al. 2004); *Corynebacterium auris* from patients with ear infections (Funke et al. 1995a); *Corynebacterium confusum* from a blood culture, patients with foot infections, and a breast abscess (Funke et al. 1998a; Bernard et al. 2002); *Corynebacterium coyleae* from blood cultures of patients with previous surgical intervention and a pancreatic abscess (Funke et al. 1997a; Taguchi et al. 2006); *Corynebacterium durum* from respiratory tract specimens, sputum, and a neck abscess (Riegel et al. 1997b; von Graevenitz et al. 1998; Bernard et al. 2002); *Corynebacterium falsenii* from blood cultures and a clinically significant bacteremia occurring in an infant while on vancomycin therapy (Sjödén et al. 1998; Tam et al. 2010); *Corynebacterium freneyi* from various clinical sources and a case of bacteremia following vascular surgery (Renaud et al. 2001; Auzias et al. 2003); *Corynebacterium glucuronolyticum* from the urogenital tract of male patients, peritoneal fluid, and prostate fluid (Funke et al. 1995b; Riegel et al. 1995a; Bernard et al. 2002); *Corynebacterium hansenii* from a pus swab of a patient with liposarcoma (Renaud et al. 2007); *Corynebacterium imitans* from nasopharyngeal specimens of patients with suspected diphtheria (Funke et al. 1997e); *Corynebacterium jeikeium* from various clinical sources and the human skin, including the axilla (Jackman et al. 1987; Tauch et al. 2004; Mookadam et al. 2006; Barzantny et al. 2012); *Corynebacterium kroppenstedtii* from a lung biopsy, the sputum of a patient with pulmonary disease, patients with granulomatous mastitis, and the retroauricular crease (Collins et al. 1998; Paviour et al. 2002; Taylor et al. 2003a; Riegel et al. 2004; Bernard et al. 2002; The Human Microbiome Project Consortium 2012b); *Corynebacterium lipophiloflavum* from a vaginal swab from a patient with the clinical diagnosis of bacterial vaginosis (Funke et al. 1997c); *Corynebacterium macginleyi* from conjunctival swabs, from the urine of a patient with a permanent bladder catheter, and from a central venous catheter in a case of sepsis (Riegel et al. 1995b; Funke et al. 1998b; Joussen et al. 2000; Villanueva et al. 2002; Mosele et al. 2012); *Corynebacterium massiliense* from blood and articular hip fluid (Merhej et al. 2009); *Corynebacterium matruchotii* from the oral cavity, cases of severe caries in young permanent teeth, the reproductive tract of women, and a mattress dust sample (Collins 1982a; Gross et al. 2010; Cherkasov and Gladysheva 2010; Täubel et al. 2009); *Corynebacterium minutissimum* as the causative agent of erythrasma in humans and from the axilla (Collins and Jones 1983a; Holdiness 2002; Troccaz et al. 2004); *Corynebacterium mucifaciens* from blood cultures, a wound swab, joint fluid, ear and nasal specimens, and the axillary microflora (Funke et al. 1997d; Morinaka et al. 2006; Taylor et al. 2003b); *Corynebacterium mycetoides* as the etiologic agent of a tropical ulcer

(Mungelluzzi and Caprilli 1965; Collins 1982b); *Corynebacterium pilbarense* from an ankle aspirate (Aravena-Roman et al. 2010); *Corynebacterium propinquum* from blood cultures and respiratory tract specimens (Riegel et al. 1993b; Motomura et al. 2004); *Corynebacterium pseudodiphtheriticum* from urine, respiratory specimens, blood, catheter tips, peritoneal fluid, and surgical wounds (Camello et al. 2009); *Corynebacterium pyruviciproducens* from a groin abscess (Tong et al. 2010); *Corynebacterium resistens* from blood cultures and samples taken from patients with acute myelocytic leukemia and myelodysplastic syndrome (Otsuka et al. 2005); *Corynebacterium riegelii* from female patients with urinary tract infections (Funke et al. 1998c); *Corynebacterium simulans* from a foot abscess, a boil, a biopsy sample of an axillar lymph node, and a mattress dust sample (Wattiau et al. 2000; Täubel et al. 2009); *Corynebacterium singulare* from semen and blood specimens (Riegel et al. 1997a); *Corynebacterium sputi* from the sputum of a patient with pneumonia (Yassin and Siering 2008); *Corynebacterium stationis* from blood cultures (Bernard et al. 2010); *Corynebacterium striatum* from a variety of human material (Eberson 1918; Martínez-Martínez et al. 1997); *Corynebacterium sundsvallense* from an intrauterine device in a woman with suspected endometritis or pyosalpingitis and from a sinus drainage from the left groin of a male patient (Collins et al. 1999a); *Corynebacterium thomssenii* from the pleural fluid of a patient with chronic renal failure (Zimmermann et al. 1998); *Corynebacterium timonense* from blood and articular hip fluid (Merhej et al. 2009); *Corynebacterium tuberculostearicum* from various clinical specimens, human axilla, tuna, and industrial environment (Brown et al. 1984; Feurer et al. 2004; Troccaz et al. 2004); *Corynebacterium tuscaniense* from blood cultures of a patient with endocarditis (Riegel et al. 2006); *Corynebacterium urealyticum* from human skin and the urinary tract of patients with underlying urological diseases (Pitcher et al. 1992; Soriano and Tauch 2008); *Corynebacterium ureicelerivorans* from blood cultures and other normally sterile sites in humans and from mattress dust samples (Yassin 2007; Fernández-Natal et al. 2009; Täubel et al. 2009); and *Corynebacterium xerosis* from various clinical samples and a brain abscess (Wooster et al. 1999). In the case of the latter species, it has to be considered that strains reported in the literature as *Corynebacterium xerosis* are probably misidentified and correspond to the species *Corynebacterium amycolatum* (Funke et al. 1996). *Turicella otitidis* was isolated from patients with otitis media, a cervical abscess, and a composted pine bark biofilter (Funke et al. 1994; Fernández-Pérez et al. 1999; Strauss et al. 2000).

Most strains of *Corynebacterium* species associated with humans were initially recovered from a variety of clinical specimens or sterile body sites. Therefore, the isolation procedure did not reveal information about the natural habitats of *Corynebacterium* species on the human body. This lack of knowledge was recently covered by meta-analyses in the course of the human microbiome project (Turnbaugh et al. 2007; Grice et al. 2009; Costello et al. 2009). To obtain an integrated view on the temporal and spatial distribution of the human microbiota, bacteria were surveyed from up to 27 body sites in healthy adults (Costello et al. 2009). The composition of the bacterial community was determined primarily by the body habitat, with high interpersonal variability and variations in time of the personalized microbiota. The colonization of the human skin is most probably driven by the highly variable ecology on the skin surface (Grice and Segre 2011). The complexity and stability of the microbial community are dependent on the specific characteristics of the skin site (Grice et al. 2009). Metagenomic analysis revealed that *Staphylococcus* and *Corynebacterium* species are the most abundant organisms colonizing moist areas of the human skin (Costello et al. 2009; Grice et al. 2009). The diversity of the microbiota of the human axilla was investigated in more detail by the cosmetic industry, as axillary malodor is attributable to the microbial biotransformation of odorless, natural secretions (James et al. 2004; Egert et al. 2011; Barzantny et al. 2012). A range of *Corynebacterium* species was detected on the axillary skin, as so-called operational taxonomic units based on amplified 16S rRNA gene sequences revealed similarities to sequences from *Corynebacterium afermentans*, *Corynebacterium amycolatum*, *Corynebacterium appendicis*, *Corynebacterium kroppenstedtii*, *Corynebacterium mucifaciens*, *Corynebacterium riegelii*, *Corynebacterium striatum*, and *Corynebacterium tuberculostearicum* (Taylor et al. 2003b; Egert et al. 2011). *Corynebacterium minutissimum* and *Corynebacterium tuberculostearicum* were isolated from the axilla and shown to produce an authentic axillary odor from collected sweat material (Troccaz et al. 2004). A probable route of human body odor formation proposed in the literature is the conversion of long-chain fatty acids into volatile short-chain fatty acids, probably by lipophilic corynebacteria which exhibit strong external lipase activities (James et al. 2004; Barzantny et al. 2012). The volatile branched-chain fatty acid (*E*)-3-methyl-2-hexenoic acid, for instance, is released from an odorless precursor by the action of a N^{α}-acylglutamine aminoacylase expressed in *Corynebacterium striatum* (Natsch et al. 2005). Moreover, the release of the malodor component 3-methyl-3-sulphanylhexan-1-ol from its odorless Gly-Cys-(S)-precursor was demonstrated in vitro by combining two enzymes (TpdA and AecD) which were cloned from axilla isolates of the species *Corynebacterium striatum* or *Corynebacterium jeikeium* (Emter and Natsch 2008). Knowledge of the putative habitat of a *Corynebacterium* species can be deduced also from metabolic analyses of the genome sequence, as demonstrated in the cases of *Corynebacterium aurimucosum* (Trost et al. 2010a), *Corynebacterium resistens* (Schröder et al. 2012a), and *Turicella otitidis* (Brinkrolf et al. 2012).

Corynebacterium species also appear to be commensals in animals or birds, and some species can cause significant infections. Others have been implicated in the transmission from animals to humans probably causing zoonotic infections. *Corynebacterium* species associated with animals or birds include the following: *Corynebacterium amycolatum* from dairy cows with mastitis (Hommez et al. 1999); *Corynebacterium aquilae* from the mouth of a Spanish Imperial eagle (*Aquila adalberti*) and the choanae of the Golden eagle (*Aquila chrysaëtos*)

(Fernández-Garayzábal et al. 2003); *Corynebacterium auriscanis* isolated from dogs with ear infections and associated with canine otitis externa (Collins et al. 1999b; Aalbæk et al. 2010); *Corynebacterium bovis* from milk samples of infected mammary glands of dairy cows (Watts et al. 2000); *Corynebacterium camporealensis* from the milk of sheep suffering from subclinical mastitis (Fernández-Garayzábal et al. 1998); *Corynebacterium canis* from a wound infection caused by a dog bite (Funke et al. 2010a); *Corynebacterium capitovis* from skin scrapings from the infected head of a sheep (Collins et al. 2001a); *Corynebacterium caspium* from the penis of a Caspian seal (*Phoca caspica*) (Collins et al. 2004; Kuiken et al. 2006); *Corynebacterium ciconiae* from the tracheas of healthy black storks (*Ciconia nigra*) (Fernández-Garayzábal et al. 2004); *Corynebacterium cystitidis* from cows with cystitis and pyelonephritis (Yanagawa and Honda 1978; Rosenbaum et al. 2005); *Corynebacterium epidermidicanis* from the skin of a dog with pruritus (Frischmann et al. 2012); *Corynebacterium falsenii* from the mouth of a Spanish Imperial eagle (*Aquila adalberti*), the trachea of the Golden eagle (*Aquila chrysaëtos*), and bioaerosols from duck houses (Fernández-Garayzábal et al. 2003; Martin et al. 2010); *Corynebacterium felinum* from a Scottish wild cat (*Felis sylvestris*) that had died from feline influenza (Collins et al. 2001b); *Corynebacterium freiburgense* from a patient's wound obtained from a dog bite (Funke et al. 2009); *Corynebacterium glucuronolyticum* from the urogenital tract of pigs (Devriese et al. 2000); *Corynebacterium kutscheri* from mice, rats, and probably hamsters (Bernard and Funke 2012; Amano et al. 1991); *Corynebacterium mastitidis* from the milk of sheep with subclinical mastitis and from preputial gland abscesses in mice (Fernández-Garayzábal et al. 1997; Radaelli et al. 2010); *Corynebacterium minutissimum* from dairy cows with mastitis (Hommez et al. 1999); *Corynebacterium mustelae* from lung tissue, the liver, and kidneys of a male ferret with lethal sepsis (Funke et al. 2010b); *Corynebacterium phocae* from the nasal cavities of common seals (*Phoca vitulina*) (Pascual et al. 1998); *Corynebacterium pilosum* from horse, dog, and cows, in the latter host associated with urinary tract infections (Yanagawa and Honda 1978; Thomas and Gibson 1981; Higgins and Messier 1997; Yeruham et al. 2006); *Corynebacterium pseudotuberculosis* predominantly from sheep, goats, horses, and cattle and associated with caseous lymphadenitis or ulcerative lymphangitis (Eberson 1918; Baird and Fontaine 2007); *Corynebacterium renale* from cattle, goats, macaques, and cows, in the latter associated with pyelonephritis (Ernst 1906; Fatihu and Addo 1991; Altmaier et al. 1994; Rosenbaum et al. 2005; Venezia et al. 2012); *Corynebacterium sphenisci* and *Corynebacterium spheniscorum* from the cloacae of healthy wild penguins (*Spheniscus magellanicus*) (Goyache et al. 2003a; Goyache et al. 2003b); *Corynebacterium suicordis* from pigs with pericarditis, pleuritis, pneumonia, and enlarged lymph nodes (Vela et al. 2003): *Corynebacterium testudinoris* from necrotic lesions in the mouth of a tortoise (Collins et al. 2001b); *Corynebacterium ulcerans* from dairy cows, dromedary camels, cats, dogs, goats, pigs, squirrels, free-living otters, macaques, and roe deer (Tiwari et al. 2008; Schuhegger et al. 2009; Bonmarin et al. 2009; Rau et al. 2012; Trost et al. 2011); *Corynebacterium ulceribovis* from the udder of a cow with a profound ulceration (Yassin 2009); *Corynebacterium urealyticum* from urinary tract infections in dogs and cats (Gomez et al. 1995; Suarez et al. 2002; Bailiff et al. 2005; Cavana et al. 2008); *Corynebacterium vitaeruminis* from the rumen of a cow and from the reproductive tract of women (Bechdel et al. 1928; Cherkasov and Gladysheva 2010); and *Corynebacterium xerosis* from clinical specimens of cows, pigs, goats, and sheep and probably in bioaerosols from duck houses (Palacios et al. 2010; Martin et al. 2010).

Some *Corynebacterium* species which have been detected in animals have also been documented to be the cause of infections in humans, probably by zoonotic transmission. These infections usually occurred in patients who had close contact with domestic or wild animals, i.e., during the occupational handling of animals, by animal bites, or by other means. *Corynebacterium amycolatum*, *Corynebacterium falsenii*, *Corynebacterium glucuronolyticum*, *Corynebacterium minutissimum*, *Corynebacterium urealyticum*, and *Corynebacterium xerosis* are usually associated with human disease, and zoonotic transmission has not been described. A case of zoonotic transmission of *Corynebacterium auriscanis* to humans has been reported, describing a leg wound infection following a dog bite in a previously healthy human patient (Bygott et al. 2008). *Corynebacterium freiburgense* was probably transmitted from the mouth of a dog to a female who had been bitten by her dog on her forearm, and *Corynebacterium canis* was also isolated from a wound infection caused by a dog bite (Funke et al. 2009, 2010a). Moreover, an infection with *Corynebacterium kutscheri* in a human has been reported after the patient had been bitten by a rat (Holmes and Korman 2007). Human infections of unknown means include a fatal case of prosthetic valve endocarditis caused by *Corynebacterium pilosum* (Sobrino et al. 1991), a fatal case of endocarditis caused by *Corynebacterium bovis* (Vale and Scott 1977), and a case of *Corynebacterium bovis* shoulder prosthetic joint infection (Achermann et al. 2009). The latter species was also isolated from a persistent leg ulcer, a human blood culture, and other clinical samples (Vale and Scott 1977; Bernard et al. 2002), whereas *Corynebacterium mastitidis* was detected in human ophthalmic samples (Eguchi et al. 2008). Human infections with *Corynebacterium pseudotuberculosis* can occasionally occur in adults who have close contact with animals (Peel et al. 1997), although a rare case of necrotizing lymphadenitis was described in a 12-year-old French girl (Join-Lambert et al. 2006). *Corynebacterium ulcerans* has been detected as a commensal in domestic and wild animals, and both groups may serve as reservoirs for the zoonotic transmission of this pathogen (Hogg et al. 2009; Wagner et al. 2010). Pet animals can also be included as carriers and potential infectious sources of *Corynebacterium ulcerans*, as a non-diphtheria toxin-producing strain caused a rare skin infection after transmission from a pet cat. The presence of *Corynebacterium ulcerans* in the mouth of the cat was confirmed by 16S rRNA gene analysis (Corti et al. 2012). Likewise, a single strain of *Corynebacterium ulcerans* was isolated from a female diphtheria patient and a chronic labial ulcer of her dog (Lartigue et al. 2005). The possible zoonotic transmission of

toxigenic *Corynebacterium ulcerans* from companion animals was also observed in a case of fatal diphtheria (Hogg et al. 2009). This observation is of particular interest, as toxigenic strains of *Corynebacterium ulcerans* can be regarded as an emerging zoonotic agent causing diphtheria-like illness in humans (Bonmarin et al. 2009; De Zoysa et al. 2005; Zakikhany and Efstratiou 2012). A case of toxigenic *Corynebacterium ulcerans* infection in a fully immunized veterinary student was reported from London, United Kingdom (Taylor et al. 2010). The case had been in close contact with a number of domestic animals. However, none of these were reported to have exhibited any clinical signs suggestive of *Corynebacterium ulcerans* infection. Moreover, toxigenic *Corynebacterium ulcerans* strains were isolated from domestic dogs in Japan. Comparative analysis of the isolates using PFGE and toxin gene typing suggests that transmission between asymptomatic dogs might have occurred (Katsukawa et al. 2009, 2012). On the other hand, *Corynebacterium diphtheriae* has been isolated from dairy cattle (Greathead and Bisschop 1963), a cow with chronic active dermatitis (Corboz et al. 1996), and domestic cats with severe otitis (Hall et al. 2010). The feline strains differed phenotypically from previously described biotypes but were regarded as typical *Corynebacterium diphtheriae* (Hall et al. 2010). Toxigenic *Corynebacterium diphtheriae* strains have been associated with wound infection in horses (Henricson et al. 2000; Leggett et al. 2010) which may act as reservoirs for toxigenic *Corynebacterium diphtheriae* strains and may facilitate the potential transmission of the pathogen to humans.

Corynebacterium ilicis was reported to be a plant-pathogenic member of the genus *Corynebacterium*, but the taxonomic position of this species has been a matter of debate (Judicial Commission of the International Committee on Systematics of Prokaryotes 2008). The Judicial Commission ruled that the features associated with *Corynebacterium ilicis* are represented by the type strain ICMP 2608 (ICPB CI144) and that *Arthrobacter ilicis* is not a homotypic synonym of *Corynebacterium ilicis*. However, *Corynebacterium ilicis* was later reassigned to *Curtobacterium flaccumfaciens* (Young et al. 2004; Bernard and Funke 2012). Therefore, the genus *Corynebacterium* is currently devoid of plant-pathogenic species.

Other *Corynebacterium* species have been described as being recovered from food products or from the environment, such as soil, water, marine ecosystems, or plant surfaces: *Corynebacterium callunae* from heather (Yamada and Komagata 1972; Bernard and Funke 2012); *Corynebacterium casei* from the surface of smear-ripened cheeses (Brennan et al. 2001; Mounier et al. 2005); *Corynebacterium deserti* from a mixed sand sample collected in a desert in the west of China (Zhou et al. 2012); *Corynebacterium doosanense* from activated sludge taken from a wastewater treatment plant in Yeongdeuk-gun, Republic of Korea (Lee et al. 2009); *Corynebacterium efficiens* from soil, vegetables, and onion bulbs (Fudou et al. 2002); *Corynebacterium flavescens* from dairy products (Bernard and Funke 2012); *Corynebacterium glaucum* from a cosmetic dye (Yassin et al. 2003); *Corynebacterium glutamicum* from soil contaminated with bird feces, sewage, manure, vegetables, and fruits (Abe et al. 1967; Liebl 2005); *Corynebacterium halotolerans* from a saline soil sample that was collected in Xinjiang Province, China (Chen et al. 2004); *Corynebacterium humireducens* from the anode of a microbial fuel cell fed with artificial wastewater (Wu et al. 2011); *Corynebacterium lubricantis* from a coolant lubricant (Kämpfer et al. 2009); *Corynebacterium marinum* from coastal sediment close to a coal-fired power station in Qingdao, China (Du et al. 2010); *Corynebacterium maris* from the mucus of the coral *Fungia granulosa* from northern Red Sea, Gulf of Eilat, Israel (Ben-Dov et al. 2009); *Corynebacterium nuruki* from *nuruk* which is a Korean alcohol fermentation starter (Shin et al. 2011a); *Corynebacterium terpenotabidum* from soil (Takeuchi et al. 1999); and *Corynebacterium variabile* from the surface of smear-ripened cheeses (Brennan et al. 2001; Mounier et al. 2005).

The detection of *Corynebacterium* species in various habitats has been broaded by recent scientific discoveries based on culture-independent studies and the systematic application of next-generation DNA sequencing technologies, for instance, in the course of the human microbiome project (Turnbaugh et al. 2007; Lewis et al. 2012). These metagenomic approaches provide huge sets of DNA sequences assigned to the bacterial 16S rDNA (Handelsman 2004) and provide new insights into the ecology of *Corynebacterium* species. Metagenome projects have been performed with various environmental samples (Pagani et al. 2012), and it is therefore helpful to screen the NCBI database for the presence of operational taxonomic units with significant similarity to the species under investigation. For instance, *Corynebacterium aurimucosum* 16S rDNA was detected in a bacterial population collected in the entrance area of a clean room environment in the Johnson Space Center (La Duc et al. 2007; Moissl et al. 2007) and in dust samples taken from office rooms in buildings located in central Finland (Rintala et al. 2008). This species was detected previously only in clinical specimens from patients with acute or chronic joint or bone infections, in infected diabetic foot wounds, in a biopsy sample from a patient with rheumatoid arthritis, and in samples from female urogenital sources (Trost et al. 2010a). *Turicella otitidis* was almost exclusively isolated from the human ear (Jeziorski et al. 2009), but 16S rDNA of this species was detected in the microbial community of the human ileum (Li et al. 2012), in floor dust, and in mattress dust (Täubel et al. 2009). The latter study indicates that human-derived bacteria account for a large part of the Gram-positive bacterial content in house dust.

Pathogenicity, Clinical Relevance

Corynebacterium diphtheriae, *Corynebacterium ulcerans*, and *Corynebacterium pseudotuberculosis* are the only species of the genus *Corynebacterium* which may produce potent exotoxins, diphtheria toxin and phospholipase D, both playing significant roles in pathogenicity (Barksdale et al. 1981; Groman et al. 1984; Wong and Groman 1984; Holmes 2000; Dorella et al. 2006). Due to their importance in human and veterinary medicine, the pathogenicity and virulence factors of these related species

have been studied intensively. Potential virulence factors of other species from human clinical sources, including *Corynebacterium jeikeium* (Tauch et al. 2005), *Corynebacterium urealyticum* (Tauch et al. 2008b), *Corynebacterium aurimucosum* (Trost et al. 2010a), and *Corynebacterium resistens* (Schröder et al. 2012a), were deduced from their annotated genome sequences, although the physiological importance of these factors remains largely unknown. The annotated genome sequences of *Corynebacterium kroppenstedtii* (Tauch et al. 2008a) and *Turicella otitidis* (Brinkrolf et al. 2012) did not reveal any apparent virulence factors. The evaluation of the genomic data indicated that lipophilism as a metabolic feature is probably involved in the pathogenicity of *Corynebacterium kroppenstedtii* and associated with granulomatous mastitis (Paviour et al. 2002; Taylor et al. 2003a). The organisms were detected in lipid-rich spaces surrounded by neutrophils and histiocytes in a histological specimen from the left breast of a Samoan woman (Taylor et al. 2003a). The lack of apparent virulence factors in *Turicella otitidis* is consistent with the low pathogenic potential of this species (Holzmann et al. 2002). Virulence factors and mechanisms of pathogenicity of other *Corynebacterium* species of medical origin are largely unknown.

Corynebacterium diphtheriae is the primary cause of the communicable disease diphtheria which is an upper respiratory tract illness initially characterized by sore throat, low-grade fever, and an adherent membrane (pseudomembrane) on the tonsils, the pharynx, or the nasal cavity (Hadfield et al. 2000). The major virulence factor of toxigenic *Corynebacterium diphtheriae* strains is diphtheria toxin, a potent A-B exotoxin that inhibits protein biosynthesis by ADP-ribosylation of the elongation factor EF-2 and kills susceptible host cells (Holmes 2000; Yates et al. 2006). As the diphtheria toxin is encoded by the *tox* gene present in the genome of a family of genetically similar corynephages, the toxigenicity of *Corynebacterium diphtheriae* is dependent on the lysogenization by a tox^+ corynephage (Holmes 2000). The regulation of diphtheria toxin expression is under bacterial control, as the corresponding iron-sensing regulator DtxR is encoded in the chromosome of *Corynebacterium diphtheriae*. Transcription of the *tox* gene is directly linked to bacterial iron homeostasis, as low iron concentrations induce the expression of diphtheria toxin (Tao et al. 1994). The enhanced synthesis of diphtheria toxin in *Corynebacterium diphtheriae* PW8 is most likely caused by a gene-dosage effect, as two ω^{tox+} phages are integrated into two non-tandem attachment sites of the chromosome (Rappuoli et al. 1983b; Trost et al. 2012). Since 1923, a diphtheria toxoid vaccine is produced from purified diphtheria toxin treated with formalin to inactivate the toxicity of the protein and to maintain its immunogenicity (Kitchin 2011). *Corynebacterium diphtheriae* PW8 and derivatives are widely used for the production of diphtheria toxin by submerged fermentation due to their ability to secrete high amounts of the toxin into the culture supernatant. Diphtheria is very effectively controlled in developed countries by an efficient immunization program (Vitek 2006), and only sporadic cases are observed in Europe (Zakikhany and Efstratiou 2012). The largest outbreak since the advent of mass immunization took place in Russia and the newly independent states of the former Soviet Union in the 1990s (Dittmann et al. 2000). The complete genome sequence of a clinical isolate related to this outbreak (*Corynebacterium diphtheriae* NCTC 13129) provided genetic information about potential virulence factors besides the diphtheria toxin, like iron transport systems and subunits of adhesive pili (Cerdeño-Tarrága et al. 2003). A genome comparison based on genomic hybridization showed remarkable differences in the distribution of putative pathogenicity islands of *Corynebacterium diphtheriae* PW8 and *Corynebacterium diphtheriae* C7(−) and provided first insights into the plasticity of the genome and the diversity of clinical isolates (Iwaki et al. 2010). This view was broadened by a comprehensive comparative genome analysis of toxigenic and non-toxigenic strains and the characterization of the pan-genome of *Corynebacterium diphtheriae* (Trost et al. 2012). This study revealed a diverse genome architecture of tox^+ corynephages and indicated that different phages can contribute to the spread of the *tox* gene in humans. Most pathogenicity islands revealed typical characteristics of horizontal gene transfer, and the majority of these islands encode subunits of a diverse set of adhesive pili which can probably play prominent roles in adhesion of *Corynebacterium diphtheriae* to different host tissues. This result implies that important variations exist on the cell surface of toxigenic and non-toxigenic *Corynebacterium diphtheriae* strains which are relevant for the initial step of an infection. Therefore, variation in the distributed gene content of individual strains is a common strategy of *Corynebacterium diphtheriae* to establish differences in host-pathogen interactions (Trost et al. 2012). However, virulence factors other than adhesive pili can contribute to the adhesion of *Corynebacterium diphtheriae* to host cells, including proteins of the resuscitation-promoting factor-interacting protein family and the cell-wall-associated hydrolase family (Ott et al. 2010; Kolodkina et al. 2011). Moreover, experimental data points to the unusual lipoarabinomannan of *Corynebacterium diphtheriae* as an adhesin to human respiratory epithelial cells, thereby contributing to the pathogenicity of this bacterium (Moreira et al. 2008). In contrast to the lipoarabinomannans of other corynebacterial species, the lipoglycan of *Corynebacterium diphtheriae* presents an unusual substitution of the α-1→6-mannan backbone by α-*D*-Ara*f*.

Toxigenic *Corynebacterium ulcerans* was first isolated from a throat lesion of a patient with respiratory diphtheria-like illness (Gilbert and Stewart 1926). Respiratory diphtheria-like illness caused by toxigenic strains of *Corynebacterium ulcerans* is now being described as an emerging, possibly zoonotic disease (Hogg et al. 2009; Dias et al. 2011; Zakikhany and Efstratiou 2012). It is increasingly reported from various industrialized countries and recently became more common than *Corynebacterium diphtheriae* infections in the United Kingdom (Tiwari et al. 2008; Wagner et al. 2010). *Corynebacterium ulcerans* strains that produce diphtheria toxin can cause infections of the human skin that completely mimic typical cutaneous diphtheria (Wagner et al. 2001). Toxigenic *Corynebacterium ulcerans* isolates produce diphtheria toxins with variations in the amino acid sequence when compared with that encoded by

Corynebacterium diphtheriae (Sing et al. 2003, 2005). Both species can harbor different corynephages carrying the *tox* gene for the diphtheria toxin (Trost et al. 2012; Sekizuka et al. 2012). Infections with toxigenic *Corynebacterium ulcerans* usually occur in adults, who consumed raw milk (Bostock et al. 1984) or had close contact with domestic animals (Wagner et al. 2010). *Corynebacterium ulcerans* has been detected also in wild animals, implying that both groups of animals may serve as reservoirs for zoonotic transmissions (Hogg et al. 2009). Ribotyping of *Corynebacterium ulcerans* strains isolated from a female diphtheria patient and from an ulcer of her dog revealed that both isolates correspond to a single clone (Lartigue et al. 2005). This example demonstrated that a distinct *Corynebacterium ulcerans* strain can infect different hosts. Besides diphtheria toxin, phospholipase D can be produced by *Corynebacterium ulcerans* (Barksdale et al. 1981). This toxin might play a role in pathogenicity of so-called non-toxigenic strains which were isolated from human clinical specimens not fitting reporting criteria for cases of diphtheria (Dewinter et al. 2005). The knowledge of virulence factors in non-toxigenic strains was recently extended by sequencing the genomes of two *Corynebacterium ulcerans* isolates from human and animal specimens, respectively (Trost et al. 2011). A bioinformatic screening for candidate virulence factors revealed the presence of genes coding for phospholipase D, neuraminidase H, endoglycosidase E, and subunits of adhesive pili in both *Corynebacterium ulcerans* genomes. The *rbp* gene encoding a putative ribosome-binding protein with striking structural similarity to Shiga-like toxins was detected only in the genome of the human isolate. The enzymatic activity of the ribosome-binding protein Rbp can probably lead to inhibition of protein biosynthesis, as is the case with diphtheria toxin (O'Loughlin and Robins-Browne 2001).

Corynebacterium pseudotuberculosis is a facultative intracellular pathogen (Dorella et al. 2006) that can be distinguished into the biovar *ovis* (from sheep and goats; negative nitrate reduction) and the biovar *equi* (from horses and bovines; positive nitrate reduction) according to the results of a nitrate reduction test (Biberstein et al. 1971). In sheep and goats, *Corynebacterium pseudotuberculosis* biovar *ovis* strains are responsible for causing the infectious, contagious, chronic disease caseous lymphadenitis which is mainly characterized by the presence of caseous necrosis on the lymphatic glands or abscess formation in superficial lymph nodes and subcutaneous tissues (Baird and Fontaine 2007). The disease is generally transmitted through direct contact with superficial wounds. The visceral form of caseous lymphadenitis can affect internal organs, resulting in weight loss and death of the infected animals (Hodgson et al. 1999). *Corynebacterium pseudotuberculosis* can be rapidly detected among diseased animals by the use of a multiplex PCR assay directed to the 16S rDNA and the *rpoB* and *pld* genes of this bacterium (Pacheco et al. 2007). Licensed vaccines intended for the use in sheep herds showed a variable efficiency in goat immunization (Williamson 2001; Dorella et al. 2009). Infections in horses by *Corynebacterium pseudotuberculosis* biovar *equi* display different disease patterns: external abscesses, ulcerative lymphangitis of the limbs, and a visceral form that affects the internal organs (Pratt et al. 2005). Regarding at least bulls and buffalo, there is evidence of the mechanical transmission of *Corynebacterium pseudotuberculosis* by houseflies, in addition to the transmission via skin contact between animals (Addo 1983; Yeruham et al. 2003). Phospholipase D is the primary virulence factor of *Corynebacterium pseudotuberculosis* (Dorella et al. 2006). It promotes the hydrolysis and degradation of sphingomyelin in endothelial cell membranes which increases vascular permeability and contributes to the spread and persistence of the bacterium in the host (Williamson 2001). The expression of phospholipase D is regulated by multiple environmental stimuli, including heat, and plays a role in the reduction of macrophage viability following infection (McKean et al. 2007a, b). Moreover, some strains of *Corynebacterium pseudotuberculosis* can express the diphtheria toxin (Funke et al. 1997b). The *fagABC* operon and the *fagD* gene are involved in iron acquisition and play a supportive role in virulence of *Corynebacterium pseudotuberculosis* (Billington et al. 2002). The *fag* genes are found on a pathogenicity island along with the *pld* gene which encodes phospholipase D (Ruiz et al. 2011). More recently, the presence of two pilus gene clusters has been reported in the genome of *Corynebacterium pseudotuberculosis* FRC41 (Trost et al. 2010b). Adhesive pili play an important role in bacterial virulence as they enable pathogens to bind to molecules on various host tissues (Ton-That and Schneewind 2003). After attaching to the host cell surface, the pathogen is able to initiate specific biochemical processes, such as extracellular and intracellular invasion, that will result in its proliferation in and dissemination among the host tissues (Wilson et al. 2002). Strain FRC41 from a human clinical specimen also encodes the virulence factors endoglycosidase E, neuraminidase H, nitric oxide reductase, an invasion-associated protein, and secreted serine proteases (Trost et al. 2010b). The cAMP-sensing transcription regulator GlxR plays a key role in controlling the expression of several genes contributing to virulence (Trost et al. 2010b).

Corynebacterium jeikeium is an opportunistic human pathogen and usually multidrug resistant (Funke et al. 1997b). It is considered part of the normal human skin flora, and colonization is predominantly found in the axillary, inguinal, and perineal areas (Coyle and Lipsky 1990). *Corynebacterium jeikeium* has been recognized as the causative agent of a variety of severe nosocomial infections (Funke et al. 1997b; Belmares et al. 2007; Schoen et al. 2009), most frequently associated with immunocompromised patients with malignancies, in-place medical devices, breaks in the skin barrier, and therapy with broad-spectrum antibiotics (Funke et al. 1997b). Antimicrobial susceptibility studies revealed that *Corynebacterium jeikeium* isolates are substantially resistant against clinically relevant antibiotics and that the glycopeptides vancomycin and teicoplanin remain universally active against this species (Lagrou et al. 1998; Traub et al. 1998). Metabolic analyses of the complete genome sequence indicated that *Corynebacterium jeikeium* is strictly dependent on the presence of exogenous fatty acids for growth which is consistent with the lipophilic phenotype of this species (Tauch et al. 2005). Potential virulence factors of *Corynebacterium jeikeium*, i.e., neuraminidase, alkaline ceramidase,

cholesterol esterase, cholesterol oxidase, and acid phosphatase, are apparently involved in ensuring the availability of fatty acids by damaging host tissue (Tauch et al. 2005). Bacteriocin-producing plasmids have been detected in *Corynebacterium jeikeium* which may facilitate the colonization of the human skin and prohibit the invasion of other corynebacterial species into the same habitat (Tauch et al. 2005; Swe et al. 2007). Multidrug resistance of *Corynebacterium jeikeium* strains is mediated by transposable elements, defective derivatives thereof, drug: H^+ antiporters, and Na^+-driven multidrug efflux pumps encoded in the chromosome and conferring resistances to macrolides, lincosamides, aminoglycosides, chloramphenicol, tetracycline, and fluoroquinolones (Tauch et al. 2005).

Corynebacterium urealyticum is a multidrug-resistant organism with a potent urease activity (Pitcher et al. 1992; Soriano and Tauch 2008) which is the major virulence factor of this pathogen. The bacterium is a common skin colonizer of hospitalized individuals who are receiving broad-spectrum antibiotics and also an opportunistic pathogen causing acute cystitis, pyelonephritis, encrusted cystitis, and encrusted pyelitis (Soriano and Tauch 2008; Famularo et al. 2008). *Corynebacterium urealyticum* is frequently isolated from the groin of elderly inpatients (Soriano et al. 1988) which favors the colonization of urinary catheters. It has been demonstrated experimentally that *Corynebacterium urealyticum* adheres efficiently to urinary catheters, thereby providing access to the human urinary tract (Soriano et al. 1993). Once the bacterium adheres to the epithelium of the urinary tract, it can grow under the stimulation of the urea present in the urine. The enzymatic activity of urease leads to hyperammoniuria and alkalinization of the urine which in turn causes hypersaturation with struvite and calcium phosphate with the consequent crystallization of struvite and apatite (Soriano and Tauch 2008). Both types of urinary stone have been reproduced by in vitro and in vivo experiments (Soriano et al. 1986, 1987). In addition to stone formation and associated obstructive complications, the potent urease activity of *Corynebacterium urealyticum* is also considered to be responsible for an ammonia-induced cytotoxicity for the renal epithelium. Options to neutralize such effect with acetohydroxamic acid were investigated, showing that this drug is able to neutralize the urease of *Corynebacterium urealyticum*, thereby also preventing the formation of struvite stones (Soriano et al. 1987). Multidrug resistance of *Corynebacterium urealyticum* is mediated by transposable elements present in the chromosome, conferring resistances to macrolides, lincosamides, aminoglycosides, chloramphenicol, and tetracycline (Tauch et al. 2008b).

Corynebacterium aurimucosum is detected rarely in human clinical specimens and has been recovered from blood cultures of a patient with bronchitis (Yassin et al. 2002b), from patients with acute or chronic joint or bone infections, from infected diabetic foot wounds, and from a biopsy sample from a patient with rheumatoid arthritis (Trost et al. 2010a). Unusual black-pigmented variants of *Corynebacterium aurimucosum* were isolated in Canada and the United States from female urogenital sources, mostly from vaginal and cervical swabs (Shukla et al. 2001; Bernard et al. 2002), and from the genital tract of women who had complications during pregnancy (Shukla et al. 2003b). According to their pigmentation, the name *Corynebacterium nigricans* was proposed for these isolates (Shukla et al. 2003a). However, biochemical analyses and sequencing of the 16S rRNA gene suggested that *Corynebacterium nigricans* is a later synonym for *Corynebacterium aurimucosum* (Daneshvar et al. 2004). The genome sequence of a black-pigmented strain (*Corynebacterium nigricans* CN-1) provided insights into the physiology and lifestyle of this potential pathogen in pregnant women (Trost et al. 2010a). The data obtained by the genome project suggest that *Corynebacterium aurimucosum* could be both a resident of the human gut and a pathogen in the female genital tract causing complications during pregnancy. Biosynthesis of the black pigment is probably required for colonization of the female genital tract by protecting the bacterial cells against the high hydrogen peroxide concentration in the vaginal environment. The 29,037-bp plasmid pET44827 was found to code for a putative non-ribosomal peptide synthetase which appeared to play a key role in the synthesis of the black pigment. Another possibility is that black-pigmented strains are sexually transmitted from infected men to some women who engage in sex during pregnancy (Trost et al. 2010a).

Corynebacterium resistens, a highly multidrug-resistant bacterium, was recovered from blood samples, bronchial aspirates, and abscess specimens in a Japanese hospital (Otsuka et al. 2005). Bacteremia associated with this organism in immunocompromised patients was rapidly fatal as therapies with the antibiotic minocycline failed (Otsuka et al. 2005). Strains obtained from inpatients revealed high levels of resistance to macrolides, aminoglycosides, tetracyclines, quinolones, and β-lactams, whereas a single isolate from an outpatient was susceptible to imipenem and minocycline. Susceptibility assays showed that the glycopeptides vancomycin and teicoplanin remain universally active against all isolates (Otsuka et al. 2005). The complete genome sequence of *Corynebacterium resistens* helped to identify genes contributing to virulence and multidrug resistance of this bacterium (Schröder et al. 2012a). Metabolic features probably related to the natural habitat indicate that *Corynebacterium resistens* might colonize the inguinal or perineal regions of the human body. The lipophilic lifestyle is linked with the enzymatic functions of several predicted virulence factors which probably ensure the availability of external fatty acids for growth by causing damage to membranes of host cells. Plasmid pJA144188 harbors several genes contributing to a broad spectrum of antibiotic resistance of *Corynebacterium resistens*, including a tetracycline-minocycline resistance region of the Tet W type known from *Lactobacillus reuteri* and *Streptococcus suis* (Schröder et al. 2012a).

Antibiotic Susceptibility and Mechanisms of Antibiotic Resistance

Antibiotic resistance of *Corynebacterium* species and *Turicella otitidis* may hamper patient management and the efficient medical treatment of infectious diseases. This has been documented

with the detection of highly resistant *Corynebacterium resistens* strains and the apparent failure of minocycline therapy which probably contributed to the subsequent death of a patient from sepsis (Otsuka et al. 2005). A life-threatening infection with daptomycin-resistant *Corynebacterium jeikeium* has been documented in a neutropenic patient (Schoen et al. 2009), and a multidrug-resistant *Corynebacterium striatum* strain was associated with bilateral pneumonia and pulmonary embolism in a heart transplant patient (Tarr et al. 2003). An earlier example is an outbreak of diphtheria in Seattle between 1972 and 1982 involving 1,100 cases, primarily adults with cutaneous lesions (Coyle et al. 1989). Erythromycin- and clindamycin-resistant *Corynebacterium diphtheriae* isolates were recovered from skin lesions, and erythromycin-inducible cross-resistance to vernamycin B_{α} was demonstrated (Coyle et al. 1979). This is remarkable as erythromycin had been considered the drug of choice for diphtheria patients in Seattle since the epidemic began in 1972 (Coyle et al. 1979). Multidrug resistance of *Corynebacterium diphtheriae* has been reported recently in a biovar *mitis* strain isolated from a skin abscess of an unvaccinated patient (Mina et al. 2011). *Corynebacterium amycolatum*, *Corynebacterium jeikeium*, and *Corynebacterium urealyticum* are generally regarded as multidrug resistant (Philippon and Bimet 1990; de Miguel-Martinez et al. 1996; Funke et al. 1997b; Soriano and Tauch 2008; Fernandez-Roblas et al. 2009). Multidrug resistance was also observed in *Corynebacterium xerosis* (Lortholary et al. 1993; Wallet et al. 1994) and *Corynebacterium striatum* (Dobler and Braveny 2003; Tarr et al. 2003; Otsuka et al. 2006; Iaria et al. 2007; Campanile et al. 2009).

The in vitro activity of various commonly used antibiotics against multidrug-resistant *Corynebacterium* species was evaluated by detecting minimum inhibitory concentrations (MICs) and time-death curves according to the recommendations of the Clinical and Laboratory Standards Institute. *Corynebacterium resistens* was described as being susceptible to the glycopeptides vancomycin and teicoplanin (Otsuka et al. 2005), as it is universally the case with *Corynebacterium amycolatum*, *Corynebacterium jeikeium*, *Corynebacterium striatum*, and *Corynebacterium urealyticum* (Lagrou et al. 1998; Traub et al. 1998; Gómez-Garcés et al. 2007). The semisynthetic glycopeptide telavancin (TD-6424) was shown to be active against *Corynebacterium amycolatum* and *Corynebacterium jeikeium* (Goldstein et al. 2004). High in vitro activity against clinical *Corynebacterium* isolates was observed with quinupristin/dalfopristin, tigecycline, linezolid, and daptomycin (Goldstein et al. 2003; Fernandez-Roblas et al. 2009). Tigecycline was very effective against multidrug-resistant species, indicating that this antibiotic is a good alternative for the therapy of corynebacterial infections (Salas et al. 2008; Fernandez-Roblas et al. 2009; Dinleyici et al. 2010). The two fluorinated oxazolidinones, U-100592 and U-100766, were positively evaluated against *Corynebacterium jeikeium* (Jones et al. 1996). Likewise, the oxazolidinone linezolid was very active against multidrug-resistant *Corynebacterium* species and *Turicella otitidis* (Jones et al. 2002; Gómez-Garcés et al. 2007). Time-death curves revealed that this antibiotic is effective as bacteriostatic agent. In contrast, β-lactams, macrolides, lincosamides, aminoglycosides, and fluoroquinolones showed generally high MICs (Gómez-Garcés et al. 2007). The cyclic lipopeptide antibiotic daptomycin was used as antibiotic therapy for the treatment of *Corynebacterium striatum* endocarditis (Shah and Murillo 2005; Fernández-Guerrero et al. 2012) and *Corynebacterium jeikeium* prosthetic valve endocarditis (Lappa et al. 2012), although daptomycin resistance has been observed in both species (Tran et al. 2012; Schoen et al. 2009).

Resistance to erythromycin and other macrolide-lincosamide-streptogramin B (MLS) antibiotics was associated with the presence of the *erm*(X) gene in *Corynebacterium afermentans* subsp. *afermentans* (Ortiz-Pérez et al. 2010), *Corynebacterium amycolatum* (Yagüe Guirao et al. 2005; Ortiz-Pérez et al. 2010), *Corynebacterium aurimucosum* (Ortiz-Pérez et al. 2010), *Corynebacterium coyleae* (Ortiz-Pérez et al. 2010), *Corynebacterium diphtheriae* (Schiller et al. 1980; Tauch et al. 2003a), *Corynebacterium jeikeium* (Rosato et al. 2001; Tauch et al. 2005; Yagüe Guirao et al. 2005; Ortiz-Pérez et al. 2010), *Corynebacterium pseudodiphtheriticum* (Olender and Niemcewicz 2010), *Corynebacterium resistens* (Schröder et al. 2012a), *Corynebacterium striatum* (Tauch et al. 2000a; Campanile et al. 2009; Ortiz-Pérez et al. 2010), and *Corynebacterium urealyticum* (Tauch et al. 2008b; Ortiz-Pérez et al. 2010). This gene encodes a 23S rRNA methyltransferase and can be associated with plasmids and mobile DNA elements, for instance, with the widely distributed transposon Tn*5432* (Tauch et al. 2000a). The related *erm*(B) determinant was detected in an unclassified *Corynebacterium* species (Luna et al. 1999) and in two *Corynebacterium urealyticum* strains (Ortiz-Pérez et al. 2010), as was the *mef* resistance gene in *Corynebacterium jeikeium* which encodes a macrolide efflux pump (Luna et al. 1999). However, resistance to MLS antibiotics is mainly due to the presence of the Erm(X) methylase in *Corynebacterium* species (Ortiz-Pérez et al. 2010).

Resistance to tetracycline and minocycline in *Corynebacterium resistens* is encoded by the *tet*(W) gene encoding a ribosomal protection protein (Schröder et al. 2012a). The ribosomal protection determinant *tet*(M) was detected by DNA-DNA hybridization in *Corynebacterium striatum* (Roberts et al. 1992). The unusual resistance gene pair *tetAB* encoding a tetracycline efflux system was also detected in this species (Tauch et al. 2000a; Campanile et al. 2009). Chloramphenicol resistance is mediated by the *cmx* gene encoding a specific antibiotic efflux system in *Corynebacterium jeikeium* (Tauch et al. 2005), *Corynebacterium resistens* (Schröder et al. 2012a), *Corynebacterium striatum* (Tauch et al. 2000a; Campanile et al. 2009), and *Corynebacterium urealyticum* (Tauch et al. 2008b). Resistances to a broad spectrum of aminoglycosides is conferred by a variety of genes encoding different enzymatic functions or at least different substrate specificities (Shaw et al. 1993; Vakulenko and Mobashery 2003). The *aphA1-IAB* gene encodes an enzyme of the aminoglycoside 3′-phosphotransferase protein family, APH (3′)-Ic. It is active against kanamycin, neomycin, lividomycin, paromomycin, and ribostamycin, and was found in *Corynebacterium striatum* (Tauch et al. 2000a; Campanile et al. 2009) and *Corynebacterium urealyticum* (Tauch et al. 2008b).

The resistance gene *aadA1a* encodes the aminoglycoside 3″-adenyltransferase ANT(3″)-Ia with a specific substrate profile comprising only streptomycin and spectinomycin, whereas the *aacA1* gene encodes the aminoglycoside 6′-acetyltransferase AAC(6′)-Ia that can confer cross-resistance against kanamycin, amikacin, dibekacin, netilmicin, sisomicin, and tobramycin. Both resistance genes were detected in *Corynebacterium resistens* (Schröder et al. 2012a). The *strA-strB* genes occur as a tandem pair in *Corynebacterium* species (Tauch et al. 2005, 2008b; Schröder et al. 2012a) and encode the aminoglycoside 3″-phosphotransferase APH(3″)-Ib and the aminoglycoside 6-phosphotransferase APH(6)-Id, respectively. Both enzymes can specifically confer streptomycin resistance (Chiou and Jones 1995). The sulfonamide resistance gene *sul1*, encoding a sulfonamide-insensitive dihydropteroate synthase, was detected in *Corynebacterium resistens* (Schröder et al. 2012a), and both the trimethoprim resistance gene *dfrA16* and the quaternary ammonium compound resistance gene *qacH* were found in a *Corynebacterium diphtheriae* biovar *mitis* strain (Barraud et al. 2011).

Resistances against quinolones and fluoroquinolones are often caused by mutations in the so-called quinolone resistance-determining region (QRDR) of the gyrase gene *gyrA* (Hooper 1999). Single amino acid substitutions in the GyrA protein are sufficient to generate quinolone resistance in corynebacteria, but double mutations in the *gyrA* gene are necessary for high-level resistances (Sierra et al. 2005; Eguchi et al. 2008). The amino acid sequence motif LAIYG of the GyrA protein from *Corynebacterium resistens*, for instance, contains the distinct leucine and glycine residues which were associated with high-level resistances to ciprofloxacin, levofloxacin, and norfloxacin in clinical isolates of *Corynebacterium macginleyi* (Eguchi et al. 2008; Schröder et al. 2012a). Likewise, specific double mutations in the QRDR of the *gyrA* genes from *Corynebacterium striatum* and *Corynebacterium amycolatum* resulted in amino acid changes of the GyrA proteins and in high levels of fluoroquinolone resistance (Sierra et al. 2005). The resistance of corynebacteria against β-lactams might be associated with the presence of antibiotic-insensitive types of penicillin-binding proteins. The high-molecular weight penicillin-binding protein PBP2C and the L,D-transpeptidase Ldt1 of *Corynebacterium jeikeium* are two examples of ampicillin-insensitive cross-linking enzymes involved in peptidoglycan biosynthesis (Lavollay et al. 2009).

Antibiotic resistances were detected also in environmental *Corynebacterium glutamicum* isolates during a systematic survey for the presence of plasmids, revealing that the R-plasmids pAG1, pCG4, pTET3, and pXZ10145 harbor antibiotic resistance determinants against tetracycline, streptomycin, spectinomycin, chloramphenicol, and sulfonamides (Tauch et al. 2003b; Tauch 2005). Tetracycline resistance in *Corynebacterium glutamicum* is due to the presence of the *tet*(Z) and *tet*(33) genes encoding efflux proteins (Tauch et al. 2000b, 2002), whereas aminoglycoside resistance is mediated by the *aadA2* and *aadA9* genes encoding aminoglycoside 3″-adenyltransferases of the ANT(3″)-I protein family (Nešvera et al. 1998; Tauch et al. 2002, 2003b). Plasmid pAG1 possesses its own tylosin resistance determinant *rlmAII*, but its host strain showed resistance against this macrolide antibiotic only in conjunction with the *tlrD* gene from *Streptomyces fradiae*, although *tlrD* alone is generally insufficient to confer tylosin resistance (Liu and Douthwaite 2002).

Application

Several *Corynebacterium* species recovered from food products or from the environment have been used in industrial applications. The most prominent example is *Corynebacterium glutamicum* that is widely used in the industrial production of amino acids, especially L-glutamic acid and L-lysine, which are important in human and animal nutrition, respectively (Kimura 2005; Kelle et al. 2005). Both L-glutamic acid and L-lysine are produced on a large industrial scale by genetically modified high-performance strains of this species (Leuchtenberger et al. 2005; Becker et al. 2011; Hirasawa et al. 2012; Ikeda and Takeno 2013). Besides these traditional products, considerable progress has been made in recent years in the fermentative production of other amino acids by metabolically engineered *Corynebacterium glutamicum* strains, including alanine, arginine and citrulline, cysteine, methionine, serine, and branched-chain amino acids (Ikeda and Takeno 2013). The biotechnological potential of *Corynebacterium glutamicum* goes beyond amino acid synthesis and includes the targeted production of organic acids (lactate and succinate), alcohols and fuels (ethanol, butanol, isobutanol, and xylitol), aromatic compounds (phenol and terephthalate), biopolymers (polylactate, polysuccinate, and poly-(3-hydroxybutyrate)), polyamines (cadaverine and putrescine), industrial enzymes, and therapeutic proteins (Vertès et al. 2013; Jojima et al. 2013). *Corynebacterium glutamicum* has been studied also for a variety of bioremediation uses, mining purposes, and the biosorption of toxic heavy metals (Vertès et al. 2013).

Corynebacterium efficiens was originally detected during a systematic search for new glutamic acid-producing bacteria that can grow at higher temperatures than *Corynebacterium glutamicum* (Fudou et al. 2002). Production strains derived from a wild-type strain of *Corynebacterium efficiens* by genetic engineering might reduce the need for cooling during fermentation (Fudou et al. 2002). A comparison between orthologous proteins of *Corynebacterium glutamicum* and *Corynebacterium efficiens* revealed a tremendous bias in amino acid substitutions in the latter species, particularly towards arginine and glycine (Nishio et al. 2003). The analysis of amino acid substitutions in 13 pairs of orthologous enzymes relevant for the production of L-glutamic acid or L-lysine in both *Corynebacterium* species revealed that three substitutions, lysine to arginine, serine to alanine, and serine to threonine, are important for thermostability. Therefore, *Corynebacterium efficiens* seems to have acquired thermostability through the accumulation of specific amino acid substitutions which, in part, correlate with the increased G+C content of the genomic DNA (Nishio et al. 2004).

Corynebacterium terpenotabidum Y-11 is capable of degrading squalene which is a naturally abundant linear triterpene (C_{30}) and an important biosynthetic precursor of steroids and triterpenes in many living organisms (Yamada et al. 1975; Takeuchi et al. 1999). The *Corynebacterium terpenotabidum* Y-11 strain cannot grow on glucose as a sole carbon source, but is able to grow in synthetic media containing squalene or fatty acids or their esters and triglycerides (Yamada et al. 1977). Microbial degradation of squalene can provide optically active precursors for the industrial production of physiologically active compounds. Metabolically engineered strains of *Corynebacterium ammoniagenes* were shown to be useful for the overproduction of deoxycytidine (Lee et al. 2011), uridine 5′-monophosphate (Wang et al. 2007), and 5′-inosine monophosphate (Abbouni et al. 2004). A large-scale production system of *N*-acetyllactosamine, a core structure of various oligosaccharides, was established by a whole-cell reaction through the combination of recombinant *Escherichia coli* strains and *Corynebacterium ammoniagenes* (Endo et al. 1999). Likewise, UDP-galactose and globotriose can be produced on a large scale by coupling *Corynebacterium ammoniagenes* and metabolically engineered *Escherichia coli* strains (Koizumi et al. 1998). Another example is the production of riboflavin by *Corynebacterium ammoniagenes* (Koizumi et al. 2000)

Corynebacterium ammoniagenes was found in significant numbers in ripening cultures on the surface of German brick cheeses and might contribute to the ripening process (Valdés-Stauber et al. 1997). It is therefore a potential candidate for designing a defined ripening culture for rennet red-smear cheese. *Corynebacterium casei* and *Corynebacterium variabile* are part of the complex microflora on the surface of smear-ripened cheeses and contribute to the development of flavor and textural properties during cheese ripening (Beresford et al. 2001). *Corynebacterium variabile* can produce acetoin, butanediol, and methanethiol which are important flavor compounds in smear-ripened cheeses (Schröder et al. 2011). Analyses of the biodiversity of the microflora on the surface of a farmhouse smear-ripened cheese at four stages of ripening showed that the bacterial flora of inoculated and non-inoculated cheeses was dominated by *Corynebacterium casei* and *Corynebacterium* variable (*Corynebacterium mooreparkense*). In addition, *Corynebacterium flavescens* was detected on the surface of the inoculated cheeses (Brennan et al. 2002). *Corynebacterium casei* and *Corynebacterium variabile* are present not only on the surface of various types of Irish farmhouse smear-ripened cheeses but also in the dairy environment and on the skin of the cheese personnel, indicating that a distinct "in-house" microflora exists in cheese plants (Mounier et al. 2005, 2006). Both species have also been detected on the surface of Gubbeen, a red-smear cheese (Rea et al. 2007).

Corynebacterium nuruki was isolated from food-related material, i.e., the Korean alcohol fermentation starter nuruk (Shin et al. 2011a). *Nuruk* is used as an amylolytic substance in the manufacture of traditional Korean rice wine and is a mixture of grains and various microorganisms including mold, yeast, and bacteria. These components execute fermentation of rice and make an alcoholic beverage named *makgeolli* (Aidoo et al. 2006). *Corynebacterium vitaeruminis*, *Corynebacterium xerosis*, and *Corynebacterium amycolatum* were found in varying amounts in the microbial population present in the fermented beverage *cauim* produced by Brazilian Amerindians (Almeida et al. 2007). This beverage is produced with the substrates cassava, rice, corn, maize, and peanuts, and it is the main staple food for infants until the age of 2.

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13 The Family *Cryptosporangiaceae*

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Abstract

Cryptosporangiaceae is a family within the order "*Frankiales*," which includes the genera *Cryptosporangium* and *Fodinicola*. Members of this family are characterized by formation of aerial and substrate hyphae, *meso*-A_2pm as a cell-wall diamino acid, iso-$C_{16:0}$ as the major cellular fatty acid, phosphatidylethanolamine as a diagnostic polar lipid, and MK-9(H_4), MK-9(H_6), and MK-9(H_8) as major isoprenoid quinones. However, regardless of the reliability of algorithms used to analyze 16S rRNA gene sequences for type strains of *Cryptosporangium* and *Fodinicola*, the position of the genus *Fodinicola* must be considered tentative. Members of this family are found in soil, on rocks, and in leaf litter.

Taxonomy: Historical and Current

Short Description of the Families

The family description is based mainly on phylogenetic positions and patterns of 16S rRNA gene sequence signatures. However, the family *Cryptosporangiaceae* exhibits some diagnostic phenotypic characteristics differing from each other and from four neighboring families of the order "*Frankiales*" (◉ *Table 13.1*).

Cryptosporangiaceae Zhi, Li, and Stackebrandt 2009 596^{VP}

Cryp′to.spo.ran.gi.a′ce.ae. N.L. neut. n. *Cryptosporangium*, type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Cryptosporangiaceae*, the *Cryptosporangium* family (Zhi et al. 2009).

Phylogenetically, a member of the order "*Frankiales*" (Ludwig et al. 2012), phylum Actinobacteria.

The family contains the genera *Cryptosporangium* (Tamura et al. 1998) and *Fodinicola* (Carlsohn et al. 2008). Although most members of the order "*Frankiales*" do not form both substrate and aerial mycelium, members of this family can form both these structures.

Sporangiospores may occur. Motile or nonmotile. Cross-linkage of peptidoglycan is by the A type; diagnostic diamino acids include *meso*-diaminopimelic acid. Each peptidoglycan is directly linked without interpeptide bridges, and the muramic acid in peptidoglycan is N-acetylated. While methyl 14-methylpentadecanoate (iso-$C_{16:0}$) is the prominent fatty acid, $C_{17:1}$, 10-methyl $C_{17:0}$, and $C_{18:1}$ may also be present. Menaquinones MK-9(H_6), MK-9(H_4), and MK-9(H_8) are the predominant isoprenoid quinones, and MK-9(H_2) may also be present. The pattern of 16S rRNA signatures consists of nucleotides at positions 66:104 (G-C), 158:163 (G-C), 186:191 (G-C), 195 (U), 196 (C), 293:304 (G-C), 600:638 (G-C), 601:637 (A-U), 841 (U), 952:1229 (C-G), 986:1219 (A-U), 1042 (U), 1251 (G), and 1003:1037 (A-C). Predominant polar lipids include phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, and several phospholipids and glycolipids. Mycolic acids and teichoic acids are absent. Xylose, as a diagnostic whole-cell sugar, is present, and acofriose (3-*O* methyl rhamnose), as a whole-cell sugar, may also be present. G+C values are in the DNA range between 65 and 76 mol%. Members of the genus *Cryptosporangium* have been isolated from soil and cultivated soil, and members of the genus *Fodinicola* have been isolated from rocks from a medieval alum slate mine. Both the genera *Cryptosporangium* and *Fodinicola* are affiliated with the suborder *Frankineae*, but not affiliated with any family according to the original report (Tamura et al. 1998; Carlsohn et al. 2008). The family *Cryptosporangiaceae* was proposed by Zhi et al. (2009);

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_181,

Table 13.1

Diagnostic properties in which the family *Cryptosporangiaceae* differs from each other and four neighboring families of the order *Frankiales* (Carlsohn et al. 2008, amended)

Taxon	*Cryptosporangiaceae*	*Acidothermaceae*	*Frankiaceae*	*Geodermatophilaceae*	*Nakamurellaceae*	*Sporichthyaceae*
Cellular morphology	Substrate and aerial hyphae or aerial mycelia and sporangia	Slender rods, filaments	Substrate hyphae; no aerial mycelium; multilocular sporangia	Thallus consisting of cuboid to oval cells; rudimentary hyphae; no aerial mycelium or cocci, rods, vibrios; pairs, tetrads; clusters	Cocci; pairs; clusters	Short aerial hyphae; no substrate mycelium
Spore/bud formation	Sporangiospores or fragmentation of aerial hyphae	–	Sporangiospores	Zoospores, buds	–	Coccoid to rod-shaped spores
Motility	+ or –	–	–	+ or –	–	+
Cell-wall diamino acid(s)	*meso*-A_2pm	A_2pm, Ser, Ala	*meso*-A_2pm	*meso*-A_2pm	*meso*-A_2pm	LL-A_2pm
Major menaquinone(s)	MK-9(H_6), MK-9(H_4), MK-9(H_8)	ND	MK-9(H_4), MK-9(H_6), MK-9(H_8)	MK-9(H_4), MK-8(H_4), MK-9(H_6), MK-9	MK-8(H_4), MK-9(H_4)	MK-9(H_8), MK-9(H_6), MK-8(H_6)
Polar lipid(s)[a]	DPG, PE, PS, PI, PL, GL	ND	PI, PIM, DPG	PE, PIM, PI, DPG, PG	DPG, PE, PE-dimethyl	PI, PG, DPG, PL
Predominant fatty acid(s)	iso-$C_{16:0}$, $C_{17:1}$, $C_{18:1}$, 10-methyl $C_{17:0}$, $C_{17:1}$ cis9	ND	iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{17:1}$	iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{16:1}$, $C_{18:1}$v9c, $C_{17:1}$v8c, $C_{17:0}$, anteiso-$C_{17:0}$	iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{18:1}$; anteiso-$C_{15:0}$, $C_{17:0}$	$C_{16:0}$, iso-$C_{16:0}$, $C_{17:1}$, $C_{17:0}$
DNA G + C content (mol %)	65–76	61	66–71	68–75	68–73	71

Data for reference genera were taken from Lechevalier (1994), Mirza et al. (1991) (*Frankiaceae*), Luedemann and Fonseca (1989), Kroppenstedt (1985), Collins et al. (1984), Urzi et al. (2004), Mevs et al. (2000) (*Geodermatophilaceae*), Yoshimi et al. (1996), Yoon et al. (2007), Yoon et al. (2007) (*Nakamurellaceae*), Tamura et al. (1999) and Rainey et al. (1993) (*Sporichthya*), Mohagheghi et al. (1986) (*Acidothermaceae*), Tamura et al. (1998) (*Cryptosporangium*)

+ present, – absent, *ND* no data available

[a]*DPG* Diphosphatidylglycerol, *GL* unknown glycolipid(s), *PC* phosphatidylcholine *PE* phosphatidylethanolamine, *PE*-dimethyl, phosphatidyldimethylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PS* phosphatidylserine, *PL* unknown phospholipid(s)

Table 13.2

Diagnostic properties of the genera *Cryptosporangium* and *Fodinicola* belonging in the family *Cryptosporangiaceae* (Carlsohn et al. 2008, amended)

Taxon	*Cryptosporangium*	*Fodinicola*
Cellular morphology	Substrate and aerial mycelia; sporangia	Substrate and aerial hyphae
Spore/bud formation	Sporangiospores	− (Fragmentation of aerial hyphae)
Motility	+	−
Cell-wall diamino acid(s)	*meso*-A2pm	*meso*-A2pm
Major menaquinone(s)	MK-9(H6), MK-9(H4), MK-9(H8)	MK-9(H4), MK-9(H6), MK-9(H8)
Polar lipid(s)[a]	PE	DPG, PE, PS, PI, PL, GL
Predominant fatty acid(s)	iso-C16: 0, C17: 1, C18: 1	iso-C16: 0, 10-methyl C17: 0, C17: 1 cis9
DNA G + C content (mol%)	70	65

+ present, − absent

[a]*DPG* Diphosphatidylglycerol, *GL* unknown glycolipid(s), *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PS* phosphatidylserine, *PL* unknown phospholipid(s)

the genera *Cryptosporangium* and *Fodinicola* are assigned to this family. Diagnostic properties of the genera *Cryptosporangium* and *Fodinicola* are shown in *Table 13.2*.

Phylogenetic Structure of the Family and Its Genera

The family *Cryptosporangiaceae* currently includes two genera, *Cryptosporangium* and *Fodinicola*. Phylogenetic trees constructed using both neighbor-joining and maximum-likelihood methods indicate that members of the genus *Cryptosporangium* form a coherent clade, but that the genera *Cryptosporangium* and *Fodinicola* do not form a coherent clade (*Fig. 13.1a, b*). Carlsohn et al. (2008) reported *Fodinicola* has a phylogenetically deep branching point within the family of the suborder *Frankineae*, similar values were observed with respect to type strains of both *Cryptosporangium* and *Sporichthya* (92.9–94.8 % and 93.9–94.5 %, respectively), and members of the genera *Frankia* and *Acidothermus* are slightly less closely related to *Fodinicola feengrottensis* (92.5–93.0 %) than members of the genus *Cryptosporangium*. The genus *Fodinicola* was not proposed to be affiliated with any families in the original paper. However, Zhi et al. (2009) updated the 16S rRNA gene sequence-based definition for higher ranks of the class *Actinobacteria* and proposed the novel family *Cryptosporangiaceae*. The genus *Fodinicola* was not assigned to any families in this paper. Ludwig et al. (2012) proposed that the monospecific genus *Fodinicola* (type species *Fodinicola feengrottensis*) is tentatively classified in the family *Cryptosporangiaceae* as a genus incertae sedis based upon similarities in 16S rRNA gene sequence. Depending on the algorithm used to construct the phylogenetic tree, the relationship between the genera *Cryptosporangium* and *Fodinicola* may change. A number of *Fodinicola* strains should be analyzed to determine phylogenetic position.

Molecular Analyses

DNA-DNA Hybridization Studies

DNA-DNA hybridization (DDH) studies have been performed on all *Cryptosporangium* type strains. DDH relatedness has been reported between the type strains of *Cryptosporangium mongoliense* and the other four type strains of the genus (5–20 %; Ara et al. 2012); between *Cryptosporangium minutisporangium* and the other three type strains without *Cryptosporangium mongoliense* (14–22 %; Tamura and Hatano 2001); between *C. aurantiacum* and the other three type strains without *Cryptosporangium mongoliense* (15–21 %; Tamura and Hatano 2001); and between *Cryptosporangium arvum* and *Cryptosporangium japonicum* (38–44 %; Tamura et al. 1998). Although *Cryptosporangium* sp. YU655-31 and *Cryptosporangium* sp. YU656-31 showed 40–57 % DDH relatedness to *Cryptosporangium arvum* and *Cryptosporangium japonicum*, strains YU655-31 and YU656-31 showed diagnostic differences in phenotypic characteristics from the type species of *Cryptosporangium arvum* and *Cryptosporangium japonicum*. Therefore, the strains YU655-31 and YU656-31 are not assigned as species.

Genome Analyses

The incomplete genome sequence of the type strain of *Cryptosporangium arvum* DSM 44712^T = YU 629-21^T (GOLD ID Gi02260) has been released and the genome sequencing of the type strain of *Cryptosporangium japonicum* DSM 44713^T (GOLD ID Gi11347) is underway. The genome of the type strain of *Cryptosporangium arvum* contains 9,195,993 bp, 8,650 open reading frames (orfs), and a mol% G+C content of 72 %.

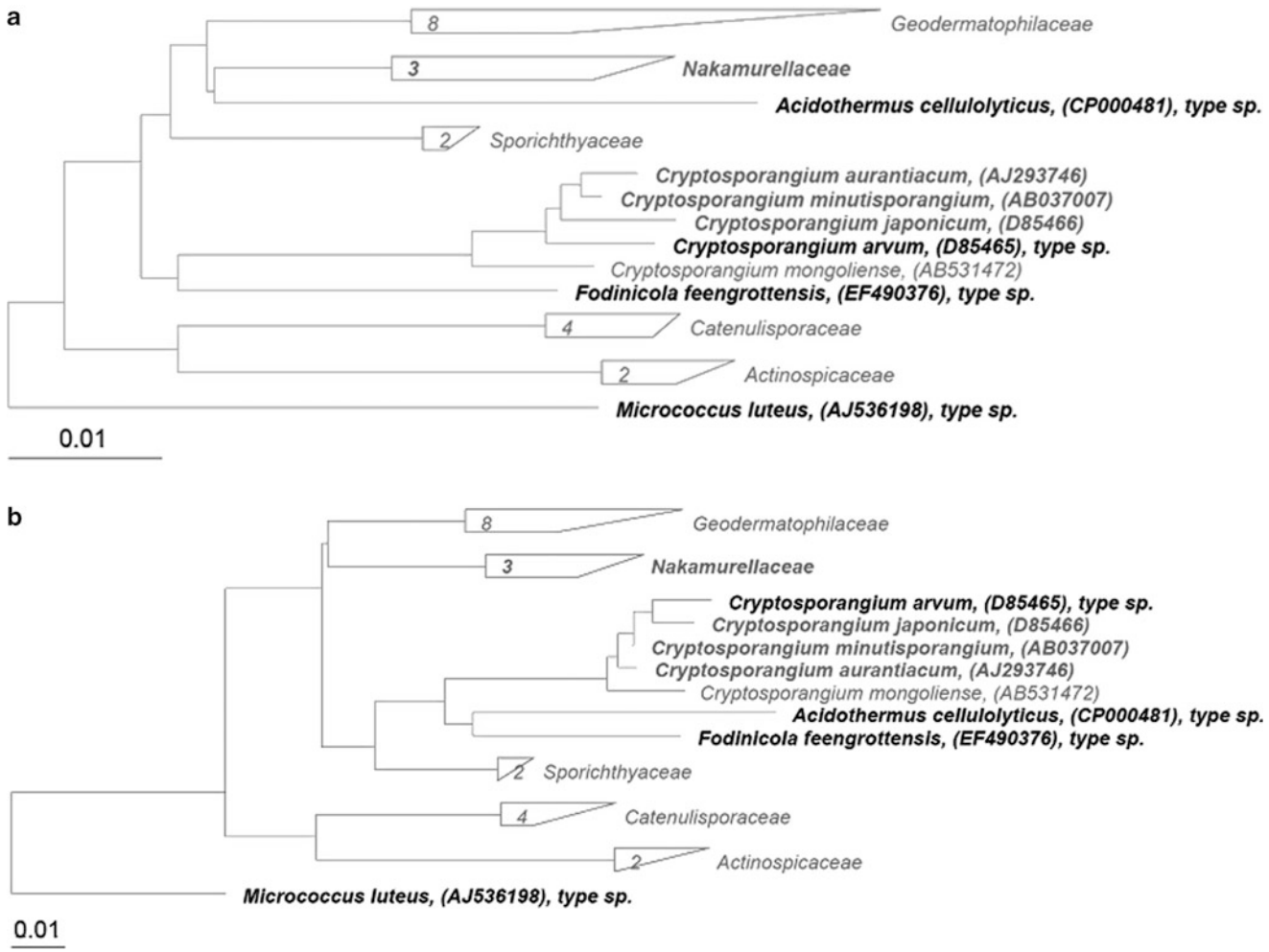

Fig. 13.1

Phylogenetic reconstruction of the family *Cryptosporangiaceae* based on 16S rRNA. Tree (a) created using the neighbor-joining algorithm with the Jukes-Cantor correction, 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. Tree (b) created using the maximum-likelihood algorithm RaxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, on both trees, 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

Phages

No phages have been observed to lyze strains of *Cryptosporangium* and *Fodinicola*.

Phenotypic Analyses

Cryptosporangium Tamura Hayakawa and Hatano 1998, 1003[VP]

Cryp'to. spo .ran'gi.um. Gr. adj. *kruptos*, hidden; N.L. n. *sporangium* [from Gr. n. *spora*, a seed (and in biology a spore), and Gr. n. *angeion* (Latin transliteration *angium*), vessel], sporangium; N.L. neut. n. *Cryptosporangium*, an organism with sporangia (spore containing vessels) covered or hidden by mycelium.

Cryptosporangium strains form branching hyphae. Non-fragmentary substrate mycelia and aerial mycelia are present. These organisms develop round or irregularly shaped sporangia that are 3–10 μm in diameter. Light microscopy has shown that their features resemble those of *Actinoplanes* strains. *Cryptosporangium aurantiacum* and *Cryptosporangium minutisporangium* were originally reported as "*Actinoplanes aurantiacus*" (Ruan et al. 1976) and *Actinoplanes minutisporangius* (Ruan et al. 1986), respectively. The muramic acid in peptidoglycan allows differentiation between the genera *Cryptosporangium* (acetyl) and *Actinoplanes* (glycolyl).

Table 13.3
Differentiating cultural features between type strains of *Cryptosporangium*[a]

	C. arvum	*C. japonicum*	*C. minutisporangium*	*C. aurantiacum*	*C. mongoliense*
ISP medium 2 (Yeast extract-malt extract agar)	Good	Good	Good	Good	Good
	Strong orange to light to moderate orange-yellow	Light to moderate orange-yellow	Light grayish brown, dark reddish brown to black	Strong orange	Honey
ISP medium 3 (Oatmeal agar)	Poor	Poor	Moderate	Moderate	Good
	Light to moderate orange-yellow	Moderate yellowish pink	Strong brown to dark reddish brown	Moderate orange-yellow	Honey
ISP medium 4	Poor	Poor	Good	Moderate	Moderate
	Light yellow	Moderate yellowish pink	Grayish olive	Light to moderate orange-yellow to moderate orange-yellow	Buff, pale yellow
ISP medium 5	Moderate	Moderate	Moderate	Moderate Light to moderate	Moderate
	Moderate orange-yellow to light to moderate orange-yellow	Light to moderate orange-yellow to light yellow	Dark reddish brown	orange-yellow to Strong orange	Hazel, light moderate yellowish brown
ISP medium 6	Poor	Poor	No growth	Scant	No growth
	Moderate orange-yellow	Moderate orange-yellow		Colorless	
ISP medium 7	Poor	Poor	Good	Moderate	Moderate
	Dark greenish yellow to strong brown	Strong brown to light grayish brown	Dark Reddish brown to dark red	Deep orange	Isabelline, moderate yellowish brown
Nutrient agar	Poor	Poor	ND	ND	ND
	Pale yellow	Pale yellow			
Brain Heart	ND	Moderate	ND	ND	ND
Infusion (Difco)		Orange-yellow			
Bennett's agar	Good	Good	Good	Good	Good
	Ochreous, moderate orange	Ochreous, moderate orange	Chestnut, dark reddish brown	Cinnamon, moderate orange	Honey, moderate yellow
Yeast extract-starch agar	Good	Good	Good	Good	Good
	Ochreous, moderate orange	Ochreous, moderate orange	Umber, strong brown	Ochreous, moderate orange	Ochreous, moderate orange
Water agar	Poor	Poor	Moderate	Moderate	Poor
	Colorless	Colorless	Buff, pale yellow	Colorless	Buff, pale yellow

[a]Data from Ara et al. (2012), Tamura et al. (1998), Tamura and Hatano (2001)
+ present, − absent, *ND* no data available

Some sporangia, particularly on the central region of the colony, are submerged under thick mycelia. Sporangiospores are motile when they are suspended in water.

Good growth occurs at 20–25 °C. The organism shows good growth on yeast extract-malt extract agar, Bennett's agar, and yeast extract-starch agar. Generally, the vegetative mycelia are yellow to orange and the aerial mycelia are white. Different cultural features and physiological characteristics between type strains of the genus *Cryptosporangium* are shown in *Tables 13.3* and *13.4*, respectively.

The cell walls contain glutamic acid, glycine, alanine, and *meso*-diaminopimelic acid. Wall chemotype is II according to

Table 13.4

Differentiating physiological characteristics between type strains of *Cryptosporangium*

	C. arvum	*C. japonicum*	*C. minutisporangium*	*C. aurantiacum*	*C. mongoliense*
Utilization of carbohydrates					
Glucose	+	+	w	+	+
d(+)mannose	w	w	+	+	–
d(+)lactose	w	+	+	+	–
d(+)xylose	+	+	w	+	W
dulcitol	–	–	–	–	ND
l-erythritol	–	–	–	–	ND
l-inositol	+	+	w	w	ND
d(+)galactose	w	w	+	+	ND
Adonitol	–	–	–	–	ND
α-methyl-D-glucoside	–	–	+	–	ND
d(+)raffinose	+	–	w	–	ND
d(–)mannitol	+	w	w	+	ND
d(+)maltose	+	+	w	+	+
l(+)arabinose	–	–	+	+	ND
l(+)rhamnose	–	+	w	+	ND
d(+)melibiose	–	–	+	+	ND
d-sorbitol	–	–	–	w	ND
Decomposition of					
Calcium-malate	+	+	–	–	–
Hydrolysis of					
Urea	+	+	–	–	–
Starch	+	+	–	–	+
Gelatin	–	–	+	–	–
Esculin	+	+	ND	ND	ND
Degradation of					
Hypoxanthine	+	+	–	+	+
Tyrosine	+	+	+	+	–
Casein	w	+	–	–	+
Utilization of organic acids					
Succinate	–	–	+	–	ND
Citrate	–	–	–	–	ND
Oxalate	–	–	–	+	ND
Malate	–	–	–	+	ND
Resistance to lysozyme	–	–	ND	ND	ND
Nitrate from nitrite	–	–	+	+	–
Resistance to NaCl					
0 %	+	+	+	+	+
4 %	–	–	–	–	–
Pigmentation in					
ISP-7	Pale reddish brown	Pale reddish brown	–	Moderate red	–
Growth at					
10 °C	–	–	+	–	
15 °C	+	+	+	w	
30 °C	+	+	+	+	
37 °C	–	+	–	+	

Data from Ara et al. (2012), Tamura et al. (1998), Tamura and Hatano (2001)

+ present. – absent, *w* weakly positive, *ND* no data available

Lechevalier and Lechevalier (1970), and the peptidoglycan type is thought to be A1γ according to Schleifer and Kandler (1972). Glucose and acofriose are detected as whole-cell sugars. The major fatty acids $C_{17:1}$, $C_{18:1}$, and iso-$C_{16:0}$ are present. The major menaquinones include MK-9(H_6), MK-9(H_4), and 9(H_8). Phosphatidylethanolamine is present as the diagnostic phospholipids (phospholipid pattern type PII).

The mol% G + C content is 56–58 %.

The type species is *Cryptosporangium arvum* (Tamura et al. 1998). The type strain is YU629-21 = DSM 44712 = HUT 6619 = JCM 10424 = NBRC 15965 = NCIMB 13630.

Actinoplanes minutisporangius (Ruan et al. 1986) is a synonym of *Cryptosporangium minutisporangium* (Tamura and Hatano 2001). "*Actinoplanes aurantiacus*" (Ruan et al. 1976) is a synonym of *Cryptosporangium aurantiacum* (Tamura and Hatano 2001).

Fodinicola Carlsohn Groth Saluz Schumann and Stackebrandt 2008, 1534[VP]

Fo.di.ni'co.la. L. n. *fodina*, a pit, mine; L. suff. *-cola* (from L. n. *incola*), dweller; N.L. masc. n. *Fodinicola*, a mine dweller.

Fodinicola strains form branched substrate mycelium and have sparse to abundant white aerial mycelium. Aerial hyphae break up into irregular rod-like elements. Phenotypic characteristics of the genus *Fodinicola* are shown in Table 13.5. Cell-wall sugars include xylose and minor amounts of an unknown compound. The predominant menaquinones include MK-9(H_4), MK-9(H_6), and MK-9(H_8). Polar lipids comprise diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and several unknown phospholipids and glycolipids, together with unknown ninhydrin-positive compounds. The cellular fatty acid profile is characterized by the predominance of iso-$C_{16:0}$, 10-methyl $C_{17:0}$, $C_{17:1}$ cis9, 10-methyl iso-$C_{18:0}$, and $C_{17:0}$.

The mol% G + C content of the type strain of the type species is 65 %.

The type species is *Fodinicola feengrottensis* Carlsohn et al. 2008. Type strain is HKI 0501 = DSM 19247 = JCM 14718.

"*Speleomyces feengrottensis*" is a synonym of *Fodinicola feengrottensis*.

Table 13.5
Phenotypic characteristics of *Fodinicola feengrottensis*[a]

Growth temperature (optimum)	20–28 °C	Activity (API ZYM tests)	
Growth pH (optimum)	pH 5.0–6.0	α-chymotrypsin (weakly)	w
Tolerance of NaCl(%)		Cystine arylamidase	+
1 %	+	Leucine arylamidase	+
2 %	–	Valine arylamidase	+
Hydration of		Esterase (C4)	+
Aesculin	+	Esterase lipase (C8)	+
Casein	+	α-galactosidase	+
Gelatin	+	β-galactosidase	+
Potato starch	+	*N*-acetyl-β-glucosaminidase	+
Urea	+	α-glucosidase	+
Reduction of Nitrate to nitrite	–	Lipase (C14) (weakly)	w
Degradation of		Mannosidase	+
Adenine	–	Naphthol-AS-BI-phosphohydrolase	+
Hypoxanthine	–	Acid phosphatase	+
Tyrosine	–	Alkaline phosphatase	+
Utilization as sole carbon sources		α-fucosidase	–
L-arabinose	+	β-glucosidase	–
D-fructose	+	β-glucuronidase	–
D-glucose (weakly)	w	Trypsin	v
D-mannitol	+		
Raffinose	+		
L-rhamnose	+		
Sucrose	+		
D-xylose	+		
Myo-inositol	–		
Cellulose	–		

[a]Data from Carlsohn et al. (2008)
+ positive, – negative, *w* weakly positive, *v* variable, *R* resistant, *S* susceptible

Isolation, Enrichment, and Maintenance Procedures

Cryptosporangium arvum and *Cryptosporangium japonicum* were isolated from soil samples collected from a vegetable field in Kofu, Yamanashi Prefecture, Japan, and from a sugar cane field on Miyako Island, Okinawa Prefecture, Japan, respectively (Tamura et al. 1998). The capillary method (Hayakawa et al. 1991) was used to isolate both strains. An air-dried soil sample (0.5 g) was placed in each of the cylindrical wells of a Lucite plate and sterile distilled water was then carefully added to the wells and connecting channel until they were full. After incubating the plate for 1 h at 30 °C, a 1-μL capillary filled with chemotaxis buffer containing vanillin as attractant was set in the Lucite plate. After incubating the plate for 1 h at 30 °C, solution in the capillary was serially diluted and spread onto humic acid/vitamin (HV) agar (Hayakawa and Nonomura 1987) containing 1.0 g/L humic acid, 0.02 g/L $CaCO_3$, 0.01 g/L $FeSO_4.7H_2O$, 1.71 g/L KCl, 0.05 g/L $MgSO_4.7H_2O$, 0.5 g/L Na_2HPO_4, 5 ml/L B-vitamin solution, 50 mg/L cycloheximide, and 18.0 g/L agar (pH 7.2). The B-vitamin solution contained 0.1 mg/mL each of thiamin-HCl, riboflavin, niacin, pyridoxin-HCl, inositol, Ca-pantothenate and *ρ*-aminobenzoic acid, and 0.05 mg/mL biotin. Agar plates were incubated at 28 °C for approximately 3 weeks. Subcultivation of the isolate was conducted in yeast extract-malt

extract agar (ISP 2 medium: Shirling and Gottlieb 1966) containing 4 g/L yeast extract, 10 g/L malt extract, 4 g/L glucose, and 20 g/l agar (pH 7.3), and yeast extract-soluble starch (YS) agar containing 2 g/L yeast extract, 10 g/L soluble starch, and 15 g agar (pH 7.3).

Cryptosporangium minutisporangium (Ruan et al. 1986; Tamura and Hatano 2001), the original name of which was *Actinoplanes minutisporangius*, was isolated from a soil sample from China (Ruan et al. 1986).

Cryptosporangium aurantiacum (Tamura and Hatano 2001), the original name of which was "*Actinoplanes aurantiacus*," was isolated from a soil sample and lake mud, collected near Bi Yun-Shi, Beijing, China (Ruan et al. 1976).

Cryptosporangium mongoliense (Ara et al. 2012) was isolated from a soil sample, collected from close to Khuvsgul Lake, Khuvsgul Province, Mongolia, using the rehydration and centrifugation (RC) method (Hayakawa et al. 2000) using HV agar supplemented with 20 mg/L trimethoprim and 10 mg/L nalidixic acid at 28 °C. Subcultivation was conducted on YS agar and maltose-Bennett's agar containing 1 g/L yeast extract, 1 g/L beef extract, 2 g/L NZ amine type A, 10 g/L maltose monohydrate, and 15 g/L agar (pH 7.3) at 28 °C for 3 weeks.

Hayakawa et al. (2010) reported that *Cryptosporangium* strains were isolated from soil samples collected from Iriomote Island, Okinawa, Japan. Additionally, Hop et al. (2011) reported that actinomycetes belonging to the genus *Cryptosporangium* were frequently isolated from leaf-litter samples collected from various locations in Vietnam, using the RC method and HV agar supplemented with nalidixic acid (20 mg/L), cycloheximide (50 mg/L), and kabicidin (20 mg/L).

Fodinicola feengrottensis (Carlsohn et al. 2008) was isolated from acidic and heavy metal-containing rocks collected in the "Barbara Grotto" of the Feengrotten medieval alum slate mine in Saalfeld, Thuringia, Germany. Material from the rock surface was scraped off using a sterile cotton swab and the adhering bacteria were dispersed in approximately 1-mL sterile distilled water. Aliquots of the resultant suspension were spread over starch-casein agar plates (Küster and Williams 1964) supplemented with cycloheximide (50 μg/mL). Agar plates were incubated at 28 °C for approximately 4 weeks. Subcultivation of the isolate was conducted on solidified organic medium 79 (Prauser and Falta 1968) containing 10.0 g/L dextrose, 10.0 g/L peptone, 2.0 g/L casein peptone, 2.0 g/L yeast extract, 6.0 g/L NaCl, 15.0 g/L agar (pH 7.8), and ISP 2 medium.

Members of the family *Cryptosporangiaceae* grow in complex liquid or on solidified media, such as ISP-2 medium, Bennett's agar containing 1 g/L yeast extract, 1 g/L beef extract, 2 g/L NZ amine, 10 g/L glucose, and 20 g/L agar (pH 7.3), YS agar and yeast extract-glucose broth containing 10 g/L yeast extract, and 10 g/L glucose. Members of these families do not require special procedures for maintenance or preservation. Cultures can be maintained by serial transfers onto the appropriate solid media. Growth on agar slants can be maintained at 4 °C for over 1 month. Medium-term preservation is in 12–15 % (v/v) glycerol suspensions at −80 °C. Long-term preservation of liquid cultures supplemented with 12–15 % (v/v) glycerol or 7 % (v/v) dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−150 °C). Freeze drying and L-drying methods can be also applied for long-term storage.

Ecology

Members of the family *Cryptosporangiaceae* were isolated from soils of vegetable fields, lake muds, acidic and heavy metal-containing rocks, and leaf litter. However, ecological information is insufficient since most known species are type strains. The following strains have been registered the 16S rRNA gene sequence to GenBank as *Cryptosporangium* strains: *Cryptosporangium* sp. LW-09 (AB607852) was isolated from Vietnam (Hop et al. 2011); *Cryptosporangium* sp. HBUM171259 (EU119260) and HBUM83975 (EU119259) were isolated from soil at China; *Cryptosporangium* sp. YIM 75710 (FJ911536) was isolated from Whitestone Quarry at Betamcherla, Kurnool, Andhra Pradesh, India; and *Cryptosporangium* sp. RS-53 (FM998040) was isolated from a rhizosphere soil of a plant *Peucedanum japonicum* Thunb. In addition, uncultured bacterium clone ncd393a10c1 (HM322140) having 98–99 % of similarities to validly published *Cryptosporangium* species by BLAST search was collected by swab from skin on antecubital fossa (Kong et al. 2012). *Fodinicola* sp. HKI 0511 (EU232177) having 99 % of similarity to *Fodinicola feengrottensis* was isolated from a medieval mine. Further studies are required to elucidate the ecological niche and function of members of the family.

Pathogenicity and Clinical Relevance

Pathogenicity has not been reported for any members of the family *Cryptosporangiaceae*.

Fodinicola feengrottensis cells are susceptible to chloramphenicol (30), ciprofloxacin (5), imipenem (10), kanamycin sulfate (30), norfloxacin (10), novobiocin (5), oxytetracycline hydrochloride (30), streptomycin sulfate (10), sulfonamide (200), and vancomycin hydrochloride (30). They are not susceptible to ampicillin (10), lincomycin hydrochloride (2), meticillin (5), nalidixic acid (30), penicillin G (10 IU), polymyxin B (300 IU), and rifampicin (30).

No information regarding antibiotic sensitivity and resistance is available for members of the genus *Cryptosporangium*.

Application

There have been several reports regarding the special use of members of the family *Cryptosporangiaceae*.

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14 The Family *Dermabacteraceae*

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Abstract
The family *Dermabacteraceae* is a phylogenetically and chemotaxonomically well defined taxon within the order *Micrococcales*, consisting at present of 4 genera and 17 species, with the majority of species described in *Brachybacterium*. *Dermabacter*, *Helcobacillus*, and *Devriesea* are monospecific. Cell morphology is rod shaped in young growth stage, forming coccoid to ovoid cells in older growth phases. Cells are nonmotile, lack mycolic acid, and do not form endospores. Strains are aerobic to facultative anaerobic. Members have a peptidoglycan type A4γ, major fatty acids are ai-$C_{17:0}$ and ai-$C_{15:0}$, predominant menaquinones (except for *Helcobacillus massiliensis*) are MK 7, MK8, or MK9 and major phospholipids are phosphatidylglycerol and diphosphatidylglycerol. The range of habitats from which the type strains were isolated is broad. Species include commensals of human and lizard's skin with the potential to be opportunistic pathogens, marine strains associated to invertebrates, strains isolated from milk and milk products, and soil organisms that are able to thrive in various conditions. One strain is able to fix nitrogen non-symbiotically. DNA-based studies reveal that, except for brachybacteria, members of the family are rare in actinomycetes 16S rRNA clone libraries.

Taxonomy: Historical and Current

The origin of the type genus of the family, *Dermabacter*, goes back to the Jones and Collins (1988) who investigated Gram-positive, asporogenous, and rod-shaped bacteria isolated from human skin. With the advent of chemotaxonomic methods the status of these "coryneform" organisms were elucidated, resulting in the delineation of the genus *Dermabacter* from other meso-diaminopimelic acid (A_2pm) containing taxa, such as *Corynebacterium*, *Caseobacter*, and *Brevibacterium*. In the same year, Collins et al (1988) described Schefferle strain 6-10^T (Schefferle 1966) from poultry deep litter as *Brachybacterium faecium* with similar chemotaxonomic properties than *Dermabacter* but without giving reference to this taxon. The first fragmentary 16S rRNA gene sequences of *Brachybacterium faecium* strains from clinical origin (CDC group 3 and 5) were published by Funke et al. (1994) and by Cai and Collins (1994) for a comparative analysis between *Dermabacter* and *Brevibacterium*. The first indication that *Dermabacter* and *Brachybacterium* are actually phylogenetic neighbors was published by Schubert et al. (1996) in their description of two novel *Brachybacterium* species from cheese surfaces.

Phylogenetic Structure of the Family and Its Genera

Dermabacteraceae Stackebrandt, Rainey, and Ward-Rainey 1997, 485^{VP}, emend Zhi et al. 2009.

Der.ma.bac.te.ra'ce.ae. M.L. masc. n. *Dermabacter*, type genus of the family; *-aceae*, ending to denote a family; M.L. fern. pl. n. *Dermabacteraceae*, the *Dermabacter* family.

The family *Dermabacteraceae* was described by Stackebrandt et al. (1997) on the basis of the isolated position of the16S rRNA gene sequences of type strains in the phylogenetic dendrogram of actinobacteria available at that time and by a set of signature nucleotides which was revised by Zhi et al. (2009). This set of signatures are (position : composition) 120 : A; 131-231 : C-G; 196 : U; 342-347 : C-G; 444-490 : A-U; 580-761 : U-A; 602-636 : C-G; 670-736 : A-U; 822-878 : G-C; 823-877 : G-C; 826-874 : C-G; 827: U; 843 : C; 950-1231 : U-G; 1047-1210 : G-C; 1109 : C; 1145 : G; 1309-1328 : G-U; 1361 ; G; 1383 : C. The signature information was not used to affiliate newly described genera to the family, i.e., *Devriesea* (Martel et al. 2008) and *Helcobacillus* (Renvoise et al. 2009), and deviations from the published set may have occurred.

The family presently comprises three monospecific genera and *Brachybacterium* with presently 14 species and the family

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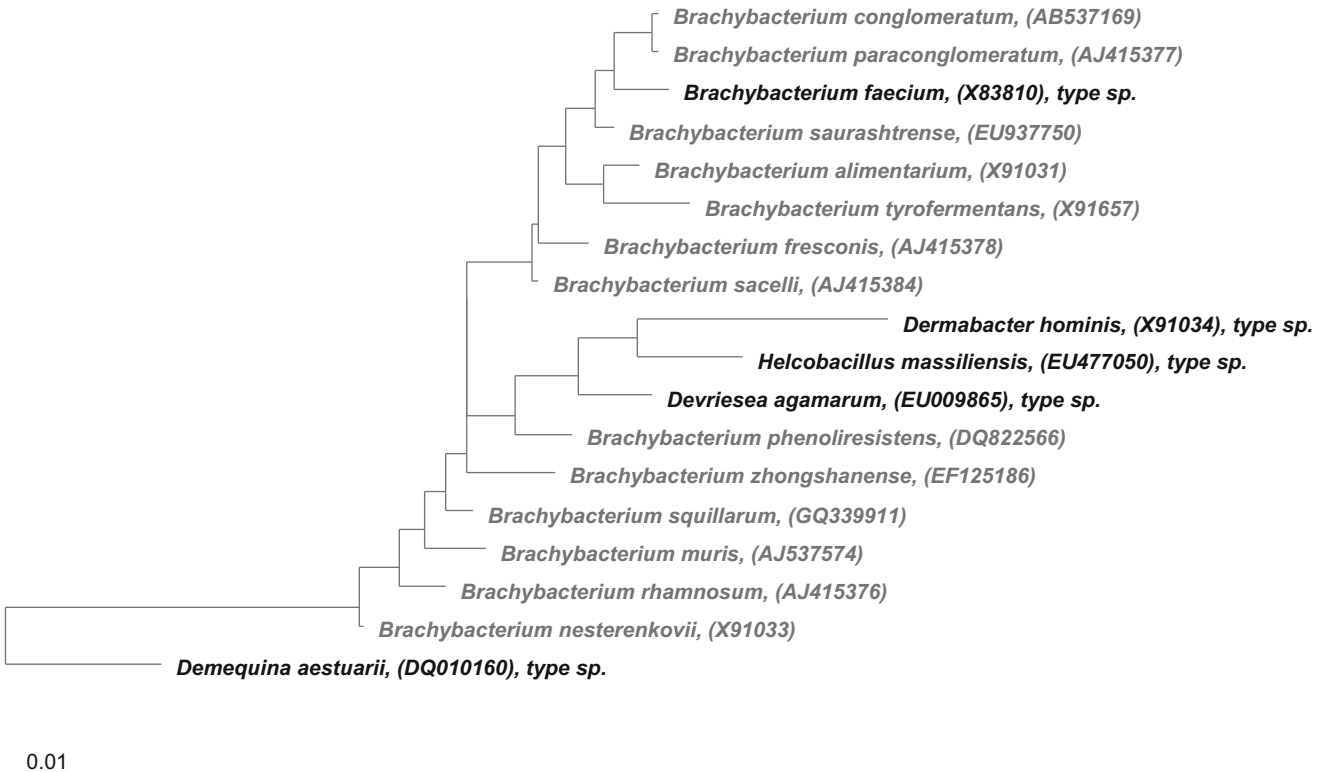

Fig. 14.1
Maximum-likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the family *Dermabacteraceae* present in the LTP_106 (Yarza et al. 2010). The tree was reconstructed by using a subset of sequences. The type strains of *Brachybacterium* used for dendrogram construction are indicated in the heading of *Table 14.2*. Those for the other type strains are: *Devriesea agamarum* IMP2^T, *Dermabacter hominis* DSM 7083^T, *Helcobacillus massiliensis* strain 6401990^T. The *bar* indicates 1 % sequence divergence

appears to be a sister clade of the family of *Jonesiaceae* (Ludwig et al. 2012) within the order *Micrococcales*.

The family *Dermabacteraceae* is well defined by molecular criteria: All genera form a phylogenetic tight cluster irrespective of the algorithms used for treeing calculation (*Fig. 14.1*), with lower 16S rRNA gene similarity values around 95–96 %. The species-rich genus *Brachybacterium* sees several smaller subbranches, but only a few of these clusters are supported by higher than 70 % bootstrap values. The largest of these is defined by *B. faecium*, *B. conglomeratum*, *B. paraconglomeratum*, and the recently described *B. saurashtrense* (Gontia et al. 2011), another one by *B. nesterenkovii*, *B. muris*, *B. squillarum*, and *B. rhamnosum* (although with low bootstrap values; Park et al. 2011a), while the other type strain sequences change their position within the genus limits. While the neighbor-joining tree (not shown), groups all species of *Brachybacterium* in a coherent cluster, the maximum-likelihood tree separates this genus by the lineage containing *Devriesea*, *Helcobacillus* and *Dermabacter* (*Fig. 14.1*).

Morphologically and chemotaxonomically, the genus is also well defined (*Table 14.1*). Cell morphology is often rod shaped in young growth stage, forming coccoid to ovoid cells in older growth phases. V-form arrangement of cells may occur. Cells are nonmotile, lack mycolic acid, and do not form endospores. Strains are aerobic to facultative anaerobic. If investigated, members have a peptidoglycan type (A4γ, according to Schleifer and Kandler 1972), defined by meso-A_2pm in position 3 of the peptide subunit and either glutamic acid (often substituted by glycine) or aspartic acid (see *Table 14.2* for a more revised typing system of Schumann (2011)). Major fatty acids are ai-$C_{17:0}$ and ai-$C_{15:0}$ (ai, anteiso) with variations in some iso-branched and straight chain fatty acids. Except for *Helcobacillus massiliensis*, in which menaquinones could not be detected, the predominant menaquinones are full unsaturated, mainly MK 7, MK8, or MK9. Major phospholipids are phosphatidylglycerol and diphosphatidylglycerol (phosphoethanolamine may occur) as well as unidentified other components (e.g., glycolipids, phospholipids, and other rare lipids). *Dermabacter* and *Devriesea* have a lower DNA G+C content of about 61–63 mol% than *Helcobacillus* and *Brachybacterium* (68–73 mol%). In contrast to the coherent chemotaxonomic properties, the habitat of the organisms enclosed in the family varies widely (see below and *Table 14.2*)

Table 14.1

Morphological, chemotaxonomic, and metabolic properties differentiating the type strains of type species of the family *Dermabacteraceae* (Data for *Dermabacter* were from Jones and Collins (1988), for *Brachybacterium* from Collins et al. (1988) and Takeuchi et al. (1995), for *Helcobacillus* from Renvoise et al. (2009) and for *Devriesea* from Martel et al. (2008))

Properties	*Dermabacter hominis* NCFB 2769^T	*Brachybacterium faecium* NCIB 9860^T	*Helcobacillus massiliensis* 6401990^T	*Devriesea agamarum* IMP2^T
Morphology	Short rods, some with rod-coccus cycle	Rod-coccus cycle	Short rods	Short rods, in pairs or short chains
Relation to oxygen	F	A, F(w)	A, F(w)	A, F
Peptidoglycan type (see *Table 14.4*)	A4γ, A31.3	A4γ, A31.1, A31.2, A31.3	Meso-A_2pm (no further analysis)	A4γ, A31.3
Major fatty acids	ai-$C_{17:0}$, ai-$C_{15:0}$, i-$C_{16:0}$	ai-$C_{17:0}$, ai-$C_{15:0}$, i-$C_{16:0}$ (most strains)	ai-$C_{17:0}$, ai-$C_{15:0}$, i-$C_{16:0}$	ai-$C_{17:0}$, ai-$C_{15:0}$, i-$C_{16:0}$, $C_{16:0}$
Major menaquinones (minor)	MK9, MK8 (MK7)	MK8 (MK7)	Not detected	MK8 (MK7, MK9)
Polar lipids	PG, DPG, (+ uPL, uGL	PG, DPG (+ uGL, uPL)	PG, DPG, PE, PC (+uGL)	PG(+uPL, uGL)
Range of DNA mol% G+C	61–63	68–73	68.6	61
Habitat	Skin sample of healthy human adults, clinical material	Wide range of habitats	Human discharge cutaneous sample from erythrasma	Skin and organ lesion of different lizards
Physiological properties	See *Table 14.4* for type strains			
Catalase	+	+	+	+
Oxidase	–	–	–	–
Gelatin hydrolysis	+	–	+	+
Nitrate reduction	–	+(v)	+	+
Aesculin	+	+	–	+
Hippurate	–	nd	nd	–
Starch	+	+	–	–
Indole production	nd	w	nd	nd
Casein	+	v	nd	nd
H_2S production	v	–	–	nd
Urease	–	v	–	+
Voges–Proskauer	–	–	nd	+
Acid production from				
Amygdalin	+	–	–	–
Cellobiose	+	v	–	–
D-arabinose	–	w	–	–
D-Fucose	–	nd	–	–
D-Galactose	+	–	–	+
D-Ribose	+	+	–	–
D-Xylose	–	–	+	–
Erythritol	–	nd	w	–
Fructose	+	–	+	+
Gentobiose	+	–	–	–
Glycerol	–	–	w	+
Inositol	–	–	+	–
Inositol	–	–	+	–
Lactose	+	–	–	–

Table 14.1 (continued)

Properties	*Dermabacter hominis* NCFB 2769^T	*Brachybacterium faecium* NCIB 9860^T	*Helcobacillus massiliensis* 6401990^T	*Devriesea agamarum* IMP2^T
Lactose	+	–	–	+
L-Arabinose	–	+	+	–
L-arabinose	–	w		–
Mannitol	–	–	+	–
Mannose	+	+	–	+
Melibiose	+	nd	–	+
Melibiose	–	–	–	+
Melizitose	+	w	w	–
Raffinose	+	–	–	+
Trehalose	+	–	w	+
Turanose	+	nd	+	+

Abbreviations: += positive, – negative, *w* weak, *v* variable, *nd* not determined, *A* aerobic, *F* facultative anaerobic, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine
MGDG monogalactosyl diglyceride, *PGL* ninhydrin-positive phosphoglycolipid, *upL* unidentified polar lipid, *iGl* unidentified glycolipid, *uPL* unidentified phospholipid

Table 14.2
Comparison of 16S rRNA gene sequence similarity values (%, lower left triangle) and DNA-DNA similarity values (%, upper right triangle) obtained for type strains of *Brachybacterium*. *1 B. faecium* DSM 48100^T, *2 B. paraconglomaratum* LMG 19861^T, *3 B. conglomeratum* JCM 11608^T, *4 B. saurashtrense* JG 06^T, *5 B. fresconis* LMG 20336^T, *6 B. tyrofermentans* CNRZ 926^T, *7 B. alimentarium* CNRZ 925^T, *8 B. sacelli* LMG 20345^T, *9 B. zhongshanense* JB^T, *10 B. nesterenkovii* DSM9573^T, *11 B. rhamnosum* LMG19848^T, *12 B. muris* C3H-21^T, *13 B. squillarum* M-6-3^T, *14* B. *phenoliresistens* phenol-A^T. Multiple values obtained for the same strain pair are indicated by an oblique(/)

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	x	19	12/19/10	36	<50	36/<11–38	19/<11–38	<50	33	5/7/22	11/28			
2		x	19	36					41	12/21	3/11			
3			x	37			<50			10/13	17/22			
4	98.2	99.2	99.0	x	28	28	28	29				11		
5	98.0				x			46	43				13	
6						x	11–18	<50		19				
7	97.5		97.4	98.2		98.7	x	<50						
8	98.1				98.7	97.9	98.3	x	50					
9		97.1			97.5		97.6	97.6	x	48	46	54		
10					97.6	97.2		98.1	97.7	x				15
11									97.7		x			21
12	96.8	96.8	96.8		97.2	96.8	97.2	97.8	97.9	97.7	97.7	x	14	11
13	97.4	97.5	97.2		97.6	96,9	97.4	97.9	97.5	98.1	98.5	98.3	x	
14	96.5	96.8			96.3	96.3	96.4	97.6		97	96.9	96.3	97.3	x

Molecular Analyses

DNA-DNA Hybridization Studies

Extensive DNA-DNA hybridization studies (DDH) have been performed on strains of individual species as well as among species as the phylogenetic relationships are often around 98 % (*Table 14.2*). A large variety of different hybridization formats were used and data are not always comparable. Nevertheless, the genospecies circumscription definition of Wayne et al. (1987) of about 70 % DDH similarity and the recommendation of Stackebrandt and Goebel (1994) to perform DDH analysis at 16S rRNA gene similarities above 97 % were followed.

The membership of several CDC groups 3 and 5 strains, many of which originated from blood samples, to the species *Dermabacter hominis* (Gruner et al. 1994), was evaluated by the hydroxyapatite method (Brenner et al. 1982) and the affiliation was supported by chemotaxonomic evidence (Funke et al. 1994). For the affiliation of milk strains to the species *B. nesterenkovii* (Gvozdyak et al. 1992), the membrane-filter method was used (Meyer and Schleifer 1978), Schubert et al. (1996) used the nick-translation method (Kelly et al. 1970) to compare novel *Brachybacterium* type strains from cheese surface with other type strains of the genus, the renaturation method of De Ley et al. (1970) was used to differentiate *B. zhongshanense* (Zhang et al. 2007) and *B. saurashtrense* (Gontia et al. 2011) from other *Brachybacterium* type strains, while Park et al. (2011a) used Cy5-labeled DNA probes in a genome-spotted microarray format (Bae et al. 2005) for defining *B. squillarum*. The fluorimetric method of Ezaki et al. (1989) was used in a number of investigations, e.g., *B. conglomeratum, B. paraconglomeratum* and *B. rhamnosum* (Takeuchi et al. 1995), *Brachybacterium* strains from medieval wall paintings (Heyrman et al. 2002), and *B. phenoliresistens* (Chou et al. 2007).

Other Methods

In addition to biochemical profiling (all studies), DDH, and serological investigation (Gvozdyak et al. 1992), a few studies included other molecular methods to display the degree of variations among strains. REP PCR fingerprinting was used to demonstrate the genomic homogeneity among isolates from wall paintings (Heyrman et al. 2002) and despite small differences in these patterns, the authors decided on the basis of highly similar 16S rRNA sequences and high DDH similarities to combine strains with different REP-PCR patterns into the same species. The same method, as well as SDS page electrophoresis, was used by Buczolits et al. (2003) to show the distinctness of *B. muris* DSM 15640^T versus *B. sacelli* DSM 14566^T; *B. nesterenkovii* CCM 2432; *B. rhamnosum* DSM 10240^T; *B. alimentarium* CCM 4520^T; and *B. fresconis* DSM 14564^T. Insertion-sequence PCR fingerprint patterns were generated to discriminate *B. zhongshanense* JB^T, *B. muris* DSM 15640T, and *B. nesterenkovii* LMG 19549^T (Zhang et al. 2007).

Genome Comparison

Two genome sequences of the family have been published recently: one complete sequence of *Brachybacterium faecium* DSM 4810^T (Lapidus et al. 2009, accession number CP001643), a draft genome sequence of *B. squillarum* M-6-3^T (Park et al. 2011b, and shotgun fragments AGBX01000001-AGBX01000008). The genomes differ in several aspects, especially *B. squillarum* lacks annotated genes for defense mechanisms (*B. faecium*: 53), intracellular trafficking and secretion (*B. faecium*: 25), and RNA processing and modification (*B. faecium*: 1). *Table 14.3* gives a comparison of features of the two sequences available, a more complete analysis for *B. faecium* DSM 4810^T has been published (Lapidus et al. 2009).

Table 14.3
Comparison of some features of the genomes of the type strains of *Brachybacterium faecium* and *B. squillarum*

Properties	*B. faecium* DSM 4810^T closed	*B. squillarum* M-6-3^T draft genome
Genome size (bp)	3,614,992	3,191,479
DNA G + C content	72.05 (reported 69.4[a])	72.8 (reported 71.5[b])
rRNA operons	3	2
Protein-coding genes	3,129	2,935
Genes assigned to COGs functional categories	2,371	2,145

[a]Collins et al. (1988), thermal denaturation method
[b]Park et al. (2011a), real-time PCR method

Phenotypic Analyses

Table 14.1 lists the main features of family specific features. The differentiating properties of the species are listed in *Tables 14.1* and *14.4* and only additional salient characteristics will be indicated in the text below. A comparative study suffers from the lack of a coherent set of data evaluated for all type strains. Morphological and cultural properties, as well as other results of commercial test system characterization, are listed in the original species descriptions. Most strains are catalase-positive and all strains are oxidase-negative. If investigated, acid is produced from glucose, maltose, and sucrose (not the type strain of *B. faecium* NCIB 9860^T) but not from adonitol, dulcitol, gluconate, glycogen, inulin, L-arabitol, rhamnose, sorbitol, tatgatose, and xylitol, though some strains can oxidize these compounds. Some diverging properties are recorded in the literature when reactions of previously described type strains were repeated in the characterization of novel type strains.

Brachybacterium Collins et al. 1988, 46^{VP}

Bra.chy.bac.te'ri.um. Gr. adj. *brachy* short; L. neut. dim. n. *bacterium* rod; M.L. neut. n. *Brachybacterium* a small rodlet.

Strains of the type species *Brachybacterium faecium* were originally labeled *Corynebacterium* ssp. and *Arthrobacter* spp, but when included in a numerical taxonomic study (Jones 1975) they grouped with *Listeria monocytogenes*. It was not until a comparative chemotaxonomic study (Collins et al. 1988), including a wide range of coryneform taxa, that the uniqueness of three of the Schefferle strains became apparent which were subsequently described as *Brachybacterium faecium* gen. nov., sp. nov. (Collins et al. 1988). Since then, a large number of new species, well defined by DNA-DNA similarity studies and other molecular methods, as well as by metabolic

Table 14.4

Phenotypic properties distinguishing type strains of *Brachybacterium* type strains. The type strain numbers are as follows: *1 B. faecium* DSM 48100^T, *2 B. paraconglomaratum* LMG 19861^T, *3 B. conglomeratum* JCM 11608^T, *4 B. saurashtrense* JG 06^T, *5 B. fresconis* LMG 20336^T, *6 B. tyrofermentans* CNRZ 926^T, *7 B. alimentarium* CNRZ 925^T, *8 B. sacelli* LMG 20345^T, *9 B. zhongshanense* JB^T, *10 B. nesterenkovii* DSM9573^T, *11 B. rhamnosum* LMG19848^T, *12 B. muris* C3H-21^T, *13 B. squillarum* M-6-3^T, *14* B. *phenoliresistens* phenol-A^T. Data compiled from the respective species descriptions and comparative studies therein. Deviations in recorded results are indicated by a *hyphen*. The value left of the hyphen refers to the original record. All strains contain ai-$C_{15:0}$ as major fatty acids and, if investigated, diphosphatidylglycerol and phosphatidylglycerol as major polar lipids

Properties	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Morphology[a]	C, O to SR	C, O to SR	C, O to SR	C, O	C, O to SR	C, to CSR	C, to CSR	C, O to SR	C, O to SR	C, O to SR	C, O to SR	Co to O	C	C to O
Relation to oxygen[b]	A	F	F	A	A	F	F	A	F	F	F	F	A	F
Peptidoglycan variation[c]	A31.2	A31.2	A31.2	A4γ	A31.2	A31.3	A31.3	A31.2	nd	A31.1	A31.2	A31.3	A31.1	A31.3
Menaquinone	MK7	MK7	MK7	MK7	MK7, MK8	MK7	MK7, MK8	MK7, MK8	nd	MK7	MK7	MK7	MK7	MK7
Predominant fatty acids	ai-$C_{17:0}$, $C_{16:0}$	ai-$C_{17:0}$i, $C_{15:0}$, i-$C_{16:0}$	ai-$C_{17:0}$i $C_{15:0}$, i-$C_{16:0}$	ai-$C_{17:0}$i $C_{15:0}$, i-$C_{16:0}$	ai-$C_{17:0}$, $C_{16:0}$	See Table heading	See Table heading	ai-$C_{17:0}$, $C_{16:0}$	ai-$C_{17:0}$, i-$C_{16:0}$	i-$C_{14:0}$, i-$C_{15:0}$	i-$C_{16:0}$, ai-$C_{17:0}$, i-$C_{14:0}$	ai-$C_{17:0}$ i-$C_{15:0}$, i-$C_{16:0}$, i$C_{14:0}$, $C_{19:0}$	i-$C_{16:0}$, ai-$C_{17:0}$, i-$C_{14:0}$	ai-$C_{17:0}$, i-$C_{16:0}$, i-$C_{14:0}$
Polar lipids[d]	uPL, uGL	uGL, uPGL	uGL, uPGL	MDGD,3 uGL, 3UPl	1 uGL,	nd	nd	1 uGL,	nd	uGL	uGL,	uGL, uP, upL	1uGL, 1uPL	4 uGL, uPl
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Oxidase	–	–	–	–	–	+/–	+/–	–	–	–/+	–	–	–	–
Growth at														
4 °C	+	–	–	–	–	+	+	–	–	–	+	–	–	+
37 °C	+	nd	+	+	(+)	–	–	(+)	+	+	–	+	+	
42 °C	(+)	(+)	(+)	+	–	–	–	–	?	–	–	(+)	–	(+)
pH 5.0	–	–	–	–	+	–	(+)	+	+	(+)	+	–	–	+
pH 10.0	–	+	+	+	+	–	–	–	(+)	–	+	+	–	+
15% NaCl	(+)	(+)	(+)	+	+	+	+	+	–	–	–	–	–	–
Indole formation	(+)	+	(+)	+	–	–	–	–	–	–	–	–	–	
Arginine dihydrolase	nd	nd	nd	nd	–	–	–	–	+	nd	nd	nd	–	–
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	nd	–
Urease production	–	+	+	–	+	–	+	–	+	–	+	–	–	–
H_2S production	–	+	+	–	–	–	–	–	nd	+	+	–	–	–

Hydrolysis of														
Starch	+	+	+	+	–	–	–	–	–	+/–	+	+	nd	+
Aesculin	+	+	+	nd	+	+	+	+	+	+	+	+	nd	+
Gelatin	–	–	–	+	+	+	+	–	+	v	+	nd	+	nd
Cellulose	?	–	?	–	–	nd	nd	–	+	–	–	–	nd	nd
Tween 20	–	–	–	nd	–	–	+	–	n.d	–/+	–	+	–	+
Tween 80	–	–	(+)	+	–	–	–	–	n.d	–	–	(+)	–	–
Acid production from														
D-Arabinose	(+)	–	–	nd	–	–	(+)	–		–	–	+	nd	+
D-Fructose	–	+	–	nd	+	–	–	+	+	+/–	+	+	nd	+
D-Glucose	+/–	+	+	nd	+	+	+	+	+	+	+	+	–	+
D-Galactose	+/–	+	+	nd	+/–	+	+	+	nd	+	+	+	nd	+
L-arabinose	+	+	+	nd	+	+	+	+	nd	+	+	+	nd	
DL-Lactose	–	(+)	–	nd	–/+	–	–	–/+	nd	+	–	+	nd	+
Maltose	(+)	+	+	nd	–/+	–	–	–/+	+	+	+	+	nd	+
D-Mannose	–	+	–	nd	+	+	(+)	(+)	+	v	+	(+)	nd	+
Melibiose	+	nd	+	nd	+	+	+	–		v	+	–	–	–
Raffinose	–	–	–	nd	+	(+)	+	–		(+)	+	–	nd	+
L-Rhamnose	–	–	–	nd	(+)	(+)	+	(+)	+	–	–	+	+	+
D-Ribose	(+)	–	(+)	nd	–	(+(+)	(+)	–		+	(+)	+	nd	–
D-Sorbitol	–	–	–	nd	–	–	–	–	+	–	+	–	–	–
Sucrose	–	+	–	nd	–/+	–/+	–	–/+	+	+	+	+	+	+
D-Xylose	–	–	–	nd	(+)	–	(+)	–		v	(+)	–	nd	+
DNA mol% G+C	69.4	68.6	70.6	73.0	70.4	73.0	73.0	70.3	71.2	71.2	71.5	nd	71.5	70.8

Abbreviations: += positive, – negative, + weak, *v* variable, *nd* not determined

[a]*C* coccoid, *O to SR* ovoid to short rods, *CSR* club-shaped rods

[b]*A* aerobic, *F* facultative anaerobic, *M* microaerophilic

[c]All types refer to A4γ according to Schleifer and Kandler (1972). A more discriminating type system has been published by Schumann (2011), A31.1 (*meso*-Dpm-D-$Glu_{2)}$, A31.2 (*meso*-Dpm-D-Glu_2; α-carboxyl group of D-Glu substituted by Gly), A31.3 (*meso*-Dpm-D-Asp-D-Glu; α-carboxyl group of D-Glu substituted by Gly). See also www. peptidoglycan-types.info

[d]*MGDG* monogalactosyl diglyceride, *PGL* ninhydrin-positive phosphoglycolipid, *upL* unidentified polar lipid, *iGl* unidentified glycolipid, *uPL* unidentified phospholipid

properties, have been described, extending the range of phenotypic and habitat diversity. These are in the order of their description:

B. nesterenkovii strains, isolated from milk products (Gvozdyak et al. 1992), contain large amount of galactose, glucose, and rhamnose on their cells. The presence of rhamnose and the absence of glycine in the peptidoglycan differentiate *B. nesterenkovii* from *B. faecium* and certain other *Brachybacterium* strains (Table 14.4).

The species *B. conglomeratum, B. paraconglomeratum*, and *B. rhamnosum* were described by Takeuchi (1995). All strains contain galactose and glucose and only *B. rhamnosum* contains large amounts of rhamnose in its cell wall. The latter species can be distinguished, among other properties, from *B. nesterenkovii* by a different peptidoglycan composition.

Schubert et al. (1996) described two species from the surface of French cheese, *B. alimentarium* and *B. tyrofermentans*. These two species contain aspartic acid in the peptide subunit of their peptidoglycan (as also seen in *B. phenoliresistens*) and contain an erthrytol teichoic acid that contains the rare component diaminoglucuronic acid as a substituent. However, except for *B. faecium* (Schubert et al. 1996), teichoic acids have not been looked for in other *Brachybacterium* strains. Galactose, galactose and glucose are found as cell wall sugars in *B. tyrofermentans*. The two cheese-derived species differ in the relative proportion of the menaquinones MK-7 and MK-8.

Two novel *Brachybacterium* species, *B. fresconis* and *B. sacelli* (Heyrman et al. 2002), each comprising several strains, were isolated from medieval wall paintings. In contrast to other species of the genus, the polyamine pattern was determined for *B. muris* (Buczolits et al. 2003) as well as for *B. faecium* CCM 4372^T. Like in other members of *Propionibacteriaceae*, it consisted mainly of spermine and spermidine (Busse and Schumann 1999) and minor amounts of putrescine, but brachybacteria can be easily distinguished from propionibacteria on the basis of menaquinone and peptidoglycan composition.

The cellulolytic species *B. zhongshanense* (Zhang et al. 2007), *B. phenoliresistens* (Chou et al. 2007), and *B. squillarum* (Park et al. 2011a) do not display specific features except for *B. squillarum* that was tested as being catalase-negative. The species *B. saurashtrense* (Gontia et al. 2011) is the only species for which nitrogen fixation has been reported. It grows in nitrogen-free medium, produces indol-3-acetic acid and siderophores, and utilizes 1-aminocyclopropane1-carboxylate (ACC) as the sole source of nitrogen and possesses ACC deaminase enzymes. On NFb semisolid medium and on nutrient broth medium, growth was observed at NaCl concentrations of 4% and 15% (w/v), respectively. The type strain possesses monogalactosyl diglyceride as polar lipids, not found in other (Table 14.4).

Dermabacter Jones and Collins 1988, 54^{VL}, Validation List N^o. 28

Der.ma.bac'ter. Gr.n. *derma* skin; M.L. masc.n. *bacter* the masculine equivalent of the Gr.neut.n. *bactrum* a rod.

Strains of *D. hominis* are part of the healthy skin flora of humans but can act as opportunistic pathogens as well, see below and Funke et al. (1994); Gruner et al. (1994). Strains resemble members of *Corynebacterium* and *Brevibacterium* but can be distinguished chemotaxonomically from these by the lack of mycolic acids and dehydrogenated menaquinones, respectively.

Helcobacillus Renvoise et al. 2009, 2349^{VP}

Hel.co.ba.cil'lus. Gr. n. *helkos*, wound; L. masc. n. *bacillus*, rod; N.L. masc. n. *Helcobacillus*, a rod found in wounds.

Chemotaxonomically, *H. massiliensis* is defined by the presence of phosphatidylethanolamine and phosphatidylcholine, absent in other members of the family. Whole cell sugars are predominantly galactose and ribose. It grew between 25 °C and 44 °C, as well as under microaerophilic conditions and in the presence of 5 % CO_2. Anaerobic growth was weak. Non-hemolytic after 24 h, but alpha-hemolytic after 48 h. As compared to its closest phylogenetic neighbor, *D. hominis*, it is characterized by a higher mol% DNA G+C content of almost 7 % and a higher amount of ai-$C_{15:0}$ fatty acids.

Devriesea Martel et al. 2008, 2209^{VP}

De'vrie.se.a. N.L. fem. n. *Devriesea* referring to the veterinary microbiologist L. A. Devriese.

This monospecific genus with *D. agamarum* as the type species is inasmuch unique as it comprises isolates which were exclusively found to be involved in skin infections of agamid lizards (see clinical significance). All strains are phenotypically and metabolically almost identical, and they grew well under aerobic, microaerophilic, and anaerobic conditions on Columbia agar with 5 % sheep blood, with colonies surrounded by a narrow zone of hemolysis.

Isolation, Enrichment, and Maintenance Procedures

Considering the wide range of habitats and the number of research groups involved in the isolation procedures, it is not surprising that strains were isolated and maintained by almost as many methods as there are type strains. Most isolates were recovered from serial dilutions, but some strains were obtained from culture collections without reference of the isolation procedure. As indicated by Jones (1975), the type strain of *Brachybacterium faecium* and other "coryneform" organisms grew on neopeptone (Difco), 2–5 g; yeast extract (Difco), 2.5 g; glucose, 2.5 g; Tween 80 I g; agar 15 g; soil extract, 250 ml; and distilled water to a final volume of 1 litre, pH 6.8. The soil extract was prepared by suspending 500 g air-dried garden soil in 1 l of tap water and autoclaving at 120 °C for 15 min. After sedimentation of the larger particles, the hot supernatant was decanted

and clarified by filtration through paper pulp in a Buchner funnel until the soil solution was clear. Clinical isolates of *Dermacoccus hominis* (Funke et al. 1994), *Helcobacillus massiliensis* (Renvoise et al. 2009) as well as *Devriesea agamarum* (Martel et al. 2008) were grown on Columbia agar with 5 % sheep blood with and without a 5 % CO_2 atmosphere, or, for dermacocci, on heart infusion agar with 5 % rabbit blood (Gruner et al. 1994). Strains of *Dermabacter* were also isolated from blood cultures on pre-enriched *Bartonella* growth medium (Cadenas et al. 2007). Chemotaxonomic and metabolic testing of some *Brachybacterium* type strains (Collins et al. 1988; Takeuchi et al. 1995; Schubert et al. 1996; Heyrman et al. 2002) was done with cells grown in tryptone, trypticase soy agar, and peptone-yeast extract medium for 2 days at 28–30 °C, sometimes supplemented with brain heart infusion. Other *Brachybacterium* strains were cultivated on beef extract agar (Gvozdyak et al. 1992), while marine agar was used for the isolation of *B. phenoliresistens* (Chou et al. 2007) *B. squillarum* (Park et al. 2011a) and for the maintenance of strains from medieval wall paintings (Heyrman et al. 2002). *B. muris* was isolated in *Brucella* broth (Buczolits et al. 2003) and grew best in an anaerobic jar on sheep blood agar and in *Brucella* broth supplemented with 8 % fetal bovine serum in an atmosphere of 90 % N_2, 5 % CO_2, 5 % H_2 and residual oxygen. *B. zhongshanense* (Zhang et al. 2007) was recovered from congo red agar medium (gl^{-1}: KH_2PO_4, 0.5; $MgSO_4$ $7H_2O$, 0.25; cellulose powder, 1.88; gelatin, 2.0 and agar, 16; Hendrick et al. 1995) after 7 days at 30 °C. The isolation of *B. saurashtrense* from roots of *Salicornia brachiata* was done on nitrogen-free semisolid NFb medium (Baldani and Döbereiner 1980).

Most strains grow on DSMZ medium 92, i.e., trypticase soy yeast extract medium (gl^{-1}: trypticase soy broth, 30.0; yeast extract 3.0; agar 15.0; pH 7.0–7.2). A few *Brachybacterium* strains require DSM medium 514, i.e., Bacto Marine broth (DIFCO 2216). *Devriesea* can be grown and maintained on DSM medium 215, i.e., brain heart infusion (Difco).

No difficulties in short- or long-term maintenance have been reported and isolates can be stored at 4 °C as 20 % (v/v) glycerol suspensions. Long-term storage includes lyophilization or in straws under N_2 vapor.

Ecology

As mentioned above, members of the family have been isolated from a wide range of habitats but their function and interactions in the environment are hardly known. *Dermacoccus* and *Helcobacillus* seem to be part of the skin flora but can turn into opportunistic pathogens (the case is not clear for *H. massiliensis*); also *Devriesea agamarum is* responsible for dermatitis and septicemia, but the only reports are from lizards. Due to the higher number of species in *Brachybacterium*, the range of habitats is significantly broader, ranging from soil, sediment, and poultry litter to medieval paints, milk and milk products, corn steep liquor, and the rhizosphere. Besides the original species descriptions coverage of family members in the literature is sparse, concentrating on *Dermabacter hominis* as pathogen (see below; one rare report lists *Dermacoccus* spp. as part of the microflora involved in coffee cherry maturation [Silv et al. 2000]). Reports on *Brachybacterium* ssp. reinforces the widespread occurrence of these organisms, mainly in the marine environment, e.g., associated with a sea urchin (Huang et al. 2009) and a sea anemone (Xiao et al. 2009), as an oil-degrader in the Atlantic ocean (Wang et al. 2010), or as a manganese-oxidizing/-removing strain from a Pacific deep-sea Mn nodule sediment (Wang et al. 2009). Strains of *Brachybacterium* were among the isolates retrieved from aerial spraying of bovine slurry waste (Murayama et al. 2010) and in a freshwater lake sediment (Krett and Palatinszky 2009). *Brachybacterium conglomeratum* was among the most resistant bacteria surviving on copper surfaces (Santo et al. 2010).

BLAST search analysis of 16S rRNA gene sequences similarities slightly expends the spectrum of habitats. Several entries with high BLAST scores between 99 % and 96 % are listed, reinforcing the role of *Dermacoccus* and *Helcobacillus* as pathogens (see below). By far the highest number of sequences highly related to *Dermacoccus hominis* originate from large sequencing studies from human skin (Kong et al. unpublished) and the microflora of the human mucosa-associated ileum (Li et al. unpublished). There are a few closely moderately related non-pathogenetic relatives of *Helcobacillus*: two sequences with 96 % identity which are endophytes from Chinese cabbage and young radish (accession numbers EU373383 and EU373420, respectively; Cho et al. unpublished) and an entry indicating the presence of dermabacters in house dust (Taubel et al. 2009), indoor environment (Rintala et al. 2008), clean rooms (Probst et al. 2010) and urban aerosols (Brodie et al. 2007). No close relatives of *Devriesea* are recorded. A large number of sequences of cultured and uncultured organisms with high BLAST identities (98–99 %) are related to *Brachybacterium* supporting the abundance of members of this genus in a wide range of habitats.

Pathogenicity: Clinical Relevance

The clinical significance of strains related to *Dermabacter hominis* is known since the 1970s when "coryneform" isolates from blood or normally sterile body sites were collected in the Center of Disease Control and Prevention, Atlanta, Ga. as CDC group 3 and group 5. In the 1990s, many of these strains were affiliated to *D. hominis* by polyphasic studies (Funke et al. 1994; Gruner et al. 1994), a taxon that consisted of human cutaneous strains only. Since the description of this species, the literature shows a number of reports which clearly identifies *D. hominis* as an opportunistic pathogen: the finding of *D. hominis* strains in a cerebral (Bavbek et al. 1998) and in a recurrent abscess (Martin et al. 2009), in a peritoneal dialysis-associated peritonitis (Radtke et al. 2001), in blood cultures of patients with chest diseases (Babay and Kambal 2004) in bacteremia (Goméz-Garcés et al. 2001), in fatal septicemia (Lee et al. 2011), and from aseptically obtained human blood (Cadenas et al. 2007).

Antibiotic sensitivity testing (MIC 90 % values) revealed that rifampicin showed excellent activity, while resistance was observed against penicillin and especially chloramphenicol, erythromycin, and clindamycin (Funke et al. 1996; Troxler et al. 2001). Though also listed as being almost resistance to cefuroxime in vitro (Funke et al. 1996; Radtke et al. 2001), the peritonitis patient could be cured by this cephalosporin antibiotic (Radtke et al. 2001).

The clinical significance of *Devriesea agamarum* is restricted to proliferative dermatitis and septicemia in desert lizards of the genera *Uromastyx*, *Pogona*, and *Agama*, notably to cheilitis in Pogona. Koch's postulates were fulfilled using the bearded dragon *P. vitticeps* as a test object (Hellebuyck et al. 2009). Survival of the bacterium on dry surfaces is limited, but it persists and remains viable for up to two months in dermal crust and up to five months in moist sand or distilled water (Hellebuyck et al. 2011).

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15 The Family *Dermacoccaceae*

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Abstract
The family *Dermacoccaceae*, a member of the order *Micrococcales*, comprises eight genera and 14 species. Irrespective of the algorithm used for the generation of 16S rRNA gene sequence trees, *Kytococcus* does not cluster with the other members of the family but shows a closer relationship to members of the family *Intrasporangiaceae*. Chemotaxonomic evidence supports the phylogenetic separateness of the genus *Kytococcus*. *Dermacoccaceae* genera, except *Kytococcus*, will be referred to as authentic members of the family in the following. Neighbor-joining (NJ) and maximum likelihood (ML) dendrograms do not agree in their clustering of the authentic members of *Dermacoccaceae*. While they constitute a coherent cluster in the NJ tree, four lineages emerge from the ML analysis. In this tree, the genera *Serinicoccus* and *Ornithinimicrobium*, though defined by a different, ornithine-based instead of lysine-based peptidoglycan type (Groth et al. Int J Syst Evol Microbiol 51:81–87, 2001; Hana et al. Int J Syst Evol Microbiol 54:1585–1589, 2004), cluster with certain genera of *Dermacoccaceae*.

Taxonomy: Historical and Current

Dermacoccaceae Stackebrandt and Schumann 2000; emend. Zhi et al. 2009; emendRuckmani et al. 2011.

The family contains the type genus *Dermacoccus* (Stackebrandt et al. 1995) and the genera *Demetria* (Groth et al. 1997), *Luteipulveratus* (Ara et al. 2010), *Yimella* (Tang et al. 2010), *Calidifontibacter* (Ruckmani et al. 2011), *Branchiibius* (Sugimoto et al. 2011), *Flexivirga* (Anzai et al. 2011), and *Kytococcus* (Stackebrandt et al. 1995).

In contrast to the emended family description by Ruckmani et al. (2011), the one given here does not include characteristics which are considered genus or species specific and may change readily with more taxa added to the family (e.g., NaCl tolerance, Tween hydrolysis, antibiotic sensitivity and resistance, and sugar oxidation reaction). The main diagnostic properties are indicated in *Table 15.1*.

Gram-positive, mainly coccoid to short rods, sometimes comma shaped or in irregular clusters. Nonmotile and nonencapsulated. Endospores are not formed. Cells rarely longer than 3.0 μm. One species forms a rudimentary aerial mycelium. Aerobic to microaerophilic, most strains are catalase-positive. Chemoorganotrophic. Mycolic acids and teichoic acids are absent. Non-acid fast. The peptidoglycan variation is of the A4α type with lysine at position 3 of the peptide side chain and an interpeptide bridge containing mono- and a dicarboxylic amino acids. The fatty acid composition is variable and includes iso-methyl and anteiso-methyl branched types as well as monounsaturated chain fatty acids. Phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol are present; other polar lipids may occur. The menaquinones are either partially hydrogenated or completely unsaturated (genus *Kytococcus*). When tested, muramic acid is acetylated. The pattern of 16S rRNA signatures will not be shown here as the set defined for the family (Zhi et al. 2009) still included sequences of *Kytococcus* species but lacked the sequences of taxa described after 2009. The DNA G+C content ranges between 66 % and 77 % (T_m, HPLC).

A member of the order *Micrococcales*, class *Actinobacteria*. The type genus is *Dermacoccus*.

Phylogenetic Structure of the Family and Its Genera

Comparative analysis of the 16S rRNA gene sequences of *Micrococcus* species led to the dissection of the genus, leaving only *M. luteus* and *M. lylae* as authentic members of the genus (Stackebrandt et al. 1995). Though morphologically resembling authentic *Micrococcus* species, the phylogenetic distance of the other species was so large and the chemotaxonomic differences so profound that it was justified to reclassify, among others, *M. nishinomiyaensis* as a member of *Dermacoccus* and *M. sedentarius* as a member of *Kytococcus*.

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Table 15.1

Properties that differentiate genera of the family *Dermacoccaceae*. Data were taken from the original taxon descriptions

Properties	*Dermacoccus*	*Luteipulveratus*	*Demetria*	*Branchiibius*	*Caldifontibacter*	*Yimella*	*Flexivirga*	*Kytococcus*
Morphology	Coccoid	Coccoid to rod-shaped, rudimentary short aerial mycelium-like formation	Irregular coccoid to rod-shaped	Coccoid	Short rods	Coccoid	Irregular coccoid to comma shaped	Coccoid, in pairs, tetrads, or cubical packets
Major fatty acids	iso-$C_{16:0}$, iso-$C_{16:1}$, iso-$C_{17:1}$, iso-$C_{17:0}$ [a]	iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{16:1}$, $C_{17:1}$ ω9c, $C_{17:0}$ 10-methyl [b]	$C_{18:1}$, iso-$C_{16:0}$, $C_{17:1}$, $C_{16:0}$ [a]	iso-$C_{16:0}$, $C_{16:0}$, $C_{17:1}$, $C_{17:0}$, $C_{18:1}$, $C_{19:1}$ [c] or iso-$C_{16:0}$, $C_{18:0}$ br [d]	iso-$C_{16:0}$, iso-$C_{16:1}$, anteiso-$C_{17:0}$, iso-$C_{17:1}$ [a]	iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ [e]	iso-$C_{16:0}$, iso-$C_{16:1}$ [f]	iso-$C_{17:1}$, anteiso-$C_{17:0}$, iso-$C_{17:0}$ [a]
Major menaquinone	MK-8(H_2)	MK-8(H_4), MK-8(H_6)	MK-8(H_4)	MK-8(H_2), MK-8(H_4) [c] or MK-8(H_6), MK-8(H_4) [d]	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-7, MK-8; MK-6, MK-9, MK-10 may be present
Polar lipids	PI, PG, DPG	PI, PG, DPG	PI, PG, DPG, PE, uPL	PI, PG, DPG, uPL	PG, PI, DPG, PIM, PS, uPL, uL	DPG, PI, GlcN-PL, uPL	PG	PI, PG, DPG; PS, PIM; uPL and uGL may be present
Major whole-cell sugars	Gal	Gal, Glc, Man, Rha, Rib	Gal	Ara, Gal, Glc, Man, Rha, Rib	nd	Fuc, Gal	Gal, Rib, Glc, Rha, Man	nd
Peptidoglycan type	L-Lys-L-$Ser_{1,2}$-D-Glu or L-Lys-L-$Ser_{1,2}$-L-Ala-D-Glu	Lys-Ser-Asp [g]	L-Lys-L-Ser-D-Asp	L-Lys-Gly-Ser_2-D-Glu [c, g] or L-Lys-D-Ser-L-Glu [d] with L-Ser at position 1 of the peptide subunit	L-Lys-Gly-L-Ser-D-Asp	L-Lys-L-Ser-D-Asp	L-Lys-Gly-L-Ser_2-D-Glu [g]	Lys-D-Glu_2
DNA G+C content	66–71	68	66	68	77	66	67	68-69

Abbreviations: $C_{18:0}$ *br* unknown saturated branched-chain fatty acid with 18 carbon atoms (Tomida et al. 2011, supplementary table), *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *DGP* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PS* phosphatidylserine, *uPL* unknown phospholipids, *uGL* unknown glycolipids, *uL* unknown lipids, *GlcN-PL* glucosamine-containing phospholipid, *Ara* arabinose, *Rha* rhamnose, *Fuc* fucose, *Rib* ribose, *Man* mannose, *Gal* galactose, *Glc* glucose

[a]Data from Ruckmani et al. (2011)

[b]Data from Ara et al. (2010)

[c]Data from Sugimoto et al. (2011)

[d]Data from Tomida et al. (2011)

[e]Data from Tang et al. (2010)

[f]Data from Anzai et al. (2011)

[g]Concluded from the amino acid composition indicated by the authors of the respective publication

When the family *Dermacoccaceae* (Stackebrandt and Schumann 2000) was described, it was placed in the suborder *Micrococcineae* (Stackebrandt et al. 1997), originally established on the basis of 16S rRNA gene sequence signatures. This suborder has recently been elevated to the order level (Ludwig et al. 2012). The genera *Dermacoccus* and *Kytococcus*, described as members of the family *Dermatophilaceae* (Austwick 1958[AL], emend. Stackebrandt et al. 1997), as well as the genus *Demetria* (Groth et al. 1997) were transferred into *Dermacoccaceae* when the topology of the enlarged 16S rRNA gene tree required readjustment of taxa above the genus level. As the overall degree of sequence similarity shared between type strains of genera of *Micrococcineae* is high and the inclusion of novel type strains frequent, the topologies of phylogenetic trees are prone to the number and selection of sequences of the order and of outside reference organisms. Also, in none of the 16S rRNA trees displaying the position of novel species, all type strains of the order are included but only subsets of sequences, subjectively based upon sequence similarity values and systematic consideration. More recently, the membership of *Kytococcus* is under discussion. The LTP 16S rRNA tree (Yarza et al. 2010) clearly sees *Kytococcus* species branching outside the authentic members of *Dermacoccaceae*, grouping with some genera of the paraphyletic family *Intrasporangiaceae* (*Fig. 15.1*). The argument to retain *Kytococcus* within the family *Dermacoccaceae* in the last edition of *Bergey's Manual of Systematic Bacteriology* (Ludwig et al. 2012) is that some chemotaxonomic properties (menaquinone composition and peptidoglycan type), which are unique for *Kytocoocus*, are absent from the other authentic members as well as from members of the family *Intrasporangiaceae* (Kageyama et al. 2008). The logic conclusion would be the description of a family of its own, *Kytococcaceae*, but in the absence of a formal description, we will also discuss properties of *Kytococcus* and its species within the scope of this chapter.

In addition to the three genera of the family described between 1995 and 1997, several monospecific genera were added to the family since 2010: *Luteipulveratus* (Ara et al. 2010), *Yimella* (Tang et al. 2010), *Branchiibius* (Sugimoto et al. 2011), *Flexivirga* (Anzai et al. 2011), and *Caldifontibacter* (Ruckmani et al. 2011). Interestingly, all trees published in the original description to depict the position of the respective new type strain included members of *Kytococcus* and in each case, members of this genus branched either within the radiation of *Dermacoccaceae* type strains or as their nearest neighbor. According to the phylogenetic branching of actinobacterial type strains in the RaxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010) (*Fig. 15.1*), the family is not a coherent taxon but its members are found in four different lineages. One major cluster contains all genera but *Kytococcus* which is a neighbor of *Marihabitans asiaticum* and more closely related to *Intrasporangiaceae* (which do not appear as a coherent family, either). The second group contains members of *Dermacoccus*, while *Calidifontibacter, Yimella, Branchiibius* (the species *B. cervicis* has not been included because of its recent description), *Flexivirga*, and *Luteipulveratus* define the third group. *Demetria terragena* branches between the *Serinicoccus*/*Ornithinimicrobium* group and species of group three. This situation is not seen in the neighbor-joining (NJ) tree (not shown), in which the *Serinicoccus*/*Ornithinimicrobium* lineage and the *Dermacoccus* cluster are sister clades. The NJ tree, too, sees *Kytococcus* species clustering apart from authentic dermacocci but with *Marihabitans asiaticum*. Chemotaxonomically, *Serinicoccus* (Yi et al. 2004) and *Ornithinimicrobium* (Groth et al. 2001) species contain ornithine as the diagnostic amino acid of the peptidoglycan belonging to the A4β or to a new type. Only *Serinicoccus chungangensis* (Traiwan et al. 2011) has been reported to contain *meso*-diaminopimelic acid, otherwise not found in any member of *Serinicoccus*, *Ornithinimicrobium*, or *Dermacoccaceae*. With respect to the composition of polar lipids, menaquinones and major fatty acids members of all these taxa (except for *Kytococcus*) are similar.

Molecular Analyses

DNA-DNA Hybridization Studies

DNA reassociation studies (DDH), mostly done with the microplate method of Ezaki et al. (1989), have only been performed between type strains of the genera *Dermacoccus* and *Kytococcus* (Pathom-aree et al. 2006a, b; Becker et al. 2003; Kämpfer et al. 2009). The phylogenetic distance among the monospecific genera and among these and type strains of *Dermacoccus* and *Kytococus* was below 96 % 16S rRNA gene sequence similarity, making it unnecessary to perform hybridization studies (Stackebrandt and Goebel 1994). The DDH values obtained for the pairwise analysis of the four closely related *Dermacoccus* species (above 98.5 % 16S rRNA gene sequence similarity) ranged at most between 7 % and 25 % DNA similarity; even the genomes of type strains of *D. barathri* and *D. profundi*, sharing 99.9 % 16S rRNA gene sequence similarity, did not hybridized higher than 25 % (Pathom-aree et al. 2006b). The genomes of the three *Kytococcus* type strains, sharing gene sequence similarities between 97.9 % and 98.6 %, were less than 46 % similar by DDH analysis (Becker et al. 2003; Kämpfer et al. 2009). The two type strains of *Branchiibius* (99.6 % sequence similarity) share <35 % DDH similarity (Tomida et al. 2011). All values were clearly below the threshold values for the delineation of genospecies (Wayne et al. 1987).

RiboPrinting

Though *Pvu* II is generally suited for generating multiband RiboPrint patterns of members of the order *Micrococcales*, only few members of the family *Dermacoccaceae* could be cut successfully with this restriction enzyme (*Fig. 15.2*). The patterns of the type strains of *Dermacoccus abyssi* and *Dermacoccus profundi* are highly similar and agree in major

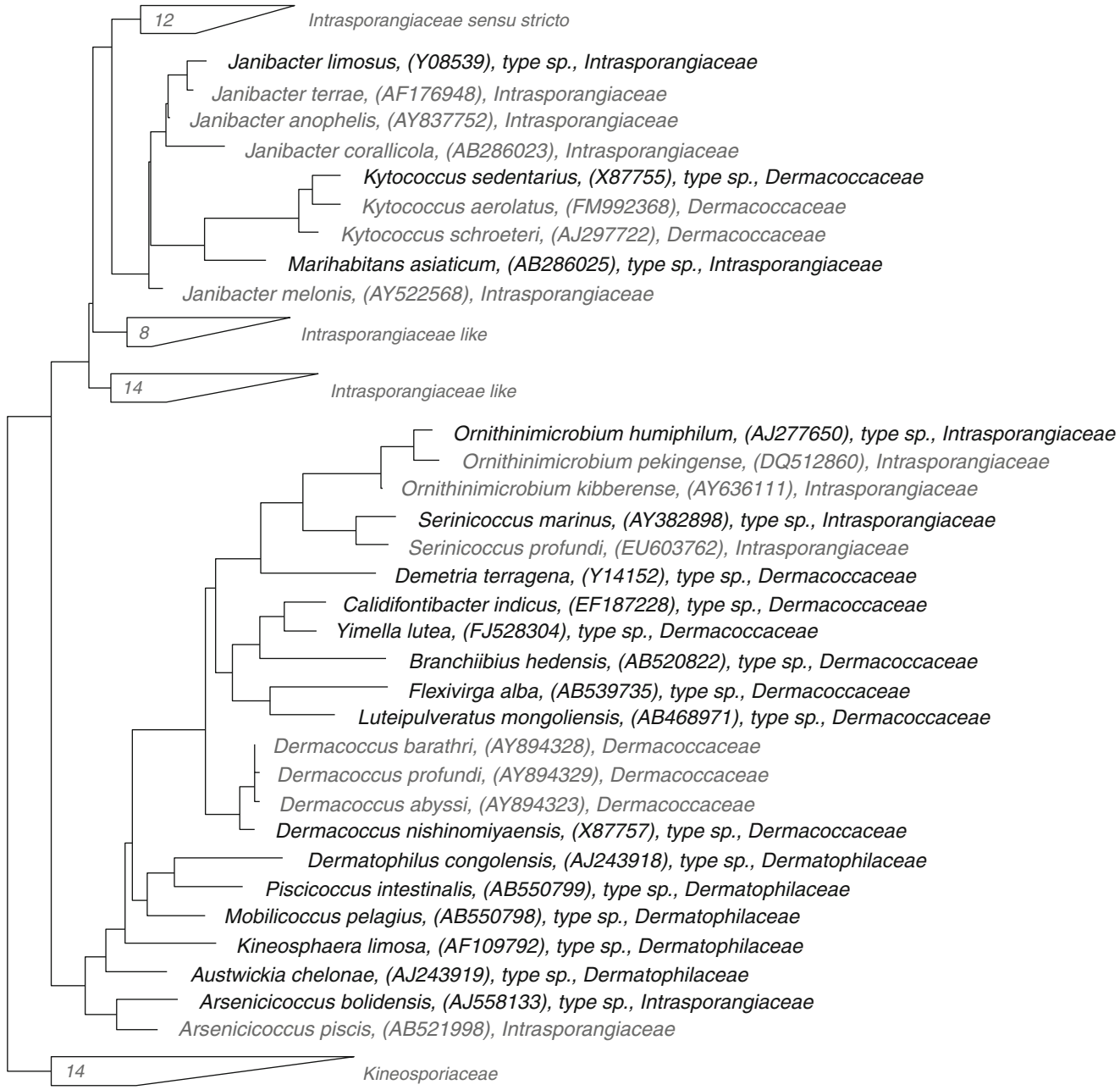

Fig. 15.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the family *Dermacoccaceae* and some neighboring taxa present in the LTP_106 (Yarza et al. 2010). The tree was reconstructed by using a subset of sequences. List of type strains used for dendrogram construction: *Dermacoccus nishinomiyaensis* DSM 20448^T, *D. abysii* MT1.1^T, *D. barathi* MT2.1^T, *D. profundi* MT2.2^T, *Demetria terragena* HKI 0089^T, *Luteipulveratus mongoliensis* MN07-A0370^T, *Flexivirga alba* ST13^T, *Branchiibius hedensis* Mer 29717^T, *Calidifontibacter indicus* PC IW02^T, YIM 45900^T, *Kytocoocus sedentarius* DSM 20547 T, *K. schroeteri* Muenster 2000, *K. aerolatus* 02-St-019/1^T

bands with the pattern of *Dermacoccus barathri* DSM 17574^T. This finding confirms the high relationship of these three *Dermacoccus* type strains as detected by 16S rRNA gene sequence comparison and MALDI-TOF mass spectrometry (See *Figs. 15.1* and *15.3*).

MALDI-TOF

The dissimilarity of whole-cell MALDI TOF mass spectra of almost all type strains indicates that this method is appropriate for identification of species of this family. Only the mass spectra

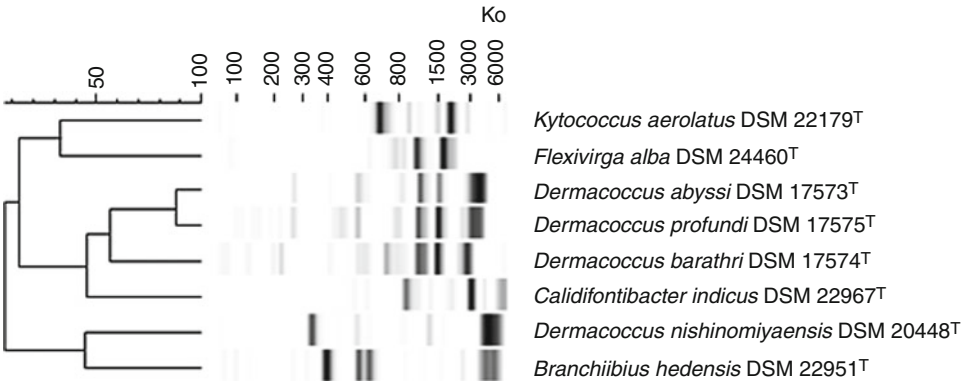

Fig. 15.2

RiboPrint patterns of those type strains of the family *Dermacoccaceae* which are cut by *Pvu*II. The dendrogram has been generated with the BioNumerics software (Applied Math, Kortrijk, Belgium)

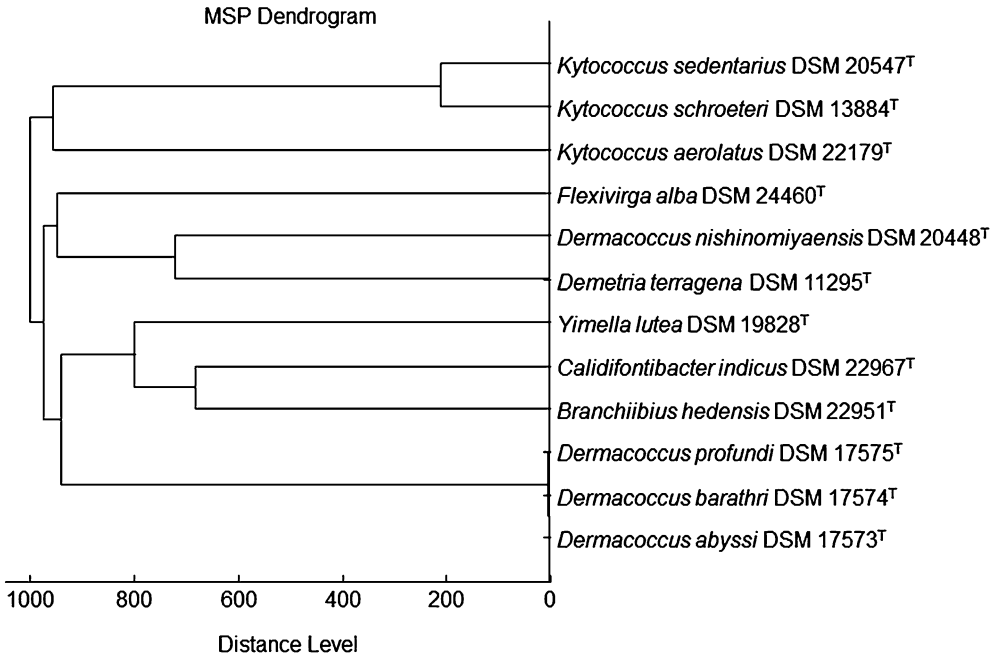

Fig. 15.3

Score-oriented dendrogram generated by the BioTyper software (version 2.0, Bruker Daltonics) showing the similarity of MALDI-TOF mass spectra of cell extracts of selected type strains of the family *Dermacoccaceae*

of the type strains of *Dermacoccus abyssi, Dermacoccus barathri*, and *Dermacoccus profundi* are almost identical (Fig. 15.2). While *Kytococcus* type strains cluster together, apart from other members of the family, *Dermacoccus nishinomiyaensis* DSM 20448^T groups with *Demetria terragena* and not with other members of the genus.

Genome Analysis

Presently the only available complete genome sequence of a member of *Dermacoccaceae* is that of *Kytococcus sedentarius* DSM 20547^T (accession number CP001686) (Sims et al. 2009), selected by the Genomic Encyclopedia of Bacteria and Archaea (GEBA) project for its production of oligoketide antibiotics as well as for its role as an opportunistic pathogen (see below). The single replicon genome is 2,785,024-bp long, annotated to contain 2,639 protein-coding, among these 1,948 with function, 1,851 of which were assigned to COGs: 64 RNA genes and only two rRNA operons are present. The mol% G+C of DNA is 71.63 % GC content which is slightly higher than that determined by the thermal denaturation method (Kloos et al. 1974). In accordance with the report Pospišil et al. (1998) on the production of polyether antibiotics (monensins), the genome contains genes involved in the synthesis of antibiotics, such as polyketide cyclases, putative exporters of

polyketide antibiotics, genes involved in polyketide biosynthesis, antibiotic biosynthesis monooxygenes, and ABC-type bacteriocin/antibiotic exporter with N-terminal double-glycine peptidase domain. Additional information can be retrieved from the JGI/DOE webpage http://img.jgi.doe.gov/cgi-bin/geba/main.cgi?section=Taxon-Detail&taxon_oid=644736380

Phenotypic Analyses

The main features of members of *Dermacoccaceae* are listed in *Tables 15.1* and *15.2*. Most of the phenotypic information is restricted to the type strain of a species. It is thus questionable whether these reactions are indeed species specific. Though membership of genera to the family has been acknowledged in the descriptions of new genera, a broad spectrum of neighboring type strains was included in hardly any of the recent descriptions, except for *Caldifontibacter indicus* (Ruckmani et al. 2011). The use of different approaches to analyze metabolic properties, except for API Zyme reactions, and the failure to perform tests used before for the characterization of other type strains results not only in the presence of many undetermined (or unrecorded) properties but also in a high number of conflicting reactions (See *Table 15.2* for some prominent examples). The recommendation of an ad-hoc committee for the reevaluation of the species definition in bacteriology (Stackebrandt et al. 2002) to obtain diagnostic or differentiating properties by comparable methods applied to reference strains of closely related taxa was in most cases ignored, making it almost impossible to find family-specific metabolic traits. In contrast, chemotaxonomic properties are evaluated by more standardized methods and chemical constituents are less subject to subjective interpretation. The exception is the fatty acid composition, especially their minor components, which differ among all studies due to differences in the growth conditions of reference material and to different designation of gas chromatographic peaks with IUPAC or trivial names. As the listing of the range of minor fatty acid components obtained in individual species descriptions is outside the scope of the chapter, the indication of fatty acids is restricted to major components as listed in *Table 15.1*. Additional data on colony morphology and colors, production of soluble pigments, acidification of growth media, antibiotic resistance and susceptibility as well as utilization of substrates for growth, acid formation, and enzymatic activities are given in the respective original descriptions and are not listed in detail.

Dermacoccus Stackebrandt et al. 1995, 689^{VP}, emend Ruckmani et al. 2011.

Der.ma.coc´cus. N.L. n. *derma*, skin: Gr. n. *coccus*, a grain, berry: M.L. masc. n. *Dermacoccus*, coccus living on skin).

In addition to properties listed in *Tables 15.1* and *15.2*, cells are nonhalophilic and mesophilic. The cytochromes are cytochromes *aa*3, *c*549, *c*555, *b*559, *b*564, and *d*626 (one type strain analyzed; Faller et al. 1980). Iso-branched saturated and monounsaturated fatty acids with 16 and 17 carbon acids dominate the cellular fatty acid profile. If present, the long-chain aliphatic hydrocarbons are C22 and C23 hydrocarbons, as well as minor amounts of C25, C26, and C27 hydrocarbons (Kloos et al. 1974).

Metabolic properties of diagnostic value of type strains of the four species are indicated in *Table 15.2*. The most extensive study on *D. nishinomiyaensis* isolates have been performed by Kloos et al. (1974), Kocur et al. (1975). D. *abysii* MT1-1^T grows well at 40 Mpa and can be considered a piezotolerant actinobacterium. At this pressure, strains of *D. nishinomiyaensis* showed a decrease in cell numbers (Pathom-aree et al. 2006a); information of the other two type strains are not available (Pathom-aree et al. 2006b). All strains grow on complex organic media but poorly only on inorganic nitrogen agar. Most auxotrophic *D. nishinomiyaensis* strains required cysteine or methionine and niacin, and were further stimulated by tryptophan, valine, aspartic acid, glutamic acid, proline, and lysine (Kloos et al. 1974). If indicated, growth occurs at 10 °C and 37 °C with an optimum of 28 °C.

The type species is *Dermacoccus nishinomiyaensis*, the type strain is DSM 20448^T, ATCC 29093^T.

Demetria Groth et al. 1997, 1132^{VP}

De.me.tria Gr. M. n. Demeter, Greek female god of agriculture and wives; -ia, M.L. fem., *Dernetria*, a bacterium being responsible for fertility.

This genus has been described on the basis of the phylogenetic distance to neighboring taxa, the presence of aspartic acid instead of glutamic acid in the peptidoglycan and menaquione MK-8(H_4) instead of MK-8(H_2), found in the most closely related taxon (at the time of the original description), *D. nishinomiyaensis*. This taxon is the only one in the family for which phosphatidylethanolamine (PE) has been reported. Metabolic differences, obvious at the time of its description, became blurred with more descriptions of *Dermacoccaceae* species being published.

The type species is *Demetria terragena*, the type strain is HKI 0089^T.

Luteipulveratus Ara et al. 2010, 578^{VP}

Lu.te.i.pul.ve.ra'tus. L. adj. *luteus* yellow; L. part. adj. *pulveratus* scattered with dust; N.L. masc. n. *Luteipulveratus* a bacterium forming white powdery aerial mycelium on yellow colonies.

This taxon is the only one within the family that produces an abundant grayish white, powdery, and sterile aerial mycelium-like structure on ISP media 3, 5, and 7, even a rudimentary aerial mycelium on ISP medium 4. Metabolic properties fall into the range of those reported for other members of the family (*Table 15.2*).

The type species is *Luteipulveratus mongolensis*, the type strain is MN07-A0370^T.

Yimella Tang et al. 2010, 662^{VP}

Yi.mel'la. L. fem. dim. suff. -ella; N.L. fem. dim. n. *Yimella* arbitrary name formed from the acronym of Yunnan Institute of Microbiology, YIM, where the first taxonomic studies of this taxon were performed.

This taxon differs from other members by a number of chemotaxonomic features, such as the presence of anteiso-C17:0 fatty acid, glycine bound to the α-carboxyl group of

Table 15.2

Properties that differentiate species of the *Dermacoccaceae* genera. Data were taken from the original type strain descriptions and from comprehensive comparative studies including previously described type strains

Properties	*D. nishinomiyaensis* (several strains)	*D. abyssi* DSM 17573^T	*D. barathri* MT2.1^T	*D. profundi* MT2.2^T	*Luteipulveratus mongoliensis* MN07-A0370^T	*Demetria terragena* HKI 0089^T	*Yimella lutea* YIM 45900^T
Cell size (μm)	Diameter 0.9–1.6	Diameter 0.8–1.5	Diameter 0.8–1.5	Diameter 0.8–1.5	0.5 × 1.2	0.8 × 1.2 or 08 × 3.0	0.7–0.9 × 1.3–1.8
Colony color	Bright orange	Pale yellow	Light yellow	Brilliant yellow	Pale to bright yellow	White to pale yellow	orange
Catalase	+	+	+	+	+	+	+
Oxidase	+	−	+	−	nd	−/+[a]	−
Degradation/decomposition of							
Arbutin	−	+	−	−	+	nd	nd
Casein	−	+	+	+	+	+	−
Cellulose	nd	+	nd	nd	nd	nd	+
DNA	−/+[b]	+/−[c]	+/−[c]	−	nd	−	−
Gelatin	+	−	−/+[c]	−	+	−	+
Starch	+	+/−[c]	−	−	+	+/−[a]	−
Tween 80	−	+	−	−	−	+	+
Urea	v	−	+/−[c]	+/−[c]	+	−/+[a]	−
Hypoxanthine	nd	+	+	+	+	−	nd
Production of							
Acetoin	−/+[b]	−	−	−	nd	−	−
Indole	−	nd	nd	nd	nd	−	−
H_2S	−	−	−	+	+	nd	−
Utilization of							
Sucrose	−	+	−	−	nd	−	nd
Lactose	−	nd	nd	nd	nd	nd	nd
Galactose	−	+	−	−	nd	−	nd
Rhamnose	−	−	−	−	nd	+	nd
Xylose	−	−	+	+	nd	+	nd
Glycerol	v	+	−	−	nd	+	nd
Mannitol	−	nd	nd	nd	nd	nd	nd
Glucose	v	+	−	−	nd	+	+
Galactose	v	nd	nd	nd	nd	nd	−
Acid production from							
Adonitol	−	−/+[d]	−	−	nd	−	−
D-Arabinose	−	−	−	−	nd	nd	−
D-Arabitol	−	−	−	−	nd	−	−
L-Arabitol	−	−	−	−	nd	−	−
D-Cellobiose	−	−	+	−	nd	−	−
Dextran	−	−	−	−	nd	nd	nd
Dextrin	−	−	nd	nd	nd	−	nd
Meso-erythritol	−	−	−	−	nd	nd	+
D-Fructose	−	−	+	−	−	+	−/+
D-Galactose	−	−	+	−	nd	+	+
D-Glucose	+/−[c]	−	+	−	+	−	+
D-Glycerol	−	−	−	−	nd	+	−

Table 15.2 (continued)

Properties	*D. nishinomiyaensis* (several strains)	*D. abyssi* DSM 17573^T	*D. barathri* MT2.1^T	*D. profundi* MT2.2^T	*Luteipulveratus mongoliensis* MN07-A0370^T	*Demetria terragena* HKI 0089^T	*Yimella lutea* YIM 45900^T
Glycogen	−	−	−	−	−	nd	−
myo-inositol	−	−	−	−	nd	nd	−
Inulin	+/−[c]	−	−	−	nd	+	
Lactose	−	−	+	−		−	+
D-Maltose	+/−[c]	+/−[c]	+	+	+	−	−/+
D-Mannitol	−	+	+	−	−	+/−[a]	+/−
D-Mannose	−	−	−	−	+	+/−[a]	−/+
D-Melizitose	−	−	−	−	nd	nd	−
D-Melibiose	−	−	−	−	nd	−	−
D-Raffinose	−	−	−	−	nd	−	−
D-Rhamnose	−	−	−	+	nd	−	−
D-Salicin	−	−	−	−	nd	−	+
Sucrose	−	−	−	−	+	+′	−/+
D-Sorbitol	+/−[c]	+/−[c]	−	−	nd	−.	+
D-Trehalose	−	−	−	+	nd	−	−
L-Sorbose	−	−	−	−	nd	nd	−
Xylitol	−	−	−	−	nd	nd	−
D-Xylose	−	+/−[c]	−	−	−	−	−
API Zym							
α-Fucosidase	+	+	+	−	w	−	−
β-Glucosidase	+	+	+	−	−	−	−
Lipase (C14)	+	+	+	−	+/−	w	+/−
Trypsin	+	−	+	+	+/−	w	+
Nitrate reduction	v	−	−	−	−	−	−
Growth on							
5 % NaCl	+	+/	+/−[c]	+	−	+	+
10 % NaCl	−	+/−[c]	+/−[c]	+	−	+	−
12.5 % NaCl	−	−	−	+	−	w	−

Properties	*Branchiibius hedensis* Mer 29717^T	*Branchiibius cervicis* PAGU 1247^T	*Caldifontibacter indicus* PC IW02^T	*Flexivirga alba* ST13^T	*Kytococcus sedentarius* DSM 20547^T	*Kytococcus schroeteri* Muenster 2000^T	*Kytococcus aerolatus* 02-St-019/1^T
Cell size (μm)	0.7–0.9		1.0–2.0	0.6–1.2	0.8–1.1	1.1–1.5	1.3
Colony color	Pale yellow	White to pale yellow	Creamish-white to yellow	white	Creamish-white to buttercup yellow	yellow	beige
Catalase	nd	+	+	−	+	+	+
Oxidase	nd	nd	+	−	−/+[b]	−	+
Degradation/decomposition of							
Arbutin	nd	nd		nd			
Casein	nd	nd	+	−	+	+	+
Cellulose	nd	nd	nd	nd	nd	nd	nd
DNA	nd	nd	−	nd	−	−	−
Gelatin	+	nd	+	nd	+/−[e]	+	−
Starch	w	nd	−	nd	−/+[e]	−	−
Tween 80	nd	nd	+	nd	−	+	nd

Table 15.2 (continued)

Properties	*Branchiibius hedensis* Mer 29717^T	*Branchiibius cervicis* PAGU 1247^T	*Caldifontibacter indicus* PC IW02^T	*Flexivirga alba* ST13^T	*Kytococcus sedentarius* DSM 20547^T	*Kytococcus schroeteri* Muenster 2000^T	*Kytococcus aerolatus* 02-St-019/1^T
Urea	nd	+	–	nd	–	–	–
Hypoxanthine	nd	nd	nd	+	nd	nd	nd
Production of							
Acetoin	nd	nd	–	nd	–	–	–
Indole	nd	nd	nd	nd	nd	nd	nd
H_2S	–	nd	–	nd	nd	nd	nd
Utilization of							
Sucrose	nd	nd	–	nd	+/–[d]	+/–[d]	–
Lactose	nd	nd	nd	nd	nd	nd	nd
Galactose	nd	nd	+	nd	–	–	–
Rhamnose	nd	nd	–	nd	–	–	–
Xylose	nd	nd	+	nd	+	–	+
Glycerol	nd	nd	–	nd	–	–	–
Mannitol	+	nd	nd	nd	–	–	–
Glucose	+	nd	–	nd	+/–[d]	+/–[d]	–
Acid production from							
Adonitol	nd	nd	–	nd	–	–	–/+[d]
D-arabinose	nd	nd	nd	w	nd	nd	nd
D-arabitol	nd	nd	nd	w	–	–	–
L-arabitol	nd	nd	nd	w	nd	nd	nd
D-cellobiose	nd	–	–	w	–	–	–
Dextran	nd	nd	nd	nd	nd	nd	nd
Dextrin	nd	nd	nd	nd	nd	nd	nd
meso-Erythritol	nd	nd	nd	nd	–	–/+[e]	–
D-fructose	+	+	+	v	v	+	+
D-galactose	nd	nd	+	nd	–	–	–
D-glucose	+	+	+	w	–/+[b, d]	–	–/+[d]
D-glycerol	nd	nd	nd	w	–	nd–	nd–
Glycogen	nd	nd	nd	nd	nd	nd	nd
myo-Inositol	nd	nd	nd	nd	–	–	–
Inulin	nd	nd	+	nd	–	–	–
Lactose	nd	nd	+	nd	–	–	–
D-maltose	+	–	+	nd	–/+[b, d]	–	–
D-mannitol	–	–	+	w	–	–/+[d]	–/+[d]
D-mannose	+	–	+	nd	–	–	–
D-melizitose	nd	nd	nd	nd	nd	nd	nd
D-melibiose	nd	nd	–	nd	–/+[b, d]	–/+[d]	–
D-raffinose	nd	–	+	nd	–	–	–
D-rhamnose	nd	nd	–	w	–	–	–
D-salicin	nd	nd	–	nd	–/+[d]	–	–/+[d]
Sucrose	+	–	+	w	–	–	–/+[d]
D-sorbitol	nd	nd	–	nd	–	–/+[d]	–
D-trehalose	nd	–	+	w	–/+[d]	–/+[d]	–
L-sorbose	nd	nd	nd	nd	nd	nd	nd

Table 15.2 (continued)

Properties	*Branchiibius hedensis* Mer 29717^T	*Branchiibius cervicis* PAGU 1247^T	*Caldifontibacter indicus* PC IW02^T	*Flexivirga alba* ST13^T	*Kytococcus sedentarius* DSM 20547^T	*Kytococcus schroeteri* Muenster 2000^T	*Kytococcus aerolatus* 02-St-019/1^T
Xylitol	nd	nd	nd	nd	nd	nd	nd
D-xylose	nd	nd	–	w	–/+[b, d]	–/+[d, e]	–
API Zym							
α-Fucosidase	nd	nd	–	w	nd	nd	nd
β-Glucosidase	+	+	–	–	nd	nd	nd
Lipase (C14)	–	+	+	–	nd	nd	nd
Trypsin	+	w	–	+	nd	nd	nd
Nitrate reduction	+	+	+	+	+	–	–
Growth on							
5 % NaCl	+	nd	–	+	+	+/–[d]	+
10 % NaCl	+	nd	–	–	+/–[b]	+/–[e]	+/–[e]
12.5 % NaCl	–	nd	–	–	–	w	–

Abbreviations: + positive, – negative, *v* variable, *w* weak, *nd* not determined
Several conflicting entries are observed which are indicated by superscript alphabets. For example:
[a]Groth et al. (1997), Ruckmani et al. (2011)
[b]Stackebrandt et al. (1995), Ruckmani et al. (2011)
[c]Ruckmani et al. (2011), Pathom-aree et al. (2006a,b)
[d]Ruckmani et al. (2011), Kämpfer et al. (2009)
[e]Becker et al. (2002), Ruckmani et al. (2011)

D-glutamic acid of the peptide subunit of peptidoglycan, fucose as whole-cell sugar and a glucosamine-containing phospholipid.

The type species is *Yimella lutea*, the type strain is YIM 45900^T.

Flexivirga Anzai et al. 2011, 616. Validation List N^o 143 in IJSEM online.

Fle.xi.vir'ga. L. adj. *flexus*, bent; L. fem. n. *virga*, a rod; N.L. fem. n. *Flexivirga*, a bent rod.

Besides the formation of comma-shaped cells seen on ISP medium 2, it is mainly the distinct phylogenetic position and the combination of chemotaxonomic properties that distinguished this taxon from other members of the family.

The type species is *Flexivirga alba*, the type strain is ST13^T.

Calidifontibacter Ruckmani et al. 2011, 2422^{VP}.

Ca.li.di.fon.ti.bac'ter. L. adj. *calidus* hot; L. n. fons, *fontis* spring, fountain; N.L. masc. n. bacter a rod;N.L. masc. n. *Calidifontibacter* a rod isolated from hot spring.

The main distinguishing properties of this taxon are the markedly higher DNA G+C content of 77 % and the presence of phosphatidylserine (disregarding strains of *Kytococcus*) and phosphatidylinositol mannosides. The considerable amount of the fatty acid iso-C16:1H (17.2 %), reported to be a discriminating feature is found at even higher amounts in three species of *Dermacoccus*. Though isolated from a warm spring (though the term, "hot" is a component of the genus name), the type strain does not grow at 42 °C.

The type species is *Calidifontibacter indicus*, the type strain is PC IW02^T.

Branchiibius Sugimoto et al. 2011, 199^{VP}.

Bran.chi.i.bi'us. L. pl. n. *branchiae* the gills of fish; N.L. masc. n. *bius* (from Gr. masc. n. bios) life; N.L. masc. n. *Branchiibius* a life existing in gills of fish.

The presence of L-serine at position 1 of the peptide subunit of the peptidoglycan (the other members of the family possess L-alanine) and arabinose as whole-cell sugar are the chemotaxonomic peculiarities of this taxon, consisting of two species. Menaquinone MK-8(H_6) and unknown branched-chain (other than 10-methyl, iso, and anteiso) fatty acids have only been reported for *Branchiibius cervicis* (Tomida et al. 2011).

The type species is *Branchiibius hedensis*, the type strain is Mer 29717^T.

Kytococcus Stackebrandt et al. 1995, 687^{VP}.

Ky.to.coc´cus. Gr. neut. n. *kytos*, skin; Gr. masc. n. *kokkos*, a grain; M.L. masc. n. *Kytococcus*, a coccus from skin.

Phylogenetic and chemotaxonomic evidence speak against the inclusion of this genus into the family *Dermacoccaceae*. Above all, the menaquinones are fully unsaturated and the peptidoglycan variation is different from all other family members. The original description of this species was reported by ZoBell and Upham (1944) and was based on one strain, designated 541. Many strains were subsequently isolated from the skin of humans and the study of Kloos et al. (1974) gives the most comprehensive view on the properties and the variation on strains labeled *Kytococcus* [*Micrococcus*] *sedentarius*.

Table 15.3
Physiological reactions differentiating *Kytococcus* type strains (All data are from Kämpfer et al. 2009)

Reaction	*K. sedentarius* DSM 20547^T	*K. schroeteri* DSM 13884^T	*K. aerolatus* 02-St-019/1^T
Hydrolysis of			
pNP-α-D-glucopyranoside	+	–	–
Bis-pNP-phosphate	+	+	–
pNP-phenyl-phosphate	+	+	–
pNP-phosphoryl-choline	+	–	–
L-Alanine-pNA	w	+	–
Assimilation of			
D-Fructose	w	–	–
D-Mannose	w	–	–
D-Maltose	+	w	–
D-Trehalose	+	+	–
Propionate	w	+	–
Glutarate	–		–
DL-3-Hydroxybutyrate	–	+	–
L-Malate	(+)	–	–
Oxoglutarate	–	+	–
D-Aspartate	+	+	–
L-Histidine	+	–	–
L-Leucine	–	+	–
L-Ornithine	–	+	–
L-Phenylalanine	–	–	–
L-Proline	+	+	–

Abbreviations: See *Table 15.3*

Table 15.3 serves for comparison of properties (excluding those which are shown in *Table 15.2*), reported to distinguish the type strains of the three species. According to the study of Kämpfer et al. (2009), Kloos et al. (1974, for *K. sedentarius*), strains are inactive in acid production from carbohydrates. *K. aerolatus* only uses a few carbon sources.

The type species is *Kytococcus sedentarius*, the type strain is strain 541^T, DSM 29547^T, ATCC 14392^T.

Clinical Significance

A few members of *Dermacoccaceae*, especially *Kytococcus schroeteri* and *K. sedentarius*, have clinical significance. The latter species is described to be a causative agent of pitted keratolysis of the foot (Nordstrom et al. 1987). This species, however, has also been discussed in a case of endocarditis (Old and McNeill 1979) and reports indicate its likely cause of airway illnesses (Savini et al. 2011). It has been identified in a case of peritoneal dialysis-associated peritonitis (Chaudhary and Finkle 2010) and in bacteremic (Mohammedi et al. 2005) and hemorrhagic pneumonia, assuming that in immunocompromised patients, this commensal may act as an opportunistic pathogen once it has spread through the blood stream (Levenga et al. 2004; Hodiamont et al. 2010; Blennow et al. 2012). More reports are available for the involvement of *K. schroeteri* in bacterial infections of blood in patients with endocarditis in both children and adults (Becker et al. 2003; Mnif et al. 2006; Aepinus et al. 2008; Renvoise et al. 2008; Jourdain et al. 2009; Yousri et al. 2010) and in shunt infection (Leport et al. 1989). The species has also been found to cause spondylodiscitis (Jacquier et al. 2010) and the formation of crusted erythematous papules in the groin of a man with acute myelocytic leukemia (Nagler et al. 2011).

Recently, strains of the novel species *Branchiibius cervicis* have been isolated from the skin of the neck of atopic dermatitis patients (Tomida et al. 2011).

Treatment of kytococci infections was successfully achieved with vancomycin or a combination of vancomycin, rifampicin, gentamicin, or pristinamycin (Mnif et al. 2006; Aepinus et al. 2008; Jourdain et al. 2009; Savini et al. 2011). Blennow et al. (2012) successfully treated a *Kytococcus* infection with linezolid and trimethoprim-sulfamethoxazole. Hodiamont et al. (2010) report that *Kytococcus* strains are often resistant to penicillin, while Szczerba (2003) indicates that micrococcal isolates, including dermacocci and kytococci, are resistant to ampicillin and erythromycin. Several descriptions of type strains, indicating resistance and susceptibility patterns, confirm these findings. *K. schroeteri* is susceptible to chloramphenicol, ciprofloxacin, gentamicin, tetracycline, vancomycin, teicoplanin, and bacitracin but resistant to penicillin, oxacillin, nitrofurantoin, and erythromycin (Becker et al. 2002). A few of these antibiotics only have been tested for the type strains of *Dermacoccus*, *Kytococcus*, *Demetria*, and *Calidifontibacter* (Ruckmani et al. 2011): The reaction toward rifampicin and ciprofloxacin is strain dependent and all dermacoccus type strains are sensitive to oxacillin.

In contrast, reports on the presence of *Dermacoccus* species in the vascular system are rare. A single study (Marques da Silva et al. 2006) reports the presence of oral bacteria, including *Dermacoccus* spp., in several 16S rRNA gene clone libraries obtained from arterial walls of aortic anaeurysms.

Isolation, Enrichment, and Maintenance Procedures

Due to the broad range of isolation sources, the isolation procedures for members of the family differed widely. *Kytococous sedentarius* and *Dermacoccus nishinomiyaensis* have been isolated frequently from the mouth

(Szczerba and Krzeminski 2001) and the skin of healthy people (Kloos et al. 1974) The nonselective medium had the following composition per liter: peptone (Difco), 10 g; yeast extract (Difco), 5 g; sodium chloride, 5 g; glucose, 1 g; agar (Difco), 15 g; distilled water, 1,000 ml, supplemented with cycloheximide (50 μg/ml). This agar was also used for the maintenance and propagation of all strains.

The other three *Dermacoccus* species (Pathom-aree et al. 2006a, b) were isolated from a 2-ml sediment sample from the Mariana Trench (Challenger Deep). A suspension of the sample (sent to the UK at 4 °C) was plated onto raffinose-histidine agar plates (Vickers et al. 1984), supplemented with cycloheximide and nystatin. Cultures were maintained on glucose-yeast extract agar plates (Gordon and Mihm 1962).

Demetria terragena (Groth et al. 1997) was isolated from a frozen compost sample on nutrient agar, containing per liter: peptone, a pancreatic digest of meat of fish, 20 g; NaCl, 5 g; agar, 15 g.

Yimella lutea (Tang et al. 2010) was isolated as a contaminant of ISP medium 5 and maintained on ISP 2 agar (Shirling and Gottlieb 1966).

Luteipulveratus mongoliensis (Ara et al. 2010) has been recovered from soil which has been dried at room temperature for 5–7 days, then rehydrated, and centrifuged (Hayakawa et al. 2000). The organism was isolated on humic acid-vitamin agar (Hayakawa and Nonomura 1987) containing 20-mg trimethoprim and 10-mg nalidixic acid per liter. Maintenance was on Bennet agar (Jones 1949).

The *Branchiibius* strains were isolated from eukaryotic specimen. *B. hedensis* was recovered from a Japanese codling (Sugimoto et al. 2011) by spreading dilutions of grind branchia onto humic acid-vitamin agar. Incubation was for 21 days at 28 °C. Maintenance was on ISP 2 medium. *B. cervicis* strains were isolated from the neck of patients with atopic dermatitis and grown on tryptic soy agar (Difco) for 1–3 days at 30 °C (Tomida et al. 2011).

Strains of *Kytococcus schroeteri* were isolated from blood of a patient with endocarditis, using the Bactec9240 System (Becton Dickinson). Growth occurred after 24 h. *K. aerolatus* was isolated from a gelatin filter set up to recover organisms from a room with walls colonized with molds (Kämpfer et al. 2009). The filter was placed on ISP3 agar medium and a colony of the species recovered after 2 weeks at 28 °C. Maintenance was on medium 79 containing (per liter) 10 g of dextrose, 10 g of Bacto Peptone (Difco), 2 g of Casamino Acids (Difco), 2 g of yeast extract (Serva), and 6 g of NaCl (pH 7.5) (Prauser and Falta 1968).

The organisms can be cultivated on the following media numbers as indicated in the DSMZ catalog of strains (http://www.dsmz.de/catalogues.html): strains of *Dermacoccus* and *Kytococcus*: (53 and 92), *Demetria* (736), *Yimella* (65), *Branchiibius* and *Calidifontibacter* (92). Strains are on a medium-term maintained as glycerol suspensions (20 %, v/v). Long-term preservation is by lyophilization in skim milk or under N_2 atm. at −196 °C.

Ecology

Habitat

As indicated in the Isolation subchapter, the habitat of type strains of *Dermacoccaceae* is broad, ranging from soil, sea sediment (Pathom-aree et al. 2006c), and compost to the branchia of fish and the skin and blood of humans. Obviously, *D. nishinomiyaensis* and *K. sedentarius*, originally isolated from water used for brewing sake (Oda 1935) and from sea water (ZoBell and Upham 1944), respectively, are part of the normal skin microflora as indicated by the studies of Kloos and Musselwhite (1975) and of Kloos et al. (1974) who found that of 115 human skin samples, 13 % and 28 % contained *K. sedentarius* and *D. nishinomiyaensis* strains, respectively. *Dermacoccus* spp. have also been isolated from salt used in the production of Spanish dry-cured ham (5.4 % of 369 strains isolated) (Cordero and Zumalacarregui 2000). As these findings conflict with the lack of growth of *D. nishinomiyaensis* strains in 7.5 % NaCl (Kocur et al. 1975), it can be assumed that either the Spanish isolates belong to a new taxon (only this species was described in the year 2000) or these organisms are transient but not actively growing on the surface of salted ham. BLAST search for the presence of *Dermacoccaceae*-related 16S rRNA sequences in public databases resulted in only few hits with high BLAST scores: Isolates have been reported in a soil sample of a dairy research station in Victoria, Australia (Ellin 185, accession number AF409027, Schoenborn et al. 2004), in soil of Barrientos Island, Antarctica (JF905611, Lee et al. unpublished), in the phyllosphere of apple trees (AB697155, He and Sano unpublished) and as a rhizosphaere strain (JN585681, Sanadhya and Jha unpublished). A *Dermacoccus*-related clone has also been identified in a 16S rRNA clone library obtained from microorganisms associated with the leaf-cutting ant *Acromyrmex* spp. (Haeder et al. 2009).

The sequence of a strain tentatively named "*Barrientosiimonas humi*" is close to that of *Demetria terragena* (JF346171, Lee et al. unpublished); another entry, close to *D. terragena*, lists the invalid name "*Demetria marina*," isolated from seawater (FM882229, Lee unpublished). *Kytococcus* isolates with 99 % BLAST scores to *K. sedentarius* were reported, among other entries, from sediments of the eastern Arabian Sea (Divya et al. 2010), Bay of Bengal (JQ068781, Kumar unpublished) and from the intertidal zone around the Republic of Palau (Gontang et al. 2007), among pelagic bacteria from the east coast of South Korea (Bhattarai et al. 2006), in association with a marine sponge (FJ999585, Liu et al. 2008) and in nonsaline groundwater environment (one strain, Tiago et al. 2004). The clinically relevant species *Kytococcus schroeteri* has also been detected among airborne isolates collected by the BioCapture BT-550 device (Mesosystems Technology Inc., Kennewick, WA, USA) during commercial airline flights (Osman et al. 2008), as well as member of the midgut microbial community of *Culex* spp. mosquitoes (Chandel et al. unpublished). Not unexpectedly, a high number of 16S rRNA clone sequences related to *Kytococcus* originate from the NIH Intramural Skin

Microbiome Consortium/NISC Comparative Sequencing Program (Kong et al. unpublished).

Application

The finding that several strains of *Kytococcus sedentarius* and *Dermacoccus nishinomyaensis* from the Czech Culture Collection of Microorganisms (CCM, Brno) produce monensins (Pospišil et al. 1998) stimulated the search for other secondary metabolites. During an isolation campaign for novel actinomycetes from Challenger Deep sediment of the Mariana Trench (Pathom-Aree et al. 2006c), 38 isolates were recovered, 19 of which belonged to *Dermacoccus* (among others *D. abysii, D. barathri*, and *D. profundi*). Screening of culture filtrates resulted in the detection of metabolites identified as phenanzine compounds, named dermacozines after high-resolution mass spectrometry and structural elucidation. Seven structures of these compounds with antitumor, antiprotozoal, and free radical scavenging activities (Abdel-Mageed et al. 2010) have been identified. As outlined by Goodfellow and Fiedler (2010), knowledge about proper taxonomic diversity and phylogenetic relationships at the species level can guide the search for new metabolites though the outcome of these studies cannot be predicted as secondary metabolites are often strain specific, rather than species specific. Nevertheless, attempts to improve classification and identification, hence nomenclature, by providing an array of genomically stable criteria have always been the goal (though not always achieved) of systematics. Secondary metabolites have rarely been included in species descriptions because of the unknown genomic stability of genes involved in their synthesis. Experience, however, has shown that phylogenetic and chemical diversities by and large mirror each other and knowledge about properties even not used in species description but in characterization can support chemists to specifically target potentially valuable biological resources.

A second example for an application is the production of keratinases from *Kytococcus sedentarius*. Two keratin-degrading serine proteases have been isolated from strain NCIMB 40287 that independently biodegrade a range of keratin polymers, including insoluble human callus (Longshaw et al. 2002). The two proteins differ in that one is constitutive while the other was highly produced under low growth rate but not under high growth rates. Maximum proteinase production occurs between pH 8.0 and 9.0. This strain is specifically mentioned in US patent (5.213.978) for a process for degradation of keratin, keratinaceous material, collagen, and collagenaceous material.

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16 The Family *Dermatophilaceae*

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Abstract
The family *Dermatophilaceae* embraces Gram-positive, high G+C, facultative anaerobic or strictly aerobic chemoorganotrophic bacteria with a wide range of habitats such as animal and human skin, fish guts, and wastewater treatment reactors. Up to date, it consists of five genera: *Dermatophilus*, *Austwickia*, *Kineosphaera*, *Mobilicoccus*, and *Piscicoccus*. Its first member, *Dermatophilus congolensis*, was isolated as the causative agent of bovine streptotrichosis, at the time named dermatose contagieuse. The disease can be described as an acute or chronic exudative dermatitis, affecting a wide range of animals and man. It has been known with a variety of names throughout time, mostly depending on the host animal, but all of them will hereafter be called under the broader name dermatophilosis. With its complex life cycle, unique morphology, and pathogenicity, *D. congolensis* has led to significant turmoil in the history of the taxonomic placement of itself and its relatives. *Austwickia chelonae*, which was originally thought to belong to the genus *Dermatophilus*, was recently reclassified as a new genus within the *Dermatophilaceae* family. *Kineosphaera limosa* was isolated from a deteriorated enhanced biological phosphorus removal reactor, and is able to accumulate polyhydroxyalkanoates. *Mobilicoccus pelagius* and *Piscicoccus intestinalis* constitute the latest additions to the family and were both isolated from the intestinal tracts of two species of fish. Thus, it can be concluded that members of the family *Dermatophilaceae* constitute a phenotypically versatile group.

Taxonomy: Historical and Current

The family *Dermatophilaceae* was first proposed by Austwick (1958) and subsequently emended a number of times (Hamada et al. 2010; Zhi et al. 2009; Stackebrandt and Schumann 2000; Stackebrandt et al. 1997; Gordon and Edwards 1963). It was originally classified in the order *Actinomycetales* and consisted of only the genus *Dermatophilus* and three species, i.e., *Dermatophilus congolensis*, *Dermatophilus dermatonomus*, and '*Dermatophilus pedis*'. The three species differed mainly on their respective hosts and the type of lesions they exhibited, a fact that rendered the rightness of their taxonomic differentiation doubtful and finally led to their grouping into one species under the name *Dermatophilus congolensis* (Gordon 1964). *Geodermatophilus obscurus*, a soil-isolated microorganism, was erroneously assigned to the *Dermatophilaceae* family based mainly on morphological characteristics (Luedemann 1968), but its chemotaxonomic characteristics led to its reclassification as a member of the *Geodermatophilaceae* family (Stackebrandt 1983; Samsonoff 1977a).

Based on the phylogeny tree (*Fig. 16.1*) constructed using 16S rRNA gene sequences, the family *Dermatophilaceae* was reclassified into the class *Actinobacteria*, subclass *Actinobacteridae*, order *Actinomycetales*, suborder *Micrococcineae* (Stackebrandt et al. 1997). It contained the type genus *Dermatophilus* and the genera *Kytococcus* and *Dermacoccus*. Using a set of signature nucleotides, the genera *Kytococcus* and *Dermacoccus* were proved to cluster separately from the genus *Dermatophilus*, leading to the division of the three genera into two families: *Dermatophilaceae* with *Dermatophilus* as the sole genus and *Dermacoccaceae*, embracing the genera *Dermacoccus*, *Kytococcus* and *Demetria* (Stackebrandt and Schumann 2000). The higher taxonomic levels were left unchanged. *Austwickia chelonae*, which was originally assigned to the genus *Dermatophilus* based mainly on growth and biochemical characteristics and molecular analysis (Masters et al. 1995; Trott et al. 1995), was reclassified outside of the *Dermatophilus* genus based on 16S rRNA sequence analysis and chemotaxonomic characterization (Hamada et al. 2010; Stackebrandt and Schumann 2000). With the isolation of

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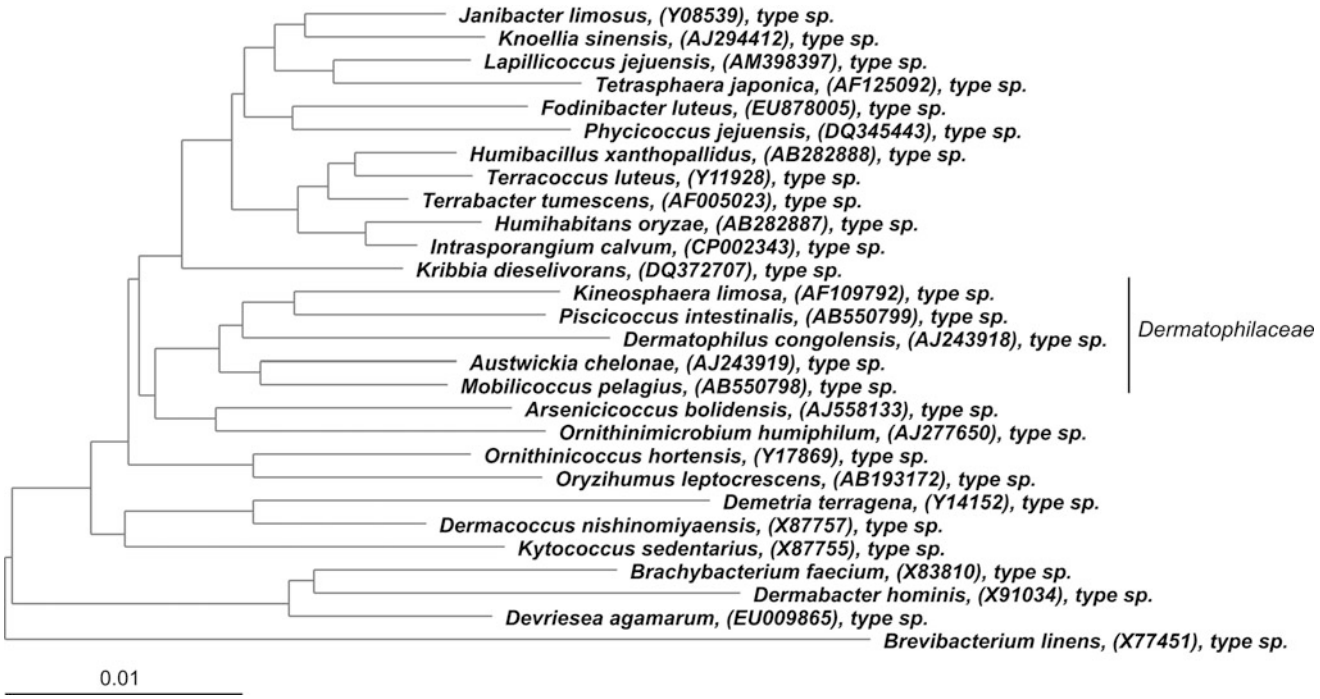

Fig. 16.1

Phylogenetic reconstruction of the family *Dermatophilaceae* based on 16S rRNA gene sequences, 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

K. limosa, *M. pelagius*, and *P. intestinalus*, the family was further emended to form the new genera/species and reclassify *Dermatophilus chelonae* as *Austwickia chelonae* (Hamada et al. 2010; Zhi et al. 2009). The distinction between *D. congolensis* and *K. limosa* was supported by the presence of signature nucleotides within the 16S rRNA gene sequence, in addition to chemotaxonomic and morphological characteristics (Stackebrandt et al. 1997).

Molecular Analyses

D. congolensis is the most well-studied member of the family *Dermatophilaceae*. Its clinical relevance has led to the application of a number of molecular techniques (i.e., sodium dodecyl sulfate polyacrylamide gel electrophoresis, western blotting, multilocus enzyme electrophoresis, restriction enzyme analysis, random amplified polymorphism DNA, and pulsed field gel electrophoresis), aiming to elucidate the variation among *D. congolensis* strains and their infective routes (Larrasa et al. 2004; Larrasa et al. 2002; Makinde and Gyles 1999; Trott et al. 1995; Gogolewski et al. 1992). They all revealed that significant genetic differences exist among *D. congolensis* isolates that do not correlate with geographic origin but sometimes correlate with host animal and properties such as hemolytic or phospholipase activity. Such methods, though effective in typing different *D. congolensis* strains, are of little use to bacterial taxonomy. Taxonomic characterization via molecular techniques such as DNA-DNA hybridization or genome comparisons has not been reported in the literature. Whole genome sequence projects have been undergone for *D. congolensis*, *A. chelonae*, *K. limosa*, and *M. pelagius*, but the results are not published yet.

Phages

Phage pDM1 was isolated from infected wool samples in Western Australia and was shown to possess a *D. congolensis*–specific lytic activity (Patten et al. 1995). The optimal medium for phage propagation is unsupplemented bovine blood agar, and clear circular plaques (1-mm diameter) are consistently produced. Chloroform, thymol, hydrogen peroxide, and ethanol, all result in significant reduction of the plaque-forming units, indicating the sensitivity of the phage to these compounds. Its lytic activity is applicable to isolates from various locations and host animals and does not extend outside the *Dermatophilus* genus level. Concurrent inoculation of the host and the phage results in the greatest reduction, indicating that early developmental stages of *D. congolensis* might be more susceptible.

Table 16.1
Major phenotypic characteristics of members of the family *Dermatophilaceae*

	D. congolensis	*A. chelonae*	*K. limosa*	*M. pelagius*	*P. intestinalis*
Oxygen relationship	Facultative anaerobic	Facultative anaerobic	Strictly aerobic	Facultative anaerobic	Facultative anaerobic
Cell shape	Coccoid	Coccoid	Coccoid	Coccoid	Cuboid/coccoid
Cell motility	Motile	Poor motility	Motile	Actively motile	Not observed
Optimal temperature (°C)	37	27	30	28	28
Optimum pH	NA	NA	7.0	7.0	6.0–7.0
NaCl tolerance (w/v)	NA	NA	3 %	7 %	5 %
Gram staining	+	+	+	+	+
Catalase[a]	+	+	+	+	+
Oxidase[a]	NA	NA	–	–	–
Methyl red[a]	–	–	NA	NA	NA
Indole[a]	–	–	NA	–	–
Casein hydrolysis[a]	+	+	NA	NA	NA
Urea hydrolysis[a]	+	Variable	NA	+	–
Gelatin hydrolysis[a]	Varies among strains	+	NA	+	–
Nitrate reduction[a]	–	Weak	–	+	+
G+C content (mol%)	57.4–59.8	66.5	71.3	71.6	71.5
Peptidoglycan type	A1γ	A1γ	A1γ	A1γ	A1γ
Phospholipids[b]	PG, PI, lyso-PE	DPG, PG, PI, lyso-PE	PG, PI, DPG, PE, PC	DPG, PG, PI	PG, PI, lyso-PE
Major fatty acids[c]	$C_{17:1}$ - $C_{15:0}$ - $C_{14:0}$ - $C_{16:0}$	$C_{15:0}$ - $C_{17:1}$ - $C_{17:0}$ - $C_{16:0}$	$C_{16:0}$ - $C_{17:1}$ - $C_{18:1}$ - $C_{17:0}$	$C_{17:1}$ - $C_{15:0}$	$C_{17:1}$ - iso-$C_{16:0}$ - iso-$C_{14:0}$
Major menaquinone	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	Exclusively MK-8(H_2)	MK-8(H_4)

[a]*NA* not available, + positive, – negative
[b]*PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PI* phosphatidylinositol *PE* phosphatidylethanolamine, *lyso-PE* lyso-phosphatidylethanolamine, *PC* phosphatidylcholine
[c]Fatty acids are listed in order of decreasing abundance

The specificity of the phage further enhances its potential as a biological control agent. However, the in vitro results need to be examined in vivo before its large-scale application, as environmental conditions (e.g., temperature, physical and chemical parameters) may alter its effectiveness. No phages infecting other members of the family have been reported so far.

Phenotypic Analyses

All genera of the *Dermatophilaceae* family are aerobic, and most of them are facultatively anaerobic, Gram-positive, and catalase-positive cocci with a high G+C content (57–72 %). The family embraces both sporulating and non-sporulating members that have MK-8 as the dominant respiratory quinone, $C_{17:1}$ as a major fatty acid, and a cell wall peptidoglycan of the A1γ type. The major phenotypic characteristics of the type species in the family are summarized in *Table 16.1*.

Dermatophilus congolensis

Etymology: *Dermatophilus*: dermato from the Greek word *derma* skin, phil from the Greek word *philos* friend, loving, *congolensis*: because it was first found in Belgian Congo.

D. congolensis colonies, depending on growth conditions, are observed as being moist and dominated by coccoid cells or dry and dominated by filamentous ones (Roberts 1957a). Intra- and inter-strain variations in the colony morphology (i.e., shape and consistency) of *D. congolensis* were confirmed by obtaining isolates from different hosts (El-Nageh 1971). Stained smears, depending on age and staining process, can exhibit a variety of cell morphologies, including lots of coccoid spores, cocci at the stage of germination (alone or in clusters), branching mycelia in the segmentation process, non-mucoid clusters, or any combination of the above (Gordon 1964). Each cell can have a few to more than fifty flagella, 8–9 μm in width (Richard et al. 1967).

The differences in colony and cell morphology can be attributed to the complex life cycle of *D. congolensis* (Roberts 1961),

which largely depends on growth conditions (temperature, oxygen supply, and nutrient availability). In brief, motile cocci (zoospores) that are reproductively inactive lose their motility and start enlarging. The cell wall yields to accommodate a bud, and the hyphae extension starts and carries on with a continuous process of division by transverse septation. Lateral bud appearance can also occur, leading to a branching pattern. If nutrients become limiting, the extension ceases and division throughout the mycelium becomes quicker. In the next step, septum formation stops, each segment starts enlarging and division in planes parallel to the hypha's axis (or also perpendicular if no physical restrictions apply) commences. The occurring cocci are not motile, and each one can go through the whole aforementioned germination process upon nutrient supplementation. At some point, division stops and a new round of enlargement of the cocci begins. Finally, cocci regain their mobility and escape (zoospores). If the growth medium is nutritionally restricted in the first place, the zoospores will not go through the germination process, while, in case nutrients are not enough for the completion of all the life cycle stages, the mycelium dies before giving rise to a new zoospore generation.

D. congolensis is an aerobic or facultatively anaerobic bacterium. Its growth substrates include fructose, glucose, and laevulose, but not adonitol, dulcitol, glycerin, inositol, lactose, mannitol, mannose, raffinose, saccharose, salicin, sorbitol, and sucrose, and transient acid production from galactose has been reported (El-Nageh 1971; Gordon 1964; Roberts 1957a). Most *D. congolensis* isolates can hydrolyze casein and digest BCP milk, and some differ in gelatin liquefaction capability and proteolytic properties. The organism is Gram-positive, catalase-positive, methyl red- and indole-negative; does not reduce nitrate to nitrite; and hydrolyzes urea and starch but not tyrosine or xanthine (Gordon 1964; Roberts 1957a). The cell-wall peptidoglycan is of the A1γ type, the major menaquinone is MK-8(H_4), the dominant polar lipids are phosphatidylglycerol, phosphatidylinositol, and lyso-phosphatidylethanolamine, and cellular fatty acids are predominantly unsaturated and straight-chain saturated ones (Hamada et al. 2010). The G+C content ranges from 57.4 to 59.8 mol% (Hamada et al. 2010; Samsonoff 1977b).

D. congolensis exhibits hemolytic activity, and its hemolytic complex is suggested to comprise of at least two components (Skalka and Pospísil 1992). *D. congolensis* can produce extracellular serine proteases with maximum activity in the pH range of 7–10 and variation between isolates seems to be host-specific (Ambrose et al. 1998).

The type strain is *Dermatophilus congolensis* DSM 44180^T (=NBRC 105199^T = ATCC 14637^T = JCM 8106^T = NCTC 13039^T = NRRL B-2350^T = DSM 44180^T).

Austwickia chelonae

Etymology: *Austwickia:* in honor of Peter K. C. Austwick who proposed the family *Dermatophilaceae*, *chelonae* from the Greek word *chelona* turtle.

Like *D. congolensis*, members of the genus *Austwickia* go through life cycle stages ranging from coccoid zoospores to branching mycelia exhibiting transverse and longitudinal septum formation, but zoospores exhibit poor motility (flagella number ranging from zero to six) (Masters et al. 1995). They are facultative anaerobic and Gram-positive. The peptidoglycan components are alanine, glutamic acid, and meso-diaminopimelic acid (molar ratio of 2.0:1.0:0.7), proving the cell-wall peptidoglycan to be of the A1γ type. The major menaquinones are MK-8(H_4), MK-8, and MK-8(H_2). The dominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and lyso-phosphatidylethanolamine. Cellular fatty acids are predominantly unsaturated and straight-chain saturated ones (Hamada et al. 2010).

The type species *A. chelonae* (former *Dermatophilus chelonae*) grows optimally at 27 °C, and exhibits different hemolytic patterns based on growth conditions (bovine, ovine, or equine blood agar). Its colonies produce a distinctive putrid odor. Its growth substrates include glucose and often fructose and galactose, but not dulcitol, lactose, mannitol, salicin, sorbitol, sucrose, or xylose. *A. chelonae* hydrolyzes casein and gelatin; is catalase-positive and indole- and methyl red-negative; does not always produce urease; and exhibits a weak reduction of nitrate to nitrite. Additionally, *A. chelonae* shows chondroitinase activity with chondroitin 4-sulfate but not with chondroitin 6-sulfate and in vitro collagenase activity (Masters et al. 1995). Its major cellular fatty acids are $C_{15:0}$, $C_{17:1}$ ω9, $C_{17:0}$, and $C_{16:0}$, and its G+C content is 66.5 mol% (Hamada et al. 2010).

The type strain is *A. chelonae* W16 (=NBRC 105200^T = ATCC 51576^T = CCUG 47447^T = CIP 104541^T = DSM 44178^T = JCM 9706^T = DSM 44178^T).

Kineosphaera limosa

Etymology: *Kineosphaera*: kineo- from the Greek word *kino* = move, -sphaera from the Greek word *sphera* = sphere, *limosa* from the Latin word *limosus*: muddy because of the connection to sludge.

The genus *Kineosphaera* embraces bacteria that are strictly aerobic, non-spore-forming, Gram-positive motile cocci with a diameter ranging from 2 to 3 μm. The cocci can grow singly, in pairs, or in clusters. Members of the genus are catalase-positive, oxidase-negative; do not produce nitrite from nitrate; and have a DNA G+C content of 71 mol%. The major diaminoacid is meso-diaminopimelic acid, the cell-wall peptidoglycan is of the A1γ type, and the dominant respiratory quinone is MK-8 (H_4). The dominant fatty acids are unsaturated and straight-chain saturated ones.

The type strain *K. limosa* is Neisser-negative and can accumulate polyhydroxyalkanoates (PHAs) up to approximately 13 % in dry cell weight with 3-hydroxybutyrate and 3-hydroxyvalerate as the major monomers. Its growth temperature range spans from 10 °C to 35 °C, with an optimum

at 30 °C. Its pH range is from 6.0 to 10.2, with an optimum at 7.0. It can grow in a NaCl concentration up to 3 % (w/v). Its growth substrates include *N*-acetyl-D-mannosamine, adenosine, D-arabitol, dextrin, fructose, α-D-glucose, glycerol, γ-hydroxybutyric acid, maltose, D-mannose, 3-*O*-methylglucose, methyl β-D-glucoside, methylpyruvate, palatinose, propionic acid, psicose, salicin, D-sorbitol, succinamic acid, succinic acid, sucrose, trehalose, D-trehalose, turanose, Tween 40, Tween 80, and xylitol. Its major fatty acids include $C_{16:0}$, $C_{17:1}$, $C_{18:1}$, $C_{17:0}$ and $C_{16:1}$. The polar lipid profile comprises of phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylcholine.

The type strain is *K. limosa* Lpha5^T (=JCM 11399^T = DSM 14548^T).

Mobilicoccus pelagius

Etymology: *Mobilicoccus*: mobile- from the Latin word *mobilis* = mobile, -coccus from the Greek word *kokkos* = grain, *pelagius* from the Greek word *pelagos* = sea.

Members of the genus *Mobilicoccus* are Gram-positive, aerobic or facultatively anaerobic, non-spore-forming, motile cocci with peritrichous flagella. Peptidoglycan is made of D-alanine, D-glutamic acid, and meso-diaminopimelic acid (molar ratio of 1.6:1.0:0.7), suggesting the cell-wall peptidoglycan as the A1γ type. Only menaquinone MK-8(H_2) is detected, and the dominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. The dominant fatty acids are unsaturated and straight-chain saturated ones, and major cell wall sugars are ribose and mannose.

The type species *M. pelagius* can grow between 10 °C and 37 °C, with an optimum around 28 °C. It has a pH range from 6.0 to 9.0, with an optimum around 7.0 and a NaCl range from 0% to 7 % (v/w) with an optimum around 1 %. No growth is observed above 10 % NaCl. Its colonies are smooth and orange-yellow in color, with an approximate size of 1–2 mm, while the cell size range is 0.7–1.2 μm in diameter. It is catalase-positive and oxidase-negative. Its growth substrates include N-acetyl-glucosamine, fructose, galactose, glucose, inositol, maltose, mannose, ribose, sucrose, and trehalose but not adonitol, amygdalin, arabinose, arabitol, arbutin, cellobiose, dulcitol, erythritol, fucose, gentiobiose, gluconate, glycerol, inulin, 2-keto-gluconate, 5-keto-gluconate, lactose, lyxose, mannitol, melezitose, melibiose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, raffinose, rhamose, salicin, sorbitol, sorbose, starch, tagatose, xylitol, and xylose. In terms of enzymatic activities, alkaline phosphatase, α-glucosidase, leucine arylamidase, and pyrazinamidase are detected, while acid phosphatase, N-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, lipase (C14), lysine decarboxylase, α-mannosidase, ornithine decarboxylase, phosphohydrolase, pyrrolidonyl arylamidase, trypsin, tryptophan deaminase, and valine arylamidase are not. The species is indole-negative, produces acetoin but not H_2S, reduces nitrate, and does not hydrolyze urea, gelatin, or esculin. Its dominant fatty acids are $C_{17:1}$ ω9 and $C_{15:0}$, and its DNA G+C content is 71.6 mol%.

The type strain is *M. pelagius* Aji5-31^T (=NBRC 104925^T = DSM 22762^T).

Piscicoccus intestinalis

Etymology: *Piscicoccus*: pisci- from the Latin word *piscis* = fish, -coccus from the Greek word *kokkos* = grain, *intestinalis*: from the Latin word *intestinum* = gut, intestine.

Members of the genus *Piscicoccus* are Gram-positive, aerobic or facultatively anaerobic, non-spore-forming and nonmotile cocci, growing in clusters of cuboid or coccoid shape. Peptidoglycan components are D- and L-alanine, D-glutamine, and meso-diaminopimelic acid (molar ratio of 1.7:1.0:0.7), suggesting the cell-wall peptidoglycan as the A1γ type. Menaquinones MK-8(H_4) and MK-8(H_6) are detected in a ratio of 96:4, and the genus's dominant polar lipids are phosphatidylglycerol, phosphatidylinositol, and lyso-phosphatidylethanolamine. The dominant fatty acids are unsaturated, iso-branched saturated, and straight-chain saturated ones, while the major cell wall sugars are ribose and mannose.

The type species *Piscicoccus intestinalis* can grow between 10 °C and 37 °C, with an optimum around 28 °C. It has a pH range from 5.0 to 9.0, with an optimum around 6.0–7.0 and a NaCl range from 0 % to 5 % (v/w), with an optimum around 0–1 %. No growth is observed above 7 % NaCl. Its colonies are rough and orange-yellow in color, ranging from 0.7 to 1.0 μm in diameter. Its growth substrates include arbutin, fructose, galactose, glucose, maltose, mannose, raffinose, sucrose, and trehalose, but not N- acetyl-glucosamine, adonitol, amygdalin, arabinose, arabitol, cellobiose, dulcitol, erythritol, fucose, gentiobiose, gluconate, glycogen, inositol, inulin, 2-keto-gluconate, 5-keto-gluconate, lactose, lyxose, melezitose, melibiose, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, rhamose, ribose, sorbitol, sorbose, starch, tagatose, xylitol, and xylose. In terms of enzymatic activities, acid phosphatase, alkaline phosphatase, β-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, and pyrazinamidase are detected, while N-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C14), lysine decarboxylase, α-mannosidase, ornithine decarboxylase, phosphohydrolase, pyrrolidonyl arylamidase, trypsin, tryptophan deaminase, and valine arylamidase are not. The species is indole-negative, does not produce H_2S, hydrolyzes esculin but not urea or gelatin, and is able to reduce nitrate. The dominant fatty acids include $C_{17:1}$ ω9 iso-$C_{16:0}$ and iso-$C_{14:0}$. The DNA G+C content is 71.5 mol%.

The type strain is *P. intestinalis* Ngc37-23^T (=NBRC 104926^T = DSM 22761^T).

Isolation, Enrichment, and Maintenance Procedures

Dermatophilus congolensis was first isolated as the causative agent of bovine streptotrichosis (Van Saceghem 1915). It was later isolated from cases of mycotic dermatitis and strawberry foot rot–infected sheep without knowing that it was the same microorganism (Coleman 1967; Thompson 1954). *D. congolensis* can be readily isolated from infected skin. The adjacent material is ground in broth, resulting in suspensions containing a lot of cocci and sometimes also fragments of branched mycelia. Plating this suspension on blood agar and incubating at 37 °C leads to the growth of mostly pure cultures of branching filaments (Roberts 1957a).

Suitable culturing media for *D. congolensis* are blood agar, Brain Heart Infusion (BHI) agar, Tryptone Broth (1 % Difco Tryptone, 0.5 % NaCl, pH 7.2), and beef infusion peptone (F5A) broth. Growth in all of them is observed under aerobic conditions at 36 °C and changing the environment to anaerobic (brewer jars with illuminating gas and varying CO_2 content at 37 °C) leads to substantial but much less growth (Gordon 1964). Growth has also been reported on sheep blood agar containing 3.9 % w/v Columbia agar base and 6.7 % v/v defibrinated sheep blood (Oxoid) in an environment containing 5 % CO_2 and 95 % air at 37 °C for 48 h (Ambrose et al. 1998) as well as on BHI agar plates in a candle jar at 37 °C for 48 h (Larrasa et al. 2002). No growth was observed on Sabouraud Dextrose Agar (Difco), Potato Dextrose, Czapek's solution, and tomato paste oatmeal agar (Pridham et al. 1957). Growth can also be achieved in DSM medium 535 (30-g Trypticase Soy Broth, 15-g agar 15.0, and 1,000-ml distilled water) with 5 % (v/v) blood at pH 7.3 and 37 °C.

For maintenance purposes, serial transfers on BHI Agar slants can be employed under aerobic incubation at 36 °C for 3–7 days, followed by storage at room temperature. In this method, survival time varies from a few weeks to 2 years, and the maximum time is achieved at room temperature. For long-term storage, cultures grown for 4 days in F5A broth (0.1 % glucose, 0.5 % NaCl) can be lyophilized in skim milk suspension. Easy recovery from lyophilization can be achieved in F5A broth (Gordon 1964). Maintenance for about 1 year has been reported (Richard et al. 1967) with semimonthly transfers in 10 % (v/v) serum broth. Long-term storage can also be achieved at −80 °C in 20 % (v/v) glycerol if isolates are first grown on 8 % sheep blood agar for 2 days at 37 °C and then sub-cultured in BHI broth for 18 h at 37 °C (Gogolewski et al. 1992). Alternatively, isolates can be stored at −20 °C in BHI/neutralized soya peptone broth with 15 % glycerol (Ambrose et al. 1998). For recovery from freeze-dried isolates, dissolution of the freeze-dried ampoule in 1 mL of BHI broth and culturing for 24–48 h at 37 °C on blood agar in 5 % CO_2 in humidified conditions has been proposed (Makinde and Gyles 1999).

Austwickia chelonae has been isolated from the nose scab of a snapping turtle, an abscess in a tortoise, and from skin lesions on a turtle, all from the Perth zoo in Western Australia (Masters et al. 1995). It has also been isolated from an infected king cobra at the Central Florida Zoo, Florida, USA (Wellehan et al. 2004). *A. chelonae* has been successfully cultured on 9 % bovine blood agar (Oxoid Columbia agar base no. 2), in the presence of 10 % CO_2 for 2–3 days at 37 °C, or in the ambient atmosphere at 27 °C for the same amount of time (Masters et al. 1995). Growth was also reported on polymyxin B blood agar (1,000 IU/mL) at both 27 °C and 37 °C, but it seemed to always be faster at 27 °C. Growth can also be achieved in DSM medium 535 (see *D. congolensis* media) with 5 % (v/v) blood at pH 7.3 and 37 °C.

Other successful growth conditions tested were inoculation of tryptose-phosphate-10 % bovine serum broth with a dense zoospore suspension (observation after 3 days of incubation at 37 °C and after additional incubation at room temperature), incubation in thioglycollate broth (Oxoid) at 37 °C for 3 days, and incubation at 37 °C and 27 °C for 2–3 days on bovine, bovine, and equine agar media (Masters et al. 1995). In the last three types of media, various hemolytic patterns could be observed based on both temperature and media type. No long-term preservation processes were reported.

Kineosphaera limosa has only been isolated from an engineered environment (a deteriorated enhanced biological phosphorus removal activated sludge reactor) enriched with PHA-accumulating microorganisms. In the beginning of each cycle, the reactor was supplemented with substrate composed of acetate, peptone, and other nutrients. The isolation process consisted of taking samples from the end of the aerobic phase, washing them two to three times with 0.5 % NaCl solution by natural settling, adjusting the biomass to 500–1,000 mg/L, dispersing the sludge flocs with an ultrasonicator, serially diluting the dispersed sludge (10^{-2}–10^{-8}), and plating onto solid growth media. Specifically, GM1 medium in distilled water at pH 7 was used, with the following composition per liter: 0.50-g glucose, 2.00-g $NaCH_3COO{\cdot}3H_2O$, 0.50-g peptone, 0.50-g yeast extract, 0.44-g KH_2PO_4, 0.50-g $MgSO_4{\cdot}7H_2O$, 0.50 g $(NH_4)_2SO_4$, 10-mL vitamin solution, 100-mL autoclaved and filtered sludge extract, and 16-g agar. For maintenance and growth purposes, broth medium was used. Cultures were incubated for 1–3 weeks at room temperature, random colonies were picked and screened for polyphosphate (Neisser staining) and PHA (PHB dyes) inclusions, and the positive ones were further purified at least once on GM1 media (Liu et al. 2000). Growth can also be achieved in DSM medium 776 (0.5-g glucose, 0.5-g peptone, 0.5-g yeast extract, 0.5-g Na-glutamate, 0.5-g KH_2PO_4' 0.1-g $(NH_4)_2SO_4$, 0.1-g $MgSO_4 \times 7H_2O$ and 1,000-ml distilled water) at pH 7.0 and 28 °C. No long-term preservation processes were reported.

Mobilicoccus pelagius **and** ***Piscicoccus intestinalis*** have only been isolated from the intestinal tracts of two species of fish (*Trachurus japonicus* and *Repomucenus richardsonii*, respectively) from Kyonan Beach, Tokyo Bay, Japan (Hamada et al. 2010). The intestinal tract suspensions were serially diluted in saline (10^{-1} to 10^{-10}), and 0.1-mL of each dilution was spread on LYPm agar and incubated at room temperature for a minimum of 1 month in a sealed nylon bag containing an O_2-absorbing and CO_2-generating agent (Hamada et al. 2009). The LYPm medium contained 10-g α-lactose, 10-g yeast extract,

20-g NaCl, 5-g polypeptone, 0.025-g Tween 80, 5-ml salts solution, and 1,000-ml distilled water, while the salts solution comprised of 40-g $MgSO_4 \times 7H_2O$, 2-g $MnSO_4 \times 4H_2O$, 2-g $FeSO_4 \times 7H_2O$, and 2-g NaCl in 1,000-ml distilled water (Iino et al. 2007). Colonies were then transferred to new LYPm agar plates, and the process was repeated several times to obtain pure cultures. Subsequently, NBRC medium 802 was used for further study (general cultivation, morphology, optimal growth parameters). The medium contained 1.0 % polypeptone (Wako), 0.2 % yeast extract (Difco), 0.1 % $MgSO_4 \cdot 7H_2O$ with the addition of 1.5 % agar if solid media were required and it had a pH of 7.0 (Hamada et al. 2009). Growth can also be achieved in DSM medium 514 (5.00-g Bacto peptone, 1.00-g Bacto yeast extract, 0.10-g Fe(III) citrate, 19.45-g NaCl, 5.90-g $MgCl_2$, 3.24-g Na_2SO_4, 1.80-g $CaCl_2$, 0.55-g KCl, 0.16-g $NaHCO_3$, 0.08-g KBr, 34.00-mg $SrCl_2$, 22.00-mg H_3BO_3, 4.00-mg Na-silicate, 2.40-mg NaF, 1.60-mg $(NH_4)NO_3$, 8.00-mg Na_2HPO_4, and 1,000-ml distilled water) at pH 7.0 and 25 °C. No long-term preservation processes were reported.

Ecology

D. congolensis in nature is found on the site of infection of animals (and man) suffering from dermatophilosis. The disease is distributed worldwide, but is most intense in regions with high humidity and a tropical or subtropical climate (Zaria 1993). Humidity can play a dual role. Water can serve as a medium for zoospore transmission and/or promote leaching of the skin's protective wax barrier and bacteriostatic substances. In both cases, *D. congolensis* infection is facilitated (Zaria 1993; Kingali 1990; Roberts 1967b). *D. congolensis* has not been found in the soil, probably indicating its inability to grow outside of the conditions provided by an infected host (Roberts 1967a).

D. congolensis is not only isolated from cattle, sheep, goats, and horses, but also from a wide range of animals, such as mules, donkeys, pigs, deer, raccoons, giraffes, antelopes, buffaloes, zebras, rodents, monkeys, snakes, lizards, polar bears, cats, dogs, and even more (Amor et al. 2011; Shaibu et al. 2011; Hamada et al. 2010; Stackebrandt and Schumann 2000; Zaria 1993). In order for it to thrive, it must penetrate the skin protective barriers, such as sebaceous wax and stratum corneum (Roberts 1963). Multiplication begins deep in the epidermis, resulting in infiltration with exudate, which leads to the dermis/epidermis layers separation and is followed by sequential cycles of bacterial proliferation, leading to the formation of thick superficial scabs (Gogolewski et al. 1992; Hyslop 1979). If hair follicles rupture as a result of infection, *D. congolensis* might also penetrate the dermis. The hyphal developmental stage is considered to be particularly invasive in terms of epidermal cell penetration (Roberts 1965).

The natural habitat of *A. chelonae* is the skin of chelonids, where it forms scabs (Wellehan et al. 2004; Masters et al. 1995). *A. chelonae* type strains DSM 44178^T and NBRC 105200^T (Hamada et al. 2010; Stackebrandt and Schumann 2000) were isolated from nose scabs on a snapping turtle (*Chelydra serpentine*), and another strain was isolated from a subcutaneous mass in a male king cobra (*Ophiopagus hannah*) (Wellehan et al. 2004).

K. limosa is isolated from a deteriorated EBPR reactor wastewater treatment process. Its ability to accumulate PHA but not cellular polyphosphate suggests that *K. limosa* can compete with polyphosphate-accumulating organisms (PAOs) for carbon substrates, and can play an important role in phosphorus removal processes. In addition, one *Kineosphaera* species was isolated from *Homo sapiens* sputum, but was not associated with human disease (Keller et al. 2010).

M. pelagius has so far only been isolated from fish intestinal tracts (*Trachurus japonicas*), and no additional ecology-relevant reports have been published on this species (Hamada et al. 2010). On the contrary, more information is available on the ecology of *P. intestinalis*, the type species of which was also isolated from fish guts (*Repomucenus richardsonii*) (Hamada et al. 2010; Anceno et al. 2009; Nasidze et al. 2009; Xin et al. 2008).

Pathogenicity: Clinical Relevance

D. congolensis is not highly pathogenic by itself, and is proposed most likely as an opportunistic pathogen (Ambrose et al. 1999; Zaria 1993). Infection likelihood can be significantly enhanced by malnutrition, stress, damaged/traumatized skin, or immune deficiency (Ambrose et al. 1999; Zaria 1993; Abu Samra 1981; Stewart 1972; Egerton 1964; Memery 1961; Hudson 1937). Up to date, no single treatment is widely accepted. Generally, the treatment approaches can be grouped into three major categories, i.e., topical medication, systemic medication, and biological approach. None of them have however gained wide acceptance (Zaria 1993). Because of the large scale of dermatophilosis infection in cattle, a prospective treatment needs to be easy to apply, rapid, and economical in order for cattle owners to be likely to use it (Coleman 1967). The thus far existing approaches do not fully satisfy one or all of these requirements.

The sensitivity of *D. congolensis* to antibiotics is important in the search of a dermatophilosis treatment. It exhibits a slight sensitivity to kanamycin and no sensitivity to polymixin, ampicillin, amoxicillin, gentamycin, cefoperazone, and negram. It is sensitive to tetracyclin, terramycin B, erythromycin, chloromycetin, neomycin, chloramphenicol, sigmamycin, natromycin, septrin, stapylomycin, and oxytetracyclin. There are contradictory results on the sensitivity to penicillin and streptomycin (Kaya et al. 2000; El-Nageh 1971; Roberts 1957b).

No vaccine has been developed due to variation in host response and strain virulence, as well as because of dosage and environmental factors (Zaria 1993). *D. congolensis* virulence is connected to its lipases and proteases, which may aid in penetration of the skin barrier (Ambrose et al. 1998). Recently, caprylic acid was proposed as a means to treat dermatophilosis, as it seems to disrupt the plasma membrane through binding on aromatic amino acids on membrane proteins (Valipe 2011).

Dermatophilosis is reported to be closely connected to infestations with *Ambylomma variegatum*, an ixodid tick. Resistance to *A. variegatum* seems to be correlated with dermatophilosis resistance. Specifically, *A. variegatum* is suggested to have a systemic effect on dermatophilosis and this effect seems to be present only for infestation with adult ticks (Ambrose et al. 1999; Ambrose 1996; Lloyd and Walker 1993; Walker and Lloyd 1993).

There is little pathogenicity-relevant information on *A. chelonae*. The species also causes scabs and lesions on chelonids, and it is resistant to polymyxin B, streptomycin, neomycin, and sensitive to penicillin G, tetracycline, chloramphenicol, and sulfafurazole (Masters et al. 1995).

There is no report on the pathogenicity of the other members of the *Dermatophilaceae* family.

Application

The only member of the family *Dermatophilaceae* with important economic ramifications is *D. congolensis*. Dermatophilosis is listed as one of the top three cattle affecting diseases in the tropics (DeRyke et al. 1991). Losses in hides/skin and decrease in meat production are the major dermatophilosis-related economic impacts, while secondary ones include lower milk production, wool devaluation, and difficulties in upgrading breeds (Zaria 1993).

Dermatophilosis causes local weaknesses at the site of infection, devaluating skin quality. After tanning, there appear to be lower quality fibers, resulting in rejection by exporters (Zaria 1993; Gbolagunte and Mshelbwala 1991).The local nature of the disease on the epidermis has no direct effect on meat quality, but the indirect consequences are pronounced. Infected animals can have a significant loss of weight and subsequently die. In such a case, the owners prefer to cull or sell the animals when they are still young, before full market price is reached (Zaria 1993). Losses as high as 20 % have been reported in milk production (Nobel et al. 1976). Moreover, the disease poses a restriction in upgrading lower productivity–infected breeds by cross-breeding them with more productive exotic ones (Zaria 1993). Finally, dermatophilosis also affects wool production (Zaria 1993). Apart from wool devaluation, significant losses may also stem from problems caused to shearing machines by scabs on animals suffering from the disease (Austwick and Davies 1958).

Numerical estimates of dermatophilosis associated losses are scarce, but it has been estimated that, in 1984–1985, more than $2 million were lost due to dermatophilosis in Western Australia (Edwards et al. 1986). It is thus important to develop an effective, quick, and economical treatment for *D. congolensis* infections.

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17 The Family *Dietziaceae*

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Abstract
Members of *Dietziaceae*, actinomycetes characterized by the presence of mycolic acids, especially high molecular weight 3-hydroxy fatty acids substituted in the two positions with a long alkyl branch, have previously been classified in the suborder *Corynebacterinae*. The suborder *Corynebacterinae* was originally placed in the order *Actinomycetales*. According to Bergey's road map of the Actinobacteria, the six families *Corynebacteriaeceae*, *Tsukumarellaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Segniliparaceae*, and *Dietziaceae* were removed from the order *Actinomycetales* and assigned to the order *Corynebacterales*. The family *Dietziaceae* comprises solely the genus *Dietzia* which consists of 13 species with validly published names. The morphology and physiology of *Dietzia* species is similar to that of *Rhodococcus equi*, which in the past often led to misidentification of *Dietzia* strains by traditional identification techniques. Nowadays molecular based methods like 16S rRNA gene sequencing can be used to discriminate *Dietzia* strains from the type strain of *Rhodococcus equi*. This is of ecological significance, as members of *Dietzia* have been isolated from diverse environments including clinical specimens, which led to conclusion that *Dietzia* species may act as an opportunistic pathogen.

Taxonomy: Historical and Current

Short Description of the Family and the Genus *Dietzia*

Dietziaceae Rainey, Ward-Rainey and Stackebrandt 1997, 486[VP], emend. Zhi, Li and Stackebrandt 2009, 595[VP]

N.L. fem. n. *Dietzia*, type genus of the family; -aceae, ending to denote a family; N.L. fem. pl. n. *Dietziaceae*, the *Dietzia* family.

The family *Dietziaceae* was proposed by Rainey et al. (1997) in the course of the hierarchical classification system of the *Actinobacteria*. The family description was mainly based upon the phylogenetic position and the presence of defined 16S rRNA gene sequence signature oligonucleotides. Rainey and colleagues defined the following 16S rRNA signatures for members of the genus *Dietzia* (Rainey et al. 1995), the only genus within the family: positions 70–98 (U-A), 293–304 (G-U), 307 (U), 418–425 (U-A), 508 (U), 614–626 (U-G), 631 (G), 661–744 (A-U), 771–808 (A-U), 824–876 (C-G), 825–875 (G-C), 843 (C), 1049–1198 (U-A), and 1122–1151 (A-U). In 2009, an emended description of the family was published by

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Zhi et al. (2009) by extending the signature oligonucleotides considering all species of the genus *Dietzia* for which the names were validly published at that time. The pattern was specified for the following positions: 241 : 285 (U–G), 250 (U), 316 : 337 (C–G), 418 : 425 (U–A), 599 : 639 (C–G), 662 : 743 (C–G), 987 : 1218 (A–U), 1000 : 1040 (A–U), 1059 : 1198 (U–A), and 1115 : 1185 (C–G).

Type genus: *Dietzia*, Rainey et al. 1995, 33^{VP,} emend. Kämpfer et al. 2010, 394^{VP}

Diet'zi.a. M.L. dim. ending –ia,; M.L. fem. n. *Dietzia*, in honor of Alma Dietz, an American microbiologist.

Type species: *Dietzia maris* (Nesterenko et al. 1982; Rainey et al. 1995).

The taxon proposed by Rainey et al. 1995 was originally described as "*Flavobacterium maris*" (Harrison 1929) and later assigned to the genus *Rhodococcus* as *Rhodococcus maris* (Nesterenko et al. 1982). The type strain of the species has probably been isolated from soil and others strains from intestinal tract of carp (*Cyprinus carpi*).

Members of the genus *Dietzia* are aerobic, non-acid fast, non-spore-forming Gram-positive bacteria. They are mostly characterized by cocci that germinate into short rods or rod-shaped cells which may produce V-shaped forms and exhibit snapping division. Strains are chemoorganotrophic. Circular, convex yellow to orange or reddish to pink colonies are formed on agar media. The diagnostic amino acid of the A1γ type peptidoglycan is meso-diaminopimelic acid. The major cell wall sugars are arabinose and galactose. Short chain mycolic acids are present and have 34–40 carbon atoms. The long-chain cellular fatty acids are predominantly straight-chain saturated and monounsaturated fatty acids. Tuberculostearic acid is present. Polar lipids of most species consist of diphosphatidylglycerol (DPG), and/or phosphatidylglycerol (PG). A few species have phosphatidylethanolamine (PE) available and others are characterized by the presence of phosphatidylinositol (PI) or phosphatidylinositol mannoside (PIM).

The predominant dehydrogenated menaquinone with eight isoprene units is MK-8(H_2). Minor amounts of MK-7(H_2) or MK-9(H_2) were also detected in some of the *Dietzia* species. Phylogenetically, the genus is placed into the family *Dietziaceae*. According to Bergey's road map of the Actinobacteria, the six families *Corynebacteriaeceae*, *Tsukamurellaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Segniliparaceae*, and *Dietziaceae* were transferred from the order *Actinomycetales* to the new order *Corynebacterales* (Ludwig et al. 2012). The order *Actinomycetales* is now restricted to members of the family *Actinomycetaceae*. The DNA G+C content of *Dietziaceae* strains varies between 64 and 73 mol%. They have been isolated from various environmental habitats as well as from clinical specimen.

Phylogenetic Structure and Molecular Analyses

At present thirteen species with validly published names are included in the genus *Dietzia*. Representatives of the genus share 16S rRNA gene sequence similarity values of 99.8–98.0 % as compared to the type species *Dietzia maris*, with the exception of the type strains of the species *D. timorensis* and *D. papillomatosis*, which are distantly related to *D. maris* only, showing similarity values of 96.89 % and 96.52 %, respectively. As shown in Fig. 17.1, the three species *D. maris*, *D. schimae*,

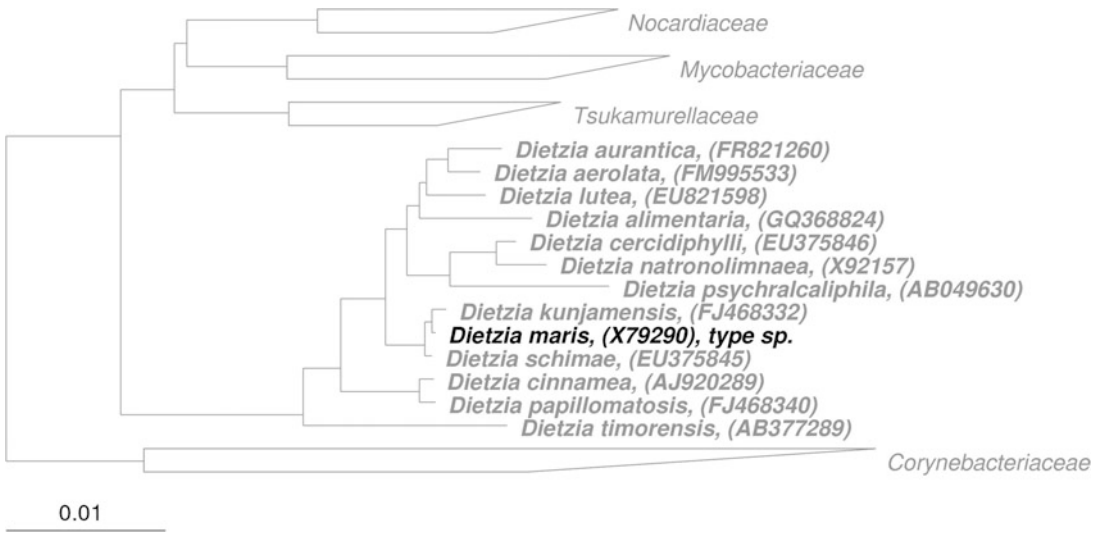

Fig. 17.1

Phylogenetic reconstruction of the family *Dietziaceae* 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. Scale bar indicates estimated sequence divergence

and *D. kunjamensis* are closely related. The 16S rRNA gene sequence of *D. schimae* differs only at three nucleotide positions as compared to that of *D. maris*. The sequence from the type strain of *D. kunjamensis* (AY972480) shows 14 additional nucleotides in the second part of the sequence and likely contains several sequencing errors. Analyses of two additional sequences, available at Genbank, confirmed the close relationship of *D. kunjamensis* to *D. maris* and *D. schimae*. However, *D. kunjamensis* could clearly be differentiated from *D. maris* and *D. schimae* by *gyr*B sequence analysis (Niwa et al. 2012). The *gyrB* protein sequence from *D. schimae* differs from *D. maris* at one position only, at which the amino acid alanine is replaced by valine. The genomic relatedness of *D. schimae* and the related phylogenetic neighbors *D. maris* and *D. kunjamensis* was determined by DNA:DNA reassociation studies (Li et al. 2008). DDH displayed low levels of DNA-DNA relatedness to both species (42.1 % and 44.0 %, respectively) and confirmed their separate species status. Further, a DNA-DNA relatedness value of 59.2 % between *D. kunjamensis* and *D. maris* was described by Mayilraj et al. (2006), as determined by the membrane filter method.

Figure 17.1 indicated also the close relationship of the two species *D. natronolimnaea* and *D. cercidiphylii*. Their 16S rRNA gene sequences are nearly identical (99.5 %), and the protein sequences of the DNA gyrase subunit B, as published by Niwa et al. 2012, also showed 100 % identity. However, DDH was determined according to a modified fluorometric micro-well method (He et al. 2005; Li et al. 2008) and led to detection of a low level relatedness (27.8 %) for *D. cercidiphylii* and *D. natronolimnaea*.

A third cluster is noticeable in *Fig. 17.1* which consists of *D. cinnamea* and *D. papillomatosis*. The 16S rRNA gene sequence of the type strain *D. papillomatosis* N1280 is accessible in Genbank as AY643401. The sequence shows several differences to *D. cinnamea*, especially behind the stretch >900 bp. The sequence of strain N1280 was reanalyzed in 2010 and submitted to Genbank again as FJ468340. This sequence showed significant differences to the original sequence (AY643401) and did not possess the various differences in the backmost part of the sequence and was found to be closely related to *D. cinnameae*. The close relationship was confirmed by *gyr* B sequence analysis (Niwa et al. 2012). The protein sequences of the DNA gyrase subunit B from both strains are nearly identical, but differ at one position, where isoleucine is replaced by valine. DDH studies have not been performed in order to confirm the separate species status of the type strains N1280 and IMMIB RIV-399.

Genome Analysis

Draft genome sequences from *Dietzia alimentaria* 72^{T} and *Dietzia cinnamea* strain P4 have been published in 2011 and 2012, respectively. *Dietzia alimentaria* strain 72 was originally derived from a traditional fermented Korean food called clam jeotgal. The genome of strain 72 has a G+C content of 67.34 % (Kim et al. 2011b). The genome sequence data are accessible via the SEED viewer (www.theseed.org, Overbeek et al. 2005). The 3,352,817 bp long genome includes 3,178 predicted protein-coding sequences, and 51 rRNA genes. The distribution of genes into subsystem categories shows that the highest numbers of genes are involved in carbohydrates (281), followed by genes coding for amino acid and derivatives (261), cofactors, vitamins, prosthetic groups, pigments (230) and fatty acids, lipids, isoprenoids (159). The whole-genome shotgun project has been deposited in GenBank under the accession number AGFF01000000.

The draft genome sequence of strain *Dietzia cinnamea* strain P4 has been deposited in GenBank under the accession number AEKG00000000. The 3,555,295 bp long genome contains 55 rRNA genes, including 50 tRNA genes. The G+C content is 70.96 %. In total, 3,593 genes were predicted, of which 3,538 were protein-coding genes: 62.82 % of the genes could be assigned to a putative function, and 72 % of these could be assigned to clusters of orthologous groups 2,587 protein coding genes with COGs are indicated in the Integrated Microbial Genomes platform (IMG, Markowitz et al. 2009). The highest number of genes is involved in amino acid transport and metabolism (220), followed by genes coding for lipid transport and metabolism (216), energy production and conversion (213), inorganic ion transport and metabolism (189). *Dietzia cinnamea* P4 derived from a study on hydrogen carbon degraders in tropical rainforest soil. A third draft genome sequence became available in 2013 (Diep et al. 2013). Strain *Dietzia* UCD-THP has originally been isolated from a residential toilet handle and shows the largest genome with 3,915,613 bp and a G+C content of 69.5 %. The whole-genome shotgun project has been deposited in GenBank under the accession number AOSR00000000: 3,614 protein-coding sequences and 50 non-coding RNAs were predicted within the RAST-Server-based annotation (Aziz et al. 2008).

Phenotypic Analysis

Phenotypic properties that distinguish *Dietzia* species from another are indicated in *Table 17.1*. Characteristics specific for the genus have been listed above.

Dietzia maris Rainey, Klatte, Kroppenstedt and Stackebrandt 1995, 33^{VP}; *Rhodococcus maris* Nesterenko, Nogina, Kasumova, Kvasnikov and Batrakov 1982, 11

mar'is. L. gen. n. *maris*, of the sea.

Gram-positive coccoid cells which germinate into short rods. Cells may exhibit snapping division and V-forms. Cells are 0.6–1.0 μm in diameter and 1.0–2.0 μm in length. Colonies grown on nutrient agar are raised, butyrous, glistening, and circular with an entire edge. Catalase activity is detectable.

Table 17.1

Phenotypic properties and other characteristics of the type strains of *Dietzia*

Characteristic	*Dietzia aerolata*	*Dietzia alimentaria*	*Dietzia aurantiaca*	*Dietzia cercidiphylii*	*Dietzia cinnamea*	*Dietzia kunjamensis*	*Dietzia lutea*	*Dietzia maris*	*Dietzia natronolimnaea*	*Dietzia papillomatosis*	*Dietzia psychralcaliphila*	*Dietzia schimae*	*Dietzia timorensis*
Colony color	Orange-yellow	Coral-red	Orange	Red-orange	Yellow-orange	Coral-red	Orange-yellow	Orange	Coral red	Orange	Coral red	Pink	Orange-red
Cell morphology	Coccoid	Rods	Coccoid	Rods, V-forms	Rods, V-forms	Coccoid, rods	Coccoid, rods, V-forms	Short rods, V-forms	Short rods, V-forms	Coccoid, rods, V-forms	Rods, snapping type division	Rods, V-forms	Coccoid, rods
Growth temperature (°C)	10–30	15–37	4–37	10–37	22–45	10–37	10–45	10–40	10–37	10–37	5–30	10–45	10–37
NaCl tolerance (%)	nd	0–10	nd	10	12	5	15	5–7	10	8	10	15	7
pH range	nd	7–10	5.5–12.5	6.0–9.0	nd	7–10	5.0–9.0	nd	6–10	nd	7–10	6–9	nd
Carbon source utilized:													
Adonitol	–	–	–	–	–	–	–	–	–	+	+	–	+
L-Arabinose	–	–	+	+	–	+	+	–	–	+	–	–	+
Cellobiose	–	–	+	–	–	+	–	–	+	+	+	+	+
D-Fructose	–	w	+	+	nd	–	+	+	–	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	nd	+	nd	+	–	+	–	–	+	–	–	+	+
Maltose	–	+	–	+	+	–	–	+	+	+	+	–	+
D-Mannose	+	+	–	+	nd	+	+	–	+	+	–	+	+
N-acetylglucosamine	–	+	+	–	nd	+	–	–	+	nd	–	–	+
Raffinose	–	–	nd	–	–	–	–	–	+	+	+	–	+
Sucrose	–	+	+	w	–	+	–	–	+	+	+	+	+
Trehalose	–	+	–	–	–	+	–	–	+	+	+	–	+
Predominant menaquinones	MK-8(H_2), MK-7(H_2)	MK-8(H_2)	MK-8(H_2), MK-7(H_2)	MK-8(H_2)	MK-8(H_2), MK-7(H_2)	MK-8(H_2)	MK-8(H_2)	MK-8(H_2)	MK-8(H_2)	MK-8(H_2), MK-7(H_2)	MK-8(H_2)	MK-8(H_2)	MK-8(H_2)
Major polar lipids	DPG, PG, PI, PIM	DPG, PG, PI, PIM	DPG, PG, PI	DPG, PG, PI, PIM	DPG, PG, PE	DPG, PG, PI	DPG, PG, PI, PIM, PE	DPG, PG, PE	DPG, PG, PE	DPG, PG, PE	nd	DPG, PG, PI	PG, PI (minor)
GC content (mol%)	nd	64.7	nd	72.6	72.3	67	70.5	73	66.1	nd	69.6	71.9	65.5

Strains: *D. aerolata* Sj14a^T (Data from Kämpfer et al. 2010); *D. alimentaria* 72^T (Kim et al. 2011); *D. aurantiaca* CCUG 35676^T (Kämpfer et al. 2012); *D. cercidiphylli* YIM 65002^T (Kim et al. 2011b); *D. cinnamea* IMMB RIV-399^T (Yassin et al. 2006); *D. kunjamensis* K30-10^T (Mayilraj et al. 2006); *D. lutea* YIM 80766^T (Li et al. 2009); *D. maris* IMV 195^T (Nesterenko et al. 1982; Rainey et al. 1995, Lie et al. 2008); *D. natronolimnaea* 15LN1^T (Duckworth et al. 1998; Kim et al. 2011b); *D. papillomatosis* N 1280^T (Jones et al. 2008); *D. psychralcaliphila* ILA-1^T (Yumoto et al. 2002; Koerner et al. 2009); *D. timorensis* ID05-A0528^T (Yamamura et al. 2010)

+, positive; –, negative; *nd* not determined. Some characteristics may differ from the original description of the strain, due to variation within the methods used for biochemical testing as published in other studies (see below)

Does not attack casein, cellulose, hypoxanthine, starch, tyrosine, and xanthine are not affected, but tween 80 is decomposed. Able to reduce nitrate. Acid is produced from glycerol, but not from galactose, inositol, mannitol, sorbitol, sorbose, and xylose. Additional properties are shown in Table 17.1. Utilizes butyrate, fumarate, and succinate in addition. Able to grow with C8 and C13 *n*-alkanes. Composition of whole cellular fatty acids is listed in Table 17.2. The type strain was originally deposited as strain IMV 195 = DSM 43672 = ATCC 35013.

Dietzia aerolata Kämpfer, Langer, Martin, Jäckel and Busse 2010, 395[VP]

ae.ro.la'ta. Gr. n. *aer* air, L. fem. part. adj. *lata* carried; N.L. fem. part adj. *aerolata*, airborne.

Gram-positive coccoid cells, 1.0–1.5 μm in diameter. Positive for catalase and oxidase activity. Phenotypic properties and other characteristics are summarized in Table 17.1. Menaquinone MK-9(H_2) is detectable in minor concentrations only (~2 %). Does not contain phosphatidylethanolamine within the polar lipid profile. The polyamine pattern consists of spermin and spermidine. Composition of whole cellular fatty acids is given in Table 17.2. The type is strain Sj14a = DSM 45334 = CCM 7659.

DNA:DNA hybridization experiments against the type strains of the species *D. schimae* DSM 45139, *D. cercidiphylii* DSM 45140, and *D. maris* DSM 43672 resulted in a relatedness value of 28, 19, and 26 %, respectively.

Dietzia alimentaria Kim, Roh, Choi, Jung, Nam, Kim, Park, Shin and Bae 2011, 2255[VP]

a.li.men.ta'ri.a. L. fem. adj. *alimentaria*, pertaining to food.

Gram-positive, nonmotile rods, 1.0–1.5 μm in length. Catalase activity positive, but oxidase negative. No growth occurred at 45 °C. Hydrolysis of Tween 20, 40, 60, and 80 is positive, but casein and starch are not hydrolyzed. Other enzyme activities with positive reactions (Api ZYM) are esterase (C4), alkaline phosphatase, esterase lipase, and naphthol-AS-BI-phosphohydrolase. Additional characteristics are indicated in Table 17.1. Also able to utilize the following substrates as tested in the API 50CH kit: methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, arbutin, aesculin, salicin, inulin, starch and glycogen. Assimilates adipic acid, malic acid, and trisodium citrate (API 20NE). Composition of whole cellular fatty acids is listed in Table 17.2. The type strain is 72^T = JCM 1630 = KACC 21126.

DNA-DNA hybridization experiments (microarray technique) showed low level relatedness to the type strains of *D. maris* JCM 6166 (17.8 %), *D. schimae* DSM 45139 (18.5 %), *D. psychralcaliphila* DSM 44820 (21.3 %), *D. kunjamensis* JCM 13325 (17.0 %), *D. cercidiphylii* DSM 45140 (26.7 %), *D. natronolimnaeae* JCM 11417 (9.6 %), and *D. cinnamea* JCM 13663 (21.9 %).

Dietzia aurantiaca Kämpfer, Falsen, Frischmann and Busse 2012, 486[VP]

au.ran.ti.a'ca N.L. fem. adj. *aurantiaca*, orange-colored

Gram-positive coccid cells up to 1.5 μm in diameter. Oxidase and catalase activity is positive. Colonies grown on TSA are circular, convex, and pigmented. Phenotypic properties are shown in Table 17.1. Utilizes ribose, acetate, azelate, fumarate, glutarate, hydroxybutyrate, DL-lactate, malate, and pyruvate in addition. Composition of whole cellular fatty acids is listed in Table 17.2. The quinone system also consists of MK-9(H_2) ~ 2 %. The type strain is CCUG 3576 = JCM 17645.

DNA:DNA reassociation experiments with the following type strains were performed: *D. aerolata* Sj14a (15 %), *D. schimae* DSM 45139 (26 %), *D. cercidiphylii* DSM 45140 (34 %), *D. maris* DSM 43672 (28 %).

Dietzia cercidiphylli Li, Zhao, Zhang, Klenk, Pukall, Qin, Xu and Li 2008, 2552[VP]

cer.ci.di.phyl'li. N.L. gen. n. *cercidiphylli* of the plant genus *Cercidiphyllum*, isolated from root sample of *Cercidiphyllum japanicum*.

Gram-positive short rods. Colonies on TSA are circular, smooth, opaque, and reddish orange. Cells may exhibit snapping division and V-forms. Positive for catalase activity, oxidase negative. Hydrolyses Tween 20, 40, 80 and urea, but not gelatin or starch. H_2S production and nitrate reduction negative. Biochemical characteristics and other properties are given in Table 17.1. Utilizes the following substrates in addition: arbutin, D-lyxose, D-mannose, potassium 5-ketogluconate, D-tagatose, and turanose. The whole cellular fatty acid profile is listed in Table 17.2. The type strain is YIM 65002 = CCTCC AA 207016 = DSM 45140.

DNA:DNA hybridization experiments displayed the following values for reassociation to. *D. kunjamensis* K30-10 (59.6 %), *D. psychralcaliphila* ILA-1(42.7 %), *D. natronolimnaea* CBS 107.95 (27.8 %), and *D. maris* DSM 43672 (32.9 %).

Dietzia cinnamea Yassin, Hupfer and Schaal 2006, 644[VP]

cin.na.me'a. L. fem. adj. *cinnamea* of/from cinnamon referring to the color of the cellular biomass.

Gram-positive, rod-shaped cells with snapping division and V-forms. Colonies on BHI agar are smooth, and yellow pigmented. Catalase activity present, oxidase activity absent. Hydrolyzes testosterone and urea, but does not attack casein, gelatin, xanthine, hypoxanthine, or tyrosine. Comparative properties of the strain are indicated in Table 17.1. Assimilates acetate, 1, 2 propanediol as carbon source in addition. The fatty acid profile is shown in Table 17.2. Phosphatidylethanolamine is the diagnostic polar lipid. Type strain is strain IMMIB RIV-399 = DSM 44904 = CCUG 50875.

Table 17.2

Comparative analysis of whole cellular fatty acid compositions (%) for the various species within the genus *Dietzia*

Fatty acids (%)	*Dietzia aerolata*	*Dietzia alimentaria*	*Dietzia aurantiaca*	*Dietzia cercidiphylii*	*Dietzia cinnamea*	*Dietzia kunjamensis*	*Dietzia lutea*	*Dietzia maris*	*Dietzia natronolimnaea*	*Dietzia papillomatosis*	*Dietzia psychralcaliphila*	*Dietzia schimae*	*Dietzia timorensis*
C14:0	0.5	–	0.9	1.2	0.8	0.5	1.0	0.8	1.0	–	0.8	2.9	–
C15:0	–	–	2.5	–	8.3 (4.6)	–	3.7	– (6)	–	5.4	–	–	–
C16:0	22.7	15.5	21.7	18.9	28.9 (22.9)	13.0 (14.4)	15.4	15.3 (33)	14.1	21.1	16.9 (25)	22.2	48.0
C17:0	14.0	10.8	25.9	–	11.7 (4.0)	8.8 (12.9)	22.4	13.2 (6)	–	6.1	13.8	–	–
C18:0	6.9	–	7.9	–	2.3	–	8.0	12.3	–	–	13.9	–	–
C19:0	8.4	9.1	4.4	–	–	–	2.6	–	–	2.6	9.6	–	–
C16:1ω6c/ω7C	5.6	15.1	9.8	18.7	2.8	12.9 (2.5)	6.7	10.6 (13)	33.0	3	10.1 (18)	25.8	–
C17:1ω7c	–	4.9	2.2	2.2	5.3	7.6	–	–	–	–	–	19.2	–
C17:1ω8c	5.0	–	–	3.7	– (11.3)	8.7	12.2	17.2	–	2.7	7.5	–	–
C17:1 ai-B/i-I	–	–	–	–	–	–	–	–	–	–	1.7	6.1	–
C18:1ω7c	–	–	–	3.5	–	–	1.7	–	4.5	–	– (25)	–	–
C18:1ω9c	19.7	7.4	17.2	27.9	4.8 (13.5)	27.3	10.2	13.9 (18)	15.7	9.0	–	–	39
C20:1ω9c	–	4.5	3.9	–	–	–	–	–	–	–	–	–	–
C20:4ω6,9,12,15c	1.4	–	1.9	–	–	–	–	–	–	–	11.7	–	–
10-methyl C16:0	–	–	0.5	1.1	3.2	–	–	0.6	0.7	–	0.3	0.8	–
10-methyl C17:0	0.7	–	1.8	0.4	11.1	1.5	2.9	5.3	0.8	–	0.6	2	–
10-methyl C18:0	13.2	14.6	18.8	17.9	28.8 (20.7)	19.8 (10.5)	7.8	10.3	30.2	22.1	9.7 (22)	13.4	8

Strains: *D. aerolata* Sj14a^T (Data from Kämpfer et al. 2010); *D. alimentaria* 72^T (Kim et al. 2011); *D. aurantiaca* CCUG 35676^T (Kämpfer et al. 2012); *D. cercidiphylli* YIM 65002^T (Li et al. 2008); *D. cinnamea* IMMB RIV-399^T (Yassin et al. 2006; Li et al. 2009); *D. kunjamensis* K30-10^T (Mayilraj et al. 2006; Li et al. 2009); *D. lutea* YIM 80766^T (Li et al. 2009); *D. maris* IMV 195^T (Rainey et al. 1995; Lie et al. 2009); *D. natronolimnaea* 15LN1^T (Li et al. 2009); *D. papillomatosis* N 1280^T (Jones et al. 2008); *D. psychralcaliphila* ILA-1^T (Yumoto et al. 2002; Li et al. 2009); *D. timorensis* ID05-A0528^T (Yamamura et al. 2010)

For some species, the values differ within the publications cited; so, differences are indicated in brackets

DNA:DNA hybridization studies revealed low levels of DNA relatedness to *D. natronolimnaea* DSM 44860 (34.2 %), *D. psychralcaliphila* DSM 44820 (35.7 %), and *D. maris* DSM 43672 (40.3 %).

Dietzia kunjamensis Mayilraj, Suresh, Kroppenstedt and Saini 2006, 1670[VP]

kun.ja.men'sis. N.L. fem. adj. *kunjamensis* pertaining to Kunjam Pass of the cold dessert of the Indian Himalayas.

Gram-positive, aerobic, nonmotile cells, which are coccoid or rod like. Cells are 1.0–1.2 in diameter and 1.1–2.0 μm in length. Colonies on TSA are small, smooth, glistening, and convex. Optimal growth temperature is 25 °C. Oxidase activity present, positive for nitrate reduction. Does not hydrolyze gelatin or urea. Acid is produced from mannitol. Phenotypic characteristics and other properties are given in Table 17.1. The following substrates can be utilized as carbon source as tested with the Biolog GP2 microplate system in addition: dextrin L-fucose, gentobiose, maltotriose, turanose, D-xylose, hydroxybutyric acid, L-asparagine, L-glutamic acid, glycerol, thymidine, and D-fructose-6-phosphate. The type strain is strain K30-10 = MTCC 7007 = DSM 44907.

DNA:DNA hybridization (membrane filter method) was performed against *D. maris* MTCC 7011, and the DNA relatedness was determined as 59.2 %.

Dietzia lutea Li, Chen, Zhao, Klenk, Pukall, Zhang, Tang, and Li 2009, 122[VP]

lu.te'a. L. fem. adj. *lutea*, orange-yellow colored.

Gram-positive, aerobic nonmotile cells which are coccoid or short rods (1.0–1.2 by 1.1–2.4 μm). Cells exhibit snapping division and V-forms. Colonies on TSA are circular, smooth, opaque, and convex. Catalase activity is positive, but negative for oxidase. Hydrolysis of Tween 20, 40, 80 is positive. API ZYM testing showed positive enzyme reactions for alkaline phosphatase, α-galactosidase, β-glucuronidase, and α-glucosidase. Utilizes the following substrates from the API 50CH kit: D-arabinose, aesculin, galactose, glycerol, inositol, D-lyxose, D-mannitol, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, L-rhamnose, D-ribose, L-sorbose, D- and L-xylose. Additional characteristics are shown in Table 17.1. The major fatty acids are given in Table 17.2. The type strain is strain YIM 80766 = KCTC 19232 = DSM 45074 = CCTCC AA 207008.

DNA:DNA hybridization experiments (fluorometric micro-well method) showed low level DNA relatedness to *D. maris* DSM 43672 (49.4 %), *D. kunjamensis* K30-10 (44.8 %), *D. natronolimnaea* CBS 107.95 (21.1 %), *D. psychralcaliphila* ILA-1 (30.2 %), *D. schimae* YIM 65001 (57.6 %), and *D. cercidiphylii* YIM 65002 (39.6 %).

Dietzia natronolimnaea Duckworth, Grant, Grant, Jones and Meijer 1998, 365[VP]; corrig. Duckworth et al. 1999

na.tro.no.lim.na'e.a. N.L. n. *natron* (arbitrarily derived from the Arabic n. natrun or natron) soda, sodium carbonate; N.L. fem. adj. *natronolimnaea*, of or from a soda lake (marsh).

Gram-positive, nonmotile rods (0.9–1.1 μm by 1.2–2.3 μm). Catalase activity is detectable, but oxidase activity not. Rod-shaped cells may exhibit snapping division and V-forms. Growth occurs in BHI medium and also on alkaline agar media. Colonies are circular, convex, glistening with entire margin. Optimal pH for growth is pH 9.0. Under neutral conditions, the following substrates are utilized within the API ATB 32N panel: acetate, fumarate, glutamate, succinate, mannitol, propionate, suberate, valerate, hydroxybutyrate, citrate, glycogen, L-serine, L-proline, L-asparagine, L-arginine, methionine, phenylalanine, L-glycine, and L-valine. Under alkaline conditions, fumarate, D-fructose, D-lactose, and D-xylose are not utilized. The following enzyme activities are detectable with the Api Zym kit: alkaline phosphatase, esterase (C4), esterase/lipase (C8), leucine arylamidase, cysteine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and α-glucosidase. Additional characteristics are given in Table 17.1 including data from Li et al. 2009, which derived from API 50CH testing. Positive reactions also occurred for amygdalin, glycerol, D-melezitose, D-melibiose, and methyl α-D-glucopyranoside. Whole cellular fatty acid profile is shown in Table 17.2. The type strain is strain 15LN1 = CBS 107.95.

DNA:DNA reassociation experiment against *D. maris* was performed with the membrane filter method using [35S] dCTP labeled DNA and revealed a low DNA relatedness of 8 %.

Dietzia papillomatosis Jones, Koerner, Natarajan, Perry and Goodfellow 2008, 71[VP]

pa.pil.lo.ma.to'sis. N.L. gen. n. *papillomatosis*, of papillamatosis.

Gram-positive, aerobic nonmotile rods or coccoid cells that show snapping division and V-forms. Colonies on modified Bennett's agar are convex, shiny, and pigmented. Tween 20, 40, and 80 are attacked, but not adenine. Degrades chitin and L-tyrosine. Utilizes isoamylalcohol as sole carbon source. Biochemical and other characteristics are listed in Table 17.1. Fumaric acid, hydroxyl benzoic acid, β-hydroxybutyric acid, sodium acetate, sodium benzoate, sodium *n*-butyrate, sodium propionate, sodium pyruvate, and sodium DL-malate are also used as carbon sources. Whole cellular fatty acid composition is given in Table 17.2. Growth is inhibited in the presence of bacitracin (10U), ciprofloxacin (5 μg/mL), cotrimoxazole (25 μg/mL), fusidic acid (10 μg/mL), and penicillin (1 μg/mL).

The type strain is strain N 1280 = DSM 44961 = NCIMB 14145.

Dietzia psychralcaliphila Yumoto, Nakamura, Iwata, Kojima, Kusumoto, Nodasaka and Matsuyama 2002, 89[VP]

psy.chral.ca.li.phil'a. Gr. adj. *psychros* cold; N.L. *alcali* alkali, from Arabic *alqali* potash soil; Gr. adj. *philos* friendly to; N.L. fem. adj. *psychralcaliphila* loving cold, alkaline environments.

Gram-positive, nonmotile rods. Cells are 0.8–1.0 μm in diameter and 1.0–2.2 μm in length. Cells may exhibit snapping division. Colonies are circular, glistening, and convex. Catalase and oxidase activity is present. Negative for indole or H_2S production, and hydrolysis of urea. Tween 20, 40, 60, and 80 can be hydrolyzed, but not casein, gelatin, or starch. Utilizes propionate, valerate, hydroxybutyric acid, pyruvate, acetate, *n*-butyrate, isobutyrate, ethanol, *n*-tridecane, *n*-pentadecane, *n*-hexadecane, *n*-eicosane, *n*-tetracosane, and pristine in addition to the substrates listed in ❯ *Table 17.1*. Composition of cellular fatty acid is given in ❯ *Table 17.2*. The type strain is strain ILA-1 = JCM 10987 = IAM 14896 = NCIMB 13777.

The level of DNA-DNA relatedness to *D. maris* and *D. natronolimnaea* was determined as 38.4 % and 49.7 %, respectively.

Dietzia schimae Li, Zhao, Zhang, Klenk, Pukall, Qin, Xu and Li 2008, 2552[VP]

schi'ma.e. N.L. gen. N. *schimae* of the plant genus *Schima*, isolated from stem of *Schima* sp.

Gram-positive, aerobic, and nonmotile rod-shaped cells which exhibit snapping division and V-forms. Catalase activity present, but negative for oxidase. Negative for hydrolysis of gelatin, urea, and starch, but able to hydrolyze Tween 20, 40, and 80. Positive for nitrate reduction. Utilizes aesculin, glycerol, in addition to the substrates listed in ❯ *Table 17.1*. The fatty acid composition of the type strain is shown in ❯ *Table 17.2*. The type strain is strain YIM 65001 = CCTCC AA 207015 = DSM 45139.

The type strain displayed low levels of DNA-DNA relatedness to *D. maris* DSM 43672 (42.1 %), *D. cercidiphylii* YIM 65002 (43.2 %), *D. kunjamensis* K30-10 (44 %), *D. natronolimnaea* CBS 107.95 (53.3 %), *D. psychralcaliphila* ILA-1 (51.1 %).

Dietzia timorensis Yamamura, Lisdiyanti, Ridwan, Ratnakomala, Sarawati, Lestari, Triana, Kartina, Widyastuti and Ando 2010, 452[VP]

ti.mo.ren'sis. N.L. fem. adj. *timorensis* pertaining to West Timor, Indonesia, from where the organism was first isolated.

Gram-positive, aerobic, nonmotile coccoid to rod-shaped cells. Colonies are circular, convex, and glistening. Aesculin is hydrolyzed; arbutin and urea are not hydrolyzed. Adenine, casein, elastin, hypoxanthine, testosterone, tyrosine, uric acid, and xanthine are not attacked. Utilizes the following substrates within the Api 50 CH-Kit: aesculin, glycerol, amygdalin, L-arabitol, D-arabitol, arbutin, dulcitol, erythrol, L-fucose, D-fucose, D-galactose, gentobiose, glycogen, inositol, inulin, D-lyxose, D-mannitol, melezitose, melibiose, methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, xylitol, L-xylose, and X-xylose. Additional characteristics are indicated in ❯ *Table 17.1*. Whole cellular fatty acid profile is summarized in ❯ *Table 17.2*.

Isolation, Enrichment, and Maintenance Procedures

Isolation and Enrichment

Dietzia maris IMV 195^T (Rainey et al. 1995), classified by Nesterenko et al. 1982 as *Rhodococcus maris*, and originally known as *Flavobacterium maris*, was isolated from soil. Additional strains have also been isolated from skin and intestinal tracts of carp (*Cyprinus carpio*). Growth occurs on nutrient agar, trypticase soy broth agar, or ISP2 medium (Shirling and Gottlieb 1966) incubated at 28 °C.

Dietzia natronolimnaea 15LN1^T (Duckworth et al. 1998) was isolated from littoral sediment of the East African soda lake (Lake Oloidien; little lake Naivasha), which is a moderately saline and alkaline soda lake with a pH of. 8.5. Strain 15LN1 was enriched in alkaline broth which contained the following compounds (g per liter): glucose 10.0, peptone 5.0, yeast extract 5.0, KH_2PO4 1.0, $MgSO_4 \times 7\ H_2O$ 0.2, NaCl 40.0, and Na_2CO_3 10.0. The strain grows also well on brain-heart-infusion agar (pH 9.0) incubated at 30 °C.

Dietzia psychralcaliphila ILA-1^T (Yumoto et al. 2002) was isolated from a drain of a fish egg processing plant. The strain represents a cold-adapted alkaline bacterium that utilizes petroleum hydrocarbons and was isolated on AT-medium that consisted of (g/L): KNO_3 5.0, KH_2PO_4 0.5, $MgSO_4 \times 7H_20$ 0.5, $FeSO_4 \times 7H_20$ 0.01, $CaCl_2 \times 2H_2O$ 0.02, $MnSO_4 \times n\ H_2O$ 0.001, $ZnSO_4 \times 7H_2O$ 0.0005, and agar 15.0 in 1 L 100 nM $NaHCO_3/Na_2CO_3$ buffer, supplemented with vaporized *n* -tetradecane as sole carbon source. Strain ILA-1 was isolated from AT medium after 1 month of aerobic incubation at 4 °C. The strain is also able to grow on R agar (pH 9.0) at 27 °C.

Dietzia cinnamea IMMB RIV-399^T (Yassin et al. 2006) was isolated from a perianal swab of a patient with a bone marrow transplant. Strain IMMB RIV-399 is able to grow on Columbia blood agar (5 % sheep blood), GPHF medium or brain-heart-infusion agar, incubated at 37 °C under aerobic conditions. GPHF medium (g/L): glucose 10.0, peptone from Casein 5.0, yeast extract (Oxoid) 5.0, beef extract (Oxoid) 5.0, $CaCl_2 \times 2H_2O$ 0.74, agar (Oxoid) 15.0; distilled water 1,000 mL, pH 7.2.

Dietzia kunjamensis K30-10^T (Mayilraj et al. 2006) was isolated from cold dessert soil, 45 cm below an ice glacier at 4,200 m at Kunjam Pass, Himachal Pradesh, India. Strain K30-10 was enriched on tryptic soy agar medium (TSA) incubated at 25 °C.

Dietzia papillomatosis N 1280^T (Jones et al. 2008) was isolated on glucose-yeast extract agar from skin scrapings of a patient suffering from confluent and reticulated papillomatosis. The strain is also able to grow on ISP2 agar or modified Bennett's agar after 5 days of incubation at 30 °C.

Dietzia cercidiphylli YIM 65002^T and *Dietzia schimae* YIM 65001^T (Li et al. 2008) were isolated from surface sterilized roots of *C. japanicum* and surface sterilized stem of *Schima* sp., respectively. Both strains were maintained on trypticase soy agar medium (TSA) at 28 °C.

Dietzia lutea YIM 80766^T (Li et al. 2009) was isolated from a soil sample collected from the Eastern dessert of Egypt. The soil sample was diluted in sterile water and after vigorous shaking for 30 min, an aliquot of the sample was spread-plated onto Horikoshi agar. Plates were incubated at 28 °C for 2 weeks. Strain YIM 80766 is also able to grow on tryptic soy agar medium (TSA).

Dietzia aerolata Sj14a^T (Kämpfer et al. 2010) was isolated on tryptone soy agar (TSA) at 26 °C from the air collected in a duck barn. Good growth occurs also on R2A agar and nutrient agar.

Dietzia timorensis ID05-A0528^T (Yamamura et al. 2010) was isolated from a soil sample collected under mahogany trees (*Swietenia mahogany*) in West Timor, Indonesia. After pretreatment with SDS yeast extract, the strain was enriched on Humic acid vitamin agar, but the strain is also able to grow on modified Bennett's agar incubated at 28 °C for 14 days or on trypticase soy broth agar.

Dietzia alimentaria 72^T (Kim et al. 2011a) was isolated on marine agar from a salt-fermented seafood sample, which was made by fermented clams mixed with rock salt. Growth occurs also on tryptic soy agar, incubated up to 5 days at 30 °C.

Dietzia aurantiaca CCUG 35676^T (Kämpfer et al. 2012) was isolated on blood agar from a cerebrospinal fluid sample from a 24-year-old woman in Gothenborg, Sweden. The strain grows also on tryptone soy agar, nutrient agar, or R2A agar.

Maintenance

Standard procedures can be applied for members of the genus *Dietzia*. Serial transfer of subcultures grown on appropriate media (every 6–8 weeks) is possible. Strains can also be achieved in glycerol stocks (50 % (v/v) stored at −20 °C or for better survivability at −80 °C). For long-term storage, freeze drying or storage in liquid nitrogen should be used. Detailed protocols are given in the Cabri guidelines, accessible at www.cabri.org.

Ecology and Pathogenicity

Strains from the genus *Dietzia* have been isolated from various environmental habitats around the world, but increasing numbers of isolates were also obtained from clinical specimen. *Dietzia maris* strains or its DNA were often detectable in soil and sediment, including petroleum- or oil-contaminated habitats (sequence accession numbers KC189154, KC514120, JF727664, EF619406; Al-Awadhi et al. 2012), were found in seawater, associated with red algae, the dinoflagellate *Pyrodinium bahamense*, soft corals or fishes, in spring water or activated sludge (HQ425656, EF469496, JF792051, Azanza et al. 2006, Ruckmani and Chakrabarti 2011, Sun et al. 2012). A few strains have been isolated from clean room environments in Brazil (FJ876398), from Phoenix associated spacecraft surfaces (USA) and also from the assembly building in Kourou (Ghosh et al. 2010; Moissl-Eichinger et al. 2012). One strain was isolated from snow and floor debris of internal surfaces from the Moon-1 Rover (JX571065). Further strains have been isolated from clinical material like blood (DQ386308, DQ286854), throat, or thoracic fluid (Niwa et al. 2012), bone biopsy (Pidoux et al. 2001) as well as from a patient with bacteremia (Dinakaran et al. 2012).

The type strain of *D. cinnamea* was isolated from a perianal swab sample of a patient with bone marrow transplant (Yassin et al. 2006), Together with *D. maris* and *D. papillomatosis*, the species *D. cinnamea* is in individual cases identified as or suspected to be an opportunistic pathogen. *D. cinnamea* was also isolated from a dog bite wound in an adult patient (Hirvonen et al. 2012). Additional strains of the species are described as hydrogen carbon degrader (von der Weid et al. 2007) or found to be associated with *Phaseolus vulgaris* (sequence accession no. HM355703) the common bean, with biofouling material (JF514328) or on fresco surface (KC429622). The type strain of *D. papillomatosis* was originally isolated from skin of an immunocompetent patient with papillomatosis (Jones et al. 2008). *D. papillomatosis* was for the first time detected in a case of infection in a 2-year-old boy with known syringomyelia (Rammer et al. 2013). Another strain affiliated to *D. papillomatosis* was originally isolated from oat bran and found to be able to produce folate (Herranen et al. 2010).

Pilares et al. 2010 have reanalyzed a set of clinical strains, which were originally identified as *Rhodococcus equi* or *Rhodococcus* like by traditional techniques using the API Coryne identification kit. Reanalysis was done by 16S rRNA sequence analysis. The study revealed that seven of the strains could be assigned to the genus *Dietzia*. Four strains were identified as *D. maris*, two strains as *D. natronolimnaea*, and one strain as *D. timorensis*. A similar study was published by Niwa et al. in 2012, reanalyzing 16 strains previously identified as *Rhodococcus equi*. Also this study showed that the strains have previously been misidentified by biochemical testing and could be assigned to the genus *Dietzia*, based on 16S rRNA – and gyrB gene sequence analysis. Most of the strains sequenced were members of the *D. cercidiphylii*/*D. natronolimnaea* cluster and five isolates were found to be related to the *D. maris*/*D. schimae* cluster. Additional reports showing that *D. cercidiphylii* strains have been isolated from humans are not available at present. The type strain of *D. natronolimnaea* has been isolated from an East African Soda Lake (Naivasha) (Duckworth et al. 1998). Additional strains have been isolated from alkaline groundwater (Tiago et al. 2004), sediment of Lonar Lake in India (Joshi et al. 2008), from waste water of a chemical plant in China (Jin et al. 2012), from soil in Japan (Ueda et al. 2001), and from reed periphyton (Rusznyák et al. 2008).

Table 17.3

16S rRNA gene sequences available in Genbank, which can be assigned to the genus *Dietzia*

Strain/DNA isolated from	Accession number	Reference
Oral cavity	GU430732	Dewhirst et al. 2010
Air sample, China	GU933571	Unpublished
Altitude wetland, Argentina	AM882683	Unpublished
Fresh water, South Korea	JQ687118	Unpublished
Arsenic ground water sediment	JX961606	Unpublished
Deep Sea sediment, China	HM222663	Unpublished
Sub-seafloor sediment	AB094465	Inagaki et al. 2003
Marine sediment	DQ344847	Biddle et al. 2005
Mangrove sediment, Thailand	AB818673	Unpublished
Permafrost ice	AB272789	Katayama et al. 2007
Sponge, South East India	DQ001306	Anand et al. 2006
Red algae	EU278344	Unpublished
Oil-pollution	DQ521380	Unpublished
Oil production, water	KC209818	Unpublished
Petroleum-contaminated soil	HM449701	Unpublished
Diesel fuel in saline environments	AY918101	Kleinsteuber et al. 2006
Solid waste from oil-shale industry	EF540468	Unpublished
Ciliate Collinia, endoparasite	EU090135	Unpublished
Swine effluent impacted environment	DQ337506	Unpublished
Bovine dung	GQ246709	Unpublished
Limestone quarries, India	FJ911544	Unpublished
Peritoneal fluid, clinical	FJ468338	Niwa et al. 2012
Blood, clinical	FJ468337	Niwa et al. 2012
Endodontic infection	AF481211	Munson et al. 2002
Horned beetle, larvae	AB266603	Takeishi et al. 2006
Plant root	JN120941	Kim et al. 2012
Smear ripened cheese	AJ969176	Unpublished
Uncultured clones:		
Waste water	AY438789	McGarvey et al. 2004
Waste, steel plant	EU151500	Freitas et al. 2008
Showerhead, swab sample	EU631293	Feazel et al. 2009
Soil	JF411345	Unpublished
Soil, phenol degrader	JN039338	Unpublished
Soil, petroleum contaminated	JN038211	Unpublished
Sediment	JQ178130	Rotaru et al. 2012
Volcanic deposits, Japan	AB366289	Lu et al. 2008
Crude oil	JN882176	Gong et al. 2012
Gypsum-treated oil sands Tailing Pond	HQ035378	Ramos-Padrón et al. 2011
Stink bug midgut	JQ927512	Zucchi et al. 2012
Rat gastrointestinal microbiota	DQ856787	Dalby et al. 2006
Vaginal microbiota	JF480085	Unpublished
Raw cow milk	EU029309	Raats et al. 2011
Cow teat, skin	JN834337	Verdier-Metz et al. 2012
Bioaerosol, hog lagoon	JQ478541	Unpublished

For *D. schimae* and *D. timorensis*, DNA sequences were only detectable from environmental samples (JQ282810, JQ409503, JX429817, JX429818 and HE578791). The same is true for *D. psychralcaliphia* strains, which have been isolated from deep sea sediment (Chen and Shao 2009), petroleum-contaminated soil (Mathe et al. 2012), or from canine dental plaque (Elliott et al. 2006).

Several sequences are presently available in GenBank which have not been assigned to one of the *Dietzia* species or which derived from not yet cultivable strains; these are summarized in *Table 17.3*. Many of these sequences are indicated as unpublished, but may be the data set entry was not updated from the authors.

Application

As shown above, strains of the genus *Dietzia* have mainly been isolated from various environmental habitats. They are common in soil and marine sediment and some of the strains are able to degrade hydrocarbons, which may play an important role for the bioremediation of hydrocarbon-contaminated environmental sites. *Dietzia cinnamea* P4 was isolated from tropical rainforest soil and is able to degrade hydrocarbons. In 2012, its whole genome sequence was published by Procópio et al. (2012) which offers the possibility to study the genetic background for degradation of *n*-alkanes in more detail.

As indicated in *Table 17.1*, all type strains of the genus *Dietzia* are able to produce pigments. The carotenoid canthaxanthin is responsible for the reddish color of the *Dietzia natronolimnaeae* colonies. Strain *D. natronolimnaea* HS-1 has extensively been used for optimization of canthaxanthin production in the past (Khodaiyan et al. 2007).

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18 The Family *Frankiaceae*

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Abstract
The family *Frankiaceae*, within the order *Actinomycetales*, contains bacteria isolated mainly from root nodules and occasionally from soil. Members of the genus *Frankia* have been found associated with the roots of 23 genera of dicots belonging to eight families. Historically, strains isolated in pure culture were grouped into two physiological categories, those that use carbohydrates and those that do not. Newer genomic information indicated that frankiae in general differ markedly in their complements of genes. Besides physiological grouping, these isolates were placed into four plant-compatibility groups (1-infective on *Alnus* and *Myrica*, 2-infective on *Casuarina* and *Myrica*, 3-infective on *Elaeagnaceae* and *Myrica*, 4-infective only on *Elaeagnaceae*). A 16S rRNA gene-based phylogenetic study, comprising non-isolated endophytes, yielded four clusters or clades, three of which are symbiotic (1-infective on *Alnus* and *Casuarinaceae* except *Gymnostoma*, 2-non-isolated strains in nodules of *Rosaceae-Datisca-Coriaria-Rhamnaceae*, 3-infective on *Elaeagnaceae* and *Gymnostoma*) and a fourth cluster that groups non-infective and non-effective strains. These groupings have been confirmed on the whole by analysis of other loci. DNA-DNA hybridization studies have yielded 12–15 genospecies, only one of which has been named, *Frankia alni*; one *Candidatus* Frankia datiscae was recently named to accommodate the genome of an endophyte in nodules of *Datisca glomerata*.

The family *Frankiaceae* is close to *Acidothermus, Cryptosporangium, Geodermatophilaceae* (*Geodermatophilus, Modestobacter, Blastococcus*), *Nakamurella, Sporichthya*, and *Fodinicola* and was grouped into suborder *Frankineae*. A recent rearrangement has resulted in the elevation of suborder *Frankineae* to order *Frankiales* (Normand and Benson 2012b) containing families *Acidothermaceae, Cryptosporangiaceae, Frankiaceae, Geodermatophilaceae, Nakamurellaceae*, and *Sporichthyaceae* as well as the *incertae sedis Fodinicola feengrottensis*.

Introduction

Plants have developed interactions with a variety of soil microbes that are either pathogens, loosely associated rhizosphere bacteria, or in rare instances that can generate positive durable interactions, mutualisms, or symbioses, that modify the fitness of plants, allowing them to thrive in nitrogen-poor environments. Actinobacteria grouped in the genus *Frankia* comprise one of these symbiotic lineages that form root nodules in which biological nitrogen fixation takes place.

Following the first descriptions at the end of the nineteenth century of structures on roots of some plant species, including legumes and non-legumes (Woronin 1866), that were correlated with the ability to thrive in nitrogen-poor soils (Hiltner 1896) and with nitrogen fixation (Hellriegel and Wilfarth 1888), *Rhizobium* strains were rapidly cultured and studied (Beijerinck, 1888). It took however close to 90 years for the first *Frankia* isolates to become available (Callaham et al. 1978) due to a slow growth rate, inappropriate media, incomplete sterilization, and

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the likelihood that several strains may be obligate symbionts progressively evolving to have complex growth requirements that cannot be met outside root tissues.

The plants, collectively called "actinorhizal plants" that enter symbioses with *Frankia* are phylogenetically grouped within the so-called Nitrogen-Fixing Clade (NFC, Soltis et al. 1995), but as a group are more deeply diverged than is the case with those infected by "rhizobia." The first ones that were described were those growing in Europe, *Alnus* and *Elaeagnus*, about which Frank in 1887 was hypothesizing that the root "swellings" could be caused by fungi (Frank 1887). This description was followed by other scientists of plants growing in more distant locales, reaching the present number of 23 genera. There may remain a few cryptic actinorhizal plants in poorly explored regions of the world such as New Guinea, central Amazonia, or central Africa. Some plants may also harbor rhizospheric populations of frankiae as in the case of birch (Mirza et al. 2009a), a phylogenetic neighbor of *Alnus* in the *Betulaceae*. Finally, some plants may harbor endophytic *Frankia* in a manner similar to that reported for some actinobacteria such as *Micromonospora* in various plants (Zhao et al. 2011; Kim et al. 2012) including the actinorhizals *Casuarina* (Valdes et al. 2005) and *Coriaria* (Trujillo et al. 2006).

In the past, grouping of *Frankia* strains was attempted based on *in planta* morphology (Becking 1970), on plant compatibility (Baker 1987), on physiology (Lechevalier and Lechevalier 1990), on protein patterns (Gardes and Lalonde 1987) or proteomics (Hahn et al. 2011), on DNA-DNA hybridization (Fernandez et al. 1989), or using sequence comparisons of various genes. These approaches have yielded low resolution clusterings; however, an integrated vision of the structure and of evolutionary history of the genus remains incomplete.

Taxonomy: Historical and Current

Fran.ki.a'ce.ae. N.L. fem. n. *Frankia* type genus of the family; suff. -aceae ending to denote a family; N.L. fem. pl. n. *Frankiaceae* the *Frankia* family. The description is an emended version of the one given in the *Bergey's Manual*, 2nd edition (Normand and Benson, 2012a).

After several failed isolation attempts over more than 60 years (Baker and Torrey, 1979), Pommer (1956), then working at the Botanischen Institut der Technischen Hochschule in Aachen, Germany, succeeded in isolating an actinobacterial strain in pure culture. His drawings of that culture clearly depict a *Frankia* strain comprising typical features such as branched hyphae, typical vesicles, and spores arranged in multilocular sporangia that are formed by cells dividing in three planes. Unfortunately, this strain was subsequently lost. So for a long period of time, scientists interested in this interaction had to be content with studying the microbe *in planta*. Eventually, Becking (1970) created the *Frankiaceae* family containing a single genus and ten species based on cross-inoculations using crushed nodules containing unisolated symbionts and morphological description of symbiotic microbial cells. The description of the *Frankiaceae* was emended by Hahn et al. (1989) to include, besides the genus *Frankia* that contains root symbionts, the genus *Geodermatophilus* whose members thrive in soils and on rock surfaces, and an uncharacterized *Blastococcus* genus containing a single sea sediment isolate. Although its sporangia had a similar morphogenesis, the skin pathogen *Dermatophilus* was deemed too distant and not included in the family. These genera have multilocular sporangia; however, this feature was judged insufficient to warrant placing them in the same family by Normand et al. (1996), particularly considering their many ecological and biochemical differences and the fact that members of the genus *Acidothermus*, although phylogenetically closer to *Frankia* than the others as assessed by 16S rDNA similarity, did not form multilocular sporangia. The *Geodermatophilaceae* is now considered distinct from the *Frankiaceae* and treated in a different chapter of the present book. The *Frankiaceae* presently contains only one genus, *Frankia*. This situation may change since it is also known that several related, presumably slow-growing, soil-inhabiting actinobacteria exist (Normand and Chapelon 1997).

The first confirmed isolation of a *Frankia* strain in pure culture, designated CpI1 (Callaham et al. 1978), was accomplished using a complex procedure involving an enzymatic digestion of host cell wall components of nodules of *Comptonia peregrina*, 8 years after Becking's taxonomic proposals. This success paved the way for the isolation of many additional strains from several plant species using protocols that got progressively simpler. When more strains became available, it became clear that Becking's proposed species based on cross-inoculation groups were untenable. Cross-inoculations with pure cultures showed that isolated strains had broader host ranges than indicated by crushed-nodule suspensions and that *in planta* morphology was under control of the host plant (Lalonde 1979).

Signature sequences have been used to gauge the distribution of frankiae. For example, the 5′-TGCAAGTCGAGCGAGGGGCTT-3′ has been proposed as being genus-specific (Normand and Chapelon 1997), although phylogenetically related uncharacterized soil actinobacteria have been later detected by PCR using this oligonucleotide as a primer. In another approach, a Blast search of the NCBI database yielded several hits in a study targeting nitrogen-incorporating bacteria in petroleum-contaminated arctic soils using ^{15}N DNA-SIP (DNA-Stable Isotope Probing) and pyrosequencing (locus JF397791, Bell et al., unpublished) (Yergeau et al. 2012). A study of *Alnus viridis* rhizosphere using a single *Frankia*-targeted primer yielded two nearly full length 16S rRNA genes that could be from cryptic soil *Frankiaceae* (Normand and Chapelon 1997).

Type genus: *Frankia* Brunchorst 1886, 174. (Brunchorst 1886)

Genus *Frankia*

Fran'ki.a. N.L. fem. n. *Frankia* is named after Albert Bernhard Frank (1839–1900), a German plant biologist working in Leipzig

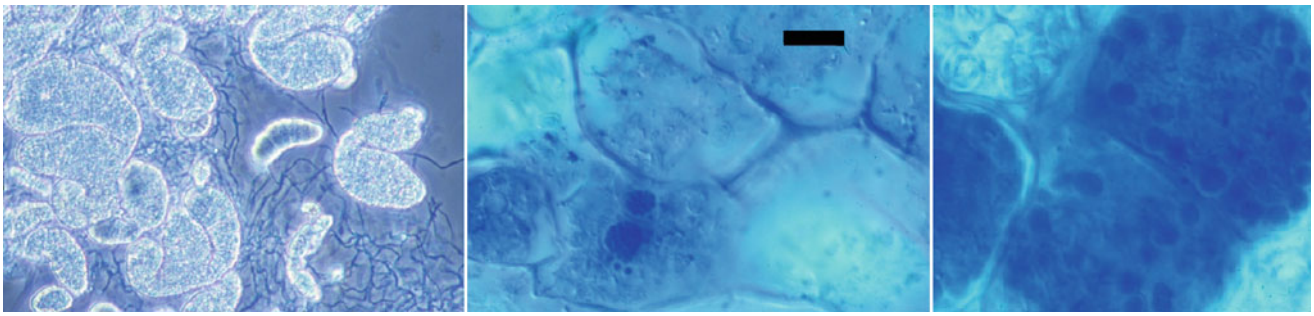

Fig. 18.1
Phase contrast micrograph of *Frankia* strain ARgP5 (clade 1b), isolated from *Alnus incana* subsp. *rugosa* (Normand and Lalonde, 1982) showing hyphae and multilocular sporangia that can reach 50 μm across (1000x). Cotton-blue stained sections of an *Alnus incana* subsp. *rugosa* old nodule induced by strain ARgP5 showing (*center*) unstained senescent vesicles in cortical cells and two stained sporangia, bar is 5 μm, and (*right*) healthy nitrogen-fixing vesicles on the periphery of cortical cells and in neighboring cells unstained amyloplasts. Photos P. Normand

and Berlin, who devoted a major part of his career, from 1877 to 1892, to the study of nitrogen nutrition in legumes and the micro-organisms that caused nodulation on roots. He is also credited with coining the term "symbiosis."

Vegetative hyphae have limited to extensive branching (*Fig. 18.1*), 0.5–2.0 μm in diameter, and occasionally wider in older cultures. No aerial mycelium is formed on solid media. Intra- and extracellular orange, red, or black pigments may be formed. Gram-positive in exponential phase cultures; Gram-variable in older cultures. Aerobic to microaerophilic, with a respiratory type of metabolism. No growth factors are required. Does not grow under anaerobic conditions. Catalase-positive due to two catalases, both expressed in pure culture at a high base level (Santos et al. 2007). Mesophilic. Chemoorganotrophic. Usually very slow-growing with doubling times of 20 h to several days. Most strains are capable of fixing N_2 both in vitro under conditions of nitrogen starvation, and *in planta*, both at atmospheric oxygen concentration and under microaerophilic conditions, a physiological adaptation that has been followed by proteomics (Alloisio et al. 2007). Nonpathogenic to humans and animals (Gordon et al. 1983). Round to irregularly shaped multilocular sporangia are borne terminally, laterally, or in an intercalary position on vegetative hyphae (*Fig. 18.1*). Lateral sporangia are usually borne on sporangiophores; some are sessile. Sporangia are up to 100 μm in length formed by septation in three planes of the cytoplasm of preexisting thin-walled swellings. Sporangiospores are nonmotile, irregular (often somewhat polygonal) in shape, usually colorless, sometimes black, showing multilamellar outer membrane–like layers in thin section. Spores are not thermally resistant. Sporangiospores usually do not develop and mature simultaneously, resulting in the presence of spores of different maturity levels in the same sporangium. Unique terminal or laterally formed "vesicles" or "diazovesicles" may be formed. These structures are terminal hyphal swellings that become increasingly septate with age. They possess envelopes with up to 90 laminated layers composed chiefly of bacteriohopanetetrol and its derivatives (Berry et al. 1993). They are the site of nitrogen fixation in cells deprived of combined nitrogen in culture and, in symbioses, where vesicles are formed (Benson and Silvester 1993). Cell walls contain meso-diaminopimelic acid (meso-DAP), glutamic acid, alanine, muramic acid, and glucosamine. No mycolates are present. Phospholipids comprise phosphatidylinositol mannosides, phosphatidylinositol, and diphosphatidylglycerol.

Vesicles occasionally form in culture, especially under low ammonium aerobic conditions where nitrogen fixation does occur. Vesicles may separate from subtending hyphae upon homogenization, and these vesiclesmay then germinate (Schultz and Benson 1989) (*Fig. 18.2*).

Fatty acids are normal, branched-chain, and mono-unsaturated. Whole-cell sugar patterns show xylose (without arabinose), madurose, or fucose or cells may contain only glucose or galactose. All tested strains contain 2-O-methyl-D-mannose, a very rare "diagnostic" sugar, and most contain rhamnose (Mort et al. 1983). Most strains are symbiotic with certain angiospermous plants, inducing nodules on the roots of suitable hosts. They may be found free-living in soil.

DNA G+C content (mol%): 70–73 (Tm) (as deduced from complete genomes analysis, *Table 18.1*).

Type species: *Frankia alni* (Woronin 1866) Von Tubeuf (1895), 118 (*Schinzia alni* Woronin (Woronin 1866), p. 6).

Species of the genus *Frankia*

1. *Frankia alni* (Woronin 1866) Von Tubeuf 1895, 118AL (*Schinzia alni* Woronin 1866, p. 6) al'ni. L. gen. n. *alni* of alder, the original source of isolation.

This is the only species designated in the genus; all other species proposed previously by Becking (1970) have been shown not to correspond to later descriptions of the genomic species (Akimov and Dobritsa 1992; Akimov et al. 1991; An et al. 1983, 1985; Bloom et al. 1989; Fernandez et al. 1989; Lumini et al. 1996). Hence, the only species still valid is the first ever described

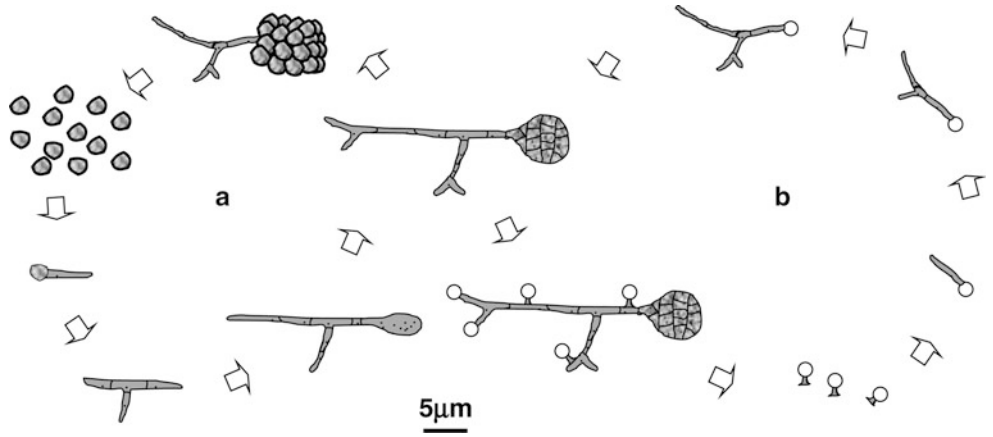

Fig. 18.2

Cell cycles of *Frankia alni*. (a) On the left from the top and counterclockwise, spores germinate and grow into a hypha that will in turn branch and divide from growth at the tip. From one hyphal tip or from an inside cell, an enlargement occurs that divides in all planes to form a sporangium from which individual spores mature. (b) Under conditions of low nitrogen solutes concentrations, vesicles appear at the tip of stalks. If the culture is homogenized, some vesicles may break free, these may germinate, form hyphae that eventually thicken and develop into sporangia. The cycle involving hyphae and spores is the dominant one while the one involving vesicles is much rarer. Drawn from Pommer (1959) and Schultz and Benson (1989)

that corresponds to the one described by Woronin (1866) as present in the roots of alder and shown later by Fernandez et al. (1989) to correspond to the numerically dominant group of strains infective on *Alnus*. Substrate hyphae, chalky white, 0.5–1.2 µm in diameter. Based on DNA-DNA hybridization and direct sequencing of 16S rDNA genes, it can be evaluated there are probably more than 20 genomic species among the isolates described so far and a similar number of unisolated strains living in the soil (Normand and Chapelon, 1997), the rhizosphere of various plants, or other environments. However, given their slow growth rates, it is likely that a rigorous species designation is still a long way in the future and may be based on genome descriptions (below). Nevertheless, 16S rRNA gene sequence characterization work continues to be carried out Clawson et al. 1998; Huguet et al. 2001; Jeong and Myrold 1999; Lumini and Bosco 1999; Navarro et al. 1999; Ritchie and Myrold 1999; Simonet et al. 1999; Wolters et al. 1997; (Clawson et al. 2004; Dai et al. 2004; Gtari et al. 2004; Huguet et al. 2004; Vanden Heuvel et al. 2004; Huguet et al. 2005b; Gtari et al. 2007b, Gtari et al. 2007a) and will be correlated with ecological investigation.

Source: root nodules of host plants belonging to families *Betulaceae* and *Myricaceae*, free-living cells may also be found in soil.

DNA G+C content (mol%): 72.8 [as determined by complete genome sequencing (Normand et al. 2007b); see the Genoscope website for details (http://www.genoscope.cns. fr/externe/English/Projets/Projet_HF/HF.html)].

Type strain: Since the publication of the Approved Lists of Bacterial Names, several strains of *Frankia alni* have been isolated. However, no type strain has been designated. The first strain isolated was HFPCpI1 (Callaham et al. 1978) with HFP standing for "Harvard Forest in Petersham" and CpI standing for "C*omptonia* p*eregrina* i*solate*"; it was deposited in the ATCC but, as is the case with several of the slow-growing *Frankia* strains, it could not be revived (although it is still available from diverse laboratories) and is no longer available there. Another strain, AvcI1 is available from the ATCC, but this strain has not been studied in much detail. For these reasons, another strain, ACN14a isolated from *Alnus crispa* growing in Tadoussac, Canada (Normand and Lalonde 1982), that has only two substitutions in the 16S rRNA gene (Normand et al. 1996) relative to HFPCpI1, has been widely distributed in different laboratories, and was chosen as model organism for whole genome sequencing. Sequence accession numbers: M88466 (16S rRNA gene; *Frankia alni* strain ACN14a), CT573213 (complete genome; *Frankia alni* strain ACN14a). More recently, the genome of cluster#1 *Alnus*-infective strain QA3 (NZ_CM001489.1) has also become available but in a non-finished state. It thus remains non-published as of August 2012 and it remains uncertain if this strain belongs to the same species as ACN14a; however, the 16S rRNA of the two strains diverges by only 1.1 % (unpublished).

There have been several studies devoted to defining genomic species among isolated strains. The first one (An et al. 1985) studied a limited number of infective strains and proposed one species with nine *Alnus*-infective isolates (cluster 1), one with a single *Elaeagnus*-infective (cluster 3) isolate and several unclustered isolates. Later, Fernandez et al. (1989) proposed 9 genomospecies among the 43 isolates analyzed, three of them in the *Alnus*-infective group of strains (cluster 1), five in the *Elaeagnus*-infective group (cluster 3), and one in the *Casuarina*-infective group (cluster 1). There were however few common strains between the two studies making it impossible to say if these numbers are to be added to those previously described except for *Frankia alni* strains CpI1 and ArI3. Lumini et al. (1996) also used this approach with *Elaeagnus*-infective

Table 18.1

Characteristics of genomes of genus *Frankia*[a]

Trait	Fa *Frankia alni* strain ACN14a	Fc *Frankia* sp. strain CcI3	Fe *Frankia* sp. strain EAN1pec	Fd *Frankia* symbiont of *Datisca glomerata* (Dg)	EuI1c *Frankia* sp. strain	EUN1f *Frankia* sp. strain	QA3 *Frankia* sp. strain	CN3 *Frankia* sp. strain	BCU110501 *Frankia* sp. strain	BMG5.12 *Frankia* sp. strain
Clade	Ia	Ib	III	II	IV	III	Ia	IV	III	III
Genome size (nt)	7,497,934	5,433,628	8,982,042	5,323,336	8,815,781	9,322,173	7,590,853	9,978,592	7,891,711	7,589,313
G+C%	72.83	70.08	71.15	70.04	70.82	72.31	72.56	71.81	72.39	71.67
# of genes	6,795	4,621	7,250	4,579	7,264	7,833	6,493	8,333	6,742	6,253
# of tRNA	46	46	47	45	46	47	46	68	47	51
# of rRNA operons	2	2	3	2	3	3	2	3	2	2
Protein coding density %	86.31	84.94	83.71	78.15	86.10	84.06	82.04	83.73	84.66	85.50
Genome accession number	CT573213	CP000249.1	CP000820.1	CP002801	ADGX00000000	NC_014666	CM001489.1	AGJN00000000	ARDT00000000	ARFH00000000

[a]Characteristics of all of the *Frankia* genome projects including published databases (Normand et al. 2007, Persson et al., 2011) and released databases that are publicly available on the Integrated Microbial Genomes (IMG) website (http://img.jgi.doe.gov) as of July 1, 2012. Several other genome sequencing programs are presently underway, targeting strains with unusual phenotypic characteristics (shaded)

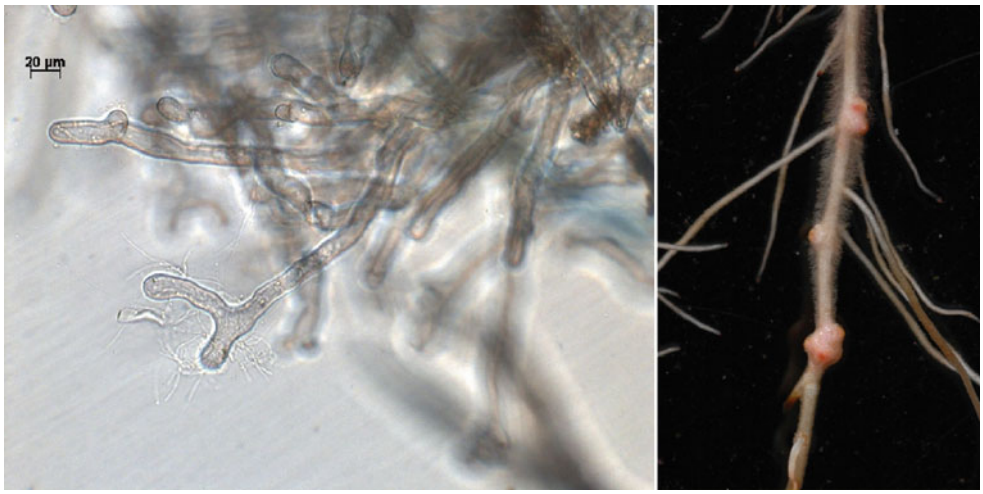

Fig. 18.3
***Alnus glutinosa* root hairs deformed and branched following inoculation (7 dpi) with *Frankia alni* strain ACN14a in growth pouches. After 3 weeks, a succession of small nodules appears on the roots; their red color is not due to hemoglobin, rather to anthocyanins. Photos courtesy of Petar Pujic, Université Lyon1**

strains, in particular those able to cross the inoculation barrier between *Elaeagnus* and *Alnus*, resulting in three further species. None of these publications used phenotypic approaches and as a consequence, none proposed naming species.

A "*candidatus*" species has been proposed for the strain present in nodules of *Datisca glomerata*, named *Candidatus* Frankia datiscae (Persson et al. 2011). Numerous isolation attempts have been made for strains of cluster 2; however, none were successful. A few attempts at cross-inoculation among potential host groups have been made, and successful nodulation of *D. glomerata* has been achieved with nodule inoculum from *Ceanothus griseus* nodules (Okubara et al. 1999).

Other species, *F. elaeagni* and subspecies *F. alni* subsp. *pommerii* and *F. alni* subsp. *vandijkii*, have been proposed by Lalonde and coworkers (1988), but these were not based on recognized approaches and have thus been abandoned.

Phylogenetic Structure of the Family and Its Genera

16S rRNA sequence analyses (*Fig. 18.3*) have resulted in genus *Frankia* being divided into four clusters with *Acidothermus* as closest neighbor and *Geodermatophilaceae* together with *Sporichthya, Nakamurellaceae*, and *Cryptosporangium* forming the Frankineae (Normand and Benson 2012b).

The cluster identified as the root of the *Frankia* lineage was #4 (non-infective, non-efficient *Alnus*-infective), then cluster #2 infective on *Rosaceae-Datiscaceae-Coriariaceae-Rhamnaceae* with clusters 3 (*Elaeagnaceae, Gymnostoma, Myricaceae*), and #1 (*Alnus, Casuarinaceae, Myricaceae*) emerging later (Normand et al. 1996). A similar topology, without considering cluster #4, was obtained by Clawson et al. (2004) using the same marker concatenated with *glnA*. A reassessment based on 16S rRNA gene and a large number of outsiders positioned cluster #4 at the root (Normand and Benson 2012b). A reassessment using the highly variable intergenic 16S-23S rRNA intergenic region also positioned cluster #4 at the root, but followed by cluster #3 and then clusters #2 and #1 (Ghodhbane-Gtari et al. 2010), a topology also recovered using a concatenate of *gyrB, nifH* and *glnII* genes (Nouioui et al. 2011). The topology obtained has been found to vary according to outgroups used, to the strains studied, to the genes regions used, and to the tree-reconstruction algorithm used. All in all, the four clusters are consistently recovered, but their respective positions vary and thus should be clarified using genome-wide approaches when a sufficiently large number of such genomes would have become available. A similar inconsistency was found to occur in the *Frankineae* forming or not a clade together comprising *Acidothermus* (*Acidothermaceae*), *Cryptosporangium* (*Cryptosporangiaceae*), *Geodermatophilaceae*, (*Geodermatophilaceae*), *Nakamurella* (*Nakamurellaceae*), and *Sporichthya* (*Sporichthyaceae*). This phylogenetic proximity between *Frankia, Geodermatophilus obscurus* and *Blastococcus aggregatus* was initially detected through reverse transcriptase sequencing and oligonucleotide cataloging of their 16S ribosomal RNA (Hahn et al. 1989). However, Barabote et al. (2009) found that although *Acidothermus* is the closest neighbor of *Frankia* when using the 16S rRNA gene sequences, BBH (Bidirectional Best Hits) protein sequences from *Acidothermus* were approximately equivalent to the more remote *Streptomyces* spp. Wu et al. (2009) studying a concatenate of 31 "universal" proteins could not recover *Frankia* and *Geodermatophilus* together in a clade. This question thus also remains open until a sufficient number of genomes from these neighbors become available.

These differences illustrate the difficulty of using a single marker to reconstruct the phylogeny of the genome as a whole, presumably because of numerous lateral transfers including those of the ribosomal genes that occur when microbes share a common niche following migrations. Presumably such transfers are more

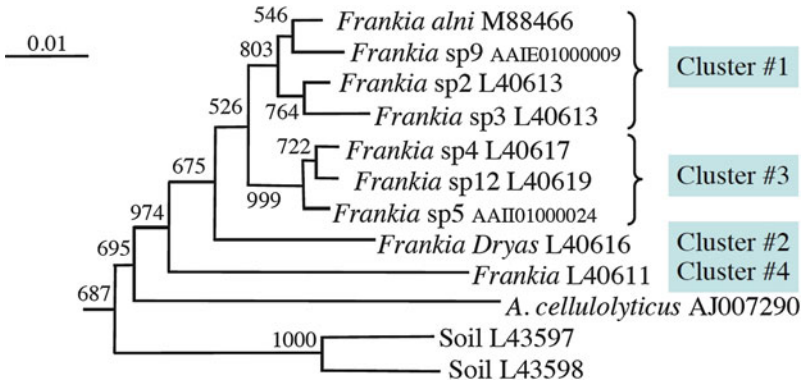

Fig. 18.4

Phylogenetic tree of *Frankia* and related taxa based on 16S analysis. The tree-reconstruction algorithm used was the Neighbor-Joining and the bootstrap results are indicated at branches (out of 1,000 replicates) and the scale bar represents 0.01 substitution per site. Unpublished

probable when genomes are large, as occurs in soil microbes. It appears inevitable that with the mounting number of genomes and with appropriate software, the complex evolutionary history of gene transfers, gene duplications, and gene loss will become clearer, although perhaps never satisfactorily. In this respect, it will be interesting to study genomes of microbes that coexist with *Frankia* in root tissues such as *Micromonospora* present in *Casuarina* (Valdes et al. 2005) and *Coriaria* (Trujillo et al. 2006).

Another marker that has been studied is the *nif* gene cluster, because it is emblematic of the symbiosis and because it is highly conserved and thus easy to recover through PCR or hybridization. NifH was used as early as 1989 to discern whether genes had been laterally transferred since the topology was distinct from that of 16S rRNA. The conclusion reached was "probably" (Normand and Bousquet 1989). A study of NifK yielded similarly uncertain results (Hirsch et al. 1995). The topology of NifH shows the gene in *Frankia* to be monophyletic, close to the Cyanobacteria while the Proteobacteria are further away and the Firmicutes even more so (*Fig. 18.4*). However, the vision that has recently emerged is that Nif determinants have undergone a complex series of events including duplications, modification of the metal cofactor (Fe, V, Mo), divergent adaptation to aerobic or anaerobic biotopes (Fani et al. 2000; Raymond et al. 2004; Boyd et al. 2011), illustrating the danger of using any single marker to infer the history of the genome as a whole. Nevertheless, the monophyly of actinobacteria and their placement as sister group to cyanobacteria appears to be a solid conclusion (Hartmann and Barnum 2010). This placement would suggest that other actinobacterial lineages have lost their *nif* genes over the time since the emergence of the Actinobacteria phylum, an event that could have happened as early as 2.4 BY ago before the rise of oxygen in the atmosphere, based on proteins molecular analyses and cell wall structure (Lake 2009), a conclusion also reached by considering physiological innovations present in actinobacteria such as the proteasome, sterol synthesis, and exospores (Cavalier-Smith 2002).

The current order *Frankiales*, as described in the latest edition of the Bergey's (Normand and Benson 2012b), thus presently comprises *Geodermatophilaceae, Nakamurellaceae, Sporichthyaceae, Acidothermaceae*, and the *Cryptosporangiaceae* as well as the *incertae sedis* species *Fodinicola feengrottensis* isolated from a medieval mine rock. The *Cryptosporangiaceae* family was previously positioned in the *Kineosporiaceae*, together with *Kineosporia* (Lilburn and Garrity, 2004) but this last family was split and the *Kineosporia* excluded to the newly created *Kineosporiaceae* together with genus *Quadrisphaera* in the latest *Bergey's Manual of Systematic Bacteriology*, 2nd edition.

Molecular Analysis

Three *Frankia* genomes sequences were published in 2007, for strains belonging to three of the known cross-inoculation groups, the first infective on *Alnus*, the second on *Casuarina* (both cluster #1), and the third on *Elaeagnus* (cluster #3) (Normand et al. 2007b). Analysis of these genomes provides some insight of these bacteria and of their evolutionary history. Besides the lack of canonical *nod* genes, a major conclusion from that study was that the three genomes had major differences in their size (5.4–7.5–9.0 Mb, see *Table 18.1*) despite having high similarity in 16S rRNA genes (1–2 % difference). Genome size correlated with host-plant range, as the genomes underwent major insertion-sequences-supported recombinations.

Since the first published report on the three *Frankia* genomes (Normand et al. 2007b), several sequencing projects on *Frankia* genomes representing the four major lineages have been initiated and are in various stages of completion including a report on a cluster 2 strain in symbiosis with *Datisca glomerata*. At present there are 11 *Frankia* genomes that have been or currently being sequenced. These sequencing projects include 3 genomes from Cluster I (ACN14a, CcI3 and QA3), 1 from Cluster II (Fd or Dg), 4 for Cluster III (EAN1pec, EUN1f, BCU110501, and BMG5.12) and 3 for Cluster IV (EuI1c, CN3 and DC12). As of July 2012, only five of these genome databases (strains ACN14a, CcI3, EAN1pec, Fd and EUI1c) have been completed and added

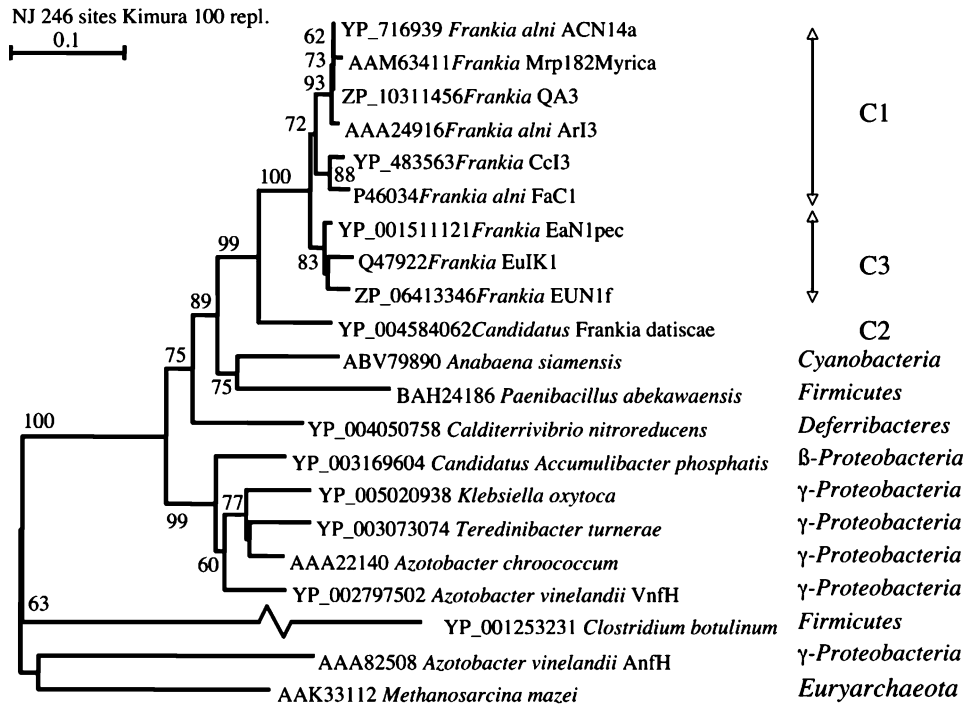

Fig. 18.5

Neighbor-Joining phylogenetic tree of NifH of *Frankia* sequences present in the NCBI data bank as well as representative sequences of other phyla. The bar represents 0.1 substitution/site. Unpublished

to the NCBI GenBank. The *Frankia* EAN1pec draft genome first reported by Normand et al. (2007) was completed and subsequently updated in GenBank. Three genomes (EUN1f, QA3, and CN3) are close to completion and have draft genome information publicly available. Two more genomes (BMG5.12 and BCU110501) are in production and the DC12 genome is in post-draft sequence finishing (*Fig. 18.5*).

An initial analysis of these eight *Frankia* genomes has provided further insight on these bacteria and their diversity. First, the correlation between host-specific range and genome size appears to be further supported. Those strains that have a wide-host-plant range had very large genomes (8.8–9.1 Mb range), while those strains with a narrow host-plant range or are potentially obligate symbionts have the smallest genomes (5.3 Mb). The medium host-range strains had a genome size that was in between the previous two (7.5 Mb). The cluster #4 strain studied had the largest genome sizes (10 Mb). Furthermore, the metabolically diverse *Frankia* groups (Cluster III and IV) were larger than the other two clusters (*Fig. 18.6*).

Five *Frankia* genomes are in databases and there should soon be about a dozen, allowing to discern better and better the core genome as new ones are added. Using the first four genomes (excluding EuI1c that does not fix nitrogen), we could extract at a threshold of 30 % of similarity in AA sequences 2,966 genes (43 %), of which 862 are characterized as "unknown function" (*Fig. 18.6*). When those genes that are also present in the 16S rRNA phylogenetic neighbors *Acidothermus cellulolyticus*, *Blastococcus saxobsidens*, *Geodermatophilus obscurus*, and *Modestobacter marinus* as well as in *Streptomyces coelicolor* are removed, the number of genes in the *Frankia*-specific core genome drops to 291 genes (4.2 %), presumably containing those genes necessary for establishment of the symbiosis and nitrogen fixation. When these are mapped onto the genome of *Frankia alni*, no strong clustering is observed (*Fig. 18.6*).

Plasmids

Prior to the genome sequencing, a few *Frankia* strains had been shown to possess plasmids (Normand et al. 1983) and several of these plasmids were sequenced (John et al. 2001; Lavire et al. 2001; Xu et al. 2002). In the post-genomic era, integrative conjugative plasmids have been identified as being present in these *Frankia* genomes (te Poele et al. 2008; Ghinet et al. 2011).

Phages

No phage has been described for the *Frankia* genomes; however, no specific work has been done for that purpose. A search in the *Frankia* genome databases for "phage" features revealed several occurrences. Since these genes are not conserved among the genomes and there is no major syntenic region, they are thus likely to represent either events that have occurred since the emergence of the different clades, or are undergoing pseudogene conversion events. The *Frankia alni* ACN14a genome has two clusters of 5–7 genes each (FRAAL 2679–2684, FRAAL 2743–51) that contain mycobacteriophage genes (*Fig. 18.6*),

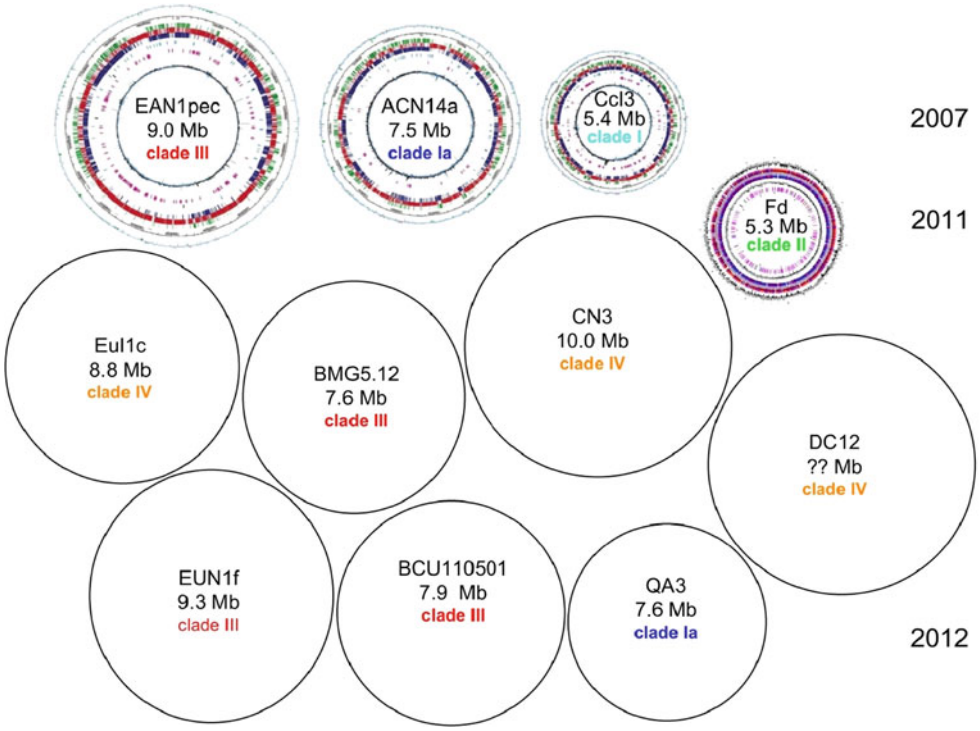

Fig. 18.6

Schematic representation of *Frankia* genomes already published in 2007 and 2011 (*top*) and those not yet published (*bottom*). The color code is clade 1 in *blue*, clade 2 in *green*, clade 3 in *red*, and clade 4 in *orange*. The size of the genomes are proportional to their real size with the largest being the clade 4 (10 Mb), followed by the clade 3 (9 Mb), the clade 1a (7.5 Mb), the clade 1 (5.4 Mb), and the smallest being the clade 2 (5.3 Mb) (Courtesy of Nick Beauchemin, University of New Hampshire, Durham, NH)

while the *Frankia* QA3 genome has one cluster (FraQA3DRAFT_2742-58) (*Table 18.2*). In the case of *Frankia* ACN14a, the similarity is low and several intervening genes are present, reducing the likelihood these clusters could be functional prophages.

The availability of genomic tools may allow an understanding how the symbiotic partners modify their expression pattern upon establishment of the symbiosis and such studies have been started. Whole genome arrays have been used on *Frankia alni* to compare symbiotic cells to in vitro grown cells, showing several clusters upregulated such as those coding for *nif*, *hup*, and *suf* (Alloisio et al. 2010).

On the host plant side, no genome project has been undertaken so far; however, an EST collection of 15,000 unigenes for both *Casuarina* and *Alnus* has permitted to compare roots to nodules 21 days after inoculation and to detect various putative symbiotic genes (Hocher et al. 2011).

Phenotypic Analysis Symbiotic Relationships

Symbiotic Relationships

Virtually all *Frankia* strains available in culture have been isolated from or described in the context of the plant hosts from which they have been isolated. Many additional strains have been identified by gene sequences obtained from field-grown root nodules during studies on strain diversity or population structure (Benson et al. 1996; Clawson et al. 1998, Clawson et al. 1999; Ritchie and Myrold 1999; Simonet et al. 1999; Huguet et al. 2001; Clawson et al. 2004; Huguet et al. 2004; Oakley et al. 2004; Vanden Heuvel et al. 2004; Huguet et al. 2005a; Huguet et al. 2005b; Mirza et al. 2009b; Welsh et al. 2009; Ghodhbane-Gtari et al. 2010; Kennedy et al. 2010; Mishra et al. 2010; Pokharel et al. 2011).

There are 23 genera of dicotyledonous plants, belonging to eight families, that have established symbiotic N_2-fixing root nodules with *Frankia* (Benson and Silvester 1993). The symbiosis is called "actinorhizal" and infected plants are referred to as "actinorhizal plants" (Torrey 1983). Suitable plant hosts (*Table 18.3*) are readily infected by most frankiae, giving rise to nitrogen-fixing (effective) nodules on the roots. Some strains give rise to non-effective nodules and still others are not infective on the host plant from which they have been isolated, under tested conditions. The latter strains are often infective on plants belonging to the family *Elaeagnaceae* (Gauthier et al. 1981; Clawson et al. 2004). Infection in the *Casuarinaceae*, *Myricaceae*, and *Alnus* takes place through root hairs (*Fig. 18.7*) after an induced deformation occurs (Callaham et al. 1979; Berry 1983; Berry and Sunell 1990; Liu and Berry 1991), whereas infection in other plants proceeds by intercellular penetration into the root (Miller and Baker 1985;

Table 18.2
Phages features in *Frankiaceae* genomes

Feature	*Fa*	*Fc*	*Fe*	*Fd*	*EuI1c*	*EUN1f*	*QA3*	*CN3*
Phage occurrence (# of CDS containing word "phage")	11	16	11	5	7	20	13	8
Phage clusters	2						1	

Liu and Berry 1991). In cases where an "elaeagnus strain" infects plants from other families, the infection route is dictated by the plant be it via intercellular penetration or through root hair (Miller and Baker 1986; Racette and Torrey 1989). The molecular mechanisms whereby the plant recognizes the microbial symbiont and forms nodules are not currently known, except for the recently demonstrated expression of homologs of SymRK, the receptor-like kinase that is necessary for legume nodule and mycorrhiza formation, in nodules of *Casuarina* (Gherbi et al. 2008) and *Datisca* (Markmann et al. 2008), and the demonstration of the presence in *Alnus* and *Casuarina* transcribed genes of homologs of the whole symbiotic cascade described in Legumes (Hocher et al. 2011).

In *Frankia* genomes, canonical *nod* genes have not been identified (Normand et al. 2007a) and the factor that deforms *Alnus* root hairs, that may or not be the symbiotic effector, has only been characterized as hydrophilic, thermostable, and sensitive to some enzymes such as pronase (Ceremonie et al. 1999). This root hair deforming factor is also known to be specific to actinorhizals, as the *Rhizobium* Nod factor did not deform *Alnus* root hairs nor did the *Frankia* deforming factor deform Legumes hairs (Van Ghelue et al. 1997).

The synthesis of plant hormones such as indole-3-acetic acid or IAA (Berry et al. 1989), 2-phenylacetic acid or PAA (Hammad et al. 2003), or cytokinins (Stevens and Berry 1988) may play a role in modification of the plant root developmental program.

Lectins may help binding of the bacterial cells to the plant surfaces (Pujic et al. 2012).

The phylogenies of actinorhizal plants (Swensen and Mullin 1997) and the associated bacteria do not overlap exactly, which has led some to conclude that coevolution may have occurred (Jeong et al. 1999), especially in the case of *Casuarina*/*Allocasuarina* host plants (Simonet et al. 1999) but geographical co-occurrence may have been a major factor in the case of *Frankia* Cluster #2 strains in Western US (Oakley et al. 2004), as may also have been the case of the *Casuarinaceae Gymnostoma* nodulated by Cluster #3 *Frankia* (Navarro et al. 1997).

The time of appearance of the symbiosis has been debated and two types of evidence have been presented (Clawson et al. 2004; Swensen and Benson 2008). In the fossil record, eudicots appear before 125 Myr at the Barremian-Aptian boundary of the lower Cretaceous as triaperturate pollen grains that are diagnostic for the eudicots (Crane et al. 1995). The oldest identifiable actinorhizal lineages leading to the *Betulaceae* and *Myricaceae* occur in pollen dated 95-90MY (Thomas and Spicer 1987; Maggia and Bousquet 1994; Sims et al. 1999). Using molecular clock estimates, the Rosid I clade, containing all nitrogen-fixing lineages of Angiosperms, is thought to have radiated over a ~17 Myr period, 108–91 Myr ago (Wang et al. 2009). Given uncertainties in constructing a molecular clock, and the vagaries of the fossil record, the origin of actinorhizal plants is not readily resolvable. In a recent analysis of the origins of nodulation in the entire NFC, Doyle (2012) posits multiple origins of nodulation within the larger plant clade. Within the actinorhizal members of the NFC, the most likely scenario seems to be a single origin of the capability to nodulate (Hocher et al. 2011) followed by losses and, more rarely gains of the phenotype (Swensen and Benson 2008). As noted by Clawson et al. (1998), the lack of identifiable fossils from an actinorhizal lineage does not mean that the symbiosis did not exist prior to the deposition of fossils. Likewise, the absence of any recognizable actinorhizal lineage in the mid-Cretaceous does not mean that the ancestors of *Frankia* were not participating in plant symbioses before the lineages became established. In effect, it is difficult if not impossible to draw strong conclusions about the early evolution of the actinorhizal symbiosis.

Ecology

Natural Habitats

Actinorhizal plants generally thrive in open spaces, especially where soils are poor in nitrogen. Many of these biotopes are natural, for instance, forests after fires, beach dunes, mountain screes, glacier moraines, and volcanic lava fields (*Fig. 18.8*). Many others are man-made, for instance, road sides or rail tracks, mine spoils, rubble heaps, forests or fields after harvests, and channel banks.

Glacier Retreat

One of the best documented instances of plant succession following glacier retreat is the case of Glacier Bay, Alaska, that has been followed over close to a century (Cooper 1923; Crocker and Major 1955; Lawrence et al. 1967; Reiners et al. 1971). The recorded succession goes from pioneer species (lichens and liverworts), to nitrogen-fixing creeping shrubs such as avens (*Dryas*), to bush trees such as alders (*A. viridis* subsp. *crispa*), and finally to the climax spruce forest community over a span of

Table 18.3
Actinorhizal plant genera and associated groups of *Frankia* strains

Order	Family (nod genera/total)	Genus (# species)	Geographical distribution[1]	16S rRNA cluster[2]	Penetration mode[3]	Common English name
Fagales	*Betulaceae* (1/6)	*Alnus* (30)	Northern hemisphere temperate, tropical mountains	1, 3, 4	RHI	Alder
	Myricaceae (3/4)	*Comptonia* (1)	Eastern NA	1,3	RHI	Sweet fern
		Morella (20)	Global ≠ Australia	1,3 1		Bayberry, yumberry, wax myrtle
		Myrica (2)	Circumboreal			Sweetgale, bog myrtle,
	Casuarinaceae (4/4)	*Allocasuarina* (58)	Australia	1	RHI	Sheoak, tamma
		Casuarina (17)	Australia	1		Sheoak, filao, ironwood
		Ceuthostoma (1)	Malaysia	nd		
		Gymnostoma (10)	Malaysia to western Pacific	3		Rhu Bukit
Rosales	*Elaeagnaceae* (3/3)	*Elaeagnus* (10)	Europe, Asia, NA	3	ICP	Olive, oleaster, silverberry
		Hippophae (2)	Eurasia	3		Sea-buckthorn, sandthorn, sallowthorn, seaberry
		Shepherdia (2)	NA	3		Buffaloberry, bullberry
	Rhamnaceae (6/55)	*Ceanothus* (55)	Western NA	3, 2	ICP	Buckthorn,snowbrush, California lilac, New Jersey tea
		Colletia (17)	Southern SA	3		Crucifix thorn, anchor plant
		Discaria (15)	SA, Australia, NZ	3		Matagouri
		Kentrothamnus (1)	Southern SA	3		NA
		Retanilla (4)	Southern SA	3		NA
		Trevoa (1)	Southern SA	3		NA
	Rosaceae (4/100)	*Cercocarpus* (6–10)	Western NA	2	ICP	Mountain mahogany
		Chamaebatia (2)	Western NA	2		Mountain misery
		Dryas (2–3)	Circumboreal	2		Aven
		Purshia (8)	Western NA	2		Bitterbrush, cliff-rose, antelope Bush
Cucurbitales	*Coriariaceae* (1/1)	*Coriaria* (5–20)	Mexico to SA, W Mediterranean, NZ, Papua NG, SE Asia	2	ICP	Redoul, Tutu
	Datiscaceae (1/1)	*Datisca* (2)	Western NA, South Asia	2	ICP	Durango root

The *Rhamnaceae Adolphia* has also been suggested to be actinorhizal but this report has not been confirmed. *Adolphia infesta* (Kunth) Meisn. and *Colubrina infesta* (Kunth) Schltdl. are synonymous with basionym *Ceanothus infestus* Kunth.
Notes
1-NA stands for North America, SA for South America, NZ for New Zealand, NG for New Guinea.
2-The numbers refer to those attributed by Normand et al. (1996).
3-RHI stands for "root hair deformation" and ICP for "intercellular penetration."

more than 100 years. The soil underwent a concomitant evolution, going from a nitrogen-poor sandy soil to a nitrogen-rich sandy loam. The ecological role of the nitrogen-fixing plants is considered to be that of facilitation, rather than required for succession (Chapin et al. 1994).

In that regard, other glaciers in other parts of the world have been thus studied but actinorhizals there have so far not been found to play a similar key role, for instance, in the case of the Alpine Evettes glacier (Pech et al. 2007) or the Scandinavian Svalbard glacier (Cannone et al. 2004).

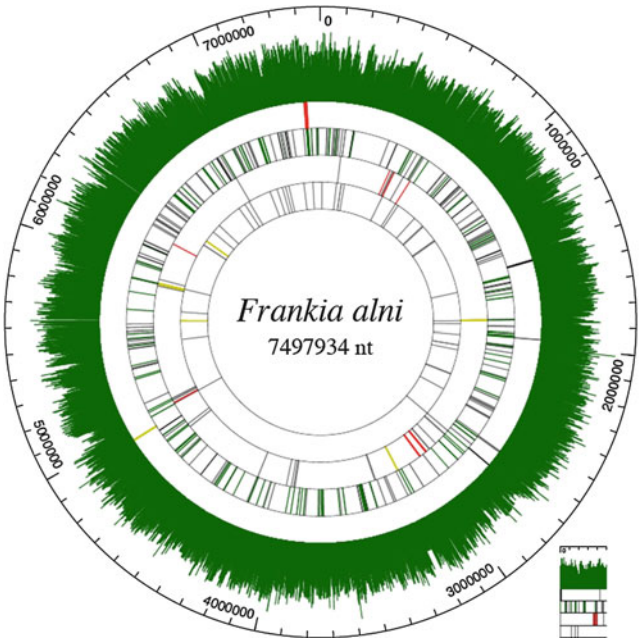

Fig. 18.7
Frankia **circular map with from the outside 1-GC (range 60–80 %, in *green*) 2-*Frankia* symbiotic genes (*shc* genes in *black*, *hup* genes in *gray*, *suf* genes in *yellow*, *nif* genes in *red*) 3-*Frankia* specific genes (at a threshold of 30 % identities over 80 % of the length of the shortest, present in ACN14a, CcI3, EaN1pec and Dg (Fd), absent in *A. cellulolyticus, B. saxobsidens, G. obscurus, M. marinus*, and *S. coelicolor*; unknown in *gray*; conserved in *black*; others in *green*) 4-Transposases (*gray*), phage genes (*red*) and plasmid genes (*yellow*) 5-rRNA (*yellow*) and tRNA genes (*gray*). Unpublished**

Forest Fires

Forests can evolve from pioneer stages to climax, storing enormous amounts of carbon and other nutrients in their biomass and that of the fauna they shelter. They nevertheless burn following dry spells, volcanic eruptions, or even human interventions, resulting in massive loss of nitrogen from the site. If left to themselves, forests may then initiate again a drawn-out succession, that will start with pioneer plants, nitrogen-fixers being among the most successful ones. In northwestern North America, for example, sitka alder (*A. viridis* subsp. *sinuata*) is among the first species to invade following fires (Uchytil, 1989).

Volcanoes

Volcanoes have shaped our planet for millions of years and continue to do so to this day. One of the largest eruptions recorded in human history occurred on the Island Krakatau in 1883 and sent ashes into the upper atmosphere that colored the sky of the whole planet for several months. Ten years later, botanical studies showed that the actinorhizal *Casuarina* was recolonizing the island (Bordage 1916). Hawaii is an archipelago in the Pacific situated atop a hot point, resulting in the regular emission of very fluid lava that covers the ground. This nitrogen-poor substrate is presently being colonized by the actinorhizal *Myrica faya*, because native plants are too demanding to be competitive (Vitousek et al. 1987). Sakurajima in the Kagoshima Prefecture on the Japanese Southern Island of Kyūshū, Japan, is a stratovolcano that has erupted regularly and has been followed by teams of botanists, who have observed *Alnus firma* as among the first to colonize the ashes and establish thickets (Kucho et al. 2010).

Applications

Plantations

Few actinorhizal plants are large enough or solid enough to be used for furniture, although red or black alder, planted on numerous sites, are used by in the Western USA (http://redalder.net/; http://www.alibaba.com/showroom/red-alder-furniture.html).

Few actinorhizal plants produce edible parts, the exceptions being *Hippophae rhamnoides*, *Elaeagnus angustifolia* var. *orientalis*, and *Myrica rubra* that are planted on a large scale for that purpose. *H. rhamnoides* (sea buckthorn) ranges naturally from Western Europe to Japan and is planted on a large scale in Europe and North America for its fruits that are processed as juice, jelly, fruit paste, wine, or into liquor. The vitamin C, E, and A contents are high, and this constitutes a marketing argument for this health food (Beveridge et al. 1999). Various *Elaeagnus* species produce edible seeds, few of which are commercialized, except for *E. angustifolia* var. *orientalis* sold on markets in Turkey and Iran as mountain ash or Trebizond date. *M. rubra* known as Chinese bayberry or yumberry is an economically important crop in China and Japan, and is sold fresh or dried, and can be canned, pressed for juice, or for alcoholic beverages. There are also numerous websites that give long lists of health benefits proposed to be accrued from its use, ranging from antioxidants, treatment of arthritis, high blood pressure, diabetes, whitening skin, etc. A related species, *Myrica nagi*, grows in the Northeastern hills of India, producing fruits, locally called kaphal, that are harvested in the forests.

Secondary Metabolites

Although many actinobacteria have been utilized for their production of chemically diverse and biologically potent natural products, the potential of *Frankia* is only slowly being uncovered. Prior to genome analysis, only one antimicrobial agent, frankiamide, had been identified (Haansuu et al. 2001; Klika et al. 2001). Bioinformatic analysis of the first three *Frankia* genomes revealed a metabolic potential for production of novel secondary metabolites (Udwary et al. 2011). The existence of lectins in the genomes of *Alnus*-infective *Frankia alni*

Fig. 18.8
The *Elaeagnaceae* buffaloberry (*Shepherdia canadensis*) growing on a railroad track ballast alongside the St-Lawrence river in Les Eboulements, PQ, Canada. The *Myricaceae* sweet fern (*Comptonia peregrina*) growing on a beach dune in Ogunquit, ME, USA. Photos P. Normand

strain ACN14a may also find applications (Pujic et al. 2012), in particular, as some ricin-type lectins have been shown to bind viruses (Tanaka et al. 2009). The promise of this metabolic potential warrants further consideration.

The host plants also are a source of metabolites. Alder species in particular have been used traditionally for the treatment of various diseases, they have been used as an astringent, cathartic, emetic, febrifuge, hemostatic, parasiticide, skin tonic, vermifuge, etc., and were found recently to contain hundreds of metabolites causing NO and TNF-α production, HIV inhibition, antibacterial activity, antitumor activity, etc. (Sati et al. 2011).

Windbreaks

Production of fruits, vegetables, and cereals in coastal areas is hampered by salt-laden and sand-carrying winds. Windbreaks are thus often planted to shelter crops and many papers have been written to describe these assays. One site that was thus protected early is the seafront in Senegal between Dakar and St-Louis, planted over an area of 500 km by 500 m (25,000 ha) and that, to this day, is still thriving (Dommergues 1995). Similar protection has been conferred on the Southern China coast (Zhong et al. 2010) and elsewhere (Dawson 1986). At the moment, there are 300,000 ha planted in southern coastal China (Zhong et al. 2011), 500,000 ha in coastal India (Rawat et al. 2011), and 100,000 ha in coastal Vietnam (Nghia et al. 2011). These plantations that would amount to about a million hectares are also used as a source of fuel and construction wood.

Mine Spoils

Mining operations are among the most environmentally damaging industries, yielding, among other problems, spoils that affect the countryside, pollute streams, and impact and damage wildlife populations. Some places on earth have become highly impacted by toxic fumes and topsoil removal, as is the case of the nickel mining operations around Sudbury, Canada, or in New Caledonia. Alder, for instance, has been found to withstand severe soil conditions, *Alnus glutinosa* is used to establish cover on coal mine spoils in Ohio (Funk 1973), *Alnus viridis* subsp. *sinuata* on copper mine spoils in western USA (Uchytil 1989), *Alnus rubra* on coal mine spoils in Washington, and *A. viridis* subsp. *crispa* on tar sand spoils in Alberta (Roy et al. 2007). Various *Gymnostoma* species have been widely planted in New Caledonia on nickel spoils (Hery et al. 2005) and *Casuarina* and *Myrica* on bauxite spoils in Jamaica. However, no comprehensive assessment of the effect of such plantations on water quality or wildlife health or may be has been made so far.

Hydroelectric Projects

Hydroelectric dams, if they are considered on the whole environmentally friendly, are nevertheless industrial scale projects that entail moving millions of tons of rock to create dykes that in turn direct water toward turbines to generate electricity (*Fig. 18.9*). These dykes must be stabilized to ensure durability and should also be covered rapidly by vegetation for aesthetic and wildlife management purposes.

Few plants can grow on these rocky substrates at polar latitudes because they are poor in nutrients, especially in nitrogen. Yet, a few subarctic pioneer plants are known to colonize open surfaces, in particular green alder (*A. viridis* subsp. *crispa*), which is why they were used in attempts at initiating ecological successions, with more than seven million seedlings inoculated with *Frankia* and planted (Périnet and Lalonde 1983). Therefore, hundreds of thousands of nodulated seedlings were planted, for instance, in the Marguerites project where 765,000 seedlings of alder, spruce, and pine were used on the shores to speed up establishment of an extensive plant cover

Fig. 18.9
On the *left* is an aerial photo of a HydroQuebec dam in Northern Quebec (Nemaska, Cree Nation territory, James Bay area, Canada) with dykes stabilized with plantations of green alder (*Alnus viridis* subsp. *crispa*). On the *right* is a close-up of green alder seedlings planted on a nitrogen-poor rocky substrate. Photos Martin Lessard, Montreal

(http://www.hydroquebec.com/sustainable-development/documentation/pdf/autres/pop_25_01.pdf).

Ornamental Plants

Several actinorhizals are planted for ornamental purposes, especially when the soil is poor and there is little money to add topsoil. Russian olive (*Elaeagnus angustifolia*) and autumn olive (*E. umbellata*) with their lush silvery foliage are but two of the long list of species of the genus that are planted as hedges around houses, parks, and highways in Europe and North America. They thrive so well that they are now considered invasive and several US states advise their removal. *Hippophae rhamnoides* is also planted for similar reasons in Europe and in California, Oregon, and Washington; growers sell alders, bitterbrush, and ceanothus species. Italian alder (*A. cordata*) is a sturdy tree with a lush foliage and large female catkins that is planted along roads and parking lots in Europe. Casuarinas are planted for the same reason in tropical countries, for instance, *C. cunninghamiana* planted around parking lots and along highways. Several Ceanothus hybrids are sold in garden shops for planting in gardens, for instance, *Ceanothus* x *delileanus* Gloire de Versailles with its large blue inflorescences.

Construction

The city of Venice was built starting from the time of the Roman Empire over hundreds of muddy islands by driving millions of alder poles through the mud. This permitted to form piles to support construction of massive buildings that have withstood some fifteen centuries and have resisted decay to this day. The Roman architect Vitruvius thus spoke of using alder poles to establish piles in muddy soil (De Architectura, II, IX, 10 e 16 – (I sec. a.C.); http://www.turismo.ravenna.it/eng/Discover-the-area/People_-history_-traditions/Famous-personages/Strabo-and-Vitruvius) : "The alder, which grows on the banks of rivers, and is to appearance an almost useless wood, possesses nevertheless most excellent qualities, inasmuch as it contains much air and fire, not a great deal of earth, and less water. Its freeness from water makes it almost eternal in marshy foundations used for piling under buildings, because, in these situations, it receives that moisture which it does not possess naturally. It bears immense weights and does not decay. Thus we see that timber which above ground soon decays, lasts an amazing time in a damp soil....."

Conclusion

Frankia offers the curious distinction of being one of the first microbes to have been hypothesized to exist, at the end of the nineteenth century, because it caused visible nodules on the roots of *Alnus* and *Elaeagnus*, yet could not be obtained in pure culture before 1978 when the strain CpI1 was obtained and distributed worldwide to be studied (Callaham et al. 1978).

Another dubious distinction of *Frankia* is that it is probably the taxon with the lowest number of described species despite having thousands of isolated strains studied and described (Normand and Benson 2012a). This is partly due to the fact that *Frankia* contains several strains that have defied isolation in pure culture, that many isolated strains that can be cultivated nevertheless grow very slowly making these difficult to treat with approaches such as DNA-DNA hybridization and to study

phenotypically, which is recognized as the standard to define and name species.

The study of the genomes of these microbes is also hindered by the lack of a genetic transformation system. Nevertheless, as more and more strains are isolated and classified into new species, as more and more genomes are obtained, as more and more genes are studied in heterologous hosts, as several soil metagenomes are studied, we should see the evolutionary history of one of the most contrasted microbial lineage unfold.

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19 The Family *Gaiellaceae*

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Abstract
Gaiellaceae, a family within the order *Gaiellales*, comprises the genus *Gaiella*. The order *Gaiellales* represents a distinct phylogenetic lineage based on 16S rRNA gene sequence analysis. The Actinobacteria include several deep-branching lineages that consist of species of the genera *Rubrobacter*, *Solirubrobacter*, *Patulibacter*, *Conexibacter*, *Thermoleophilum*, and more recently *Gaiella* (Albuquerque et al. Syst Appl Microbiol 34:595–599, 2011; Reddy and Garcia-Pichel. Int J Syst Evol Microbiol 59, 87–94, 2009). This organism was isolated from a deep mineral water borehole, it is strictly aerobic, catalase- and oxidase-positive, utilizing several organic compounds. The most distinctive characteristic is the presence of iso internally branched fatty acids found in no other bacterium (Albuquerque et al. Syst Appl Microbiol 34:595–599, 2011). Since only one species is known, we will treat the order *Gaiellales* and the family *Gaiellaceae* on equal terms. The type strain is F2-233^T (=CECT 7815^T = LMG 26412^T) and strain F2-223 (=CECT 7816) is an additional strain of this species.

Taxonomy: Historical and Current

Short Description of the Order and Family

Gaiellales Albuquerque et al. 2012

Gaiellales (Ga.i.el.la'les, N.L. fem. dim. n. *Gaiella*, type genus of the order; suff. -ales, ending to denote an order; N.L. fem. pl. *Gaiellales*, the *Gaiella* order).

Members of the order *Gaiellales* are non-spore-forming. The order represents a distinct phylogenetic lineage based on 16S rRNA gene sequence analysis. The order *Gaiellales* belongs to the class *Actinobacteria*. The order contains the family *Gaiellaceae* (Albuquerque et al. 2011).

Gaiellaceae Albuquerque et al. 2012

Gaiellaceae (Ga.i.el.la.ce'a.e. N.L. fem. dim. n. *Gaiella*, type genus of the family; suff. -*aceae*, ending to denote a family; N.L. fem. pl. *Gaiellaceae*, the *Gaiella* family).

The members of the sole family *Gaiellaceae* of the order *Gaiellales* stain Gram-negative and form rod-shaped cells. These organisms are strictly aerobic and chemoorganotrophic. The peptidoglycan contains *meso*-Dpm as diagnostic diamino acid. Internally methyl-branched iso-fatty acids are present. Menaquinone 7 (MK-7) is the major respiratory lipoquinone. The type and only genus of this family is *Gaiella* (Albuquerque et al. 2011).

Phylogenetic Structure of the Order, Family, and Its Genus

Comparison of the 16S rRNA gene sequence of *Gaiella occulta* with sequences in the public data bases demonstrated that this genus represents a deep-branching lineage of the Actinobacteria that branches within the radiation of the genera *Rubrobacter* (Carreto et al. 1996; Chen et al. 2004; Suzuki et al. 1988), *Thermoleophilum* (Zarilla and Perry 1984; Zarilla and Perry 1986), *Patulibacter* (Kim et al. 2012; Reddy and Garcia-Pichel 2009; Takahashi et al. 2006), *Conexibacter* (Monciardini et al. 2003; Seki et al. 2012) and *Solirubrobacter* (An et al. 2011; Kim et al. 2007; Singleton et al. 2003). These genera currently form four orders *Rubrobacterales* (genus *Rubrobacter*), *Thermoleophilales* (genus *Thermoleophilum*), *Solirubrobacterales* (genera *Solirubrobacter*, *Conexibacter*, and *Patulibacter*), and *Gaiellales* (*Gaiella occulta*). *Gaiella occulta* shares between 87 % and 88 % pairwise 16S rRNA gene sequence similarity with the species of the orders *Thermoleophilales* and *Solirubrobacterales* and just 85 % with the species of the order *Rubrobacterales* (*Fig. 19.1*). This lack of a close relationship with these existing orders is also supported by the low bootstrap value for the branching of the *Gaiella oculta* lineage with the other deep-branching actinobacterial lineages including the order *Coriobacteriales* (Albuquerque et al. 2011).

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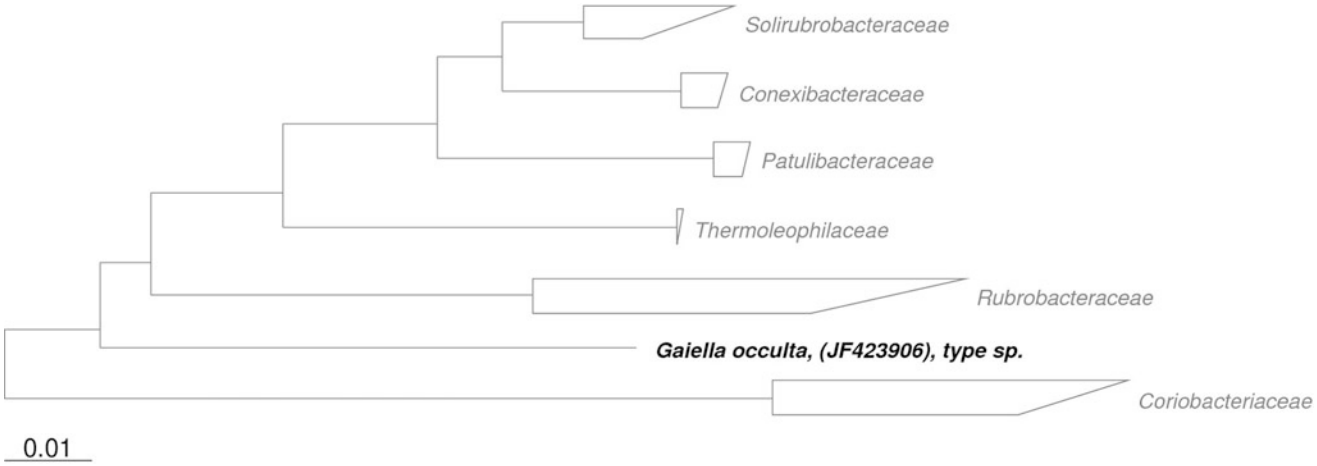

Fig. 19.1

Phylogenetic reconstruction of the family *Gaiellaceae* 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

Phenotypic Analyses

The main features of members of the orders *Gaiellales, Solirubrobacterales, Rubrobacterales, and Thermoleophilales* are listed in *Table 19.1*.

The sole species of the order *Gaiellales* can be distinguished from the species of the other deep-branching orders of the Actinobacteria by several characteristics that seem to be order specific and to reflect the phylogeny based on 16S rRNA gene sequence analysis. For example, *Gaiella occulta* (strains F2-233^T and F2-223) are mesophilic, like the species of the order *Solirubrobacterales*, while the members of the order *Rubrobacterales* and *Thermoleophilales* are thermophilic with optimum growth temperatures ranging between about 45 °C and 60 °C. The order *Gaiellales* can be differentiated from these three orders by their novel internally branched iso-fatty acids. The species of the *Rubrobacterales* have straight chain internally branched fatty acids, namely, $C_{16:0}$ 12-methyl and $C_{18:0}$ 14-methyl instead of internally branched iso-fatty acids; the members of the order *Solirubrobacterales* possess primarily straight chain monounsaturated fatty acids. However, the fatty acids of the *Thermoleophilales* have not been examined and a full comparison of these chemotaxonomic markers cannot be made at this time. The members of the *Solirubrobacterales* possess MK-7(H_2), MK-7(H_4) or DMK-7 instead of MK-7 found in strains F2-233^T and F2-223, while the species comprising the *Rubrobacterales* possess MK-8. The peptidoglycan of *Gaiella occulta* contains *meso*-Dpm like the species of the orders *Solirubrobacterales* and *Thermoleophilales*, while species of the order *Rubrobacterales* have a different type, containing L-Lys.

Gaiella Albuquerque et al. 2012

The family *Gaiellaceae* comprises one genus composed of a sole species represented by strains F2-233^T (=CECT 7815^T = LMG 26412^T) and F2-223 (=CECT 7816) isolated from a deep mineral water aquifer in Portugal.

Gaiella [Ga.i'el.la. N.L. dim. fem. n. *Gaiella*, named after Gaia, Greek goddess of the earth, referring to the origin of the organism (i.e., the earth)]. This genus comprises one species *Gaiella occulta*.

Gaiella occulta forms nonmotile short rod-shaped cells with 1.0–3.0 μm in length and 0.3–0.5 μm in width and stain Gram-negative. The members of this species are strictly aerobic, and oxidase- and catalase-positive. The optimum growth temperature is about 35–37 °C in liquid R2A medium and the cultures are non-pigmented; growth does not occur at 15 and 45 °C. The optimum pH for growth is between 6.5 and 7.5; growth does not occur at pH 5.0 or pH 9.0. No growth occurs in media containing 1 % NaCl. Poor growth occurs on medium R2A solidified with agar. Nitrate is reduced to nitrite. Gelatine is degraded; esculin and hippurate are not degraded. Alkaline phosphatase, esterase (C 4), esterase lipase (C 8), acid phosphatase, naphthol-AS-BI-phosphohydrolase are positive in the API ZYM, other activities are negative. The organism assimilates a few sugars, as well as *myo*-inositol, organic acids, and amino acids, namely, D-glucose, D-fructose, D-mannose, D-ribose, D-xylose, α-ketoglutarate, DL-lactate, acetate, pyruvate, glutamate, L-alanine, L-asparagine, L-lysine, proline, L-glutamine, L-arginine, L-serine, and ornithine. A large number of sugars, organic acids, and amino acids are not utilized: D-galactose, L-rhamnose, L-fucose, L-sorbose, D-arabinose, L-arabinose,

Table 19.1

Phenotypic and chemotaxonomic characteristics of the orders *Gaiellales, Solirubrobacterales, Rubrobacterales, and Thermoleophilales*

	Gaiellales[a]	*Solirubrobacterales*[a, b, c]	*Rubrobacterales*[a]	*Thermoleophilales*[a, d]
Morphology	Rods	Rods	Pleomorphic	Rods
Pigmentation	Non-pigmented	Non-pigmented/pink/yellow	Pink	Non-pigmented
Gram-stain	Negative	Positive	Positive	Negative
Motility	–	Variable	–	–
Temperature for growth (°C)				
Range	20–42.5	5–46	30–70	45–70
Optimum	35–37	28–38	46–60	60
pH for growth				
Range	5.5–8.5	5.0–10.0	6.0–11.0	6.0–7.5
Optimum	6.5–7.5	6.5–9.0	7.0–8.0	6.8–7.0
NaCl for growth (%)				
Range	0	0–4	0–6	0–nd
Metabolism	Aerobic	Aerobic	Aerobic	Aerobic
Reduction of nitrate	+	Variable	+	nd
Presence of				
Oxidase	+	Variable	+	nd
Catalase	+	+	+	+
Assimilation aliphatic hydrocarbons	–	nd	nd	+
Resistant to radiation	–	nd	+	nd
Peptidoglycan type	A1γ′	A1γ′	A3α′	nd
Diagnostic peptidoglycan amino acids[e]	*meso*-Dpm	*meso*-Dpm	L-Lys	*meso*-Dpm
Major fatty acids	Internally branched iso-	Unsaturated straight chain, iso-, anteiso-	Straight chain internally branched	nd
Polar lipids[f]	PL(s), PGL(s)	PI, DPG, PG, PGL, PL(s), GL(s), AL(s), UL(s)	DPG, PG, PGL, GL, PL(s)	nd
Major respiratory lipoquinone[g]	MK-7	MK-7(H_4)/MK-7(H_2)/DMK-7	MK-8	MK-7(H_4)
G + C content (mol%)	71.6	70.6–75.0	64.9–68.5	70.0–70.4

Symbols: +, positive; –, negative; nd, not determined

[a]Albuquerque et al. 2011

[b]Kim et al. 2012

[c]Seki et al. 2012

[d]Collins et al. 1986

[e]*meso-dpm meso*-diaminopimelic acid, l-*Lys* L-lysine

[f]*DGP* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *AL(s)* unknown aminolipid(s), *GL(s)* unknown glycolipid(s), *PGL(s)* unknown phosphoglycolipid(s), *PL(s)* unknown phospholipid(s), *UL(s)* unknown lipid(s)

[g]*MK* menaquinone, *DMK* demethylmenaquinone

sucrose, maltose, lactose, D-cellobiose, D-trehalose, D-raffinose, glycerol, ribitol, xylitol, sorbitol, mannitol, erythritol, D-arabitol, L-arabitol, succinate, malate, citrate, benzoate, fumarate, formate, D-gluconate, D-glucoronate, aspartate, glycine, L-histidine, valine, L-phenyalanine, L-leucine, L-isoleucine, methionine, and threonine. Aliphatic hydrocarbons, namely, hexane, heptane, *n*-decane, dodecane, *n*-hexadecane, petroleum ether, L-chlorobutane, and toluene, are not used either. The peptidoglycan type is A1γ' (*meso*-Dpm directly cross-linked; glycine at position 1 of the peptide subunit). Major fatty acids are iso-$C_{17:0}$ 10-methyl, iso-$C_{17:0}$, and iso-$C_{15:0}$ 8-methyl. Polar lipids consist of three unidentified phospholipids and two unidentified phosphoglycolipids. The G + C content of the DNA is 71.6 mol%. Unlike the species of the genus *Rubrobacter*, this organism does not survive exposure to a 1 kGy dose of gamma ionizing radiation (Albuquerque et al. 2011).

Isolation, Enrichment, and Maintenance Procedures

Gaiella occulta was isolated from the vent water of a deep mineral water aquifer (150 m) (Albuquerque et al. 2011). Large sample volumes (250–1,000 ml) were filtered through membrane filters (Gelman type GN-6; pore size 0.1 μm; diameter 47 mm). The filters were placed on the surface of solidified R2A agar medium. This organism grew very poorly on medium solidified with agar, growth was better in liquid R2A medium. The strain was routinely cultured in liquid R2A medium at 37 °C.

Gaiella occulta does not require special procedures for maintenance and long-term storage. Can be stored frozen at −70 °C in R2A medium containing 15 % glycerol without loss of viability. Long-term preservation is by lyophilization.

Ecology

Habitat

The only strains known were isolated from one deep aquifer in Portugal, supplying a mineral water bottling plant. The borehole water has a temperature of 28 °C, a pH of 5.9, and is very poor in mineral ions (Albuquerque et al. 2011). Other environments are unknown. *Gaiella occulta*, unlike the species of the genus *Rubrobacter*, was not resistant to 1 kGy dose of gamma ionizing radiation. Other closely related environclones (about 98 % 16S rRNA sequence similarity) have been identified in soil (EU335248), water distribution systems (JF922500, JF922567), and a shallow lake (GQ472411).

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20 The Family *Geodermatophilaceae*

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Abstract
Members of the family *Geodermatophilaceae*, order *Actinomycetales*, contain bacteria isolated mainly from soils, seawater, and stone surfaces that have been grouped into the three genera *Geodermatophilus*, *Blastococcus*, and *Modestobacter*. Members of *Geodermatophilus* have been found mainly in soils, *Blastococcus* spp. have been found in a marine sediment and in stone interiors and *Modestobacter* spp. have been found on stone surfaces and in polar regolith. Members of the genera *Geodermatophilus* and *Modestobacter* have been found to be unusually resistant to oxidative stresses while those of *Blastococcus* were found resistant to some heavy metals and metalloids. This family is close to the genera *Acidothermus, Cryptosporangium, Frankia, Nakamurella, Sporichthya*, and *Fodinicola* and was grouped with them into suborder *Frankineae*. A recent rearrangement has resulted in the elevation of suborder *Frankineae* to order *Frankiales* (Normand and Benson, Order VI. *Frankiales* ord. nov. In: *Bergey's Manual of Systematic Bacteriology*, vol 5. The Actinobacteria. Bergey's Manual Trust, Athens, pp 509–511, 2012) containing families *Acidothermaceae*, *Cryptosporangiaceae*, *Frankiaceae*, *Geodermatophilaceae*, *Nakamurellaceae*, *Sporichthyaceae*, and *Fodinicola.*

Introduction

Common sense tells us that stones are devoid of life, given there are basically no organic substrates for organotrophs and little for chemolithotrophs. Many stones also contain high concentrations of chemicals that constitute fierce challenges to microbial cells such as reactive metals and metalloids. Besides, there is, in general, little free water available to microbial life. Finally, stones have varied origins but most have undergone episodes of high pressure and/or high temperature that are adverse to most microbial taxa. Yet stones, especially sedimentary ones such as those of calcareous origin, crumble, eventually forming soils and sediments. Stone degradation is a time-requiring process that comprises colonization of outer layers, local pH modifications together with physicochemical processes such as acid secretion and exudates that attack calcium carbonate, thus forming niches for sturdy, non-demanding microbes.

Stone surfaces exposed to air constitute a biotope generally considered as punishing to microbial life. On top of being nutrient poor, they are exposed to direct or indirect solar radiations, to temperature extremes, and to desiccation cycles. Yet, there are nutrients in rain water that washes the stones, there is wind-borne carbon-laden dust, and there is some percolation from the soil solution carrying nutrients. Furthermore, the surfaces are full of micro-depressions where microbial cells can get a grip. Therefore, robust radiation-resistant microbes can colonize stone surfaces as is well known by city managers who have to fight deterioration of old stone buildings brought about by a combination of physicochemical and biological agents.

Soils in general constitute very permissive biotopes, inhabited by an extremely varied menagerie considered to be the richest

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_180,

among biotopes (Torsvik et al. 2002). However, dry desert soils are an exception to this vision as the lack of water is a strong selection factor for microbes, killing many, and also for plants. Since plants constitute the dominant source of carbon in soils, desert soils are not only dry but also carbon poor and thus select for an adapted microflora of sturdy, non-demanding microbes able to withstand long episodes of famine. Regolith, the crumbly material originating from stone flaking brought about by cycles of thawing, water infiltration, and freezing that occur under polar conditions, is also a very inhospitable biotope when compared to soil.

These three biotopes, stone interiors, stone surfaces, and desert soils, have been found to be colonized by strains phylogenetically related, belonging to the *Geodermatophilaceae*. They represent unusual examples of adaptation to extreme biotopes. They are also unusual in that they belong phylogenetically to the actinobacteria; yet, they have no or only rudimentary hyphae. Nevertheless, the three lineages are related as storms can uplift desert soils and carry particles over long distances (Chuvochina et al. 2011a, b), as stones surfaces are attacked by physicochemical factors, leading to cracks, fissures, and flaking (Gomez-Alarcon and de la Torre 1994; Welton et al. 2003; De Graef et al. 2005) and these detritus are in turn released, fall into the soil, or are carried away by winds.

Taxonomy: Historical and Current

Geo.der.ma.to.phi.la′ce.ae. N. L. masc. n. *Geodermatophilus* type genus of the family; -aceae ending to denote a family; N. L. fem. pl. n. *Geodermatophilaceae* the *Geodermatophilus* family (Modified from *Bergey's Manual*). The description is an emended version of the one given in the *Bergey's Manual*, 2nd edition (Normand and Benson 2012a).

The genus *Geodermatophilus* was grouped together with the genus *Frankia* into family *Frankiaceae* that was thus emended based on the similarity in 16S rRNA sequences as well as similarity in a morphological feature, multilocular sporangia (Hahn et al. 1989). This arrangement was maintained by subsequent works such as the second edition of *The Prokaryotes* (Akkermans et al. 1992). The family *Geodermatophilaceae* was created by default in 1996 (Normand et al. 1996) based on 16S rRNA gene analysis when the family *Frankiaceae* that initially contained *Geodermatophilus* was split to be left with only genus *Frankia*. That arrangement was maintained the following year (Stackebrandt et al. 1997) when the two families, each then containing a single genus, were pooled into a suborder, Frankinae, in the new organizational scheme proposed for the Actinobacteria. A formal description of family *Geodermatophilaceae* was finally published (Normand 2006) to contain three genera *Geodermatophilus* (Luedemann 1968) found in soils, *Blastococcus* (Ahrens and Moll 1970) found in sea sediments, and *Modestobacter* (Mevs et al. 2000) found in polar regolith, three genera that have strikingly different physiological features (*Table 20.1*).

After a long period with a modest rate of description of new species, the last two years have seen the description of several new species, most of them based on a single isolate. As this chapter goes to press, this rush of new species (three in genus *Geodermatophilus* all in the year 2012, two in genus *Modestobacter*, one in 2011 and the other in 2012) has made the task of the authors more difficult as it was impossible to get all data in time.

Genus *Geodermatophilus*

Geo.der.ma.to.phi.lus. Gr. n. *ge* earth; Gr. n. *derma*, *atos* skin; Gr. adj. *philos* loving; N.L. masc. n. *Geodermatophilus*, a group of microorganisms that live in the soil, yet that supposedly love the skin, by analogy to the genus *Dermatophilus* (*Micrococcineae*; *Dermatophilaceae*), another actinobacterial genus that causes a skin disease and has similar morphological features.

The genus *Geodermatophilus* was the first member of the family to be described by Luedemann (1968) from microbiological work on soils from the Amargosa Desert of Nevada, USA. Colonies of a black-pigmented, friable organism appeared repeatedly from cultures of such soil samples from various deserts. The name given was based on the fact the organism was from soils (Geo from the greek γή), had a morphological feature reminiscent of another actinobacterium, *Dermatophilus*, namely, multilocular sporangia and the fact its pigmentation was intense and black (*obscurus*). There were no true hyphae, only a few small fragmented filaments, cuboid cells, and motile zoospores. Division was found to occur by a budding process. The germinating spores become septate. They also produce either a short tube or longer, flexible hyphae with transverse septations.

Since *Dermatophilus* is a known animal pathogen, causing skin infections, *Geodermatophilus* was also tested on animals (Gordon and Perrin 1971). Infection studies in rabbits and guinea pigs did not permit to establish the pathogenicity of the different isolates available at the time and it is now considered nonpathogenic.

The morphology of *Geodermatophilus* is similar to that of *Dermatophilus*. It is now considered these morphological similarities constitute a case of convergent evolution of a few striking features rather than phylogenetic similarity as the genetic distance in the 16S rRNA genes between *Geodermatophilus* and *Dermatophilus* is very large, between 10 % and 12 % (unpublished). Several types of cell are found (*Fig. 20.1*).

The morphology appears to consist of two forms called R-forms (motile bud-forming rods) and C-forms (coccoids) according to Ishiguro and Wolfe (1970). The cell wall of the R-form consists of two layers with an inner transparent membranous layer (10–12.5 nm thick) and an outer dense diffuse layer (7.5–10 nm). In addition to these, the C-form has a supplementary thick fibrous layer (30 nm) over the dense layer that would act as a mortar to hold coccoid cells together. An unidentified factor present in Difco Tryptose appears necessary to maintain the cells in the C-form as well as to trigger change from the R-form to the C-form. The C-form consists of sporangia and vegetative cells that would be resting forms able

Table 20.1
Characteristics of the three genera of the family *Geodermatophilaceae*

Characteristic	*Geodermatophilus*	*Blastococcus*	*Modestobacter*
Morphology			
Hyphae	Rudimentary	–	–
Spores	Motile	Motile	Motile
Sporangia	+	–	–
Buds	+	+	+
Colony pigmentation	Orange, red to black	Orange	Initially pink turning black
Physiology			
O_2 requirements	Aerobic	Aerobic/microaerophilic	Aerobic
Temperature range (°C)	18–40	3–40	0–35
Temperature opt (°C)	24–28	25	19–21
Tolerance to 3 % NaCl	+	+	+
Hydrolysis of starch	+	–	–
Hydrolysis of D-glucose	+	+	–
Chemistry			
Menaquinone	9 (H_4)	9 (H_4)	9 (H_4)
DNA G + C content (mol%)	73–76	70–74	69–74
Resistance			
Gamma radiation (D_{10}, Gy)	9,000	900	6,000
UV radiation (D_{10}, kJm^{-2})	3,500	6	900
Desiccation (60 days)	20 % viability	0 % viability	10 % viability
Mitomycin C (40 min)	100 % viability	0 % viability	100 % viability
H_2O_2 (lethal dose, %)	30	5	30
Ag^{2+} (MIC in mM)	0.1	0.1	0.1
AsO_4^{3-} (MIC in mM)	50	85	10
Co^{2+} (MIC in mM)	0.3	0.5	0.5
Cr^{3+} (MIC in mM)	0.5	20	2
Cu^{2+} (MIC in mM)	1	3	1
Ni^{2+} (MIC in mM)	0.5	1	1
Pb^{2+} (MIC in mM)	30	30	30
Biotope	Desert sands, soil, rhizosphere, deteriorated stones	Marine sediments, internal part of rocks	Regolith, beach sands, rock surfaces

[a]From Mevs et al. (2000), Urzi et al. (2004), Lechevalier (1989), and Gtari et al. (2012)

to withstand adverse conditions while the R-form is motile and dividing and would be more active (*Fig. 20.2*).

DNA G+C content (mol%): 72.9–75.9.

Type species: *Geodermatophilus obscurus* Luedemann 1968, 1857[AL].

Recently, a second species *G. ruber* was described for a single isolate obtained from the rhizosphere soil of the medicinal plant *Astragalus membranaceus* (Zhang et al. 2011) growing in Qinghai Province, in northwest China. The name chosen to designate the species was related to the most prominent distinguishing feature, colony pigmentation that was red instead of black as in *G. obscurus*. However, since this new species was based on the study of a single isolate, it remains to be seen whether this feature is constant and indeed if all features are general. In particular, *G. obscurus* was found to have higher than usual resistance to gamma irradiation (Rainey et al. 2005; Ryjenkov et al. 2005; Xiang et al. 2005; Cash et al. 2006; Chanal et al. 2006; Chevrot et al. 2006; Nakabachi et al. 2006; Amato et al. 2007a, b; Bordenave et al. 2007; Cheng and Foght 2007; Liao et al. 2007; Markert et al. 2007; Meyer and Kuever 2007; Bagwell et al. 2008; Callegan et al. 2008; Fredrickson et al. 2008; Hoehenwarter et al. 2008; Kumar et al. 2008; Lapouge et al. 2008; Manickam et al. 2008; Hervas et al. 2009; Hervas and Casamayor 2009; Yuan et al. 2009; Login et al. 2011; Gtari et al. 2012) and it should be possible to determine if this resistance is a generic feature or if it is linked to the habitat.

More recently, a third species, *G. nigrescens*, has been proposed for a single soil isolate obtained from a dry-hot river valley

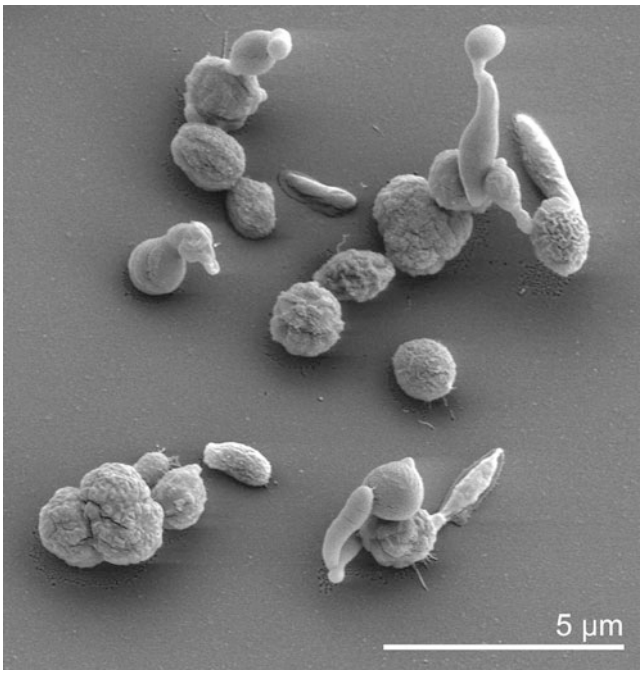

Fig. 20.1
Scanning electron micrograph of *Geodermatophilus obscurus* G-20T, courtesy of Prof. Manfred Rohde, Helmholtz Centre for Infection Research, Braunschweig, Germany (Ivanova et al. 2010)

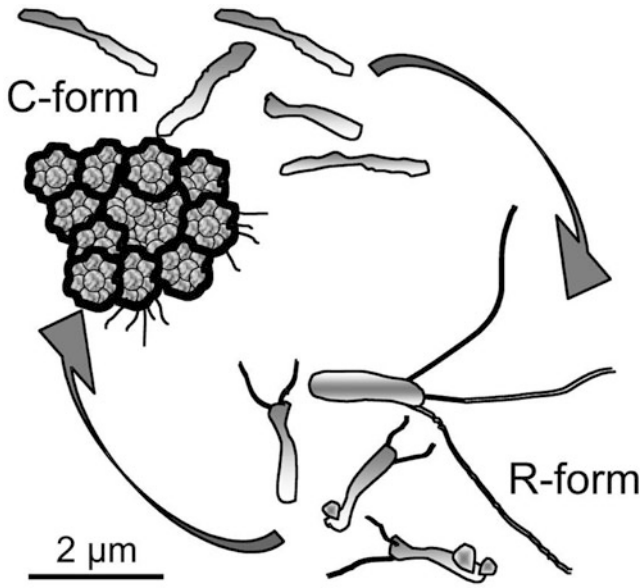

Fig. 20.2
Drawing of *Geodermatophilus obscurus* inspired from Ishiguro and Wolfe (1970) and others showing the two forms, C-forms or coccoid cells and R-forms or motile budding rods

in Dongchuan county, Yunnan province, in southwest China (Nie et al. 2012). Because once again a single isolate was studied, it is impossible to determine if the discriminating features listed are indeed common to the species as a whole. This species was proposed in the journal *Antonie van Leeuwenhoek* and later validated in the IJSEB (Official publication date 17 September 2012 Validation List N° 147 in IJSEM Online), the name is thus considered validly published.

Lastly, a fourth, a fifth, and a sixth species have been proposed by the same authors to describe isolates obtained from arid sands from the African country of Chad. Two of these species, called *G. arenarius* (Montero-Calasanz et al. 2012) and *G. siccatus* (Montero-Calasanz et al. 2013), are single-isolate species that can grow at high temperature, form pigmented colonies, and have very high G+C% with *G. arenarius* having seemingly the highest known G+C% with 75.9 %. A sixth species, *G. telluris*, DSM 45421, has been isolated from a desert soil sample and has just been accepted for publication in IJSEM (HP Klenk, pers. comm.).

Other species isolated from dry sandy soils are under study and should be published in the coming years (HP Klenk, pers. comm.).

DNA G+C content (mol%): 72.8–75.9.

Type species: *Geodermatophilus obscurus* Luedemann 1968, 1857AL (Table 20.2).

Species of the Genus *Geodermatophilus* Luedemann 1968, 1857[AL]

1. *Geodermatophilus obscurus* Luedemann 1968, 1857[AL]

ob.scur'us. L. masc. adj. *obscurus* dark, obscure, indistinct.

Black-pigmented colonies (Fig. 20.3). Gram-positive. Forming highly irregular tuber-shaped non-capsulated multilocular thalli. These structures contain highly irregular vibrioid or cuboid cells, 0.5–2.0 mm in diameter. Under favorable environmental conditions, the thalli will release individual nonmotile cells. A proportion of these cells will develop into elliptical zoospores propelled by a terminal tuft of long flagella. Mycelium is rudimentary. No aerial mycelium is produced. Cell walls contain meso-DAP as well as glutamic acid, alanine, glucosamine, and muramic acid. Aerobic. Chemoorganotrophic. Mesophilic. Thalli appear greenish-black and vary in size with the strain. Colonies after 30 day incubation at 28 °C are flat, black, (Fig. 20.3) granular, dry, with an earthy smell. Good growth on yeast extract-starch-sucrose-malt extract agar.

DNA/DNA hybridization was not minimum standard practice when the known isolates of *Geodermatophilus obscurus* were simply characterized morphologically and trophically into five subspecies: *obscurus*, *amargosae*, *utahensis*, *dictyosporus*, and *everesti* (Table 20.3).

Source: the type strain was isolated from soil of the Amargosa Desert, Nevada, USA.

DNA G+C content (mol%): 72.9–74.6 (Luedemann and Fonseca 1989).

Type strain: ATCC 25078, DSM 43160, NBRC 13315, JCM 3152, NRRL B-3577, VKM Ac-658, CBS 237.69, IAM 14282, JCM 3152, KCC A-0152, NBRC 13315.

Sequence accession no. (16S rRNA): L40620.

2. *Geodermatophilus ruber* Zhang 2011, 192[VP]

(ru'ber. L. masc. adj. ruber red).

Table 20.2

Discriminating physiological and morphological features of the five species of genus *Geodermatophilus*

	G. arenarius	*G. nigrescens*	*G. obscurus*	*G. ruber*	*G. siccatus*
Pigmentation	Light-red, brown	Red turning black	Black	Light-red to red	Light-red to black
Colony surface on R2A/GYM	Moist	Moist	Dry	Moist	Moist
Reduction of nitrate	−	+	−	+	+
Gelatin hydrolysis	+	−	+	−	ND
Starch hydrolysis	+	+	+	−	+
Degradation of tween 60	ND	−	+	ND	ND
Milk peptonization	ND	+	−	−	ND
Utilization as sole C-source					
D-arabitol		ND	+	−	
D-glucose		−	+	+	
D-mannitol	−	+/−	+	−	+
D-mannose	+	−	+	+	+
D-sorbitol	−	+	+	−	+
Glycerol	+	+	+	−	+
Maltose		+	−	−	
Raffinose		+	+	−	
Raffinose		+	+	−	
Rhamnose	+	+	+	−	−
Succinic acid		−	+	+	
Trehalose		−	+	−	
Oxidase activity	−	−	+	−	−
Utilization as sole N-source					
L-alanine	+	−	+	+/−	+
L-arginine	−	−	+	+	+
L-cysteine		+	−	+	
Cystine		−	+	−	
Histidine	−	−	−	+	−
Hypoxanthine		−	+	−	
Pectin	−	+	+	−	+
L-phenylalanine		−	+	−	
Xanthine		−	+	−	
Acid production from					
Cellobiose	+	+	+	+	−
D-fructose		ND	−	+	
D-arabinose		ND	+	−	
D-galactose		−	+	−	
D-glucose		−	+	−	
D-mannitol		−	+	−	
D-sucrose		−	−	−	
D-xylitol	ND	−	+	ND	ND
Predominant menaquinone(s)	MK-9(H_4), MK-8(H_4), MK-9(H_0)	MK-9(H_4)	MK-9(H_4), MK-9	MK-9(H_4)	MK-9(H_4), MK-8(H_4), MK-9
Polar lipids	DPG, PE, PG, PC, PI	DPG, PE, PG, PC, PI	DPG, PE, PG, PC, PI	DPG, PE, PG, PC, PI, PL, PGL	DPG, PE, PG, PC, PI
Major fatty acids (>10 %)	i-$C_{15:0}$, i-$C_{16:0}$	i-$C_{15:0}$, i-$C_{16:0}$, $C_{16:0}$, $C_{17:0}$	i-$C_{16:0}$, i-$C_{15:0}$, i-$C_{17:1\omega8c}$	i-$C_{16:0}$, i-$C_{15:0}$, i-$C_{17:1\omega8c}$, i-$C_{18:1\omega8c}$	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:1\omega8c}$

From Lechevalier (1989), Nie et al. (2012) and from (Montero-Calasanz et al. 2012, 2013)

[a]++ Strongly positive, + positive, +/− weak, − negative, *nd* not determined

Red-pigmented. Newly formed colonies are light red, becoming deeper red after growth for 4 days on complex medium agar. No diffusible pigments are produced on any medium tested. Gram-positive. Coccoid cells are motile with flagella. Mycelium is rudimentary. No aerial mycelium is produced. Cell walls contain meso-DAP. Chemoorganotrophic. Mesophilic. Colonies after 30 day incubation at 28 °C are flat, granular, and moist. Good growth on R2A agar.

Source: The type strain was isolated from a rhizosphere soil of the medicinal plant, the *Fabaceae Astragalus membranaceus* growing in the mountainous areas around Xining (37°35′N, 101°49′E; elevation 2,800 m), Qinghai Province, northwest China (Zhang et al. 2011).

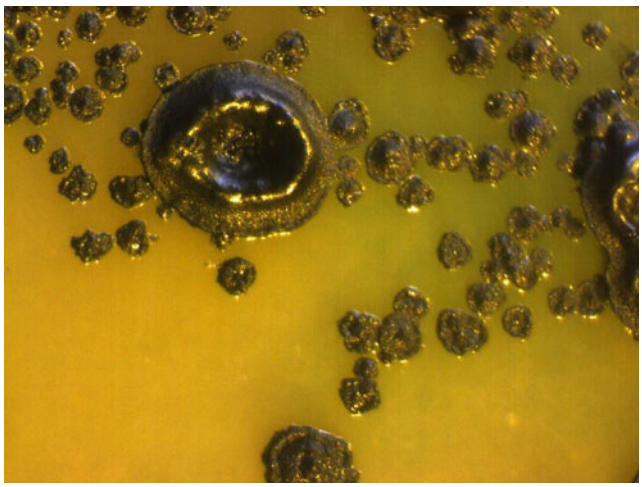

Fig. 20.3
Colonies of *G. obscurus* strain G20 growing on a complex medium with a black-pigmented shine and a concave center (Photo courtesy of Jerome Gury (Université de Pau))

DNA G+C content (mol%): 72.8 (HPLC; (Mesbah et al. 1989)).

Type strain: CPCC 201356^T.

Sequence accession no. (16S rRNA gene): EU438905.

3. *Geodermatophilus nigrescens* Nie et al. 2012, 811.

(ni.gres'cens. L. v. *nigresco* to become black; L. part. adj. *nigrescens* becoming black).

Newly formed colonies are light red, becoming black after growth on complex medium agar such as nutrient agar, Czapek, potato-glucose, yeast extract-malt ISP2 or ISP3. No diffusible pigments are produced on any medium tested. Gram-positive. Mycelium is rudimentary. No aerial mycelium is produced. Cell walls contain meso-DAP. Chemoorganotrophic. Mesophilic. Colonies after 30 day incubation at 37 °C are flat, granular, and moist. Good growth on yeast extract-malt extract (ISP2) and oatmeal (ISP3).

Source: the type strain was isolated from a soil sample collected from a dry-hot river valley in Dongchuan county, Yunnan province, southwest China (Nie et al. 2012).

DNA G+C content (mol%): 73.1 (fluorometric micro-well method, (Ezaki et al. 1989)).

Type strain: YIM 75980^T.

Sequence accession no. (16S rRNA gene): JN656711.

4. *Geodermatophilus arenarius* Montero-Calasanz et al. 2013 (Montero-Calasanz et al. 2012)

(a.re.na'rius. L. masc. adj. *arenarius* related to *arena*, sand, from which the organism has been isolated).

Light red- to brown-pigmented. Newly formed colonies are light red, turning deeper red and brown, convex with a moist surface, and an entire margin. No diffusible pigments are produced on any medium tested. Gram-positive. Coccoid cells are motile with periplasmic flagella. Mycelium is rudimentary. No aerial mycelium is produced. Cell walls contain meso-DAP.

Table 20.3
Discriminating physiological features of the five subspecies of *Geodermatophilus obscurus*

	G. obscurus	*G. amargosae*	*G. utahensis*	*G. dictyosporus*	*G. everesti*
Pigmentation	Dark brown to black	Black	Black	Black	Orange to red
Reduction of nitrate	–[a]	–	++	–	+/–
Gelatin hydrolysis	–	+/–	–	++	–
Utilization of					
D-arabinose	–	–	+	–	–
L-arabinose	++	++	+/–	++	nd
Glycerol	++	++	+/–	++	nd
Inositol	++	+	+	–	–
β-lactose	–	+/–	–	++	–
Melezitose	–	+/–	+/–	++	nd

From Lechevalier (1989)

[a]++ Strongly positive, + positive, +/– weak, – negative, *nd* not determined

Chemoorganotrophic. Mesophilic. Colonies after 30 day incubation at 28 °C are flat, granular.

Source: the type strain was isolated in 2007 from a Saharan desert soil collected near Ouré Cassoni in the Republic of Chad (Montero-Calasanz et al. 2012).

DNA G+C content (mol%): 75.9 (HPLC; (Mesbah et al. 1989)).

Type strain: CF5/4^T (=DSM45418^T).

Sequence accession no. (16S rRNA gene): HE654547.

5. *Geodermatophilus siccatus* Montero-Calasanz et al. 2012 (Montero-Calasanz et al. 2013)

(si.cca'tus. L. part. masc. adj. *siccatus* dried referring to the survival under xerophilic conditions).

Young colonies are light red becoming black-pigmented. Convex and circular colonies. No diffusible pigments are produced on any medium tested. Gram-positive. Catalase-positive and oxidase-negative. Circular to elliptical cells are motile with flagella. Septate mycelium from germinating zoospores is rudimentary. No aerial mycelium is produced. Cell walls contain meso-DAP. Chemoorganotrophic. Mesophilic with no growth observed below 15 °C.

Source: The type strain was isolated in 2007 from a Saharan desert soil collected near Ourba in the Republic of Chad (Montero-Calasanz et al. 2013).

DNA G+C content (mol%): 74.9.

Type strain: CF6/1^T (=DSM45419^T).

Sequence accession no. (16S rRNA gene): HE654548.

Genus *Blastococcus* Ahrens and Moll 1970, 264^{AL} emend. Urzì, Salamone, Schumann, Rohde and Stackebrandt 2004b, 257 emend. Lee 2006, 2394

Blas.to.coc'cus. Gr. n. *blastos* bud, shoot; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Blastococcus* budding coccus.

Gram-stain-positive, coccoid cells that occur singly or in pairs, often reproducing by budding and multiple fission, giving rise to several cell forms. Single cells may be motile rods and vibroid or nonmotile cocci that have a tendency to form aggregates. Strains may form motile zoospores. Formation of buds on rods is common, and gave the genus its name but are not universal. Membranous bodies, 1–4 per cell, that are linked to the cytoplasmic membrane, may be present. Oxidase-negative, catalase-positive, and aerobic; some strains may be microaerophilic. Strains from surfaces of marble and limestone utilize a broader spectrum of organic compounds than the only known strain from a marine sediment. Predominant fatty acids are C16:0 iso, and, in some strains, C16:1 iso,C18:1ω9c,C17:1 ω8c, C15:0 iso, and C17:0. Peptidoglycan contains meso-DAP as diagnostic diamino acid. Predominant menaquinone is MK-9(H4); MK-9 may also occur in high amounts. Polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine; phosphatidylcholine, phosphatidylethanolamine, and two unknown phospholipids may occur. Arabinose and galactose have been identified as whole-cell sugars in one species.

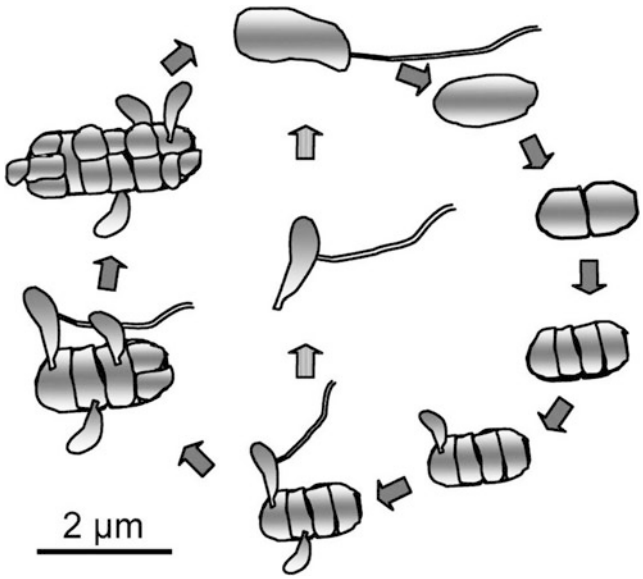

Fig. 20.4
Schematics of the life cycle of *Blastococcus aggregatus* starting at the top with a motile rod with one or two motile flagella. Motile cells lose their flagella (*clockwise*) and start dividing in several planes. At the same time, budding starts and some of the budding cells become motile and detach from the mother cell (*vertical arrows*) (Drawing from Ahrens and Moll (1970), Urzi et al. (2004))

Phylogenetically, a member of the family *Geodermatophilaceae*, genus *Blastococcus* was described as a single isolate and single species genus, containing *Blastococcus aggregatus*, an isolate obtained from sediments at the bottom of the Baltic Sea (Ahrens and Moll 1970). The colonies obtained were pigmented, divided by budding (gr. *blastos*), and formed coccoid cells (from Gr. masc. n. kokkos, grain, seed), that remained aggregated. The life cycle of this unusual bacterium has been studied by Ishiguro and Wolfe (1970) and found to consist of two types of cells, motile individual rods, and nonmotile aggregated cells (*Fig. 20.4*).

DNA G+C content (mol%): 72.3–74.

Type species: *Blastococcus aggregatus* Ahrens and Moll 1970, 264AL emend. Urzì, Salamone, Schumann, Rohde and Stackebrandt 2004b, 257.

Species of the Genus *Blastococcus*

1. *Blastococcus aggregatus* Ahrens and Moll 1970, 264^{AL} emend. Urzì, Salamone, Schumann, Rohde and Stackebrandt 2004b, 257 ag.gre.ga'tus. L. masc. part. adj. *aggregatus* added to, joined together, referring to the tendency to form coccoid aggregates.

Gram-positive, highly irregular vibroid or rod-shaped cells occurring singly or in three-dimensional coccoid aggregates. The manifestation of these stages is influenced by environmental conditions; low salt concentrations, temperatures of 5–10 °C, and microaerophilic conditions favor the motile rod stage.

Table 20.4

Discriminating physiological and morphological features of the type strains of the three species of genus *Blastococcus* using morphological and physiological parameters[a]

	B. aggregatus	*B. saxobsidens*	*B. jejuensis*
Cell shape	Coccoid, rods, vibrios	Coccoid	Coccoid, rods
Bud formation	+	–	–
Germ tube	+	–	–
Oxygen requirements	Aerobic/microaerophilic	Aerobic	Aerobic
Pigmentation	Pink	Pink-orange	Apricot
$C_{15:0}$ iso-fatty acid	<1 %	>5 %	>10 %
$C_{17:1}$ ω8c fatty acid	<2 %	>9 %	>10 %
API 20 NE			
Arginine dihydrolase	–	+	–
β-galactosidase	–	+	–
Assimilation of arabinose	–	+	–
API ZYM			
Valine arylamidase	–	–	w
Alkaline phosphatase	–	+	–
Cystine arylamidase	–	–	w
Naphthol-AS-Biphosphohydrolase	–	w	–
α-glucosidase	w	+	nr
β-glucosidase	–	w	–
β-glucuronidase	–	+	–
Biolog GP2			
Adenosine	+	+	–
D-malic acid	+	–	nr
Tween 40	+	+	–
Methylsuccinate	+	+	–
Acetic acid	+	–	+
α-Ketoglutaric acid	+	–	–

[a]Data from Urzi et al. (2004) and Lee (2006)

Single cells are either motile vibroids (0.3–1.5 × 0.4–3.0 μm) or rods or ellipsoid (1.2–1.5 × 1.5–3.0 μm). Rods are often separated by disk-like septa. These motile or nonmotile rods carry 1–6 vibrioform buds, attached by a tapered pole onto the mother cell. Increased temperature and salt concentrations induce the formation of nonmotile coccoid aggregates (1.2–2.5 μm in diameter), appearing as linear, band-like, or column-like three-dimensional forms. Larger aggregates (1 mm in diameter) are separated by rectangular and/or radial partitions. Cell types of different sizes occur mostly side by side. After 5 days on peptone-yeast extract agar at 20 °C, colonies are pink, turbid, round, and convex with shiny surfaces. In liquid media, thin turbidity and formation of pink sediment are observed. Catalase-positive, oxidase-negative. No growth is observed in mineral medium; weak growth is seen in the presence of ethanol, but not with glucose, acetate, citrate, methanol, ethylamine, or paraffin. Good growth is observed in mineral medium plus peptone (0.5 %) and yeast extract (0.1 %); in these media, addition of glucose, acetate, citrate, ethylamine, or paraffin (at 1 %) reduces growth. Growth is enhanced in peptone-yeast extract medium by the addition of 1 % NaCl. Increasing the salt concentration leads to increased formation of aggregates. No acid or gas is produced from glucose, galactose, fructose, xylose, sucrose, maltose, lactose, or glycerin. Methyl red and Voges–Proskauer reactions are negative. Does not hydrolyze starch, chitin, alginate, or cellulose. Reactions toward substrates provided by the API ZYM, API 20 NE, and Biolog GP2 panels are indicated in ❯ *Table 20.4*. Peptidoglycan diamino acid, menaquinone, and polar lipids are as described for the genus. Two unknown phospholipids may occur. Major fatty acids are 14-methylpentadecanoic acid (C16:0 iso) and 14-methylpentadecenoic acid (C16:1 iso); smaller amounts of C18:0, C16:1 ω7c, C18:1 ω9c, C16:0, and C14:0 iso occur. Extinction maxima of carotenoids are at 470 and 500 nm.

Source: isolated from sediment of Station Breitengrund at a depth of 20 m in the Western Baltic Sea.

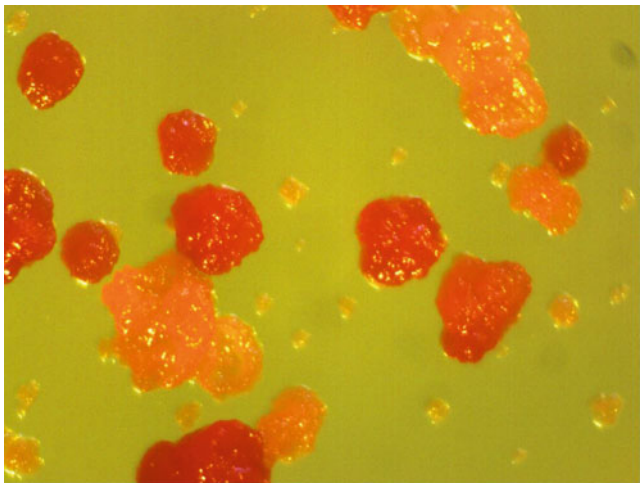

Fig. 20.5
Colonies of *Blastococcus saxobsidens* strain DD2 growing on a complex medium. Color ranges from *orange* to *red* (Photo courtesy of Jerome Gury (Universite de Pau). Source: the isolation site of the type strain was limestone sampled in Malta. Other strains were isolated from calcarenite and marble from Italy and Greece)

DNA G+C content (mol%): 73.9 (HPLC).

Type strain: DSM 4725, ATCC 25902. Sequence accession no. (16S rRNA gene): AJ430193.

A second species, *Blastococcus saxobsidens*, was created in 2004 (Urzi et al. 2004) to accommodate isolates obtained from stones around the Mediterranean.

Blastococcus saxobsidens Urzì, Salamone, Schumann, Rohde and Stackebrandt 2004b, 258[VP].

sax.ob'si.dens. L. neut. n. *saxum* rock; L. part. adj. *obsidens* staying, remaining, occupying; N.L. masc. part. adj. *saxobsidens* rock-occupying.

Colonies are pink-orange pigmented, irregular, and convex with a smooth to rough surface (2–3 mm in diameter) (*Fig. 20.5*). Aerobic, Gram-stain-positive. Motile and nonmotile cells (1.0–1.7 μm in diameter) are coccoid, occurring in tetrads with a tendency to remain aggregated. Sparse growth on Luedemann medium, malt agar, and yeast extract-casein hydrolyzates starch-glucose agar. No growth on potato-dextrose agar or yeast extract-glucose-glycerol agar. Grows between 20 °C and 37 °C and at pH 5–8.6; optimum growth is at 32 °C and pH 6.8. No growth in 3 % NaCl, except strain BC448, which can grow at 5 % NaCl. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Carbon sources utilized for growth are: D-glucose, D-fructose, L-arabinose, ribose, myo-inositol, and lactose. No acid production is observed from D-fructose, myo-inositol, or lactose. Casein, gelatin, and starch are not hydrolyzed. DNA, tyrosine, xanthine, and hypoxanthine are not decomposed. Tweens 20, 40, 60, and 80 are not hydrolyzed. The peptidoglycan diamino acid, major menaquinone, and polar lipids are as described for the genus. Major fatty acids are 14-methyl-pentadecanoic acid (C16:0 iso), *cis*-9-heptadecanoic acid (C17:1 ω8c), and 13-methyl-tetradecanoic acid (C15:0 iso), with smaller amounts of C16:0, C17:0 ante, C16:1 iso, C18:1 ω9c, and C16:1 ω7c.

DNA G+C content (mol%): 72.95 (genome sequence (Chouaia et al. 2012)).

Type strain: BC444, DSM 44509, NRRL B-24246.

Sequence accession no. (16S rRNA gene): AJ296061.

A third species, *Blastococcus jejuensis*, was created in 2006 (Lee 2006) to accommodate a single isolate from a beach sediment in Korea.

Blastococcus jejuensis Lee 2006, 2395[VP]

je.ju.en'sis. N.L. masc. adj. *jejuensis* of or belonging to Jeju Island, Republic of Korea, the site from which the type strain was isolated.

Aerobic, motile, non-spore-forming, oxidase-negative, catalase-positive, Gram-positive. Cells are cocci that occur in pairs or rods. Bud formation is observed for rod-shaped cells. Colonies are circular, smooth, transparent, and apricot in color. Starch and casein are hydrolyzed, but not elastin. Hypoxanthine, tyrosine, and xanthine are not decomposed. In API 20NE tests, glucose fermentation and indole production from tryptophan are not observed. Activities of arginine dihydrolase, urease, and β-galactosidase are not present. Nitrate is not reduced to nitrite. Esculin degradation and gelatin hydrolysis are not detected. Caprate, adipate, citrate, and phenylacetate are not assimilated. In API ZYM tests, results for leucine arylamidase and α-glucosidase are positive and weakly positive, respectively, whereas results for esterase lipase (C8), lipase (C14), trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are negative. The temperature range for growth is 10–37 °C, with optimum growth at 30 °C. Growth occurs at pH 6.1–10.1, with optimum growth at pH 7.1. Growth is observed in the presence of 0–1 % NaCl, but not in 2 % NaCl. The following substrates are used as sole carbon and energy sources: mannan, *N*-acetyl-β-D-mannosamine, amygdalin, cellobiose, D-fructose, D-galactose, D-galacturonic acid, gentiobiose, myo-inositol, melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl-D-glucoside, palatinose, L-rhamnose, sedoheptulosan, D-sorbitol, sucrose, D-tagatose, turanose, xylitol, acetic acid, γ-hydroxybutyric acid, α-ketovaleric acid, D-lactic acid methyl ester, succinic acid, L-asparagine, putrescine, 2,3-butanediol, adenosine, and D-glucose 6-phosphate. Tween 40, D-ribose, a-ketoglutaric acid, and methyl succinate are not utilized. The polar lipid profile contains phosphatidylcholine, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, and phosphatidylinositol. Mycolic acids are not present. The major cellular fatty acids are C16:0 iso, C17:1ω8c, and C15:0 iso. The predominant menaquinone is MK-9(H4). Whole-cell hydrolyzates contain meso-DAP as the diagnostic diamino acid and arabinose and galactose as diagnostic sugars.

Source: isolated from sand sediment from Gwakji beach on Jeju Island, Republic of Korea.

DNA G+C content (mol%): 72.3 (method not reported).

Type strain: KST3-10, NRRL B-24440, KCCM 42251. Sequence accession no. (16S rRNA gene): DQ200983.

Stone surfaces are likely to host *Blastococcus* lineages that remain to be described as it was recently found by studying Madrid and Mallorca stone walls comprising sandstone, granite, and slate material (Salazar et al. 2006).

Genus *Modestobacter* Mevs, Stackebrandt, Schumann, Gallikowski and Hirsch 2000, 344^{VP} emend. Reddy, Potrafka and Garcia-Pichel 2007, 2018

Mo.des.to.bac′ter. L. adj. *modestus* modest, humble; N.L. masc. n. *bacter* a rod or staff; N.L. masc. n. *Modestobacter* a rod with modest growth requirements.

Gram-positive, non-spore-forming. Short rods or cocci with a tendency to remain aggregated and form short, multiseptate filaments. These produce slender buds. Aerobic heterotrophs able to grow in oligotrophic medium. Typically psychrotolerant. Major fatty acids include C18:1, C16:0 iso, and C17:0 anteiso. The major respiratory quinone is MK-9(H4). The cell wall peptidoglycan contains meso-DAP as diamino acid, with alanine, glutamate, and meso-DAP present in a 2:1:1 stoichiometric ratio. DNA G+C content (mol%): around 70. Type species: *Modestobacter multiseptatus* Mevs et al. 2000, 344VP. Further descriptive information: The genus was created in 2000 to accommodate strains isolated from Antarctic "soil" or regolith. The morphology of the strains was found to be sufficiently special to warrant the creation not only of a novel species, but also of a new genus that was later positioned in the *Geodermatophilaceae* on the basis of its 16S rRNA gene sequence (Mevs et al. 2000). The word "soil" is debatable because the fine granular material found in the McMurdo dry valley being almost completely devoid of organic matter can better be called regolithic. The recent description of three other species, *M. versicolor* (Reddy et al. 2007), *M. marinus* (Xiao et al. 2011) and *M. roseus* Qin et al. (2013), has permitted a more elaborate description. The four species are found in markedly different biotopes and thus have a number of distinguishing features.

Modestobacter multiseptatus Mevs et al. 2000, 344^{VP} emend. Reddy et al. 2007, 2018 mul.ti.sep.ta'tus. L. adj. *multus* much; L. adj. *septatus* fenced; N.L. masc. adj. *multiseptatus* much fenced, with many septa.

Colonies are irregularly shaped, shiny, and beige to pinkish. Cells are Gram-positive, short rods, or cocci with a tendency to remain aggregated. Cells show cross and longitudinal wall growth and multiply by budding and swarmer formation. Cell sizes vary (1.0–2.8 × 1.0–3.0 μm), with a mean size of 1.7 × 1.6–1.9 μm. Slender buds may become motile. Aerobic heterotroph; can grow on oligotrophic medium PYGV (Staley 1968) or on DSMZ medium 65. Growth occurs between 0 °C and 28 °C. Can tolerate pH 3–12, with optimum growth at pH 7.5–8.5. Positive for catalase, cytochrome oxidase, phosphatase, and amylase. Shows type II restriction endonuclease activity. H_2S is not formed from cystine or sulfate. Does not utilize fructose, xylose, or trehalose, but can utilize D-glucose, D-galactose, lactose, sucrose, mannitol, succinate, and malate. Utilization of maltose, mannose, melibiose, fucose, ribose, rhamnose, sorbitol, and *N*-acetylglucosamine is variable. Adenine, hypoxanthine, xanthine, hippurate, cellulose, chitin, dextrin, xylan, arbutin, and casein are not hydrolyzed. Utilizes peptone or yeast extract as nitrogen source and reduces nitrate aerobically or anaerobically. The main respiratory quinone is MK-9(H4); MK-8(H4) and MK-9(H6) are present in small amounts. Main fatty acids are C18:1 and C16:0 iso. meso-DAP is present. Cell wall sugars are composed of galactose, glucose, and ribose.

Source: isolated from Antarctic surface "soil" from Linnaeus Terrace (1,600 m) of the Asgard Range in the Transantarctic Mountains. DNA G+C content (mol%): 68–70 (melting spectrometry; Mandel and Marmur 1968).

Type strain: AA-826, DSM 44406, CIP 106529, JCM 12207.

Sequence accession no. (16S rRNA gene): Y18646.

Species of the Genus *Modestobacter*

Modestobacter versicolor Reddy et al. 2007, 57^{VP} ver.si.co'lor. L. masc. adj. *versicolor* that changes its color, of changeable color, of various colors, particoloured.

Colonies are dark brown on oligotrophic medium and pink to white on copiotrophic medium, 1–4 mm in diameter, convex, entire, smooth to rugose, and slightly mucoid. Cells are short, small rods (straight, lightly curved, irregular, or even tapering), often developing into multiseptate cells, occurring singly or in pairs, sometimes remaining aggregated and only rarely forming filaments longer than several cells. Single rods are 0.5–1.0 × 1.0–3.0 μm. Short filaments are up to 7.0-mm long. Septation is transversal (orthogonal to the long cellular axis), and apical cells may resemble buds. Motility is variable, by means of polar flagellation. Growth is observed at 4–30 °C (but not at 37 °C) and pH 5–9, with optimum growth at 25 °C and pH 7. Produces copious amounts of melanin under oligotrophic conditions. Tolerates NaCl at concentrations less than 3 %. Cells are positive for catalase, β-galactosidase, phosphatase, urease, and lipase, but negative for oxidase, gelatinase, arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, and phenylalanine deaminase. Negative for methyl red, Voges–Proskauer and indole tests. Hydrolyzes casein and aesculin, but not cellulose or starch. Reduces nitrate to nitrite. Does not produce H_2S gas and cannot grow on DNA or Simmons' citrate test plates. Utilizes a wide variety of sugars, low-molecular-mass organic acids and amino acids, and all four nitrogenous bases. Respiratory quinones and cell-wall peptidoglycan, fatty acid, and polar lipid compositions are given in *Table 20.5*.

In the original description, the species had been proposed as dinitrogen fixer, but no experimental details have been provided and the claim should thus be seen as unconfirmed.

Source: The type strain is CP153-2T (5ATCC BAA-1040T 5DSM 16678T), it was isolated from a biological soil crust on the Colorado Plateau, USA (Reddy et al. 2007).

Table 20.5

Discriminating physiological and morphological features of the type strains of the four species of genus *Modestobacter* species using morphological and physiological parameters[a]

	M. multiseptatus	*M. marinus*	*M. roseus*	*M. versicolor*
Colony color on solid medium	Pale pink	Pink-deep orange to dark	Pink	Pink-deep orange to dark
Diffusible pigment	ND[b]	Melanin	None	Melanin
Biochemical characteristic (API 20 NE)				
Urease activity	ND	−	−	+
Reduction of nitrate	+	w	−	+
Assimilation (API 20NE):				
Arabinose	+	+	−	−
Fucose	+	ND	ND	ND
Glucose	+	+	+	+
D-mannose	w	w	+	w
N-acetyl-D-glucosamine	w	−	+	w
Maltose	w	−	w	w
Gluconate	ND	+	+	−
Malate	+	+	+	+
Phenyl acetate	ND	w	−	−
Acid produced from(API 50CH)				
Adonitol	ND	+	−	+
D-Arabinose	w	+	−	+
Arbutin	−	−	+	−
Benzoate	+	ND	ND	ND
Cellobiose	−	+	−	−
Citrate	+	ND	ND	ND
Erythritol	w	w	−	−
Glycerol	ND	+	−	+
Glycogen	ND	−	+	−
Inositol	−	+	−	+
Lactose	+	+	+	+
Maltose	w	+	+	+
Mannitol	+	+	w	+
Melibiose	−	−	−	+
Raffinose	ND	−	−	+
Saccharose	+	+	−	+
Sorbitol	+	+	−	−
Sorbose	ND	−	−	w
Xylose	−	+	−	−
Assimilation of sole nitrogen sources				
L-Cysteine	ND	+	−	−
L-Lactamine	ND	−	−	+
L-Proline	ND	+	−	+
L-Valine	ND	+	w	w
L-Histidine	ND	w	−	+
Growth at 4 °C	+	+	−	+
Growth on 7 % NaCl	+	−	+	+
Growth on 10 % NaCl	−	−	+	w
DNA G+C content (mol %)	68.4–69.9	72.3 ± 1	71.7	73 ± 2.5

[a]According to Mevs et al. (2000), Reddy et al. (2007), Xiao et al. (2011), Qin et al. (2013)

[b]*ND* not determined, *w* weak response

DNA G+C content (mol%): 73 ± 2.5 (spectrophotometry; (De Ley et al. 1970)).

Sequence accession no. (16S rRNA gene): AJ871304.

Modestobacter marinus Xiao et al. 2011, 1710^{VP} ma.ri'nus. L. masc. adj. *marinus* concerning the sea, the ocean, where the type strain was found.

Colonies are orange to red on copiotrophic medium at the initial stage of growth, turning black after 14 days. Colonies are dark throughout growth on oligotrophic medium. Cells are non-spore-forming, Gram-positive, short rods (straight, lightly curved, about 0.5–0.8 × 1.5–2.5 mm), motile by means of flagella. Psychrotolerant, growing at 4–35 °C (but not at 37 °C), with optimal growth at 28 °C. Tolerant of only narrow variations in pH, with fast growth occurring at pH 6–8 and very slow growth at pH 9. Tolerates NaCl at concentrations less than 5 % (w/v). Hydrolyzes casein, cellulose, and starch. Does not produce H_2S and cannot grow on DNase test plates. In API 20NE tests, positive for aesculin hydrolysis, *p*-nitrophenyl β-D-galactopyranosidase, and assimilation of L-arabinose, D-mannitol, potassium gluconate, malic acid, and phenylacetate. Negative for indole production, acid from glucose, arginine dihydrolase, urease and gelatinase. Nitrate reduction is weakly positive. Tested by the API ZYM enzyme assay, positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-Biphosphohydrolase, α-glucosidase, and β-glucosidase; weakly positive for cystine arylamidase, trypsin, α-chymotrypsin, and β-galactosidase; negative for esterase (C4), lipase (C14), α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. In the API 50 CH test system, acids are produced from glycerol, L-arabinose, D-ribose, D-galactose, D-fructose, D-mannose, D-mannitol, aesculin, maltose, sucrose, trehalose, turanose, and L-fucose. Weakly positive for acid production from L-rhamnose, salicin, cellobiose, lactose, and D-xylose. All other API 50 CH test results are negative. The cell-wall peptidoglycan contains meso-diaminopimelic acid as the diamino acid. Major fatty acids are iso-$C_{16:0}$ and $C_{17:1v8c}$; the major respiratory quinone is MK-9(H_4). The diagnostic phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, and an unknown aminophospholipid (Xiao et al. 2011).

Source: The type strain, 42H12-1^T (=DSM45201^T = CGMCC 4.5581^T), was isolated from deep-sea sediment collected from the Mid-Atlantic Ocean at coordinates 14.75°N 44.97°W close to the Mid-ocean ridge (Xiao et al. 2011).

DNA G+C content (mol%): 72.3 ± 1 (HPLC; (Mesbah et al. 1989)).

Sequence accession no. (16S rRNA gene): EU181225.

Modestobacter roseus Qin et al. (2013))^{VP} ro'se.us. L. masc. adj. roseus rose-colored, pink.

As this chapter goes to press (Nov 2012), the paper describing *M. roseus* has just been accepted for publication. Colonies are rose-colored on oligotrophic and copiotrophic medium. Cells are Gram-positive, aerobic, non-spore-forming, short rods (straight, lightly curved), or cocci with a tendency to remain aggregated, motile by means of flagella. Growth is observed at 10–37 °C and pH 6–11, with an optimum at 28 °C and pH 7–8. The NaCl range for growth is 0–10 % (w/v). In API 20NE tests, positive for aesculin hydrolysis, paranitrophenyl-β-D-galactopyranosidase, assimilation of arabinose, glucose, mannitol, mannose, *N*-acetyl-D-glucosamine, maltose, malate, and gluconate. Uses L-valine and L-lysine as sole nitrogen sources. In the API 50 CH test system, acids are produced from L-arabinose, arbutin, esculin, D-fructose, glycogen, lactose, maltose, D-mannitol, D-mannose, and D-ribose. The cell wall peptidoglycan contains meso-diaminopimelic acid as the diamino acid. Major fatty acids are iso-C_{16}: 0, iso-$C_{15:\ 0}$, and $C_{17:1\omega8c}$; major respiratory quinone is MK-9(H_4). The diagnostic phospholipids are DPG, PE, PI, PIM, two unknown aminophospholipids and an unknown phospholipid.

Source: The type strain is KLBMP 1279T (=KCTC 19887T = NBRC 108673T), it was isolated from surface-sterilized roots of a coastal halophyte *Salicornia europaea* Linn. collected from the city of Nantong, Jiangsu Province, east of China. A single isolate has been studied.

DNA G+C content (mol%): 71.7 (HPLC; (Mesbah et al. 1989)).

Sequence accession no. (16S rRNA gene): JQ819258.

Phylogenetic Structure of the Family and Its Genera

The family is quite coherent, distinct from the other families in order Frankiales with a long branch length. However the three genera *Geodermatophilus, Blastococcus*, and *Modestobacter* are close to one another with short branch lengths between genera and long branch lengths within genera (Fig. 20.6).

16S rRNA sequence analyses have positioned genus *Geodermatophilus* at the root of the tree (Normand and Benson 2012b) with *Modestobacter* and *Blastococcus* in derived positions. A different topology had been obtained by Stackebrandt and Schumann (2012) with *Modestobacter* at the root of the clade. Previously, Normand and coworkers (1996), using the same marker but different outgroups, placed *Blastococcus* at the root. This last topology was obtained using a large number of conserved proteins (in preparation). This topology would be coherent with *Blastococcus* being the ancestor of the clade as its physiology is closer to that of other actinobacteria, regarding in particular resistance to reactive oxygen species. *Blastococcus* would thus have colonized stones interior as well as marine and beach sediments. Nevertheless, the uncertainties concerning the topology and the bushiness of the tree point to a rapid differentiation of the three genera. The present treatment based on the single 16S RNA gene positions *Modestobacter* at the root but this topology may not be the definite one.

A similar inconsistency was found to occur in the *Frankia-Geodermatophilaceae* forming or not a clade together with *Acidothermus, Sporichthya*, and *Cryptosporangium*. This phylogenetic proximity between *Frankia, Geodermatophilus obscurus* and a strain of "*Blastococcus*" was initially detected through reverse transcriptase sequencing and oligonucleotide cataloging

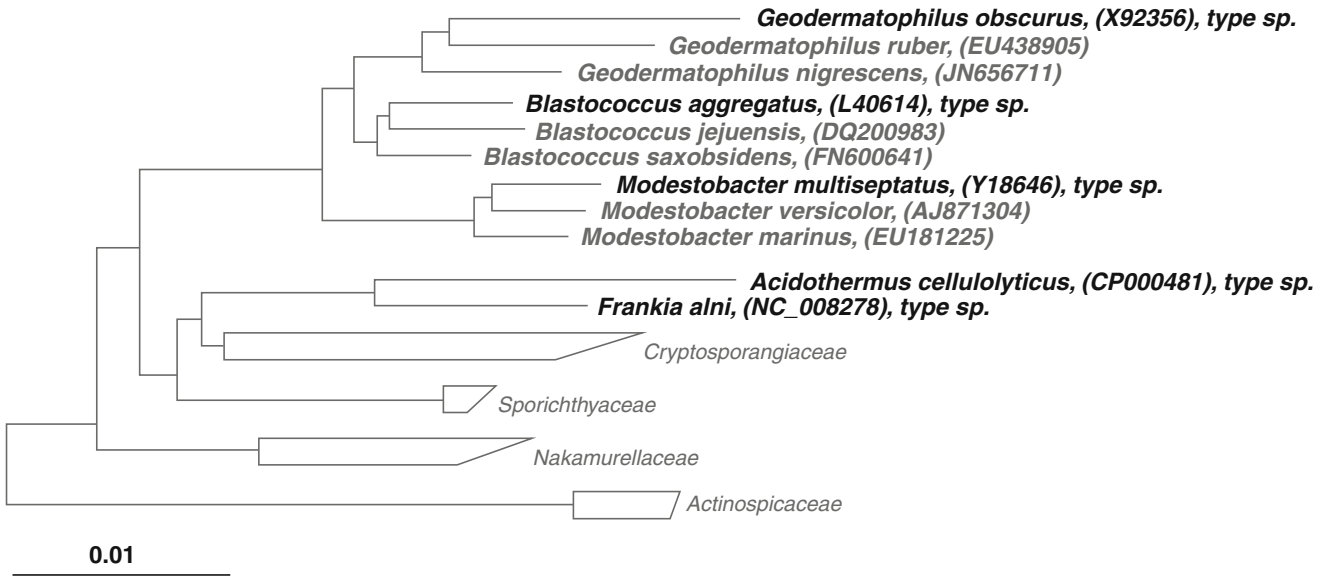

Fig. 20.6

Phylogenetic reconstruction of the family *Geodermatophilaceae* 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

of their 16S ribosomal RNA (Hahn et al. 1989). This clade was completed and confirmed with *Modestobacter* by sequencing the full 16S rRNA gene (Normand et al. 1996). However, Wu et al. (2009) studying a concatenate of 31 "universal" proteins could not recover *Frankia* and *Geodermatophilus* together in a robust clade.

These differences illustrate the difficulty of using a single marker to reconstruct the phylogeny of the genome as a whole, presumably because of numerous lateral transfers including those of the ribosomal genes that occur when microbes share a common niche following migrations. Presumably such transfers are more probable when the number of gene copies is low, as occurs in slow-growing microbes. It appears inevitable that with the mounting number of genomes and with appropriate software, the detailed evolutionary of gene transfers, gene duplications, and gene loss will be possible to recreate.

Molecular Analysis

Three genomes have been recently undertaken, one for each of the three recognized genera. The first one, that of *Geodermatophilus obscurus*, was published in 2010 (Ivanova et al. 2010) and was used to reassess by multi-genes analysis the phylogeny of bacteria (Wu et al. 2009). The second one was that of *Blastococcus saxobsidens* (Chouaia et al. 2012) and the third one was that of *Modestobacter marinus* (Normand et al. 2012), both of which were recently published allowing to define a core genome (*Fig. 20.7*, *Table 20.6*).

Phages

No phage has been described for the three genera; however, no specific work has been done for that purpose. A search in the three genomes for "phage" as keyword revealed several occurrences; however, these genes are not conserved and there is no extensive syntenic region and are thus likely to represent events that have occurred since the emergence of the three genera (*Table 20.7*).

Phenotypic Analysis

Isolation and Maintenance Procedures

Several media have been used for the isolation of the *Geodermatophilaceae*. In general, the media are based on rich organic ingredients like yeast extract, peptone, and malt extract. Among sugars, glucose is the most used. The isolation can be a relatively long process with a rather long time for the appearance of the first colonies that may be visualized on plates after 2–4 weeks. In certain cases, especially for testing at low temperatures, incubations last up to several months. In several

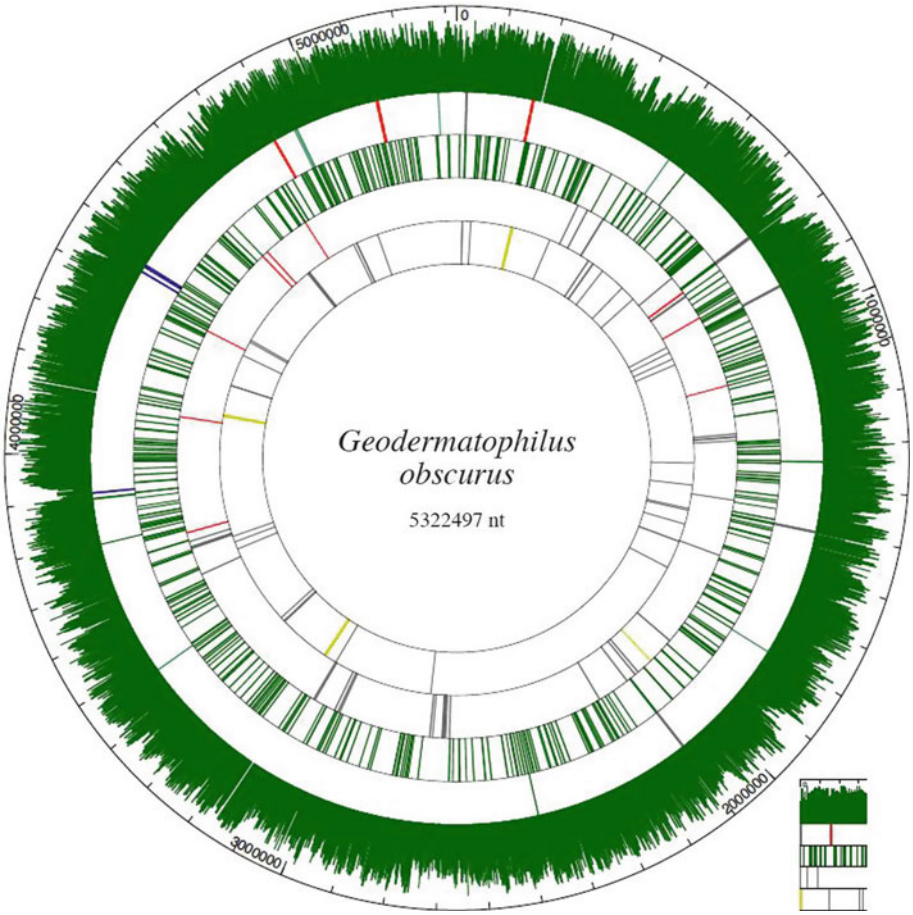

Fig. 20.7

Geodermatophilus **circular map with from the outside. 1 GC (range 60–80 %); 2 duplicated genes, *cox* (*red*), *trwC* relaxase (*gray*), *arsR* (*sea green*), *copR* (*dark green*), uvrD (*navy blue*); 3 *Geodermatophilus* specific genes (at a threshold of 30 % identities over 80 % of the length of the shortest, present in *B. saxobsidens, G. obscurus, M. marinus* but absent in *A. cellulolyticus, Frankia* ACN14a, Ccl3, EaN1pec and Dg (Fd), and *S. coelicolor*; unknown in gray; conserved in black; others in green); 4 Transposases (*gray*), phage genes (*red*), and plasmid genes (*yellow*); 5 rRNA (*yellow*) and tRNA genes (*gray*)**

Table 20.6

Characteristics of the genomes of the three genera of family *Geodermatophilaceae*

Characteristic	*Geodermatophilus obscurus*	*Blastococcus saxobsidens*	*Modestobacter marinus*
Genome size (nt)	5,322,497	4,875,340	5,569,024
G+C%	73.98	72.95	74.09
n. of genes	5,334	4,818	5,468
n. of tRNA	48	48	48
n. of rRNA operons	3	3	3
Protein coding density (%)	85.66	89.59	89.53

instances, rich but diluted media were used to simulate the oligotrophic conditions occurring in the starting source of the inocula like barren soils or stones. In other cases, standard microbiological media like TSA or R2A have been used for isolation. In several studies, considering the slow growth rate of the *Geodermatophilaceae*, antifungal compounds have been used in the medium to inhibit fungal growth and favor the *Geodermatophilaceae*.

Maintenance of the cultures can be done by storing for some weeks on slants at 4 °C, while for longer periods the cultures are

Table 20.7
Phages features in *Geodermatophilaceae* genomes

Feature	*Geodermatophilus*	*Blastococcus*	*Modestobacter*
Phage occurrence	9	15	15
Phage clusters	0	1	1

generally conserved in 20 % (w/v) glycerol suspensions at −20 °C and −80 °C in a suitable medium.

The isolation procedure to obtain the original strains is described in more detail below.

Geodermatophilus obscurus (Luedemann 1968). The isolation procedures are not described in the original study that isolated the first *Geodermaptophilaceae* species (Luedemann 1968), but *G. obscurus* was maintained on the so-called Luedemann medium consisting of 0.5 % Yeast Extract (Difco), 0.5 % NZ Amine type A, 1 % glucose, 2 % Soluble Starch (Difco), 0.1 % CaCO3, and 1.5 % agar.

Geodermatophilus arenarius (Montero-Calasanz et al. 2012) and *G. siccatus* (Montero-Calasanz et al. 2013). The isolation procedure comprised resuspension of desert sand in physiological saline, shaking for 1 h at 26 °C, keeping overnight at 4 °C, and finally shaking for 2 h before streaking the supernatant on R2A and TSA medium. Plates were incubated at 25 °C for 3–10 days. Maintenance of the isolates was originally performed by using freezing in MicrobankTM Blue Colour Beads (Pro-Lab Diagnostics, Richmond, Canada).

Geodermatophilus nigrescens (Nie et al. 2012). Two grams of air-dried soil were shaken in a flask with 18-mL sterile water and glass beads at 30 °C for 1 h. The suspension was serially diluted 100 times and 200 µL were spread on yeast extract-malt extract agar ISP2 medium (Shirling and Gottlieb 1966) supplemented with nalidixic acid (25 mg L^{-1}) and nystatin.

Geodermatophilus ruber (Zhang et al. 2011). *G. ruber* has been isolated on an R2A medium following the dilution plating method and incubating at 28 °C for 3 weeks. The isolate was maintained on R2A slant agar at 4 °C and as suspensions of cells in 20 % (v/v) glycerol.

Modestobacter marinus (Xiao et al. 2011). The isolation conditions for *M. marinus* were not reported in the original description. However, the strain was routinely cultured in liquid or solid ISP2 medium (Shirling and Gottlieb 1966) for 3–5 days at 28 °C. The strain was cultured in solid ISP2 or TSA media within 7 days.

Modestobacter multiseptatus (Mevs et al. 2000). Solid PYGV medium (pH 6.5; Staley 1968) was inoculated directly with soil particles (0.9 g) and incubated for 1–5 months at 4 or 9 °C. Pure cultures were kept on PYGV agar slants or lyophilized or frozen at −71 °C. Good growth was also observed on DSMZ medium 65.

Modestobacter versicolor (Reddy et al. 2007). The biological soil crust sample for the original isolation of *M. versicolor* was suspended in Ringer's solution and plated on oligotrophic BG11-PGY plates (10 % strength BG11 mineral medium, 0.025 % peptone, 0.025 % yeast extract, 0.025 % glucose, 1.5 % agar). BG11 mineral medium is made of $NaNO_3$ 1.5 g L^{-1}, $K_2HPO_4 \cdot 3H_2O$ 40 mg L^{-1}, $MgSO_4 \cdot 7H_2O$ 75 mg L^{-1}, $CaCl_2 \cdot 2H_2O$ 36 mg L^{-1}, citric acid 6 mg L^{-1}, ferric ammonium citrate 6 mg L^{-1}, EDTA 1 mg L^{-1}, Na_2CO_3 20 mg L^{-1}, and 1 mL L^{-1} of trace element solution.

Blastococcus aggregatus (Ahrens and Moll 1970). Peptone-yeast medium (peptone 5 g L^{-1}, yeast extract 1 g L^{-1}, $FePO_4$ 0.1 g L^{-1} in tap water, adjusted to pH 7.2) added with 0.8 % NaCl was used for the isolation. Colonies appear small (1 mm diameter after 21 days) with a pink color. The colony surface looks like an orange peel under a microscope at low magnification (Normand and Benson 2012a).

Blastococcus saxobsidens (Urzi et al. 2004). Isolation was performed by growing the cells at 28 °C after dilution plating on Bunt and Rovira medium (1955) that was modified by adding 0.5 % glucose, 0.5 % NaCl, and 0.03 % Na_2CO_3 and by setting pH at 8.6. The stone samples used for isolation were ground to a powder in a sterile mortar and suspended (ratio 1:10, w/v) in physiological saline (0.85 % NaCl). For favoring the cell detachment from the stone material, 0.001 % (w/v) Tween 80 was supplemented to the solution and the stone powder suspension was vortexed for 60 min. The isolated strain was maintained on Luedemann medium (Luedemann 1968).

Blastococcus jejuensis (from Lee 2006). *B. jejuensis* was isolated from a 1-m deep beach sediment. Samples were placed into sterile 50-mL Falcon tubes containing seawater. One gram of sand sediment was mixed with 9-mL sterile distilled water and mixed in a tube rotator for 30 min at a moderate speed. A subsample (0.1 mL) was serially diluted and transferred onto SC-SW agar plates supplemented with 60 % (v/v) sterilized natural seawater. The SC-SW agar medium contained 1 % soluble starch, 0.03 % casein, 0.2 % KNO_3, 0.2 % NaCl, 0.002 % $CaCO_3$, 0.005 % $MgSO_4 \cdot 7H_2O$, 0.001 % $FeSO_4 \cdot 7H_2O$, and 1.8 % agar in 60 % sterilized natural seawater and 40 % distilled water (pH 7.2). The isolate was maintained on ISP2 medium (Shirling and Gottlieb 1966) supplemented with 60 % sterilized natural seawater, and in a 20 % (v/v) glycerol suspension supplemented with 60 % (v/v) sterilized natural seawater, at −20 °C and −80 °C.

Biotypes and Ecology

Common characteristics of the genera in the *Geodermatophilaceae* are modest growth requirements and the ability to grow as a pioneer in biotopes commonly considered as nutrient-poor like mineral rock substrates or dry soils. The three genera have been isolated from very different regions, latitudes, and climatic conditions including desert environments, rocks, dry soils, monument surfaces, but all characterized by dry conditions and scarcity of organic matter (Taylor-George et al. 1983; Eppard et al. 1996; Garrity et al. 1996; Urzì and Realini 1998; Urzì et al. 2001; Essoussi et al. 2010). They typically grow as endoliths on and in stone materials where they contribute in forming the so-called subaerial biofilms (Gorbushina 2007).

Geodermatophilaceae have been frequently retrieved from extreme environments with very hot or cold and dry climates.

Several *Geodermatophilaceae* and in particular species of the genus *Geodermatophilus* have been isolated from sand and stones in hot deserts from different continents (Eppard et al. 1996; Garrity et al. 1996; Nie et al. 2012). Indeed the original description of the genus *Geodermatophilus* arises from isolates obtained from soil of the Amargosa desert in Nevada, US (Luedemann 1968). Recently, Montero-Calasanz et al. (2012, 2013) reported the description of two new species of *Geodermatophilus, G. arenarius*, and *G. siccatus*, isolated from Sahara sands in Chad, while *G. nigrescens* has been isolated from a dry-hot river valley in southwest China (Nie et al. 2012) and *Geodermatophilus* sp. has been isolated from the Sonoran desert in North America (Rainey et al. 2005). Of the genus *Modestobacter, M. versicolor* has been isolated from biological soil crusts of arid soils from the Colorado Plateau in USA and showed a variable pigmentation from pink to brown-black depending on the level of organic matter available (levels of carbon and nitrogen) in the medium (Reddy et al. 2007).

Besides the hot desert environments, several reports indicated that *Geodermatophilaceae* colonize dry and cold soils. Strains of the genus *Geodermatophilus* were isolated from hyperarid areas from the Qinghai-Tibet plateau in China at altitudes between 3,000 and 3,900 m (Zhang et al. 2011). Other *Geodermatophilaceae* have been isolated or detected through cultivation-independent approaches also in Antarctica like in the Asgard Range (Transantarctic Mountain) where *M. multiseptatus* has been originally isolated (Mevs et al. 2000) and in the Miers Valley where *Geodermatophilaceae* have been evidenced by PCR and sequencing (Babalola et al. 2009).

An interesting aspect of *Geodermatophilaceae* is that they are apparently capable of surviving prolonged periods in the atmosphere associated to soil dust and cover long transcontinental distances. Following a 16S rRNA-based sequence diversity survey of high altitude snows in the Alps (Col du Dome, Mt. Blanc, 4,250 m), several clones of *Geodermatophilaceae*, associated to *Geodermatophilus* and *Blastococcus* were detected and associated to depositions of Saharan dust (Chuvochina et al. 2011a, b). These observation indicates that *Geodermatophilaceae* have a large range dispersal pattern through the atmosphere that makes the family ubiquitous in different environmental conditions.

Besides extreme soils of hot and cold desert, *Geodermatophilaceae* have been also found in standard soils and rhizospheres. For instance, *Geodermatophilus ruber* has been isolated from the rhizosphere soil of the medicinal plant *Astragalus membranaceus* (Zhang et al. 2011). Some *Blastococcus* sp. strains have been proposed as endophytes being found to be associated to plant tissues of medicinal plants from the tropical rain forests of Xishuangbanna in China (Qin et al. 2013).

Another interesting biotope *Geodermatophilaceae* has been associated to the surface of stone monuments (Urzì et al. 2001; Salazar et al. 2006). In several studies, *Geodermatophilaceae* of the three genera have been isolated from monument surfaces, for instance, in the Mediterranean basin (Eppard et al. 1996; Urzì and Realini 1998; Brusetti et al. 2008). In particular, cultivation experiments evidenced the presence of *Modestobacter* and *Blastococcus* genera on calcarenite stones (Urzì et al. 2001; Brusetti et al. 2008), while *Geodermatophilus* has been recovered more from desert environments (Eppard et al. 1996; Garrity et al. 1996).

As endoliths growing in stone materials, members of *Geodermatophilus, Modestobacter* and *Blastococcus* species have evolved as parapatric taxa (ecotypes) among the family *Geodermatophilaceae*. These hypotheses about the evolutionary paths and the ecological microniches among *Geodermatophilaceae* have been formulated based on ROS-generating resistance patterns data (Gtari et al. 2012) as well as microniche colonization (Brusetti et al. 2008; Gtari et al. 2012). Whereas *Blastococcus saxobsidens* was found to be sensitive to gamma radiation (D10 = 900 Gy; 10 % survival at 900 Gy), *Modestobacter marinus* was moderately tolerant (D10 = 6,000 Gy) and *Geodermatophilus obscurus* was highly tolerant (D10 = 9,000 Gy). The high resistance to gamma ionizing radiation of *Geodermatophilus* sp. has been also reported by Rainey et al. (2005). A difference in resistance to short wavelength (254 nm) UV was shown by *B. saxobsidens*, *M. multiseptatus*, and *G. obscurus*, being sensitive, tolerant, and highly tolerant (D10 of 6, 900, and >3,500 kJ m^{-2}, respectively, Gtari et al. 2012). Tolerances to desiccation, mitomycin C, and hydrogen peroxide correlated with the ionizing radiation and UV resistance profiles of the three species as well as with the pigments synthesized. Resistance to heavy metals/metalloids did not follow the same pattern, with resistance to Ag^{2+} and Pb^{2+} being similar for *B. saxobsidens, M. marinus*, and *G. obscurus*, whereas resistance to AsO_4^{3-}, Cr^{2+}, or Cu^{2+} was greater for *B. saxobsidens* than for the other two species (Gtari et al. 2012). These distinguishable physiological features were reflected by diverse preferences of members of the three *Geodermatophilaceae* genera in colonizing stone material and to drive the stringencies caused by solar radiation, temperature, drought, and lack of nutrients. On rocks and stone materials, *Geodermatophilus* and *Modestobacter* appear as inhabitants respectively of altered and surface stone materials and are subsequently adapted to extensive environmental changes in contrast to *Blastococcus* which inhabits the deeper part of stone material and relatively stable conditions.

The original biotopes where *Blastococcus* strains were first isolated are marine biotopes. Ahrens and Moll (1970) proposed the description of the new genus *Blastococcus* and the species *Blastococcus aggregatus* following the isolation from sediments of the Baltic Sea. Other *Geodermatophilaceae* isolates derive from marine sediments: *Blastococcus jejuensis* has been isolated from beach sediments in South Korea (Lee 2006) and *Modestobacter marinus* was isolated from a 2,983-m deep-sea sediment sample collected in the Atlantic Ocean (Xiao et al. 2011). Marine sediments represent oxic/anoxic interfaces where steep gradients of oxygen exist and microaerophilic conditions may occur. The isolation of *Geodermatophilaceae* from such environment suggests an ecological assembly of *Geodermatophilaceae* selected to thrive at relatively low oxygen tensions, a concept further supported by the capability of growth under microaerophilic conditions especially within the *Blastococcus* genus (Normand 2006).

Applications

Some features of the *Geodermatophilaceae* have been studied for various applications.

The adaptations of *Geodermatophilaceae* to thrive in harsh environments characterized by low water and nutrient availability, exposure to high irradiation rates, and for strains adapted to mineral rocky substrates with exposure to heavy metals have selected features of interest for potential applications.

The capability of resistance to high irradiation rates and the tolerance to heavy metals/metalloids as reported in the previous paragraph make these bacteria potential candidates for the primary colonization of substrates with low organic inputs, for instance, in the formation of the soil-protective biological soil crusts in barren sterile soils, or for the bioremediation of polluted environments even in the presence of radiations.

Geodermatophilaceae appear equipped with robust enzymes capable of being active in relatively harsh conditions. For instance, it has been shown that the esterases (EC 3.1.1.x) of *Geodermatophilaceae*, which constitute a diverse group of enzymes that play central roles in most catalytic pathways, are highly tolerant to stressful conditions. Esterases were studied for a collection of *Geodermatophilaceae* and other actinobacteria obtained from Sahara desert stone and monument in Tunisia and Egypt (Essoussi et al. 2010, 2012). *Blastococcus, Geodermatophilus*, and *Modestobacter* are distinguishable from *Arthrobacter, Micromonospora, Actinoplanes, Kineococcus*, and *Nocardia* by a rich and particular pool of esterase activities that could be directly linked to harsh conditions characterizing their ecological habitat including high level of aridity, temperature, ionic strength, and low nutrient availability. As an example, esterases from *Geodermatophilus* strains display a high resistance to thermal inactivation and alkaline pH and retain up to 30 % of activity after heating for 20 min at 120 °C and pH 12. The activity was strongly activated in the presence of Ca^{2+} and Mg^{2+} ions and moderately by Zn^{2+} and was markedly inhibited by Cu^{2+} and Co^{2+} ions. Jaouani et al. (2012) purified an intracellular esterase from *Geodermatophilus obscurus* strain G20 of approximately 55 kDa. The enzyme was highly thermostable, with a residual activity greater than 90 % after incubation at 80 °C for more than 10 h. The enzyme showed an optimal activity between pH 8.0 and 9.0 and was stable in the pH range 7.0–10.0. This esterase showed preference for esters of *p*-nitrophenol with short chain fatty acid. When the *p*-nitrophenyl acetate (C2) was used as substrate, the Michaelis–Menten constant (Km) and maximum velocity for the reaction (Vmax) of esterase were 400 μM and 2,500 U/mg protein, respectively. The effect of phenylmethanesulfonyl fluoride (PMSF), a serine-specific inhibitor, on the enzyme activity suggested that the thermostable esterase belongs to the serine hydrolase group. The high thermostability, activity at alkaline pH, tolerance to methanol and various metal ions, and specificity for short chain fatty acids make this enzyme highly interesting for applications in biocatalysis.

Geodermatophilaceae have been also associated to negative traits. When recovered from monument stones,

Fig. 20.8
Degradation of a calcareous stone wall in Carthage, Tunisia, where several isolates of the three *Geodermatophilaceae* genera have been isolated. Biopitting and stains are associated with these bacteria

Geodermatophilaceae have been frequently associated with mechanical alterations of the stone material with biopitting and powdering and alterations of the surfaces (*Fig. 20.8*) with orange, grayish, greenish, and black spots and patinas (Urzì and Realini 1998). However, clear demonstrations of causative roles in the alterations have been not documented, and until now, the *Geodermatophilaceae* have been shown to be simply associated to the surfaces alterations.

Conclusion

Geodermatophilaceae are isolated from extreme biotopes such as polar regoliths, gamma-irradiated sands, or from the inside of stone monuments. They are also present in less extreme locales such as rhizosphere soils. However, they do not appear as dominant in molecular catalogs and thus have a selective advantage only when environmental conditions are very punitive for more common bacteria. They constitute a very coherent clade, recovered by many approaches including phylogeny of several markers and morphology.

Given the *Geodermatophilaceae* are slow growers and that they inhabit extreme environments that have been less studied through metagenomics, it is expected new taxa should be recovered soon with the constantly rising sequencing power and the numerous metagenomics programs underway. Already, hints of new lineages have been obtained through studying with 16S rRNA targeted primers the microbial diversity of stones surfaces (Salazar et al. 2006).

The study of the genomes of these microbes, only recently obtained, is not complete. It is not yet understood, for instance, how two of the three strains (*Geodermatophilus* and *Modestobacter*) could have developed such high resistance to oxidative stress (Gtari et al. 2012). It is expected that the

genomes should yield clues permitting to understand how the *Geodermatophilaceae* did adapt to their respective biotopes. As more and more strains are isolated and classified into new species, as more and more genomes are obtained, we should see the evolutionary history of some of the most contrasted microbes unfold.

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21 The Order *Glycomycetales* and the Genus *Actinocatenispora*

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Abstract
The order *Glycomycetales* comprises the family *Glycomycetaceae* with its genera *Glycomyces*, *Haloglycomyces* and *Stackebrandtia*. Also covered in this chapter is the genus *Actinocatenispora* which, in some 16S rRNA gene sequence-based trees shows a weak affiliation to this order. The type strains were either directly isolated from soil or they were recovered as endophytes. All strains form a vegetative mycelium and some strains form an aerial mycelium on some media. Antibiotic substances have been identified in one species, and the presence of PKS I genes have been demonstrated in an unnamed species. Members of the order can be isolated routinely on standard growth media but are considered "rare actinomycetes" as their presence is hardly detected in non-culture studies.

Taxonomy, Historical and Current

Short Description of the Family

The family *Glycomycetaceae* was originally described by Stackebrandt et al. (1997) on the basis of the isolated position of the *Glycomyces* line of descent within the radiation of members of the order *Actinomycetales* and a set of 16S rRNA gene-specific nucleotides. In the same publication the suborder *Glycomycineae* was described. Since then the family *Glycomycetaceae* was emended twice. Firstly by Labeda and Kroppenstedt (2005) incorporating the new phenotypic information of the second genus of the family, *Stackebrandtia*, secondly by Zhi et al. (2009), who also emended *Glycomycineae*, after many new genera and families were added to the *Actinobacteria*, expanding the diversity of 16S rRNA gene sequences. Recently, a third genus has been added to the family, named *Haloglycomyces* (Guan et al. 2009) without additional emendation of the family. In the 2nd. edition of *Bergey's Manual of Systematic Bacteriology*, vol 5, Actinobacteria, the family *Glycomycetaceae* has been placed in the order *Glycomycetales* (Ludwig et al. 2012). Phenotypic and chemotaxonomic properties of the three genera are compiled in ❯ *Table 21.1*.

Glycomycetaceae Rainey et al. 1997, 487^{VP}, emend Labeda and Kroppenstedt 2005, emend Zhi et al. 2009.

Gly.co.my.ce.ta'ceae. N.L. masc. n. *Glycomyces* type genus of the family; -taceae ending to denote a family; N.L. fem. pl. n. *Glycomycetaceae* the *Glycomyces* family.

Aerobic. Gram-positive, non-acid-fast, non-motile actinomycetes. Branched substrate mycelium (approx. 0.35–0.5 μm in diameter) and, on some media, aerial mycelia are produced. Catalase positive. Mycolic acids are absent. Contains meso-diaminopimelic acid (A_2pm) as the diamino acid. Cell walls contain *N*-glycolylmuramic acid. When searched for, teichoic acids are present (Potekhina et al. 1993). Whole-cell sugar pattern contains ribose as a diagnostic sugar along with other genus-specific sugars. Iso(i)- and anteiso(ai)-branched fatty acids. Phospholipid content includes phosphatidylglycerol and diphosphatidylglycerol in addition to other genus-specific phospholipids (Labeda and Kroppenstedt 2005).

The 16S rRNA gene sequence pattern of 16S rRNA signature nucleotides consists of nucleotides at positions 657 : 749 (G–U), 672 : 734 (C–G), 681 : 709 (A–U), 831 : 855 (U–G), 832 : 854 (G–U), 833 : 853 (G–C), 840 : 846 (C–U), 952 : 1229 (C–G), 1064 : 1192 (G–G) and 1117 : 1183 (A–U) (Zhi et al. 2009).

The family comprises the genera *Glycomyces, Stackebrandtia* and *Haloglycomyces*.

The nomenclatural type is the genus *Glycomyces* Labeda et al. (1985).

Phylogenetic Structure of the Family and Its Genera

The position of the family in the 16S rRNA gene tree depend upon the selection of outgroup sequences used

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Table 21.1

Properties differentiating the genera *Glycomyces, Stackebrandtia and Haloglycomyces*. Properties of the distantly related genus *Actinocatenispora* is included for comparison (Data are from Labeda and Kroppenstedt (2005), Wang et al. (2009), Guan et al. (2009) and Seo and Lee (2009))

Characteristics	*Glycomyces*	*Stackebrandtia*	*Haloglycomyces*	*Actinocatenispora*
Spore –like bodies	Oval spherical, or rod-shaped spores on vegetative hyphae or chains of cylindrical spores on aerial hyphae	Non-fragmenting vegetative and aerial mycelium	Short or elongated rods on vegetative hyphae (approx 0.45 mm in diameter)	Cylindrical spores on vegetative hyphae or Chains of cylindrical spores on aerial hyphae
Aerial mycelium	On some media Two species without aerial mycelium	On some media in *S. nassauensis* No aerial mycelium in *S. albidiflava*	Well developed on most media	No aerial mycelium in *A. sera*
Diagnostic amino acid(s) in peptidoglycan	meso- A_2pm, gly[a]	meso- A_2pm	meso- A_2pm	meso- A_2pm, Gly
Major menaquinone	MK10, MK11 and/or MK12 isoprene units, but the degree of saturation varies within each species	MK10(H_4), MK10(H_6), MK11(H_4), MK11(H^6)	MK-9(H_4), MK-9(H_2), MK-8(H_4), MK-10(H_4)	MK-9(H_4). MK-9(H_6), and MK-9(H_8) are strain dependent
Presence of $C_{16:0}$ 10-methyl and iso-branched 2 hydroxy fatty acids	–	+	–	–[b]
Major polar lipids	PI, DPG, PIM, PG (some strains)[c]	PG, DPG, PE, PME[c]	PG, PE, DPG, PI, PIM[c]	PI, DPG, PE, PIM, PG[c]
Whole cell sugars	Strain specific, very variable, see Table 21.3	Rib, xyl, glu or rib, ino	Rib, xyl, glu	Ara, xyl, other sugars are strain specific
DNA mol% G+C	70–73	69–73	60.8	72–74.3
NaCl tolerance	Most strains tolerate 5 %	Up to 9 %	Up to 18 %	4 %, one strain 7 %

Abbreviations: *PIM* phosphatidylinositol mannosides, *DGP* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PME* phosphatidylmethylethanolamine, *PG* phosphatidylglycerol and additional unknown phospholipids. *ara* arabinose, *gal* galactose, *glu* glucose, *ino* inositol, *man* mannose, *rib* ribose, *xyl* xylose

[a]Glycine, as well as *N*-glycolylmuramic acid, has been determined in a few species only

[b]Fatty acid composition of strains often differs significantly in different publications

[c]Additional non-identified phospholipids, aminophospholipids and phosphoglycolipids may be present, either individually or in combination

(Labeda and Kroppenstedt 2005; Guan et al. 2009). In the maximum-likelihood and maximum-parsimony trees of Zhi et al. (2009) *Glycomycetaceae*, together with *Actinomycetaceae* and *Actinopolysporaceae*, form a clade, though with different nearest neighbors. The editors (Whitman et al. 2012) of the vol. 5 of *Bergey's Manual*, *Actinobacteria* (www.bergeys.org) elevated the former ranks of subclass and suborder to the ranks of class and order, respectively, to make the taxonomy of the *Actinobacteria* more consistent with that of other prokaryotes. In this outline *Glycomycetales* is a member of a larger clade that also includes the orders *Actinopolysporales*, *Corynebacteriales*, *Jiangellales*, *Micromonosporales*, *Propionibacteriales*, and *Pseudonocardiales*. In the curated tree of The All-Species Living Tree (http://www.arb-silva.de/fileadmin/silva_databases/livingtree/LTP_release_106/LTPs106_SSU_tree.pdf) the nearest neighbor of the *Glycomycetaceae* is the genus *Actinocatenispora* (Thawai et al. 2006; Matsumoto et al. 2007; Seo and Lee 2009). As mentioned by Ludwig et al. (2012), genera of *Glycomycetaceae* and *Actinocatenispora* have certain properties in common (Table 21.1) and the higher taxon affiliation of *Actinocatenispora* should be reconsidered once the branching position of this lineage within the actinobacterial tree has been consolidated. In contrast to the phylogeny depicted in *Bergey's Manual*, the topology of the LTP tree sees *Glycomycetaceae* to be more closely related to *Streptosporangiaceae, Nocardiopsaceae and Thermomonosporaceae*. As pointed out by Munk et al. (2009), NCBI BLAST server search of the *S. nassauensis* 16S rRNA gene sequence indicated the absence of type strains of *Glycomyces* within the 250 top hits. Reanalysis of the search in 2012 confirmed that the closest relatives of *S. nassauensis* and *S. albidiflava* are strains of *Luedemanella, Actinoplanes, Krasilnikovia, Catenuloplanes, Pseudosporangium and Catellatospora* but not the sequences of either *Glycomyces* or *Haloglycomyces* strains. Using the sequence of *Glycomyces harbinensis* as a reference, *Haloglycomyces albus* and *S. albidiflava* are among the 40 top hits while *S. nassauensis* is absent among

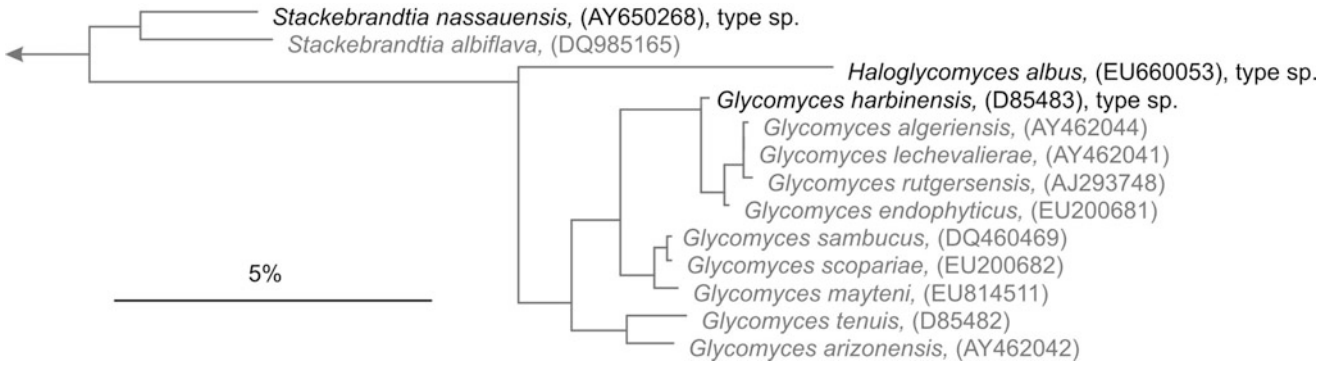

Fig. 21.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the family *Glycomycetaceae* 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 5 % sequence divergence. List of type strains used for dendrogram construction; *Glycomyces harbinensis* IFO (NBRC) 14487^T, *G. algeriensis* NRRL B-16327^T, *G. lechevalierae* NRRL B-16149^T, *G. rutgersensis* IMSNU 22074^T, *G. endophyticus* YIM 56134^T, *G. sambucus* E71^T, *G. scopariae* YIM 56256^T, *G. mayteni* YIM 61331^T, *G. tenuis* NBRC 15904^T, *G. arizonensis* NRRL B-16153^T, *Haloglycomyces albus* YIM 92370^T, *Stackebrandtia nassauensis* DSM 44728^T, *S. albidiflava* YIM 45751^T

the top 250 hits. Undiscovered sequence idiosyncrasies may be the reason for this unusual observation.

The intrafamily relationship sees type strains of *Stackebrandtia* as the deepest branching members, while *Haloglycomyces* and *Glycomyces* are sister lineages. *Stackebrandtia* type strains show 95.8 % 16S rRNA gene sequence similarity among each other and around 92 % similarity with type strains of the other two genera. Within *Glycomyces* the pair *G. tenuis* and *G. arizonensis* form a separate branch. In contrast to other species of the genus these two species are the only ones for which an aerial mycelium has never been observed. A second group comprises the highly related pair *G. sambucus*, and *G. scopariae* (99.7 %) and, more distantly related, *G. mayteni* (98.4–98.8 %), while a third group encompasses the close relatives *G. algeriensis*, *G. lechevalierae* and *G. rutgersensis* (>99.5 %), *G. endophyticus* and *G. harbinensis* (>98.9–99.1 %) (Fig. 21.1). *Haloglycomyces albus* branches between the two generic lineages (Fig. 21.1) and more closely to *Glycomyces* species. The two type strains of *Stackebrandtia* are moderately related among each other (95.4 %) and they are distantly related to both, *Glycomyces* (88.9–90.7 %) and *Haloglycomyces* type strains (89 %). The three *Actinocatenispora* type strains, being 97.6–99.3 % related among themselves, are slightly more close to *Stackebrandtia* strains (90.4–91.1 %) than to those of *Haloglycomyces* and *Glycomyces* (86.4–87.5 %).

Additional species names have been proposed during the deposition of 16S rRNA gene sequences in public databases but none of them have yet been validly named: "*Glycomyces mongolensis*" (Tsetseg et al. unpublished, AB367437), "*Glycomyces xinjiangensis*" from a salt field in Xinjiang, China (Shi and Tang, unpublished, HQ833642), "*Glycomyces donghensis*" and "*Actinocatenispora albus*" from a salt soil (Gorden unpublished, JN252407 and HQ412605, respectively) and "*Glycomyces albus*" (Tang unpublished GU119912). A strain of "*Glycomyces illinoisensis*" has been deposited by D. Labeda in the DSMZ as DSM 44734. It should be noted that the sequence AM980986 is entered in public data repositories as "*Actinocatenispora silicis*" and not under *A. rupis*.

Molecular Analyses

DNA-DNA Hybridization Studies

As the 16S rRNA gene sequences of several *Glycomyces* strains are highly similar, DNA-DNA hybridization (DDH) experiments were performed in order to elucidate whether or not they represent genomic species. Even at very high sequence similarities at 99.7 % sequence similarity corresponding DHH values were not higher than 58 % (Table 21.2), thus distinctly below the 70 % threshold value recommended for species delineation (Wayne et al. 1987). The only other highly related strain pair is *Actinocatenispora thailandica and A. sera* (99.3 % 16S similarity) sharing 45–475 DDH similarity (Matsumoto et al. 2007).

Genome Analysis

At present only the full genome sequence of *Stackebrandtia nassauensis* DSM 44728^T is available for members of *Glycomycetaceae*. The genome which has been sequenced within the Genomic Encyclopedia of Bacteria and Archaea project (Munk et al. 2009) has a circular chromosome of 6,841 Mb. 6.450 genes were predicted of which 6.487 code for proteins. In addition 2 rrn operons were identified and 53 genes coding for RNAs. 68 % of the protein-coding genes were associated with the

Table 21.2

Relationship between 16S rRNA gene sequence similarities (lower left triangle) and DNA-DNA reassociation values (upper right triangle) within groups of highly related *Glycomyces* species. Similarity values were calculated on the basis of the restricted set of sequences of strains: *G. algeriensis* NRRL B-16327^T; *G. lechevalierae* NRRL B-16149^T; *G. rutgersensis* IMSNU 22074^T; *G. endophyticus* YIM 56134^T; *Glycomyces harbinensis* IFO 14487^T; *G. sambucus* E71^T; *G. scopariae* YIM 56256^T; *G. mayteni* YIM 61331^T; *G. tenuis* NBRC 15904^T; *G. arizonensis* NRRL B-16153^T

	NRRL B-16327^T	NRRL B-16149^T	IMSNU 22074^T	YIM 56134^T	IFO 14487^T	E71^T	YIM 56256^T	YIM 61331^T	NBRC 15904^T 9
NRRL B-16327^T	X	57	40	50	–	–	–	–	–
NRRL B-16149^T	99.9	X	54	53	–	–	–	–	–
IMSNU 22074^T	99.9	99.9	X	44	–	–	–	–	–
YIM 56134^T	99.1	99.1	99.0	X	36	–	–	–	–
IFO 14487^T	98.8–98.9			98.9	X	–	–	–	–
E71^T	97.1–97.5					X	58	42	–
YIM 56256^T	96.7–97.2					99.6	X	38	–
YIM 61331^T	96.7–97.3					98.8	98.4	X	
NBRC 15904^T	94.7–96.0								X
NRRL B-16153^T	94.0–95.2								96.4

– not determined

general COG functional categories. The majority of these genes were annotated as belonging to transcription (13.5 %), carbohydrate- (9.0 %) and amino acid transport and metabolism (7.6 %), as well as signal transduction mechanisms (7.2 %). The mol% G + C content of 68.1 % is 4.3 % lower than that determined by HPLC (Labeda and Kroppenstedt 2005).

Phenotypic Analyses

Glycomyces Labeda et al. 1985, 419^{VP}, emend Labeda and Kroppenstedt 2004, 2345.

Gly'co.my.ces. Gr. adj. *glykus* sweet; Gr. n. *myke* fungus; N.L.n. *Glycomyces* sweet (glycolipid-containing).

The emendation became necessary with the description of four novel species (Evtushenko et al. 1991; Labeda and Kroppenstedt 2004) when the original circumscriptions of morphological and chemotaxonomic properties were too narrow. With the description of additional four species between 2007 and 2009 (Gu et al. 2007; Qin et al. 2008, 2009) an even higher variation among some of the genus-specific properties were noticed without, however, emending the genus description. Type strains of the species were not cultivated on the same range of growth media and not all morphological (e.g., spore morphology, dimension of vegetative mycelium), physiological (e.g., lysozyme sensitivity, catalase) and chemotaxonomic properties (e.g., presence of glycine in peptidoglycan, mol% G+C of DNA) were investigated for all type strains. In addition to entries listed in ❯ *Table 21.1* the range of described properties for type strains are indicated in ❯ *Tables 21.3* and ❯ *21.4*. Strains are Gram-positive, aerobic, and filamentous. The *N*-acyl group of the cell-wall muramic acid is glycolyl. All strains produce acid from L-arabinose, maltose, D-xylose as well as from D-glucose, salicin, D-mannose (*G. tenuis* not tested), and α-methyl-D-glucoside (*G. tenuis* and *G. arizonensis* not tested), but not from dulcitol. All strains decompose casein. Additional reactions which were only tested in a few species are not listed here and should be looked at in the original descriptions. The growth temperature ranges from 10 °C to 42 °C and most strains grow between 15–20 °C and 37 °C.

Genus-specific 16S rRNA gene signature nucleotides have not been determined. The type species is *Glycomyces harbinensis*.

Stackebrandtia Labeda and Kroppenstedt 2005, 169^{VP}, emend. Wang et al. 2009.

Stack.e.brandt'i.a. N.L. fem. n. *Stackebrandtia* named for Erko Stackebrandt.

Gram-positive, aerobic non-motile, filamentous. Branched and non-fragmenting substrate mycelium are produced. Catalase-positive. Growth range between 17 °C and 37 °C. The *N*-acyl group of the cell-wall muramic acid is glycolyl. Other genus-specific as well as species-specific properties are indicated in ❯ *Tables 21.1* and ❯ *21.5*. Genus-specific 16S rRNA gene signature nucleotides have not been determined. The type species is *Stackebrandtia nassauensis*.

Haloglycomyces Guan et al. 2009, 1299^{VP}

Ha'lo.gly.co.my'ces. Gr. n. *hals* salt; N.L. masc. n. *Glycomyces* a bacterial genus name; N.L. masc. n. *Haloglycomyces* a salt-(loving) *Glycomyces*-like bacterium.

Most of the salient generic features are indicated in ❯ *Table 21.1*. Aerobic, Gram-positive, halophilic and filamentous. Oxidase-negative and catalase-positive. The menaquinones

Table 21.3

Range of morphological and chemotaxonomic properties in Glycomyces type strains (Data are from Labeda et al. (1985), Labeda and Kroppenstedt (2004), Evtushenko et al. (1991), Qin et al. (2008, 2009), and Gu et al. (2007)) 1 *G. harbinensis* IFO 14487^T, 2 *G. algeriensis* NRRL B-16327^T, 3 *G. lechevalierae* DSM 44724^T, 4 *G. rutgersensis* IFO 14488^T, 5 *G. endophyticus* YIM 56134^T, 6 *G. scopariae* YIM 56256^T, 7 *G. sambucus* DSM 45047^T, 8 *G. mayteni* YIM 61331^T, 9 *G. tenuis* VKM Ac-1250^T, 10 *G. arizonensis* NRRL B-16153^T

Characteristics	1	2	3	4	5
Color vegetative mycelium	Pale yellow to Yellowish white	White to yellowish white	White to yellowish white	Yellowish white to tan	Yellowish to cream
Aerial mycelium	On some media	On some media	On several media	On some media	On the media tested
Spore morphology	Square-ended conidia	nd	nd	Square-ended conidia	Square-ended conidia
Lysozyme sensitive	+	nd	nd	+	nd
Catalase	+	nd	nd	+	nd
Peptidoglycan	meso-A_2pm, Gly	meso- A_2pm	meso- A_2pm	meso- A_2pm, Gly	meso- A_2pm,
Cell wall sugar (minor)	Xyl, ara	Xyl, rib, man, gal	Xyl, rib, man, gal	Xyl, ara	Xyl, gal, glu (rib)
Phospholipid	PI, DPG, PIM	PI, PG, DPG, PIM. PL[a]	PI, PG, DPG, PIM, PL[a]	PI, DPG, PIM	PI, PG, DPG, PIM, PL[a]
Major Fatty acids Differ slightly from study to study	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, ai-$C_{15:0}$[b]	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, ai-$C_{15:0}$	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, ai-$C_{15:0}$	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, ai-$C_{15:0}$[b]	ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$
Major menaquinones	MK10(H_2), MK10(H_4)	MK10, MK11, MK12	MK10, MK11, MK10(H_2), MK10(H_4), MK11(H_2), MK11(H_4)	MK10(H_2), MK10(H_4)	MK10(H_4), MK11(H_4)
G+C mol% DNA	71	nd	nd	73 or 70, depending on method	72
Characteristics	6	7	8	9	10
Color vegetative mycelium	Yellowish white to yellow orange	Yellowish white to tan	Yellowish white to cream yellow	White to cream	White to yellowish white
Aerial mycelium	On the media tested	On most media	On the media tested	none	none
Spore morphology	Square-ended conidia	Square-ended conidia	Square-ended conidia	Oval to rod shaped	nd
Lysozyme sensitive	nd	nd	nd	+	nd
Catalase	nd	nd	nd	nd	nd
Peptidoglycan	meso- A_2pm,	meso- A_2pm	meso- A_2pm,	meso- A_2pm, Gly	meso- A_2pm
Cell wall sugar (minor)	Xyl, Gal, Glu	Xyl, Gal, Glu (Rib)	Xyl, Gal, Glu	Ara, Xyl	Xyl, Rib, Man, Gal
Phospholipid	PI, PG, DPG, PIM, PL[a]	PI, PG, DPG, PIM, PL[a]	PI, PG, DPG, PIM, PL[a],	PI, PIM, cardiolipin	PI, PG, DPG, PIM. PL[a]
Major Fatty acids Differ slightly from study to study	ai-$C_{15:0}$, i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, i-$C_{14:0}$	iso-C16:0, ai-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, i-$C_{14:0}$	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, ai-$C_{15:0}$[1]	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, ai-$C_{15:0}$
Major menaquinones	MK10(H_4), MK11, MK11(H_4)	MK11, MK11(H_4), MK10	MK10, MK11, MK11(H_4)	MK9(H_6), MK10(H_6), MK11(H_6)	MK10(H_2), MK10(H_4), MK11(H_2), MK11(H_4)
G+C mol% DNA	72.2	70	71.5	72	nd

Abbreviations see *Table 21.1*. *ai* anteiso, *i-* iso

[a]*PI* phospholipids of unknown composition

[b]Evtushenko et al. 1991

are MK-9(H4), MK-9(H2), MK-8(H4), MK- 10(H4) and MK-10(H2). Major cellular fatty acids are iso-C16 : 0, iso-C17 : 0 and anteiso-C17 : 0. The 16S rRNA signature nucleotides are 126 : 235 (A–U), 137 : 226 (U–G), 146 : 176 (G–U), 379 : 384 (G–C), 407 : 435 (G–C), 408 : 434 (A–U), 418 : 425 (U–A), 501 : 544 (U–G), 615 : 625 (U–A), 617 : 623 (A–U), 896 : 903 (U–G), 1124 : 1149 (A–U), 1165 : 1171 (U–A), 1263 : 1272 (G–U) and 1265 : 1270 (U–G). The type species is *Haloglycomyces albus*.

Table 21.4

Differential metabolic reactions of *Glycomyces* type strains (Data are from Labeda et al. (1985), Labeda and Kroppenstedt (2004), Evtushenko et al. (1991), Qin et al. (2008, 2009), and Gu et al. (2007)) 1 *G. harbinensis* NRRL B-15337^T, 2 *G. algeriensis* NRRL B-16327^T, 3 *G. lechevalierae* DSM 44724^T, 4 *G. rutgersensis* NRRL B-16106^T, 5 *G. endophyticus* YIM 56134^T, 6 *G. scopariae* YIM 56256^T, 7 *G. sambucus* DSM 45047^T, 8 *G. mayteni* YIM 61331^T, 9 *C. tenuis* VKM Ac-1250^T, 10 *G. arizonensis* NRRL B-16153^T

Reactions	1	2	3	4	5	6	7	8	9	10
Acid produced from										
Adonitol	+	–	+	–	–	–	–	–	–	+
Cellobiose	+	+	+	+	+	+	+	–	nd	+
Dextrin	+	+	+	+	+	–	+	+	nd	+
D-fructose	+	+	+	+	+	–	+	+	+	+
D-galactose	+	+	+	+	+	+	+	–	+	+
D-lactose	+	+	+	w	+	–	+	–	+	+
D-mannitol	–	–	–	+	–	+	–	+	–	+
D-rhamnose	+	+	+	+	+	–	+	–	+	+
D-sorbitol	+	–	w	+	–	–	–	–	–	+
Erythritol	–	–	–	–	–	+	–	–	nd	+
Glycerol	+	+	+	+	nd	nd	+	nd	nd	+
Inositol	–	+	+	–	+	–	–	–	–	+
Mannitol	–	–	–	+	nd	nd	nd	nd	nd	+
Melibiose	–	–	w	–	–	–	+	–	–	–
Melizitose	–	–	–	w	–	–	–	–	nd	+
Methyl β-xyloside	+	–	–	+	+	+	+	–	nd	–
Raffinose	+	–	–	+	–	–	+	–	+	–
Sorbitol	+	–	w	+	nd	nd	nd	nd	nd	+
Sucrose	–	–	+	–	+	–	+	+	+	+
Trehalose	+	–	+	+	w	–	–	+	nd	+
Assimilation of sole carbon sources										
Acetate	+	w	+	+	–	–	+	–	+	–
Citrate	+	–	+	–	–	–	w	–	–	–
Oxalate	–	w	+	–	–	–	–	–	–	–
Succinate	+	–	+	–	+	+	+	–	–	–
Malate	+	w	+	+	+	+	+	+	–	–
Propionate	+	w	–	+	w	+	–	–	nd	–
Lactate	–	–	–	+	nd	nd	nd	nd	nd	–
Tartrate	–	–	–	–	–	–	+	–	–	–
Decomposition of										
Adenine	+	+	+	+	+	+	+	+	nd	–
Aesculin	+	–	+	+	+	+	+	+	+	+
Gelatin	–	–	–	+	–	–	–	–	nd	–
Hypoxanthine	+	–	+	+	+	+	–	–	+	–
L-tyrosin	–	–	+	–	+	–	–	+	–	–
Starch	+	–	+	+	+	–	+	+	+	–
Urea	–	–	–	–	+	+	–	+	–	–
Xanthine	–	–	–	–	–	–	–	+	nd	–
Reduction of nitrate	w	w	+	+	+	–	w	+	nd	w
Growth at 42 °C	+	–	–	–	–	–	–	–	–	–
Growth on 5 % NaCl	+	+	+	+	+	+	–	+	+	+

+ positive, 2 negative, *w* weak reaction, *nd* not determined

Table 21.5

Differentiating properties of *S. nassauensis* NRRL B-16338T and *S. albidiflava* YIM 45751^T. Both type strains of *Stackebrandtia* produce acid from galactose, mannose, arabinose and cellobiose, but not from inositol, adonitol, dulcitol, melezitose or erythritol. Both strains are positive for starch, casein and gelatin hydrolysis, but negative for adenine degradation. Neither strain assimilates tartrate, benzoate or mucate (Data are from Labeda and Kroppenstedt (2005) and Wang et al. (2009) who also lists additional morphological properties and metabolic reactions)

Characteristics	NRRL B-16338^T	YIM 45751^T
Aerial mycelium	On some media	On no medium tested
Catalase	+	+
Peptidoglycan	meso-Dpm	Meso-Dpm
Whole cell sugars	Rib, Ino (ara, man)	Rib, Xyl, Glu
Phospholipids[a]	PG, DPG, 2 PI	PG,DPG, PE, PME, PI, 3 PL, 1APL
Menaquinones	MK10(H_4), MK10(H_6), MK11(H_4), MK11(H_6)	MK10(H_4), MK10(H_6), MK11(H_4), MK11(H_6)
Fatty acids	ai-$C_{17:0}$, ai-$C_{17:0}$-2OH, 10-methyl-$C_{16:0}$, i-$C_{15:0}$	ai-$C_{17:0}$, i-$C_{17:0}$, i-$C_{15:0}$
Mol% DNA G+C content	72.4 (HPLC)	69.4 (HPLC)-
Hydrolysis of		
Allantoin	+	nd
Aesculin	+	–
Hypoxanthine	+	–
Xanthine	–	+
Tyrosine	+	–
Utilization of		
Citrate	–	w
Propionate	–	+
Acetate	w	–
Malate	w	–
Acid from		
Mannitol	–	+
Glycerol	+	–
Raffinose	+	–
Glucose	+	–
Trehalose	+	–
Sucrose	+	–
Nitrate reduction	w	+
Grows up to 9 % NaCl	+	–

For other abbreviations see *Tables 21.1* and *21.3*. *APL* aminophospholipid + positive, – negative, *w* weak reaction, *nd* not determined

[a]The phospholipid pattern determined for *S. nassauensis* by Wang et al. (2009) is different to that determined for the same strain by Labeda and Kroppenstedt (2005)

In addition to properties listed in *Table 21.1* morphological descriptions and metabolic reactions are indicated by Guan et al. (2009).

The type strain *H. albus* YIM 92370^T is defined by the following metabolic reactions: utilizes cellobiose, glucose, maltose, mannitol, mannose, rhamnose and sorbitol as sole carbon sources, but not D-arabinose, dextrin, fructose, galactose, glycerol, inositol, lactose, melibiose, raffinose, ribose, xylitol or xylose. Positive for gelatin hydrolysis, starch hydrolysis, milk coagulation and peptonization, but negative for urease, cellulose hydrolysis, production of H_2S and nitrate reduction. additional morphological properties and metabolic reactions.

Actinocatenispora Thawai et al. 2006, 1792^{VP}, emend Seo and Lee 2009

The genus *Actinocatenispora* is not an authentified member of *Glycomycetaceae* but included here as family *insertae sedis* because of its isolated phylogenetic position.

Ac.ti.no.ca.te.ni.spo′ra. Gr. n. *actinos* ray; L. n. *catena* chain; Gr. n. *spora* seed; N.L. fem. n. *Actinocatenispora* spore chain-producing ray (fungus).

In addition to properties indicated in *Table 21.1* Gram-positive, non-acid-fast, non-motile and aerobic organisms with branching substrate hyphae. Aerial hyphae are formed in two species. Spore chains are borne on the aerial mycelium or produced directly from the vegetative mycelium. Spores (0.4–0.5 × 0.9–1.6 μm or 0.3–0.4 × 0.5–1.0 μm) are cylindrical and the spore surface is smooth. Cell wall contains glutamic acid, glycine, alanine and *meso*-diaminopimelic acid. The *N*-acyl group of the cell-wall muramic acid is glycolyl. Mycolic acids are not detected. Habitat is soil. The type species is *Actinocatenispora thailandica*.

The three type strains of the species *A. thailandica* TT2-10^T (Thawai et al. 2006), *A. sera* KV-744^T (Matsumoto et al. 2007) and *A. rupis* CS5-AC17^T (Seo and Lee 2009) differ in the metabolic properties (*Table 21.6*) as indicated by the latter authors. Additional metabolic, chemotaxonomic and morphological characteristics are indicated in the respective species descriptions.

Isolation, Enrichment and Maintenance Procedures

Several strains were taken as freeze-dried culture from public culture collections and information about the original isolation method is not available. For the isolation of *G. arizonensis* B-16153^T, a 1 g soil sample from a kangaroo rat (*Dypodymus* sp.) burrow was suspended in 9 ml sterile tap water and shaken with two 5 mm glass beads in a 25 × 150 mm. After heating at 60 °C for 5 min and further dilutions samples were spread on the surface of Czapek's sucrose agar (Waksman 1950) amended with 50 μg each of cycloheximide and nystatin ml^{-1}, 10 mg streptomycin ml^{-1} and 25 mg novobiocin ml^{-1}. Antibiotics were also

Table 21.6
Characteristics differentiating the three type strains of *Actinocatenispora*. All strains utilize D-glucose but not D-fructose as sole carbon source

Characteristic	TT2-10^T	KV-744^T	CS5-AC17^T
Utilization of			
L-arabinose	–	–	+
Cellobiose	+	–	+
Glycerol	+	–	+
myo-Inositol	+	–	+
D-mannitol	+	–	+
Melibiose	+	–	–
Raffinose	+	–	–
L-rhamnose	–	+	+
Trehalose	+	–	+
D-xylose	+	+	–
Gelatine liquefication	w	–	+
Nitrate reduction	+	+	–
Growth at/with			
42 °C	–	–	+
pH4.5	+	–	–
pH12.1	+	–	+
7 % NaCl	+	–	–

+ positive, – negative, *w* weak reaction

used in the isolation of *Stackebrandtia albiflava* (raffinose-histidin agar, (Vickers et al. 1984), supplemented with cycloheximide and nyastin,50 and 25 µg ml^{-1}, respectively) and for the endophyte *G.sambucus*. This organism was obtained by placing slices of surface sterilized root samples on BL-2 agar plates containing per liter 5 g each of glucose, soluble starch, NaCl and $CaCO_3$, 2 g acid casein hydrolysate, 1 g yeast extract, 15 g agar, and 100 µg penicillin ml^{-1} (Gu et al. 2006). The organism, seen around the root sample under a light microscope, was transferred onto fresh yeast extract/malt extract (ISP medium 2) agar (Shirling and Gottlieb 1966). As an alternative, pulverized surface-sterilized roots were pulverized and tap water suspensions spread ontop of tap water/yeast extract medium (Crawford et al. 1993). Isolation of *Haloglycomyces albus* was achieved on cellulose-casein agar containing 10 g microcrystalline cellulose, 0.3 g casein, 0.2 g KNO_3, 0.5 g K_2HPO_4, 0.02 g $CaCO_3$, 0.01 g $FeSO_4$, 100 g NaCl, 30 g $MgCl_2.6H_2O$, 20 g KCl and 15 g agar per litre distilled water, pH 7.5 (Tang et al. 2008). *Actinocatenispora thailandica* was isolated on starch-casein nitrate agar. A. sera was isolated on agar media containing per litre 10 g L-proline, and 15 g agar, supplemented with the anti-fungal reagent (Benlate® 20 *m*g ml^{-1} Dupont Co., DE, USA). *A. rupis* was recovered on starch casein agar (1 % soluble starch, 0 · 03 % casein, 0 · 2 % KNO_3, 0 · 2 % NaCl, 0 · 002 % $CaCO_3$, 0 · 005 % $MgSO_4.7H_2O$, 0 · 001 % $FeSO_4.7H_2O$ and 1 · 8 % agar [pH 7 · 2]) after 2 weeks at 30 °C. Cultivation was done at about 27–30 °C for 1–4 weeks. Subcultivation and maintenance were done on a wide range of media conventionally used for the growth of streptomycetes, such as various ISP media, medium ATCC 172, Czapek agar, oatmeal agar or yeast extract-malt extract agar.

No difficulties in short or long term maintenance have been reported and isolates can be stored at 4 °C as 20 % (v/v) glycerol suspensions, freeze-dried in sterile serum at 4 °C, lyophilized or kept in straws under N_2 vapor.

Ecology

Four of the *Glycomyces* type strains are endophytes, i.e., *G. endophyticus* (Qin et al. 2008), *G. sambucus* (Gu et al. 2007), and *G. scopariae* and *G. mayteni* (Qin et al. 2009) isolated from the roots of *Carex baccans* and *Sambucus adnata*, *Scoparia dulcis* and *Maytenus austroyunnanensis*, respectively; all other type strains were soil isolates. *G. tenuis* was isolated from a sample of *Streptomyces galilaeus* which itself is a soil organisms (Evtushenko et al. 1991). The type of soil varied from greenhouse to farm soils and origin of the soils and plants from China, North Africa and the USA indicates a wide geographic distribution. Soil is also the habitat of *Stackebrandtia* (Bahamas, China), *Haloglycomyces* (China) species, the latter being isolated from a hypersaline sample, as well as of *Actinocatenispora* (soil surface of peat swamp forest, Thailand; cliff of Korean island and soil, Niigata Prefecture, Japan).

Browsing through the NCBI BLAST server only a few sequences are found which are closely to moderately related to members of the three genera. Several of the strains/DNA originate from soil but also from sediments, plant material (e.g., seagrass, Zhang et al. unpublished, GQ163474.1) and waste (e.g., composted swine manure Hayakawa et al. unpublished; AB562484). Besides strains with invalid names mentioned above, several entries do not link the origin of the habitat to the sequence information. *Glycomyces* strains have been reported from a study on rare actinomycetes from shallow water of Trondheim Fjord, Norway (e.g., EF212018, EF216364; Bredholdt et al. 2007). A large number of entries (>20, e.g., GQ262917, GQ263583) belong to *Glycomyces*-related clone sequences (97–98 % similarity) from a simulated low-level radioactive waste side in Idaho, USA (Field et al. 2010). This simulated waste layer (not soil) was covered with an overlying soil layer using local unsaturated soils. The same study also reports the presence of a single clone related to *Actinocatenispora* spp (97 % BLAST hit; GQ263629).

The only record for a sequence moderately related (95 %) to *Haloglycomyces albus* originate from a highly alkaline saline soil (Valenzuela-Encinas et al. 2009).

A few entries of as-yet uncultured strains were found to be moderately (96 %) related to *Stackebrandtia* strains such as those found in pig manure (Snell-Castro et al. unpublished AM982657), from fungus garden material of the leaf-cutter ant *Atta colombica* (Suen et al. 2010) and in a report from the NIH Intramural Skin Microbiome Consortium (Kong et al. unpublished).

Medical Relevance and Application

All members of the four genera are listed as risk group 1 organisms according to the German Technical Rules for Biological Agents and internal DSMZ assessment as indicated in the DSMZ catalogue of strains (www.dsmz.de).

Antibiotic sensitivity has only been tested for *G. tenuis* VKM Ac-1250^T (Evtushenko et al. 1991). The strain did not grow in the presence of (per ml^{-1})10 μg of streptomycin, 5 μg of tobramycin, 10 μg of erythromycin, 1 μg of ristomycin; ir is resistant to (per ml^{-1}) 5 μg of oligomycin, 1 μg of novobiocin, and the following antibiotics at concentrations less than 1 μg/ml: carbenicillin, erythromycin, streptomycin, tetracycline, rifampin, and ristomycin. No antibiotic activity is observed against *Bacillus mycoides, Escherichia coli, Staphylococcus aureus, Sarcina lutea*, and *Saccharomyces cerevisiae.*

Of the two *Glycomyces* isolates isolated from the Trondheim Fjord (Bredholdt et al. 2007) and tested for antimicrobial activity and the presence of polyketide synthase and non-ribosomal peptide synthetase genes only one strain gave a positive signal for the presence of a PKS I genes. Neither strain showed antimicrobial activity.

Screening of the complete genome of *Stackebrandtia nassauensis (accession number* CP001778) revealed several KEGG-defined entries of proteins related to polyketide synthases and non-ribosomal peptide synthetase (several for non-ribosomal peptide synthetase/polyketide synthase; several for putative exporter of polyketide antibiotics-like protein; putative cyclase/dehydrase; putatively involved in polyketide/antibiotic biosynthesis; and coronafacic acid polyketide synthase I and II). This suggests that under favorable conditions *S. nassauensis* is capable of antibiotic production, possibly including lantibiotics, as the genome analysis gave several hints for proteins involved in their synthesis such as putative lantibiotic modification protein, lantibiotic dehydratase domain protein, and other proteins involved in lantibiotic biosynthesis.

G. harbinensis is the producer of the antitumor antibiotic LL-DO5139β (Lee et al. 1987). This substance is part of the LL-D05139 complex, containing LL-DQ5139β and azaserine. Chemical analysis revealed LL-DO5139β to be *N*-L-alanyl-L-serine diazoacetate with similar but greater antibacterial activities than serine diazoacetate (azaserine). The dipeptide antibiotic is active against gram-positive and -negative bacteria and tumors. The fermentation process and antimicrobial spectrum of LL-D05139/3 and azaserine has been shown by Lee et al. (1987).

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22 The Family *Iamiaceae*

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Abstract
A family within the order *Acidimicrobiales* embraces the genera *Iamia* and *Ilumatobacter*. Members of the family are defined by a wide range of morphological and chemotaxonomic properties, such as isoprenoid menaquinones, fatty acids, amino acids of peptidoglycan and whole cell sugars, which are used for the delineation of genera and species. Members of the family are mainly found in marine environments, but they have been isolated from soils, activated sludge.

Taxonomy: Historical and Current

Short Description of the Family and Their Genera

I.a.mi.a'ce.ae. N.L. fem. n. *Iamia* type genus of the family; -aceae ending to denote a family; N.L. fem. N. *Iamiaceae* the family of *Iamia*.

Phylogenetically a member of the order *Acidimicrobiales* (Stackebrandt et al. 1997; Zhi et al. 2009), phylum Actinobacteria.

The family was originally proposed for the genus *Iamia* (Kurahashi et al. 2009), but nearly at the same time, a new genus *Ilumatobacter* (Matsumoto et al. 2009), which should be included in this family, was independently proposed in 2009. "*Candidatus* Microthrix parvicella" (Blackall et al. 1996) and "*Candidatus* Microthrix calida" (Levantesi et al. 2006), which has been proposed for filamentous bacteria isolated from activated sludge sewage treatment plants and their tasxonomic category not covered by the Rules of the Bacteriological Code, could be included in this family. Taxonomic characterization for these species is still incomplete (*Fig. 22.1*).

Gram-staining positive. Non-sporing and nonmotile rods. Aerial mycelium is not formed. Grow aerobically. Some species require NaCl or seawater for their growth. Diagnostic diaminoacids are either *meso*-diaminopimelic acid or LL-diaminopimelic acid. Hexadecanoic acid (C16:0) and hexadecenoic acid (C16:1) are the predominant fatty acids. Menaquinone MK-9(H_4) or MK-9(H_8) is the predominant isoprenoid quinone. The set of 16S rRNA gene sequence signature is comprised of positions 408–434 (G-C), 722–733 (G-G), 1118–1155 (U-A), 443–491 (U-A), 1165–1171 (G-C), and 1263–1272 (A-U). G+C values of DNA range between 68 and 74 mol%. Usually found in marine environments.

Phylogenetic Structure of the Family and Its Genera

According to the phylogenetic branching of actinobacterial type strains in the 16S rRNA gene tree of the Living Tree Project (Munoz et al. 2011), the family *Iamiaeae* is moderately related to the family *Acidmicrobiaceae* (Stackebrandt et al. 1997; Zhi et al. 2009). In the *Iamiaceae* cluster, genus *Iamia*, *Ilumatobacter*, "*Candidatus* Microthrix parviella" (Blackall et al. 1996), and "*Candidatus* Microthrix calida" (Levantesi et al. 2006) are included.

Molecular Analyses

Genome Comparison (Genome Analyses)

Genome sequences are not available for any strain in this family.

Phages

Any phages are not known for the strains in this family.

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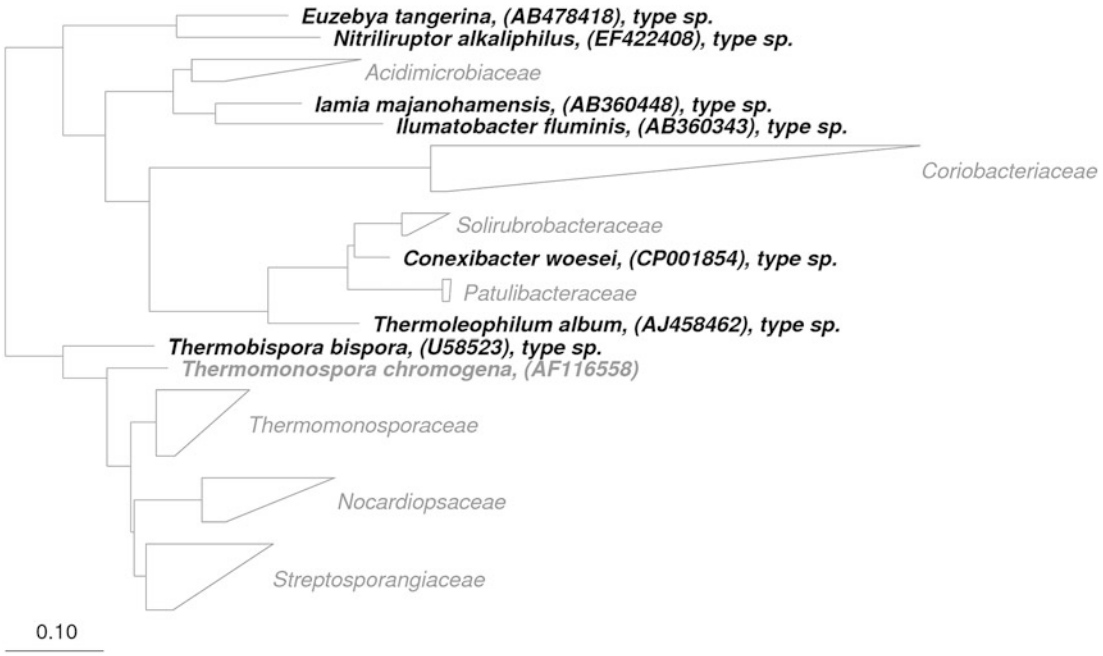

Fig. 22.1

Phylogenetic reconstruction of the family *Iamiaceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as 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

Phenotypic Analyses

Differentiating cultural and physiological characteristics between *Iamia* and *Ilumatobacter* and neighboring taxa are indicated in *Table 22.1*.

Iamia Kurahashi et al. 2009, 871[VP]

Iamia (I.a'mi.a. N.L. fem. N. *Iamia* arbitrary name formed from the acronym of the Institute of Applied Microbiology at the University of Tokyo, which has made significant contribution to Microbiology).

Cells are Gram-positive, non-endospore-forming rods that are nonmotile. Colonies grow aerobically, but not anaerobically. In media devoid of sodium chloride, growth is observed. Oxidase- and catalase-positive. The predominant menaquinone is MK-9(H_6). Cell wall contains glutamic acid, alanine, and *meso*-diaminopimelic acid. The peptidoglycan is presumed to be A1γ. The peptidoglycan is of the glycolyl type. The major cellular fatty acids are heptadecanoic acid (C17:0), heptadecenoic acid (C17:1ω8c), pentadecanoic acid (C15:0), and hexadecanoic acid (C16:0). The type species is *Iamia majanohamensis*.

In addition to the properties given in the genus description, the type strain of *Iamia majanohamensis* F12^T shows following characteristics: cells are 1.2–1.7 × 0.3–0.5 μm in size. Colonies on marine agar are small, white, circular, convex, smooth, shiny, and 0.2–0.3 mm in diameter after 3-week incubation at 30 °C. Optimal growth temperature is 28–30 °C. No growth occurs at temperatures above 45 °C or below 10 °C. Hydrolysis of gelatin is observed. Cell-wall sugars are rhamnose, mannose, arabinose, galactose, and xylose. Positive for catalase, oxidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, production of acetoin and gelatinase, and reduction of nitrate to N_2, but negative for β-galactosidase, urease, tryptophan deaminase, utilization of citrate, production of H_2S and indole, and assimilation of amygdalin, arabinose, D-glucose, inositol, D-mannose, melibiose, rhamnose, sucrose, and sorbitol. The DNA G+C content is 74.4 mol%.

The type strain of *Iamia majanohamensis* F12^T was isolated from the ventral epidermis of the sea cucumber *Holothuria edulis* at Aka Island, Okinawa, Japan.

Ilumatobacter Matsumoto et al. 2009[AL]

Ilumatobacter (I.lu.ma.to.bac'ter. Gr. N. *iluma* –atos, sediment deposited in water; N.L. masc. n. *bacter*, a rod, a bacterium; N.L. masc. n. *Ilumatobacter* a rod isolated from a sediment).

Cells are Gram-positive, aerobic, nonmotile rod. The cell-wall peptidoglycan contains LL-diaminopimelic

Table 22.1
Differential characteristics of members of the family *Iamiaceae* and related genera

	Acidimicrobium	*Ferrimicrobium*	*Ferrithrix*	*Aciditerrimonas*	*Iamia*	*Ilumatobacter*	"*Candidatus* Microthrix parvicella"	"*Candidatus* Microthrix calida"
Cell shape	Rod	Rod	Filament	Short rod	Rod	Rod	Filament	Filament
Pigmentation	ND	ND	ND	non-pigment	non-pigment	non-pigment	ND	ND
Gram stain	+	−	−	+	+	+	+	+
Motility	+	+	−	+	−	−	ND	ND
Temperature for growth								
Range (◦C)	25–50	37	50	35–58	28–30	26–31	7–25	10–36.5
Optimum (◦C)	45–50	35	43	50	ND	ND	ND	ND
pH for growth	2	2	1.8	3	7	7–11	ND	ND
Oxidation of Ferrous ion	+	+	+	−	ND	ND	ND	ND
Diamino acid in CW	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	LL-DAP	ND	ND
Major fatty acid	i-C16:0, ai-C17:0 i-C18:0	i-C16:0, i-C14:0, ai-C15:0	i-C16:0	i-C16:0, ai-C17:0 i-C18:0	C17:0, C17:1w8c	i-C16:0, i-C17:1 w9c, i-17:0	ND	ND
Major quinone	MK-9(H8)	MK-8(H10)	ND	MK-9(H8)	MK-9(H6)	MK-9(H8)	ND	ND
G+C content (mol%)	67–69	55	50	74	74	68	ND	ND
Isolated from geothermal/mine habitat	+	+	+	+	−	−	−	−
	Pyrite of Icelandic geothermal site	Mine water in North Wales	Mine water in North Wales	Solfataric field of Hakone	Sea cucumber epidermis	Sediment of estuary	Activated sludgesewage treatment plants	Activated sludge sewage treatment plants

acid, glycine, alanine, and hydroxyglutamate (molar ratio, ca. 1.4: 2.4: 1.1: 1.0). The acyl type is glycolyl. Predominant menaquinone is MK-9(H_8). Mycolic acids are not detected. The major cellular fatty acids are 14-methyl-pentadecanoic acid (iso-C16:0), 15-methyl- hexadecenoic acid (iso-C17:1ω9c), 15-methyl-hexadecanoic acid (iso-C17:0), 14-methyl-pentadecenoic acid (iso-C16:1), heptadecenoic acid (C17:1ω8c), octadecenoic acid (C18:1ω7c), 16-methyl-hexadecenoic acid (iso-C18:1), and 14-methyl-hexadecanoic acid (anteiso-C17:0). The type species is *Ilumatobacter fluminis*.

In addition to the properties given in the genus description, the type strain of *Ilumatobacter fluminis* shows the following characteristics: The colonies are colorless. The cells are about 0.4–0.5 × 1.3–1.6 μm. Sea water or artificial sea water is needed for growth. The temperature range for growth is 26–31 °C. Growth occurs at pH 7–11. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and β-glucosidase are present, but α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase are absent. The G+C content of the genomic DNA of the type strain is 68 mol%. Habitat is marine. The type strain is YM22-133^T.

Isolation, Enrichment, and Maintenance Procedures

Iamia majanohamensis F12^T was isolated from abdominal epidermis of a sea cucumber, *Holothuria edulis*, collected at seawater off the coast of Okinawa, Japan. The medium used for isolation of the strain F12^T was SN medium, consisting of 770-mg $NaNO_3$, 15.9-mg K_2HPO_4, 5.6-mg disodium EDTA dehydrate, 10.4-mg Na_2CO_3, 1-mg vitamin B_{12}, and 1-mg Cyano trace metal solution in filtered 75 % seawater. Cyano trace metal solution is composed of 6.25-g citric acid. H_2O, 6.0-g ferric ammonium citrate, 1.4-g $MnCl_2{\cdot}4H_2O$, 0.39-g $Na_2MoO_4{\cdot}2H_2O$, 0.025-g $Co(NO_3)_2{\cdot}6H_2O$, and 0.222-g $ZnSO_4{\cdot}7H_2O$ in l L of distilled water. The isolate also grows on marine agar (MA) but very slowly, and colonies on MA after 3 weeks at 30 °C were 0.2–0.3 mm in diameter. The strain grows on a medium devoid of sodium chloride. The cultures of *Iamia majanohamensis* F12^T were maintained on marine agar (MA) at 25 °C.

Ilumatobacter fluminis YM22-133^T was isolated from the sediment of an estuary collected at the mouth of the Kuira River, Okinawa, Japan. The sample was homogenized with a glass rod, and the homogenate (50 μL) was placed on "R" medium plates, and was incubated at 25 °C for 30 days.

The isolate also grows on marine agar (MA). The strain requires seawater or artificial seawater for its growth. The culture of *Ilumatobacter fluminis* YM22-133^T was maintained on "R" medium agar at 25 °C.

Ecology

As the habitat of species of two genera in the family *Iamiaceae* is only known for mainly the type strains, information on the ecological niche and on ecological function of members of the family is lacking.

Although the members of the genus *Iamia* were isolated from sea cucumber and the type strain of *Ilumatobacter fluminis* was isolated from sediment of an estuary at mouth of river in Okinawa, Japan, soil or seawater appears to be the natural environment for the member of family *Iamiaceae*.

A high number of environmental clone sequences which are affiliated to the family *Iamiaceae* were obtained from soils and marine circumstances and listed in The NCBI taxonomy Browser (http://www.ncbi.nlm.nih.gov/guide/taxonomy/) and the Green-Gene database (DeSantis et al. 2006), e.g., from Australia caves (AF317769, Holmes et al. 2001), farm soil (AY921876, Tringe et al. 2005), Lake freshwater in Japan (AB154320), heavy oil seeps (EF157223, Kim and Crowley 2007), soil sample collected under a glacier (EU421859), and pasture soil (AY395396). Several clones from marine environments are also listed: Gulf of Mexico sediments (DQ521825, Lloyd et al. 2006), deep-sea octacoral (DQ395467), Antarctic sea water (DQ295238, Grzymski et al. 2006), marine basalt (DQ070822, Mason et al. 2007), and carbonate chimney in hydrothermal field (DQ270650, Brazelton et al. 2006).

The strain of *Iamia majanohaensis* does not require seawater or NaCl for its growth, and therefore, it could not be a marine species. As the strain of *Ilumatobacter* requires seawater for its growth, this species seems to be a marine species.

Strains of "*Candidatus* Microthrix" were isolated from industrial activated sludge wastewater treatment plants. "Microthrix" strains are known to cause solids separation problem known as foaming and bulking. A high number of uncultured clone sequences which have high similarity to "*Candidatus* Microthrix" have been isolated from many environments including soils, activated sludge, wastewater treatment, etc.

Pathogenicity: Clinical Relevance

No information on pathogenicity is available for *Iamia majanohamensis* and *Ilumatobacter fluminis*.

Application

No information on application is available for *Iamia majanohamensis* and *Ilumatobacter fluminis*.

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23 The Family *Intrasporangiaceae*

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Abstract
The family *Intrasporangiaceae*, proposed on the basis of phylogenetic evidence, is a genus-rich taxon of the order *Micrococcales*, comprising more than 50 species with a wide range of phenotypic and chemotaxonomic properties and thriving in a wide range of habitats. Even at the genus level some taxa are taxonomically heterogeneous. While several genera contain more than a single species, more recently described genera are defined by only their type strain. In contrast to earlier phylogenetic analysis, recent evidence shows the polyphyletic structure of the family with some genera branching outside the core of the family. *Tetrasphaera*, *Janibacter*, and *Terrabacter* species are of ecological importance as they are involved in biological phosphate removal from wastewater and bioremediation processes. The clinical significance is low though the number of reports including members of the family being involved in infections as opportunistic pathogens is increasing.

Taxonomy, Historical and Current

The family *Intrasporangiaceae* was established on the basis of the phylogenetic position of members of the genera *Intrasporangium*, *Sanguibacter*, and *Terrabacter* and a set of unique 16S rRNA gene sequence signatures. It was placed within the suborder *Micrococcineae*, order *Actinomycetales* (Stackebrandt et al. 1997). Later, the genus *Sanguibacter* was excluded as a family of its own (Stackebrandt and Schumann 2000) and the genera *Terracoccus* and *Janibacter* were included into the emended family *Intrasporangiaceae*. Since then, a large number of newly described genera have been affiliated to the family which at the time of writing harbors 19 genera and more than 55 species. In the latest edition of *Bergey's Manual of Systematic Bacteriology* (Goodfellow et al. 2012) *Micrococcineae*, covering 16 genera, was elevated to order rank, *Micrococcales* (Busse 2012), phylum Actinobacteria. The set of signature nucleotides given originally for the family (Stackebrandt et al. 1997) was emended by Stackebrandt and Schumann (2000), Zhi et al. (2009), who also gave the signatures for the 16 genera covered. Members of the family have been isolated from sea and lake sediments, soil, ultrapure water, salt mines and mine waste, activated sludge, corals, insects, rotten melons, marine waters, and the upper stratosphere. It is therefore not surprising that the family is heterogeneous with respect to morphology (cocci, rods, rod-coccus cycle, and mycelia), relation to oxygen (aerobic to facultatively anaerobic), and the diagnostic amino acid composition of peptidoglycan

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(meso-A_2pm [type A1γ], LL-A_2pm [A3γ], and ornithine [A4β]). The majority of strains encompass the menaquinones MK-8(H_4), but MK-10(H_4) and the fully saturated MK-8 type are also represented. The complex cellular fatty acids include iso- and anteiso-branched chain acids and monounsaturated straight-chain acids. Phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol are the major polar lipids. Table 23.1 lists all validly published species names, together with the type strain designation and their isolation source. A comparison of morphological and chemotaxonomic traits is given in Table 23.2, while Tables 23.3–23.11 compile some cultural, metabolic, and some additional chemotaxonomic data. It should be noted that several species descriptions are incomplete with respect to presenting chemotaxonomic properties as devised by Schumann et al. (2009) in the Minimal Standards list of recommended properties for characterization and differentiation of genera and species classified in the suborder *Micrococcineae* (now order *Micrococcales*).

Phylogenetic Structure of the Family and Its Genera

Phylogenetic analysis of genera based upon the RAxML algorithm (Fig. 23.1) analyses of 16 S rRNA gene sequences of nearly all type strains of the family sees a clustering of most species according to their genus affiliation. The genera, however, do not cluster exclusively according to the diagnostic amino acid of peptidoglycan: *Terrabacter* (note that *T. aeriphilus* appears to be more closely related to *Terracoccus luteus*), *Terracoccus*, *Humibacillus*, and *Intrasporangium* all contain LL-A_2pm, but *Lapillibacillus*, also possessing LL-A_2pm, groups with genera containing meso-A_2pm. *Phycicoccus* is not a coherent genus, as the type species *P. jejuensis* groups with *Ornithinibacter aureus* and *Fodinibacter luteus*. Members containing ornithine in their peptidoglycan are found at different positions in the dendrogram. This relationship, however, is not supported by chemotaxonomic evidence (Table 23.2). The family *Intrasporangiaceae* appears not to be a coherent taxon: both RAxML and NJ (not shown) analyses see the family to consist of two sister clades, the first one embracing the majority of genera and the second one not only containing the pairs *Ornithinimicrobium* and *Serinicoccus* and *Marihabitans* and *Arsenicicoccus*, but also, in addition, members of the families *Dermacoccacea* and *Dermatophilaceae*. The relationship of one or more members of *Intrasporangiaceae* to *Dermatophilus* and *Dermacoccus* has been noted before in the literature, e.g., Stackebrandt (2012), in the original description of *Arsenicicoccus piscis* (Hamada et al. 2009), and the phylogenetic closeness between *Intrasporangiaceae* and *Dermatophilaceae* with *Marihabitans* clustering with members of the latter family is also obvious in the dendrogram shown by Schumann et al. (2009). *Arsenicicoccus* and *Marihabitans* contain A_2pm isomers as diagnostic amino acids of peptidoglycan, while *Ornithinimicrobium* and *Serinicoccus* possess ornithine. Members of *Dermacoccaceae* contain L-Lys at position 3 of the peptidoglycan subunit, and the interpeptide bridge contains a dicarboxyl amino acid, while *Dermatophilus* contains meso-A_2pm, like some other members of *Intrasporangiaceae* which cluster in the main clade. From the viewpoint of phylogenetic consistency either families *Dermacoccaceae* and *Dermatophilaceae* need to be included in *Intrasporangiaceae*, or a new family could be created for the genera *Ornithinimicrobium* and *Serinicoccus* (which would be supported by chemotaxonomic evidence), *Arsenicicoccus* could be described as a member of *Dermatophilaceae*, while *Marihabitans* would constitute a family of its own. Family-specific 16S rRNA signature nucleotide sets would have to be searched for in order to support this decision.

A more detailed view of the relationship among species is depicted in Fig. 23.2.

Molecular Analyses

DNA-DNA Hybridization (DDH) Studies

This method has been used extensively to delineate novel strains from validly named species (<70 %) and to allocate strains to the same species (>70 %). The latter tests included, among others, strains of *Kribbia dieselivorans* (Jung et al. 2006), *Oryzihumus leptocrescens* (Kageyama et al. 2005), *Janibacter melonis* (Yoon et al. 2004), *Tetrasphaera jenkinsii* (McKenzie et al. 2006), and *Janibacter terrae* (Lang et al. 2003). A wide range of DDH methods has been used which explains the lack of correlation between DHH values and 16S rRNA gene sequence similarity values. Two main DDH methods dominate, the fluorimetric method of Ezaki et al. (1989) and the thermal reassociation method of De Ley et al. (1970), modified by Huss et al. (1983). Other methods were the radioactive filter method of Seldin and Dubnau (1985), the membrane filter method of Tourova and Antonov (1987), the method of Takahashi et al. (1993), the modified hydroxyapatite method of Ziemke et al. (1998), and the genome-based microarray approach (Bae et al. 2005).

The intraspecific relatedness of *Tetrasphaera jenkinsii* was also determined by 16-23S RNA intergenic fingerprinting (McKenzie et al. 2006). *rpoC1* gene analysis was performed on some *Tetrasphaera* type strains (McKenzie et al. 2006). In situ FISH probes have been designed on the basis of strains described by Maszenan et al. (2000) and are useful to detect these strains in wastewater and to differentiate them from related, yet undescribed members of the genus (Kong et al. 2005).

Riboprinting

The restriction enzyme *Pvu*II is superior to *Eco*RI in generating multiband patterns for members of the family *Intrasporangiaceae*. However, DNA may remain partially undigested, causing the band at ca. 50 kbp in some patterns (see Fig. 23.2).

Table 23.1

Alphabetical list of genera and species, designated type strains, effective publication, and the habitat of type strains

Genus and species	Type strain	Authors	Habitat of isolation
Aquipuribacter hungaricus	IV-75^T	Tóth et al. (2012)	Ultrapure water, power plant purification system, Hungary
Arsenicicoccus bolidensis	CCUG47306^T	Collins et al. (2004)	Lake mine waste sediment, Boliden, Sweden
Arsenicicoccus piscis	Kis4-19^T	Hamada et al. (2009)	Intestinal tract of Japanese fish
Fodinibacter luteus	YIM C003^T	Wang et al. (2009)	Wall of a salt mine, Yunnan, China
Humibacillus xanthopallidus	KV-663^T	Kageyama et al. (2008a)	Paddy field soil, Saitama Prefecture, Japan
Intrasporangium calvum	7KIP^T	Kalakoutskii et al. (1967)	Air in a school dining room, Russia
Intrasporangium chromatireducens	Q5-1^T	Liu et al. (2012a)	Manganese mine soil, Hunan, China
Intrasporangium mesophilum	YIM 49065^T	Yang et al. (2012)	Soil, *Jatropha curcas*, Yunnan, China
Intrasporangium oryzae	KV-657^T	Yang et al. (2012)	Soil, paddy field, Saitama, Japan
Janibacter alkaliphilus	SCSIO 10480^T	Li et al. (2012)	Gorgonian coral *Anthogorgia* sp., Weizhou Island, China
Janibacter anophelis	H2.16B^T	Kämpfer et al. (2006)	Midgut of *Anopheles arabiensis*, Kenya
Janibacter corallicola	04PA2-Co5-61^T	Kageyama et al. (2007)	Hard coral *Acropora gemmifera*, Angauru, Palau
Janibacter hoylei	PVAS-1^T	Shivaji et al. (2009)	40–41.4 km altitude, Hyderabad, India
Janibacter limosus	HKI 83^T	Martin et al. (1997)	Wastewater sludge, Jena, Germany
Janibacter melonis	CM2104^T	Yoon et al. (2004)	Spoiled oriental melon, Korea
Janibacter terrae	CS12^T	Yoon et al. (2000)	Soil from around a wastewater treatment plant in Korea
Knoellia aerolata	5317S-21^T	Weon et al. (2007a)	Air sample, Suwon, Korea
Knoellia flava	TL1^T	Yu et al. (2012)	Pig manure, Wuhan, China
Knoellia locipacati	DMZ1^T	Shin et al. (2012)	Soil sample, demilitarized Zone, Korea
Knoellia sinensis	HKI 0119^T	Groth et al. (2002)	Soil from a cave, Guilin, China
Knoellia subterraneae	HKI 0120^T	Groth et al. (2002)	Soil from a cave, Guilin, China
Kribbia dieselivorans	N113^T	Jung et al. (2006)	Tidal flat sediment, Kwangyang, Korea
Lapillicoccus jejuensis	R-Ac013^T	Lee and Lee (2007)	Stone from agricultural field, Jeju, Korea
Marihabitans asiaticum	HG667^T	Kageyama et al. (2008b)	Surface seawater, Kesennuma port, Japan
Ornithinibacter aureus	HB09001^T	Xiao et al. (2011a)	Seawater South China Sea, Hainan, China
Ornithinimicrobium humiphilum	HKI 0124^T	Groth et al. (2001)	Garden soil, Giessen, Germany
Ornithinimicrobium kibberense	K22-20^T	Mayilraj et al. (2006)	Soil, Lahaul-Spiti Valley, Himalayas, India
Ornithinimicrobium murale	01-Gi-040^T	Kämpfer et al. (2013)	Mold-colonized cellar wall, Giessen, Germany
Ornithinimicrobium pekingense	LW6^T	Liu et al. (2008)	Activated sludge from a sequential batch reactor
Ornithinicoccus hortensis	HKI 0125^T	Groth et al. (1999)	Garden soil, Giessen, Germany
Oryzihumus leptocrescens	KV-628^T	Kageyama et al. (2005)	Paddy field soil, Saitama Prefecture, Japan
Phycicoccus aerophilus	5516 T-20^T	Weon et al. (2008)	Air sampler, Taean region, Korea
Phycicoccus bigeumensis	MSL-03^T	Dastager et al. (2008)	Soil sample, Bigeum Island, Korea
Phycicoccus badiiscoriae	Sco-B23T	Lee (2013)	Scoria layer, Darangshi Oreum mountain, Jeju, Korea
Phycicoccus cremeus	V2M29^T	Zhang et al. (2011)	Forest soil, Changbai Mountains, China
Phycicoccus dokdonensis	DS-8^T	Yoon et al. (2008)	Soil, Dokdo, Korea
Phycicoccus ginsenosidimutans	BXN5-13^T	Wang et al. (2011)	Soil of ginseng field, Baekdu Mountain, Korea
Phycicoccus jejuensis	KSW2-15^T	Lee, (2006)	Dried seaweed, Gwakji beach, Jeju, Korea
Serinicoccus chungangensis	CAU 9536^T	Traiwan et al. (2011)	Tidal flat sediment, Seogmo Island, Yellow Sea, Korea
Serinicoccus marinus	JC1078^T	Yi et al. (2004)	Surface sea water, East Sea, China
Serinicoccus profundi	MCCC 1A05965^T	Xiao et al. (2011b)	Deep sea sediment (5,368 m), Indian Ocean
Terrabacter aeriphilus	5414 T-18^T	Weon et al. (2010)	Air sampler, Taean region, Korea
Terrabacter aerolatus	5516 J-36^T	Weon et al. (2007b)	Air sampler, Jeju, Korea

Table 23.1 (continued)

Genus and species	Type strain	Authors	Habitat of isolation
Terrabacter carboxydivorans	PY2^T	Kim et al. (2011)	Soil sample, Yonsei University, Seoul, Korea
Terrabacter ginsenosidimutans	Gsoil 3082^T	An et al. (2010)	Soil of ginseng field, Korea
Terrabacter lapilli	LR-26^T	Lee et al. (2008)	Stone from agricultural field, Jeju, Korea
Terrabacter terrae	PPLB^T	Montero-Barrientos et al. (2005)	Soil mixed with Iberian pig hair, Spain
Terrabacter terrigena	ON10-^{T}	Yoon et al. (2009)	Soil around a wastewater treatment plant, Korea
Terrabacter tumescens	NCIB 8914^T	Collins et al. (1989)	Soil, Australia Jensen (1934, 1933)
Terracoccus luteus	IMET 7848^T	Prauser et al. (1997)	Bank of duck pond, Hiddensee, Germany
Tetrasphaera australiensis	Ben 109^T	Maszenan et al. (2000)	Activated sludge, Glenelg, Australia
Tetrasphaera duodecadis	IAM 14868^T	Ishikawa and Yokota (2006)	Arable soil
Tetrasphaera elongata	Lp2^T	Hanada et al. (2002)	Activated sludge reactor, Japan
Tetrasphaera japonica	T1-X7^T	Maszenan et al. (2000)	Activated sludge, Japan
Tetrasphaera jenkinsii	Ben 74^T	McKenzie et al. (2006)	Activated sludge, Glenelg, Australia
Tetrasphaera remsis	3-M5-R-4^T	Osman et al. (2007)	Life support module simulator, Pasadena, USA
Tetrasphaera vanveenii	Ben 70^T	McKenzie et al. (2006)	Activated sludge, Carrum, Australia
Tetrasphaera veronensis	Ver 1^T	McKenzie et al. (2006)	Activated sludge, Verona, italy

Table 23.2

Morphological and chemotaxonomic characteristics of genera of *Intrasprongiaceae*. Following the type genus *Intrasporangium* the order of other genera is according to peptidoglycan types: *Arsenicoccus* (Collins et al. 2004), *Terracoccus* (Prauser et al. 1997), *Humibacillus* (Kageyama et al. 2008a), *Lapillicoccus* (Lee and Lee 2007), *Terrabacter* (Collins et al. 1989), *Janibacter* (Martin et al. 1997), *Fodinibacter* (Wang et al. 2009), *Knoellia* (Groth et al. 2002), *Tetrasphaera* (Maszenan et al. 2000), *Phycicoccus* (Lee 2006), *Marihabitans* (Kageyama et al. 2008b), *Kribbia* (Jung et al. 2006), *Aquipuribacter* (Tóth et al. 2012), *Oryzihumus* (Kageyama et al. 2005), *Ornithinibacter* (Xiao et al. 2011a), *Ornithinicoccus* (Groth et al. 1999), *Ornithinimicrobium* (Groth et al. 2001), and *Serinicoccus* (Yi et al. 2004). Major polar lipids and fatty acids are those common to all species of the respective genus, as compiled (with the exception of compilations for *Ornithinimicrobium* and *Terrabacter* species) from the original species descriptions. Species-specific variations are listed in *Tables 23.3–23.10* and in the original genus descriptions

Properties	*Intrasporangium*	*Arsenicicoccus*	*Terracoccus*	*Humibacillus*	*Lapillicoccus*
Number of species	4	2	1	1	1
Morphology	Branching mycelium	Cocci, clusters	Cocci, singly or packets	Irregular rods	Cocci
Metabolism	Aerobic	Facultative anaerobic	Aerobic	Aerobic	Aerobic
Peptidoglycan type according to Schleifer and Kandler (1972) (nomenclature according to www.peptidoglycan-types.info)	A3γ (A41.2)	A3γ (A41.1)	A3γ (A41.2)	A3γ (no term available)	A3γ (ND)
Diagnostic peptidoglycan diamino acid-interpeptide bridge[a]	LL-A_2pm–Gly_3 (α-carboxyl group of D-Glu substituted by Gly)	LL-A_2pm-Gly	LL-A_2pm-Gly_3 (α-carboxyl group of D-Glu substituted by Gly)	LL-A_2pm-Gly_2	LL-A_2pm-ND
Major polar lipids[b]	PG, DPG, PI, PIM	PG, DPG, PI, PE[c]	PG, DPG, PI, PE	ND	DGP, PI
Major fatty acids (>10 %) common to all members of the genus[d]	i-$C_{15:0}$, ai-$C_{16:0}$, i-$C_{16:0}$	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	i-$C_{15:0}$, ai-$C_{15:0}$, $C_{16:0}$	i-$C_{15:0}$	i-$C_{16:0}$, i-$C_{15:0}$, $C_{17:1}\omega$8c

Table 23.2 (continued)

Properties	*Intrasporangium*	*Arsenicicoccus*	*Terracoccus*	*Humibacillus*	*Lapillicoccus*
Menaquinone	[j]MK-8, MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
Cell-wall/whole-cell sugars[e]	Gal, Man, Glc[c]	Gal[c]	Rha, Rib, Gal, Glc, Man	Gal, Glc, Rha	ND
G+C content	68	71–72	73	69–70	74
Properties	***Terrabacter***	***Janibacter***	***Fodinibacter***	***Knoellia***	***Tetrasphaera***
Number of species	8	8	1	5	8
Morphology	Coccoid to short rods, rods	Cocci, cluster; rods when young	Irregular rods, singly or in clusters	Rod, cocci, life cycle	Cocci, often in cluster, or in pairs, clusters of elongated rods to septate filaments
Metabolism		Aerobic	Aerobic		
Peptidoglycan type according to Schleifer and Kandler (1972) (nomenclature according to www.peptidoglycan-types.info)	A3γ (A41.2)	A1γ (A31)	A1((A31)A1((A31) A3(	A1((A31) A1(((A31)A3(	A1((A31)A1((A31)6A3(
Diagnostic peptidoglycan diamino acid-interpeptide bridge[a]	LL-A_2pm-Gly_3 (α-carboxyl group of D-Glu substituted by Gly)	Meso-A_2pm – none	Meso-A_2pm – none	Meso-A_2pm – none	Meso-A_2pm – none or 3-hydroxy meso-A_2pm – Asp-containing interpeptide bridge[f]
Major polar lipids common to all members of a genus	DGP, PI, PE	DPG, PG, PI[c]	PG, DPG, PI,PE, 2 uPL	DPG, PI, PE	DGP, PI
Major fatty acids (>10 %), common to all members of a genus	i-$C_{15:0}$, ai-$C_{15:0}$	i-$C_{16:0}$	$C_{16:0}$, $C_{18:1}\omega9c$	i-$C_{15:0}$, i-$C_{16:0}$	i-$C_{16:0}$,ai-$C_{17:0}$, i-$C_{15:0}$, i-$C_{14:0}$, i-$C_{16:1}$, $C_{16:1}$
Menaquinone	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
Cell-wall/whole-cell sugars[e]	Variable, see *Table 23.5*	Man, Rib, Glc[c]	Rib, Glc	Glc, Rib[c]	ND
G+C content	69.2–75.6	70–73	72	68–73	68–71
Properties	***Phycicoccus***	***Marihabitans***	***Kribbia***	***Aquipuribacter***	***Oryzihumus***
Number of species	6	1	1	1	1
Morphology	Cocci	Short coccoid rods	Cocci, short rods	Rod-coccus cycle	Irregular rods
Metabolism		Aerobic	Aerobic, nitrate fermentation	Aerobic	Aerobic to microaerophilic
Peptidoglycan type according to Schleifer and Kandler (1972) (nomenclature according to www.peptidoglycan-types.info)	A1γ (A31)	A1γ (A31)	A1γ (A31)	A1γ (A31)	A1γ (A31)
Diagnostic peptidoglycan diamino acid-interpeptide bridge[a]	Meso-A_2pm – none	Meso-A_2pm – none	Meso-A_2pm – none	Meso-A_2pm – none	Meso-A_2pm – none
Common major polar lipids	DGP	PG, DPG, PIMs, uPL	ND	PG, 2uPL, uGL	PG, DPG, uPL, uPGL
Major fatty acids (>10 %) common to all members of the genus	i-$C_{15:0}$, i-$C_{16:0}$,	i-$C_{17:0}$, $C_{18:1}\omega9c$, $C_{17:1}\omega8c$,	10-Me $C_{18:0}$, i-$C_{16:0}$, $C_{18:1}\omega9c$, $C_{16:0}$, $C_{18:0}$	$C_{18:3}$ ω6c (6,9,12), ai-$C_{15:0}$	i-$C_{16:0}$, i-$C_{15:0}$, i-$C_{14:0}$,
Menaquinone	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-10(H_4)	MK-8(H_4)

Table 23.2 (continued)

Properties	*Phycicoccus*	*Marihabitans*	*Kribbia*	*Aquipuribacter*	*Oryzihumus*
Cell-wall/whole-cell sugars[e]	Glc, Rib[c]	ND	ND	ND	ND
G+C content	71–74	70	69–70	75	72–73

Properties	*Ornithinibacter*	*Ornithinicoccus*	*Ornithinimicrobium*	*Serinicoccus*
Number of species	1	1	3	3
Morphology	Braching rods	Cocci, singly, in pairs, or clusters	Cocci, short rods,	Cocci
Metabolism	Aerobic	Aerobic to microaerophilic	Aerobic to microaerophilic	Aerobic
Peptidoglycan type according to Schleifer and Kandler (1972) (nomenclature according to www.peptidoglycan-types.info)	ND	A4β (A21.13)	A4β (no term available)	ND or A1γ (A31)[g]
Diagnostic peptidoglycan diamino acid-interpeptide bridge[a]	L-Orn – ND	L-Orn – Gly_{1-2} – D-Glu	L-Orn – L-Ala – Gly – D-Asp[h]	L-Orn or meso-A_2pm[g]
Major polar lipids	PG, DGP, PE, PI, uGL	PG, DPG, PI, PS, uPL	PG, DPG, PI, uPL, uGL	PG, DGP, PI, diverse see *Table 23.9*
Major fatty acids (>10 %) common to all members of the genus	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$, $C_{18:1}\omega9c$	i-$C_{15:0}$, ai-$C_{15:0}$	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$, i-$C_{17:1}\omega9c$[i]	i-$C_{15:0}$, i-$C_{16:0}$
Menaquinone	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
Cell-wall/whole-cell sugars[e]	ND	Glc	Glc, Ara, Xyl[c]	Glc, Rib[c]
G+C content	70	72	69–71	72–74

Symbols and abbreviations: + positive, − negative, *w* weakly positive, nd not determined, *v* variable

[a] A_2pm, diaminopimelic acid; Gly, glycine; Asp, aspartic acid; Orn, ornithine. The interpeptide bridge composition has not always been investigated

[b] *PG* phosphatidylglycerol, *DGP* diphosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PE* phosphatidylethanolamine, *u* unknown, *GL* glycolipid, *PL* phospholipid, *PGL* phosphoglycolipid

[c] Not all species investigated

[d] A number before a colon indicates the number of carbons; the number after the colon is the number of double bonds; iso- indicates a methyl branch at the iso position; anteiso- indicates a methyl branch at the anteiso position

[e] Ara, arabinose; Gal, galactose, Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose

[f] *meso*-A_2pm and 3-hydroxy *meso*-A_2pm as well as aspartic acid as constituent of the interpeptide bridge were reported by Ishikawa and Yokota (2006)

[g] *S. chungangensis* displays the peptidoglycan type A1γ (A31) according to Traiwan et al. (2011)

[h] *O. pekingense* contains lysine as additional diamino acid according to Liu et al. (2008) and *O. murale* contains no aspartic but glutamic acid in the interpeptide bridge (Kämpfer et al. 2013)

[i] Data from Kämpfer et al. (2013) who compared all four species in a single study

[j] MK-8 in *I. calvum* was reported by Collins et al. (1984) and Kageyama et al. (2007), while Yang et al. (2012) found MK-8(H_4); see section Genus *Intrasporangium* Kalakoutskii et al. (1967)

The *Pvu*II RiboPrint patterns served for the classification of eight dibenzofuran-degrading strains that agreed in their *Pvu*II RiboPrint patterns with this of *Janibacter brevis* DSM 13953^T (Lang et al. 2003). Though the species *Janibacter brevis* and *Janibacter terrae* were considered synonymous due to agreement in phenotypic characteristics and a high DNA-DNA similarity value, RiboPrinting revealed that their type strains are not identical and differ in their strain-specific patterns from one another and both from this of *Janibacter limosus* DSM 11140^T (Lang et al. 2003; *Fig. 23.2*). However, the type strains of *Janibacter terrae* DSM 13876^T and *Janibacter anophelis* DSM 18333^T (98.4 % 16S rRNA gene sequence similarity) display almost identical *Pvu*II RiboPrint patterns (*Fig. 23.2*).

MALDI-TOF

The MALDI-TOF mass spectra of species of the family *Intrasporangiaceae* differ sufficiently to be well suited for their identification. The most similar spectra were obtained for the type strains of the synonymous species *Janibacter terrae* and *Janibacter brevis* which share 80.6 % DNA-DNA similarity (Lang et al. 2003) but differ in their *Pvu*II RiboPrint patterns (*Fig. 23.3*; Lang et al. 2003). Though there are several exceptions, most spectra of type strains of *Intrasporangiaceae* species cluster according to their genus affiliation (*Fig. 23.4*). The MALDI-TOF mass spectra of *Terrabacter*, *Terracoccus*, *Intrasporangium*, and *Humibacillus* species cluster together in agreement with the respective

Table 23.3

Additional properties apt to differentiate type strains of *Intrasporangium* species (see Table 23.1). 1. *I. calvum*; 2. *I. mesophilum*; 3. *I. oryzae*; 4. *I. chromatireducens*

Properties	1	2	3	4
Morphology	Branching and fragmenting mycelium	Branching and fragmenting mycelium	Fragmentary vegetative mycelium	Branching hyphae
Diameter of cells (μm)	0.4–1.2	ND	0.5	0.3–0.5
Aerial mycelium	None	White	None	ND
Substrate mycelium	Whitish, cream whitish	White, pale orange yellow	Pale yellow	Pale yellow to cream whitish
Temperature range (optimum) °C	10–42 (28–37)	20–37 (28)	8–40 (ND)	10–45 (37)
pH range (optimum)	6–8 (ND)	6–8 (7–8)	5–11 (ND)	5–10 (8)
Growth up to NaCl%	0–3	0–3	0–3	0–4
Catalase	+	+	+	+
Oxidase	–	ND	ND	–
NO_3 reduction	+	+	+	+
Whole-cell sugars other than those listed in Table 23.1	None	Ara	Rib	ND
Fatty acids (>5 %) other than those listed in Table 23.2[a]	i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{15:0}$, i-$C_{16:1}$H, i-$C_{14:0}$	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$, i-$C_{16:1}$H	i-$C_{15:0}$ i-$C_{14:0}$, i-$C_{16:0}$, ai-$C_{15:0}$, i-$C_{16:1}$H
Mol% G+C of DNA	68.2	69.6	70	71.5
H_2S production	–	–	–	–
Hydrolysis of				
Tween 80	ND	–	ND	ND
Gelatin	–	+	–	ND
Utilization of				
Cellobiose	+	–	–	ND
Mannitol	–	+	+	+
Raffinose	+	–	–	–
L-Rhamnose	+	–	–	–
D-Ribose	–	+	–	+
Sorbose	–	–	+	ND
Trehalose	–	–	+	ND

nd not determined, *Ara* arabinose, *Rib* ribose

[a]Fatty acid data of *I. calvum*, *I. mesophilum*, and *I. oryzae* from Yang et al. (2012) and Kageyama et al. (2007), data of *I. chromatireducens* from Liu et al. (2012a)

clustering in Fig. 23.2 though MALDI-TOF mass spectrometry does not demonstrate the total phylogenetic structure of the family *Intrasporangiaceae* when compared to the 16S rRNA sequence-based dendrograms (Fig. 23.4 vs. Figs. 23.1 and 23.2).

Genome Comparison

As of January 2013, the Genomes OnLine Database (Pagani et al. 2012) contained 26 registered genome projects of *Intrasporangiaceae*, *Dermacoccaceae*, and *Dermatophilaceae*. Thereof, genome sequences of nine species were available with protein sequences in GenBank with seven type strains among them. These and the outgroup (*Kineococcus*) genomes were *Austwickia chelonae* NBRC 105200^T (BAGZ00000000), *Dermacoccus* sp. Ellin185 (AEIQ00000000), *Intrasporangium calvum* DSM 43043^T (Del Rio et al. 2010; CP002343), *Janibacter hoylei* PVAS-1^T (Pawar et al. 2012; ALWX00000000), *Janibacter* sp. HTCC2649 (Thrash et al. 2011; AAMN00000000), *Kineosphaera limosa* NBRC 100340^T (BAHD00000000), *Kytococcus sedentarius* DSM 20547^T (Sims et al. 2009; CP001686), *Mobilicoccus pelagius* NBRC 104925^T (BAFE00000000), *Serinicoccus profundi* MCCC 1A05965^T (Xiao et al. 2011c; AFYF00000000), and *Kineococcus radiotolerans* SRS30216^T (Bagwell et al. 2008; CP000750-CP000752). The genome sequences were phylogenetically investigated as described in Spring et al. (2010),

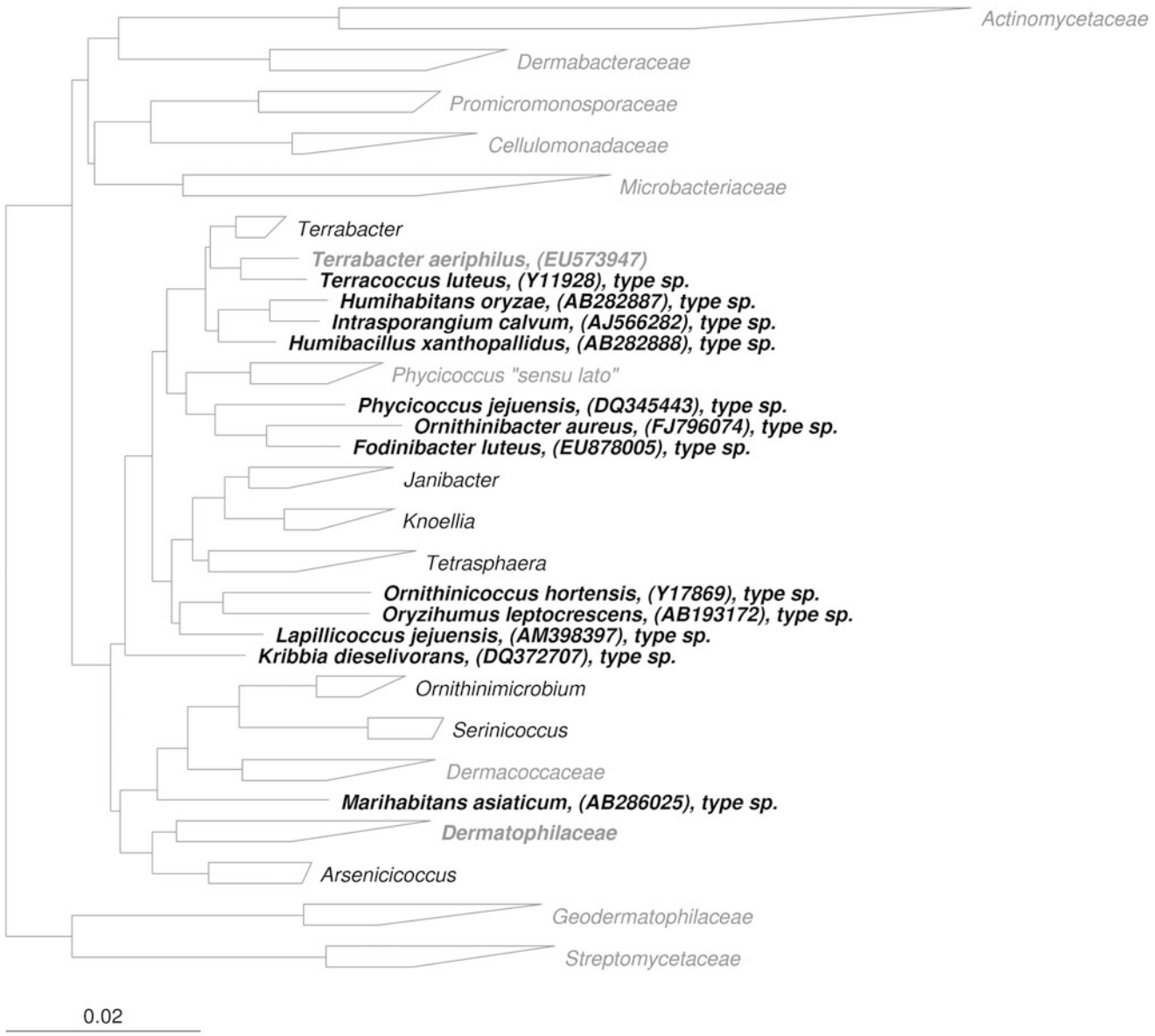

Fig. 23.1

Phylogenetic reconstruction of the family *Intrasporangiaceae* and related families based on16S rRNA gene sequences and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence data sets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). The tree topology was stabilized with the use of a representative set of nearly 750 high-quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

Anderson et al. (2011), Göker et al. (2011), and Abt et al. (2012). That is, maximum likelihood (ML) and maximum-parsimony (MP) phylogenetic trees were inferred from two distinct supermatrices (concatenated alignments), a "full" matrix using all alignment comprising at least four sequences and a matrix using the "core genes" only, i.e., those alignments containing 10 sequences, as well as from the ortholog-content and from the gene-content matrix.

The "full" supermatrix contained 2,778 genes and 847,312 characters, whereas the core-gene supermatrix comprised 744 genes and 229,331 characters. Both matrices were analyzed under ML with the suggested model, PROTGAMMAJTTF. The resulting trees had an identical topology, which is shown in *Fig. 23.5a* together with ML and MP bootstrap support values. The best MP core-gene tree was topologically identical to the ML trees, whereas the MP tree inferred from the "full" supermatrix positioned the clade comprising *Kytococcus sedentarius* DSM 20547^T and *Serinicoccus profundi* MCCC 1A05965^T as sister clade of the other ingroup organisms and grouped *Dermacoccus* sp. Ellin185 as sister taxon to the

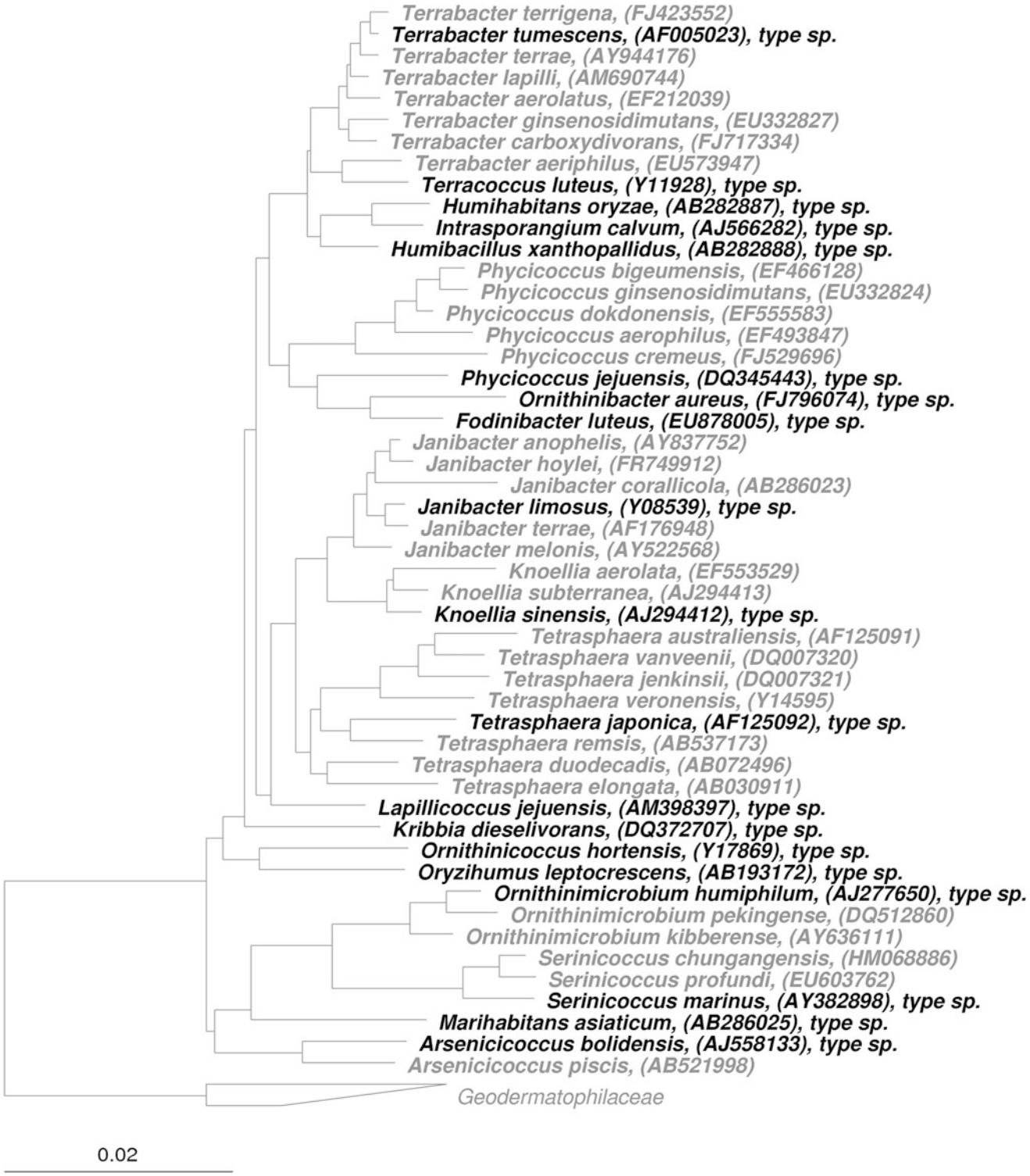

Fig. 23.2

Phylogenetic reconstruction of *Intrasporangiaceae* species based on16S rRNA gene sequences and created using the neighbor-joining algorithm with the Jukes-Cantor correction. For details of tree generation, see legend to *Fig. 23.1*

other ingroup clades except the previously described one (data not shown). Support was maximum (100 %) for all branches except for the two previously described branches under ML and MP (*Fig. 23.5a*).

The gene-content matrix comprised 8,499 characters, the ortholog-content matrix 11,943 characters. For both matrices, the BINGAMMA model was used as implemented in RAxML (Stamatakis 2006). ML and MP trees from both matrices were

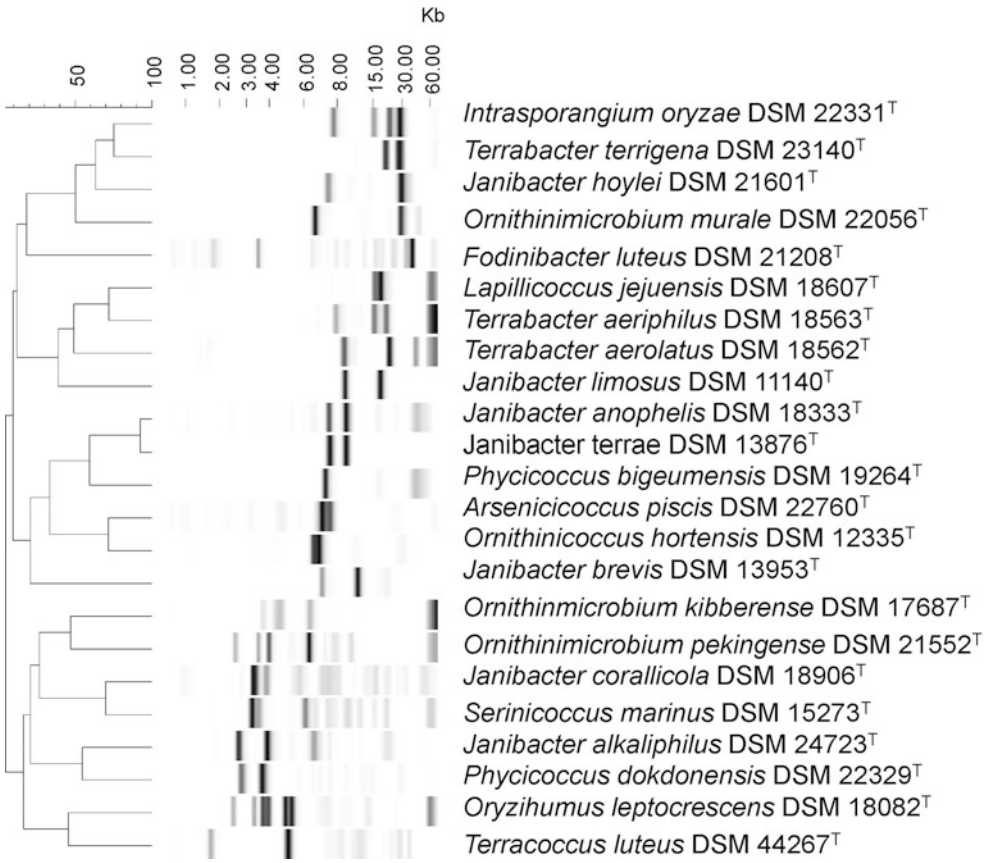

Fig. 23.3

***Pvu*II RiboPrint patterns of selected type strains of *Intrasporangiaceae* species. The dendrogram has been generated with the BioNumerics software (Applied Math, Kortrijk, Belgium)**

topologically identical and are shown in *Fig. 23.5b* with bootstrap support values. In contrast to the supermatrix trees, a maximally supported clade comprising *Janibacter* sp. HTCC2649 and *Intrasporangium calvum* DSM 43043^T was revealed.

Both phylogenetic trees (*Fig. 23.5a*, *b*) support the monophyly of *Dermatophilaceae* (which are, however, only represented by *Mobilicoccus pelagius* NBRC 104925^T, *Kineosphaera limosa* NBRC 100340^T, and *Austwickia chelonae* NBRC 105200^T), whereas the family *Intrasporangiaceae* did not form a monophyletic group in either tree. Rather, they clustered into two distinct clades, each together with *Dermatococcaceae* species, confirming the 16S rRNA analysis shown above.

FISH Probes

An oligonucleotide probe, combined with microautoradiography and histochemical staining, was designed to monitor the diversity of *Tetrasphaera* strains in wastewater treatment systems with enhanced biological phosphorus removal (EBPR). Quantitative FISH analyses showed that the *Tetrasphaera*-specific probe displayed different morphologies, and positively reacting cells constituted up to 30 % of the total biomass (Xia et al. 2008).

Phages

Using the type strains of *Tetrasphaera elongata, T. japonica, T. australiensis*, and *T. jenkinsii* as target strains, a plaque was observed on *T. jenkinsii* lawn using an enriched sample from the wastewater treatment plant in Carrum (Victoria, Australia). The phage TJE1, belonging to the *Caudovirales*, produced lytic plaques on *T. jenkinsii* DSM 17519^T but not on the other tested strains of *Tetrasphaera*, nor on any of the other 65 actinobacterial cultures screened. The complete genome sequence of 49,219 bp and 66 putative open reading frames could be divided into three functional modules comprising (i) a DNA modification, replication, and cell lysis, (ii) a DNA packaging and structure, and (iii) a module encoding host interacting proteins. Only 16 ORFs (24 %) could be assigned a putative function on the basis of their amino acid identity with other proteins in the GenBank database or by the presence of functionally conserved motifs (Petrovski et al. 2012).

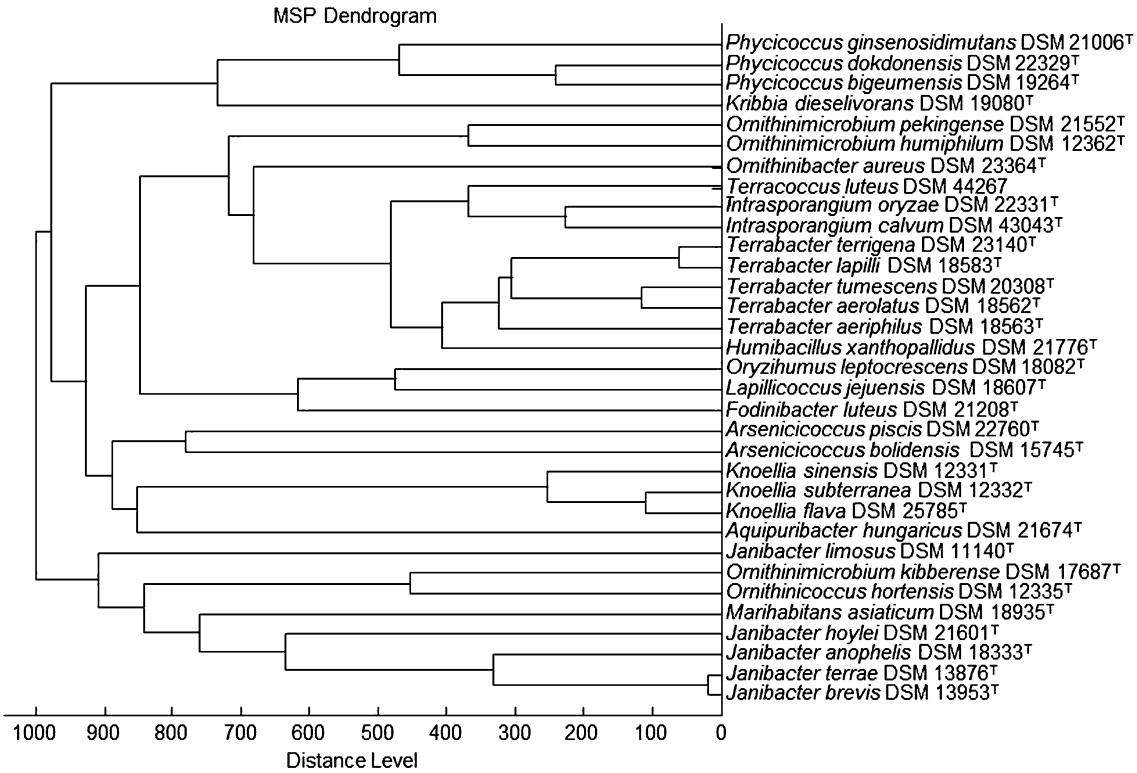

Fig. 23.4

Score-oriented dendrogram generated by the BioTyper software (version 2.0, Bruker Daltonics) showing the similarity of MALDI-TOF mass spectra of cell extracts of selected type strains of the family *Intrasporangiaceae*

Phenotypic Analyses

The main features of members of *Intrasporangiaceae* are listed in Table 23.2. Rather than giving detailed phenotypic traits, only properties used in the differentiation of species within individual genera and some common properties are given in Tables 23.3–23.11. The original descriptions are indicated in Table 23.1.

Genus *Intrasporangium* Kalakoutskii et al. 1967

In.tra.spo.ran'gi.um. L.prep. *intra* within; Gr. n. *spora* a seed; gr. h. *angelon* a vessel; N.L. neut. N. *Intrasporangium* a name coined to emphasize the possibility of intercalary formation of sporangia in mycelium filaments.

The genus includes the species *Intrasporangium calvum* (Kalakoutskii et al. 1967), *Intrasporangium mesophilum* (Yang et al. 2012), *Intrasporangium chromatireducens* (Liu et al. 2012a), and *Intrasporangium oryzae* (Yang et al. 2012), the latter resulting from the reclassification of the species *Humihabitans oryzae* (Kageyama et al. 2007). Menaquinone MK-8(H_4) was detected as major isoprenoid quinone in *I. chromatireducens*, *I. mesophilum*, and *I. oryzae*. However, there are conflicting data on the major menaquinone of the type strain of *I. calvum*: While Kalakoutskii (1989), Collins et al. (1984), and Kageyama et al. (2007) agreed in reporting MK-8 as menaquinone of the type strains *I. calvum* KIP 7^T, IMET 7816^T, and NBRC 12989^T, Yang et al. (2012) found MK-8(H_4) as the major menaquinone of the type strain *I. calvum* DSM 43043^T. The latter observation and additional information on the fatty acid and polar lipid composition gave rise to the emendation of the description of the genus *Intrasporangium* (Yang et al. 2012). A recent reexamination of the menaquinone compositions by HPLC and mass spectrometry of *I. calvum* type strains with identical 16S rRNA gene sequences from different culture collections by Hamada and Schumann (unpublished) confirmed the presence of MK-8(H_4) as the major menaquinone in the type strains *I. calvum* DSM 43043^T and NRRL B-3866^T. However, after cultivation under the same conditions, MK-8 was the predominant menaquinone in *I. calvum* NBRC 12989^T, while ca. 70 % MK-8 and 30 % MK-8(H_4) co-existed in strain IMET 7816^T that represented the oldest genuine deposition of the type strain KIP 7^T in a service culture collection (IMET Jena, German Democratic Republic) and had been maintained as stab culture above liquid nitrogen since 1966. This study supports the view that the type strain of *I. calvum* has the potential to produce both menaquinones MK-8 and MK-8(H_4) and it might depend on the way of long-term storage and reactivation in which menaquinone occurs as the major component. Additional data than those shown in Table 23.3 are listed in the original species descriptions.

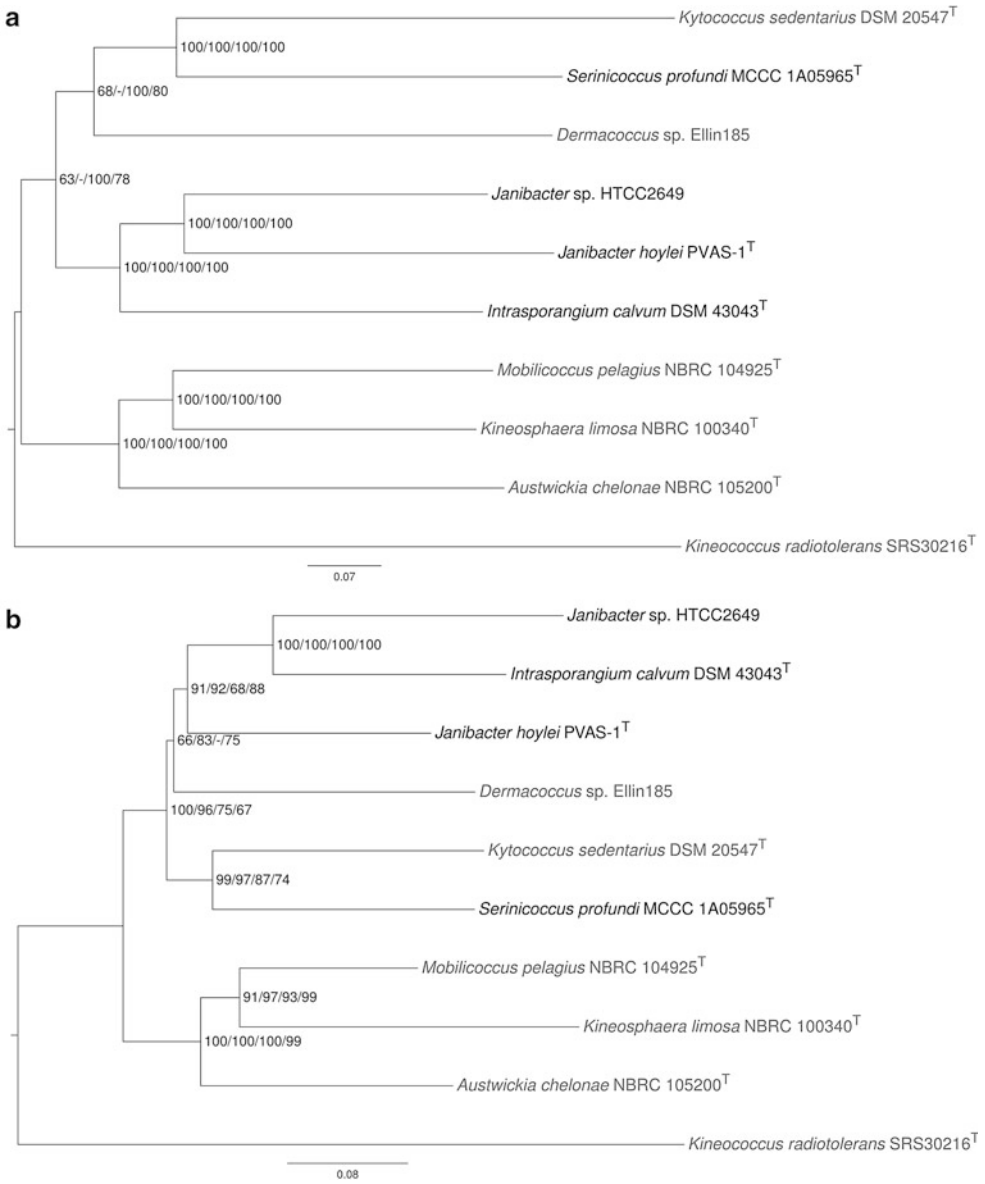

Fig. 23.5

(a) Phylogenetic tree inferred from the ''full'' matrix under the maximum likelihood (ml) criterion and rooted with kineococcus radiotolerans srs30216t. The branches are scaled in terms of the expected number of substitutions per site. Intrasporangiaceae are labelled in black, other taxa in grey. Numbers on the nodes (from left to right) are bootstrapping support values (if larger than 60 %) from (i) ml ''full'' supermatrix, (ii) maximum-parsimony (mp) ''full'' supermatrix, (iii) ml core genes, and (iv) (mp) core-gene analysis. (b) Phylogeny inferred from the gene-content matrix under the maximum likelihood (ml) criterion and rooted with kineococcus radiotolerans srs30216t. The branches are scaled in terms of the expected number of substitutions per site. Intrasporangiaceae are labelled in black, other taxa in grey. Numbers on the nodes (from left to right) are bootstrapping support values (if larger than 60 %) from (i) ml gene-content matrix, (ii) mp gene-content matrix, (iii) ml ortholog-content matrix, and (iv) mp ortholog-content matrix analysis

Genus *Janibacter* Martin et al. 1997

Ja.ni.bac'ter. L. n. *Janus*, a god in roman mythology, who is said to have had two faces; M. L. masc. n. *bacter*, a rod; L. n. *Janibacter*, referring to the changing morphology of the microorganisms.

Table 23.4 lists some properties useful to differentiate among species. Additional data other than those shown in *Table 23.4* are listed in the original species descriptions. The study of Kageyama et al. (2007) compiles the fatty acid profiles of five species performed under the same conditions. By and large the profiles agree with those listed in the original species description though some deviations are obvious (e.g., the presence of 10-methyl-$C_{17:0}$ in *J. terrae*, the absence of ai-$C_{17:0}$ in *J. terrae*, or the absence of $C_{17:1}$ in *J. limosus*).

Table 23.4

Properties apt to differentiate type strains of *Janibacter* species (see Table 23.1). 1. *J. limosus*; 2. *J. terrae*; 3. *J. melonis*; 4. *J. anophelis*; 5. *J. hoylei*; 6. *J. corallicola*; 7. *J. alkaliphilus*

Properties	1	2	3	4	5	6	7
Morphology	Coccoid, rods, singly, pairs, or clusters	Coccoid or short rods	Coccoid	Coccoid, short rods when young	Coccoid	Coccoid	Coccoid, singly, pairs, or clusters
Dimensions of cocci (rods)	0.4–1.1 (0.4 × 1.2)	0.6–1.1	0.8–1.0	1.0–1.5	0.4–0.7	0.9–1.1	ND
Colony color	White or yellow	White, pale cream, or yellowish	Creamish	ND	Creamish	Pale yellow	White, pale cream, or yellowish
Temperature range in °C (optimum)	ND- 37 (28)	10–40 (28–30)	10–40 (30–37)	20–40 (35)	20–40 (30)	16–38 (23–36	20–40 (25–30)
pH range (optimum)	ND (ND)	5–10 (7–8)	6–9 (7–8)	ND (ND)	5–10 (9)	5–9 (6–8)	7–11 (9–10)
Growth up to NaCl%	10	8	7	ND	10	7	17
Catalase	+	+	ND	ND	+	ND	+
Oxidase	+	w	–	+	+	ND	–
NO_3 reduction							
Cell sugars other than those listed in Table 23.2	Man, Glc	Gal	ND	Glc	ND	ND	Glc, Glc-NH_2, Man, Rib
Fatty acids (>5 %) other than those listed in Table 23.2[a]	C17:1ω8c[b], $C_{17:0}$, $C_{17:1}$, $C_{18:1}$ω9c	$C_{17:1}$ω8, i-$C_{17:0}$, ai-$C_{17:0}$, $C_{18:1}$ ω9c, ai-$C_{15:0}$	$C_{17:1}$ω8, $C_{17:0}$, $C_{15:0}$, i-$C_{15:0}$	$C_{17:1}$ω8, $C_{17:0}$, $C_{15:0}$	$C_{17:1}$ω8, $C_{18:1}$ω9c, i-$C_{18:0}$, 10-methyl-$C_{17:0}$	$C_{17:1}$ω8, $C_{17:0}$, $C_{18:1}$ω9c, 10-methyl-$C_{17:0}$	i-C17:0, i-$C_{15:0}$, $C_{18:1}$ω9c, ai-$C_{17:0}$
Mol% G+C of DNA	70	69	73	ND	73	70–71	70
H_2S production	+	+	–	ND	–	ND	–
Hydrolysis of							
Tween 80	+	+	+	ND	–	ND	+
Gelatin	+	+	+	ND	+	ND	–
Starch	–	–	–	ND	+	ND	–
Utilization of							
D-Mannose	–	ND	–	+	–	+	–
D-Glucose	–	ND	–	+	–	+	–
Maltose	–	ND	–	+	–	+	–
Na-acetate	+	+	+	+	ND	ND	–

ND not determined, *Glc* glucose, *Man* mannose, *Rib* ribose, *Glc-NH_2* glucosamine

[a]Cell mass harvested on TSA agar

[b]Determined by Lang et al. (2003)

Genus *Terrabacter* Prauser et al. 1997

Ter.ra.bac'ter. L. n. terra, earth; M. L. masc. n. bacter, masc. equivalent of Gr. neut. n. bacterium, a rod; *Terrabacter*, earth [soil] rod.

Table 23.5 lists some properties useful to differentiate among species. Additional data than those shown in Table 23.5 are listed in the original species descriptions.

Genus *Tetrasphaera* Maszenan et al. 2000

Te.tra.sphae'ra. Gr. n. *tetra* four; M.L. fem. n. *sphaera* sphere; M.L. fem. n. *Tetrasphaera*, four spherical bacterial cells.

In addition to the described species *T. japonica*, *T. australiensis*, and the rod-shaped *T. elongata*, several organisms fell into the radiation of the genus which grew as clumps of cocci on R2A medium, while irregular twisting filaments and swollen

Table 23.5

Properties apt to differentiate type strains of *Terrabacter* species (see Table 23.1). 1, T. *tumescens*; 2, *T. terrae*; 3, *T. lapilli*; 4, *T. aerolatus*; 5, *T. terrigena*; 6, *T. aeriphilus*; 7, *T. ginsenosidimutans*; 8, *T. carboxydivorans*

Properties	1	2	3	4	5	6	7	8
Morphology	Rod-coccus cycle, primary branching	Rods	Short rods	Coccoid, rods	Short rods	Coccoid, rods	Short rods	Rods
Dimensions of rods (cocci)	0.6–1.2 × 1.2–2.0 (0.5–0.8)	2.0 × 6.0	0.3–0.5 × 1.0–1.4	0.9–1.0 × 1.0–3.5	0.3–0.6 × 1.0–5.0	0.9 × 1.0–1.6	0.6–1.0 × 1.0–1.6	ND
Motility	+	+	–	+	–	–	–	–
Colony color	White grey	Yellow	Yellow	White	Greyish yellow	White	White	White
Temperature range in °C (optimum)	10–35 (25–30)	ND	10–40	5–35 930)	10–37	5–35 (30)	10–40	15–40 (30)
pH range (optimum)	ND	ND	4–12	4–9 (7–8)	5–8.5 (6.5–7)	4–9 (6–7)	5–10 (7)	4–12
Growth up to NaCl%	5	7	3	5	3	7	5	5
Catalase	+	+	+	+	ND	+	ND	+
Oxidase	–	–	–	–	ND	+	ND	–
NO_3 reduction	+	–	+	+	–	–	+	ND
Whole-cell sugars	ND	Fuc, Gal	Glc, Rha, Rib, Xyl, Ara	Glc, Rib, Rha, Xyl, Gal	Glc, Man, Ara, Xyl	Glc, Man, Rib	Gal, Rib, Fuc, Rha	ND
Fatty acids (>5 %) than those listed in Table 23.2[a]	$C_{14:0}$, i-$C_{14:0}$, $C_{16:0}$, i-$C_{16:0}$, C17:0, C18:0, $C_{18:1}\omega$ 9c, summed feature 3[b]	$C_{16:0}$, i-$C_{16:0}$, ai-C17:0, summed feature 3[b]	i-$C_{16:0}$, C17:1ω8c,	i-C14:0, i-$C_{16:0}$, i-C17:0	i-C14:0, i-C16:1, i-$C_{16:0}$	i-C14:0, i-$C_{16:0}$, C17:1ω8c, i-C16:1H	i-$C_{16:0}$, $C_{16:0}$, i-C14:0	i-$C_{16:0}$, C17:1ω8c, i-C14:0
Assimilation of								
N-Acetylglucosamine	+	–	–	–	+	+	+	+
Adipate	w	–	–	–	w	+	–	+
L-Arabinose	+	–	–	–	ND	–	–	+
Caproate	w	–	–	+	ND	–	–	+
Citrate	–	+	+	+	–	+	–	+
Gluconate	+	–	–	+	+	+	+	+
Malate	–	–	–	+	+	+	+	+
Maltose	+	–	+	+	+	+	+	+
D-Mannitol	+	+	–	+	+	+	+	+
D-Mannose	+		–	–	ND	+	+	+
Enzyme activities								
Alkaline phosphatase	–	–	–	–	–	–	+	+
Cystine arylamidase	–	–	+	–	+	+	–	w
β-Glucosidase	–	–	–	w	–	–	=	+
α-Mannosidase	–	–	–	w	–	–	–	–
Naphthol-AS-BI-phosphohydrolase	+	+	–	+	–	+	+	+
Valine arylamidase	–	–	+	+	ND	+	–	w
α-Glucosidase	+	+	+	+	+	+	–	+

ND not determined, *Ara* arabinose, *Gal* galactose, *Fuc* fucose, *Glc* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose

[a]Six type strains were analyzed in parallel for the composition of fatty acids (Weon et al. 2010), which may differ from that given in the original decriptions

[b]i-C15:0 2OH/C16:1ω7c

cocci of Gram-positive and Gram-negative staining cells were observed on glucose sulfide (GS) medium. These strains, belonging to the cultured organisms of the "*Candidatus* Nostocoida limicola" taxon. (Blackall et al. 2000), were described as members of the species *T. jenkinsii*, *T. vanveenii*, and *T. veronensis* (McKenzie et al. 2006).

All members of the genus (excepting *T. duodecadis* and *T. remsis*) store polyphosphate either as granules (grown on R2A) or throughout the trichomes (grown on GS).

Table 23.6 lists some properties useful to differentiate among species. Additional data than those shown in *Table 23.6* are listed in the original species descriptions.

Genus *Knoellia* Groth et al. 2002

Knoell'i.a. N.L. fem. n. *Knoellia* after Hans Knöll (1913–1978), a German pioneer in antibiotic research.

Table 23.7 lists some properties useful to differentiate among species. Additional data than those shown in *Table 23.7* are listed in the original species descriptions. Yu et al. (2012) compared the fatty acid patterns of four species, grown under the same condition. Significant differences in both quality and quantity are obvious when compared to the patterns listed in the original description, e.g., the presence of i-C14:0 and C17:1ω9c in *K. sinensis* and *K. subterraneae* and absence of i-C17:1 in *K. sinensis*.

Genus *Phycicoccus* Lee 2006

Phy.ci.coc'cus. L. n. phycos -i from Gr. n. phukos seaweed; N.L. masc. n. coccus from Gr. n. kokkos a grain or berry; N.L. masc. n. *Phycicoccus* coccus from seaweed).

*Phycicoccus ochan*gensis (Kim et al. 2012) and *Phycicoccus badiiscoriae* (Lee 2013) have been effectively published but are not yet validated. *Phycicoccus aerolatum*, isolated from air, has been mentioned in public databases (EF493848; Weon, unpublished) but is not validly named.

Table 23.8 lists some properties useful to differentiate among species. Additional data than those shown in *Table 23.8* are listed in the original species descriptions. All strains are nonmotile and positive for catalase reaction and assimilation of glycogen, sucrose, β-galactosidase, β-glucosidase, and urease. All strains are negative for indole production, acid production from glucose, and assimilation of L-arabinose, adipate, caprate, citrate, phenylacetate, lipase (C14), valine arylamidase, trypsin, β-glucuronidase, *N*-acetyl-b-glucosaminidase, and α-fucosidase. Other reaction are listed in *Table 23.2* and in the original species descriptions. Wang et al. (2011) compared the fatty acid composition of four species performed under the same conditions. As in other such studies, remarkable differences in the composition are found when compared to the original descriptions, e.g., the presence of significant amounts of $C_{17:0}$ in *P. dokdonensis* and of $C_{15:0}$, $C_{16:0}$, and ai-$C_{15:0}$ in *P. aerophilus*.

Genus *Serinicoccus* Yi et al. 2004

Ser.in'i.coc.cus. N.L. n. *serinum* serine; N.L. masc. n. coccus from Gr. masc. n. *kokkos* a grain, seed; N.L. masc. n. *Serinicoccus* a coccus with serine in the cell wall.

Table 23.9 lists some properties useful to differentiate among species. Additional data than those shown in *Table 23.9* are listed in the original species descriptions. All strains are positive for catalase and negative for oxidase. Nitrate is reduced to nitrite. All strains show esterase (C4) and valine arylamidase activity but are negative for α-mannosidase and urease and negative for the utilization of L-arabinose, D-mannitol, and *N*-acetylglucosamine.

Genus *Ornithinimicrobium* Groth et al. 2001

Or'ni.thi.ni.mic.ro.bi.um. Gr. n. *ornithos* bird, ornithine an amino acid named after birds; Gr. adj. *micros* small; Gr. masc. n. *bios* life; N.L. neut. n. *Ornithinimicrobium* a microbe with ornithine.

Table 23.10 lists some properties useful to differentiate among species. Additional data than those shown in *Table 23.10* are listed in the original species descriptions.

Genus *Arsenicicoccus* Collins et al. 2004

Ar.sen.i.ci.coc'cus. L. n. arsenicum arsenic;N.L. masc. n.coccus berry; N.L. masc. n. *Arsenicicoccus* arsenic coccus, because the type species was recovered from an arsenic enrichment.

Table 23.11 lists some properties useful to differentiate among species. Additional data than those shown in *Table 23.11* are listed in the original species descriptions.

The two species share many metabolic properties (Hamada et al. 2009), such as presence of β-galactosidase, α-glucosidase, and β-glucosidase and hydrolysis of aesculin and gelatin. Acid is produced from D-fructose, D-galactose, D-glucose, glycogen, D-maltose, D-mannose, D-melibiose, D-raffinose, starch, D-sucrose, and D-trehalose. They differ from each other in that *A. bolidensis* produces acids from *N*-acetyl-glucosamine, arbutin, D-cellobiose, gentobiose, D-mannitol, and salicin, while *A. piscis* does not, but produces acid from D-ribose. The latter species is positive for alkaline and acid phosphatase, α-chymotrypsin, phosphohydrolase, and pyrazinamidase, while *A. bolidensis* reacts negatively. Both type strains grow in the presence of 0.45 mM arsenic.

The following genera contain a single species only. Their main phenotypic properties are indicated in *Tables 23.2* and *23.11*. Other metabolic traits are indicated in the original species descriptions. The composition of fatty acids may vary when the type strains have been included in comparative analysis with newly described species. The origin of the species is compiled in *Table 23.1*.

Table 23.6

Properties apt to differentiate type strains of *Tetrasphaera* species (see Table 23.1). 1. *Tetrasphaera japonica*; 2. *Tetrasphaera remsis*; 3. *Tetrasphaera duodecadis*; 4. *Tetrasphaera elongata*; 5. *Tetrasphaera jenkinsii*; 6. *Tetrasphaera vanveenii*; 7. *Tetrasphaera australiensis*; 8. *Tetrasphaera veronensis*

Properties	1	2	3	4	5	6	7	8
Morphology	Cocci in clusters of tetrades	Cocci in diploid, tetrad or cluster arrangements	Rod-coccus growth cycle	Oval to short rods, forming elongated irregular clumps	Long irregular filaments or cocci/ discoid cells depending on medium; irregular septation	Long irregular filaments or cocci depending on medium	Cocci in clusters of tetrades	Long irregular filaments or cocci/ discoid cells depending on medium; irregular septation
Colony color	ND	Beige colored	Cream colored to pale brown, somewhat translucent	Beige colored	ND	Beige colored	ND	Whitish, shiny
Temperature range (optimum) °C	20–37 (25)	15–45 (25)	At 10 but not at 37 (20–32)	20–35 (25)	ND	ND	15–37 (25)	15–35 (ND)
pH range (optimum)	6.0–8.0 (7.0)	6–9 (7)	ND	6–9 (7)	ND	ND	6.0–8.0 (7.0)	ND
Oxidase	+	–	–	–	+	+	+[a]	+
β-Galactosidase	+	+	+	ND	ND	ND	+	ND
NO_3 reduction	–	–	+	+	+	+	–	+
H_2S production	–	–	–	ND	ND	ND	–	ND
Hydrolysis of								
Gelatin	–	+	+	ND	ND	ND	–	ND
Acetamide	ND		+	ND	ND	ND	ND	ND
Aesculin	ND	+	+	ND	ND	ND	ND	ND
Urea	–	–	–	ND	ND	ND	–	ND
Arginine	ND	–	–	ND	ND	ND	ND	ND
Fatty acids (>5 %) than those listed in Table 23.2[b]	i-$C_{15:0}$, ai-$C_{15:0}$, i-$C_{14:0}$	$C_{18:1}$, $C_{18:0}$, i-$C_{15:0}$, $C_{17:0}$, i-$C_{17:0}$, $C_{16:0}$, $C_{17:1}$	10-Methyl-$C_{17:0}$, i-$C_{15:0}$, 10-methyl-$C_{18:0}$, *cis*-9-$C_{17:1}$, $C_{17:0}$, *cis*-9-$C_{18:1}$	i-$C_{15:0}$, ai-$C_{15:0}$,	i-$C_{15:0}$, $C_{16:0}$, ai-$C_{17:0}$,	i-$C_{15:0}$, i-$C_{16:1}$, $C_{17:1}$, $C_{15:0}$	i-$C_{15:0}$, i-$C_{16:1}$, $C_{16:0}$, *cis*-9-$C_{16:1}$	i-$C_{16:1}$, i-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:1}$, i-$C_{14:0}$
Mol% G+C of DNA	71 (Tm)	69.2 (Tm)	73 (HPLC)	70 (Tm)	ND	ND	68–70 (Tm)	ND
Utilization of								
Acetate	+	ND	ND	+	+	+	+	+
Pyruvate	+	ND	ND	+	+	+	ND	+
Propionate	+	ND	ND	ND	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Fructose	ND	+	+	ND	+	+	ND	+
Lactose	ND	–	ND	+	+	+	ND	+
Mannose	ND	–[a]	–	+	+	+	ND	+
Tween 80	–	+[a]	ND	ND	+	+	+	+
Glycerol	ND	+	ND	ND	+	+	ND	+

Table 23.6 (continued)

Properties	1	2	3	4	5	6	7	8
Lactate	ND	–	ND	ND	–	–	ND	–
Ethanol	ND	ND	ND	ND	–	–	ND	–
Oleate	ND	ND	ND	ND	–	–	ND	–

ND not determined. All species are positive for catalase (*T. japonica* is weakly positive). Data are from the original species descriptions and from Seviour and Maszenan (2012) and Blackall et al. (2000)

[a]Result of the type strain, other strains give different results

[b]Data for 1, 3, 4, and 7 from Ishikawa and Yokota (2006); for 5, 6, and 8 from McKenzie et al. (2006); for 2 from Osman et al. (2007)

Table 23.7

Properties apt to differentiate type strains of *Knoellia* species (see *Table 23.1*). Other chemotaxonomic properties are indicated in *Table 23.1*. The use of different API substrate galleries prevents a complete overview of metabolic reactions 1. *K. sinensis*; 2. *K. subterraneae*; 3. *K. locipacati*; 4. *K. flava*; 5. *K. aerolata*

Properties	1	2	3	4	5
Size of rods, μm	1.7–4.5 × 0.4–0.9	1.9–6.0 × 0.5–1.2	ND	1.0–1.9 × 0.6	1.5–2.0 × 1.0
Diameter of cocci, mm	0.6–0.9	0.8–1.4	ND	0.5–1.1	1.0–1.5
Colony color	White to cream	White to cream	White to cream	Yellow	White to cream
Temperature range °C (optimum)	(28)	(28–37)	10–37 (30)	4–37 (28)	5–35 (30)
pH range (optimum)	5–9	5–9	6–9 (7–8)	5–9 (7)	5–9 (6–7)
Growth up to NaCl%	4	4	5	5	2
Catalase	+	+	+	+	ND
Oxidase	–	–	–	–	ND
NO_3 reduction	+	+	ND	+	ND
Fatty acids (>5 %) in addition to those listed in *Table 23.2*	i-$C_{17:0}$,ai-$C_{17:0}$, i-$C_{17:1}$	i-$C_{17:0}$, ai-$C_{17:0}$,ai-$C_{15:0}$, i-$C_{17:1}$	i-$C_{14:0}$, $C_{17:0}$, 10-methyl-$C_{17:0}$, $C_{17:1}\omega8c$	i-$C_{14:0}$, $C_{17:0}$, $C_{17:1}\omega8c$	$C_{17:0}$, ai-$C_{17:0}$, $C_{17:1}\omega8c$, $C_{18:1}\omega9c$
Polar lipids in addition to those listed in *Table 23.1*	PG, UPL	PG, UPL	PG, 5uPL	uPLs	None
Cell sugars	None	None	Glc, Rib	ND	Glc
Hydrolysis of					
Casein	+	+		+	–
Tyrosine	+	+		–	–
API20 NE/API32GN					
Arginine hydrolase	–	–	ND	w	–
D-Mannose	–	+	ND	+	+
D-Mannitol	–	+	+	+	+
N-Acetylglucosamine	–	+	ND	–	+
Gluconate	–	–	–	–	+
Malic acid	–	+	ND	+	–
D-Ribose	–	+	–	–	–
Inositol	–	–	–	–	+
Lactic acid	w	+	ND	+	–
L-Alanine	–	+	ND	+	+
L-Serine	–	+	ND	+	–
D-Sorbitol	–	+	ND	+	+
L-Histidine	+	+	ND	+	–

Table 23.7 (continued)

Properties	1	2	3	4	5
API Zym					
Alkaline phosphatase	w	+	+	+	+
Acid phosphatase	w	–	+	+	–
Esterase (C4)	+	+	+	+	w
Cystine arylamidase	w	–	–	+	–
Trypsin	w	–	w	W	–
β-Galactosidase	–	–	w	+	+
β-Glucuronidase	–	–	–	–	w
α-Glucosidase	+	+	+	+	w
β-Glucosidase	w	+	+	+	

ND not determined, *Glc* glucose, *Rib* ribose, *PG* phosphatidylglycerol, *u* unknown, *PL* phospholipid

Table 23.8

Properties apt to differentiate type strains of *Phycicoccus* species (see Table 23.1). 1, *P. jejuensis*; 2, *P. dokdonensis*; 3, *P. aerophilus*; 4, *P. bigeumensis*; 5, *P. cremeus*; 6, *P. ginsenosidimutans*

Properties	1	2	3	4	5	6
Morphology	Coccoid	Coccoid	Short rods	Coccoid	Rods	Coccoid
Cell dimensions	ND	0.3–0.7	0.8–0.9 × 1.1–1.5	0.4–0.5	0.5–0.8 × 1.5–2.0	0.3–0.7
Colony color	Mud yellow	Greyish yellow	White	Yellow	Creamish	Greyish yellow
Temperature range in °C (optimum)	4–37 (30)	10–36 (30)	5–37 (30)	20–37 (28)	14–35 (29)	10–37 (30)
pH range (optimum)	5–10 (7)	5–8.5 (6.5–7.5)	5–9 (6–7)	7–12 (7.4)	4.1–10 (7–8)	5–10 (7)
Growth up to NaCl%	7	5	7	5	7	5
Fatty acids (>5 %) in addition to those listed in Table 23.1	$C_{17:1}\omega8c$, $C_{15:0}$, $C_{17:0}$, i-$C_{14:0}$	ai-$C_{15:0}$, i-$C_{14:0}$	$C_{17:1}\omega8c$, $C_{17:0}$	$C_{17:0}$, ai-$C_{15:0}$, 10-me $C_{17:0}$, i-$_{14:0}$, $C_{17:1}\omega8c$,	$C_{17:1}\omega8c$, 10-me $C_{17:0}$,	$C_{17:0}$, ai-$C_{15:0}$
Polar lipids in addition to those listed in Table 23.2	PE, PI	PG, 2uPl	PE, PI	PG, 2uGL	PI, 6uGL	PG, PE, PC,PI
Cell wall sugars	ND	ND	Glc, Rib	ND	ND	ND
Oxidase	–	+	–	–[a]	–	+
NO_3 reduction	+	–	–	+	+	ND
mol% G+C of DNA	74	71	71	73	72	71
API20 NE						
N-Acetylglucosamine	+	+	+	–	+	–
Gelatin hydrolysis	+	+	–	–	+	–
Gluconate	+	+	+	–	+	+
D-Glucose	+	+	+	–	+	+
Malate	+	+	+	–	+	+
Maltose	+	+	+	+	+	–
D-Mannitol	+	+	+	–	+	+
D-Mannose	+	+	w	–	+	–
API ZYM						
Acid phosphatase	+	+	+	+	+	–
Alkaline phosphatase	–	+	+	+	w	–
Esterase (C8)	+	+	+	+	–	–

Table 23.8 (continued)

Properties	1	2	3	4	5	6
Esterase lipase (C4)	+	+	+	+	w	–
Cystine arylamidase	–	+	–	–	+	–
Leucine arylamidase	+	+	–	W	–	–
α-Chymotrypsin	W	–	+	W	+	–
α-Galactosidase	+	+	–	–	+	–
α-Glucosidase	+	+	–	+	+	–
α-Mannosidase	w	+	–	–	+	–
Naphthol-AS-BI-phosphohydrolase	–	+	+	+	+	w

+ Positive, W weakly positive, 2 negative
ND not determined, *Glc* glucose, *Rib* ribose, *PC* phosphatidylcholine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PE* phosphatidylethanolamine, *u* unknown, *GL* glycolipid, *PL* phospholipid
[a]Data from Zhang et al. 2011

Table 23.9

Properties apt to differentiate type strains of *Serinicoccus* species. 1. *S. profundi*; 2. *S. marinus*; 3. *S. chungangensis*

Properties	1	2	3
Morphology	Coccoid	Coccoid	Coccoid
Cell dimensions	0.5–0.9	ND	ND
Colony color	Yellow	Yellow	Yellow
Temperature range in ˚C (optimum)	10–35 (35)	10–35 (30)	20–40 (30)
pH range (optimum)	6–11 (8)	6–11 (7)	5–11 (9)
Growth up to NaCl%	14	14	15
Fatty acids (>5 %) in addition to those listed in *Table 23.1*	ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$	i-$C_{17:1}\omega$9c, ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$
Polar lipids in addition to those listed in *Table 23.1*	uGL	PC, uGL	PC, uL
Cell sugars	ND	ND	Glc, Rib
mol% G+C of DNA	72	72	73.5
Aesculin hydrolysis[a]	w	+	+
Utilization of			
Glucose	+	+	–
Maltose	+	ND	–
Mannose	+	+	–
Gluconate	+	ND	–
Malic acid	+	ND	–
Enzyme activities[a]			
Acid phosphatase	–	–	+
Alkaline phosphatase	–	+	+
Esterase (C8)	–	+	+
Cystine arylamidase	–	+	w
Leucine arylamidase	–	+	+
α-Chymotrypsin	–	+	+
α-Galactosidase	–	–	w
α-Glucosidase	+	+	–
β-Glucosidase	–	+	w
Naphthol-AS-BI-phosphohydrolase	w	–	w
Trypsin	–	+	w

ND not determined, *Glc* glucose, *Rib* ribose, *PC* phosphatidylcholine, *u* unknown, *GL* glycolipid, *L* lipid
[a]Compiled by Traiwan et al. 2011

Table 23.10

Properties apt to differentiate type strains of *Ornithinimicrobium* species. 1. *O. humiphilum*; 2. *O. kibberense*; 3. *O. pekingense*; 4. *O. murale*

Properties	1	2	3	4
Morphology	Coccoid, short rods	Coccoid, short rods	Coccoid, short rods	Rudimentary mycelium, coccoid, short rods
Cell dimensions	Cocci 0.7–1.4, rods 0.6–1.0 × 1.4–3.2	ND	0.5–0.8 × 1.0–1.6	Width 1.3
Colony color	Whitish to bright yellow	ND	Light yellow	White to beige
Temperature range in °C (optimum)	(37–42)	20–37 (28)	26–38 (33–37)	15–30 (25–30)
pH range (optimum)	ND	ND	6–9 (7.8–8.2)	7–9 (7.5–8.5)
Fatty acids (>5 %) than those listed in Table 23.11	$C_{16:0}$, $C_{16:1}$[a], i-$C_{17:1}\omega8c$	ai-$C_{17:0}$, i-$C_{17:1}\omega8c$	$C_{16:0}$	None
Mol% G+C of DNA	70	69	71	ND
Growth up to NaCl%	7	7	7	0
Catalase	+	+	+	ND
Oxidase	–	–	–	ND
NO_3 reduction	+	+	+	ND
Utilization of				
Arabitol	–	+	+	ND
Cellobiose	–	+	+	+
D-Fructose	–	+	+	+
Raffinose	+	+	w	ND
D-Galactose	–	+	+	+
Citrate	–	+	+	–
Acetate	–	+	+	+
Benzoate	–	+	+	ND
Acid produced from				
L-Arabinose	+	+	–	–
D-fructose	–	+	–	–
D-Galactose	+	+	–	–
D-Xylose	w	–	–	–

[a]According to Kämpfer et al. (2013)
ND not determined

Genus *Ornithinicoccus* Groth et al. 1999

Or'ni.thi.ne. Gr. n. *ornithos* bird, ornithine an amino acid named after birds; Gr. n. *coccos* a grain; M.L. masc. n. *Ornithinicoccus* a coccus with ornithine.

Genus *Ornithinibacter* Xiao et al. 2011

or.ni.thi.ni.bac,ter. N.L. n. *ornithinum* ornithine; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Ornithinibacter* ornithine-containing rod.

Genus *Oryzihumus* Kageyama et al. 2005

ory.zi.hu'mus. L. fem. n. *oryza* rice; L. masc. n. *humus* soil; N.L. masc. n. *Oryzihumus* rice soil.

Genus *Kribbia* Jung et al. 2006

kribb.i'a. N.L. fem. n. *Kribbia* arbitrary name formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB.

Genus *Marihabitans* Kageyama et al. 2008

ma.ri.ha'bi.tans. L. neut. n. *mare* sea; L. part. adj. *habitans* inhabiting; N.L. adj. used as a neut. subst. *Marihabitans* inhabitant of the sea.

Genus *Terracoccus* Prauser et al. 1997

ter.ra.coc'cus. M. L. fem. n. *terra*, soil; Gr. masc. n. *coccus*, berry; M. L. masc. n. *Terracoccus*, coccus isolated from soil.

Table 23.11

Genera embracing two or a single species: morphological, chemotaxonomic, and some physiological properties which are not listed in Table 23.2. Data are from the original description

Properties	*Arsenicicoccus bolidensis*	*Arsenicicoccus piscis*	*Aquipuribacter*	*Ornithinibacter*	*Oryzihumus*	*Kribbia*
Colony color	ND	Greyish	Pale orange	Bright yellow	Pale yellow	ND
Rods cell size μm	ND		0.9–1.1	0.0–0.80 × 0.8–1.1	0.4–0.9 × 0.9–1.9	0.4–0.6 × 1.0–1.5
Cocci cell size μm	ND		3.8–1.4	Absent	Absent	Absent
Temperature range in °C (optimum)	ND	15–37 (28)	20–37 (20–28)	4–45 (34)	15–37	8–42 (30)
pH range (optimum)	ND	5–8 (6–7)	(7–8)	5–10 (7.2)	4–9	(6.5–7.5)
Growth up to NaCl%	ND	5	2.5	5	4	ND
Catalase	+	+	+	+	+	+
Oxidase	–	–	–	–	ND	–
NO_3 reduction	+	+	–	–	ND	+
Fatty acids (>5 %) other than those listed in Table 23.1	ai-$C_{15:0}$, i-$C_{16:1}$H, $C_{16:1}$ ω9*c*, $C_{18:1}$ ω9*c*	ai-$C_{15:0}$	$C_{16:0}$, $C_{18:0}$, $C_{18:3}\omega$6*c* (6,9,12), i-$C_{15:0}$	$C_{18:1}$, $C_{16:1}$ ω9*c*, $C_{17:1}$, i-$C_{14:0}$	None	10-Methyl-$C_{16:0}$
Properties	***Humibacillus***	***Fondibacter***	***Ornithinicoccus***	***Marihabitans***	***Terracoccus***	***Lapillicoccus***
Colony color	Pale yellow	Orange yellow	Cream	Light yellow	Yellow to orange	Bright yellow
Rods cell size μm	0.4–1.1 × 1.1–1.9	0.3–0.6 × 0.7–2.2	Absent	0.6–1.0 × 0.8–1.3	Absent	Absent
Cocci cell size μm	Absent	Absent	0.8–1.3	ND	0.7–1.3	0.2–0.3
Temperature range in °C (optimum)	7–32 (27)	15–37 (28)	(28–37)	18–34	15–37 (28)	20–39 (30)
pH range (optimum)	5–11 (7)	(6.5–7.5)	ND	(9)	4.5–9.5 (6–7.2)	4–11 (7.1)
Growth up to NaCl%	3	2.5	4	7	ND	2
Catalase	+	+	+	+	+	+
Oxidase	ND	+	–	–	–	–
NO_3 reduction	ND	+	+	+	–	–
Fatty acids (>5 %) other than those listed in Table 23.1	ai-$C_{15:0}$, i-$C_{16:0}$, $C_{17:1}$ ω8*c*	i-$C_{16:0}$, $C_{17:1}$ ω8*c*, i-$C_{14:0}$, $C_{18:0}$	i-$C_{17:1}$	i-$C_{15:0}$, i-$C_{17:1}\omega$9*c*	ai-$C_{17:0}$	$C_{15:0}$, 10-methyl $C_{17:0}$, ai-$C_{17:0}$

ND not determined

Genus *Lapillicoccus* Lee and Lee, 2007

la.pil.li.coc'cus. L. masc. n. *lapillus* a little stone; N.L. masc. n. *coccus* coccus; N.L. masc. n. *Lapillicoccus* a coccus attached to a little stone.

Genus *Humibacillus* Kageyama et al. 2008

hu.mi.ba.cil'lus. L. fem. n. *humus* soil; L.masc. n. *bacillus* rod; N.L. masc. n. *Humibacillus* rod isolated from soil.

Genus *Fodinibacter* Wang et al. 2009

Fo.di.ni.bac'ter. L. fem. n. *fodina* mine; N.L. masc. n. *bacter* rod; N.L. masc. n. *Fodinibacter* rod bacterium isolated from a mine.

Genus *Aquipuribacter* Tóth et al. 2012

a.qui.pu.ri.bac'ter. L. n. *aqua* water; L. adj. *purus* clean, pure; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Aquipuribacter* a rod isolated from pure water.

Isolation, Enrichment, and Maintenance Procedures

Considering the large number of species, isolation sources, and laboratories involved in isolation, isolation and enrichment strategies differ widely. Most studies used 10^{-5} dilutions or standard dilution techniques, mixing soil samples or crushed and grinded stones or corals in water or phosphate buffer. In rare

cases, the material was spread directly on enrichment plates (e.g., *Aquipuribacter hungaricus* and *Marihabitans asiaticum*). Techniques and media used can be grouped into categories. R2A medium was used widely, either undiluted (e.g., *Aquipuribacter hungaricus, Janibacter alkaliphilus, Tetrasphaera jenkinsii*) and with the addition of 200 µg cycloheximide for air-sampled organisms (*Terrabacter aeriphilus, Knoellia aerolata, Phycicoccus aerophilus, Janibacter hoylei*) or at different dilutions, e.g., 10× (*Kribbia dieselivorans, Phycicoccus bigeumensis*) or 5× (e.g., *Terrabacter ginsenosidimutans*). Marine agar (MA2216, Difco) was used for *Marihabitans asiaticum* (10× diluted), *Fodinibacter luteus, Janibacter corallicola* (supplemented with 1 % $CaCO_3$), *Serinicoccus marinus*, and *S. profundi*. Other studies used peptone-yeast extract/brain heart infusion agar (Yokota et al. 1993) (*Knoellia subterranea, Ornithinicoccus hortensis, Ornithinimicrobium humiphilum*), WAT agar (Li et al. 2002) (*Phycicoccus jejuensis, Terrabacter lapilli*), TSA (*Knoellia locipacati, Ornithinimicrobium kibberense*), glucose-yeast extract agar (*Serinicoccus chungangensis*), nutrient agar (Difco) (*Janibacter melonis, Terrabacter terrigena, Phycicoccus dokdonensis* [10× diluted]), LB agar (*Ornithinimicrobium pekingense, Intrasporangium chromatireducens*), and various other, more specialized media, such as mineral medium (Kim and Hegeman 1981), V2M (Zhang et al. 2010), xylan-nutrient agar (Wang et al. 2011), GS agar (Maszenan et al. 1997), feather agar (Williams et al. 1990), GMP agar (for the isolation of *Oryzihumus leptocrescens* with the addition of superoxide dismutase) (Takahashi et al. 2003), starch-casein agar (Küster and Williams 1964), basal salt medium (Collins et al. 2004), casein-mineral medium (Altenburger et al. 1996), LYPm medium (Iino et al. 2007), or R medium (Yamada and Komagata 1972). Several type and non-type strains of *Tetrasphaera* were isolated by micromanipulation (Skerman 1968) from activated sludge biomass and cells enriched on GS or R2A agar Plates. *T. duodecadis* (formerly *Arthrobacter duodecadis*) was isolated on medium containing vitamin B12 as essential growth factor (Lochhead 1958).

Long-term preservation followed routine techniques, e.g., at −20 °C, −70 °C, or −80 °C in 20 % glycerol, or in liquid nitrogen in the presence of 5 % DSMO (w/v).

Pathogenicity, Clinical Relevance

None of the *Intrasporangiaceae* strains is considered obligatory pathogenic for humans, though several reports are available that indicate the involvement of members of the family in clinical cases. Clones related to *Terracoccus luteus* were found in children with atopic dermatitis (Kong et al. 2012), and a clone related to *Janibacter* spp. was found among the microorganisms determined in the inflammation of the ileal pouch (McLaughlin et al. unpublished). A strain of *Ornithinimicrobium pekingense* was reported to be involved in an eye infection (Borsali et al. 2011) and a *Terrabacter* strain was detected in infectious endophthalmitis (Aarthi et al. 2012). An undescribed strain of *Janibacter* was identified in the blood of a patient with acute myeloid leukemia (Loubinoux et al. 2005), and *J. melonis* was described to cause bacteremia (Elsayed and Zhang 2005). Antibiotic sensitivity testing has been performed on a few type strains, i.e., *Phycicoccus dokdonensis, Serinibacter profundus, Ornithinimicrobium humiphilum, Knoellia sinensis, K. subterranea, Ornithinibacter aureus*, and *Ornithinicoccus hort*ensis, but not all of these species have been tested against the same set of antibiotics. Five of the seven species (two were not tested) are sensitive towards lincomycin, polymyxin B, rifampicin, and streptomycin. Most of the species are also sensitive towards choramphenicol, erythromycin, gentamicin, and neomycin (*O. aureus* is resistant against each of these) and resistant against oxacillin. Reactions against ampicillin, kanamycin, penicillin G, and tetracycline vary.

Habitat

While the habitat of type strains is shown in ❯ *Table 23.1*, information on additional habitats is available following BLAST analysis of 16S rRNA gene sequences deposited in public databases. The following listing on cultured and not-yet cultured stains with 98–99 % BLAST sequence similarity to type strains is not extensive, and the reader is encouraged to search for the most recent entries. In some cases organisms from different genera were found in the same habitat, i.e., in Australian soil, uranium-contaminated soil, and on skin of children with atopic dermatitis. Soil and the marine environment appear to be the main habitats.

Cultured strains, related to *Janibacter* species, were from alkaline groundwater (Tiago et al. 2004), fermented onions (HM439458; Park and Sa, unpub.), leaves of *Phaseolus vulgaris* (Lopez-Lopez et al. 2010) and the midgut of *Culex quinquefasciatus* (JN644568; Chandel et al. unpub.), and from deep sea sediment (HM222674; Luo et al. unpub.). Clone sequences originate from seahorses (Balcazar et al. 2010), pouchitis microflora (GQ158446; McLaughlin et al. unpub.), and arctic sea ice (Yu et al. 2009).

Knoellia-related isolates and clones originated from urban aerosols (Brodie et al. 2007), marine sediment (Bredholdt et al. 2007), plant leaves (Lopez-Lopez et al. 2010), and Caribbean sponges (Tabares et al. 2011).

Arsenicicoccus-related sequences were retrieved from a strain isolated from a salt mine (DQ358660; Xiao et al. unpub.); from glaciers (JX949811; Liu and Xin, unpub.), marine sediment (HQ858012; Dastager, unpub.), cellulose or xylan/pectin enrichments, and pig feces (JQ607653; Ziemer et al. unpub.); and from uncultured soil (Joseph et al. 2003). A clone sequence was obtained from material of an indoor environment (Rintala et al. 2008). An *Ornithinicoccus* strain was among a wide range of bacteria associated to root nodules collected from legumes (Zakhia et al. 2006).

Isolates related to *Serinibacter* species originate from marine sediments (Gontang et al. 2007) and other marine environments (DQ985072; Wang and Jiao, unpub.), sea sponges (JX007966; Sun and Li, unpub.; JN128293; Su et al. unpub.), and fermented seafood (JN187087; Jeon and Jung, unpub.) and as for

Table 23.12

Summary of *Intrasporangiaceae* strains with bioremediating capacities

Species and strain	Gene/protein	Function	Comment	Reference
Terrabacter sp. strain DBF63	dbfA and dbfBC fln-dbfA, pht, and pca gene clusters	Dibenzofuran degradation utilization of fluorene	DfdA dioxygenase degrades dibenzo-p-dioxin, carbazole, dibenzothiophene, anthracene, phenanthrene, and biphenyl	Kasuga et al. (2013)
Terrabacter sp. strain DBF63	DbfA1, DbfA2	Dibenzofuran 4,4a-dioxygenase	The DFDO system converts dibenzofuran to 2,2′,3-trihydroxybiphenyl	Takagi et al. (2005)
Terrabacter sp. strain DBF63	DbfA	Fluorene degradation to phthalate	Fluorene and 9-fluorenone are degraded to 9-fluorenol and 1,1a-dihydroxy-1-hydro-9-fluorenone, respectively	Habe et al. (2004)
Terrabacter sp. strain DBF63	flnD1 and ORF16	Fluorene degradation to phthalate. Class III two-subunit extradiol dioxygenase	9-Fluorenol and 1,1a-dihydroxy-1-hydro-9-fluorenone are degraded to 9-fluorenone and 2′-carboxy-2,3-dihydroxybiphenyl, respectively	Habe et al. (2004)
	FlnE	Serine hydrolase	Phthalate formation	
Terrabacter sp. strain FLO	ND	Aromatic-ring-hydroxylating dioxygenase	Biodegradation of polycyclic aromatic hydrocarbons to transform phenanthrene, fluorene, pyrene, and fluoranthene into the cis-dihydrodiol metabolites	Zhou and Zhou (2007)
Terrabacter spp.	PAH-RHDalpha	Alpha subunit of the PAH-ring hydroxylating dioxygenase	PCR primer set to amplify narAa, phdA/pdoA2, idA/pdoA1, nidA3/fadA1	Cébron et al. (2008)
Janibacter terrae XJ-1	dbdA	Dibenzofuran dioxygenase	Dibenzofuran is degraded to 2,2′,3-trihydroxybiphenyl, salicylic acid, gentisic acid, and other metabolites	Jin et al. (2006)
Janibacter sp. TYM3221	Gene cluster bphAaAbAcAd	1,1-Dichloro-2,2-bis(4-chlorophenyl)ethylene degradation	End product 4-chlorobenzoic acid	Nguyen et al. (2011b)
Janibacter strain YA	ND	Mono-chlorinated dibenzo-p-dioxin degradation: 1-chloro-dibenzo-p-dioxin (1-CDD) and 2-chloro-dibenzo-p-dioxin	2-chloro-2′,6-dihydroxydiphenylether as metabolite	Iwai et al. (2005)
Janibacter strain YY-1	ND	Growth on fluorene and dibenzothiophene cometabolization of dibenzo-p-dioxin, phenanthrene, and anthracene	2,3,2′-Trihydroxybiphenyl, biphenyl-dihydrodiol, dibenzothiophene 5-oxide, and coumarin as major metabolites	Yamazoe et al. (2004)
Terrabacter ginsenosidimutans strain Gsoil 3082^T	bgpA	Ginsenosidase type III	Hydrolyzes 3-O-glucoside of multi-PPD-type ginsenosides. 3-O-β-D-(1− > 2)-glucopyranosyl of Rb1 is hydrolyzed to gypenoside XVII, and the 3-O-β-D-glucopyranosyl of gypenoside XVII is hydrolyzed to gypenoside LXXV. Glucopyranosyls linked to the 3-O-position of Rb2, Rc, Rd, Rb3, and Rg3 are hydrolyzed	Jin et al. (2012)

ND not determined

Terracoccus luteus from skin of children with atopic dermatitis (Kong et al. 2012).

Several sequences of isolates and clones are reported for *Ornithinimicrobium* species, such as oyster shells (Islam et al. 2009), garbage composter (Narihiro et al. 2004), and leachate sediment (Liu et al. 2011). Several clones were obtained from skin of children with atopic dermatitis (Kong et al. 2012), and they were detected in a study on the synecology of the primary and secondary feedlot habitats of *Escherichia coli* O157:H7 (Durso et al. unpub.).

Uncultured strains moderately related to described *Tetrasphaera* species originate not only from wastewater

(HQ010781; Ji and Chen 2010) and activated sludge (EU104275; Brown and Turner, unpub.), have been found also in clean room environments (accession number EU071503; Nellen et al. unpub.), from a siliceous sedimentary rock (AB179506, Yoshida et al. unpub.), and the human skin (Grice et al. 2009). Several isolates (Ellin strains) were recovered from soil (Schoenborn et al. 2004).

Sequences of uncultured strains, related to *Terrabacter* species, originate not only from house dust (FM872945; Taubel et al. 2009), rhizosphere of Phragmites (AB240275; Nakamura et al. unpub.), apple phyllosphere (Yashiro et al. 2011), and freshwater (Baik et al. unpub.), but also some from Australian soil (Ellin strains) (Schoenborn et al. 2004).

Clone sequences related to *Terracoccus luteus* were found on the skin of children with atopic dermatitis (Kong et al. 2012), while *Intrasporangium calvum* shared close relatedness with uncultured microorganisms involved in anaerobic benzene degradation (Kunapuli et al. 2007) and in soil heavily contaminated with uranium (Brodie et al. 2006).

Phycicoccus-related bacteria were reported by Mitsui et al. (1997) and uncultured strains were mainly identified in soil samples, such as in potassium rich soil (JF428901; Huang and Sheng, unpub.), an unidentified soil (EF688365; Jia et al. unpub.), uranium-contaminated soil (Brodie et al. 2006), soils of the Tianshan Glacier No. 1 (JN662535; Wu et al. unpub.), volcanic ash (FN386744; Lee and lee, unpub.), and unvegetated soil (Sattin et al. 2009). Only two clone sequences were related to *Ornithinibacter aureus*, both from a wastewater treatment plant (HM773480; Lee et al. unpub.; Liu et al. 2012b). *Fodinibacter luteus* is related to an uncultured marine bacterium (AB522645; Kasai et al. unpub.).

Application

Especially members of *Tetrasphaera* are intrinsic members of wastewater treatment plants, and, except for *T. duodecadis* and *T. remsis*, all species accumulate polyphosphate granules (Maszenan et al. 2000; Hanada et al. 2002; McKenzie et al. 2006).

The culture extract of a *Janibacter limosus* strain showing a high biological activity against bacteria and fungi was found to contain two natural products, i.e., helquinoline (4-methoxy-2-methyl-1,2,3,4-tetrahydroquinoline-8-carboxylic acid) and *N*-acetylkynuramine (Asolkar et al. 2004).

Waste Treatment and Removal

Members of the genus *Tetrasphaera* are considered to be polyphosphate accumulating organisms (PAOs) in enhanced biological phosphorus removal (EBPR) from wastewater in which they may play a dominating role (Liu et al. 2001; Eschenhagen et al. 2003; Kong et al. 2005; Nguyen et al. 2011a). None of the type strains accumulates polyhydroxyalkanoates (Liu et al. 2001; Maszenan et al. 2000). Analysis of the annotated genome of *T. australiensis, T. japonica, T. elongata*, and *T. jenkinsii* allowed the elucidation of key features of organisms thriving under the alternating anaerobic/aerobic conditions encountered in EBPR systems. It was postulated that under anaerobic conditions these PAOs take up glucose (but also amino acids and acetate), fermenting it to succinate and other components. Glycogen is synthesized as a storage polymer, using energy generated from the degradation of stored polyphosphate and glucose fermentation. Under aerobic conditions the stored glycogen is catabolized to provide energy for growth and used to replenish the intracellular polyphosphate pool needed for the subsequent anaerobic metabolism. Denitrifying abilities were also recognized among the *Tetrasphaera* strains (Kristiansen et al. 2013). *Tetrasphaera* strains seem to occupy a slightly different ecological niche compared with "*Candidatus* Accumulibacter" contributing to a functional redundancy and stability of the EBPR process (Xia et al. 2008).

Bioremediation

Using a microcosm enrichment approach to enrich bacteria which are representative of long-term biphenyl-adapted microbial communities resulted in the isolation of more than 150 biphenyl-degrading strains. Based upon characterization of fatty acid methyl ester (FAME) analysis and 16S rRNA gene sequence comparison, one minor cluster was assigned to *Terrabacter* sp. (Wagner-Döbler et al. 1998).

Terrabacter sp. strain DBF63 is capable of degrading fluorene (FN) to intermediates of the tricarboxylic acid cycle via phthalate and protocatechuate. Genes were identified for the protocatechuate branch of the beta-ketoadipate pathway (pcaR, pcaHGBDCFIJ) by sequence analysis of a 70 kb DNA region of the FN-catabolic linear plasmid pDBF1 (Habe et al. 2004, 2005). The dbfA1A2 cistron and pht operon of *Terrabacter* sp. DBF63 were located on the two linear plasmids, pDBF1 (160 kb) and pDBF2 (190 kb), while dbfBC genes were located on the chromosome (Nojiri et al. 2002). Another *Terrabacter* strains capable of degrading dibenzofuran by the plasmid-borne dioxygenase dfdA1 gene was strain YK3 (Iida et al. 2002). *Table 23.12* gives an overview of strains and their bioremediating capacities.

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24 The Order *Jiangellales*

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Abstract
The order *Jiangellales* was created by elevation of suborder *Jiangellineae* of Tang et al. (Int J Syst Evol Microbiol 61:194–200, 2011), mainly based on signature nucleotide patterns and phylogenetic criteria (Tang et al. The order *Jiangellineae*. In: Whitman WB, Goodfellow M, Kämpfer P, Busse H-J, Trujillo M, Garrity G, Ludwig W, Suzuki K-I (eds) Bergey's manual of systematic bacteriology, vol 5, 2nd edn. Springer, New York, p 555, 2012). The order contains the sole suborder *Jiangellineae*. As such, their members are Gram-positive, aerobic, halophilic, or nonhalophilic filamentous actinomycetes. *Jiangellineae* contains the family *Jiangellaceae*, which embraces the genera *Haloactinopolyspora* and *Jiangella*, and for which one and four species each, have been described.

Ji.ang.el 'la.les. N.L. fem. n. *Jiangella* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Jiangellales* the *Jiangella* order.

Taxonomy: Historical and Current

Short Description of the Order, Suborder, and the Family

The genus *Jiangella* was first proposed by Song et al. (2005) and assigned to the family *Nocardioidaceae* within the suborder *Propionibacterineae*. Then, another novel genus, *Haloactinopolyspora*, was described by Tang et al (2011), on the basis of phylogenetic reconstruction. The genera *Jiangella* and *Haloactinopolyspora*, forming a monophyletic deep branch at the periphery of the evolutionary radiation occupied by the suborder *Propionibacterineae*, were clearly different as compared to other described suborders of the class *Actinobacteria* and showed that the genera *Jiangella* and *Haloactinopolyspora* did not belong to the suborder *Propionibacterineae* nor to any other described suborders within the phylum Actinobacteria. Representatives of this phylum showed less than 93.1 % 16S rRNA gene sequence similarities to the genera *Jiangella* and *Haloactinopolyspora*. The 16S rRNA gene sequences of all suborders of the class *Actinobacteria* (Zhi *et al.* 2009) and the genera *Jiangella* and *Haloactinopolyspora* were scanned for signature nucleotides. Both genera *Jiangella* and *Haloactinopolyspora* had several unique 16S rRNA gene signature nucleotides as compared to sequences of members of other suborders, particularly reflected in 11 different positions, namely, in 127:234 (G-C), 598:640 (C-G), 672:734 (G-C), 831:855 (U-A), 833:853 (G-C), 840:846 (A-U), 950:1231 (G-C), 952:1229 (G-C), 955:1225 (G-U), 986:1219 (U-G), and 987:1218 (C-G). Thus, the genus *Jiangella* together with the genus *Haloactinopolyspora* has been proposed to be classified as *Jiangellaceae* fam. nov. and *Jiangellineae* subord. nov (Tang et al. 2011). Later, the order *Jiangellales* was created by elevation of suborder *Jiangellineae* of Tang et al (2010), mainly based on signature nucleotide patterns and phylogenetic criteria (Tang et al. 2012).

Jiangellineae Tang, Zhi, Wang, Shi, Lou, Xu and Li 2011, 198[VP]

Ji.ang.el.li'ne.ae. N.L. fem. n. *Jiangella* type genus of the suborder; *-ineae* ending to denote a suborder; N.L. fem. pl. n. *Jiangellineae* the suborder of the genus *Jiangella*.

The pattern of 16S rRNA signatures consists of nucleotides at positions: 127 : 234 (G–C), 598 : 640 (C–G), 672 : 734, (G–C), 831 : 855 (U–A), 833 : 853 (G–C), 840 : 846 (A–U), 950 : 1231

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Table 24.1
Differential properties of the genus *Jiangella* and the genus *Haloactinopolyspora*

Characteristics	*Jiangella*	*Haloactinopolyspora*
Fragmentation of aerial mycelium	–	–
Spore chain	–	Long
Spores heap	–	+ (Root-like)
Growth in		
0 % NaCl	+	–
20 % NaCl	–	+
Gelatin liquefaction	+	–
Cell-wall sugars	variable: rib, glu[a], or glu, rha, rib, man[b], or glu, rib, gal[c], or glu, rha[d]	glcN, man, glc, ara, gal, rha, one unknown sugar
Polar lipids	variable: DPG, PG, PGL, PL, PI, PIMs[a], or DPG, PG, PC, PI, PIM, PL[b], or DPG, PG, PI, PIM, PGL, PLs[c], or DPG, PG, PC, PI, PIM[d]	DPG, PGL, PL, PI, PIMs, GL
Major fatty acids (>10 %)	variable: ai-$C_{15:0}$ (35.9 %), ai-$C_{17:0}$ (15.8 %)[a], or ai-$C_{15:0}$ (20.4 %),i-$C_{16:0}$ (18.0 %)[b], or ai-$C_{15:0}$ (26.1 %), i$C_{16:0}$(20.6 %)[c], or ai-$C_{15:0}$ (30.8 %),i-$C_{16:0}$ (12.1 %), i-$C_{16:0}$ (11.5 %)[d]	i-$C_{16:0}$ (29.4 %), ai-$C_{15:0}$ (28.4 %), ai-$C_{17:0}$ (13.7 %)
DNA G+C (mol %)	70–71.9[a–d]	70.5

Abbreviations: + positive, – negative, *GlcN* glucosamine, *man* mannose, *gal* galactose, *glc* glucose, *ara* arabinose, *gal* galactose, *rha* rhamnose, *rib* ribose, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PC* phosphatidylcholine, *PIM* phosphatidylinositol-mannoside, *PI* phosphatidylinositol, *PL* unidentified polar lipid, *GL* unidentified glycolipid, *PGL* unidentified phosphoglycolipid
Strains: *Haloactinopolyspora alba* YIM 93246^T (Tang et al. 2011)
[a] *Jiangella gansuensis* (Song et al. 2005; Kroppenstedt unpublished data)
[b] *Jiangella alkaliphila* (Lee 2008)
[c] *Jiangella alba* (Qin et al. 2009b)
[d] *J. muralis* (Kämpfer et al. 2010)

(G–C), 952 : 1229 (G–C), 955 : 1225 (G–U), 986 : 1219 (U–G) and 987 : 1218 (C–G) (Tang et al. 2011).

Jiangellaceae Tang, Zhi, Wang, Shi, Lou, Xu and Li 2011, 198^{VP}

Ji.ang.el.la.ce'ae. N.L. fem. n. *Jiangella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Jiangellaceae* the family of the genus *Jiangella*.

The 16S rRNA nucleotide signatures are as that of the suborder.

The family accommodates the genera *Jiangella* (Song et al. 2005) and *Haloactinopolyspora* (Tang et al. 2011). The pairwise similarities of 16S rRNA gene sequences between members of the two genera are about 96.6–96.9 %.

Type genus: *Jiangella* Song, Li, Wang, Chen, Zhang and Xu 2005, 883^{VP}.

The discriminating properties for these two genera are indicated in *Table 24.1*.

Phylogenetic Structure of Suborder and the Family

Although the genus *Jiangella* proposed by Song et al. (2005), was assigned to the family *Nocardioidaceae* within the suborder *Propionibacterineae*, the phylogenetic analysis based on 16S rRNA gene sequences of members of the phylum Actinobacteria revealed that the genera *Jiangella* and *Haloactinopolyspora* form a deep branch, clearly distinguished from other described suborders of the phylum Actinobacteria (*Fig. 24.1*). Representatives of members of the phylum Actinobacteria shared less than 93.1 % of 16S rRNA gene sequence similarities with the genera *Jiangella* and *Haloactinopolyspora*. Although the genera *Jiangella* and *Haloactinopolyspora* clearly belong to the order *Actinomycetales*, they do not belong to any of the described suborders within the *Actinomycetales*. Accordingly, the genera *Jiangella* and *Haloactinopolyspora* are closely related phylogenetically but distinct from other suborders. Thus, a novel family *Jiangellaceae* fam. nov. and a novel suborder *Jiangellineae* subord. nov. were proposed to accommodate the genera *Jiangella* and *Haloactinopolyspora*.

Molecular Analyses

DNA-DNA hybridization (DDH) studies have been performed on four *Jiangella* type strains, most extensively among *J. muralis* 15-Je-017^T and the other three type strains of the genus. The phylogenetic neighbors strain 15-Je-017^T and *J. alba* YIM 61503^T, sharing 99.7 % 16S rRNA gene sequence similarity, exhibit 48.1 % DDH similarity, while the other type strains

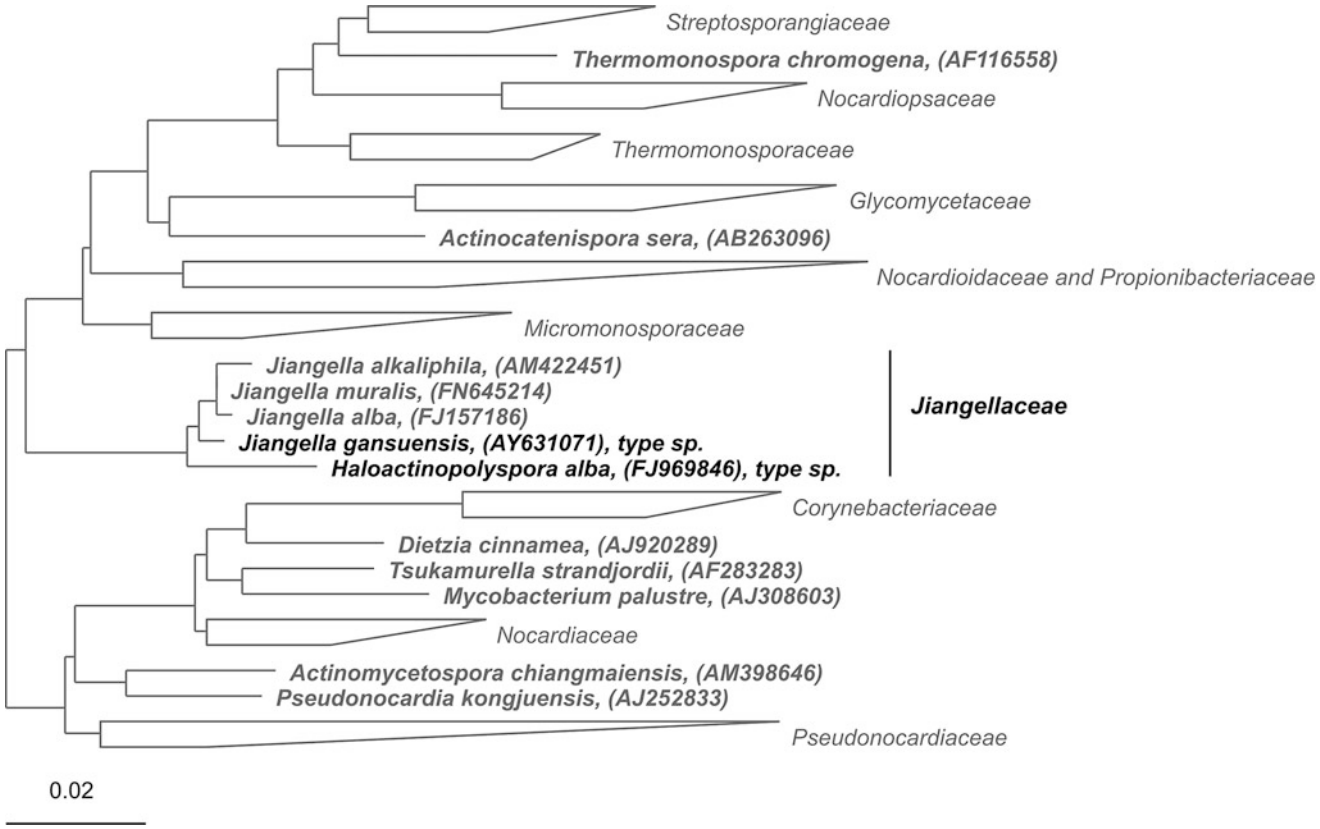

Fig. 24.1

Phylogenetic reconstruction of the family *Jiangellaceae* based on 16S rRNA and created using the maximum likelihood algorithm mRAxML(Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, a 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

show DDH values below 40 % (Kämpfer et al. 2011). Additional DDH values are available for the neighboring species *J. alba* YIM 61503^T and *J. alkaliphila* D8-87^T and *J. gansuensis* YIM 002^T, sharing 98.8 % and 98.6 % 16S rRNA gene sequence similarity (45.4 %, 41 % DDH similarity, respectively) (Qin et al. 2009b). No DDH studies were done for other *Jiangella* species nor for members of *Haloactinopolyspora* as the 16S rRNA gene sequence similarities among the type strains of the respective genera were below the threshold value of 97 %, indicative of separate genomic species (Stackebrandt and Goebel 1994).

No DNA fingerprinting method was performed on the members of the genera *Jiangella* and *Haloactinopolyspora*.

Genome Analyses

No full genome has been sequenced completely in the genera *Jiangella* and *Haloactinopolyspora*.

The four 16S rRNA gene sequences of the genus *Jiangella* were used as AY631071, AM422451, FJ157186, and FN645214 of *J. gansuensis* YIM 002^T, *J. alkaliphila* D8-87^T, *J. alba* YIM 61503^T and *J. muralis* 15-Je-017^T, respectively (Song et al. 2005; Lee et al. 2008; Qin et al. 2009b; Kämpfer et al. 2011). While, the single species of the genus *Haloactinopolyspora* was used as FJ969846 of *H. alba* YIM 93246^T.

Phenotypic Analyses

Jiangella Song, Li, Wang, Chen, Zhang and Xu 2005, 883^{VP}

Ji.ang.el'la. N.L. fem. dim. n. *Jiangella* named after the Chinese microbiologist Cheng-Lin Jiang in recognition of his work on actinomycete taxonomy.

Gram-positive filamentous actinomycete. Aerobic, with a strictly respiratory type of metabolism. Catalase positive. The substrate mycelium fragmented into short and elongated rods. Cell wall contains LL-A_2pm as the diamino acid in the peptidoglycan. MK-9(H_4) as the predominant menaquinone.

The mol% G + C of the DNA is 70–71.9 %.

The type species is *Jiangella gansuensis* Song et al. 2005. The type strain is YIM 002^T. Type strains of the other species are indicated in *Tables 24.1* and *24.2*.

Table 24.2

Physiological characteristics of type strains of the genus *Jiangella*

Characteristic	*J. gansuensis* YIM 002^T	*J. alkaliphila* DSM 45079^T	*J. alba* YIM 61503^T	*J. muralis* 15-Je-017^T
Growth at/with[a,b,c,d]				
10 % NaCl	–	–	+	ND
pH 10.0	+	+	–	ND
45 °C	–	–	+	–
Reduction of nitrate[a,b,c,d]	–	–	+	ND
Degradation of [d]				
Urea	+	–	–	ND
Hypoxanthine	–	+	+	ND
Xanthine	–	–	+	ND
pNP β-D-glucuronide	+	–	+	+
pNP α-D-glucopyranoside	+	+	–	+
pNP β-D-glucopyranoside	+	+	–	–
pNP β-D-xylopyranoside	+	–	+	+
bis-pNP phosphate	+	–	–	+
pNP phosphorylcholine	+	–	+	+
Assimilation of[d]				
N-Acetyl-D-galactosamine	(+)	–	(+)	(+)
D-Fructose	+	–	+	(+)
D-Galactose	(+)	–	–	(+)
D-Mannose	+	–	+	+
L-Rhamnose	–	–	–	(+)
Salicin	+	–	+	+
D-Xylose	(+)	–	(+)	(+)
myo-Inositol	(+)	–	–	–
Maltitol	(+)	–	+	+
D-Mannitol	+	(+)	+	–
Acetate	(+)	–	(+)	(+)
Fumarate	–	–	+	+
Glutarate	(+)	–	–	–
DL-3-Hydroxybutyrate	(+)	–	(+)	(+)
DL-Lactate	+	–	(+)	+
L-Malate	–	(+)	(+)	+
Oxoglutarate	(+)	–	–	(+)
L-Alanine	–	–	–	(+)
L-Aspartate	–	–	–	(+)
L-Histidine	(+)	–	–	(+)
L-Phenylalanine	(+)	–	–	(+)
L-Proline	(+)	–	–	(+)

+ positive, – negative, (+) weakly positive, *ND* not determined

[a] *Jiangella gansuensis* (Song et al. 2005)

[b] *Jiangella alkaliphila* (Lee 2008)

[c] *Jiangella alba* (Qin et al. 2009b)

[d] *Jiangella muralis* (Kämpfer et al. 2011)

All strains were positive for hydrolysis of aesculin, ρ-nitrophenyl (pNP) phenylphosphonate, 2-deoxythymidine-5′-pNP phosphate, L-alanine ρ-nitroanilide (pNA) and L-proline pNA and assimilation of *N*-acetyl-D-glucosamine, L-arabinose, parbutin, cellobiose, D-glucose, maltose, D-ribose, sucrose, trehalose, adonitol, and pyruvate. All strains were negative for hydrolysis of onitrophenyl b-D-galactopyranoside and L-glutamate-γ -3-carboxy pNA and assimilation of gluconate, melibiose, D-sorbitol, putrescine, propionate, *cis*- and *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, itaconate, mesaconate, suberate, b-alanine, L-leucine, L-ornithine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate, and phenylacetate (Kämpfer et al. 2011).

Haloactinopolyspora, Tang, Zhi, Wang, Shi, Lou, Xu and Li, 2011, 199^{VP}

Ha.lo.ac.ti.no.po.ly.spo'ra. Gr. n. *hals halos*, salt; Gr. n. *actis actinos*, a ray; Gr. adj. *poly*, many; Gr. n. *spora*, a seed and, in biology, a spore; N.L. fem. n. *Haloactinopolyspora*, salt-loving and the many spored ray.

Gram-positive, aerobic, moderately halophilic filamentous actinomycete. The substrate mycelium fragments into rod-like elements, and the aerial mycelium has long spore chains and forms root-like spores heap at maturity. The whole-cell hydrolysates contain LL-DAP, alanine, glycine, and glutamic acid as the cell-wall amino acids; glucosamine, glucose, galactose, mannose, and arabinose are the major cell-wall sugars. Other chemotaxonomic properties are indicated in ❯ *Table 24.1*. The genus contains a single species, *Haloactinopolyspora alba*, the type strain is YIM 93246^T.

The growth was good on GTY agar and potato agar, moderate on Czapek's agar, inorganic salts-starch agar (ISP 4), and oatmeal agar (ISP 3), weak on nutrient agar and glycerol/asparagine agar (ISP 5), but no growth was observed on yeast extract-malt extract agar (ISP 2). The color of the aerial mycelium was white and that of the substrate mycelium was white-yellow. No soluble pigments were produced. Grows at 15–45 °C (optimum 28–37 °C), at pH 4.0–9.0 (optimum pH 7.0–8.0) and with 7–23 % (w/v) NaCl (optimum 10–15 % NaCl), and no growth occurs in the absence of NaCl. Aesculin, Tweens 40, 60, and 80 are decomposed, but casein, starch, dextrin, chitin, Tween 20 and urea are not. Tests for milk peptonization and coagulation are positive, but gelatin liquefaction, nitrate reduction, starch hydrolysis, H_2S, and melanin production are negative. Utilizes the following substrates as sole carbon sources: cellobiose, dulcitol, D-fructose, inositol, lactose, maltose, D-mannose, rhamnose, sucrose, sorbitol, and trehalose. The following substrates are not utilized: erythritol, galactose, D-glucose, glycerol, glycine, mannitol, raffinose, D-ribose, sodium propionate, trisodium citrate, xylitol, and D-xylose. Adenine, L-arginine, L-histidine, hypoxanthine, L-lysine, L-methionine, L-proline, L-serine, and L-threonine are utilized as sole nitrogen sources, whereas growth on L-alanine, D-arabinose, L-asparagine, L-phenylalanine, L-tyrosine, and xanthine is not observed. In the API ZYM system, tests are positive for alkaline phosphatase, esterase(C4), esterase lipase(C8), lipase (C14), α- and β-galactosidase, α- and β-glucosidase, α-mannosidase, and *N*-acetyl-β-glucosaminidase, but negative for acid phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, β-glucuronidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase.

The type species is *Haloactinopolyspora alba*.

Isolation, Enrichment, and Maintenance Procedures

Isolation and Enrichment

Jiangella gansuensis YIM 002^T (Song et al. 2005) was isolated from a desert soil sample, by using the dilution plating method. The medium used for selective isolation was glycerol-asparagine agar (ISP medium 5), which was incubated at 28 °C for about 2 weeks.

Jiangella alba strain YIM 61503^T (Qin et al. 2009b) was isolated from surface-sterilized stems of *Maytenus austroyunnanensis*. Stem samples were air-dried at room temperature after being thoroughly washed under tap water and surface sterilized according to the five-step sterilization procedure. Samples were then pulverized in a ceramic mortar and processed with a calcium carbonate enrichment method. The samples were serially diluted in sterile distilled water and spread-plated on glycerol-asparagine agar (ISP medium 5). Strain YIM 61503^T was isolated after incubation at 28 °C for 21 days.

Jiangella alkaliphila D8-87^T (Lee 2008) was isolated from a soil of a natural cave, by using dilution plating method on starch-casein agar (SCA), which was incubated at 30 °C for 14 days.

Jiangella muralis 15-Je-017^T (Kämpfer et al. 2011) was isolated from the cellar wall of a house colonized with moulds by using agar plate containing ISP-3 medium and incubated at 28 °C for 2 weeks.

Haloactinopolyspora alba YIM 93246^T (Tang et al. 2011) was isolated from a hypersaline soil sample, after 3 week incubation at 37 °C on cellulose-casein multi-salt (CCMS) medium described by Tang et al. (2008).

Maintenance

Members of this family do not require special procedures for maintenance and medium and long-term storage. These should be stored at room or preferably refrigerator temperature. Generally, strains are maintained on isolation medium as agar slants 4 °C for a few days. GTY (Tang et al. 2010) agar slants for *Haloactinopolyspora alba* contained 15 % NaCl (w/v).

Medium-term maintenance is in 20 % (v/v) glycerol suspensions at −80 °C.

Long-term preservation is by lyophilization or in liquid nitrogen.

Ecology

As the habitats of the members of this family are very diverse: *Jiangella gansuensis* YIM 002^T was isolated from a desert soil sample, *Jiangella alba* strain YIM 61503^T was isolated from stem samples of *Maytenus austroyunnanensis* collected from a tropical rainforest, *Jiangella alkaliphila* D8-87^T was isolated from a soil of a natural cave, *Jiangella muralis* 15-Je-017^T was isolated from the cellar wall of a house colonized with moulds, while *Haloactinopolyspora alba* YIM 93246^T was isolated from a hypersaline soil sample collected from a salt lake.

Actually, as judged from the few reports on other strains affiliated to the two genera, the isolation source is much broader. Internet search on *Jiangella* reveals the presence of names of species which have no standing in taxonomy as they have never validly published: "*Jiangella ginsengisoli*" (Im and Lee 2006, unpublished) isolated from a ginseng field in Korea was related to *Jiangella gansuensis* (98 % similarity, accession no AB271058). A *Jiangella* strain related to *Jiangella gansuensis* by BLAST analysis (97 % similarity, accession no EU741189) was isolated from water of Costa Rica in Pacific and Caribbean (Solano et al. 2008). A *Jiangella* strain related to *Jiangella gansuensis* by BLAST analysis (98 % similarity, accession no GU574118) was isolated from mould-colonized water-damaged building material (Schafer et al. 2010, unpublished). A *Jiangella* strain related to *Jiangella gansuensis* by BLAST analysis (98 % similarity, accession no EU910884) was isolated from a hypersaline soil sample in Hami Lake (Cao et al. 2008, unpublished). A *Jiangella* strain related to *Jiangella alkaliphila* by BLAST analysis (99 % similarity, accession no JX035894) was isolated from animal feces (Tang et al. 2012, unpublished). The NCBI taxonomy browser lists several additional unnamed strains or clones affiliated to *Jiangella*, e.g., from an insect herbivore, USA (accession number HM559049), mould-colonized water-damaged building material, Germany (GU574028), simulated low-level-radioactive-waste site, USA (GQ263071), saline-alkali soil, China (JF727732), marine sediments in the Bay of Cadiz, Spain(GQ249574), and aliphatic hydrocarbon-contaminated soil, France (FM209114).

Application

The only evidence that any strain of the two genera is involved in application originates from a study by Qin et al. (2009a) involving a strain of *Jiangella* (YIM 61503 for antimicrobial activity). This strain inhibited growth of *Bacillus subtilis*, and did no inhibit growth of *Candida albicans, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Screening showed lack of presence of PKS-I, PKS-II, and NRPS functional genes.

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25 The Families *Jonesiaceae*, *Ruaniaceae*, and *Bogoriellaceae*

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Abstract
All three families are members of the order *Micrococcales* (Busse 2012). As such they are Gram-positive, not acid-fast, do not form endospores, and do not contain mycolic acids in their cell wall. *Jonesiacea* is a monogeneric family and contains two species, *J. denitrificans* and *J. quinhaiensis*. *Ruaniaceae* encompasses two monospecific genera, *Ruania* and *Haloactinobacterium*, while *Bogoriella* embraces the monospecific genus *Bogoriella* and *Georgenia* for which six species have been described. Phylogenetic neighbors are the families *Bogoriellaceae, Ruaniaceae, Beutenbergiaceae*, and *Actinomycetaceae*. The rationale for treating these three neighboring genera in one chapter but omitting *Actinomycetaceae* is the fact that the latter family contains seven genera with more than 50 species, deserving a chapter on its own. Except for *Jonesia denitrificans*, a former member of the genus *Listeria*, hardly any information is available for other species of these families besides their original description.

Taxonomy: Historical and Current

Short Description of the Families and Their Genera

The family descriptions are mainly based upon phylogenetic position and the presence of 16S rRNA sequence signatures. Differentiating, predominantly chemotaxonomic properties, are indicated in ❯ *Table 25.1*. The phylogenetic closeness of all members of the order *Micrococcales* together with the species richness of this order explains why branching points of lineages at almost any rank are rarely supported by high bootstrap values, hence without statistic significance. The consequence is that the addition of novel taxa will lead to rearrangements of lineages, most likely be followed by future reclassification of taxa, predominantly at the level of families.

Jonesiaceae Stackebrandt, Rainey and Ward-Rainey 1997, 485^{VP} emend. Zhi, Li and Stackebrandt 2009, 598.

Jone.si.a'ce.ae. N.L. fem. n. *Jonesia* type genus of the family; -*aceae* ending to denote a family; N.L. fem. pl. n. *Jonesiaceae* the *Jonesia* family.

The 16S rRNA gene signatures consists of:120 (A), 131:231 (A-G), 196 (C), 342–347 (C-G), 444–490 (A-U), 580–761 (C-G), 602–636 (C-G), 670–736 (A-U), 822–878 (U-C), 823–877 (A-C), 826–874 (U-G), 827 (G), 843 (C), 950–1231 (U-A), 1047–1210 (G-C), 1109 (C), 1145 (G), 1309–1328 (G-C), 1361 (G), and 1383 (C) (Zhi et al. 2009).

Type genus: *Jonesia* Rocourt, Wehmeyer and Stackebrandt 1987, 268^{VP}.

Ruaniaceae Tang et al. 2010a, 2118.^{VP}

Ru.a.ni.a'ce.ae. N.L. fem. n. *Ruania* type genus of the family; -*aceae* ending to denote a family; N.L. fem. pl. n. *Ruaniaceae* the *Ruania* family.

The 16S rRNA gene signatures consists of: 120 (A), 131:231 (A-G), 196 (C), 342–347 (C-G), 444–490 (C-U), 580–761 (C-G), 602–636 (S-K), 670–736 (A-U), 822–878 (G-C), 823–877 (G-C), 826–874 (C-G), 827 (U), 843 (U), 950–1231 (U-A), 1047–1210 (G-C), 1109 (C), 1145 (G), 1309–1328 (G-C), 1361 (G), and 1383 (U) (Tang et al. 2010a).

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Table 25.1

Diagnostic properties in which the families *Jonesiaceae, Ruaniaceae, and Bogoriellaceae* differ from each other and two neighboring families of the suborder *Micrococcineae* (Fig. 25.1)

Character	*Jonesiaceae*[a]	*Ruaniaceae*[b, c]	*Bogoriellaceae*	*Actinomycetaceae*[f]	*Beutenbergiaceae*[g–j]
Morphology	Branched rods, variable	Coccoid to short rods	Irregular coccoid rods[d] or rod-coccus cycle[e]	Highly variable: coccoid, or coccobacillary; or branching rods; or filamentous	Rod-coccus cycle[g] coccoid to rod shaped[h–j]
Aerial mycelium	–	–	–	Some species	–
Motility or motile elements	+or –	–	–	Many species	–
Peptidoglycan type	L-Lys-L-Ser-D-Glu	L-Lys-Gly–L-Glu–L-Glu[b] or L-Lys-L-Glu[c]	Variable (see Table 25.3)	Highly variable: L-Orn-L-Lys-D-Glu, or L-Lys-L-Lys-D-Glu, or L-Lys-L-Ala-L-Lys-D-Glu or L-Lys-D-Asp	L-Lys-L-Glu[g,i] or L-Orn-L-Glu[h, j]
Polar lipids	PI, DPG, PL, PGL GL	DPG, PG,GL[b] or DPG, PG, PI, PGL, PL[c]	Variable (see Table 25.3)	Variable: PIM DPG, PC, PI, PIM, PG	Variable: PI, DPG, PLs[g], or PG, DPG, PLs[h], or PG, PE[i], or PG, DPG, PI, PL[j]
Major fatty acids	ai-$C_{15:0}$, i-$C_{16:0}$, $C_{16:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{16:0}$ [b] or ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$ [c]	Variable (see Table 25.3)	Highly variable: saturated and unsaturated iso and anteiso	ai-$C_{15:0}$, i-$C_{15:0}$ [g], or ai-$C_{15:0}$, $C_{14:0}$, $C_{16:0}$ [h], or ai-$C_{15:0}$, $C_{16:0}$, i-$C_{16:0}$, ai-$C_{17:0}$ [i], or ai-$C_{15:0}$, $C_{16:0}$ [j]
Major menaquinones	MK-9	MK-8(H_4)	MK-8(H_4)	Highly variable: MK-9, MK-10, or MK-10(H4), or MK-9(H4)	MK-8(H_4)
G+C content of DNA (mol%)	56–58	68[c]–70[b]	66–73	49–68	71–75

PG phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PIM* phosphatidylinositolmannoside, *PI* phosphatidylinositol, *PL* unidentified polar lipid, *GL* unidentified glycolipid, *PGL* unidentified phosphoglycolipid, *L* unidentified lipid. The ending "s" denotes minor amounts of other such components

[a]Schumann et al. 2004; [b]*Ruania* (Gu et al. 2007); [c]*Haloactinobacterium* (Tang et al. 2010a); [d]*Bogoriella* (Groth et al. 1997); [e]*Georgenia* (Altenburger et al. 2002); [f]Schaal et al. (2006); [g]*Beutenbergia* (Groth et al. 1999); [h]*Salana* (von Wintzingerode et al. 2001); [i]*Serinibacter* (Hamada et al. 2009a); [j]*Miniimonas* (Ue et al. 2011).

The family contains the type genus *Ruania* (Gu et al. 2007) and the genus *Haloactinobacterium* (Tang et al. 2010a). The two type species share 96.2 % 16S rRNA gene sequence similarity. Discriminating properties for these two genera are indicated in Tables 25.1, 25.3, and 25.4.

Bogoriellaceae Stackebrandt and Schumann 2000, 1283[VP] emend. Zhi, Li and Stackebrandt 2009, 597 [VP] emend. Hamada, Iino, Tamura, Iwami, Harayama and Suzuki 2009, 2813.

Bo.go.ri'.el'la. M. L. dim. fem. n. *Bogoriella*, named after Lake Bogoria in Kenya, the place from which isolate HKI0088[T] originated.

The 16S rRNA gene signatures consists of: 144–178 (U-G), 258–268 (G-C), 280 (C), 293–304 (G-U), 379–384 (C-G), 479 (U), 586–755 (C-G), 589–650 (Y-R), 602–636 (C-G), 668–738 (A-U), 1003–1038 (G-C), 1027–1034 (C-G), 1409–1491 (C-G), 1414–1486 (C-G) (Hamada et al. 2009a).

The family accommodates the genera *Bogoriella* (Groth et al. 1997) and *Georgenia* (Altenburger et al. 2002). The phylogenetic distance between the two genera is about 94.2 % 16S rRNA gene sequence similarity.

Phylogenetic Structure of the Family and Its Genera

The phylogenetic tree (Fig. 25.1) indicates that none of the three families are sister clades. 16S rRNA gene sequence dendrograms published in the original genus and species descriptions do not reveal a coherent picture of the as the number and selection of reference organisms vary. The phylogenetic closeness of the two genera of *Bogoriellaceae* was confirmed in the last five species descriptions of *Georgenia* and in the description of *Haloactinobacterium album* (Tang et al. 2010a). In the publications of Tang et al. (2010a, b), the family *Beutenbergiaceae* branched closer to *Ruaniae* than to *Bogoriellaceae*, while *Jonesia denitrificans* branched deeply in the tree of the order *Micrococcales*; the latter branching is also shown in the studies of Groth et al. (1997), Li et al. (2007), and Kämpfer et al. (2010). In the description of *Ruania* (Gu et al. 2007) *R. albidiflava* branches next to members of *Georgenia* and *Bogoriella*, while *Beutenbergiellaceae* spp. branched more deeply. Neither *Ruania albidiflava* nor members of *Jonesia* were included in the

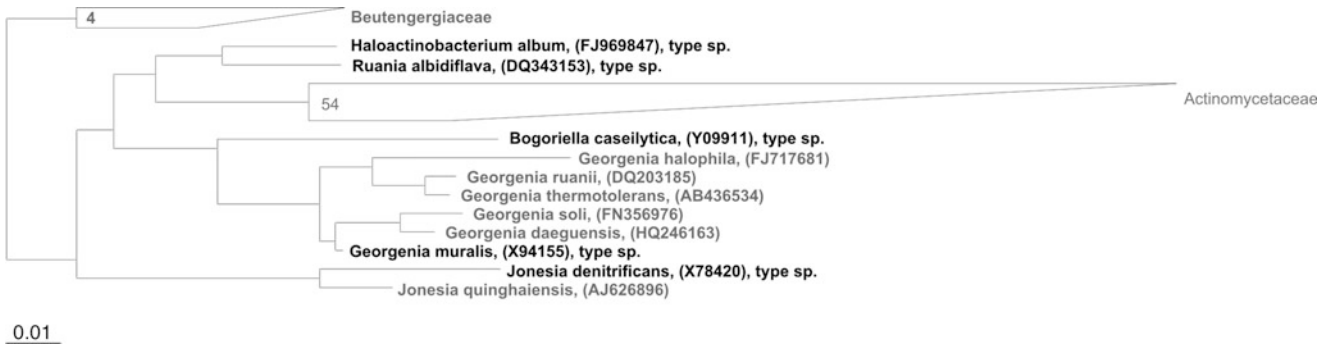

Fig. 25.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the families *Jonesiaceae, Ruaniaceae, and Bogoriellaceae* 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. Number in triangle denotes number of taxa included. The *bar* indicates 5 % sequence divergence

description of *Georgenia thermotolerans* (Hamada et al. 2009b) and of *G. daeguensis* Woo et al (2012) while *Ruania albidiflava* was not a closely related neighboring species of *Bogoriellaceae* in the study of Kämpfer et al. (2010) and Tang et al. (2010b). None of the publications included any member of *Actinomycetaceae*.

The family *Jonesiaceae* was created by Stackebrandt et al. (1997) on the basis of phylogenetic position and the presence of a unique set of 16S rRNA gene sequence signature nucleotides. Based on a modification of this set, due to newly described actinobacterial species and the availability of their 16SrRNA gene sequences, the description of *Jonesiaceae* was emended (Zhi et al. 2009). The family *Ruaniaceae* was described in 2010 (Tang et al. 2010a) after the finding that the genera *Ruania* (Gu et al. 2007) and *Haloactinobacterium* (Tang et al. 2010a) form a separate line of descent among the order *Micrococcales*. The family *Bogoriellaceae* was established for the genus *Bogoriella* by Stackebrandt and Schumann (2000), based upon a unique set of signature nucleotides of the genus *Bogoriella*. The genus *Georgenia* (Altenburger et al. 2002) was added to the emended family by Hamada et al. (2009a) on the basis of phylogenetic position and an emended set of signature nucleotides. Families containing more than a single genus are not coherent with respect to peptidoglycan type, polar lipids, or major fatty acids (*Table 25.1*). *Haloactinobacterium* (*Ruaniaceae*) and most *Georgenia* species (*Bogoriellaceae*) share the same L-Lys-L-Glu peptidoglycan type. *Jonesiaceae* differs from *Ruaniaceae* and *Bogoriellaceae* in menaquinone type and lower mol% G+C of DNA.

Jonesia denitrificans was originally classified as *Listeria denitrificans* (Prevot 1961). Results from morphological, biochemical, serological, chemical, and nucleic acid studies, however, indicated that *L. denitrificans* is not a member of the genus *Listeria* (Chatelain and Second 1976; Welshimer and Meredith 1971; Jones 1975; Stuart and Welshimer 1973, 1974; Wilkinson and Jones 1975, 1977; Collins et al. 1983; Fiedler and Seger 1983; Fiedler et al. 1984) but more closely related to coryneform bacteria. Relatedness to *Oerskovia*, *Renibacterium*, and *Arthrobacter* was excluded on the basis of individual chemotaxonomic properties (see Seeliger and Jones 1986). 16S rRNA oligonucleotide analysis then separated *Listeria denitrificans* CIP 55134^T from the other members of *Listeria*, leading to the description *Jonesia denitrificans* (Rocourt et al. 1987). Similarities in the DNA mol% G+C content, peptidoglycan structure, lipid pattern, isoprenoid quinone, and the 16S rRNA similarities supported the taxonomic placement of *J. denitrificans* within the phylum Actinobacteria. Changes in the phylogenetic affiliation of the genus *Jonesia, which included the transfer of Jonesia into Cellulomonadaceae* (Stackebrandt and Prauser 1991) and its exclusion from this family (Rainey et al. 1995) have been summarized by Stackebrandt (2012). In some published phylogenetic dendrograms *Jonesia* species appear to be related to the family *Dermabacteraceae* (Stackebrandt et al. 1997), and more distantly, to *Brevibacteriaceae*. *Jonesia* species differ from members of *Dermabacter* and *Brachybacterium* in peptidoglycan composition, but they share fully unsaturated menaquinones and similar composition in fatty acids and polar lipids; these features, however, are also frequently found in other members of *Micrococcales*. A remote relationship between *Jonesia* (*Listeria*) *denitrificans* and *Brachybacterium faecium* had been observed earlier in a numerical phenetic study (Jones 1975).

Molecular Analyses

The phylogenetic dendrogram of *Georgenia* type strains indicates the presence of two sister clades. One clade is composed of *G. soli* and *G. daeguensis* which share 98.8 % 16S rRNA gene sequence similarity with *G. muralis* branching slightly deeper (~97.5 % similarity). The second clade groups *G. ruanii* and

G. thermotolerans (99 % similarity) with G. *halophila* branching more deep (~96.5 % similarity). The two clades share ~96.2 % similarity.

DNA-DNA hybridization (DDH) studies have been performed on several *Georgenia* type strains, most extensively among *G. daeguensis* 2C6-43^T and the other five type strains of the genus. The phylogenetic neighbors strain 2C6-43^T and *G. soli* DSM 21838^T, sharing 98.8 % 16S rRNA gene sequence similarity, exhibit 40.5 % DDH similarity, while the other type strains show DDH values below 30 % (Woo et al. 2012). Additional DDH values are available for the neighboring species *G. thermotolerans* TT02-04^T and *G. ruanii* NBRC 103883^T, sharing 99.0 % 16S rRNA gene sequence similarity (45–47 % DDH similarity) and for strain TT02-04^T and *G. muralis* (7–10 % DDH similarity) (Hamada et al. 2009b), as well as for G. *ruanii* YIM 004 T and *G. muralis* DSM 14418 T (18 %) (Li et al. 2007). No DDH studies were done for other *Georgenia* species nor for members of *Jonesiaceae* and *Ruaniaceae* as the 16S rRNA gene sequence similarities among the type strains of the respective genera were below the threshold value of 97 %, indicative of separate genomospecies (Stackebrandt and Goebel 1994).

The only study including DNA fingerprinting method was that of Altenburger et al. (2002) who performed ERIC-, REP-, and BOX PBR on the type strain and two additional strains of *Georgenia muralis* to confirm their membership to the same species. Neither riboprinting, ribotyping, nor MALDI-TOF analyses is available for any member of these three families.

Genome Analyses

Jonesia denitrificans DSM 20603^T is the only strain of the three families for which the full genome sequence has been generated (INSDC ID CP001706) (Pukall et al. 2009). The single replicon genome, analyzed in the course of the *Genomic Encyclopedia of Bacteria and Archaea* project, is 2,749,646 bp long with a 58.42 % GC content. This value is only slightly higher than those determined by T_m and nuclease method performed on purified DNA (56–58 mol%, ❯ *Table 25.1*). Extrachromosomal elements were absent. Besides 47 pseudogenes, 2,629 genes have been predicted of which 2,558 were protein coding genes, and 71 were RNA genes. The majority of the genes (68.3 %) were assigned a putative function, 69.45 % of the genes were assigned to clusters of orthologous groups (COGs), while the remaining ones are annotated as hypothetical proteins. The distribution of genes into COGs functional categories indicate that the highest number of genes is involved in carbohydrate transport and metabolism (210; 8.2 %), followed by genes coding for amino acid transport and metabolism (162; 6.3 %) and transcription (160; 6.3 %); 23 genes (0.9 %) were found to code for secondary metabolites biosynthesis, transport, and catabolism. A detailed listing of COG categories is given by Pukall et al. (2009).

The sequences of the five 16S rRNA gene copies in the genome of strain DSM 20603^T are identical but differ by eight nucleotides from the published 16S rRNA gene sequence of the same strain deposited as ×78420. This sequence has also been used as a reference by Tang et al. (2010a) and in the Living tree Project which has been the basis for the tree shown in ❯ *Fig. 25.1*. Other publications used the sequence X83811 of *J. denitrificans* (Schumann et al. 2004; Tang et al. 2010b; Li et al. 2007). Whether about 0.5–0.8 % sequence difference (depending upon the 16S rRNA gene fragment analyzed) observed between these two sequences actually influences some of the discrepancies seen in the branching of *J. denitrificans*, as indicated above, needs to be evaluated.

Phages

No phages have been described to lyze strains of Jonesia, 16 *Listeria* phages isolated from environmental sources and from lysogenic *Listeria* strains were active on strains of *L. monocytogenes, L. seeligeri, L. innocua, L. ivanovii*, and *L. welshimeri* but not on those of *L. murrayi, L. grayi* (united under *M. grayi* [Rocourt et al. 1992]), or *Jonesia* (formerly *Listeria*) *denitrificans* (Loessner and Busse 1990).

Phenotypic Analyses

Jonesia Rocourt et al. 1987, 268^{VP}.

Jones'i.a L.fem. n. *Jonesia* of Jones, honoring Dorothy Jones, a British microbiologist.

Nonsporing rods showing branched Y- and club-like forms. Irregular, branching rods may occur in one species. Gram-positive, but many cells, especially in older cultures, are readily decolorized. Filamentous and coccoid cells may also occur in older cultures. Motile or nonmotile. Colonies on nutrient agar are 0.5–1.5 mm in diameter (24–48 h), convex, smooth, grayish, and translucid to opaque, becoming yellowish in 10–20 days. Colonies of may show rhizoid appearance (4 mm diameter). Catalase-positive, oxidase-negative. Chemotaxonomic properties are indicated in ❯ *Table 25.1*. Cell wall contains teichoic acid of the poly(ribitol phosphate) type (only *J. denitrificans* investigated). No lipoteichoic acid. Acid production from a wide range of mono- and polysaccharides.

The mol% G+C of DNA is 56–58.

The type species is *Jonesia denitrificans* Prevot 1961^{AL}. The type strain is CIP 55.134^T.

The two type strains *J. denitrificans* DSM 20603^T and *J. quinhaiensis* DSM 1570^T share 96.6 % 16S rRNA gene sequence similarity. According to API 50 CHE substrate panel, the type strains of both species utilize: glycerol, L-arabinose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, arbutine, aesculin, salicine, cellobiose, maltose, lactose, saccharose, trehalose, amidon, glycogen, ß-gentiobiose, D-turanose, and 5-ceto-gluconate. According to the Biolog GP2 substrate panel, the following substrates were utilized in addition: dextrin, glycerol, maltotriose, D-psicose, D-ribose, adenosine, 2'-deoxyadenosin. Additional metabolic properties are indicated in ❯ *Table 25.2* (modified from Schumann et al. 2004) and ❯ *Table 25.3*.

Table 25.2
Differentiating cultural and physiological differences between *Jonesia denitrificans* DSM 20603^T and *J. quinghaiensis* DSM 15701^T (Data from Schumann et al. 2004)

Differentiating properties	*J. denitrificans* DSM 20603^{T1}	*J. quinghaiensis* DSM 15701^{T1}
Morphology	Coccoid to irregular rods, (0.3–0.5 × 2–3 μm) Filamentous forms may develop.	Rod shaped (0.5 × 1.5–2 μm)
Staining	Gram-positive, older cells may be Gram-negative	Gram-positive
Motility	+, peritrichous flagella	–
Optimal growth temperature	~30 °C.	20–30 °C
Biolog GP2 MicroPlate		
D-cellobiose	+	–
D-galactose	+	–
3-methyl-glucose	+	–
D-sorbitol	+	–
Turanose	+	–
Acetic acid	+	–
Inosine	+	–
Thymidine	+	–
Uridine	+	–
Propionic acid	w	–
Pyruvic acid	w	–
N-acetyl L-glutamic acid	w	–
Palatinose	w	–
D-tagatose	w	–
Mannan	w	–
ß-methyl-D-galactoside	w	–
2,3 butanediol	w	–
Amygdalin	–	+
D-gluconic acid	–	+
Methyl pyruvate	–	+

+, positive; –, negative; w, weakly positive

In addition, *J. denitrificans* has the following properties: Temperature limits of growth 10–40 °C. Cells do not survive heating at 60 °C for 30 min. Grow in 5 % but not 10 % (w/v) NaCl. Catalase-positive. Oxidase-negative. Facultatively anaerobic. Acid but no gas produced from melibiose, starch, and dextrin. Weak acid production from melezitose. No acid produced from L-fucose, D-arabinose, sorbose, L-xylose, methyl β-xyloside, rhamnose, erythritol, inositol, dulcitol, sorbitol, a-methyl-d-glucoside, a-methyl-d-mannoside, *N*-acetylglucosamine, inulin, d-raffinose, xylitol, d-fucose, d-arabitol, l-arabitol, gluconate, 2-ketogluconate, and melibiose, (API50CH). Extracellular enzymes hydrolyze DNA, RNA, cellulose, and starch but not gelatin, chitin, casein, lecithin (egg yolk), xanthine, tyrosine, Tween 20, or Tween 80; slight hydrolysis of Tweens 40 and 60 takes place after 7 d. Phosphatase, sulfatase, and urease are not produced. Esculin is hydrolyzed. Sodium hippurate is not hydrolyzed or only weakly hydrolyzed (H_2SO_4 method) after 10 d. Acid produced in litmus milk. Nitrates are reduced to nitrites. H2S-negative. Indole-negative. Galactosamine is a diagnostic whole-cell sugar.

Jonesia quinghaiensis is in addition characterized by an optimal pH of 7–9; optimal salt concentration is 2.0–7.5 % (w/v) NaCl.

Source: mud of a soda lake in Qinghai, a Western province of China. The type strain is QH3A7^T.

Ruania Gu et al. 2007, 811.^{VP}

Ru.an'i.a. N. L. n. *Ruania* named after Ji-Sheng Ruan, a Chinese microbiologist.

Gram-positive, aerobic, mesophilic, moderately halotolerant, non-acid-fast, nonmotile and non-spore-forming cocci. No rod-coccus cycle observed. Chemotaxonomic properties are indicated in *Table 25.1*. The genus contains a single species, *Ruania albidiflava*, the type strain is 3-6^T.

Cells are 0.5–0.8 μm in diameter. Colonies are convex and moist, with a pale yellow color. Growth optimum at 28° C. pH range for growth is of 5.5–12.5 with an optimum at pH 6.5–10.5. Can tolerate up to 10 % (w/v) NaCl. Catalase-positive, oxidase-negative. Indole and Voges-Proskauer reactions are negative. H_2S is not produced. Potato starch is decomposed, but adenine, casein, aesculin, gelatin, hippurate, hypoxanthine, tyrosine, urea, and xanthine are not. Acids are produced from salicin, D-ribose, methyl β-D-xylopyranoside, D-fructose, aesculin, D-maltose, D-xylose, and L-fucose, but not from L-xylose, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, D-lactose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-fucose, DL-arabitol, 2-ketogluconate. Additional reactions are listed in *Table 25.3*. Utilizes the following substrates as sole carbon sources: acetate, D-lactose, D-melezitose, L-alanine, L-cysteine, L-leucine, L-methionine, L-proline, methyl a-D-glucoside, oxalate, D-fructose, D-glucose, D-glutamic acid, D-maltose, D-ribose, D-sorbitol, D-trehalose, glycerol, L-arginine, L-fucose, and sucrose. The following substrates are not utilized: citrate, D-cellobiose, D-galactose, D-inulin, D-lactulose, D-mannitol, D-mannose, D-raffinose, D-rhamnose, D-sorbose, D-xylose, dulcitol, erythritol, glycogen, inositol, L-arabinose, L-leucinamide, L-ornithine, L-phenylalanine, L-tyrosine, L-valine, malate, malonate, nicotinamide, salicin, and succinate.

In the API ZYM assay, tests are positive for cystine arylamidase, a-fucosidase, lipase (C8), acid phosphatase, β-galactosidase, b-glucosidase, N-acetyl-β-glucosaminidase, and a-mannosidase, but negative for esterase (C4), lipase (C14), trypsin, chymotrypsin, and b-glucuronidase (see also *Table 25.4*).

Whole-cell sugars are galactose and glucose.

Table 25.3

Diagnostic differences in acid production (API 50CH) determined for type strains of *Jonesia, Ruania, and Haloactinobacterium*

Character	*Jonesia denitrificans* DSM 20603[Ta]	*Jonesia. quinghaiensis* DSM 15701[Ta]	*Ruania albidiflava* 3-6[Tb]	*Haloactinobacterium album* YIM 39906[Tc]
Amygdalin	–	+	–	–
Arbutin	+	+	+	–
D-Adonitol	–	–	–	+
Cellobiose	+	+	–	+
D-galactose	+	–	–	+
D-Glucose	+	+	–	+
Gluconate	–	+	–	–
D-Lyxose	–	+	+	–
D-Turanose	+	+	–	+
D-Tagatose	–	–	–	+
Erythritol	–	–	–	+
Glycerol	+	+	–	+
5- ketogluconate	–	+	–	–
L-rhamnose	–	–	+	+
D-arabinose	–	–	+	+
L-arabinose	+	+	+	+
D-ribose	–	–	+	+
D-mannose	+	+	–	+
D-melibiose	+	+	–	–
D-mannitol	–	–	–	–
N-acetyl-glucosamine	–	–	–	+

+, positive; –, negative; w, weakly positive

[a]Schumann et al. 2004; [b]Gu et al. 2007; [c]Tang et al. 2010a

The type species is *Ruania albidiflava.*

Haloactinobacterium Tang, Zhi, Wang, Wu, Lee, Kim, Lou, Xu and Li 2010, 2118.[VP]

Ha.lo.ac.ti.no.bac.te'ri.um. Gr. n. hals, halos salt; Gr. n. actis, actinos a ray; L. neut n.bacterium a rod; N.L. neut. n. *Haloactinobacterium* a halophilic actinobacterium.

Cells are aerobic, Gram-positive, nonmotile, and moderately halophilic short rods (0.3–0.4 × 0.6–0.7 μm). No rod-coccus life cycle. The chemotaxonomic properties are indicated in ❯ *Table 25.1*.

The genus contains a single species *Haloactinobacterium album*, the type strain is YIM39906[T].

In addition to properties indicated in ❯ *Tables 25.1*, ❯ *25.3*, and ❯ *25.4*, the species is characterized as follows: Colonies are creamy white, circular, smooth, opaque and non-pigmented on GTY agar supplemented with 10 % NaCl. Grows at 10–40 °C (optimum 37 °C), at pH 6.0–10.0 (optimum pH 7.0–8.0), and with 2–16 % (w/v) NaCl (optimum 7–10 % NaCl). Catalase-positive and oxidase-negative. Voges-Proskauer tests are negative. Cellulose and aesculin are hydrolyzed, but gelatin, casein, dextrin, starch, DNA, chitin, urea, and Tweens 20, 40, 60, and 80 are not. H_2S production, melanin, and indole are negative. With API ZYM, positive for esterase lipase (C8), cystine arylamidase, β-galactosidase, β-glucosidase, α-mannosidase, and N-acetyl-β-glucosaminidase, but negative for lipase (C14), trypsin α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, and β-glucuronidase. With GP2 MicroPlates, oxidizes L-arabinose, D-fructose, L-fucose, D-galactose, a-D-glucose, maltose, D-psicose, D-ribose, sucrose, D-xylose, methyl β-D-galactoside, pyruvic acid, pyruvic acid methylester, DL-α-glycerol phosphate, D,L-lactic acid, acetic acid, glycerol, and amygdalin.

Produces acid (see also ❯ *Table 25.4*) from maltose, D-mannose, L-rhamnose, D-ribose, D-xylose, methyl β-D-xylopyranoside, aesculin, and salicin, but not from D-fucose, gentiobiose, lactose, melezitose, melibiose, raffinose, L-sorbose, starch, sucrose, trehalose, L-xylose, DL-arabitol, dulcitol, inositol, D-mannitol, D-sorbitol, xylitol, glycogen, inulin, methyl α-D-mannopyranoside, methyl a-glucopyranoside, potassium 5-ketogluconate, or potassium 2-ketogluconate.

Bogoriella Groth et al. 1997, 793.[VP]

Bo.go.ri.el'la.M. L. dim. fem. n. *Bogoriella*, named after Lake Bogoria in Kenya, the place from which isolate HKI 0088 T originated.

Cells are nonmotile, irregular, and rod shaped or coccoid and occur singly, in pairs, or in small irregular clusters. Aerobic

Table 25.4
Physiological characteristics of *Ruania albidiflava* 3-6^T and *Haloactinobacterium album* YIM 93306^T. Both type strains produce acids (API 50CHE) from D-xylose, D-fructose galactose, aesculin, maltose, lactose, sucrose, trehalose, starch, glycogen β-gentiobiose, salicin and utilize (Biolog GP2 substrate panel) dextrin, glycerol, maltotriose, D-psicose, D-ribose, and adenosine-2′-deoxyadenosine, other compounds used are listed in Table 25.3. Data from Tang et al. (2010a)

Character	*Ruania albidiflava* 3–6^T	*Haloactinobacterium album* YIM 93306^T
Facultative anaerobic	–	+
Growth with		
0 % NaCl	+	–
15 % NaCl	–	+
Range for growth		
pH	5.5–12.5	6–10
Temperature (°C)	20–37	10–40
Methyl red test	+	–
Nitrate reduction	+	–
Enzymes (API ZYM)		
Esterase (C4)	–	+
Alkaline phosphatase	+	–
Leucine arylamidase	+	–
Valine arylamidase	+	–
Naphthol-AS-BI-phosphohydrolase	+	–
α-Glucosidase	+	–
α-Galactosidase	+	–
α-Fucosidase	+	–
Acid production (API 50CH) from		
Arbutin	+	–
D-Adonitol	–	+
Cellobiose	–	+
D-Galactose	–	+
D-Glucose	–	+
D-Lyxose	–	–
D-Mannose	–	+
Turanose	–	+
D-Tagatose	–	+
Erythritol	–	+
Glycerol	–	+
N-Acetyl-glucosamine	–	+

or microaerophilic. Very poor growth can occur under anaerobic conditions. Catalase-positive and oxidase-negative. Chemotaxonomic properties are indicated in Tables 25.1 and 25.5.

The genus contains a single species, *Bogoriella caseilytica*. The type strain HKI 0088^T.

Cells are 0.5 to 0.8 by 1.0 to 2.5 µm. A few cells may be filamentous and up to 10 µm long. Colonies are round, smooth, slightly convex, glistening, and 1–3 mm in diameter. The colony color varies from pale yellow to intense yellow. Alkaliphilic. Optimal growth occurs at pH 9–10 and at 28–37 °C. NaCl tolerant. Aerobic or microaerophilic. In addition to the metabolic properties indicated in Tables 25.5, the species produces acids from D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, D-glucitol, glycerol, inulin, lactose, maltose, D-raffinose, D-ribose, salicin, sucrose, potato starch, trehalose, and D-xylose. Aconitate, benzoate, citrate, formate, succinate, and tartrate are not utilized as carbon sources. Starch, esculin, casein, and gelatin are hydrolyzed while urea, hippurate, adenine, hypoxanthine, xanthine, tyrosine, and Tween 80 are not decomposed or hydrolyzed. Hydrogen sulfide is produced; indole is not produced. Methyl red and Voges-Proskauer reactions are negative.

Altenburger et al. 2002, 880^{VP}, emend Li et al. 2007, 1426.

Ge.or.gen'i.a. suff.-ia to denote a locality; N.L. fem. n. *Georgenia* referring to the village St. Georgen in Styria, where the type strain was isolated.

Cells are nonmotile, exhibiting a rod-coccus cycle. Rods and cocci occur singly or in small clusters. Cocci are 1 µm in diameter, rods are 2 µm in length and 1 µm in width. Growth occurs under both aerobic and anaerobic conditions. Oxidase- and catalase-positive. Chemotaxonomic properties are indicated in Tables 25.1 and 25.5. In addition, a low content of polyamines is present in the type species which are spermidine and spermine as major polyamines.

The type species is *Georgenia muralis*, the type strain is 1A-C^T. Type strains of the other species are indicated in Table 25.5.

Phenotypic tests on type strains included a variety of different determination methods, such as a non-commercial microtiter plate system for carbon source utilization, sugar fermentation, and qualitative enzymes tests (Kämpfer et al. 1991) for *G. muralis* (Altenburger et al. 2002) and *G. soli* (Kämpfer et al. 2010); API ZYM, API Coryne, API 20 E, and API 50CH systems (bioMérieux) for *G. thermotolerans* (Hamada et al. 2009b) and *G. halophila* (Tang et al. 2010b); API ID32 GN and API 20NE for *G. daeguensis* (Woo et al. 2012); and, in addition to API ID32 GN, another system for *G. ruanii* (Hangzhou Tianhe Microorganism Reagent tubes) (Li et al. 2007). Reactions determined for the same strain in different studies agree by and large when the same methods were applied (Altenburger et al. 2002 and Kämpfer et al. 2010), but differed significantly when the same strain tested by a different method than the originally applied method (see Kämpfer et al. 2010 versus Hamada et al. 2009b). As the same method was used to characterize all available 6 type strains of *Georgenia* (Woo et al. 2012), the results of this study are included here to show

Table 25.5

Differential metabolic and chemotaxonomic characteristics of type strains of *Georgenia* (*G*; modified *Table 1* of Woo et al. 2012) and *Bogoriella* (*B.*, Groth et al. 1997). In substrate assimilation tests, all strains showed identical biochemical characteristics except those indicated here. For a detailed listing, see Woo et al. (2012)

Characteristic	*G. daeguensis* 2C6-43^T	*G. soli* DSM 21838^T	*G. muralis* DSM 14418^T	*G. thermotolerans* DSM 21501^T	*G. ruanii* KCTC 19029^T	*G. halophila* DSM 21365^T	*Bn caseilytica* DSM 11294^T
Reduction of nitrate	+	+	+	+	–	+	–
Reduction of nitrite	–	–	–	–	–	–	n.d.
Hydrolysis of gelatin	–	+	–	–	–	–	+
Assimilation of[a]							
3-hydroxybenzoate	–	–	–	–	–	+	n.d.
2-ketogluconate	–	+	+	–	–	–	n.d.
5- ketogluconate	+	+	+	–	–	–	n.d.
D,L-hydroxybutyrate	+	–	–	–	–	–	n.d.
L-rhamnose	–	–	–	–	+	–	+
L-arabinose	–	–	+	–	+	–	+
L-fucose	–	+	–	–	w	–	+
D-ribose	+	+	–	+	–	+	+
D-mannose	+	–	–	+	–	+	+
D-melibiose	–	–	+	–	+	–	+
D-mannitol	–	–	+	–	+	–	+
L-alanine	–	–	–	–	–	+	–
Salicin	–	+	+	+	+	+	+
Malate	–	–	–	–	–	+	–
Itaconate	–	–	–	–	–	+	n.d.
Suberate	–	–	–	–	–	+	n.d.
Acetate	–	–	+	w	–	w	–
N-acetyl-glucosamine	+	+	+	–	+	+	–
Chemotaxonomic properties							
Peptidoglycan	Lys, Ala, Glu[b]	L-Lys-L-Glu	L-Lys-L-Glu	Lys-Asp	L-Lys-L-Ala-L-Glu	Lys, Ala, Glu[b]	L-Lys-L-Ala_2-L-Glu
Polar lipids	PG, DPG, PI, PIM, PL	PGs, DPG, PI, PIMs, L	PGs, DPG, PIM, PLs, GL	DPG, PIMs	PG, DPG, Pi, PL	PG, DPG, PI, PIMs, PL	PG, DPG, PI, PL
Major fatty acids (>5 % f total)[c]	ai-$C_{15:0}$ ante, ai-$C_{15:1}$, i-$C_{15:0}$, $C_{12:0}$, $C_{16:0}$	ai-$C_{15:0}$ ai-$C_{15:1}$, i-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$	ai-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$, $C_{12:1}$	ai-$C_{15:0}$, i-$C_{15:0}$, $C_{16:0}$	ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$	ai-$C_{15:0}$, $C_{16:0}$, $C_{18:0}$ $C_{12:1}$	ai-$C_{15:0}$, ai-$C_{17:0}$, i-$C_{15:1}$
Whole-cell sugars	rham, rib, gal	n.d.	n.d.	gal	rham, gal	rham, glu, gal	gal
Menaquinone types	MK-8 (H_4)	MK-8 (H_4) Minor MK-8 (H_2)	MK-8 (H_4) Minor MK-7 (H_4)	MK-8 (H_4)	MK-8 (H_4) Minor MK-7 (H_4), MK-9 (H_4)	MK-8 (H_4)	MK-8 (H_4), MK-9 (H_4)
DNA mol% G+C	66.2	n.d.	69.7	73.0	72.9	72.9	70

+, Positive; w, weakly positive; –, negative; n.d., not determined

rham rhamnose, *rib* ribose, *gal* galactose, *glu* glucosamine, *ara* arabinose, *man* mannose. For abbreviations of polar lipids, see legend of *Fig. 25.1*.

[a]*Georgenia* type strains were analyzed by API GN and API 20NE, while the type strain of *Bogoriella* was analyzed by Biolog GP

[b]no interpeptide bridge structure was determined, proposed was L-Lys-L-Glu

[c]Different proportions of fatty acids were reported in the original descriptions of type strains by Kämpfer et al.(2010), Altenburger et al. (2002), Hamada et al. (2009b), Li et al. (2007), and Tang et al. (2010b)

differentiating properties among the type strains (*Table 25.5*) and the reader is referred to the original studies for obtaining information on additional tests of individual type strains. All strains of *Georgenia* are positive for esculin hydrolysis and p-nitrophenyl-β-D-galactopyranoside but negative for arginine dihydrolase, urease, indole production and glucose acidification. In addition to the information indicated in *Table 25.5*, all type strains utilize glucose, sucrose, maltose, gluconate, and glycogen

for growth but not L-histidine, L-serine, L-proline, D-sorbitol, inositol, caprate, adipate, citrate, phenyl-acetate, Na-malonate, lactate, propionate, valerate, trisodium citrate, and 4-hydroxybenzoate (Woo et al. 2012).

Isolation, Enrichment, and Maintenance Procedures

Isolation and Enrichment

Jonesia denitrificans CIP 55.134^T was isolated from cooked ox blood (Sohier et al. 1948), but the natural habitat of the organism is not known. It grows well on tryptic soy broth agar (TSBA, Difco, containing 1.5 % Difco agar) and on tryptose blood agar base to which 1 % glucose is added. *Jonesia qinghaiensis* QH3A7^T (Schumann et al. 2004) was isolated from a mud sample of a soda lake (ca. pH 9.0) in the west of China. Isolation was done at 28° C by dilution plating on Bacto marine broth agar (MBA), pH value 7.2.

Ruania albidiflava 3-6^T (Gu et al. 2007) originate from a cotton field farmland soil sample, Shandong Province, China. A 1-g soil sample was suspended in 10-ml sterile distilled water and mixed thoroughly by shaking overnight at room temperature. The soil-water suspension was serially diluted and spread onto yeast extract-starch agar (Riken-Japan Collection of Microorganisms medium no. 42) plates, followed by incubation for 1 week under humid conditions at 28 °C.

Haloactinobacterium album YIM 93306^T (Tang et al. 2010a) was isolated from a soil sample collected from salt Lake Qijiaojing Xinjiang province, northwest China after 3 weeks incubation at 37 °C on glucose-tryptone-yeast (GTY) agar containing (g per liter) glucose 1, tryptone 0.5, yeast extract 2, $CaCl_2$ 1, NaCl 100, and agar15, [final pH not adjusted] supplemented with 10 % (w/v) NaCl. NaCl was sterilized separately before being added to the medium.

Bogoriella caseilytica HKI0088^T (Groth et al. 1997) was isolated from a soda soil sample, near lake Bogoria, in the Kenian-Tanzanian Rift Valley by dilution plating on a medium containing (g per liter) glucose 10.0, peptone (Difco) 5.0, yeast extract (Difco) 5.0, KH_2PO 1.0, $MgSO_4 \cdot 7H_2O$ 0.2, NaCl 40.0, Na_2CO_3 10.0, and agar 20.0 (pH 9.6). The NaCl and Na_2C0_3 were autoclaved separately and were added to the organic compounds at 60 °C before the agar medium was poured.

G. daeguensis 2C6-43^T (Woo et al. 2012) originate from an activated sludge sample, Korea, that was initially incubated with 50 ppm (0.39 mM) of 4-Chlorphenole and then diluted serially in 0.85 % saline solution. Aliquots of each serial dilution were spread on R2A agar and incubated at 30 °C for 14 days. The isolate was routinely cultured at 30 °C on R2A agar containing (g per liter) glucose 0.5, soluble starch 0.5, casein hydrolysate 0.5, yeast extract 0.5, peptone 0.5, MgSO4 · 7H2O 0.05, KH2PO4 0.3, and agar 15 (7.2 prior to autoclaving).

G. ruanii YIM 004^T (Li et al. 2007) was isolated from a forest-soil sample collected from Lijiang, Yunnan Province, China, after 2-week incubation at 28 °C on ISP 2 agar (Shirling and Gottlieb 1966).

G. halophila YIM 93316^T (Tang et al. 2010b) was isolated from soil of the same area from which *Haloactinobacterium album* has been recovered (Qijiaojing Lake, Xinjiang, China) after 3 weeks of incubation at 37 °C on the same medium that was also used for the isolation of *H. album* (final pH 7.5).

G. soli CC-NMPT-T3^T (Kämpfer et al. 2010) was isolated from iron-ore-contaminated soil near New Mangalore Port, Karnataka, India, on nutrient agar (Oxoid).

G. muralis 1A-C^T (Altenburger et al. 2002) was recovered from a medieval wall painting in the church of St Georgen, Styria, Austria, as described by Altenburger et al. (1996). Approximately 1-g material was collected in a sterile tube. The homogenized samples were suspended in sterile saline with 0.001 % Tween 80 and shaken continuously for 1 h; 0.1 ml of appropriate dilutions was transferred to casein mineral medium agar plates containing (g per liter) K_2HPO_4 0.6, Na_2HPO_4 $2H_2O$ 0.5, $MgS0_4 \cdot 7H_2O$ 0.05, $MgCl_2 \cdot 7H$,O 0.1, KNO_3 0.2, $FeCl_3 \cdot 6H_2O$ 0.01, casein 0.8, yeast extract 0.4 (pH 7.0) plus 2 % sucrose.

G. thermotolerans TT02 -04^T (Hamada et al. 2009b) was isolated from a forest-soil sample in Iriomote island, Japan, using the selective SDS/yeast extract pretreatment of soil which was mixed with a solution containing yeast extract (YE) 6 % and sodium dodecyl sulfate (SDS) 0.05 %, at 40 °C for 20 min (Hayakawa and Nonomura 1987). The slurry was subsequently diluted with water and the sample was allowed to incubate for a few week on humic acid-vitamin (HV) agar plates containing nalidixic acid 20 mg/L. Humic acid-vitamin medium contains (per liter) humic acid 1 g, Na_2PO_4 0.5 g, KCL 1.7 g, $MgSO_4 \cdot 7H_2O$ 50 mg, $FeSO_4 \cdot 7H_2O$ 10 mg, $CaCO_3$ 10 mg, agar 10. Before adding, humic acids are dissolved in 10 mo of 0.2 N NaOH. The other components are dissolved in distilled water and the pH is adjusted to 7.2, then autoclaved; 1 ml of the vitamin stock solution is added by sterile filtration (Hayakawa and Nonomura 1989).

Maintenance

Members of this family do not require special procedures for maintenance and medium and long-term storage. Facultative anaerobic strains may be preserved for some months by stab inoculation in nutrient agar (Tryptose Agar for *J. denitrificans*, or other similar media) in screw-capped vials. These should be stored at room or, preferably refrigerator temperature. Generally strains are maintained on isolation medium as agar slants at 4 °C for a few days. GTY agar slants for G. halophila contained 10 % NaCl (w/v).

Medium-term maintenance is in 20 % (v/v) glycerol suspensions at −20 °C or at −70 °C.

Long-term preservation is by lyophilization or in liquid nitrogen.

Ecology

As the habitat of most species of the five genera is only known for mainly the type strain, information on the ecological niche and on ecological function of members of the three families is lacking. As for other members of the order *Micrococcales*, soil appears to be the natural environment for several species to thrive: Two type strains were isolated from forest soil, two other strains from halophilic soil. On the other hand, the isolation source cooked ox blood, activated sludge, or a medieval wall painting may point toward wind or water-borne random niche occupation and unknown functions. Actually, as judged from the few reports on other strains affiliated to the five genera, the isolation source is broader. Internet search on *Georgenia* reveals the presence of names of species which have no standing in taxonomy as they have never validly published: Chen, C., and Li, W.-J. "Georgenia deserti sp. nov. isolated from a desert soil in Egypt" and "Georgenia ferrireducens" Pukhova, N.Y., Pukhov, D.E., Poltaraus, A.B., and Rodionova, T.A. "*Georgenia ferrireducens* sp. nov., a novel Fe(III) reducing actinobacterium". Strain HM06-11, found in drilling draining fluid of a deep subsurface sample, was related to *Georgenia muralis* 3A-1 (Mayhew et al. 2008). Another strain related to *Georgenia muralis* by BLAST analysis (100 % similarity, accession no AB094466) was isolated from sub-seafloor sediment of the sea of Okhotsk (Inagaki et al. 2003). A *Georgenia* strain with high similarity to sequences of as-yet uncultured *Georgenia* strains and to *G. desertii* (98 % similarity, accession no DQ203185) was cultivated from oral squamous cell carcinoma tissue removed from a 96-year-old female patient (Hooper et al. 2006). Airway specimen analysis from acute exacerbations of chronic obstructive pulmonary disease (Huang et al. 2010) by 16S rRNA phylochip analyses resulted in the detection many actinobacteria including a single *Georgenia* signal with relationship to *G. muralis* 3A-1. The NCBI taxonomy browser lists several additional unnamed strains and clones affiliated to *Georgenia*, e.g., from the halophilic Lunar Lake, India (accession number FN995635), activated sludge, Japan (AB211016), Taklamaka desert, China (EF01725), Phoenix spacecraft associated surfaces (EU977822), and the marine environment (JF346459).

Information on additional strains of the families *Jonesiaceae* and *Ruaniaceae* is sparse. A single GenBank entry is found for an uncultured *Ruania* strain (accession number HQ646261) detected in a denitrifying sulfide removal process. A single clone remotely related to *Jonesia* spp. (accession number DQ 788537) was found in a biofilm of a recirculation aquaculture system effluent (Schneider et al. 2007).

Pathogenicity and Clinical Significance

Due to its original classification as a member of *Listeria*, *Jonesia denitrificans* is classified as a biosafety level group 2 organism. It is β-hemolysis negative and CAMP test against *Staphylococcus aureus* and *Rhodococcus equi* is also negative. It is serologically distinct from *Listeria* spp. and from *Erysipelothrix* spp. (Welshimer and Meredith 1971; Wilkinson and Jones 1975). It is pathogenic to rats and mice when injected intraperitoneally but does not cause conjunctivitis when instilled into the eyes of rabbits and guinea pigs. The finding of some strains of *Georgenia* in clinical material of human should not be used elevate their biosafety level as long as their direct involvement in pathogenicity has been proven.

J. denitrificans is sensitive by agar diffusion method to penicillin, streptomycin, chloramphenicol, aureomycin, terramycin, erythromycin, tetracycline, bacitracin, novobiocin, oleandomycin, kanamycin, vancomycin, colomycin, polymyxin B, and nitrofurantoin but resistant to sulfonamide, neomycin, and nalidixic acid.

Ruania albidoflava cells are susceptible to ampicillin (10 mg), chloramphenicol (30 mg), ciprofloxacin (5 mg), erythromycin (15 mg), gentamicin (10 mg), kanamycin (30 mg), neomycin (30 mg), polymyxin B (300 IU), rifampicin (15 mg), and streptomycin (10 mg). Not susceptible to nitrofurantoin (300 mg) or oxacillin (1 mg).

Bogoriella caseilytica cells are susceptible to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, lincomycin, neomycin, oxacillin, oxytetracycline, penicillin G, polymyxin B, rifampin, and streptomycin. Cells are resistant to nitrofuran and sulfonamid.

Georgenia ruanii ells are susceptible to penicillin G, vancomycin, polymyxin B erythromycin, terramycin, aureomycin, tetracycline, streptomycin, novobiocin, gentamicin, and chloramphenicol. Cells are resistant to oleandomycin and nalidixic acid.

No information on antibiotic sensitivity and resistance is available for *J. quinghaiensis, Haloactinobacterium album, Georgenia muralis, G. thermotolerans, G. soli, G. daeguensis*, and *G. halophila.*

Application

The only evidence that any strain of the three families is involved in application originates from a study by Sianidis et al. (2005) involving a strain of *Jonesia* (DSM14140 with restricted distribution). This strain has been used in the large-scale production of a xyloglucanase (Xeg, family 74 glycoside hydrolases). Xeg was overexpressed in *Streptomyces lividans* TK24 and is the longest heterologous polypeptide shown to be secreted from *S. lividans.*

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26 The Suborder *Kineosporiineae*

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Abstract
Kineosporiaceae is only one family within the order "*Kineosporiales,*" which includes the genera *Angustibacter*, *Kineococcus*, *Kineosporia*, *Pseudokineococcus*, and *Quadrisphaera* at this writing.

Members of the genera *Angustibacter*, *Kineococcus*, *Pseudokineococcus*, and *Quadrisphaera* are characterized by formation of cocci to rods, *meso*-diaminopimelic acid as a cell wall diamino acid, saturated, iso-branched methyl and anteiso branched methyl fatty acid as the cellular fatty acid. The members of the genus *Kineosporia* are characterized by formation of spores on substrate hypha by the intermediary of sporophores, *meso*- and LL-diaminopimelic acid as a cell wall diamino acid, saturated, unsaturated, and 10 methyl branched methyl fatty acid as the cellular fatty acid.

Members of this family are found in soil, desert sand, coast sediment, shield cell facility, aerobic granule, air sample, roof tile, sphagnum, leaf, fallen leaf, plant litter, root, on stem of plant.

Taxonomy, Historical and Current

Short Description of the Families

The family description is based mainly on phylogenetic positions and patterns of 16S rRNA gene sequence signatures.

Kineosporiaceae Zhi, Li and Stackebrandt 2009, 596^{VP}

Ki'ne.o.spo.ri.a'ce.ae. N.L. fem. n. *Kineosporia* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Kineosporiaceae* the *Kineosporia* family (Zhi et al. 2009).

Phylogenetically, a member of the order "*Frankiales*" (Ludwig et al. 2012), phylum Actinobacteria.

The family contains the genera *Angustibacter* (Tamura et al. 2010; Kim et al. 2013; Lee 2013), *Kineococcus* (Yokota et al. 1993), *Kineosporia* (Pagani and Parenti 1978; Itoh et al. 1989; Kudo et al. 1998), *Pseudokineococcus* (Jurado et al. 2011), and *Quadrisphaera* (Maszenan et al. 2005).

Substrate hypha may occur. Motile or nonmotile. Cross-linkage of peptidoglycan is by the A type; diagnostic diamino acids include *meso*-diaminopimelic acid or *meso*- and LL- diaminopimelic acid. Each peptidoglycan is directly linked without interpeptide bridges, and the muramic acid in peptidoglycan is *N*-acetylated. The cellular fatty acids are a complex mixture of straight-chain saturated, monounsaturated, and iso- and anteiso-methyl-branched acids. Predominant fatty acid is C16:0 and C18:1 for *Kineosporia*, anteiso-C15:0 for *Kineococcus*, *Pseudokineococcus*, and *Quadrisphaera*, and iso-C17:0, iso-C15:0, C16:0, iso-C16:0, anteiso-C15:0, anteiso-C17:0, and/or C16:1 for *Angustibacter*. Menaquinone MK-9(H_4), MK-9(H_2), or MK-8(H_2) is the predominant isoprenoid quinone. The pattern of 16S rRNA signatures consists of nucleotides at positions 127:234 (A–U), 142:221 (C–U), 598:640

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_179,

Table 26.1
Diagnostic properties of the genera *Kineosporia*, *Angustibacter*, *Kineococcus*, *Pseudokineococcus* and *Quadrisphaera Fodinicola* belonging in the family *Kineosporiaceae* (Jurado et al. 2011, amended)

Characteristic	*Kineosporia*	*Angustibacter*	*Kineococcus*	*Pseudokineococcus*	*Quadrisphaera*
Cell morphology	Single spores borne at tips of substrate hyphae and spore clusters on a sporophore	Irregular rods and cocci	Cocci in tetrad arrangements	Cocci in pairs, tetrads and clusters	Cocci in tetrad arrangements
Motility	Motile	Motile or Non-motile	Motile or non-motile	Motile	Non-motile
Cell-wall diamino acid(s)	meso- and LL-A2pm	meso-A2pm	meso-A2pm	meso-A2pm	meso-A2pm
Fatty acid type (predominant)	S, U, M (C16:0, C18:1)	S, I, A, M (iso-C17:0, iso-C15:0, C16:0, iso-C16:0, anteiso-C15:0, anteiso-C17:0, C16:1)	S, I, A (anteiso-C15:0)	S, I, A (anteiso-C15:0)	S, I, A, U (anteiso-C15:0)
Predominant menaquinone	MK-9(H4)	MK-9(H4)	MK-9(H2)	MK-9(H2)	MK-8(H2)
Polar lipids	PC, DPG, PI, PIM	DPG, PG, PI, PIM	DPG, PG, GL	DPG, PG, PL, GL, PGL, PI	DPG, PG, PI
Characteristic sugars	Gal, Man, Rib	Gal, Rib	Gal, Ara	Rib, Rha, Gal, Ara	ND
DNA G+C content (mol%)	69–71	71–74	73–77	77	75

Data from Tamura et al. (2010), Lee (2006) and Jurado et al. (2011)
A2pm 2,6-diaminopimelic acid, *A* anteiso-methyl-branched, *I* iso-methyl-branched, *M* 9- 10-methyl-branched, *S* straight-chain saturated, *U* monounsaturated, *DPG* diphosphatidylglycerol, *GL* unknown glycolipid, *PC* phosphatidylcholine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PL* unknown phospholipids, *PGL* unknown phosphoglycolipid, *Ara* arabinose, *Gal* galactose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *ND* no data

(U–G), 840:846 (A–C), 845 (A), 986:1218 (A–U), 1163:1173 (G–U), 1164:1172 (G–C), and 1165:1171 (G–A). Predominant polar lipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, phosphatidylcholine, and several phosphoglycolipids, phospholipids, and glycolipids. Phosphatidylethanolamine is absent. Mycolic acids and teichoic acids are absent. Galactose, as a diagnostic whole-cell sugar, is present. G+C values are in the DNA range between 69 and 77 mol%. The family *Kineosporiaceae* was proposed by Zhi et al. (2009); the genera *Kineococcus, Kineosporia, and Quadrisphaera* are assigned to this family. Diagnostic properties of the genera *Kineosporia*, *Angustibacter Kineococcus, Pseudokineococcus, and Quadrisphaera* are shown in ❯ *Tables 26.1* and ❯ *26.2*.

Phylogenetic Structure of the Family and Its Genera

The members of the family *Kineosporiaceae* form a cluster in the tree of the NJ method based on the 16S rRNA gene sequences, while those of ML the method indicate that the genus *Angustibacter* is placed outside the family. Consequently, within the family, the five genera formed separate lineages in the NJ tree and, except the genus *Angustibacter*, in the ML tree (❯ *Figs. 26.1* and ❯ *26.2*).

Molecular Analyses

DNA-DNA Hybridization Studies

DNA-DNA hybridization (DDH) studies have been performed on *Kineosporiaceae* strains by Kudo et al. (1998) to separate five species including the type species. Sakiyama et al. (2009) confirmed the homogeneity and independency of three strains of *Kineosporia babensis* by DDH. DDH was carried out between the type strain of *Kineosporia mesophila* and those of the five species of the genus and showed 59–34 % relatedness (Li et al. 2009b). DDH relatedness has been reported between type strains of *Angustibacter aerolatus* and *Angustibacter luteus* (42–47 %; Kim et al. 2013); between type strains of *Angustibacter peucedani* and *Angustibacter luteus* (22.3 ± 0.9 %; Lee 2013); between type strains of *Kineococcus gynurae* and type strains of *Kineococcus aurantiacus* and *Kineococcus radiotolerans* (25 % and 39.4 %, respectively; Duangmal et al. 2008);

Table 26.2

Differentiating cultural features between type strains of *Kineosporia* (Li et al. 2009, amended)

Characteristic	*Kineosporia aurantiaca*	*Kineosporia babensis*	*Kineosporia mesophila*	*Kineosporia mikuniensis*	*Kineosporia rhamnosa*	*Kineosporia rhizophila*	*Kineosporia succinea*
Isolation source	Soil	Plant litter	Stems of *Tripterygium wilfordii*	Sphagnum	Leaves of *Typha latifolia*	Roots of *Cyperus microiria*	Fallen leaves
Nitrate reduction	–	–	–	–	–	+	–
H_2S production	–	ND	–	+	–	–	–
Highest NaCl tolerance (%, w/v)	3	4[a]	3	1	3	5	5
Sole carbon source utilization							
D-arabinose	+	ND	+	–	–	+	+
Dulcitol	–	ND	–	+	–	–	+
Inositol	+	+	–	+	+	–	+
Lactose	–	+	–	+	–	+	+
D-mannose	+	ND	+	+	–	–	–
Melezitose	+	+	+	+	–	+	+
Melibiose	+	+	+	+	–	+	+
Raffinose	–	+	+	–	+	+	–
D-ribose	+	+	–	+	–	+	+
Sodium oxalate	+	ND	+	–	+	+	–
D-sorbitol	–	+	+	–	+	–	+
Sucrose	+	+	+	+	–	+	+
Trehalose	+	+	+	+	–	+	+
Trisodium citrate	+	ND	+	–	+	–	–
D-xylose	–	+	+	+	+	+	+
Sole nitrogen source utilization							
L-alanine	+		+	+	–	+	+
L-arginine	+		–	+	+	+	–
L-asparagine	+		+	+	–	+	+
L-hydroxyproline	+		+	+	+	+	–
L-lysine	–		–	+	–	+	–
L-phenylalanine	–		+	+	+	+	+
L-serine	+		+	+	+	+	–
Decomposition of							
L-tyrosine	–	+	–	–	–	–	–
DNA G + C content (mol%)	69.5	69–70	70.3	69.4	68.3	70.0	70.7

All strains were positive for hydrolysis of urea and starch and for utilization of L-arabinose, cellobiose, D-fructose, D-galactose, glucose, maltose, D-mannitol and L-rhamnose. All strains were negative for decomposition of adenine, hypoxanthine and xanthine. + positive, – negative. [a]One of three strains tolerates up to 3 %

between type strains of *Kineococcus glutinatus* and *Kineococcus xinjiangensis* (41.6 ± 1.8 %; Nie et al. 2012); and between type strains of *Pseudokineococcus lusitanus* and *Kineococcus marinus* (46.6 ± 0.8 %; Jurado et al. 2011).

Genome Analyses

The whole genome sequence of the type strain of *Kineococcus radiotolerans* SRS30216 (GOLD ID Gc00615)

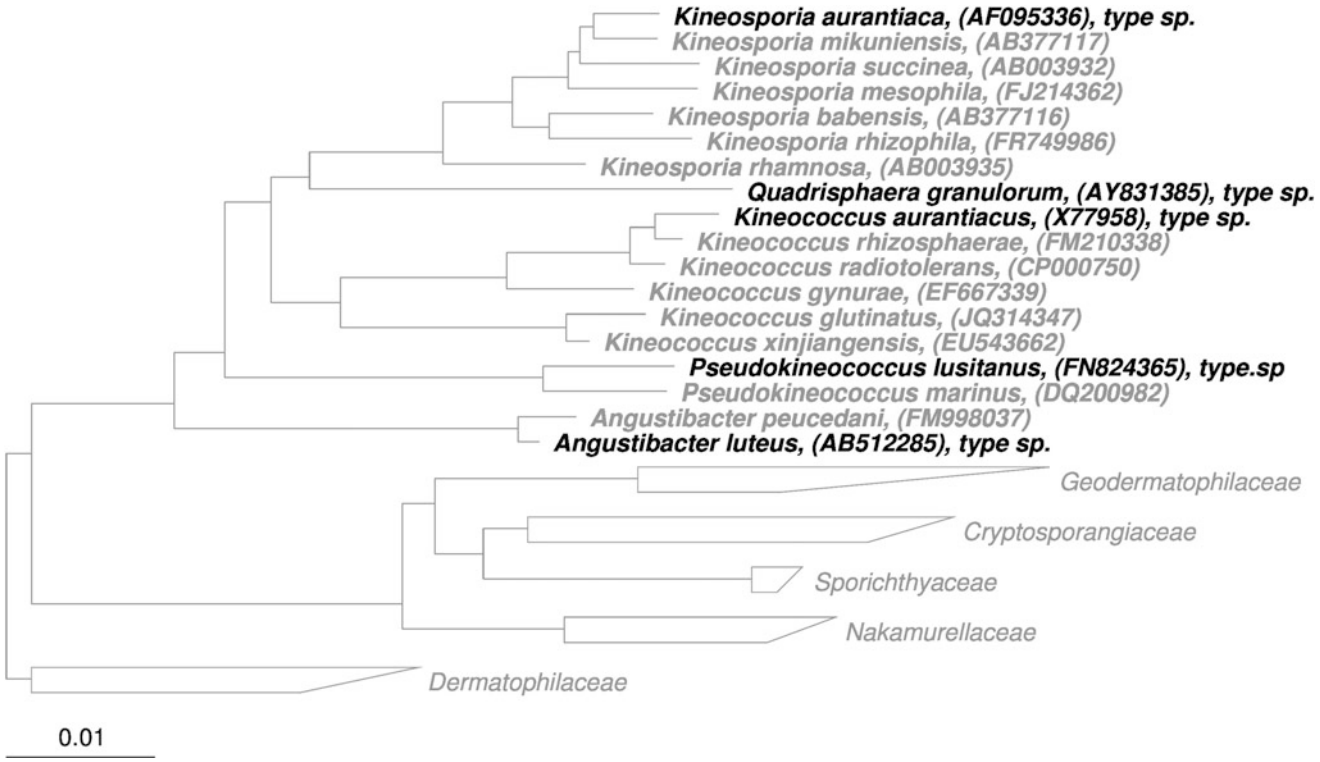

Fig. 26.1

Phylogenetic reconstruction of the family *Kineosporiaceae* 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

has been released. The genome of the type strain of *Kineococcus radiotolerans* contains 4,957 kbp, 4,785 open reading frames (orfs), and a mol% G+C content of 74 %.

Phages

No phages have been observed to lyse strains of *Angustibacter*, *Kineococcus*, *Kineosporia*, *Pseudokineococcus*, and *Quadrisphaera*.

Phenotypic Analyses

Kineosporia Pagani and Parenti 1978, 289^{AL}, Emend. Kudo Matsushima Itoh Sasaki and Suzuki 1998

Ki.ne.o.sporia. Gr. n. kinesis, motion; N.L. fem. n. spora (from Gr. fem. n. spora, a seed), a spore; N.L. fem. n. *Kineosporia*, an organism that has motile spores.

Aerobic, Gram-positive, and nonacid fast. Colonies on agar medium lack aerial mycelia, form central projections with radiating vegetative hyphae, and are occasionally accompanied by bunches of spore clusters in the agar. Mature colonies have a gelatinous matrix, which confers a glossy appearance. Spores, which are spherical to ovoid or pyriform with a long axis of 1–2 μm, are catenated around the central projection or are located singly or aggregately at the tips of hyphae. The spores are motile with polar tufts of flagella.

The peptidoglycan contains both LL- and *meso*-DAP; the ratio in the cells from submerged culture containing both mycelia and spores is dependent on strains. *meso*-DAP is generally predominant in the cells of spore fraction (Kudo et al. 1998). The content of glycine is smaller in the spore fraction (Itoh et al. 1989). The whole cells contain galactose, glucose, mannose, and ribose, but the content of mannose is negligible in the spore fraction. In addition, rhamnose and 3-O-methylrhamnose are present in the cells of *Kineopsoria rhamnosa*. Phosphatidylcholine is a diagnostic phospholipid, and MK-9(II,III-H4) is present as a main menaquinone component. The cellular fatty acid profile mainly contains 16:0, 18:1, and 10Me 18:0 and lacks iso/anteiso branched fatty acids. 2-Hydroxy fatty acids are also present in some species. Lipid compositions are not significantly different in the whole cultured organism and the spore fraction. Mycolic acids are absent.

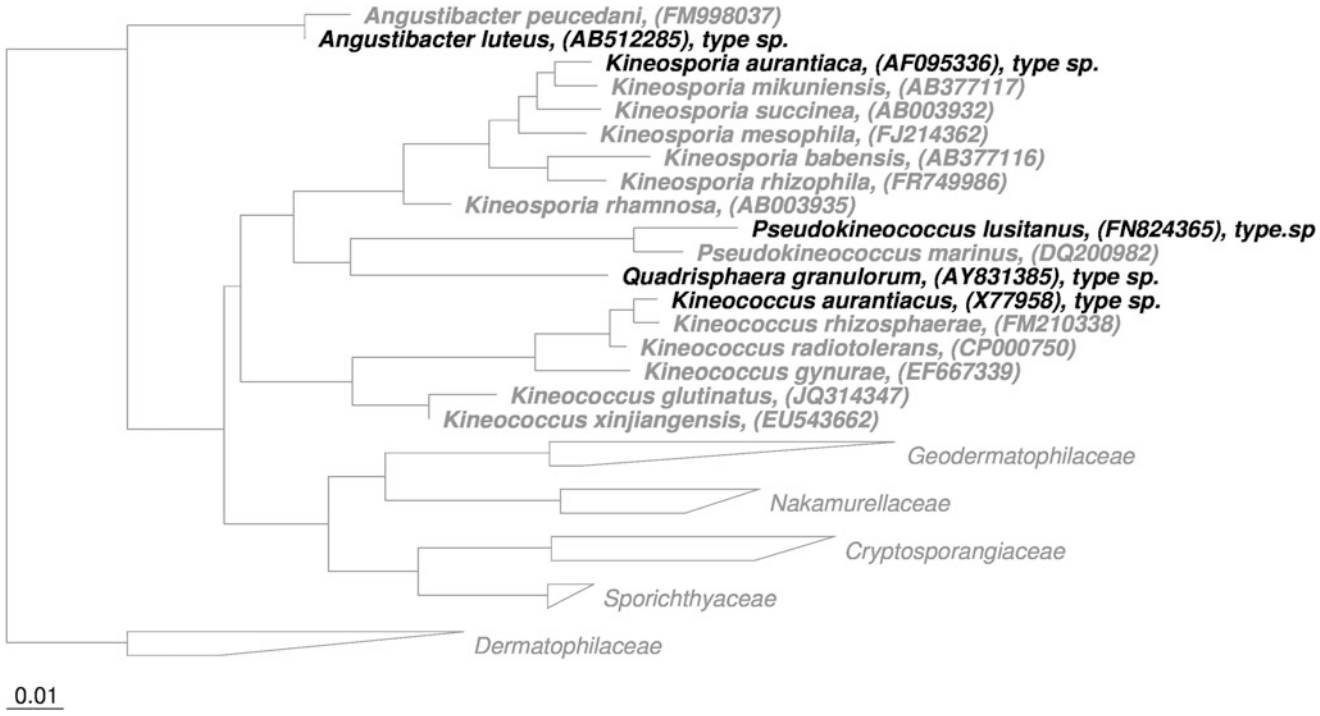

Fig. 26.2

Phylogenetic reconstruction of the family *Kineosporiaceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, a 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 mol% G+C content ranges from 68.3 to 70.8.

The type species is *Kineococcus aurantiaca* Pagani and Parenti 1978. Type strain is ATCC 29727 = DSM 43858 = NBRC 14067 = JCM 3230 = NRRL B-16913 = VKM Ac-702.

Angustibacter Tamura Ishida Otoguro Yamamura Hayakawa and Suzuki 2010, 2441^{VP}, Emend. Lee 2013[1]

An.gu.sti.bac'ter. L. adj. angustus narrow; N.L. masc. n. bacter a rod; N.L. masc. n. *Angustibacter* narrow bacterium (Table 26.3).

Gram-positive, non-spore-forming cocci to rods. Variable in motility and catalase. Aerobic or facultatively anaerobic. The peptidoglycan is of the A type (A1γ sensu; Schleifer and Kandler 1972) containing *meso*-diaminopimelic acid, alanine, and glutamic acid. Cell-wall sugars are galactose, glucose, and ribose. Mycolic acids are absent. The major menaquinone is MK-9(H4). The phospholipids comprise diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannoside. The cellular fatty acids are of the complex mixture of straight-chain saturated, monounsaturated, and iso- and anteiso-methyl-branched acids, and the composition is variable in species. Anteiso-C15:0, iso-C15:0, C16:0, iso-C16:0, and summed feature 9 (including iso-C17:1ω9c and/or C16:0 10-methyl) are predominating.

The mol% G + C content ranges from 70.9 to 73.6, and the type strain of the type species is 71 mol%.

The type species is *Angustibacter luteus* Tamura et al. 2010. Type strain is TT07R-79 = NBRC 105387 = KACC 14249 = JCM 17683.

Kineococcus Yokota Tamura Nishii and Hasegawa 1993, 52^{VP}

Ki.ne.o.coc'cus. Gr. n. kinesis, motion; N.L. masc. n. coccus (from Gr. masc. n. kokkos, a grain, seed), coccus; N.L. masc. n. *Kineococcus*, a motile coccus (Table 26.4).

Cells are Gram-positive, spherical, and 1.0–1.5 μm in diameter and occur in pairs, in tetrads, or in clusters. Motile with tufts of flagella. Endospores are not formed. Colonies are circular and rough and may be cream-colored to mange. Strictly aerobic. Catalase- and urease-positive. Oxidase-negative.

[1] The genus description was emended to contain motile, aerobic, and/or catalase-negative species by Kim et al. (2013) and Lee (2013) separately.

Table 26.3

Differentiating cultural features between type strains of *Angustibacter* (Kim et al. (2013), Lee (2013), amended)

Characteristic	*Angustibacter luteus*	*Angustibacter aerolatus*	*Angustibacter peucedani*
Cell morphology	Cocci to rods	Short rods	Rods
Cell size (μm)	0.3–0.5 × 0.3–2.0	0.9–1.2 × 1.5–1.8	0.4–0.8 × 0.9–1.4
Anaerobic growth	+	−	−
Catalase/oxidase	+/nd	−/−	+/−
Motility	−	+	+
Temperature range for growth (°C)	5–30	10–37	10–37
pH range for growth (optimum)	6.0–8.0 (6.0–7.0)	6.0–8.0 (7.0)	6.0–10.0 (7.0–8.0)
NaCl tolerance for growth (%)	0	0	0–3
Gelatin hydrolysis	+	−	+
Enyzme activity			
Alkaline phosphatase	+	+	−
α-galactosidase	+	+	−
β-glucosidase	+	+	−
Acid production from:			
D-arabitol	−	−	w
L-arabinose	+	−	−
D-xylose	+	−	+
L-xylose	−	+	−
L-rhamnose	+	−	−
D-lyxose	+	−	+
DNA G + C content (mol%)	71	73	74
Whole-cell sugars	Galactose, glucose, ribose	Glucose, rhamnose, ribose	Glucose

Data from Kim et al. (2013), Lee (2013), Tamura et al. (2010)

Strains are positive for aesculin hydrolysis and β-galactosidase (API 20NE), enzyme activation of alkaline phosphatase, acid phosphatase, esterase lipase (C8), valine arylamidase, leucine arylamidase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase (API ZYM), and acid production from D-glucose, aesculin ferric citrate, cellobiose, sucrose, raffinose, glycogen and melibiose (API 50CH). Both strains are negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease (API 20NE), utilization of malic acid, trisodium citrate, phenylacetic acid, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, 3-hydroxybutyric acid, L-serine, D-mannitol, L-fucose, D-sorbitol, propionic acid, capric acid, L-histidine, potassium 2-ketogluconate and 4-hydroxybenzoic acid (API 20NE and API 32GN), enzyme activation of lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM), and acid production from glycerol, erythritol, D-arabinose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, inulin, melezitose, xylitol, turanose, D-tagatose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate (API 50CH). + Positive, *w* weakly positive, − negative, *nd* not determined

The cells do not reduce nitrate to nitrite. Acid is produced from glucose and some other sugars. Esculin is hydrolyzed. Starch, gelatine, and casein are not hydrolyzed. The optimum growth temperature is 27 °C.

The cell-wall peptidoglycan contains *meso*-DAP, alanine, and glutamic acid. The major menaquinone is MK-9(H2). Mycolic acid is not present. The major cellular fatty acid is anteiso-C15:0. Diphosphatidylglycerol, phosphatidylglycerol, and unidentified glycolipids are present as polar lipids.

The mol% G+C content of the type strain of the type species is 74 mol%.

The type species is *Kineococcus aurantiacus* Yokota et al. 1993. Type strain is RA 333 = ATCC 51238 = CIP 105426 = DSM 7487 = IFO (now NBRC) 15268 = JCM 10180 = VKM Ac-1947.

Pseudokineococcus Jurado Laiz Ortiz-Martinez Groth and Saiz-Jimenez 2011, 2515[VP]

Pseu.do.ki.ne.o.coc'cus. Gr. adj. pseudês false; NL. masc. n. *kineococcus* a bacterial genus name; N.L. masc. n. *Pseudokineococcus* the false *Kineococcus* (Table 26.5).

Cells are spherical and 1.0–1.5 μm in diameter and occur in pairs, in tetrads, or in clusters. Cells are motile and have tufts of flagella. Endospores are not formed. Gram-positive. Colonies are circular, rough, and orange-colored. Strictly aerobic. Catalase-positive and oxidase-negative. Do not reduce nitrate to nitrite. Acid is produced from glucose and some other sugars. Aesculin is hydrolyzed. The diagnostic diamino acid of the peptidoglycan is *meso*-diaminopimelic acid. The major menaquinone is MK-9(H2). Mycolic acids are

Table 26.4

Differentiating cultural features between type strains of *Kineococcus* (Jurado et al. 2011, amended)

Characteristic	*Kineococcus aurantiacus*	*Kineococcus glutinatus*	*Kineococcus gynurae*	*Kineococcus radiotolerans*	*Kineococcus rhizosphaerae*	*Kineococcus xinjiangensis*
Acid produced from:						
D-arabitol	ND	ND	+	ND	ND	ND
D-galactose	–	ND	+	+	+	ND
Lactose	–	ND	+	–	–	ND
Maltose	–	ND	+	–	+	+
D-mannitol	–	ND	+	+	ND	–
D-mannose	–	ND	+	+	+	ND
Melezitose	ND	ND	+	ND	+	ND
Melibiose	ND	ND	+	ND	+	ND
Raffinose	–	ND	–	–	+	ND
D-ribose	–	ND	–	–	–	–
Trehalose	–	ND	+	ND	–	ND
Gentiobiose	ND	ND	+	ND	ND	ND
Glycerol	–	ND	–	+	–	ND
Inositol	-	ND	–	+	ND	+
L-rhamnose	-	ND	+	–	–	+
N-acetylglucosamine	ND	ND	–	ND	ND	ND
Growth at/in:						
7 % (w/v) NaCl	–	–	(+)	–	–	–
5 °C	–	–	–	–	–	–
Hydrolysis of:						
Aesculin	–	ND	ND	ND	+	+
Gelatin	–	–	+	ND	+	+
Starch	–	+	ND	–	–	–
Urea	+	+	+	–	+	–

Data from Lee (2009), Duangmal et al. (2008), Liu et al. (2009), Nie et al. (2012) and Maszenan et al. (2005)
+ positive, – negative, (+) weakly positive, +/– variable, *ND* not determined

not present. The characteristic phospholipid of the genus is phosphatidylglycerol. Diphosphatidylglycerol and phosphatidylinositol may be present. The major cellular fatty acid is anteiso-C15:0.

The mol% G + C content of the type strain of the type species is 77 mol%.

The type species is *Pseudokineococcus lusitanus* Jurado et al. 2011. Type strain is T2A-S27 = CECT 7306 = DSM 23768 = LMG 24148.

Kineococcus marinus (Lee 2006) was transferred to the genus *Pseudokineococcus* by Jurado et al. (2011).

Quadrisphaera Maszenan Tay Schumann Jiang and Tay 2005, 1771[VP]

Qua.dri.sphae'ra. L. pref. numer. adj. quadr- four; L. fem. n. sphaera a ball, globe, sphere; N.L. fem. n. *Quadrisphaera* four-fold balls, coccus in tetrad.

Gram-positive, non-spore-forming cocci, 1 · 2–3 · 0 μm in diameter, occurring in tetrad arrangement, fitting the morphological description of tetrad-forming organisms. MK-8(H2) is the predominant menaquinone. The major cellular fatty acid is anteiso-C15:0.

The mol% G+C content of the type strain of the type species is 75 mol%.

The type species is *Quadrisphaera granulorum* Maszenan et al. 2005 (*Table 26.6*). Type strain is AG019 = ATCC BAA-1104 = DSM 44889 = JCM 16010.

Isolation, Enrichment, and Maintenance Procedures

Kineosporia aurantiaca was isolated from soil at St. Rphael, France, by cultivating on Czapek-glucose agar and incubating at 30 °C (Pagani and Parenti 1978). Strains of this species were also isolated from plant samples in Japan (Kudo et al. 1998).

Table 26.5
Differentiating cultural features between type strains of *Pseudokineococcus* (Jurado et al. 2011, amended)

Characteristic	*Pseudokineococcus lusitanus*	*Pseudokineococcus marinus*
Acid produced from:		
D-arabitol	−	+
D-galactose	+	+
Lactose	(+)	+
Maltose	+	+
D-mannitol	+	+
D-mannose	+	+
Melezitose	−	+
Melibiose	+/−	−
Raffinose	−	+
D-ribose	−	+
Trehalose	+	+
Gentiobiose	−	+
Glycerol	+	+
Inositol	−	(+)
L-rhamnose	+	+
N-acetylglucosamine	+/−	−
Growth at/in:		
7 % (w/v) NaCl	(+)	+
5 °C	−	+
Hydrolysis of:		
Aesculin	+	+
Gelatin	+	+
Starch	V	+
Urea	−	−

Data from Jurado et al. (2011)
+ positive, − negative, (+) weakly positive, +/− variable, *ND* not determined

Strains of *Kineosporia mikuniensis, Kineosporia rhizophila, Kineosporia rhamnosa*, and *Kineosporia succinea* were isolated from plant samples such as leaves of cat-tail (*Typha latifolia*), fallen leaves, root of galingale (*Cyperus microria*), and sphagnum in Japan. The collected samples were dried at 28 °C for at least 1 week and were ground with a blender after adding sterile water. The suspension was incorporated into an agar medium containing 0.2 g yeast extract (Difco) 50 mg cycloheximide, 50 mg nystatin, and 15 g agar in 1,000 ml distilled water (pH not adjusted) and poured to petridishes for incubation at 28 °C for more than 2 weeks. Colonies were picked up with a sterile needle under a stereomicroscope and transferred to yeast extract-starch agar (2 g yeast extract, 10 g soluble starch, and 15 g agar in 1,000 ml). In addition to the four species, two strains of *Kineosporia aurantiaca* were also isolated (Kudo et al. 1998).

Kineosporia babensis was isolated from plant litter collected from the mountainside at Ba Be National Park, Bac Kan Province, in northern Vietnam. The samples were dried at room temperature for 5–7 days and treated with rehydration-centrifugation method (Hayakawa et al. 2000) and inoculated on humic acid-vitamin agar (Hayakawa and Nonomura 1987) containing nalidixic acid (20 mg/l) and kabicidin (0.75 mg/l).

Kineosporia mesophila was isolated from a surface-sterilized stem sample of *Tripterygium wilfordii* from Yunnan province, southwest China. The procedure was according to that of Coombs and Franco (2003) followed by Li et al. (2009).

Angustibacter luteus was isolated by using the yeast extract-SDS method (Hayakawa and Nonomura 1989) from broad-leaf forest soil at Rishiri Island, Hokkaido, Japan. The type strain was picked up on HV medium (Hayakawa and Nonomura 1987) containing 20 mg/L nalidixic acid and 50 mg/L cycloheximide after incubating at 28 °C for 2 weeks. Subcultivation of the isolate was conducted in yeast extract-malt extract agar (ISP-2 medium: Shirling and Gottlieb 1966) containing 4 g/L yeast extract, 10 g/L malt extract, 4 g/L glucose, and 20 g/L agar (pH 7.3), yeast extract–soluble starch (YS) agar containing 2 g/L yeast extract, 10 g/L soluble starch, and 15 g agar (pH 7.3), and NBRC medium 802 (Code No. 398-01671; Wako Pure Chemical Ind., Ltd., Osaka, Japan) containing 10 g/L Polypepton (Wako), 2 g/L yeast extract, 1 g/L $MgSO_4 \cdot 7H_2O$, 15 g/L agar (pH 7.0).

Angustibacter aerolatus was isolated from an air sample in Jeju Island, Republic of Korea. Type strain was isolated under aerobic conditions on an R2A (Difco) agar plate containing 0.5 g/L Bacto yeast extract (Difco), 0.5 g/L Bacto proteose peptone No. 3 (Difco), 0.5 g/L Bacto casamino acids (Difco), 0.5 g/L glucose, 0.5 g/L soluble starch, 0.3 g/L Sodium pyruvate, 0.3 g/L K_2HPO_4, 0.05 g/L $MgSO_4 \cdot 7H_2O$, 15 g/L agar (pH 7.2+/−0.2). The strain was purified and maintained on R2A agar plates.

Angustibacter peucedani was isolated from soil adhering to a root of a wild plant (*Pseucedanum japonicum* Thumb) collected in Mara Island, Jeju, Republic of Kore*a*. Soil samples were diluted by consecutive tenfold dilutions with sterile distilled water, and aliquots (100 ml) of the dilutions were transferred onto starch–casein agar medium containing 10 g/L soluble starch, 0.3 g/L casein, 2 g/L KNO_3, 2 g/L NaCl, 2 g/L KH_2PO_4, 0.02/L g $CaCO_3$, 0.05/L g $MgSO_4$. $7H_2O$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 18 g/L agar (pH 7.2). The agar plates were incubated for 6 weeks at 30 °C, and a tiny, orange colony was subcultured on ISP-2 medium.

Kineococcus aurantiacus was isolated from soil obtained from Indore region of India by the dilution plate method on a medium containing 10 g/L soluble starch, 1 g/L casein, 0.05 % K, HPO, and 1.5 % agar (pH 7.0–7.5) supplemented with 25 μg/ml nalidixic acid, 12.5 μg/ml kanamycin, 5.0 μg/ml cefsulodin, and 6.25 μg/ml kabicidin. Type strain was isolated from soil obtained from Indore region of India. Subcultivation of the strain was conducted in ISP-2 medium.

Kineococcus gynurae was isolated from the roots of a Thai medicinal plant, *Gynura pseudochina* (L.) DC. var. *hispida* Thwaites, Bangkok, Thailand, by using starch casein agar (Küster and Williams 1964) supplemented with antibacterial and antifungal agents (nalidixic acid and ketokonazole). The plates were incubated at room temperature for 14 days. One colony was isolated and purified on glucose yeast extract (GYE) agar containing 10 g/L glucose, 10 g/L yeast extract, and 15 g/L agar (pH 7.0). Subcultivation of the strain was conducted in GYE agar.

Kineococcus glutinatus was isolated from a soil sample collected from a dry-hot river valley in Dongchuan county, Yunnan Province, southwest China. Soil samples dissolved in sterilized water were shaken at 28 °C with 200 rpm for 1 h. The soil suspension was serially diluted to 10^{-2} fold, and 200 μL of the 10^{-2} dilution was spread on GTY medium (Tang et al. 2008) supplemented with nalidixic acid (25 mg/L) and nystatin (50 mg/L). Growth was observed after incubation at 28 °C for 2 weeks. Pure cultures of strain were obtained by repeated incubating on ISP 2 medium.

Kineococcus radiotolerans was isolated from a shielded cell facility in the Savannah River Technology Center at the Savannah River Site, in Aiken, South Carolina, USA. The radiation level of the sampling site was 0.18–3.5 Gyh^{-1}, and all the isolation procedures were carried out in the shielded cells with mechanical manipulation. The entire swab wiped the surface of the working area was placed in 10 ml PTYG nutrient solution (1 % glucose, 0.5 % yeast extract, 0.5 % tryptone, 0.5 % peptone, 0.006 % $MgSO_47H_2O$, 0.0006 % $CaCl_2$, pH 10.7) in a 15 ml centrifuge tube. The alkaline pH of the medium due to the nature of the samples (Phillips et al. 2002).

Kineococcus rhizosphaerae was isolated from rhizosphere soil of a cliff-associated plant (*Peucedanum japonicum* Thunb.) on Mara Island, Jeju, Republic of Korea. Soil samples serially diluted with sterile distilled water and transferred onto plates of starch-casein agar. Colonies were cultivated at 30 °C for 14 days (Lee 2009).

Kineococcus xinjiangensis was isolated from desert sand in Xinjiang Province, China. Medium used for isolation was ten-fold-diluted tryptic soy broth (Difco) (Liu et al. 2009).

Quadrisphaera granulorum was isolated from aerobic granules. The aerobic granules were cultivated in a laboratory-scale sequencing batch reactor as described by Dulekgurgen et al. (2003), with synthetic wastewater containing acetate as the sole carbon source (Moy et al. 2002). Granule samples were harvested 4 weeks after reactor start-up and were disrupted at 2,500 r.p.m. for 3 min with a Mini Beadbeater (Biospec Products). Microscopic observations of these granules revealed that the microbial community was dominated by coccoid cells that clustered mostly in tetrads. These coccoid cells were retrieved from the disintegrated biomass using a Skerman micromanipulator (Skerman 1968) and transferred to GS agar (Williams and Unz 1985) plates for incubation at 25 °C (Williams and Unz 1985). Colonies arising from micromanipulated cells were transferred several times to GS agar plates to obtain pure cultures; culture purity was confirmed microscopically by examining cells from single colonies.

***Pseudokineococcus* lusitanus** (Jurado et al. 2011) was isolated from a roof tile in Oportp, Portugal, on Tryptose Soy Agar (TSA) (Oxoid) after 4 weeks at 28 °C.

Pseudokineococcus marinus was isolated from a sediment sample of the coast of Jeju, Republic of Korea. Beach sediment samples were taken at a depth of 1 m below the surface and placed directly into sterilized 50 ml Falcon tubes. For bacterial isolation, each sediment sample (1 g) was placed into a sterile plastic tube containing 9 ml sterile distilled water, which was then mixed in a tube rotator for 30 min at moderate speed. Aliquots (100 ml) of the serial diluent of the samples were transferred onto SC-SW agar plates containing 10 g/L soluble starch, 0.3 g/L casein, 2 g/L KNO_3, 2 g/L NaCl, 0.02 g/L $CaCO_3$, 18 g/L agar, 0.05 g/L $MgSO_4.7H_2O$, and 0.01 g/L $FeSO_4.7H_2O$ in a 60 : 40 mixture of natural seawater and distilled water supplemented with 60 % (v/v) sterilized natural seawater. The plates were incubated at 30 °C for 14 days, and colonies were subcultured on YE-SW medium containing 4 g/L yeast extract, 10 g/L malt extract, 4 g/L glucose, and 18 g/L agar in a 60 : 40 mixture of natural seawater and distilled water.

Members of the family *Kineosporiaceae* grow in complex liquid or on solidified media, such as ISP-2 medium, Bennett's agar containing 1 g/L yeast extract, 1 g/L beef extract, 2 g/L NZ amine, 10 g/L glucose, and 20 g/L agar (pH 7.3), YS agar and yeast extract-glucose broth containing 10 g/L yeast extract, and 10 g/L glucose. Members of these families do not require special procedures for maintenance or preservation. Cultures can be maintained by serial transfers onto the appropriate solid media. Growth on agar slants can be maintained at 4 °C for over 1 month. Medium-term preservation is in 12–15 % (v/v) glycerol suspensions at −80 °C. Long-term preservation of

Table 26.6
Phenotypic characteristics of *Quadrisphaera granulorum*

Utilizes	
α-Cyclodextrin, α-dl-glycerol phosphate, Tween 40, arbutin, glucose 1-phosphate, Tween 80, glucose 6-phosphate, adonitol, L-arabinose, D-arabitol, glucuronamide, cellobiose, D-psicose, D-mannitol, D-melezitose, D-melibiose, L-serine, methyl β-D-glucoside, psicose, D-xylose, methyl pyruvate, pyruvate, 2-aminoethanol, mono-methyl succinate, glycerol, L-serine, turanose, glucuronic acid, α-ketobutyric acid, α-ketovaleric acid, dl-lactic acid, L-aspartic acid, L-glutamic acid, uroconic acid, pyruvic acid	Positive
β-Cyclodextrin, dextrin, glycogen, inulin, mannan, *N*-acetyl D-galactosamine, *N*-acetylglucosamine, *N*-acetylmannosamine, amygdalin, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-glucuronic acid, α-D-glucose, m-inositol, α-D-lactulose, α-lactose, maltose, D-mannitol, D-mannose, D-melezitose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, methyl α-D-glucoside, methyl β-D-glucoside, methyl α-D-mannoside, palatinose, D-raffinose, L-rhamnose, salicin, sedoheptulosan, D-sorbitol, stachyose, sucrose, D-trehalose, xylitol, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, α-ketoglutaric acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, itaconic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, lactamide, D-lactic acid methyl ester, D-malic acid, L-malic acid, propionic acid, succinamic acid, succinic acid, *N*-acetylglutamic acid, bromosuccinic acid, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, glycyl-L-glutamic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, glycyl-L-aspartic acid, L-histidine, hydroxyl-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, adenosine, 2′-deoxyadenosine, inosine, thymidine, uridine, adenosine 5′-monophosphate, thymidine 5′-monophosphate, uridine 5′-monophosphate, phenylethylamine, putrescine	Negative
Enzyme activities	
Esterase, esterase lipase, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase	Positive
Alkaline phosphatase, lipase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase., arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, gelatinase	Negative
Catalase	Positive
Oxidase	Negative
Production of H_2S	Negative
Production of indole	Negative
Voges–Proskauer-activity	Negative
Production of acetoin	Negative
Reduction from nitrate to nitrite	Negative

liquid cultures supplemented with 12–15 % (v/v) glycerol or 7 % (v/v) dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−150 °C). Freeze-drying and L-drying methods can be also applied for long-term storage.

Ecology

Members of the family *Kineosporiaceae* were isolated from soils, broad-leaf forest soil, an air sample, soil adhering to a root of a wild plant (*Pseucedanum japonicum* Thumb), the roots of a Thai medicinal plant, *Gynura pseudochina* (L.) DC. var. *hispida* Thwaites, soil collected from dry-hot river valley, sediment sample of the coast, shielded cell facility, rhizosphere soil of a cliff associated plant (*Peucedanum japonicum* Thunb.), desert sand, plant litter, surface-sterilized stem sample of *Tripterygium wilfordii*, fallen leaves, root of galingale (*Cyperus microiria*), sphagnum, leaves of cat-tail (*Typha latifolia*), aerobic granules, roof tile.

Pathogenicity and Clinical Relevance

Pathogenicity has not been reported for any members of the family *Kineosporiaceae*.

Application

There have been several reports regarding the special use of members of the family *Kineosporiaceae*.

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27 The Family *Micrococcaceae*

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Abstract
The family *Micrococcaceae* is well-defined family within the order *Actinomycetales*. Members of the family are defined by a wide range of morphological and chemotaxonomic properties, such as polar lipids, fatty acids, amino acids of peptidoglycan, and whole-cell sugars which are used for the delineation of genera and species. All genera of the family *Micrococcaceae* are characterized by the occurrence of L-lysine as diagnostic diamino acid of the type A peptidoglycan and predominance of *iso*- and *anteiso*-branched cellular fatty acids but differ markedly in the structures of their interpeptide bridges and in the composition of their respiratory quinones predominantly with 7–10 isoprenoid units in the side chain, which are either completely unsaturated, dihydrogenated, or a combination of both. Polar lipid profiles usually contain phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and unknown glycolipid(s). Genera affiliated with this family are *Acaricomes*, *Arthrobacter*, *Auritidibacter*, *Citricoccus*, *Enteractinococcus*, *Kocuria*, *Micrococcus*, *Nesterenkonia*, *Renibacterium*, *Rothia*, *Sinomonas*, *Yaniella*, and *Zhihengliuella*. Members of the family are mainly found in mammalian skin, clinical specimen, blood cultures, and in various soil samples as well as marine

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Taxonomy, Historical and Current

Short Description of the Family

Mic.ro.co.cca'.ce.ae. M.L. fem. n *Micrococcus*, type genus of the family; suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Micrococcaceae*, the *Micrococcus* family. Spherical or elliptical usually aerobic eubacteria that produce yellow or orange or red pigment.

The family *Micrococcaceae* proposed by Pribram (1929) was later reorganized and emended by Stackebrandt et al. (1997) exclusively based on signature nucleotides in the 16S rRNA gene sequences. At that time, in addition to the type genus *Micrococcus*, the genera *Arthrobacter* (Conn and Dimmick 1947), *Kocuria* (Koch et al. 1995), *Nesterenkonia* (Stackebrandt et al. 1995), *Renibacterium* (Sanders and Fryer 1980), *Rothia* (Georg and Brown 1967), and *Stomatococcus* (Bergan and Kocur 1982) were placed into this family. The single species of the latter genus, *Stomatococcus mucilaginosus*, was reclassified as *Rothia mucilaginosa* (Collins et al. 2000). Subsequently, seven additional genera were assigned to the family including *Citricoccus* (Altenburger et al. 2002), *Acaricomes* (Pukall et al. 2006), *Zhihengliuella* (Zhang et al. 2007), *Yaniella* (Li et al. 2008a), *Sinomonas* (Zhou et al. 2009), *Auritidibacter* (Yassin et al. 2011), and *Enteractinococcus* (Cao et al. 2012) that have also been incorporated into the family *Micrococcaceae*. Genera of this family share the characteristics of the order *Micrococcales*. Gram stain positive. Morphological forms vary from cocci to short slender, irregular rod coccobacilli with varying sizes, which may show primary branching; short filaments and coccoid forms may occur in late exponential phase cultures; or vegetative mycelia with the oldest parts of the mycelium fragment into sections of different sizes and more or less irregular shape and fragmentation may continue to yield rodlike cells in appearance and arrangement. Aerial mycelium is not formed. Species mostly are nonmotile. Aerobic to facultatively anaerobic, producing acid from a variety of carbohydrates. Cross-linkage of the peptidoglycan is of A type with a lysine diamino acid. Interpeptide bridges contain either aspartic acid or glutamic acid. The prominent fatty acids are $C_{15:0}$ anteiso, $C_{15:0}$ iso, or $C_{15:0}$ anteiso and $C_{17:0}$ anteiso. Predominant menaquinones are MK-7, MK-8, MK-9, and MK-10 isoprenoid quinone. The polyamines of the representatives of the family examined thus far, including species of the genera *Micrococcus*, *Kocuria*, *Arthrobacter*, and *Citricoccus* (Altenburger et al. 1997, 2002; Gvozdiak et al. 1998; Hamana 1994), are mainly spermidine, often with moderate amounts of spermine. Representatives of the family are characterized by a pattern of 16S rRNA gene sequence signature nucleotides at positions 41:401 (G-C), 45–396 (U-G), 69–99 (A-U), 144–178 (C-G), 140–223 (R-U), 142–221 (C-G), 157–164 (G-U), 248–276 (C-G), 258–268 (A-U), 293–304 (G-U), 379–384 (C-G), 407–435 (A-U), 502–543 (R-Y), 586–755 (C-G), 589–650 (C-G), 591–648 (U-A), 610 (G), 602–636 (C-G), 615–625 (G-C), 839:847 (A-U), 863 (U), 1133–1141 (A-U), 1244–1293 (C-G), 1254–1283 (G-C), 1263–1272 (A-U), 1310–1327 (R-Y), and 1414–1486 (C-G) (Zhi et al. 2009). Polar lipid profiles usually contain phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and unknown glycolipid(s). Usually found in soil, rhizosphere, solar salt, fish, blood cultures and mammalian skin. The genomic G+C content value varies from 57.7 to 76.0 mol% (*Table 27.1*). Regardless of the complex phylogeny, the *Micrococcaceae* is well defined in rRNA gene trees. It includes all the genera that were classified within this family in the previous road map except for *Stomatococcus*, which has since been reclassified to the genus *Rothia* (Collins et al. 2000; Garrity et al. 2005). In addition, six genera have been added, including *Acaricomes*, *Yaniella*, *Zhihengliuella*, and the recently described three genera *Sinomonas* (Zhou et al. 2009), *Auritidibacter* (Yassin et al. 2011), and *Enteractinococcus* (Cao et al. 2012).

Phylogenetic Structure of the Family and Its Genera

According to the phylogenetic branching of actinobacterial type strains in the RaxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2008, 2010), the family is moderately related to the families *Brevibacteriaceae* and *Dermabacteraceae*. A phylogenetically broad group containing *Microbacteriaceae*, *Actinomycetaceae*, and several other less species-rich families appear as a sister clade of these families (*Fig. 27.1*).

The genus *Micrococcus* forms a subclade within the family that is closely related to the genus *Citricoccus*. This relationship is supported by similarities in the cell-wall composition, major fatty acids, and polar lipids, although significant differences are present in the menaquinone composition (Busse 2012a). The genus *Micrococcus* comprises the type species *Micrococcus luteus* and five closely related species *Micrococcus antarcticus*, *M. endophyticus*, *M. flavus*, *M. lylae*, and *M. yunnanensis*. In rRNA gene trees, the monospecific genus *Acaricomes* (type species *Acaricomes phytoseiuli*) is closely related to *Arthrobacter sanguinis* within the large radiation of *Arthrobacter* species (dealt in separate chapter on "*Arthrobacter*"). However, chemotaxonomic data which might support this relationship are not available, and it remains unproven (Busse 2012a). The rRNA gene tree of the genus *Arthrobacter* is complex with many short branches and multifurcations that are difficult to resolve. Moreover, the genera *Acaricomes*, *Renibacterium*, and *Zhihengliuella* appear within the radiation that includes the type species *Arthrobacter globiformis*, making the genus *Arthrobacter* paraphyletic. Reclassification of many *Arthrobacter* species may be necessary in the future to reduce the diversity of the genus.

Table 27.1

Chemotaxonomic characteristics of genera of the family *Micrococcaceae*

Genera	Characteristic				
	Quinone system	Peptidoglycan interpeptide bridge	Major fatty acids	Major polar lipids	DNA G+C content (mol%)
Acaricomes[a]	MK-10(H2), MMK-10(H2)	L-Lys–L-Ala	$C_{15:0}$ anteiso and $C_{17:0}$ anteiso	DPG, PG, PI	57.7
Auritidibacter[b]	MK-10	L-Lys–Gly–L-Glu	iso-$C_{15:0}$ and anteiso-$C_{17:0}$	DPG, PG, PI. GL	59.7
Citricoccus[c]	MK-9(H2), MK-7(H2) MK-8(H2)	Lys–Gly–Glu	$C_{15:0}$ anteiso or $C_{15:0}$ anteiso and $C_{17:0}$ anteiso	DPG, PG, PI, GL, PL, UL	63.8–68.0
Kocuria[d]	MK-7(H2) or MK-8(H2) or MK-7(H2), MK-8(H2) or MK-8(H2), MK-9(H2), MK-7(H2) or MK-8(H2), MK-9(H2)	L-Lys–L-Ala3–4	$C_{15:0}$ anteiso	DPG, PG,PI	60.0–75.3
Micrococcus[e]	MK-8, MK-8(H2) or MK-8(H2) or MK-7(H2), MK-8(H2)	L-Lys–peptide subunit or L-Lys–D-Asp	$C_{15:0}$ anteiso or $C_{15:0}$ anteiso and $C_{15:0}$ iso	PG, DPG, PI, GL	66.4–75.5
Nesterenkonia[f]	MK-8, MK-9, MK-7	Lys–Gly–Glu, Lys–Glu, or Lys–Gly–Asp	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	DPG, PG, PI, PL, GL	64–72
Renibacterium[g]	MK-9, MK-10	Lys–Ala–Glym	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso	DPG, GL	52–54
Rothia[h]	MK-7 or MK-7, MK-6(H2)	Lys–Ala3, Lys–Ala, Lys–Ser, Lys–Gly, or Lys–Gly–Ala	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso, $C_{16:0}$	DPG, PG	49–60
Sinomonas[i]	MK-9(H_2), MK-8(H_2)	L-Lys–L-Ala3	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{17:0}$ anteiso	DPG, PG, PI, PME	71
Yaniella[j]	MK-8 and MK-9 or MK-8	L-Lys–Gly–L-Glu	$C_{15:0}$ anteiso,$C_{15:0}$ iso, or $C_{15:0}$ anteiso	DPG, PG, PL, GL	53–58
Zhihengliuella[k]	MK-9, MK-10	L-Ala–L-Glu	$C_{15:0}$ anteiso, $C_{15:0}$ iso, $C_{17:0}$ anteiso	DPG, PI, PG, PL, GL(s)	66.5–70.3
Enteractinococcus[l]	MK-7, MK-8	L-Lys–Gly–L-Glu	$C_{15:0}$ iso, $C_{15:0}$ anteiso, $C_{16:0}$ iso	DPG, PG, PI, PIM, DMDG, GL	56.2–61.6

[a]Data from Pukall et al. (2006)
[b]Data from Yassin et al. (2011)
[c]Data from Altenburger et al. (2002) and Li et al. (2005b)
[d]Data from Stackebrandt et al. (1995), Kovács et al. (1999), Reddy et al. (2003), Kim et al. (2004), Tvrzová et al. (2005a), Li et al. (2006), Mayilraj et al. (2006), and Zhou et al. (2008)
[e]Data from Kocur et al. (1972), Stackebrandt et al. (1995), Wieser et al. (2002), Liu et al. (2000), and Liu et al. (2007)
[f]Data from Stackebrandt et al. (1995), Collins et al. (2002), Li et al. (2005a, 2004b, 2008a), Delgado et al. (2006), and Yoon et al. (2006)
[g]Data from Sanders and Fryer (1980)
[h]Data from Stackebrandt et al. (1995), Collins et al. (2000), Fan et al. (2002), Li et al. (2004c), and Chou et al. (2008)
[i]Data from Zhou et al. (2009)
[j]Data from Li et al. (2004a)
[k]Zhang et al. (2007) and Zhi et al. (2009)
[l]Cao et al. (2012)
DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *DMDG* dimannosyl diacylglycerol, *GL* unknown glycolipid, *PL* unknown phospholipid, *PME* phosphatidylethanolamine

In addition, some species are more closely affiliated with type species of other genera in the family *Micrococcaceae* and should be reclassified on those grounds. A combination of rRNA gene sequence similarity and chemotaxonomic features has been used to further classify *Arthrobacter* species into four "rRNA clusters," five "subclades," and two "groups" (Busse 2012). The rRNA clusters comprise species with similar chemotaxonomic features and high rRNA gene sequence similarity that do not form a discrete clade in the phylogenetic trees. In rRNA gene trees, this latter group is affiliated with the clade containing the genera

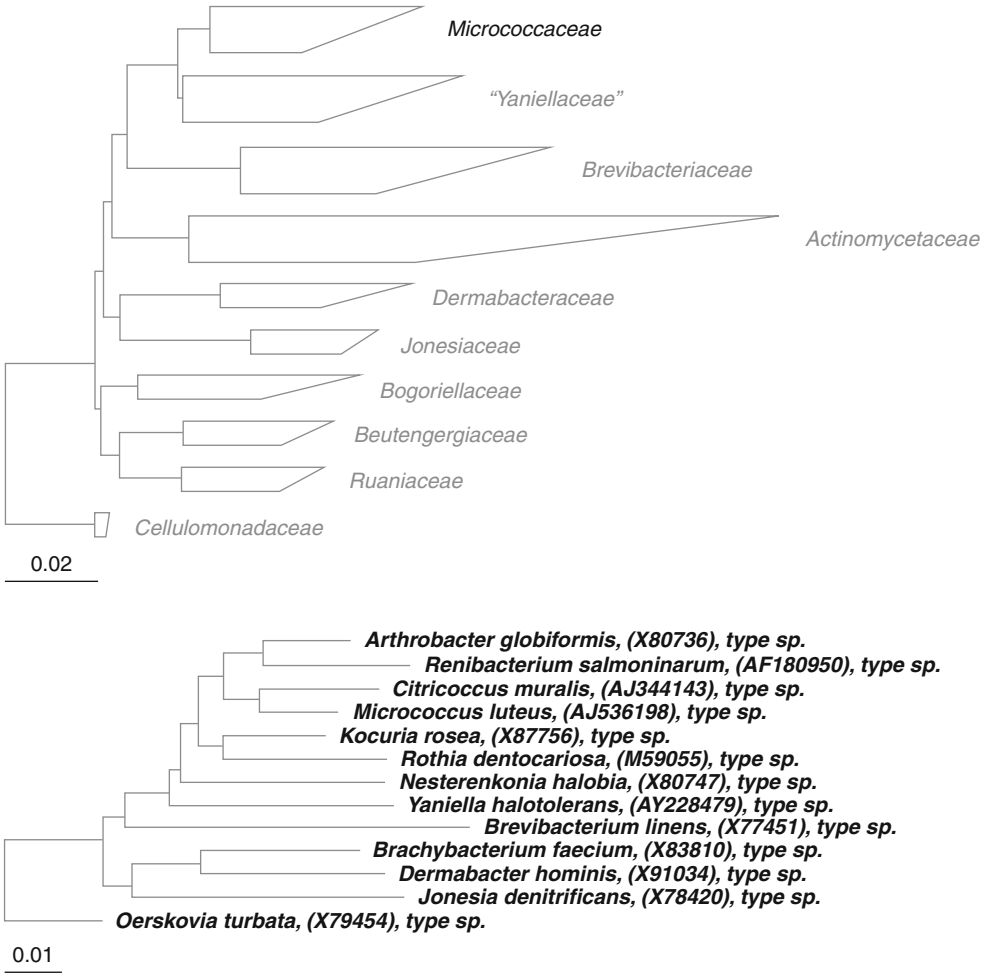

Fig. 27.1
Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the family *Micrococcaceae* present in the LTP_106 (Yarza et al. 2010). The number in triangle denotes the number of taxa included. The bar indicates 5 % sequence divergence (Stamatakis et al. 2005)

Nesterenkonia, *Sinomonas*, and *Yaniella*. Possibly, they represent a novel genus within this clade (Heyrman et al. 2005). The genus *Citricoccus* is closely related to the genus *Micrococcus* in rRNA gene trees. It comprises the type species *Citricoccus muralis* and the closely related species *Citricoccus alkalitolerans*, *C. parietis*, and *C. zhacaiensis*. The genus *Kocuria* represents one of the deepest lineages in the family *Micrococcaceae*. The genus comprises four clades. The first contains the type species *Kocuria rosea* as well as *Kocuria aegyptia*, *K. flava*, *K. himachalensis*, *K. polaris*, and *K. turfanensis*. The second clade is closely related and comprises *Kocuria halotolerans*, *K. koreensis*, and *K. kristinae* as well as the genus *Rothia*. The third clade includes only *Kocuria palustris*. The last clade appears as the deepest lineage in the family and comprises *Kocuria atrinae*, *K. carniphila*, *K. gwangalliensis*, *K. marina*, *K. rhizophila*, and *K. varians*. However, the biological significance of these clades is not currently supported by chemotaxonomic or other evidences, so their importance is not yet certain. The genus *Nesterenkonia* is in a clade containing the genera *Sinomonas* and *Yaniella* and the *Arthrobacter* species *Arthrobacter albus* and *A. cumminsii*. The genus contains two subclades. The first comprises the type species *Nesterenkonia halobia* and *Nesterenkonia aethiopica*, *N. alba*, N. *flava*, *N. halophila*, *N. lacusekhoensis*, and *N. xinjiangensis*. The second, closely related subclade comprises *Nesterenkonia halotolerans*, *N. jeotgali*, *N. lutea*, and *N. sandarakina*. Although the chemotaxonomic and physiological properties of the two clades are very similar, only members of this second subclade possess peptidoglycan containing L-Lys–Gly–D-Asp (Stackebrandt 2011). In the first subclade, the peptidoglycan contains L-Lys–Gly–D-Glu or L-Lys–D-Glu. The monospecific genus *Renibacterium* (type species *Renibacterium salmoninarum*) is related to *Arthrobacter russicus* and *Arthrobacter* Subclade IV, which includes *Arthrobacter psychrolactophilus*, *A. stackebrandtii*, and *A. psychrochitiniphilus*. Differences in the menaquinone and peptidoglycan composition of *Renibacterium* and the *Arthrobacter* species do not provide support for this affiliation, although it is possible that *Renibacterium* was derived from an

Arthrobacter ancestor by changes in these and other characters. The genus *Rothia* includes a well-defined clade composed of the type species *Rothia dentocariosa* and *Rothia aeria*, *R. amarae*, *R. mucilaginosa*, *R. nasimurium*, and *R. terrae*. These taxa are also related to some species of *Kocuria*. The genus *Sinomonas* comprises the type species *Sinomonas flava* and *Sinomonas atrocyanea*. In rRNA gene trees, it is affiliated with the clade containing the genera *Nesterenkonia*, *Yaniella*, and the *Arthrobacter* group 2 species *Arthrobacter albus* and *A. cumminsii*. The genus *Yaniella* contains the type species *Yaniella halotolerans* and *Yaniella flava*. Although originally classified in its own family (Li et al. 2008a), the rRNA gene trees calculated here suggest it is closely related to *Nesterenkonia*, *Sinomonas*, and *Arthrobacter* group 2 species *Arthrobacter albus* and *A. cumminsii*. This conclusion is consistent with similarities in cell-wall, menaquinone, and phospholipid compositions (Yassin et al. 2011). However, the DNA G+C content is quite different, 53–58 mol% in *Yaniella* and 64–72 mol% in *Nesterenkonia*. The genus *Zhihengliuella* comprises the type species *Zhihengliuella halotolerans* and *Zhihengliuella alba* and is closely related to the subclades of *Arthrobacter*. Presumably, this relationship reflects the heterogeneity of the genus *Arthrobacter* rather than the need for reclassification of the genus *Zhihengliuella*, and apart from this, two more genera have been validly published like *Auritidibacter and Enteractinococcus* where *Auritidibacter* has one species and *Enteractinococcus* has two valid species: *Enteractinococcus coprophilus* and another reclassified species from *Yaniella fodinae* to *Enteractinococcus fodinae*.

Molecular Analyses

DNA–DNA Hybridization Studies

A description of all *Citricoccus* species includes results of DNA–DNA hybridization (DDH) studies, and a few species only were found to be closely related, e.g., *C. alkalitolerans* and *C. muralis* (56 %; Li et al. 2005b) and a cluster comprising *C. parietis* (35–63 %; Schäfer et al. 2010) and *C. zhacaiensis* 39–54 % (Meng et al. 2010). Type strains of the *Kocuria* species based on DDH results revealed similarity values below 70 %, indicative of separate genomospecies. DDH values are moderately high values ranging between 31 % and 71 % similarity like *K. rhizophila* (31 %) with *K. palustris*, whereas others related to *Kocuria* shows 37–46 % (Kovács et al. 1999). *K. polaris*, *K. rosea*, *K. flava*, *K. aegyptia*, and *K. turfanensis* are close neighbors, as strains of these species share between 64 % and 75 % DNA similarity (Reddy et al. 2003; Zhou et al. 2008). DDH relatedness of *Micrococcus* species was reported in the range of 27–65 %, e.g., *M. antarcticus*, *M. luteus*, and *M. lylae* with 40 % similarity (Liu et al. 2000), *M. flavus* (36–55 %, Liu et al. 2007), *M. endophyticus* (27–54.2 %, Chen et al. 2009), *M. yunnanensis* (27–65.4 %, Zhao et al. 2009), and *M. terreus* (39–57.5 %, Zhang et al. 2010). DDH reassociation values for *Nesterenkonia* species were of 11–66 %. For example, *N. halotolerans* with strains *N. sandarakina* and *N. lutea* showed 43.3 % and 39.1 %, respectively, and between the *N. sandarakina* and *N. lutea* was 45.2 %. DDH results of *N. aethiopica* with the closest phylogenetic affiliation, i.e., *N. halobia*, *N. lacusekhoensis*, and *N. xinjiangensis*, showed between 35.7 % and 63.7 %. While DDH relatedness between strain *N. jeotgali* and other type strains of *Nesterenkonia* species showed value of 11–53 % (Stackebrandt et al. 1995; Collins et al. 2002; Li et al. 2004b, 2005a), *N. flava* with 14–66 % relatedness to its closest phylogenetic neighbor (Luo et al. 2008). *N. alba* revealed 35–60.7 % relatedness with other species (Luo et al. 2009). *Rothia* species revealed the DDH relatedness between *Rothia aeria* and *R. dentocariosa* genomovar II was 100 %, whereas they showed only 34.1 and 21.2 % relatedness with their related neighbor's like *R. mucilaginosa* and *R. dentocariosa* (Li et al. 2004c). *R. terrae* and its closest phylogenetic neighbors showed 15–21 % DDH relatedness (Chou et al. 2008). Species of *Sinomonas* have moderate DDH relatedness, e.g., *S. flava* and *S. atrocyanea* (52.2 %, Zhou et al. 2009). *Zhihengliuella* species also showed moderate level of DDH relatedness between strains *Z. alba* and *Z. halotolerans* (41.4 %, Tang et al. 2009a).

Riboprinting and Ribotyping

The study including DNA fingerprinting method was that of Yoon et al. (2006) who performed combined rep-PCR fingerprints generated by REP, BOX, and $(GTG)_5$ PCR primers. The cutoff point for the recognition of clades was set at 30 % similarity (*Fig. 27.2*) on the type strains of *Nesterenkonia* species to confirm their membership to the genus. As the number of bands is low, the phylogenetic significance is restricted, and the resulting dendrogram of ribopatterns bears no similarity with the 16S rRNA gene dendrogram. At the intraspecies level, the patterns may vary significantly, and the ribopattern of the type strain does not necessarily characterize additional strains of the species (Yoon et al. 2006). RiboPrint analysis in *Kocuria* species by the RiboPrint robot (Qualicon, DuPont) indicates that a unique pattern defines each type strain (*Fig. 27.3*). As the number of bands is low, the phylogenetic significance is restricted, and the resulting dendrogram of ribopatterns bears no similarity with the 16S rRNA gene dendrogram (Busse 2012). Neither riboprinting nor ribotyping analyses are available for any member of other genera in the *Micrococcaceae* family. At the intraspecies level, the patterns may vary significantly, and the ribopattern of the type strain has limited significance taxonomically.

Fourier-Transform Infrared (FT-IR) Spectroscopy

The method and its taxonomic application were described in detail by Oberreuter et al. (2002). The method is particularly valuable for the differentiation of related species from similar ecological niches, which display almost identical phenotypic traits in terms of traditional physiological and biochemical criteria and similar (identical) colony colors. It should be noted that spectral similarities of strains do not necessarily correspond to their

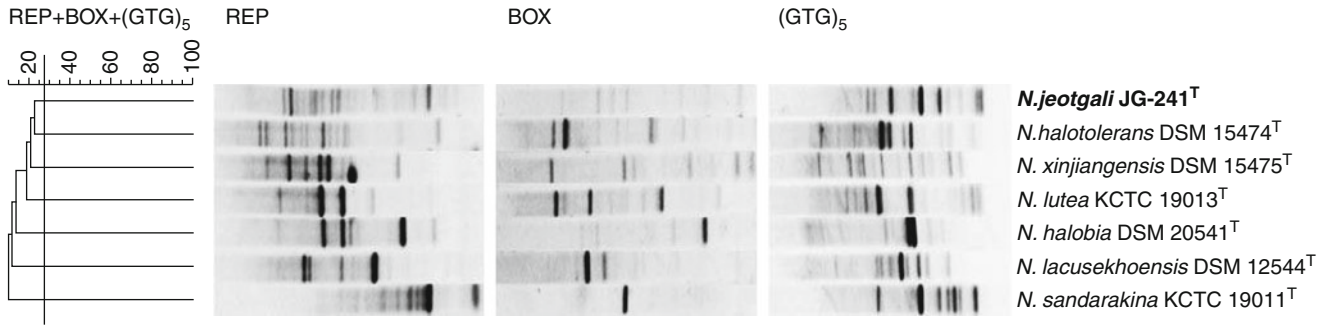

Fig. 27.2

Consensus dendrogram showing relationships among *Nesterenkonia* in the *Micrococcaceae* family based on similarity values derived by using the Jaccard correlation coefficient and the UPGMA algorithm in an analysis of the combined rep-PCR fingerprints generated by REP, BOX, and $(GTG)_5$ PCR primers. The cutoff point for the recognition of clades was set at 30 % similarity

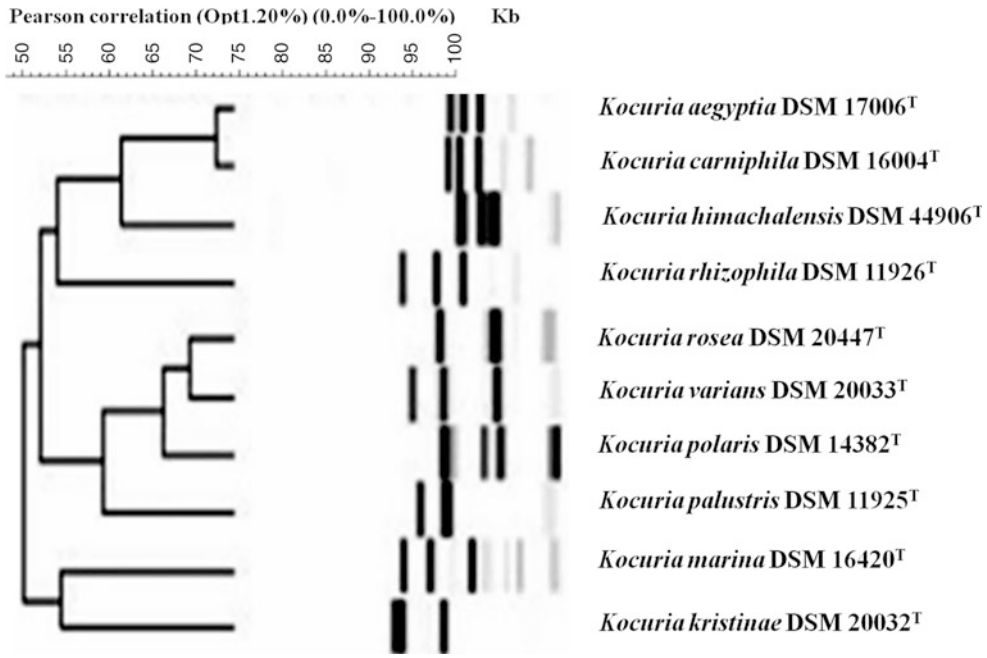

Fig. 27.3

Normalized *Eco*RI RiboPrint profiles (Qualicon, DuPont) of *Kocuria* species of *Micrococcaceae* family and a dendrogram of band pattern relatedness as generated by using BioNumerics software (Applied Maths, Kortrijk, Belgium)

phylogenetic relationships (Kümmerle et al. 1998; Oberreuter et al. 2002). The method has been tested on many representatives of the suborder *Micrococcineae* (Oberreuter et al. 2002).

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI/TOF)

MALDI/TOF mass spectrometry is a rapid and efficient identification method for intact whole bacteria (Holland et al. 1996, 1999) which has been proven to aid medical diagnostics and risk assessment. This technique may cover a broad taxonomic range depending on the instrument conditions chosen, from the genus level up to the authentication of strains. Although reports on the application of MALDI/TOF mass spectrometry for members of the suborder *Micrococcineae* (Vargha et al. 2006) are still rare, this fast method has turned out to be of great taxonomic importance when compared with gene sequence phylogenies (Stackebrandt et al. 2005), and its application as a tool for characterization and differentiation at the species level is highly encouraged.

Genome Analyses

The genomes of five members of this family have been sequenced to date, namely, *Arthrobacter aurescens* TC1 and *Arthrobacter* sp. strain FB24, *Kocuria rhizophila*, and *Renibacterium*

salmoninarum (Mongodin et al. 2006; Kim et al. 2011). Sequencing and annotation of the genome analysis of strain *K. rhizophila* DC2201 (NBRC 103217, Hiromi et al. 2008) revealed a single circular chromosome (2,697,540 bp; G+C content of 71.16 %) containing 2,357 predicted protein-coding genes. Most of the predicted proteins (87.7 %) were orthologous to actinobacterial proteins, and the genome showed fairly good conservation of synteny with taxonomically related actinobacterial genomes. On the other hand, the genome seems to encode much smaller numbers of proteins necessary for secondary metabolism (one each of non-ribosomal peptide synthetase and type III polyketide synthase), transcriptional regulation, and lateral gene transfer, reflecting the small genome size. The presence of probable metabolic pathways for the transformation of phenolic compounds generated from the decomposition of plant materials and the presence of a large number of genes associated with membrane transport, particularly amino acid transporters and drug efflux pumps, may contribute to the organism's utilization of root exudates, as well as the tolerance to various organic compounds.

Renibacterium salmoninarum is the causative agent of bacterial kidney disease and a significant threat to healthy and sustainable production of salmonid fish worldwide. The complete genome of *R. salmoninarum* ATCC 33209 was sequenced (Wiens et al. 2008) and shown to be a 3,155,250-bp circular chromosome that is predicted to contain 3,507 open-reading frames (ORFs). A total of 80 copies of three different insertion sequence elements are interspersed throughout the genome. Approximately 21 % of the predicted ORFs have been inactivated via frameshifts, point mutations, insertion sequences, and putative deletions. The *R. salmoninarum* genome has extended regions of synteny to the *Arthrobacter* sp. strain FB24 and *Arthrobacter aurescens* TC1 genomes, but it is approximately 1.9 Mb smaller than both *Arthrobacter* genomes and has a lower G+C content, suggesting that significant genome reduction has occurred since divergence from the last common ancestor. A limited set of putative virulence factors appear to have been acquired via horizontal transmission after divergence of the species; these factors include capsular polysaccharides, heme sequestration molecules, and the major secreted cell surface antigen p57 (also known as major soluble antigen). Examination of the genome revealed a number of ORFs homologous to antibiotic resistance genes, including genes encoding β-lactamases, efflux proteins, macrolide glycosyltransferases, and rRNA methyltransferases. The genome sequence provides new insights into *R. salmoninarum* evolution and may facilitate identification of chemotherapeutic targets and vaccine candidates that can be used for prevention and treatment of infections in cultured salmonids.

Micrococcus luteus (NCTC2665, "Fleming strain") has one of the smallest genomes of free-living actinobacteria sequenced to date, comprising a single circular chromosome of 2,501,097 bp (G+C content, 73 %) predicted to encode 2,403 proteins (Young et al. 2010). The genome shows extensive synteny with that of the closely related organism, *Kocuria rhizophila*, from which it was taxonomically separated relatively. Despite its small size, the genome harbors 73 insertion sequence (IS) elements, almost all of which are closely related to elements found in other actinobacteria. An IS element is inserted into the *rrs* gene of one of only two *rrn* operons found in *M. luteus*. The genome encodes only four sigma factors and 14 response regulators, a finding indicative of adaptation to a rather strict ecological niche (mammalian skin). The high sensitivity of *M. luteus* to β-lactam antibiotics may result from the presence of a reduced set of penicillin-binding proteins and the absence of a *wblC* gene, which plays an important role in the antibiotic resistance in other actinobacteria. Consistent with the restricted range of compounds it can use as a sole source of carbon for energy and growth, *M. luteus* has a minimal complement of genes concerned with carbohydrate transport and metabolism. It has very few genes associated with secondary metabolism. In contrast to most other actinobacteria, *M. luteus* encodes only one resuscitation-promoting factor (Rpf) required for emergence from dormancy, and its complement of other dormancy-related proteins is also much reduced. *M. luteus* is capable of long-chain alkene biosynthesis, which is of interest for advanced biofuel production; a three-gene cluster essential for this metabolism has been identified in the genome.

Draft genome sequence of *Citricoccus* strain CH26A genome was submitted recently (Hayano-Kanashiro et al. 2011). A total of 826,643 reads were generated and assembled de novo with the Newbler assembler, v. 2.5, yielding 67 contigs with an *N*50 of 121 kb and the largest contig of 299.376 kb. Size of the *Citricoccus* CH26A genome is 3.7 Mb, with a mean GC content of 70.96 %. Gene prediction and annotation carried out further and identified 3,030 coding sequences, grouped into 23 categories. Genome-scale metabolic reconstruction (GSMR) of central metabolic pathways using an actinomycete-focused metabolic database analysis showed the presence of all genes for the glycolytic and pentose phosphate pathways, as well as those for the biosynthesis of amino acids and nucleotides.

The sequencing of draft genome of *K. atrinae* C3-8 produced 2,116,953 reads with an average length of 123 bases covering 261 Mb (Nam et al. 2012). The sequence reads were assembled into 221 contigs (>1 kb in size; 246 contigs greater than 500 bases) with approximately 82-fold coverage. The draft genome of *K. atrinae* C3-8 is 3.19 Mbp long with a G+C content of 63.8 %. Single copies of the 5S, 16S, and 23S rRNAs and 45 tRNAs were identified using RNAmmer 1.2 (Lagesen et al. 2007) and tRNA scan-SE (Lowe and Eddy 1997), respectively. A total of 3,959 predicted protein-coding sequences were annotated (Aziz et al. 2008). Of the predicted protein-coding genes, 1,243 (31.4 %) were assigned as encoding hypothetical proteins. These included 497 genes related to carbohydrate metabolism. A total of 205 genes were related to protein metabolism. A single gene for histidinol phosphate phosphatase (EC 3.3.3.15), which catalyzes the biosynthesis of histidine, was identified. Eight genes were related to molybdopterin synthesis. A more detailed analysis of this genome will provide useful information related to the application of microorganisms in the food industry.

Genome sequencing of *Nesterenkonia* sp. strain F was performed (Sarikhan et al. 2011) and obtained a total of 201,320 random reads, covering a total of 44,347,672 bp. The approximate coverage of *Nesterenkonia* sp. strain F genome was 16-fold. The sequence reads were assembled into 138 contigs. The G+C content of the draft genome was 71.5 %, similar to that previously reported by Stackebrandt et al. (1995). The sum of the sizes of the 138 large contigs in *Nesterenkonia* sp. strain F is 2,812,133 bp. The draft genome contained 2,484 genes, with 1,794 nonhypothetical and 690 hypothetical protein-coding sequences and 50 structural RNAs. Analysis of annotated genome sequence of *Nesterenkonia* sp. strain F revealed the presence of genes involved in production of α-amylases, including maltodextrin glucosidase and alpha-glucosidase. In addition, the genes encoding proteins involved in resistance to heavy metals and toxic compounds, including copper homeostasis, cobalt–zinc–cadmium resistance, arsenic resistance, and β-lactamase, were identified. These results show the genetic potential of the *Nesterenkonia* sp. strain to adapt to extreme lifestyles. Furthermore, the genome comprises clustered regularly interspaced short palindromic repeats (CRISPR), which constitutes an effective mechanism against foreign genetic elements as an adaptive immune system. The CRISPR-associated helicase Cas3, the RecB family exonuclease Cas4b, and the CRISPR-associated protein Cas1 have been detected. The *Nesterenkonia* sp. strain has also glycolysis and gluconeogenesis systems, a pentose phosphate pathway, and a tricarboxylic acid cycle. It contains genes for response to osmotic and oxidative stresses, in addition to heat shock, cold shock, and detoxification systems, and also the genes involved in ammonia assimilation and nitrate and nitrite ammonification, which are very attractive for bioremediation studies.

Phages

The set of phages proposed to be specific of the genus *Micrococcus* (Sozzi et al. 1973) are phages N1 (ATCC 4698-B1), N4 (ATCC 4698-B2), N8 (ATCC 4698-B3), and N3 (ATCC 4698 B4) for host *Micrococcus luteus* ATCC 4698.

Phenotypic Analyses

Micrococcus Cohn (1872), 151[AL] Emend. Stackebrandt, Koch, Gvozdiak, Schumann (1995), 682 Emend. Wieser, Denner, Kämpfer, Schumann, Tindall, Steiner, Vybiral, Lubitz, Maszenan, Patel, Seviour, Radax, and Busse (2002), 635

Mi.cro.coć cus. Gr. adj. *mikros* small, little; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos* grain, seed) coccus; N.L. masc. n. *Micrococcus* small coccus.

Members of the genus share the *Micrococcaceae*-specific signature nucleotides at positions 293–304, 610, 598, 615–625, 1025–1036, 1026–1035, 1265–1270, and 1278 of the 16S rRNA gene sequence (*E. coli* numbering) and lack the signature nucleotides at positions 640, 839–847, and 859 (Stackebrandt et al. 1997).

Cells are spherical and nonmotile. Endospores are not formed. Gram stain positive, aerobic, and yellow colored. Chemoorganotrophic; metabolism is strictly respiratory. Catalase and oxidase positive, mesophilic, and non-halophilic. The peptidoglycan contains L-lysine as the diagnostic diamino acid. The peptidoglycan type is either A2, with the interpeptide bridge consisting of a stem peptide, or A4α both with lysine as the diagnostic diamino acid (Wieser et al. 2002). The predominant menaquinones are either MK-8 and MK-8(H2) or MK-8(H2) or MK-7(H2) and MK-8(H2). The cytochromes are *aa*3, *b*557, *b*567, and *d*626; cytochromes *c*550, *c*551, *b*563, *b*564, and *b*567 may be present. Polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, an unknown glycolipid, and an unknown ninhydrin-negative phospholipid. The cellular fatty acids are iso- and anteiso-branched fatty acids, with $C_{15:0}$ anteiso and $C_{15:0}$ iso predominating. The major aliphatic hydrocarbons (br-Δ-C) are C27 to C29. Mycolic acids and teichoic acids are absent; teichoic acids may be present. Mannosamine-uronic acid may be present as an amino sugar in the cell-wall polysaccharide. D-Arabinose, *p*-arbutin, D-cellobiose, D-galactose, D-melibiose, D-ribose, and salicin are not assimilated. Members of the genus share the *Micrococcaceae*-specific signature nucleotides at positions 293:304, 610, 598, 615:625, 1025:1036, 1026:1035, 1265:1270, and 1278 of the 16S rRNA gene sequence (*Escherichia coli* numbering) and lack the signature nucleotides at positions 640, 839:847, and 859 (Stackebrandt et al. 1997).

The genus *Micrococcus* is the type genus of the order *Micrococcales* Prévot 1940 and the family *Micrococcaceae* Pribram 1929. Former members of the genus including *Micrococcus varians*, *M. kristinae*, *M. roseus*, *M. agilis*, *M. sedentarius*, *M. nishinomiyaensis*, and *M. halobius* have been reclassified in other genera based on their distinct phylogenetic positions and differences in the quinone systems, interpeptide bridges of the peptidoglycans, and fatty acids (Koch et al. 1995; Stackebrandt et al. 1995). *Micrococcus varians*, *M. kristinae*, and *M. roseus* were reclassified as species of the genus *Kocuria*. *Micrococcus agilis* was transferred to the genus *Arthrobacter*. *Micrococcus sedentarius*, *Micrococcus nishinomiyaensis*, and *Micrococcus halobius* were proposed as species of the genera *Kytococcus*, *Dermacoccus*, and *Nesterenkonia*, respectively.

The genus *Micrococcus* (Cohn 1872) was emended after taxonomic dissection resulting from phylogenetic and chemotaxonomic analyses (Stackebrandt et al. 1995). *M. luteus* can be divided into three biovars that are distinguished by several chemotaxonomic and biochemical traits: biovar I, represented by *M. luteus* DSM 20030[T]; biovar II, represented by strains D7 (DSM 14234); and biovar III, represented by strain Ballarat (DSM 14235). On the basis of the results, emended descriptions of the genus *Micrococcus* and *M. luteus* into three biovars are as follows: biovar I, represented by *M. luteus* DSM 20030[T]

[peptidoglycan type A2, predominant quinones MK-8 and MK-8(H_2)]; biovar II, represented by strains D7, 3, 6, 7, 13C2, 38, 83, and 118 [peptidoglycan type A2, predominant quinone MK-8(H_2)]; and biovar III, represented by strain Ballarat [peptidoglycan type A4*a*, predominant quinone MK-8(H_2)]. Consequently selected physiological and biochemical properties appear to remain suitable for differentiating between *M. luteus* and *M. lylae* strains (Table 27.2).

The genus now consists of seven species, *Micrococcus luteus* (the type species of the genus), *M. lylae*, *M. antarcticus*, *M. endophyticus*, *M. yunnanensis*, *M. flavus*, and *M. terreus*. The type strains of the species are separated from each other at 97.8–99.8 % 16S rRNA gene sequence similarities. DNA G+C content (mol%): 69–76. The chemotaxonomic properties and other detailed characteristics of all the species are given in Table 27.3.

Kocuria Stackebrandt, Koch, Gvozdiak, and Schumann (1995), 690^{VP}

Ko.cu'ri.a. N.L. fem. n. *Kocuria*, named after Miroslav Kocur, a Slovakian microbiologist for his pioneering studies on Gram-stain-positive cocci.

The genus Kocuria was first proposed by Stackebrandt et al. (1995) on the basis of a detailed phylogenetic and chemotaxonomic analysis of the genus *Micrococcus* (Stackebrandt et al. 1995). This leaded further to the description of *Dermacoccus*, *Kocuria*, *Kytococcus*, and *Nesterenkonia*. The latter three genera are members of the family *Micrococcaceae*, order *Micrococcales*, within the emended order *Actinomycetales* (Buchanan 1917) (Stackebrandt et al. 1997). The new genus *Kocuria* embraced three former *Micrococcus* species, i.e., *Kocuria rosea* (*Micrococcus roseus* Flügge 1886), *Kocuria varians* (*Micrococcus varians* Migula 1900), and *Kocuria kristinae* (*Micrococcus kristinae* Kloos et al. 1974). Based upon 16S rRNA gene sequence similarities, *Kocuria* species appear to be equidistantly related to members of *Arthrobacter* and *Rothia*, but the bootstrap values of the deeply branching lineages within this family are low, thus without statistical significance. The genus currently comprises 17 recognized species and *Kocuria rosea* as a type species (*Micrococcus roseus* Flügge 1886 183^{AL}).

Species of the genus *Kocuria* are closely related among themselves and form four intrageneric lineages. The 16S rRNA gene sequence similarities indicate the presence of two strain clusters and two individual lineages. The branching order is supported by bootstrap values of higher than 70 % (in *Bergey's Manual of Systematic Bacteriology*, Vol. 5, p. 626). Members of these two clusters share higher than 98 % similarities among each other, while the four lineages are separated from each other by similarities of 95.5–97.5 %. The clustering of strains within each of the two clusters is correlated neither with similarities at the phenotypic level nor with the habitats from which they were isolated. However, *Eco*RI-based RiboPrint analysis by the RiboPrint robot (Qualicon, DuPont) using some members of Kocuria could not clearly differentiate between each species or any clue regarding two clusters.

Cells are coccoid, Gram positive, catalase positive, nonhalophilic (except *K. halotolerans*), mesophilic, nonencapsulated, non-endospore forming, and nonmotile. Strains are chemoorganotrophic, and metabolism is strictly respiratory. Most of them are aerobic; however, the strains of one species are slightly facultatively anaerobic. The cell-wall peptidoglycan type is A3a (L-Lys–L-Ala3–4); however, mycolic acids and teichoic acids are absent. Menaquinones are hydrogenated; the predominant menaquinones are MK-7(H2), MK-8, or MK-9(H2), either alone or in combination. Polar lipids include diphosphatidylglycerol and phosphatidylglycerol; phosphatidylinositol is present in one species. The major fatty acid is C15:0 anteiso. *DNA G+C content* (mol%): 66–75.

Kocuria species resemble each other in the majority of chemotaxonomic properties and differ from each other mostly in quantitative composition of these markers. The peptidoglycan is characterized by the presence of lysine in position 3 of the peptide subunit. The interpeptide bridge consists of 3 or 4 L-alanine residues, thus defined as the A3a variation (Schleifer and Kandler 1972). The isoprenoid quinones are hydrogenated menaquinones MK-8(H_2), MK-9(H_2), and MK-7(H_2), occurring either as a single component or in combination with each other. The major fatty acid is $C_{15:0}$ anteiso (>50 % of total), but $C_{15:0}$ iso and $C_{16:1}$ represent about 10 % each of the total. The base composition of DNA spans a rather broad range (60–75 mol%), which may be due to the different methods used for quantitation. At the interspecies level, a range of 9 % and 6 % has been determined for *Kocuria rosea* and *Kocuria varians* species, respectively, for which more than the type strain is available for investigation. The extreme values were determined for the recently described type strains of *Kocuria marina* (60 mol%) and *Kocuria himachalensis* (75.3 mol%). Chemotaxonomic characteristics have been discussed in Table 27.4.

Growth temperatures and salt tolerance parameters considerably vary. Most *Kocuria* strains grow between 20 °C and 37 °C. Strains like *Kocuria aegyptia* can grow at up to 40 °C, and *Kocuria marina* even up to 43 °C. Only the type strains of *Kocuria polaris* and *Kocuria marina* are able to grow at 5 °C. The strains of *Kocuria polaris* and *Kocuria palustris are unable to* grow and divide at 37 °C. Similarly, most strains tolerate 5 % NaCl, but only one strain tolerates 15 % NaCl. The optimal pH range required for growth may be broad (i.e., 5–12 for *Kocuria aegyptia* and 7–12 for *Kocuria polaris*) or narrow, usually slightly alkaliphilic. Main peculiar property is that all species produce acids from one or more carbohydrates which make them distinct from other authenticated members of *Micrococcus*. Majority strains do not degrade starch and DNA. Gelatinase, esterase, and lipase can be variably utilized by few species.

DNA–DNA hybridization experiments are conducted between various pairs of type strains, ultimately most of which

Table 27.2

Physiological and biochemical properties for differentiating between *M. luteus* and *M. lylae* and other biovars

Property	*M. lylae* DSM 20315^T	*M. luteus* DSM 20030^T	D7	3	6	7	13C2	38	83	118	Ballarat
Pigmentation	W	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Urease	–	+	+	–	–	+	–	–	–	–	+
Growth at pH6	–	–	+	–	(+)	+	(+)	(+)	+	(+)	–
Assimilation of											
D-Mannose	–	+	+	+	+	+	+	+	+	+	+
D-Maltose	+	–	+	+	+	+	+	+	+	+	+
D-Trehalose	+	–	+	+	+	+	+	+	+	+	+
D-Xylose	–	–	–	–	–	+	–	–	–	–	–
Adonitol	–	–	–	–	–	+	–	–	–	–	–
i-Inositol	–	–	–	+	–	–	–	–	–	–	–
Maltitol	+	+	+	+	–	+	+	+	+	+	–
D-Mannitol	–	–	–	–	–	–	–	–	+	–	–
D-Sorbitol	–	–	–	–	–	–	–	–	+	–	–
Putrescine	–	–	+	+	+	+	–	+	+	+	–
Acetate	+	–	+	+	+	+	+	+	+	+	–
Propionate	–	+	+	+	+	+	+	+	+	+	–
4-Aminobutyrate	–	–	+	+	+	+	–	–	+	+	–
Citrate	+	–	–	–	–	–	–	+	–	–	–
Fumarate	+	–	+	+	–	+	+	+	+	+	+
Glutarate	–	–	–	–	–	–	–	+	–	–	–
DL-3-Hydroxybutyrate	+	–	+	+	+	+	+	+	+	+	+
DL-Lactate	+	–	+	+	+	+	+	+	+	+	+
L-Malate	–	–	–	–	–	–	–	+	–	–	–
Oxoglutarate	–	–	–	–	–	–	–	–	–	–	+
Pyruvate	+	–	+	+	+	+	+	+	+	+	+
L-Alanine	–	–	+	+	–	–	–	+	–	–	–
L-Aspartate	+	+	+	+	–	+	+	+	+	+	–
L-Histidine	+	–	+	+	+	+	+	+	+	+	+
L-Leucine	+	–	–	–	–	–	–	–	–	–	–
L-Phenylalanine	–	–	+	+	+	+	+	+	+	+	–
L-Proline	–	–	–	–	–	+	–	–	–	–	+
L-Serine	–	–	+	+	+	+	+	+	+	+	–
3-Hydroxybenzoate	+	–	–	–	–	–	–	–	–	–	–
4-Hydroxybenzoate	+	–	–	–	–	–	–	–	–	–	–
Phenylacetate	–	–	+	+	+	+	+	+	+	+	–
Hydrolysis of:											
L-Proline pNA	+	+	+	+	+	+	+	+	+	+	–
Tween 20	+	+	+	+	+	–	+	+	+	+	+
Tween 80	+	–	–	–	–	–	–	+	+	–	–
Casein	–	–	+	+	+	+	+	+	+	+	+

Data from Wieser et al. (2002), + Positive, – negative, (+) weakly positive, *y* yellow, *w* white

share lower than 98.8 % 16S rRNA similarities. Interestingly, in each case, the corresponding DNA–DNA reassociation values were lower than 60 %. Even at a 16 rRNA gene sequence similarity of 99.8 % (*Kocuria polaris* vs. *Kocuria rosea*), the corresponding DNA reassociation value was as low as 71 %. These data clearly indicate a threshold level of 98.8 % 16S rRNA gene sequence similarity is essential to be included as a member in *Kocuria*.

Table 27.3

Differential characteristics of the species of the genus *Micrococcus*[a]

Characteristic	*M. luteus*[b]	*M. lylae*[b]	*M. flavus*[c]	*M. antarcticus*[d]	*M. endophyticus*[e]	*M. yunnanensis*[f]	*M. terreus*[g]
Pigmentation	Yellow	Cream white	Yellow	Yellow	Yellow	Yellow	Yellow
Optimum growth Temperature (°C)	37	37	31	16.8	28	28	33
Growth at 4 °C	–	–	–	+	–	+	–
Growth at 45 °C	+	nd	–	–	–	+	–
Major menaquinone(s)	MK-8 and MK-8(H_2) or MK-8(H_2)	MK-8(H_2)	MK-8(H_2) MK-(H_2)	MK-8, MK-8(H2)	MK-8(H_2), MK-7(H_2)	MK-8(H_2), MK-7(H_2)	MK-7, MK-7(H_2), MK-8 MK-8(H_2)
Nitrate reduction	–	–	–	+	+	–	–
Voges–Proskauer reaction[f]	–	–	–	+	–	–	–
Hydrolysis of							
Tween 80	d	+	–	+	–	+	+
Starch	–	–	+	+	–	–	–
Casein[g]	+	–	–	–	nd	nd	–
Assimilation of							
D-Mannose	+	–	–	–	–	nd	–
D-Trehalose	+	+	+	–	+	nd	+
Maltose	+	+	–	–	nd	nd	+
L-Malate	–	–	–	+	+	nd	+
Pyruvate	+	+	nd	–	nd	nd	nd
Acetate	d	+	nd	–	nd	nd	+
Propionate	+	–	nd	–	nd	nd	nd
L-Alanine	d	–	nd	+	nd	nd	nd
Acid production from[f]							
Adonitol	–	+	–	–	–	–	nd
Amylum	–	+	+	+	–	–	nd
D-Arabinose	+	–	–	–	+	–	nd
L-Arabinose	–	–	–	+	+	–	–
Arbutin	+	–	–	+	+	–	nd
Cellobiose	–	–	+	+	+	–	nd
Dulcitol	–	+	+	–	–	–	nd
Erythritol	–	–	+	–	–	–	nd
Aesculin	–	–	+	+	+	–	–
Fructose	+	+	–	+	+	–	nd
D-Fucose	–	+	–	–	–	–	nd
Galactose	–	–	–	+	+	–	nd
β-Gentiobiose	–	+	–	+	–	–	nd
Glycogen	–	+	–	+	–	–	nd
Gluconate	–	–	+	–	–	–	(+)
Inositol	–	–	–	+	–	–	nd
Inulin	–	+	–	–	–	–	nd
Lactose	–	+	+	+	–	–	nd
D-Lyxose	–	+	–	+	+	–	nd
Mannitol	–	+	–	–	–	–	+
Melibiose	–	–	–	+	–	+	nd

Table 27.3 (continued)

Characteristic	*M. luteus*[b]	*M. lylae*[b]	*M. flavus*[c]	*M. antarcticus*[d]	*M. endophyticus*[e]	*M. yunnanensis*[f]	*M. terreus*[g]
Melezitose	–	+	–	–	–	–	nd
Methyl-α-D-mannoside	–	–	+	–	–	–	–
Methyl-α-D-glucoside	+	–	–	–	+	–	+
N-Acetylglucosamine	–	–	–	+	+	–	–
Raffinose	–	–	–	–	+	–	nd
Rhamnose	–	+	–	+	–	–	nd
Ribose	–	–	+	+	+	–	nd
Salicin	+	+	+	+	–	–	nd
Sorbitol	–	+	–	–	+	–	nd
Sorbose	–	+	–	–	–	–	nd
D-Tagatose	–	–	–	+	–	–	nd
Trehalose	+	+	+	–	+	+	+
D-Xylose	–	–	–	+	–	–	nd

[a]Symbols: +90 % or more strains positive, −90 % or more strains negative, *d* 11–89 % of strains positive, *nd* not determined, (+) weakly positive
[b]Data from Wieser et al. (2002) and Zhao et al. (2009)
[c]Data from Liu et al. (2007) and Zhao et al. (2009)
[d]Data from Liu et al. (2000) and Zhao et al. (2009)
[e]Data from Chen et al. (2009) and Zhao et al. (2009)
[f]Data from Zhao et al. (2009)
[g]Data from Zhang et al. (2010)

Citricoccus Altenburger, Kämpfer, Schumann, Steiner, Lubitz, and Busse (2002), 2099[VP]

Ci.tri.coc'cus. L. n. *citrus* lemon, citron or citrus, an African tree; N.L. masc. n. *coccus* a sphere; N.L. masc.n. *Citricoccus* lemon-yellow-pigmented coccus.

The genus *Citricoccus* (belonging to the family *Micrococcineae* within the class *Actinobacteria*) was proposed by Altenburger et al. (2002). Presently, the genus contains four validly described strains: *C. muralis*, *C. alkalitolerans*, *C. parietis*, and *C. zhacaiensis* which were isolated from a medieval wall painting, a desert soil in Egypt, a mold-infected wall, and a wastewater bioreactor, respectively (Altenburger et al. 2002; Li et al. 2005b; Schäfer et al. 2010; Meng et al. 2010). Recently, *Citricoccus nitrophenolicus* was isolated from wastewater treatment plant; however, strains described as DSM 23311 and CCUG 59571 are not cited in the species description. The genus *Citricoccus* comprises a type species *Citricoccus muralis*, and all strains belonging to the genus are nonpathogenic in nature.

Although the type strains of *C. muralis* and *C. alkalitolerans* share more than 99.5 % 16S rRNA gene sequence similarity, the DNA–DNA relatedness value between them (56 %; Li et al. 2005b) was lower than the threshold value of 70 % for species delineation (Wayne et al. 1987). Interestingly, phenotypic differences were also noteworthy in a sense that type strain of *C. alkalitolerans* being alkalitolerant with optimum growth at pH 8.0–9.0, while that of *C. muralis* prefers neutral environments. Similar case can also be seen in case of C. zhacaiensis, where 16S rRNA gene similarity was 98.9 % and 98.8 % with *C. alkalitolerans* and *C. muralis* and DNA–DNA hybridization values were 54 % and 39 %, respectively (Meng et al. 2010). Differences in the isolation sources, closer similarity with respect to 16S rRNA similarity, and differences in DNA–DNA hybridization have been correlated with phenotypic, chemotaxonomic, and biochemical attributes by each strain in a particular habitat as summarized in *Table 27.5*.

The bacteria are Gram-stain-positive cocci, occurring singly, in short chains, or in clusters, about 1 mm in diameter; cells are nonmotile, non-spore forming, and aerobic. Oxidase-negative and catalase-positive characteristics. The members of the genus are Gram-positive cocci and have the following chemotaxonomic characteristics: MK-9(H_2) as the predominant menaquinone, along with moderate amounts of MK-7(H_2) and MK-8(H_2), is also present. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, four unknown glycolipids, two unknown phospholipids, and an unknown lipid. Recently, Nielson et al. (2005) proposed an emended description of the genus *Citricoccus* Altenburger et al. (2002) as correct, except that either MK-9 or MK-8 may be the predominant menaquinones. While spermidine is a predominant polyamine, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$, and iso-$C_{15:0}$ are the major fatty acids (Altenburger et al. 2002; Li et al. 2005b; Meng et al. 2010).

Table 27.4

Phenotypic characteristics used for assigning membership of *Kocuria* spp.

Characteristic	*K. rosea*[a] ATCC 186[T]	*K. aegyptia*[b] DSM 17006[T]	*K. carniphila*[c] JCM 14118[T]	*K. himachalensis*[d] JCM 13326[T]	*K. kristinae*[e] DSM 20032[T]	*K. marina*[e] KCTC 9943[T]	*K. palustris*[f] NBRC 16318[T]	*K. Polaris* MTCC 3702[T][g]	*K. rhizophila*[f] CIP 105972[T]	*K. varians*[e] LMG 14231[T]	*K. salsicia*[h] JCM 16361[T]	*K. atrinae*[i] KCTC 19594[T]	*K. flava*[j] JCM 15621[T]	*K. turfanensis*[j] KCTC 19307[T]	*K. koreensis*[i] KCTC 19595	*K. gwangalliensis*[k] KCCM 42914[T]	*K. halotolerans*[l] DSM 18442[T]
Biochemical characteristic																	
Oxidase	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Urease	–	–	–	–	–	+	+	–	–	–	+	+	+	–	+	–	–
Adipic acid[h]	ND	ND	+	ND	ND	+	ND	ND	+	W	+	–	ND	ND	ND	–	ND
Nitrate reduction	+	–	+	–	–	+	+	+	–	–	+	+	+	W	–	+	+
H_2S production	–	–	–	–	–	–	(+)	ND	(+)	–	ND	ND			ND	ND	ND
Pigment color	Pink/red	Pink	Yellow	Orange/ pink	Pale cream to pale orange	Orange	Pale yellow	Orange	Yellow	Yellow	Lemon yellow	Pale yellow	Yellow	Orange/ red	Pale cream to pale orange	Pink orange	Pale yellow
Hydrolysis of																	
Gelatin (protease)	–	–	–	–	(+)	+	+	–	–	+[h]	w	–, w[h]	–	–	–	+[h]	–
Starch	V	–	–	–	–	–	–	–	–	V	+	+	+	+	ND	–	–
β-Glucosidase (aesculin hydrolysis)[h]	ND	ND	–	ND	ND	–	ND	ND	–	W	–	–	ND	ND	ND	–	ND
β-Galactosidase (PNPG hydrolysis)[h]	ND	ND	+	ND	ND	+	ND	ND	–	–	–	–	ND	ND	ND	–	ND
Tween 80	–	–	–	–	+	–	+	ND	+	+	–	+	+	+	+	ND	–
Growth at/in																	
5 °C	–	ND	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
37 °C	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+

Table 27.4 (continued)

Characteristic	*K. rosea*[a] ATCC 186[T]	*K. aegyptia*[b] DSM 17006[T]	*K. carniphila*[c] JCM 14118[T]	*K. himachalensis*[d] JCM 13326[T]	*K. kristinae*[a] DSM 20032[T]	*K. marinae*[e] KCTC 9943[T]	*K. paulustris*[f] NBRC 16318[T]	*K. Polaris* MTCC 3702[T g]	*K. rhizophila*[f] CIP 105972[T]	*K. varians*[a] LMG 14231[T]	*K. salsicia*[h] JCM 16361[T]	*K. atrinae*[i] KCTC 19594[T]	*K. flava*[j] JCM 15621[T]	*K. turfanensis*[j] KCTC 19307[T]	*K. koreensis*[j] KCTC 19595	*K. gwangalliensis*[k] KCCM 42914[T]	*K. halotolerans*[l] DSM 18442[T]
5 % NaCl	+	+	+	+	+	+	(+)	–	+	–	+	+	+	+	+	+	+
10 % NaCl	–	–	–	–	+	+	–	–	+	–	–	+	+	+	+	–	+
15 % NaCl	–	–	–	–	–	–	–	–	(+)	–	–	–	–	–	+	–	–
Utilization as sole source of carbon																	
Adonitol	–	ND	ND	–	+	–	+	+	+	+	ND	ND	ND	ND	ND	ND	+
L-Arabinose	–	–	ND	+	+	–	–	+	+	+	–	–	+	–	–	ND	ND
myo-Inositol	–	–	+	–	+	–	–	+	–	–	ND	–	ND	ND	+	ND	+
D-Mannitol	–	+	+	+	+	–	–	–	–	–	–	–	ND	ND	+	ND	+
D-Mannose	–	–	–	–	–	–	–	–	–	–	+	+	ND	ND	+	ND	+
D-Sorbitol	+	+	+	–	+	–	–	+	–	+	ND	+	ND	ND	+	ND	+
Enzyme activities																	
α-Glucosidase	ND	ND	+	ND	ND	–	ND	ND	W	–	–	W	ND	ND	ND	+	ND
β-Glucuronidase	ND	ND	W	ND	ND	–	ND	ND	–	–	–	W	ND	ND	ND	W	ND
Leucine arylamide	ND	ND	W	ND	ND	W	ND	ND	+	W	–	+	ND	ND	ND	+	ND
Acid phosphatase[h]	ND	ND	W	ND	ND	–	ND	ND	W	–	–	W	ND	ND	ND	W	ND
Cystine arylamidase[h]	ND	ND	–	ND	ND	–	ND	ND	–	–	–	–	ND	ND	ND	W	ND
Esterase (C4)[h]	ND	ND	W	ND	ND	W	ND	ND	W	W	–	W	ND	ND	ND	W	ND
β-Galactosidase[h]	ND	ND	+	ND	ND	–	ND	ND	–	+	–	–	ND	ND	ND	–	ND
Acid production from																	
Galactose	–	ND	ND	–	–	–	+	(+)	–	–	ND	–	ND	ND	ND	ND	ND
D-Glucose	+	–	+	+	+	–	+	+	+	V, +[h]	+	–	ND	ND	+	–	ND
Lactose	–	–	+	–	–	–	–	+	+	+	ND	–	ND	ND	ND	–	–

Maltose	–	–	(+)	–	+	–	–	–	–	–	+	+	ND	ND	–	+	ND
D-Mannitol	+	–	–	+	–	–	–	+	–	–	–	–	ND	ND	ND	+	ND
Sorbitol	–	–	–	–	+	–	–	+	–	–	ND	+	ND	ND	ND	ND	ND
Sucrose	–	ND	(+)	–	+	+	+	+	–	–	–	–	ND	ND	ND	–	ND
D-Xylose	+	ND	–	+	–	–	–	+	–	–	ND	–	ND	ND	ND	–	–
L-Malic acid	ND	ND	+	ND	ND	+	ND	ND	+	W	+	–	ND	ND	ND	–	ND
D-Mannose			+			W			+	+	–	+				W	
Phenylacetic acid[h]	ND	ND	+	ND	ND	–	ND	ND	+	+	+	–	ND	ND	ND	–	ND
D-Arabinose	+	ND	ND	+	–	–	–	+	–	–	ND	–	ND	ND	–	–	–
Potassium gluconate[h]	ND	ND	+	ND	ND	+	ND	ND	+	+	+	–	ND	ND	ND	–	ND
Trisodium citrate[h]	ND	ND	–	ND	ND	W	ND	ND	–	W	+	–	ND	ND	ND	–	ND
Major menaquinone(s)	MK-8 (H_2)	MK-8 (H_2)	MK-7	MK-	MK-7 (H_2)	ND	MK-7 (H_2)	MK-7 (H_2)	MK-7 (H_2)	MK-7 (H_2)	MK-7	MK-7	MK-8 (H_2), MK-9 (H_2)	MK-8 (H_2), MK-9 (H_2)	MK-7 (H_2)	MK-7 (H_2), MK-6 (H_2)	MK-6 (H_2), MK-7 (H_2), MK-8 (H_2)
Major polar lipids	PG, DPG	PG, DPG	ND	PG, DPG, 2GLs	PG, DPG, PI, PL, GL	ND	PG, DPG	PG, DPG	PG, DPG	PG, DPG	ND	ND	ND	ND	ND	ND	PG, DPG
Whole-cell sugars	Galactosamine	Galactose (Glucose)	ND	ND	Glucosamine	ND	ND	Galactose, glucose, ribose	ND	Galactosamine	ND	ND	ND	ND	ND	ND	Galactose, glucose
Major fatty acid(s)	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, C 15:0 iso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, C 15:0 iso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso	$C_{17:0}$ anteiso, $C_{15:0}$ anteiso, $C_{15:0}$ iso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ anteiso	$C_{15:0}$ anteiso, iso-$C_{15:0}$ iso-$C_{16:0}$	$C_{15:0}$ anteiso	$C_{15:0}$ iso	C 15:0 anteiso, C 16:0 iso,C 17:0 anteiso	$C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso
DNA G+C content	66–75	73	71	75	67	60	70	73	70	66–72	60	70.2	71	65	65.2	65.2	68

[a]Data from Stackebrandt et al. (1995)

[b]Data from Li et al. (2006)

[d]Data from Mayilraj et al. (2006)

[e]Data from Kim et al. (2004)

[f]Data from Kovács et al. (1999)

[g]Data from Reddy et al. (2003)

[h]Data from Yun et al. (2011)

[i]Data from Park et al. (2010)

[j]Zhou et al. (2008)

[k]Seo et al. (2009)

[l]Tang et al. (2009b)

Abbreviations: +, positive reaction; –, negative reaction, *W* weakly positive, *ND* no data, [a]*K. erythromyxa* (Brooks and Murray 1981) reclassified as *Kocuria rosea* (Flügge 1886, 49, 393–396)

Table 27.5

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

Characteristic	*C. muralis*[a] DSM 14442	*C. alkalitolerans*[b] JCM 13012	*C. parietalis*[c] CCM 7609	*C. zhacaiensis*[d] JCM 15136	*C. nitrophenolicus*[e] CCUG 59571
General					
pH range for growth[e]	6–10	5.5–12	6.5–12	6–9	6.3–9.5
NaCl range for growth (%)[e]	0–10	0–15	0–10	0–5	0–17
T range for growth (°C)[e]	4–28	10–37	4–36	10–37	7–34
Biochemical characteristics					
Urease[f,e]	+	–	–	ND	–
Gelatinase[f,e]	+	–	ND	ND	–
α-Glucosidase[d]	+	–	ND	+	ND
Growth in the presence of methyl red[e,f]	+	–	ND	–	–
Presence of					
Lysine decarboxylase	+	–	ND	ND	–
α-Maltosidase	+	–	ND	ND	ND
Utilization of					
L-Asparagine[d]	+	–	ND	–	ND
Citrate[d]	+	–	ND	+	ND
D-Galactose[d]	+	–	ND	–	ND
Glucose[d]	+	+	ND	–	ND
Glycine[d]	+	–	ND	–	ND
Malonate[d]	+	–	ND	+	ND
Antibiotic sensitivity					
Rifampicin[d]	R	S	ND	R	ND
Streptomycin[d]	R	S	ND	R	ND
Utilization as sole source of carbon					
Fructose	+	–	–	–	+
Amygdalin	–	+	ND	ND	–
Glycine	–	+	ND	ND	ND
Arginine	+	–	–	ND	+
para-Nitrophenol (PNP)[e]	–	–	–	–	+
Assimilation of[c]					
Adipate, azelate, glutarate, L-histidine[g]	–	–	+	ND	ND
Putrescine, suberate	–	–	+	ND	ND
Acetate, 4-hydroxybenzoate[c]	–	–	+	ND	ND
L-Phenylalanine, propionate, L-serine[c]	+	–	+	ND	ND
trans-Aconitate, L-alanine[c]	–	+	–	ND	ND
4-Aminobutyrate[c]	+	+	–	ND	ND
DL-Lactate [c]	+	–	+	ND	ND
Major quinone	MK-9(H_2) (94 %)[e]	MK-9(H_2)	MK-9(H_2) (57 %)	MK-9(H_2)	MK-8(H_2) (54 %)

Table 27.5 (continued)

Characteristic	*C. muralis*[a] DSM 14442	*C. alkalitolerans*[b] JCM 13012	*C. parietalis*[c] CCM 7609	*C. zhacaiensis*[d] JCM 15136	*C. nitrophenolicus*[e] CCUG 59571
Second major quinone	MK-8(H_2) (6 %)[e]	ND	MK-8 (25 %)	MK-7(H_2), MK-8(H_2)	MK-9(H_2) (46 %)
Fatty acids	anteiso-$C_{17:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$	iso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$
DNA G+C content (mol%)	68	64	67.8	66.0	68.1

[a]Data from Altenburger et al. (2002)
[b]Data from Li et al. (2005b)
[c]Data from Schäfer et al. (2010)
[d]Data from Meng et al. (2010)
[e]Data from Nielsen et al. (2011)
[f]Data from *Bergey's manual* 5th edn
[g]Data congruent with those reported by Li et al. (2005) for *C. alkalitolerans* as mentioned by Schäfer et al. (2010)
ND no data, *R* resistant, *S* sensitive, + for positive and − for negative reaction

Renibacterium, Sanders and Fryer (1980), 501[VP]

Re.ni.bac.te¢ri.um. L. pl. n. *renes* the kidneys; L. neut. n. *bacterium* rod; N.L. neut. n. *Renibacterium* kidney bacterium.

Members belong to *Renibacterium* genus mainly known as kidney disease bacterium, the causative agent of bacterial kidney disease in salmonid fishes. The initial isolation report was found in two independent cases in the 1930s, bacterial kidney disease (corynebacterial kidney disease, salmonid kidney disease; also known as Dee disease) reported from Atlantic salmon. In another report, similar disease was first reported in the United States in brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) from a hatchery (Belding and Merriii 1935). Bacteria in both case reports a small, Gram-positive diplobacillus that did not grow on any available media was reported. After this report, these bacteria are commonly found in salmonid populations from parts of Europe, Canada, the United States, Japan, and Iceland; however, host of pathogenesis is restricted to only salmonid fishes.

Furthermore, attempts were made to culture and identify causal agent bacteria of kidney disease on the basis of its morphological appearance, as a species of *Corynebacterium* by Ordal and Earp (1956). After that, Smith in 1964 isolated the same bacteria and concluded that Dee disease of *sal*monids in Scotland and bacterial kidney disease were caused by the same bacterium. After this report, since these two findings, no further studies on the classification of the kidney disease bacterium (KDB) have been reported till date due to fastidious nature and slow growth rate of bacteria.

The generic classification of KDB within the coryneform group of bacteria is questioned and further complicated by the various taxonomic issues encountered with this diverse group (Rogosa et al. 1974). Prior to the description of *Renibacterium*, the organism was linked with *Brevibacterium* (Smith 1964), *Corynebacterium* (Sanders et al. 1978), *Lactobacillus* (Vladik et al. 1974), *Listeria* (Bullock et al. 1975), and *Rickettsia* (Snieszko and Griffin 1955). By 16S rRNA sequence-based cataloging, *Renibacterium salmoninarum* was assumed to comprise a member of the actinomycete subdivision, being related to *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Jonesia*, *Micrococcus*, *Promicromonospora*, *Stomatococcus*, and *Terrabacter* (Gutenberger et al. 1991; Stackebrandt et al. 1988). This was supported by Grayson et al. (2000), linking *Renibacterium* to the high-G+C group of the actinobacteria, notably *Arthrobacter*, on the basis of rRNA gene sequence comparisons (Grayson et al. 2000). The organism has been included in the family *Micrococcaceae* (Stackebrandt and Schumann 2000) with low genetic diversity (Starliper 1996). Presently, *Renibacterium* has only one type strain and same as a type species.

Short rods or coccobacilli, 0.3–1.0 × 1.0–1.5 mm, usually occur in pairs (diplococcobacilli) and short chains. Gram stain positive, nonencapsulated, nonmotile, and non-endospore forming. Aerobic, slow-growing bacterium; temperature for optimum growth 15–18 °C; no growth at 37 °C. Cysteine required for growth. Growth enhanced by addition of blood, serum (especially fetal calf serum), or charcoal to media. No acid production from sugars. Catalase positive. The cell-wall peptidoglycan contains D-alanine, D-glutamic acid, glycine, and lysine as the diamino acid. Cell-wall sugars include galactose, rhamnose, *N*-acetylglucosamine, and *N*-acetylfucosamine. No mycolic acids are present. The major fatty acid is 12-methyltetradecanoic acid ($C_{15.0}$) with 14-methylhexadecanoic acid ($C_{17:0}$ anteiso) also present in significant amounts. The major respiratory quinines are unsaturated menaquinones with nine isoprene units. Based on some biochemical, phenotypic, and chemotaxonomic properties, *Renibacterium* differs from other genera in *Micrococcaceae* family as described in *Table 27.6*.

Rothia Georg and Brown (1967), 68[AL] Brian Austin (Updated from Gerencser and Bowden 1986)

Roth'i.a. N.L. fem. n. *Rothia* named for Genevieve D. Roth, who performed basic studies with these organisms (Gerencser and Bowden 1986).

Table 27.6
Distinguishing properties of *Renibacterium* from other representatives of the *Micrococcaceae*[a]

Characteristic/ genus	*Arthrobacter*[b]	*Citricoccus*[c]	*Kocuria*[d]	*Micrococcus*[d]	*Nesterenkonia*[d]	*Rothia*[e]	*Yania*[f]	*Renibacterium*[g]
Optimum growth (°C)	25–30	4–28	22–37	25–37	20–40	35–37	28	15–18
Requirement for cysteine	–	–	(+)	–	–	–	–	+
DNA G+C content (mol%)	61–66	63–68	66–75	69–76	70–72	49–53	53	53
Major menaquinone content	MK-9(H2) or MK-9 and MK-8 or MK-9 and MK-10	MK-9(H2)	MK-7(H2) or MK-7(H2) and MK-8(H2) or MK-8(H2) or MK-8(H2) and MK-9 (H2)	MK-8 and MK-8(H2) or MK-8(H2) or MK-8(H2) and MK-7(H2)	MK-7 or MK-7 and MK-8 or MK-8 and MK-9	MK-7	MK-8 and MK-9 or MK-8	MK-9, MK-10

[a]Symbols: +, –, positive and negative reactions
[b]Data from Stackebrandt and Fiedler (1979), Koch et al. (1995)
[c]Data from Altenburger et al. (2002)
[d]Data from Stackebrandt et al. (1995), Li et al. (2004b, 2005a, 2006), Tvrzová et al. (2005), Mayilraj et al. (2006), Yoon et al. (2006)
[e]Data from Georg and Brown (1967)
[f]Data from Bergan and Kocur (1982)
[g]Data from Li et al. (2004a, 2005c)

Historically, the genus was classified within the family *Actinomycetaceae* because of morphological similarities (Schaal 1992). Later in 1997, Stackebrandt and co-workers transferred the genus to the family *Micrococcaceae* based on phylogenetic evidences. The genus currently harbors six species with *Rothia dentocariosa* as the type species.

Cells are Gram positive, nonmotile, non-acid-fast, endospore negative, catalase positive, and oxidase negative. Aerobic to microaerophilic with poor growth under obligately anaerobic conditions. Cells occur in different shapes that include cocci, diphtheroid, filamentous, and mycelial with mostly in a diameter of about 1 μm. Mixtures of the above morphologies are also a common occurrence. Mycelial forms may fragment into bacillary or coccoid forms. Growth in solid media occurs mostly in the form of filaments, whereas broth cultures usually appear coccoid in cell morphology. The chemotaxonomic properties and other detailed characteristics of all the species are given in *Table 27.1*. Young colonies are usually creamy, white, and smooth, while mature colonies are rough, dry, folded, and convex adhering to the agar medium with difficulty in picking them. However, in some cases, old colonies may also be round, convex, and smooth surfaced, reflecting a bacillary or coccoid micromorphology (Daneshvar et al. 2004).

The optimum temperature for growth is 30–37 °C. Generally nonhemolytic except for the type species wherein some strains show hemolysis on rabbit blood (Daneshvar et al. 2004). Positive for nitrate reduction, VP test (except *R. nasimurium*), and gelatin and aesculin hydrolysis but not for casein, starch, and urea. A variety of sugars are fermented for acid production, and lactic acid is the main product of this metabolism together with acetic acid and small amounts of succinic acid (Daneshvar et al. 2004). Generally, acid is produced from fructose, D-glucose, glycerol, maltose, and sucrose. Details of some other biochemical tests are given in *Table 27.1*. Negative for indole production. In the Biolog GP2 system, all species are positive for oxidation of the three substrates, α-D-glucose, D-psicose, and glycerol, and negative for α-cyclodextrin, β-cyclodextrin, inulin, mannan, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, amygdalin, L-arabinose, D-arabitol, D-cellobiose, D-galactose, D-galacturonic acid, gentiobiose, *m*-inositol, α-D-lactose, lactulose, D-mannitol, D-melibiose, α-methyl-D-galactoside, β-methyl-D-galactoside, α-methyl-D-mannoside, D-raffinose, L-rhamnose, sedoheptulosan, D-sorbitol, stachyose, D-tagatose, xylitol, D-xylose, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, lactamide, D-malic acid, *N*-acetyl-L-glutamic acid, D-alanine, L-alanine, glycyl-L-glutamic acid, L-pyroglutamic acid, adenosine, 2′-deoxyadenosine, inosine, thymidine, adenosine-5′-monophosphate, thymidine-5′-monophosphate, D-fructose-6-phosphate, α-D-glucose-6-phosphate, α-D-glucose-1-phosphate, and DL-α-glycerol phosphate.

The chemotaxonomic properties of the genus that are important for species description include fatty acids, menaquinone composition, polar lipids, and cell-wall amino acids. The most predominant fatty acid is anteiso-$C_{15:0}$ (>50 %), whereas iso-$C_{16:0}$ and anteiso-$C_{17:0}$ occur in variable amounts in different species (5–20 %). The major menaquinone is MK-7. The peptidoglycan is of the A3α type with L-Lys and D-Ala occurring at positions 3 and 4 of the peptide subunit, respectively. The interpeptide bridge generally consists of L-ala residues except in *R. mucilaginosa* where L-ala is partially replaced by L-ser. The diagnostic polar lipids of the genus are

phosphatidylglycerol and diphosphatidylglycerol. Some other lipids such as phosphatidylinositol and unknown phospholipids and glycolipids may also be present in minor quantities as in the case of *R. amarae* and *R. terrae*, respectively. These characteristics are summarized in Table 27.7. The G+C content of the genus ranges from 53.7 % to 59.0 % (Chou et al. 2008; Austin 2012).

Zhihengliuella Zhang, Schumann, Yu, Liu, Zhang, Xu, Stackebrandt, Jiang, and Li (2007), 1018^{VP} Emend. Tang, Wang, Chen, Lou, Cao, Xu, and Li (2009), 2029

Zhi.heng.li.u.el¢la. N.L. fem. dim. n. *Zhihengliuella* named after Zhi-Heng Liu (1940–), a Chinese microbiologist who devotes himself to the study of actinomycete taxonomy.

The genus *Zhihengliuella* was established by Zhang and his colleagues in 2007. The species was isolated from a saline soil sample in China. The genus was placed within the family *Micrococcaceae* and revealed 16S rRNA gene sequence similarities of 93.5–96.4 % to members of the family. Interestingly, the genus shows close proximity to certain species of the *Arthrobacter nicotianae* group (sensu Keddie et al. 1986), e.g., *Arthrobacter nicotianae*, *A. protophormiae*, *A. uratoxydans*, *A. rhombi*, *A. bergerei*, and *A. arilaitensis* in possessing A4α peptidoglycan with the interpeptide bridge Ala-Glu and the menaquinones MK-9 and MK-10. However, this close relationship based on chemotaxonomic properties is not supported by 16S rRNA-based phylogeny (Busse 2012). The genus currently encompasses four species *Z. halotolerans* (Zhang et al. 2007), *Z. alba* (Tang et al. 2009a), *Z. salsuginis* (Chen et al. 2010), and *Z. aestuarii* (Baik et al. 2011) with *Z. halotolerans* as the type species of the genus. The species *Z. salsuginis* is however not a validly published name.

The cells are Gram positive, strictly aerobic, nonmotile, and coccoid to ovoid to rods measuring 0.5–1.0 × 0.8–2.0 μm in size or 0.6–0.9 μm in diameter. Cells of *Z. salsuginis* occur in pairs, tetrads, and clusters (Chen et al. 2010). Colonies are pale yellow or white in color, circular, convex, and with entire margins and 1–3 mm in diameter. All the species described till date are catalase positive, oxidase negative, mesophilic with a growth temperature range of 4–45 °C and an optimum of 25–30 °C, slightly alkaliphilic with pH optimum of 7.5–9.0, and moderately halotolerant with growth at NaCl concentrations of 0–25 % with optimum at 5–10 %. However, the presence of NaCl is not a mandatory requirement for growth. Negative for nitrate reduction, H_2S production, MR-VP test, indole production, and urea hydrolysis. All species (except *Z. aestuarii*) produce acid from D-fructose, D-glucose, maltose, L-rhamnose, D-ribose, starch, sucrose, and D-xylose but not from dulcitol, D-galactose, inulin, melibiose, and melezitose (Chen et al. 2010). All species assimilate D-glucose and maltose and (except *Z. aestuarii*) also utilize sucrose, glycerol, gluconate, and L-glutamic acid. Negative for (except *Z. aestuarii*) utilization of acetamide, adonitol, L-arginine, butyrate, citrate, formate, fumarate, D-galactose, L-glycine, glycogen, histidine, hydroxy-L-proline, inulin, L-isoleucine, L-leucine, malate, malonate, melezitose, L-methionine, L-proline, propionate, L-rhamnose, succinate, L-valine, and D-xylose. Other characteristics are given in Table 27.8.

In addition to the above morphological and biochemical characteristics, chemotaxonomic properties like peptidoglycan structure, cell-wall sugars, fatty acids, and menaquinone system are important biomarkers for description of species within the genus. The cell-wall type is A4α (L-Lys–L-Ala–L-Glu). The most important parameter is the occurrence of cell-wall sugar tyvelose which differentiates this genus from other members of the family *Micrococcaceae*. MK-9 and MK-10 are the major menaquinones with any one of them being predominating depending upon the species. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. Anteiso-$C_{15:0}$ is the major fatty acid. G+C content ranges from 59.1 to 70.3 which is quite wide keeping in mind that there are just four species within the genus currently. All the characteristics are given in detail in Table 27.2. The species seem to grow optimally at NaCl concentrations of 2–10 % (Baik et al. 2011). However, the strains are not obligately halophilic determined by their ability to grow at 0 % NaCl concentration and are probably halotolerant since they tolerate up to 25 % salt concentration (Baik et al 2011).

Yaniella (Li, Chen, Xu, Zhang, Schumann, Tang, Xu, and Jiang 2004a; Li, Zhi, and Euzéby (2008b), 526^{VP}) (Illegitimate Homotypic Synonym: *Yania* Li, Chen, Xu, Zhang, Schumann, Tang, Xu, and Jiang 2004a, 529)

Ya.ni.el'la. N.L. fem. dim. n. *Yaniella* named after Sun-Chu Yan (1912–1994), a Chinese microbiologist who devoted his life to the study of actinomycete taxonomy and antibiotics.

The genus *Yaniella* was proposed by Li et al. in 2004a originally as *Yania*. The genus was later emended by description of one more species with proposal of a new family *Yaniellaceae* (Li et al. 2005c). The family was proposed based on some unique 16S rDNA signature nucleotides, compared to other families of the order *Micrococcales*, such as 140–223 (A–G), 142–221 (C–A), 615–625 (G–U), 839–874 (A–A), and 1134–1140 (A–U) (Li et al. 2004a, 2005c). However, Yassin et al (2011) based on observations by Zhi et al (2009) concluded that the family *Yaniellaceae* should no longer be retained and transferred the genus to the family *Micrococcaceae*. The name *Yania* has been replaced by the name *Yaniella* because of the precedence of the genus name *Yania* in zoological descriptions (Li et al. 2008a). The genus currently encompasses two species *Yaniella halotolerans* (Li et al. 2004a) and *Y. flava* (Li et al. 2005c) with the former as the type species. One additional species *Y. fodinae* proposed (Dhanjal et al. 2011) was recently reclassified as member of another genus *Enteroactinococcus* (Cao et al. 2012).

Cells are Gram positive, nonmotile, aerobic, non-spore formers with a coccoid or oval morphology, and about

Table 27.7

Comparative phenotypic characteristics of *Rothia* spp.

Characteristic	*R. dentocariosa* ATCC 17931^T	*R. amarae* JCM 11375^T	*R. aeria* DSM 14556^T	*R. mucilaginosa* DSM 20476^T	*R. nasimurium* CCUG 35957^T	*R. terrae* BCRC 17588^T
Biochemical characteristics						
Catalase	+	+	+	–	+	+
Trypsin	–	–	ND	–	+	–
Valine arylamidase	–	–	ND	–	+	–
Alkaline phosphatase	–	–	–	+	–	+
β-Glucosidase	+	–	+	–	+	+
Nitrate reduction	+[a]	+[b]	+[c]	+[d]	+[e]	+
VP test	+	+	ND	+	–	+
Acid production from						
Lactose	–	–	–	–	+	+
Ribose	+	+	–	–	–	–
Oxidation of (Biolog GP2)						
2,3-Butanediol	+	–	–	+	+	–
Glycerol	+	+	+	–	+	+
DL-α-Glycerol phosphate	–	–	–	+	–	–
α-Hydroxybutyric acid	+	–	+	–	–	–
L-Lactic acid	+	(+)	+	–	–	+
L-Malic acid	+	–	–	–	–	–
3-Methyl glucose	–	–	+	+	+	+
Methylpyruvate	+	–	–	+	–	+
Methyl-α-D-glucoside	–	+	+	–	–	–
Methyl-β-D-glucoside	–	+	+	+	+	–
Monomethyl succinate	(+)	–	–	–	–	–
D-Psicose	–	(+)	+	–	+	+
Pyruvic acid	(+)	–	–	+	+	–
Salicin	–	–	+	+	–	–
Succinic acid	+	–	–	–	–	–
Chemotaxonomic properties						
Major menaquinones	MK-7[a]	MK-6 (H_2), MK-7[b]	MK-7[c]	MK-7[d]	MK-7	MK-7
Major fatty acids	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$[a]	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$[b]	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$[c]	anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$, $C_{16:0}$[d]	anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, $C_{16:0}$
Major polar lipids	DPG, PG[a]	DPG, PG[b]	DPG, PG[c]	ND	ND	DPG, PG
Peptidoglycan type	A3α[a]	A3α[b]	A3α[c]	A3α[d]	ND	A3α
DNA G+C content	53.7–54.7[a]	54.5[b]	57.8[c]	59.0[d]	56.0	56.1

Data for all strains taken from Chou et al. (2008) except for those which are marked with symbols

[a]Data taken from Daneshvar et al. (2004), Chou et al. (2008), and Austin (2012)

[b]Data taken from Fan et al. (2002)

[c]Data taken from Li et al. (2004c)

[d]Data taken from Bergan and Kocur (1982)

[e]Data taken from Collins et al. (2000)

ND not determined, (+) weakly positive, *DPG*, diphosphatidylglycerol, *PG* phosphatidylglycerol

Table 27.8

Comparative phenotypic characteristics of *Zhihengliuella* spp.

Characteristic	*Z. halotolerans* YIM 70185^T	*Z. alba* YIM 90734^T	*Z. salsuginis* JSM 071043^T	*Z. aestuarii* KCTC 19557^T
Biochemical characteristics				
Hydrolysis of				
Casein	–	–	+	+
Starch	+	–	+	–
Tween 20	+	–	+	+
Tween 80	+	+	+	–
Aesculin	+	+	–	–
Gelatin	+	+	+	–
Oxidation of (**Biolog GP2**)				
Acetic acid	+	–	+	–
N-acetyl-D-galactosamine	–	+	–	–
D-Alanine	+	–	+	–
L-Alanine	–	–	+	+
L-Alanyl glycine	+	–	–	–
L-Arabinose	+	–	–	–
D-Arabitol	+	+	–	–
L-Asparagine	–	+	–	+
2,3-Butanediol	+	–	–	–
Cellobiose	–	+	+	–
β-Cyclodextrin	+	–	–	–
2′-Deoxyadenosine	–	+	–	–
D-Fructose-6-phosphate	+	–	–	–
Gentiobiose	+	–	+	–
α-D-Glucose-1-phosphate	+	–	–	–
D-Glucose-6-phosphate	+	–	–	–
L-Glutamic acid	+	–	–	+
DL-α-Glycerol phosphate	+	–	–	–
α-Hydroxybutyric acid	+	–	+	–
β-Hydroxybutyric acid	+	–	–	–
γ-Hydroxybutyric acid	+	–	+	–
Inosine	+	–	–	–
myo-Inositol	+	+	–	–
α-Ketoglutaric acid	+	–	–	–
α-Ketovaleric acid	+	+	–	–
Lactose	–	+	–	–
D-Malic acid	+	–	–	–
L-Malic acid	+	–	–	+
Maltose	+	+	–	–
Maltotriose	+	–	+	–
Mannan	+	+	–	–
Melibiose	+	–	+	–
Methyl-β-D-galactoside	+	–	–	–
Methyl-α-D-glucoside	+	+	–	–

Table 27.8 (continued)

Characteristic	*Z. halotolerans* YIM 70185^T	*Z. alba* YIM 90734^T	*Z. salsuginis* JSM 071043^T	*Z. aestuarii* KCTC 19557^T
Methyl-β-D-glucoside	+	−	−	−
Methyl-α-D-mannoside	+	−	−	−
Palatinose	+	+	−	−
L-Pyroglutamic acid	−	−	+	+
Pyruvic acid	+	−	+	−
Pyruvic acid methyl ester	+	−	−	−
D-Psicose	+	+	+	−
Salicin	+	−	+	−
Sedoheptulosan	+	−	+	−
L-Serine	−	+	−	+
Oxidation of (Biolog GP2)				
D-Sorbitol	+	−	+	−
Stachyose	+	−	−	−
Succinamic acid	+	−	−	−
Sucrose	+	+	−	−
D-Tagatose	+	+	−	−
Thymidine	+	+	−	−
Thymidine-5′-monophosphate	+	−	−	−
Trehalose	+	+	−	−
Turanose	+	−	−	−
Tween 40	+	+	−	−
Tween 80	+	+	−	−
Uridine	+	−	+	−
Uridine-5′-monophosphate	+	−	−	−
Enzyme activity (API ZYM)				
Acid phosphatase	−	+	−	−
Alkaline phosphatase	+	−	+	−
α-Chymotrypsin	−	+	+	−
Cystine arylamidase	−	+	−	−
Esterase lipase (C8)	+	+	+	−
α-Galactosidase	−	+	−	−
β-Glucosidase	−	+	+	−
Lipase (C14)	+	−	−	−
Leucine arylamidase	−	+	+	+
Valine arylamidase	−	+	−	−
Trypsin	−	+	+	(+)
Naphthol-AS-BI-phosphohydrolase	−	+	−	(+)
Esterase (C4)	+	+	+	−
Chemotaxonomic properties				
Major menaquinones	MK-9, MK-10[a]	MK-10, MK-9[b]	MK-9, MK-8[c]	MK-10, MK-9
Major fatty acids	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$[a]	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$[b]	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$[c]	anteiso-$C_{15:0}$, iso-$C_{15:0}$

Table 27.8 (continued)

Characteristic	*Z. halotolerans* YIM 70185^T	*Z. alba* YIM 90734^T	*Z. salsuginis* JSM 071043^T	*Z. aestuarii* KCTC 19557^T
Major polar lipids	DPG, PG, PI[a]	DPG, PG, PI[b]	DPG, PG, PI[c]	DPG, PG, PC
Peptidoglycan type	A4α[a]	A4α[b]	A4α[c]	A4α
Cell-wall sugars	Glu, Tyv	Tyv, Man	Tyv, Man	Tyv, Glu
DNA G+C content	70.3	66.5	67.8	59.1

Data for all strains taken from Baik et al. (2011) except for those which are marked with symbols
[a]Data taken from Zhang et al. (2007)
[b]Data taken from Tang et al. (2009a)
[c]Data taken from Chen et al. (2010)
(+), weakly positive *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol; *PC* phosphatidylcholine, *Tyv* tyvelose, *Glu* glucose, *Man* mannose

0.4–0.7 μm in diameter and occur singly or in clusters. Colonies are yellow in color, circular, lubricious, and opaque. Catalase positive and oxidase negative. Both species are moderately halophilic or halotolerant and negative for MR-VP, melanin production, H_2S and indole production, Tweens 20 and 80, casein, and starch hydrolysis. Positive for lysine decarboxylase, arginine dihydrolase and lipase activity. Negative for ornithine decarboxylase, L-aspartic arylamidase, α-galactosidase activities and growth on cellulose. Substrates acetamide, fructose, galactose, glucose, maltose, mannose, and salicin are utilized for growth, whereas adonitol, arabinose, arabitol, inositol, mannitol, and sorbitol are not. The type species *Y. halotolerans* YIM 70085^T grows within a temperature range of 10–40 °C, with optimum being 28–30 °C. Data for temperature range and optimum is not available for *Y. flava*. The range of pH for growth is 6.0–9.0 and optimum is 7.0–8.0 for both species. The presence of salts (especially KCl) seems to enhance the growth of both species. Optimal KCl concentration for growth is 10–15 %. Other differential physiological and metabolic characteristics of both species are given in *Table 27.3*. The cell-wall peptidoglycan is of the A4α type (L-Lys–Gly–L-Glu) with L-glutamic acid and glycine present in the interpeptide bridge. The major fatty acids are anteiso-$C_{15:0}$ and iso-$C_{15:0}$ or anteiso-$C_{15:0}$. MK-8 or MK-8 and MK-9 are the major menaquinones. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, unknown phospholipid, and glycolipid. The other properties are listed in *Table 27.9*. The G+C content of genomic DNA ranges from 53.5 to 57.9.

Acaricomes Pukall, Schumann, Schutte, Gols, and Dicke (2006), 465^{VP}

A.ca.ri.co¢mes. N.L. masc. pl. n. acarithe mites; L. masc. n. comes companion; N.L. masc. n. *Acaricomes* companion of mites.

The genus *Acaricomes* was established by Pukall et al. in 2006 and was isolated from diseased, surface-sterilized specimens of the predatory mite *Phytoseiulus persimilis* Athias-Henriot. The genus was placed within the family *Micrococcaceae* and revealed 16S rRNA gene sequence similarities of 94.0–94.8 % to members of the family. Cells are aerobic, mesophilic, Gram positive, non-spore-forming rods, catalase positive, and oxidase negative. A rod–coccus life cycle is absent. The optimum pH for growth is pH 6·0–8·0. The peptidoglycan type is A3α L-lys–L-Ala_3. The predominant menaquinone is the partially saturated menaquinone MK-10(H_2) with one of the ten isoprene units hydrogenated; in addition, MMK-10(H_2) is detectable. The major fatty acids determined are anteiso-$C_{15:0}$ (12-methyltetradecanoic acid) and anteiso-$C_{17:0}$ (14-methylhexadecanoic acid). The major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol. The G+C mol% is of is 57.7 determine by HPLC.

The genus contains a single species, *Acaricomes phytoseiuli*. The type strain DSM 14247^T.

Cells are 0.5–0.8 × 1–1.5 μm in size. Colonies on TSA are circular, convex, 1–2 mm in diameter, and colored yellowish. Growth occurs at 15–30 °C with an optimum of 25 °C. Grows at pH 6.0–9.5. The following substrates are utilized: dextrin, α-D-glucose, α-D-fructose, maltose, maltotriose, D-mannose, sucrose, turanose, L-glutamic acid, and glucose 1-phosphate. The following substrates are not utilized: α- and β-cyclodextrin; glycogen; inulin; mannan; Tween 40; *N*-acetyl-D-glucosamine; *N*-acetyl-D-mannosamine; amygdalin: L-arabinose; D-arabitol; arbutin; D-cellobiose; L-fucose; D-galactose; D-galacturonic acid; gentiobiose; D-gluconic acid; *myo*-inositol; α-D-lactose; lactulose; D-mannitol; D-melezitose; D-melibiose; methyl-α-D-galactoside; methyl-β-D-galactoside; 3-methyl glucose; methyl-α-glucoside; methyl-β-D-glucoside; methyl α-D-mannoside; D-psicose; D-raffinose; L-rhamnose; D-ribose; salicin; sedoheptulosan; D-sorbitol; stachyose; D-tagatose; D-trehalose; xylitol; D-xylose; acetic acid; α-, β-, and γ-hydroxybutyric acid; *p*-hydroxyphenylacetic acid; α-ketovaleric acid; lactamide; D-lactic acid methyl ester; L-lactic acid; D-malic acid; methylpyruvate; monomethyl succinate; propionic acid; pyruvic acid; succinamic acid; succinic acid; *N*-acetyl-L-glutamic acid; L-alaninamide; D-alanine; L-alanyl glycine; glycyl-L-glutamic acid; L-serine; putrescine; 2,3-butanediol;

Table 27.9
Comparative phenotypic characteristics of *Yaniella* spp.[a]

Characteristic	*Y. flava* YIM 70178^T	*Y. halotolerans* YIM 70085^T
Biochemical characteristics		
Optimal concentration of KCl for growth (% **w/v**)	10–15	10
Range of salt concentrations for growth (% **w/v**)		
NaCl	0.5–25	0–25
KCl	0.5–30	0–20
$MgCl_2$	0.5–30	0–15
pH range for growth	6.0–9.0	6.5–8.5
Utilization of sucrose	–	+
Enzyme activities		
Urease	–	+
β-Glucosidase	–	+
N-Acetyl-glucosaminidase	+	–
β-Galactosidase	+	–
α-Maltosidase	–	+
Chemotaxonomic properties		
Major menaquinones	MK-8, MK-9	MK-8
Major fatty acids	anteiso-$C_{15:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0}$
Major polar lipids	DPG, PG, PL, and GL	DPG, PG, PI, PL, GL
Peptidoglycan type	A4α	A4α
DNA G+C content	57.9	53.5

[a]All data are from Li et al. (2005c)
DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *GL* glycolipid

glycerol; adenosine; 2′-deoxyadenosine; inosine; thymidine; uridine; adenosine 5′-monophosphate; thymidine 5′-monophosphate; uridine 5′-monophosphate; fructose 6-phosphate; and DL-α-glycerol phosphate. Cell-wall sugars are galactose and glucose.

Auritidibacter Yassin, Hupfer, Siering, Klenk, and Schumann (2011), 228^{VP}

Au.ri.ti.di.bac'ter. L. n. auris, -is the ear; L. suff. -itis, -itidis, suffix used for inflammation; N.L. masc. n. bacter a rod; N.L. masc. n. *Auritidibacter* rod-shaped bacterium causing inflammation of the ear, also referring to the source of isolation.

The genus *Auritidibacter* was proposed by Yassin et al. (2011) with less than 95 % sequence similarity related to members of the family *Micrococcaceae*.

Cells are aerobic, Gram positive, motile, and non-spore forming and have a rod–coccus life cycle where the cells were cocci in the stationary growth phase, were rods in 11-h-old cultures, were predominantly short rods or oval-shaped after 60 h, and had a coccoid shape again after 108 h. Catalase positive and grows at 10–37 °C. Peptidoglycan type is A4α (L-Lys–Gly–L-Glu), MK-10 as the predominant menaquinone and long-chain cellular fatty acids of straight-chain and branched-chain saturated types (with iso-$C_{15:0}$ and anteiso-$C_{17:0}$ predominating). The major polar lipids included diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol in addition to unknown glycolipids. The DNA G+C content was 59.7 mol%. The pattern of the 16S rRNA gene signature nucleotides consists of nucleotides at positions 400 (U), 671 (U), 986 (U), 987 (G), 1120 (U), and 1408 (G). The pattern of signature nucleotides must be considered tentative and should be updated as the number of sequences in this lineage increases. The genus contains a single species *Auritidibacter ignavus*; the type strain is IMMIB L-1656^T.

The species has the following characteristics – cell size: cocci are 0.3–1 mm in diameter. Rods are 0.4–0.5 mm wide and 2.1–2.5 mm long. Colonies are cream colored and smooth and have an entire margin. Growth occurs on BHI agar, DST agar, Columbia agar supplemented with 5 % sheep's blood, and TSA agar. CAMP reaction is negative with *S. aureus*. Tolerates up to 12 % NaCl. Grows at pH 7.4–9.0 (optimum at pH 9.0) but not at pH 4–6. Nitrate reduction is negative. Acetoin production is positive. Hydrolyzes DNA and starch (3 days of incubation). Tyrosine is weakly hydrolyzed. The following substrates are not hydrolyzed: adenine, casein, elastin, aesculin, gelatin, guanine, hippurate, hypoxanthine, keratin, testosterone,

xanthine, and urea. Acetate, adipate, adonitol, L-arabinose, isoamyl alcohol, 2,3-butanediol, cellobiose, citrate, meso-erythritol, D-galactose, D-glucose, D-gluconate, m-hydroxybenzoate, p-hydroxybenzoate, myo-inositol, L-lactate, lactose, maltose, D-mannitol, melezitose, 1,2-propanediol, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, and D-xylose are not assimilated. Acetamide, L-alanine, gelatin, L-proline, and L-serine are not utilized as simultaneous sources of carbon and nitrogen. Activity is detected for alkaline phosphatase, ester lipase C8, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and pyrazinamidase. No activity is detected for acid phosphatase, arginine dihydrolase, esterase C4, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, lipase C14, α-mannosidase, α-fucosidase, α-chymotrypsin, trypsin, cystine arylamidase, leucine arylamidase, pyrrolidonyl arylamidase, and valine arylamidase.

Nesterenkonia Stackebrandt, Koch, Gvozdiak, and Schumann (1995), 689^{VP} Emend. Collins, Lawson, Labrenz, Tindall, Weiss, and Hirsch (2002) Emend. Li, Chen, Kim, Zhang, Park, Lee, Xu, and Jiang (2005)

Ne.ste.ren.ko¢ni.a. N.L. fem. n. Nesterenkonia honoring olga nesterenko, a Ukrainian microbiologist.

Taxonomic dissection of the genus *Micrococcus* by Stackebrand et al. in 1995 led to propose new genus *Nesterenkonia* with the type strain *Nesterenkonia halobia* (DSM 20541^T) for the strain *Micrococcus halobius* sp. *n* (Onishi and Kamekura 1972). The genus currently encompasses *Nesterenkonia lacusekhoensis* (Collins et al. 2002), *Nesterenkonia xinjiangensis* (Li et al. 2004a), *Nesterenkonia halotolerans* (Li et al. 2004), *Nesterenkonia lutea* (Li et al. 2005a), *Nesterenkonia sandarakina* (Li et al. 2005a), *Nesterenkonia jeotgali* (Yoon et al. 2006), *Nesterenkonia aethiopica* (Delgado et al. 2006), *Nesterenkonia alba* (Luo et al. 2009), *Nesterenkonia flava* (Luo et al. 2008), and *Nesterenkonia halophila* (Li et al. 2008b).

Cells are Gram positive, aerobic, mesophilic, nonencapsulated, moderately halophilic or halotolerant, motile or nonmotile, and non-spore forming which may consist of short rods, sometimes showing branching, or of cocci. Some species are alkaliphilic or alkalitolerant. Chemoorganotrophic; metabolism is strictly respiratory. They are catalase positive, and cell wall does not contain mycolic acid. The major cellular fatty acids are iso- and anteiso-branched fatty acids, with anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ and iso-$C_{16:0}$ predominating. Major phospholipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. Menaquinones are present, MK-7, MK-8, and MK-9 predominating. Peptidoglycan is of the A4α type (L-lys–gly–L-Glu, L-lys–L-glu, or lys–gly–D-Asp). The G+C content of the DNA is 64–72 mol%.

Chemotaxonomic and phenotypic properties which differentiate among the *Nesterenkonia* species are indicated in Table 27.10.

Sinomonas Zhou, Wei, Wang, and Lai (2009), 259^{VP} Emend. Zhou, Chen, Zhang, Wang, and Xu (2012), 764

Sinomonas (Si.no.mo'nas. M.L. n. Sina China; L. fem. n. monas a unit, monad; N.L. fem. n. *Sinomonas* a monad from China).

The genus *Sinomonas* (type species *Sinomonas flava*) was first proposed by Zhou et al. (2009) with the description of the newly isolated strain *S. flava* CW 108^T and the reclassification of *Arthrobacter atrocyaneus* as *Sinomonas atrocyanea* (type strain DSM 20127^T) (Zhou et al. 2009; Kuhn and Starr 1960). Shortly after the description of the genus *Sinomonas* was published, two novel species *Arthrobacter echigonensis* and *Arthrobacter albidus* were proposed (Ding et al. 2009), but strains of these species showed high 16S rRNA gene sequence similarity values (>98 %) with strains of *S. flava* and *S. atrocyanea* and low 16S rRNA gene sequence similarity (less than 95.2 %) to all other members of the related genera *Citricoccus*, *Micrococcus*, *Zhihengliuella*, *Arthrobacter*, *Kocuria*, *Rothia*, *Yaniella*, *Acaricomes*, and *Renibacterium* within the family *Micrococcaceae*.

Sinomonas flava species was isolated from a saline soil sample in China. It showed high 16S rRNA gene sequence similarity to *Arthrobacter atrocyaneus* DSM 20127^T. Furthermore, *Arthrobacter atrocyaneus* displayed well-differentiated morphological and physiological characteristics. The two strains were separated from other members of the *Micrococcaceae* by colony color or obvious growth on PYES (Wieser et al. 2002), YDC (Kuhn and Starr 1960) and TYB (containing 0.3 % yeast extract, 0.2 % beef extract, 0.6 % tryptone, 0.3 % NaCl, and 0.001 % $FeCl_3$) media at 41 °C. Both strains shared the same 16S rRNA gene signature nucleotides, and most signature nucleotides of the two strains match those of the family *Micrococcaceae* (Stackebrandt et al. 1997), except at positions 640, 839, 847, 1025, 1036, and 1278 (*E. coli* numbering), where the nucleotides U, A, U, U, C, G, and U were replaced by G, C, G, C, U, C, and C, respectively. Based on the signature nucleotide, results supported the placement of the two isolates in a new genus. The name *Sinomonas flava* genus novel and *Arthrobacter atrocyaneus* DSM 20127^T is reclassified as *Sinomonas atrocyanea* comb. nov. (Zhou et al. 2009). Recent studies carried out by Zhou et al. (2012) proposed one more new species *Sinomonas soli* and also reclassified the species *A. echigonensis* and *A. albidus* as members of the genus *Sinomonas* as *Sinomonas echigonensis* comb. nov. and *Sinomonas albida* comb. nov., respectively, and emended the description of the genus Sinomonas. The genus currently encompasses five species *S. flava*, *S. atrocyanea* (Zhou et al. 2009), *Sinomonas soli*, *Sinomonas echigonensis*, and *Sinomonas albida*, respectively (Zhou et al. 2012). All five species formed a deep separate lineage within the family *Micrococcaceae*.

Cells stain Gram positive or variable and are aerobic, mesophilic rods (or show a rod–coccus cycle) and nonmotile, bent rods (0.5–0.9 × 1.7–4.5 μm). Weak catalase activity is present and oxidase activity is absent. Colonies are circular, convex, and pale yellow colored after 2 days cultivation

Table 27.10
Comparative phenotypic characteristics of *Nesterenkonia* spp.

Characteristics	*N. aethiopica* DSM 17733^T	*N. alba* CAAS 252^T	*N. flava* CAAS 251^T	*N. halobia* ATCC *21727*^{T}	*N. halophila* YIM 70179^T	*N. halotolerans* YIM 70084^T	*N. jeotgali* JG-241^T	*N. lacusekhoensis* IFAM EL-30^T	*N. lutea* YIM 70081^T	*N. sandarakina*	*N. xinjiangensis*
Cell shape	Short rods	Short rods	Short rods	Cocci in pairs or in tetrads	Cocci	Cocci	Cocci	Short rods occasionally branching or cocci	Cocci	Cocci	Short rods
Motility	−	−	−	−	−	+	−	−	+	−	−
Colony color	Yellow	White	Yellow	Yellow or colorless	Ivory white	Deep orange to yellow	Light yellow	Bright yellow	Light yellow to primrose yellow	Orange to yellow	Light yellow
NaCl tolerance	3–12	0–6	0–10	5 to >23	0.5–30	0–25	0–16	0 to >15	0–20	1–15	0–25
pH tolerance	7–11	8–12	8–12	<6 to 10	6–10.5	7–9	6–9	7.5–9.5	6.5–10	5–12	7–12
Oxidase activity	+	−	−	+	−	−	−	−	−	−	−
Starch hydrolysis	+	−	+	+	−	−	−	−	−	−	−
Gelatin hydrolysis	+	−	+	−	−	+	−	−	−	+	+
Hydrogen sulfide production	−	−	−	−	−	−	−	W	−	−	−
Carbon utilization											
D-Fructose	nd	−	+	nd	+	+	+	+	+	+	+
D-Mannose	nd	−	+	−	+	+	w	+	+	+	+
D-Trehalose	nd	+	−	−	nd	−	+	+	−	+	−
D-Xylose	−	−	−	+	+	−	+	nd	+	+	+
Acid from											

D-Galactose	–	–	–	+	nd	–	+	–	+	+	–
D-Lactose	–	–	–	+	–	–	–	–	+	–	–
D-Mannitol	–	–	–	+	–	–	+	–	+	+	–
D-Xylose	–	–	–	+	–	–	+	–	+	+	–
Trehalose	–	w	–	–	nd	–	w	+	+	–	–
Chemotaxonomic features											
Peptidoglycan type	L-Lys–Gly–L-Glu	L-Lys–Gly–D-Asp	L-Lys–Gly–D-Asp	L-Lys–Gly–L-Glu	L-Lys–Gly–L-Glu	L-Lys–Gly-Asp	L-Lys–Gly-Asp	L-Lys–L-Glu	L-Lys–Gly-Asp	L-Lys–Gly–D-Asp	L-Lys–Gly–L-Glu
Polar lipids	nd	PG, DPG, PI, GL	PG, DPG, PI, GL	DPG, PG, PI, GL	PI, PG, DPG, GL	DPG, PG, PI, GL	DPG, PG, PI, PL	DPG, PG, PC, GL	DPG, PG, PI, GL	DPG, PG, PI, GL	DPG, PG, PL
Major menaquinones	nd	MK7, MK8, MK9	MK7, MK8, MK9	MK7, MK8, MK9	MK8, MK9, MK7	MK7, MK8	MK7, MK8, MK9	MK7, MK8	MK7, MK8	MK7, MK8	MK7, MK8, MK9
Major cellular fatty acids (>10 %)	nd	$C_{17:0}$ anteiso, $C_{15:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso,	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso, $C_{16:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso	$C_{15:0}$ anteiso, $C_{17:0}$ anteiso,	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{15:0}$ iso	$C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{15:1}$ anteiso, $C_{16:1}$ iso,	$C_{16:0}$ iso, C15:0 anteiso, C17:0 anteiso,	$C_{17:0}$ anteiso, $C_{15:0}$ anteiso,
DNA G+C content (mol%)	69	60.2	65.5	72	68.4	64	68	66	65	64	67

Data were taken from Delgado et al. (2006) (*N. aethiopica* DSM 17733^T), Luo et al. (2008, 2009) (*N. alba* CAAS 252^T *and N. flava* CAAS 251^T), Mota et al. (1997), Stackebrandt et al. (1995), Onishi and Kamekura (1972) (for strain *N. halobia* ATCC 21727^T), Li et al. (2008b) (for strain *N. halophila* YIM 70179^T), Li et al. (2004b) (for strains *N. halotolerans* YIM 70084^T and *N. xinjiangensis* YIM 70097^T), Yoon et al. (2006) (*N. jeotgali* JG-241^T), Collins et al. (2002) (*N. lacusekhoensis* IFAM EL-30 T), and Li et al. (2005a) (*N. lutea* YIM 70081^T and *N. sandarakina* YIM 70009^T)

Symbols: + positive, – negative, w weak reaction, and nd not determined. Abbreviation: *Asp* aspartic acid, *Gly* glycine, *Glu* glutamic acid, *Lys* lysine, *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol *PI* phosphatidylinositol, and *GL* unidentified glycolipids(s)

at 30–37 °C on TYB, YDC, or PYES media. Growth occurs at 15–42 °C (optimum 30–37 °C) and pH 5.0–9.0 (optimum pH 6.0–8.0). No dark-blue pigment is produced on YDC agar at 30 °C. Growth occurs with 0–3 % NaCl, but does not occur with 4 % NaCl. Displays the following results in API 20NE tests: Tween 80 is hydrolyzed, but casein, starch, and tyrosine are not hydrolyzed; urease, β-galactosidase, and lipase activities are present; arginine dihydrolase, tryptophan decarboxylase, ornithine decarboxylase, and lysine decarboxylase activities are absent; nitrate is reduced and nitrite is not reduced; citrate is not utilized and gelatin is not hydrolyzed; H_2S is not produced; the Voges–Proskauer test is positive; and indole is negative. Displays the following results in API 50CHB tests: aesculin, cellobiose, erythrito1, D-fructose, D-glucose, glycerol, inositol, 2-ketogluconate, maltose, D-mannose, mannitol, melezitose, methyl-b-D-xyloside, D-ribose, sorbitol, sucrose, and turanose are utilized as sole carbon sources. *N*-acetylglucosamine, D-adonitol, amygdalin, D- and L-arabitol, D- and L-arabinose, arbutin, dulcitol, D- and L-fucose, D-galactose, gentiobiose, gluconate, glycogen, inulin, 5-ketogluconate, D-lactose, D-lyxose, melibiose, methyl-a-D-glucoside, methyl-α-D-mannoside, raffinose, L-rhamnose, salicin, L-sorbose, starch, D-tagatose, trehalose, xylitol, and D- and L-xylose are not utilized. The major fatty acids are ai-$C_{15:0}$, i-$C_{15:0}$, and ai-$C_{17:0}$. The respiratory quinone system consists of MK-9(H_2) and MK-8(H_2) in a molar ratio of about 7:1. Polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol and a minor amount of phosphatidylmonomethylethanolamine. The peptidoglycan type is A3α, and cell-wall sugars are galactose, mannose, ribose, and glucose. The DNA G+C content is 66.9–71.8 mol%. All the characteristics are given in detail in *Table 27.11*.

Enteractinococcus Cao, Yi, Jin, Han, He, Li, Huang, and Xue (2012), 2710

Enteractinococcus (En.ter.ac.ti.no.coc'cus. Gr. n enteron intestine; Gr. n. actis actinos a ray; N.L. masc. n. coccus a grain or berry; N.L. masc. n. *Enteractinococcus* intestinal and ray coccus).

The genus *Enteractinococcus* was very recently established by Cao et al. in 2012, isolated from *Panthera tigris* amoyensis faeces Wild Animal Park, China. The genus was placed within the family *Micrococcaceae*. The genus shows close proximity to species of the *Yaniella* with 16S rRNA gene sequence similarities of 95.9–96.2 %. Currently, the genus comprises two validly proposed species *Enteractinococcus coprophilus* and another one was reclassified species from *Yaniella fodinae* (Dhanjal et al. 2011) to *Enteractinococcus fodinae* (Cao et al. 2012).

Cells are nonmotile coccoids that occur singly or in clusters. Catalase positive; negative for production of H_2S and starch and gelatin hydrolysis. Major cellular fatty acids are iso-$C_{15:0}$ and anteiso-$C_{15:0}$. The cell-wall peptidoglycan is of type A4a (L-Lys–Gly–L-Glu). The quinone system comprises menaquinones MK-7 and MK-8. Major polar lipids are DPG, PG, and DMDG. The DNA G+C content is 56.2–61.6 mol%.

In addition, cells were not shown; the rod–coccus cell cycle as observed in the members of the genera *Arthrobacter*, *Auritidibacter*, and *Sinomonas*. The menaquinones were different from those in all genera of the family *Micrococcaceae*. The polar lipid profile, namely, DPG, PG, PI, PIM, DMDG, and unknown phospholipids, was very different from all genera of the family *Micrococcaceae*. Colonies are orange yellow. Growth occurs at 10–37 °C and at pH 7.0–11.0. Optimum growth occurs at 28 °C and pH 8.0. Tolerates up to 11 %, 15 %, and 11 % (w/v) NaCl, KCl, and $MgCl_2.6H_2O$, respectively. Oxidase negative. Positive for nitrate reduction, but negative for gelatinase, cellulose, and starch hydrolysis, milk coagulation and peptonization, and H_2S and urease production. Utilizes acetic acid, acetoacetic acid, L-aspartic acid, D-aspartic acid, D-arabitol, L-arginine, a-hydroxybutyric acid, b-hydroxy-DL-butyric acid, c-aminobutyric acid, citric acid, D-fructose, D-fucose, L-fucose, D-fructose 6-phosphate, methyl-b-D-glucoside, glycerol, a-D-glucose, D-glucose-6-phosphate, D-gluconic acid, D-glucuronic acid, L-glutamic acid, *N*-acetyl-D-glucosamine, α-ketoglutaric acid, inosine, L-galactonic acid lactone, L-lactic acid, D-mannitol, D-mannose, D-malic acid, L-malic acid, mucic acid, *N*-acetyl-neuraminic acid, propionic acid, L-pyroglutamic acid, pectin, glycyl-L-proline, quinic acid, raffinose, L-rhamnose, D-sorbitol, bromosuccinic acid, L-sorbose, D-saccharic acid, sodium butyrate, sodium lactate, L-serine, D-serine, sucrose, D-salicin, D-sorbitol, Tween 40, xylitol, and D-xylose, but not L-arabinose, L-alanine, α-ketobutyric acid, cellobiose, dextrin, dulcitol, D-lactic acid methyl ester, formic acid, fusidic acid, D-galactose, *N*-acetyl-D-galactosamine, 3-methyl glucose, gelatin, gentiobiose, D-galacturonic acid, glucuronamide, L-histidine, myo-inositol, α-lactose, maltose, melibiose, *N*-acetyl-β-D-mannosamine, methylpyruvate, p-hydroxyphenylacetic acid, stachyose, trehalose, or turanose. The cell-wall peptidoglycan is of A4a type (L-Lys–Gly–L-Glu). Whole-cell sugars contain mannose, galactose, rhamnose, glucose, and ribose. The main fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, and iso-$C_{16:0}$, with lesser amounts of anteiso-$C_{17:0}$, iso-$C_{14:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$, $C_{16:0}$, anteiso-$C_{15:1}$ A, iso-$C_{13:0}$, iso-$C_{16:1}$ G, $C_{14:0}$, and $C_{14:1}$ω5c. The DNA G+C content of the type strain is 56.2 mol%. All the characteristics are given in detail in *Table 27.12*.

Isolation, Enrichment, and Maintenance

Members of the family *Micrococcaceae* grow on a wide range of media as isolation procedures for *Micrococcus* species do not exhibit any special growth requirements. Common media containing yeast extract and peptone and a pH close to 7.0 are suitable for isolation. Specific isolation of *Micrococcus antarcticus* and *Micrococcus yunnanensis* may be done at 4 °C. To suppress growth of staphylococci, the medium for isolation of *Micrococcus luteus* should be supplemented with 0.03 % (w/v) furazolidone (Baker 1984) and 10 % (w/v) NaCl

Table 27.11

Morphological and physiological characteristics of five type strains of the genus *Sinomonas*

Characteristic	*S. soli* KCTC 19389^T	*S. flava* KCTC 19388^T	*S. atrocyanea* DSM 20127^T	*S. echigonensis* IAM 15385^T	*S. albida* IAM 15386^T
Colony pigmentation YDC agar at 25 °C TYB or PYES agar	Pale yellow Pale yellow	Pale yellow Pale yellow	Dark blue Gray white	Creamy white Pale yellow	Creamy white Pale yellow
Optimum growth temperature (°C)	30–37	30–37	37	30	30
Growth at 15 °C	+	+	–	+	+
Growth at 42 °C	+	+	+	–	–
Major fatty acids (>10 %)	ai-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, i-$C_{15:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$
	i-$C_{15:0}$	ai-$C_{17:0}$	i-$C_{15:0}$	i-$C_{15:0}$	i-$C_{15:0}$
Nitrate reduction	–	+	+	–	–
API 20E Citric acid Catalase Urease Tween 80	 (+) + (+) (+)	 + (+) + +	 – + – (+)	 + (+) – +	 – – – +
API 50CHB					
Glycerol	–	+	–	(+)	+
Erythritol	–	+	–	–	–
Ribose	–	(+)	–	–	–
Methyl β-D-xyloside	–	+	+	–	+
D-Glucose	–	+	+	(+)	+
Sorbitol	–	(+)	–	–	+
N-Acetylglucosamine	(+)	–	–	–	+
Salicin	+	–	–	(+)	+
Cellobiose	–	(+)	–	(+)	+
Maltose	–	(+)	–	(+)	+
Sucrose	+	(+)	+	–	+
Melezitose	–	(+)	–	–	+
Turanose	+	(+)	(+)	–	(+)
2-Ketogluconate	–	+	–	–	–

Data from Zhou et al. (2009, 2012), Ding et al. (2009)

+ positive, – negative, (+) weakly positive. All strains are negative for citrate utilization, gelatin liquefaction, H_2S production, indole, tryptophan deaminase, ornithine decarboxylase, lysine decarboxylase. All are positive for β-galactosidase

(Wieser et al. 2002) and incubated at 45 °C. *Micrococcus* cultures may be lyophilized or stored as a glycerol culture at −80 °C by common procedures used for many bacteria. Storage on agar slants at 4 °C for several weeks and as a glycerol suspension in 20 % concentration (v/v) or lyophilized forms for several years.

Different methods are available by which *Kocuria* sp. can be cultured from variety of samples and sources. The use of P-agar is recommended for the isolation of strains from mammalian skin of Kloos and Musselwhite (1975) and of Kloos et al. (1976) using P-agar (Naylor and Burgi 1956), and also supplementation of media with cycloheximide (50 mg/ml; Kloos et al. 1976) or 7 % NaCl (Schleifer et al. 1981) was suggested in order to prevent fungal and bacterial contaminant growth. Strains of *Kocuria varians* and *Kocuria kristinae* have been isolated on *Corynebacterium* agar. Some *Kocuria* strains may be isolated from soil or sand as described by Prauser et al. (1997) in which a diluted soil suspension in phosphate buffer is mixed with solubilized organic agar medium with agar content reduced to 0.6 % (w/v) like a semisolid medium at 48 °C. *Kocuria polaris* was isolated from Antarctic bacterial medium (ABM) (Reddy et al. 2003). *Kocuria himachalensis* was obtained on tryptone soy broth (Oxoid CM 129). *Kocuria marina* was isolated on Bacto marine broth (Difco 2216). *Kocuria aegyptia* was recovered on a medium containing (g/l^{-1}) glucose, 10.0; peptone, 5.0; yeast extract, 5.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; Na_2CO_3, 10.0; and agar, 15, pH 10–10.5 (Li et al. 2006).

Table 27.12

Comparative phenotypic characteristics of *Enteractinococcus* spp.

Characteristics	*Enteractinococcus coprophilus* DSM 24083T	*Enteractinococcus fodinae* DSM 22966T
Nitrate reduction	+	–
pH range for growth	7.0–11.0	5.2–11.0
Assimilation of:		
D-Arabitol	+	–
D-Aspartic acid	+	–
L-Arginine	+	–
γ-Aminobutryric acid	+	–
α-Hydroxybutyric acid	+	–
Cellobiose	–	+
Dextrin	–	+
Fusidic acid	–	+
D-Galactose	–	+
N-Acetyl-D-glucosamine	+	–
Glycerol	+	–
Gentiobiose	–	+
D-Galacturonic acid	–	+
Glucuronamide	–	+
α-Ketoglutaric acid	+	–
L-Glutamic acid	+	–
α-Lactose	–	+
L-Lactic acid	+	–
D-Mannitol	+	–
D-Mannose	+	–
D-Malic acid	+	–
L-Malic acid	+	–
N-Acetyl-neuraminic acid	+	–
p-Hydroxyphenylacetic acid	–	+
Pectin	+	–
Glycyl L-proline	+	–
Quinic acid	+	–
Raffinose	+	–
Sucrose	+	–
D-Salicin	+	–
Stachyose	–	+
D-Sorbitol	+	–
Bromosuccinic acid	+	–
Sodium lactate	+	–
D-Serine	–	+
Major menaquinones	MK-7, MK-8	MK-8, MK-9
Major fatty acids	iso-$C_{15:0}$, anteiso-$C_{15:0}$,iso-$C_{16:0}$	anteiso-$C_{15:0}$, iso-$C_{17:0}$
Major Polar lipids	DPG, PG, PI, PIM, DMDG, GL	DPG, PG, DMDG, PI, PIM
DNA G+C content	56.2	61.6

Data for all strains taken from Dhanjal et al. (2011) and Cao et al. (2012)

Diphosphatidylglycerol (*DPG*), phosphatidylglycerol (*PG*), phosphatidylinositol (*PI*), phosphatidylinositol mannosides (*PIM*), dimannosyl diacylglycerol (*DMDG*), an unknown glycolipid (*GL*)

Recently, *K. salsicia*, *K. atrinae*, and *K. koreensis* have been isolated on marine agar 2216 (MA, BBL) (Park et al. 2010; Yun et al. 2011), whereas *K. halotolerans* was isolated on modified ISP 5 medium (Tang et al. 2009b), and *K. gwangalliensis* was isolated on nutrient agar medium (Difco) and was maintained on PPES-II medium (Seo et al. 2009). Except *K. kristinae* (optimum temperature at 37 °C), all other representatives of the genus grow optimally at 28–30 °C. Strains can be stored for some weeks as slant cultures at 4 °C and as 20 % (w/v) glycerol suspensions at −20 °C and at −80 °C or for long-term preservation by lyophilization in skim milk and in liquid nitrogen at −196 °C.

There is no report of specific growth requirement for the members of *Citricoccus*; hence, no specific isolation medium has been defined. However, good growth occurs on PYES agar (Altenburger et al. 2002), R2A agar, TS agar (Oxoid), and, a very low at nutrient medium, CasMM agar (Altenburger et al. 1996) at 10–28 °C. Moderate growth occurs at 4 °C and 37 °C, but no growth is observed at 40 °C. Strain like *C. parietalis* (Schäfer et al. 2010) can be maintained on organic medium M79 and preserved at 28 °C by mixing in a 1:1 ratio of well-grown cultures in organic medium M79 broth with glycerol preservation medium (Salser 1978), containing (w/v) 1.26 % K_2HPO_4, 0.36 % KH_2PO_4, 0.01 % $MgSO_4$. H_2O, 0.09 % sodium citrate, 0.18 % $(NH_4)_2SO_4$, and 8.8 % glycerol. Stock cultures of the isolates in liquid M79 supplemented with 5 % DMSO were additionally maintained in the vapor phase of liquid nitrogen. The strains of *C. zhacaiensis* can be maintained in ZC medium (g/l^{-1}) 10.0 g NaCl, 1.0 g KCl, 2.0 g $MgCl_2$. $6H_2O$, 2.0 g casamino acids (Difco, Becton Dickinson), and 5.0 g Bacto yeast extract (Becton Dickinson), pH 7.5 (Meng et al. 2010). Similar enrichment, isolation, and testing media for different parameters like auxotrophy are also studied using various media (Nielsen et al. 2011). *Citricoccus* cultures can be lyophilized or stored in 21 % glycerol at −80 °C.

For isolation of *Renibacterium* species, diseased kidney tissue specimens of fish can be streaked or plated on Mueller–Hinton medium supplemented with 0.1 % (w/v) L-cysteine hydrochloride (Wolf and Dunbar 1959); kidney disease medium (KDM-2) containing 1.0 % (w/v) peptone, 0.05 % (w/v) yeast extract, 0.1 % (w/v) L-cysteine hydrochloride, and 1.5 % (w/v) agar, at pH 6.5, sterilized at 121 °C for 15 min, and cooled to 45 °C, and then 20 % (v/v) sterile fetal calf serum added (Evelyn 1977); selective kidney disease medium (SKDM) containing 1.0 % (w/v) tryptone, 0.05 % (w/v) yeast extract, 0.005 % (w/v) cycloheximide, and 1.0 % (w/v) agar, at pH 6.8, sterilized at 121 °C for 15 min, and cooled to 50 °C, and then sterile fetal calf serum added to 10 % (v/v); filter-sterilized solutions containing L-cysteine hydrochloride (0.1 % w/v), D-cycloserine (0.00125 % w/v), polymyxin B sulfate (0.0025 % w/v), and oxolinic acid (0.00025 % w/v) (Austin et al. 1983); or charcoal agar containing 1.0 % (w/v) peptone, 0.05 % (w/v) yeast extract, 0.1 % (w/v) L-cysteine hydrochloride, 0.1 % (w/v) activated charcoal, and 1.5 % (w/v) agar, at pH 6.8, sterilized at 121 °C for 15 min (the charcoal may be placed in dialysis tubing prior to sterilization to obtain a clear broth medium) as suggested by Daly and Stevenson (1985). *Renibacterium* cultures may be lyophilized by common storage used for many bacteria. Broth cultures supplemented with 15–20 % (v/v) glycerol as cryopreservation agent may be frozen at −70 °C, and their viability is maintained for longer periods.

The various species of *Rothia* described thus far do not seem to be isolated in any specific medium. It appears that species can be isolated in undefined media containing complex nutrients like tryptone, yeast, or beef extract. Strains belonging to the genus have been isolated on diverse media such as blood agar, Luria broth, and humic acid vitamin agar (Hayakawa and Nonomura 1987). In fact some species such as *R. terrae* and *R. amarae* have been maintained in nutrient agar and trypticase soy agar, respectively (Fan et al. 2002; Chou et al. 2008). Cultures can be preserved at −80 °C as a glycerol suspension in 20 % concentration (v/v), TSA, or blood agar slants at 4 °C or lyophilized (Fan et al. 2002; Chou et al. 2008).

As detailed in the above section, marine agar supplemented with NaCl seems to be a good axenic medium for isolation of *Zhihengliuella* species from saline soil samples. Cultures are routinely maintained on marine agar slants at 4 °C and as 20 % (w/v) glycerol suspensions at −20 or −80 °C. *Z. alba* can also be maintained on modified ISP 5 medium containing 5 % NaCl at 4 °C. Cells of *Z. salsuginis* can also be maintained by lyophilization at 4 °C.

Species of the *Yaniella* genus, *Y. halotolerans*, and *Y. flava* have been isolated on ISP 5 media (g/l^{-1}) 1.0, asparagine; 10.0, glycerol; 1.0, K_2HPO_4; 1.0 ml trace salts solution; and 20.0, agar (Shirling and Gottlieb 1966) and SGA agar (7.5 g casamino acids, 10.0 g yeast extract, 20.0 g $MgSO_4 \cdot 7H_2O$, 3.0 g sodium citrate, 2.0 g KCl, 1.0 ml trace salts solution, and 15.0 g agar per 1,000 ml distilled water, pH 7.5–7.6; Al-Tai and Ruan 1994), respectively, by dilution plating of saline soil samples. Both the media were supplemented with 15–20 % KCl which appears to be necessary for isolation. The trace salts solution for both strains consisted of 0.1 g $FeSO_4 \cdot 7\ H_2O$, 0.1 g $MnCl_2 \cdot 4H_2O$, and 0.1 g $ZnSO_4 \cdot 7H_2O$ per 1,000 ml distilled water, pH 7.0–7.4. Both species were isolated by incubation at 28 °C for 2 weeks. Cells can be maintained on potato agar or ISP 5 agar slants containing 10 % KCl (w/v) at 4 °C or as glycerol suspension (20 % v/v) at −20 °C.

Species of the genus *Sinomonas* were isolated on PYES (Wieser et al. 2002), YDC (Kuhn and Starr 1960), and TYB (containing 0.3 % yeast extract, 0.2 % beef extract, 0.6 % tryptone, 0.3 % NaCl, and 0.001 % $FeCl_3$) media at 41 °C from surface and polluted forest soil. Strains were routinely cultivated using TYB and PYES media at 30 °C. The pure culture was preserved in 25 % (v/v) glycerol at −80 °C, and strains can also be maintained by lyophilization at 4 °C.

Acaricomes phytoseiuli may be isolated from its host (the predator mite *Phytoseiulus persimilis*) after surface sterilization on Luria–Bertani agar and incubation for 1 week at 25 °C under aerobic conditions (Pukall et al. 2006). No information is available concerning maintenance of *Acaricomes phytoseiuli*. Similarly, *Auritidibacter ignavus* IMMIB L-1656^T (Yassin et al. 2011) was isolated from an ear swab of a 28-year-old man with

fulminant otitis externa by plating on Columbia agar supplemented with 5 % sheep's blood (BD). No information is available concerning maintenance of *Acaricomes phytoseiuli* CSC^T and *Auritidibacter ignavus* IMMIB L-1656^T.

Species of the genera *Nesterenkonia* were isolated from different habitats like *Nesterenkonia aethiopica* DSM 17733^T (Delgado et al. 2006) isolated from a soiled feather sample collected on the shore of Lake Abjata in Ethiopia (7° 60′ N 38° 62′ E), growing in complex YP medium (Mota et al. 1997) at 37 °C, with shaking at 200 r.p.m. *Nesterenkonia alba* CAAS 252^T (Luo et al. 2008, 2009) was isolated from the black liquor treatment system of a cotton pulp mill in Wuhan, China, using the standard dilution plating method. The carboxymethylcellulose sodium salt medium (CMC; containing l^{-1} peptone, 10 g; yeast extract, 10 g; NaCl, 5 g; carboxymethylcellulose sodium salt, 10 g; KH_2PO_4, 1 g, pH 10, adjusted with NaOH) was used for isolation, and these plates were incubated at 42 °C. *Nesterenkonia flava* CAAS 251^T (Luo et al. 2008, 2009) was isolated from paper-mill effluent collected in Wuhan, China, using Luria–Bertani (LB) medium at 37 °C at pH 10.0. *Nesterenkonia halobia* (Onishi and Kamekura. 1972) was isolated from unrefined solar salt of unknown origin obtained from Noda, Japan, in complex medium (Sehgal and Gibbons 1960) and nutrient broth containing 1 M NaCl. *Nesterenkonia halophila* YIM 70179^T (Li et al. 2008b) was isolated from a saline soil sample collected from Xinjiang Province, northwest China, by using the dilution plating method. For isolation, MSG medium supplemented with 25 % KCl (w/v) was used. The MSG medium was modified from S-G medium (Sehgal and Gibbons 1960) and contained (per liter distilled water) 7.5 g casamino acids, 10.0 g yeast extract, 3.0 g trisodium citrate, 2.0 g NaCl, 2.0 g $MgCl_2 . 6H_2O$, 1.0 g $MgSO_4 . 7H_2O$, 0.05 g $FeSO_4 . 7H_2O$, and 0.2 mg $MnSO_4 . 7H_2O$ (pH 9.0). Sterilized saturated NaOH was used to adjust the pH. Plates were incubated at 28 °C for 2 weeks. *Nesterenkonia halotolerans* YIM 70084^T and *Nesterenkonia xinjiangensis* YIM 70097^T (Li et al. 2004b) were isolated from hypersaline soil samples from Xinjiang Province, western China, using a modified glycerol/asparagine agar (ISP 5) medium (Shirling and Gottlieb 1966) supplemented with 15 % (w/v) $MgCl_2 . 6H_2O$ and KCl, respectively. The isolation plates were incubated at 28 °C for 2 weeks. *Nesterenkonia jeotgali* JG-241^T (Yoon et al. 2006) was isolated from jeotgal, a traditional Korean fermented seafood, by the usual dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C. *Nesterenkonia lacusekhoensis* IFAMEL-30^T (Collins et al. 2002) was isolated from a 23 m deep water sample of Ekho Lake (a hypersaline, meromictic, and heliothermal lake in the ice-free Vestfold Hills, East Antarctica) by means of a Kemmerer sampler. For isolation, PYGV medium (Staley 1968) agar prepared with Ekho Lake water of 10 % salinity was used. The sample was spread directly onto these plates and incubated at 15 °C in the dark. *Nesterenkonia lutea* YIM 70081^T (Li et al. 2005a) was isolated from a saline soil sample from China using a modified glycerol/asparagine agar medium (ISP 5) (Shirling and Gottlieb 1966) supplemented with 15 % (w/v) $MgCl_2 . 6H_2O$. Isolation plates were incubated at 28 °C for 2 weeks. *Nesterenkonia sandarakina* YIM 70009^T (Li et al. 2005a) was isolated from a soil sample collected from the eastern desert of Egypt using modified medium A (supplemented with 15 % NaCl, w/v, pH 10 · 0–10 · 5), as described previously (Hozzein et al. 2004). Isolation plates were incubated at 28 °C for 2 weeks.

Nesterenkonia alba CAAS 252^T and *Nesterenkonia flava* CAAS 251^T were subcultured and maintained on peptone yeast extract agar (PYA; containing l^{-1} peptone, 8.0 g; yeast extract, 3.0 g; K_2HPO_4, 1.0 g; EDTA, 3.5 mg; $ZnSO_4.7H_2O$, 3.0 mg; $FeSO_4 . 7H_2O$, 3.0 mg; $MnSO_4 . H_2O$, 2.0 mg; $CuSO_4 . 5H_2O$, 1.0 mg; H_3BO_3, 1.0 mg; agar, 15.0 g; $NaHCO_3/NaCO_3$, 0.1 mol, pH 10.0) and Luria–Bertani (LB) plates at 42 °C (for strain CAAS 252^T) or at 40 °C (for strain CAAS 251^T).

Nesterenkonia halophila YIM 70179^T strain YIM 70179^T was maintained on MSG agar slants that contained 10 % KCl (w/v) at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C. *Nesterenkonia halotolerans* YIM 70084^T and *Nesterenkonia xinjiangensis* YIM 70097^T were cultivated and maintained on medium containing 0.1 % (w/v) asparagine, 1 % glycerol, 0.1 % $K_2HPO_4.3H_2O$, 0.5 % yeast extract, 10 % $MgCl_2.6H_2O$ (for strain YIM 70084^T), or 10 % KCl (for YIM 70097^T). The pH was adjusted to 7.2 with 1 M NaOH. *Nesterenkonia lutea* YIM 70081^T and *Nesterenkonia sandarakina* YIM 70009^T were cultivated and maintained on modified TSA medium containing 5–10 % $MgCl_2 . 6H_2O$ (w/v), pH 7.0–8.0 for strain YIM 70081^T, or 5–10 % NaCl (w/v), pH 8.0–9.0 for strain YIM 70009^T. *Nesterenkonia lacusekhoensis* IFAM EL-30^T is maintained on the modified PYGV medium described above, while *Nesterenkonia halobia* DSM 20541^T is maintained on modified Corynebacterium agar (DSM medium 53 containing 6 % NaCl) (catalogue of strains, DSMZ 2001). All strains can also be stored on medium ISP 5, containing the recommended salt (NaCl or KCl) concentration or in the Microbank system (BioLab Diagnostics, Richmond Hill, ON, Canada). For medium-term maintenance, 20 % (v/v) glycerol suspensions at −20 °C or at −70 °C were recommended, and for long-term preservation, lyophilization in liquid nitrogen was recommended.

Strains of *Enteractinococcus* were isolated on trypticase soy agar (TSA; HiMedia) and mycose-proline agar (5 g mycose, 1 g proline, 1 g $(NH_4)_2SO_4$, 1 g NaCl, 2 g $CaCl_2$, 1 g K_2HPO_4, 1 g $MgSO_4 . 7H_2O$, 3.7 mg vitamin mixture (Hayakawa and Nonomura 1987), 20 g agar, pH 7.2) supplemented with chloramine (50 mg l^{-1}) after incubation at 28 °C for 21 days. The strain was maintained on trypticase soy agar (TSA; Difco) slants at 4 °C and as 20 % (v/v) glycerol suspensions at −20 °C.

Habitat and Ecology

Members of the *Micrococcaceae* family have been isolated from various habitats, including activated sludge, medieval wall painting, meat, human and other mammal skin, marine sediment, freshwater, desert soil, cyanobacterial mat, plants, seafood, saline soil, and oral cavity from which the original cultures were isolated (Stackebrandt et al. 1995; Rainey et al. 1997; Kovács et al. 1999; Altenburger et al. 2002; Reddy

et al. 2003; Kim et al. 2004; Li et al. 2005a, b, c, 2006; Tvrzová et al. 2005; Mayilraj et al. 2006; Zhou et al. 2008; Seo et al. 2009; Tang et al. 2009; Meng et al. 2010; Park et al. 2010; Schäfer et al. 2010; Yun et al. 2011).

Optimal growth temperature reflects the natural habitat of some *Micrococcus* species. *Micrococcus antarcticus* isolated from Antarctica grows best at 16.8 °C, *Micrococcus flavus* isolated from activated sludge grows best at 31 °C, and *Micrococcus luteus* and *Micrococcus lylae* both isolated from human and other mammal skin exhibit optimal growth at 37 °C. Kloos and Musselwhite (1975) showed that *Micrococcus luteus* is the predominant organism isolated from skin of the head, legs, and arms, whereas *Micrococcus lylae* is only occasionally isolated from skin but most frequently during the colder seasons. The association of *Micrococcus luteus* with humans is also indicated by the higher frequency of recovery from airborne bacteria collected in the "Museo Correr" in Venice, Italy, during visiting hours (Camuffo et al. 1999; Wieser et al. 2002). *Micrococcus luteus* and more rarely *Micrococcus lylae* can be also isolated from different foodstuffs. During the production of cassava fish among others, *Micrococcus luteus* can be isolated early in the fermentation process (Anihouvi et al. 2007). *Micrococcus luteus* has been isolated in moderate amounts from "androlla" (a Spanish dry-fermented sausage; García Fontán et al. 2007) and detected during ripening of Camembert cheese (Addis et al. 2001) and in goat cheese (Prado et al. 2001). Micrococci are the only Gram-stain-positive bacteria which can be isolated from ice-stored fish, and strains of *Micrococcus luteus* were shown to make up almost 20 % of totally recovered strains from this source (Lakshmanan et al. 2002a). *Micrococcus endophyticus* and *Micrococcus yunnanensis* were isolated from plant tissue and root, respectively (Chen et al. 2009; Zhao et al. 2009), whereas *Micrococcus terreus* and *Micrococcus flavus* were isolated from forest soil and activated sludge (Liu et al. 2007; Zhang et al. 2010).

Type strains of species originate from various habitats, including meat (*Kocuria carniphila*, *Kocuria varians*, and *Kocuria Kristina*); mammalian skin (*Kocuria kristinae*, *Kocuria varians*); marine sediment (*Kocuria marina*) and freshwater (*Kocuria rosea*, *Kocuria varians*); cold (*Kocuria himachalensis*), temperate (*Kocuria rosea*), and desert (*Kocuria aegyptia*) soils; cyanobacterial mat (*Kocuria polaris*); phyllosphere (*Kocuria palustris*); seafood (*K. salsicia*, *K. atrinae*, *K. koreensis*); air (*K. flava* and *K. turfanensis*); seawater (*K. gwangalliensis*); and the saline soil (*K. halotolerans*) (Stackebrandt et al. 1995; Rainey et al. 1997; Kovács et al. 1999; Reddy et al. 2003; Kim et al. 2004; Tvrzová et al. 2005; Mayilraj et al. 2006; Li et al. 2006; Zhou et al. 2008; Seo et al. 2009; Tang et al. 2009b; Park et al. 2010; Yun et al. 2011).

Presently, the *Citricoccus* genus contains four validly described strains: *C. muralis* were isolated from a fragment of a stalactite in Jourmon Cave (Gifu Prefecture, Japan), from deep-sea sediment from the Western Mediterranean Sea (Fritz 2000), and from subsea floor sediment in the southwestern part of the Sea of Okhotsk (Inagaki et al. 2003). In addition, Tiago et al. (2004) found *Citricoccus*-like organisms in nonsaline, alkaline environments. *C. alkalitolerans*, *C. parietis*, and *C. zhacaiensis* were isolated from a medieval wall painting, a desert soil in Egypt, a mold-infected wall, and a wastewater bioreactor, respectively (Altenburger et al. 2002; Li et al. 2005b; Schäfer et al. 2010; Meng et al. 2010). Recently, *Citricoccus nitrophenolicus* was isolated from wastewater treatment plant. *Renibacterium salmoninarum* is the only species in the genus *Renibacterium* which was isolated from diseased fish by using diseased kidney tissue.

The type species of the genus *Rothia* were isolated from oral cavity of humans where it is believed to be a common inhabitant (Georg and Brown 1967). In a later report, Daneshvar et al. (2004) reported isolation of eight strains of *R. dentocariosa* from various clinical samples such as throat, blood, urine, mammaries, sputum, and bronchus. These investigators also transferred the species *Stomatococcus mucilaginosus* to the genus *Rothia* as *R. mucilaginosa* comb. nov. The species *R. mucilaginosa* is a regular inhabitant of the human mouth and upper respiratory tract. It has also been found in bronchial secretions and blood cultures (Bergan and Kocur 1982). This *R. nasimurium* as the name indicates was isolated from the nose of a healthy mouse (Collins et al. 2000). *R. amarae* was isolated from a sludge suspension sample collected from a water sewer in China (Fan et al. 2002). *R. aeria* was isolated from air samples from the Russian space station Mir and was described together with a novel *Arthrobacter* and *Rhodococcus* sp. (Li et al. 2004c). In a previous study by Kawamura et al. (2001), these Gram-positive isolates were suspected to be opportunistic pathogens for immunocompromised astronauts. *R. terrae* was isolated from a wasteland soil in Taiwan (Chou et al. 2008). Type species for *Acaricomes* genus *Acaricomes phytoseiuli* has been isolated from the diseased predator mite *Phytoseiulus persimilis* Athias-Henriot (Pukall et al. 2006).

The organism was originally isolated from unrefined solar salt. Recently, more species have been added to the genus *Nesterenkonia*, isolated from various sources like hypersaline *Nesterenkonia lacusekhoensis* for strains from alkaline olive oil extraction waste (Collins et al. 2002; Ntougias et al. 2006), to *Nesterenkonia halobia* for strains from decomposing rhizomes of the reed *Phragmites australis* (Borsodi et al. 2005) and a strain from alkaline water of Lake Abijata, Ethiopia (Martins et al. 2001), and to *N. halotolerans* and *N. xinjiangensis* for a strain from an alkaline groundwater environment (Tiago et al. 2004; Li et al. 2004b). *N. sandarakina*, *N. lutea* (Li et al. 2005a), and *N. aethiopica* (Delgado et al. 2006) were isolated from hypersaline soils, while *N. jeotgali* (Yoon et al. 2006) was isolated from jeotgali, a traditional Korean fermented seafood. Many research studies on microbial diversity on such ecosystems have reported the occurrence of strains belonging to this genera: extreme alkali-saline soil (Shi et al. 2012), hypersaline high Arctic spring channel (Lay et al. 2012), and saline lake of Sovata, Romania (Borsodi et al. 2010). *N. halobia* is the most thoroughly studied species. In all, 150 taxonomic properties of six strains, isolated from ponds of a saltern located in Huelva, Spain (Ventosa et al. 1998), were studied (Mota et al. 1997).

Acaricomes phytoseiuli CSC^T was isolated from an infected mite *Phytoseiulus persimilis* (Pukall et al. 2006), and *Auritidibacter ignavus* IMMIB L-1656^T was isolated from a 28-year-old man with fulminant otitis (Yassin et al. 2011). So far, the genera *Acaricomes* and *Auritidibacter* are only represented by a single strain of a single species. Hence, their habitat and ecology are not well understood and in future addition of strains will increase the knowledge regarding these aspects.

Zhihengliuella halotolerans the type species of the genus was isolated from a saline soil sample collected from Qinghai Province, northwest China (Zhang et al. 2007). The second reported species *Z. alba* was also recovered from a saline soil sample in China. The other two species *Z. salsuginis* and the recently described *Z. aestuarii* were isolated from a salt brine and tidal flat sediment, respectively (Tang et al. 2009a; Chen et al. 2010; Baik et al. 2011).

The two type species of the genus *Yaniella*, *Y. halotolerans* and *Y. flava*, have been isolated from saline soil samples of the Xinjiang and Qinghai Provinces, respectively, of China (Li et al. 2004, 2005). One additional species *Y. fodinae* proposed (Dhanjal et al. 2011) was recently reclassified as member of another genus *Enteroactinococcus* (Cao et al. 2012) which was isolated from coal mine and *Panthera tigris* amoyensis faeces. It therefore appears that saline environments may be the best place to look for these species but their specific habitat and ecological roles are not possible to discuss due to dearth of data on this aspect. Probably isolation of more strains within the genus will shed some light on this issue.

Sinomonas flava type strain of the genus *Sinomonas* was isolated from a surface layer of forest soil in Anhui Province. Then reclassified strain *Sinomonas echigonensis* was isolated as a contaminant on a plate of Sabouraud's glucose agar. Recently proposed novel species *Sinomonas soil* was isolated from a polluted forest soil sample in Anhui Province, China. *Arthrobacter echigonensis* and *Arthrobacter albidus* (Ding et al. 2009) were reclassified as *Sinomonas echigonensis* and *Sinomonas albida* which were basically isolated from a filtration substrate made from trass, a volcanic rock on NY medium.

Type species of *Enteractinococcus* genus were isolated from soil sample of coal mine in India and from suspension inoculum (suspended in 0.85 % NaCl solution) of tiger feces collected from Wild Animal Park in China.

Pathogenicity and Clinical Significance

Strains of *Micrococcus* spp. are widespread in nature and are frequently found as normal skin flora in humans and other mammals. *Micrococcus* strains such as *M. luteus* have been isolated associated with catheter-related bacteremia in patients undergoing hemodialysis or leukemia treatment and in patients with pneumonia, endocarditis, intracranial abscesses, continuous ambulatory dialysis peritonitis, septic arthritis, and meningitis. Since *Kocuria* spp. were contained in the genus *Micrococcus* prior to 1995, some of the early reports documenting the roles of *Micrococcus* strains in human infections may be attributable to *Kocuria* spp. There are only a few reports dealing with micrococci related to human infections. However, *Micrococcus luteus* in particular can be considered an opportunistic pathogen. Strains of this species were identified as causative agents of septic shock (Albertson et al. 1978); meningitis (Fosse et al. 1985); septic arthritis (Wharton et al. 1986); endocarditis (Dürst et al. 1991; Glupczynski et al. 1986; Seifert et al. 1995); infections associated with indwelling lines, continuous ambulatory peritoneal dialysis, or a ventriculoperitoneal shunt (Magee et al. 1990); intracranial suppuration (Selladurai et al. 1993); bacteremia (Peces et al. 1997; von Eiff et al. 1996); chronic cutaneous infections in HIV-positive patients (Smith et al. 1999); and catheter infection (Oudiz et al. 2004). Another threat to human health is the ability of *Micrococcus luteus* to produce cadaverine in food through the action of lysine decarboxylase (Lakshmanan et al. 2002b). Cadaverine itself has little toxicity, but it potentiates the toxicity of histamine in food by inhibiting histamine-metabolizing enzymes such as diamine oxidase and histamine *N*-methyltransferase (Taylor and Sumer 1986).

Members belonging to the genus *Kocuria* are the part of commensal microbes on human skin, mucous membranes, the oral cavity, and outer ear canal (Frank et al. 2003; Szczerba 2003b; Szczerba and Krzeminski 2002) and were detected in indoor environments and in bacterial contaminations of airplanes (Gorny and Dutkiewicz 2002; McManus and Kelley 2005). *Kocuria rosea* and *Kocuria kristinae* were reported to cause catheter-related bacteremia in patients with severe underlying diseases (Altuntas et al. 2004; Basaglia et al. 2002); *Kocuria kristinae* infection has been reported to be associated and antibiotically treated with acute cholecystitis (Ma et al. 2005). Amoxicillin with clavulanic acid, doxycycline, ceftriaxone, cefuroxime, or amikacin (Szczerba 2003a) have been recommended to cope up with infections. For diagnosis of such infections, phenotypic-based identification methods are available, but sometimes they are misleading (Ben-Ami et al. 2003). Recently, DNA-based molecular techniques involving a combination of 16S rRNA gene amplification and subsequent single-strand conformation polymorphisms (SSCP) of the variable region V3 and a combination of community-level physiological profiling and rRNA restriction analysis (ARDRA) were used to determine the change in microbial populations in goat milk (Callon et al. 2007; Borsodi et al. 2007).

Renibacterium belongs to hallmark of kidney disease bacteria (KDB), and first two cases were reported from salmonid fishes, which are the only hosts known for the infection and etiology. By intraperitoneal injection into Chinook salmon, the mortality was observed in first ever in vivo studies reported by Ordal and Earp (1956). Later, Murray et al. (1992) induced disease in same Chinook salmon animal model by immersing them in 10^4–10^6 cells/ml for 15–30 min and by cohabitation of healthy with other experimentally infected fish. After this noteworthy observation, various studies proved that pathogen becomes internalized in non-phagocytic cells (González et al. 1999) and macrophages that produce virulence factors (McIntosh et al. 1997); within phagocytic cells, the pathogen divides slowly and survives for >10 days (Gutenberger et al. 1997); respiratory burst products

generated by macrophages kill renibacteria (Campos-Pérez et al. 1997; Hardie et al. 1996). Various genetic answers came from colonization and infection causes, i.e., soluble cell surface p57 protein is responsible for agglutination of salmonid leukocytes (Senson and Stevenson 1999; Wiens et al. 1999) and the presence of proteases (Sakai et al. 1989) and hemolysins (Grayson et al. 1995a, 2001) in extracellular products (ECPs) with the mode of action reflecting inhibition of the respiratory burst but not phagocytic activity in spleen phagocytes (Densmore et al. 1998). Several virulence factors like hydrophobicity and auto-aggregation (Bruno 1988), an iron acquisition mechanism (Grayson et al. 1995b), respond to infection by the production of stress factors, including plasma cortisol and lactate, and reduced levels of plasma glucose (Mesa et al. 1999); a 70-kDa stress protein (HSP70) that has been recognized in diseased coho salmon (Forsyth et al. 1997) has been linked with severity of infection. Routine serological diagnostic methods include co-agglutination (Kimura and Yoshimizu 1981), Western blots (Lovely et al. 1994), the fluorescent antibody test, and the enzyme-linked immunosorbent assay (Griffiths et al. 1996, 1991; Jansson et al. 1996; Kozinska and Pekala 2005; O'Connor and Hoffnagle 2007; Pascho et al. 1998) that have also been suggested along with somewhat cross-reactivity. The polymerase chain reaction (PCR) targeting a 320-bp fragment of a 57-kDa protein (p57 antigen; Pascho et al. 1998), nested reverse transcriptase PCR (C.ook and Lynch 1999), quantitative PCR (Powell et al. 2005), real-time PCR, sequencing of the 16S rRNA gene (Konigsson et al. 2005), and terminal restriction fragment length polymorphism (Nilsson and Strom 2002) are specific and sensitive for testing purpose.

Out of the six species of *Rothia* described so far, three strains, *R. dentocariosa*, *R. mucilaginosa*, and *R. aeria*, have been isolated from humans, from oral cavity, mouth, and upper respiratory tract and blood, synovial fluid, and sputum, respectively. These species are now established as opportunistic pathogens especially in children and immunocompromised individuals. *R. dentocariosa* and *R. mucilaginosa* have been isolated from infections in patients diagnosed with a medical condition that results in lowered immune responses such as cancers, AIDS, transplantations, or surgery (Austin 2012). *R. dentocariosa* has been frequently associated with endocarditis in patients with periodontal disease and recovered from children with eye infections and tonsillitis (Austin 2012). Daneshvar et al (2004) had reported charcoal-black-pigmented colonies of *R. dentocariosa* isolated from the female genitourinary tract. *R. mucilaginosa* has been mainly associated with meningitis in immunocompromised patients with cancers or those who receive stem cell transplantations (Austin 2012). The species is also an etiological agent for bacteremia in patients with leukemia, pneumonia, and AIDS (Austin 2012). Recently, *R. aeria* has been established as a causative agent of sepsis in a female neonate (Monju et al. 2009) and an 88-year-old woman diagnosed with rheumatoid arthritis (Verrall et al. 2010). In both cases, the patients had a history of dental infection, thus implicating the oral cavity as the source of pathogen. The species was also associated with a case of acute bronchitis in a 66-year-old man with rheumatoid arthritis (Michon et al. 2010). In both cases of adults, immunosuppressive therapy was administered to the patients due to arthritis, and it is speculated that this resulted in the normal bacterial inhabitant of the mouth to become a pathogen. Except these three cases, there has been till date no other report of pathogenesis regarding *R. aeria*. Antimicrobial MICs determined using the broth microdilution method in accordance with National Committee for Clinical Laboratory Standards (NCCLS) revealed that the *R. dentocariosa* strains were susceptible to cefepime, cefotaxime, ceftriaxone, chloramphenicol, levofloxacin, penicillin, tetracycline, and vancomycin (Daneshvar et al. 2004). *R. mucilaginosa* is sensitive to bacitracin, ampicillin, chloramphenicol, erythromycin, fusidic acid, lincomycin, neomycin, novobiocin, oleandomycin, oxytetracycline, and penicillin G. Usually sensitive to sulfonamide and streptomycin (Bergan and Kocur 1982). *R. terrae* is sensitive by disc diffusion method to the following antibiotics: ampicillin, chloramphenicol, erythromycin, penicillin G, rifampicin, novobiocin, streptomycin, and tetracycline. It is resistant to gentamicin, kanamycin, and nalidixic acid (Chou et al. 2008). Based on Clinical and Laboratory Standards Institute interpretative criteria for staphylococci, *R. aeria* is sensitive to most of the antibiotics like penicillin, ampicillin, erythromycin, clindamycin, gentamicin, vancomycin, amoxicillin, and cefotaxime and is resistant to ofloxacin (Monju et al. 2009; Verrall et al. 2010; Michon et al. 2010). For the rest of the three species, *R. aeria*, *R. amarae*, and *R. nasimurium*, no data on antibiogram is available.

Acaricomes phytoseiuli is assumed to be the causative agent of the "non-responding syndrome" in *Phytoseiulus persimilis* (Schütte et al. 1998; Dicke et al. 2000; Bjørnson and Schütte 2003). *Auritidibacter ignavus* can cause fulminant obits externa in humans, but no information is available concerning the pathogenic potential for humans and clinical importance. The cells were sensitive to the following antimicrobial agents (mg per disc): ampicillin (10), cefazolin (30), clindamycin (2), oxacillin (5), penicillin (10), and vancomycin (30).

No pathogenic role has been reported for other members of the *Micrococcaceae* family like *Citricoccus*, *Nesterenkonia*, *Yaniella*, *Sinomonas*, *Zhihengliuella*, and *Enteractinococcus*.

Applications

Micrococci, like many other representatives of the *Actinobacteria*, can be catabolically versatile, with the ability to utilize a wide range of unusual substrates, such as pyridine, herbicides, chlorinated biphenyls, and oil. They are likely involved in detoxification or biodegradation of many other environmental pollutants. Other *Micrococcus* isolates produce various useful products, such as long-chain (C21–C34) aliphatic hydrocarbons for lubricating oils. *Micrococcus* species has potential role in bioremediation and in its importance in biotechnology. In the former sphere, it combines two properties essential to dealing with toxic wastes: the ability to degrade toxic organic pollutants and tolerance to metals (Sandrin and Maier 2003).

Often found in contaminated soils, oil spills, and sludge, *M. luteus* can degrade hydrocarbons and olefinic compounds (Zhuang et al. 2003), use biphenyl as a carbon source, and degrade phthalates (Eaton 1982). It harbors a plasmid capable of degrading malathion and chlorpyrifos (Guha et al. 1997). A whole repertoire of functions which deal with metals have been found in *M. luteus*. It carries out biosorption of strontium (Faison et al. 1990) and to a lesser degree lead, nickel, and zinc (Lo et al. 2001). The organism was utilized in a filtration system bound to gelatin beads for the biosorption of strontium. Since strontium is an end product of uranium decay, this could be a major component of atomic energy waste management. There is special interest in its ability to bind gold (Levchenko et al. 2001). Interesting variants of *M. luteus* have been isolated from gold deposits in Russia, which are able to precipitate gold by concentrating and crystallizing it on their surface. It has been suggested that these properties could be used for gold adsorption and concentration from low-abundance ores and depleted deposits (Marakushev 1991).

In the chemical and pharmaceutical industries, *M. luteus* may be exploited for its capability in isoprene synthetic reactions. This is the cornerstone of sterol, carotenoid, rubber, and fatty acid synthesis, and *M. luteus* has been the platform for isolation of important enzymes in this most basic processes, including the cis-prenyltransferase gene, whose gene product carries out the condensation of isopentenyl phosphate with allelic diphosphate (Oh et al. 2000). This is an essential step in the biosynthesis of terpenes, major components of a number of commercial products. The membranes of *M. luteus* are rich in enzymes that catalyze the synthesis of prenyl pyrophosphates at chain lengths between 15 and 45 carbon atoms (Saito and Ogura 1981). A number of interesting biological issues are also inherent in the study of *Micrococcus*, including dormancy without spore formation, resuscitation from dormancy, and the significance of tetrad formation. Although capable of survival under stress conditions, such as low temperature and starvation, *M. luteus* does not form spores as survival structures, usually thought of as a prerequisite for long-term survival in some other bacteria like Bacilli and Actinomycetes. This capability to survive for long periods in extreme environments may well explain their repeated isolation from within fossilized amber (Greenblatt et al. 2004). Of special recent interest is the elucidation of the resuscitation-promoting factor (Rpf) described in *Micrococcus luteus* (Mukamolova et al. 2002). This was the founder member of a family of secreted transglycosylase-like proteins that can resuscitate bacteria from a dormant state (Kell and Young 2000; Cohen-Gonsaud et al. 2005; Mukamolova et al. 2006). *M. luteus* Rpf can improve the cultivability of other high-G+C organisms with a low plating efficiency manyfold. Rpf has many important implications for the detection and culturing of these organisms, a number of which are important human pathogens (e.g., *Mycobacterium tuberculosis*). Genes similar to *rpf* have a widespread distribution throughout the actinobacteria, and most organisms, including *M. tuberculosis*, contain multiple gene homologues. *M. luteus* is very unusual in containing only a single, apparently essential *rpf*-like gene, which makes *M. luteus* the organism of choice for further work on the mechanism of restoration of cultivability. The tetrad of *Micrococcus luteus* may share with that of *Deinococcus radiodurans* a special function in DNA repair (Englander et al. 2004). Comparative genomics should, therefore, also provide clues to understanding developmental physiology and morphology in actinobacteria. The ability to adapt to oligotrophic environments and tolerate toxic metals and organic compounds may also relate to these morphological alterations. Finally, *M. luteus* (formerly *Micrococcus lysodeikticus*) is of historical interest in microbiology and medicine, since it played a prominent part in Fleming's discovery of lysozyme, to which it shows exquisite sensitivity (Fleming 1922). Some species of *Micrococcus* carry out biosorption of Sr (Faison et al. 1990). Indeed, these species belong to a group of bacterial strains that are particularly well adapted to environments contaminated with elevated levels of toxic metals and that are potentially useful for bioremediation applications (Young et al. 2010, and references therein). The genus *Micrococcus* now includes seven species, with one of the smallest actinobacterial genomes sequenced to date; *M. luteus* possesses unusual abilities to remediate co-contaminated sites with organic and metal pollutants through a whole repertoire of functions which deal with these pollutants (Sandrin and Maier 2003; Young et al. 2010). In addition, *M. luteus* can degrade hydrocarbons and olefinic compounds (Zhuang et al. 2003), use biphenyl as a carbon source, and metabolize dibutylphthalate by a pathway which has at least five regulatory units (Eaton 1982). As mentioned earlier, *Micrococcus luteus* has important biotechnology applications, especially in the chemical and pharmaceutical industries. *M. luteus* may be potentially exploited for its capability in isoprene and terpene synthetic reactions. *M. luteus* has been the platform for isolation of the cis-prenyltransferase gene (a *Rer2* gene homolog first found in *S. cerevisiae*). *cis*-Prenyltransferase catalyzes the sequential condensation of isopentenyl diphosphate with allylic diphosphate to synthesize polyprenyl diphosphates that play vital roles in cellular activity. Even earlier studies have shown that the membranes of *M. luteus* are rich in enzymes that catalyze the synthesis of prenyl pyrophosphates (small molecules required to make compounds such as cholesterol, carotene, and alkylamines) (Saito and Ogura 1981). Prenyl pyrophosphates are currently being looked at as possible nonpeptide antigens that stimulate certain T-cells as vaccines to prevent human infections and to treat cancer. Their activation requires their exposure to small phosphorus containing antigens in the family of prenyl pyrophosphates and their related biosynthetic precursors such as isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are naturally occurring metabolites in *Micrococcus luteus* as well as several microbial pathogens. Various prenyl pyrophosphate and diphosphonate compounds are being tested to find new vaccine candidates and thus invite the potential use of isoprenoid-pyrophosphonates as specific immunoregulatory molecules (Zgani et al. 2004). The invention discloses an application of a strain of *Micrococcus antarcticus* in low-temperature sewage disposal, *Micrococcus antarcticus* applied in low-temperature sewage disposal. At lower

temperature (10–15 °C) *Micrococcus antarcticus* efficiently removes BOD, COD, egg white, fat, and the starch in sewage, has very high application value in biological processing which low temperature sewage/wastewater (such as sewage).

Polycyclic aromatic hydrocarbons degrading activity in *Kocuria* species have been reported. Harwati et al. (2007) first time reported degradation of components of Arabian light crude oil by *Kocuria rosea* and *Kocuria aegyptia*. Nazina et al. (2002) isolated a strain *Kocuria erythromyxa* (*K. rosea* according to reclassification) from an oil field. Rauch et al. (2006) isolated *Kocuria rhizophila* from aviation fuel tank. Tumaikina et al. (2008) isolated *K. rosea* from the pondweed surface that grew on agar medium with crude oil as carbon source. Mariano et al. (2008) isolated *Kocuria palustris* from soil of a petrol pump and first time reported degradation of commercial diesel oil by *K. palustris*. Similarly, biodegradation of methyl-*tert*-butyl ether by *Kocuria* sp. has been reported by Lalević et al (2012), and 6-aminohexanoate oligomer hydrolases from the alkaliphilic *Kocuria* species have been reported (Yasuhira et al. 2007). Bacteriocin in food biopreservation is represented by variacin produced from *Kocuria varians* to control the growth of *B. cereus* in chilled dairy products (O'Mahony et al. 2001).

Potential xylanolytic actinomycete strain was reported (Kocuria RM1) from the extremely alkaline bauxite residue obtained from National Aluminum Company Ltd., Damanjodi, India (Krishna et al. 2008). Karn et al. (2011) investigated the PCP degradation activity in Kocuria which degraded 58.64 % of PCP from the sludge within 2 weeks of treatment. These results highlight the potential of these bacteria to be used in bioremediation of high-strength PCP contaminated pulp and paper-mill sludge. Lignin peroxidase enzymes from *Kocuria rosea* MTCC 1532 have been studied by Parshetti et al. (2010, 2012) and tested for its ability to decolorize different groups of dyes. And novel alkali-stable, cellulase-free xylanase from *Kocuria* sp. Mn22 has been investigated by Li et al. (2009).

Some species such as *Nesterenkonia halobia* have been shown to produce industrially relevant enzymes such as amylases (Sánchez-Porro et al. 2003), while other species secrete unique serine proteases (Yang et al. 2007). However, no detailed studies have yet been performed on the production of hydrolytic enzymes from this genus. Therefore, Lucretia et al. (2009) describe the isolation of hydrolase-producing bacteria from evaporator ponds at Sua Pan in Botswana, with more emphasis on the production of xylanase by a newly isolated *Nesterenkonia* species.

Hong et al. (2010) first time described the cloning and expression of a family 11 xylanase, Xyn11NX, from the genus *Nesterenkonia*. This xylanase had high sequence homology to the xylanases from Actinomycetes but showed much broader pH adaptability and higher thermostability. Good pH adaptability and thermal stability, cellulase-free natures, and less complex hydrolysis products make it promising for various applications in many industries, especially in pulp and paper industry. Nel et al. (2011) reported the nitrilase superfamily with amidase activity derived from a novel psychrophilic strain of the genus *Nesterenkonia*, isolated from Antarctica Dry Valley soils. Cold-active amidases have yet to find commercial application, but there remains substantial interest in the applications of cold-active enzymes. The focus of such interest is in industrial sectors where low-temperature processing is beneficial, most commonly in the food and beverage industry and in some chemical biotransformations.

Zamakhchari et al. (2011) investigated the specific microorganisms in mixed dental plaque that display potent gluten-degrading activity from *Rothia* genus. The most efficient cleaving strains were identified as *Rothia mucilaginosa* and *Rothia aeria*. In gliadin pretreatment with mammalian digestive enzymes, gliadins (and by inference glutens) serve as a good substrate for *Rothia*-associated bacterial enzymes and are rapidly cleaved. Importantly, major immunogenic epitopes that play a key role in celiac disease are also targeted by *Rothia* enzymes. These findings suggest that *Rothia* bacteria may contribute to the digestive processing of immunogenic gluten proteins. Gluten-degrading microorganisms in the gastrointestinal tract may play a hitherto unappreciated role in the digestion/detoxification of dietary gluten. They open promising new avenues in the search for novel therapies to neutralize the deleterious effects of gluten in patients with celiac disease. Furthermore, to exploit these bacteria or their enzymes is highly attractive, since they belong to the normal flora of the upper gastrointestinal tract. Patent No US20120230976 A1 disclosed are glutamine endopeptidase enzymes from *Rothia* sp. bacteria that are naturally associated with the oral cavity, formulations comprising the glutamine endopeptidase enzymes and the use thereof for the treatment, prevention of allergic reaction, and diagnosis of gluten allergy-related diseases such as celiac sprue, gluten allergy, and/or dermatitis herpetiformis. While the human digestive enzyme system lacks the capacity to cleave immunogenic gluten, such activities are naturally present in the oral microbial enzyme repertoire. The identified bacteria may be exploited for physiological degradation of harmful gluten peptides (Helmerhorst and Oppenheim 2012).

Nelly et al. (2004) isolated a new strain of *Renibacterium salmoninarum* with the capacity to grow on and degrade *n*-hexadecane and at the same time to produce surface-active compounds. And this is the first report of *Renibacterium salmoninarum* strain that produces the two typical for *Pseudomonas aeruginosa* rhamnolipids. There are several reports of pathogenic bacteria that were found to produce biosurfactants (Burd and Ward 1996; Iglewski 1989). The exact role of biosurfactants is not clear – maybe they assist the colonization of host tissues or participate in increasing the bioavailability and degradation of hydrophobic organic contaminants by the host bacteria (Finnerty 1994; Rosenberg 1986; Rouse et al. 1994). Hence, these organisms may play an important role in the natural degradation of hydrocarbon contaminants in the environment and have potential use in accelerated bioremediation processes.

Nielsen and Ingvorsen (2012) reported a Gram-positive bacterium *Citricoccus nitrophenolicus* (strain PNP1T, DSM 23311^T, CCUG 59571^T) isolated from a wastewater treatment plant was capable of effectively degrading p-nitrophenol (pNP)

as a source of carbon, nitrogen, and energy for growth strain also degraded 4-chlorophenol, phenol, and salicylate. pNP was degraded at pH values between 6.8 and 10.0 and at temperatures between 15 °C and 32 °C. Therefore, it is a potential candidate for use in subsequent bioremediation of this breakdown product at the Breakwater 42 waste dump following alkaline hydrolysis and solubilization of the original contaminants in the aquifer. An antitumor activity has been reported by Nataliya et al. (2011) from a novel actinobacterium; *Citricoccus* strain KMM 3890 was isolated from a sediment sample from the Sakhalin shallow environment. In addition to its hemolytic activity, strain exhibited inhibitory activity against Gram-positive bacteria. It was found that the marine isolate *Citricoccus* sp. KMM 3890 produced large amount of the compound, which was isolated and structurally characterized as known cyclic siderophore nocardamine on the basis of combined spectral analyses. Nocardamine showed inhibitory effects to colony formation of T-47D, SK-Mel-5, SK-Mel-28, and PRMI-7951 tumor cell lines. This study can be considered as the first report on marine isolate of the genus *Citricoccus* producing nocardamine with antitumor activity.

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28 The Family *Micromonosporaceae*

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Abstract
Since the last edition of *The Prokaryotes*, most microorganisms grouped in the chapter "The genus *Actinoplanes* and Related Genera" have been formally classified in the family *Micromonosporaceae*, order *Micromonosporales*, phylum Actinobacteria. According to the phylogenetic branching of the RaxML 16S rRNA gene tree, the members of the family represented by all type strains form well-defined clades and are related to members of the families *Glycomicetaceae* and *Jiangellaceae*. The family currently harbors 27 genera and includes microorganisms characterized by three types of sporulating structures, namely, single spores, spore chains, and sporangia which are borne directly on the substrate hyphae. Spores may be nonmotile or motile with tufts of polar flagella. They are aerobic, non-acid fast and mesophilic microorganisms. Many strains produce carotenoid mycelial pigments, giving the

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colonies an orange to red appearance; however blue-green, brown, or purple pigments are also produced.

Most members of the family *Micromonosporaceae* are characterized by a cell-wall type chemotype II. The wall peptidoglycan contains *meso*- and/or 3-hydroxy-diaminopimelic acid and is of the A1α type; L-lysine may also be found as a diagnostic amino acid. Except for Pilimelia especies which contain acetate, the first aino acid of the peptide chain is glycine in all members of the family. Whole-organism hydrolysates are rich in arabinose, xylose, and galactose, with variable amounts of other sugars. The organisms produce complex mixtures of saturated, iso-, and anteiso-fatty acids. Phosphatidylethanolamine is the diagnostic phospholipid (phospholipid type II).

Micromonosporaceae strains have been isolated from diverse habitats including soil, sediments, fresh and marine water, rhizosphere, and plant tissues. Many species degrade chitin, cellulose, lignin, and pectin, and these microorganisms play an important role in the turnover of organic plant material. In addition, many strains produce useful secondary metabolites and enzymes. They have important applications in industry, biotechnology, and agriculture.

Taxonomy: Historical and Current

Short Description of the Family

Mi.cro.mo.no.spo.ra.ce'a.e. N. L. fem. n. *Micromonospora* type genus of the family; suff.-*aceae* ending to denote a family; N. L. fem. pl. n. *Micromonosporaceae* the *Micromonospora* family.

The family *Micromonosporaceae* is a member of the order *Micromonosporales* (Genilloud 2012) in the phylum Actinobacteria. The genus *Micromonospora* (Ørskov 1923) is the type genus of the family which at time of writing includes 27 phylogenetically closely related genera (http://www.bacterio.cict.fr) that can be distinguished using a combination of chemotaxonomic, morphological, and phylogenetic characteristics.

Members of the family *Micromonosporaceae* stain Gram-positive and form non-fragmenting, branched, and septate substrate hyphae; aerial mycelium is absent or scanty. Microorganisms in this taxon are characterized by three types of sporulating structures, namely, single spores, spore chains, and sporangia which are borne directly on the substrate hyphae. Spores may be nonmotile or motile with tufts of polar flagella. These bacteria are aerobic, non-acid fast, and mesophilic. Colonies on agar media are flat to elevated with smooth or wrinkled surfaces and show a large variety of pigments. Many strains produce carotenoid mycelial pigments giving the colonies an orange to red appearance; however blue-green, brown, or purple pigments may also be produced. The wall peptidoglycan contains *meso*- and/or 3-hydroxy-diaminopimelic acid and is of the A1α type; L-lysine may also be found as a diagnostic amino acid. Except for Pilimelia especies which contain acetate, the first aino acid of the peptide chain is glycine in all members of the family. Whole-organism hydrolysates are rich in arabinose, xylose, and galactose, with variable amounts of other sugars. The organisms produce complex mixtures of saturated, iso-, and anteiso-fatty acids. Phosphatidylethanolamine is the diagnostic phospholipid (phospholipid type II) but diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol are also found as major components. Mycolic acids are absent. Menaquinone profiles are heterogeneous and may include all types of the MK-9 and MK-10 series. A family-specific pattern of the 16S rRNA gene sequence signatures was defined at positions 127 : 234 (A–U), 209 (G), 534 (G), 831 : 855 (U–G), 832 : 854 (G–Y), 833 : 853 (U–G), 840 : 846 (Y–G), 845 (G), 955 : 1225 (A–U), 986 : 1219 (U–A), and 987 : 1218 (G–C) based on 17 genera described at that time (Zhi et al. 2009).

Microorganisms classified in the family *Micromonosporaceae* have been isolated from diverse habitats including soil, sediments, fresh and marine water, rhizosphere, and plant tissues (Kawamoto 1989; Zhao et al. 2004; Valdés et al. 2005; Maldonado et al. 2005a; Trujillo et al. 2007; de Menezes et al. 2008; García et al. 2010; Carro et al. 2012; Genilloud 2012).

Molecular Analyses

Phylogenetic Structure of the Family and Its Genera

As currently defined, the family *Micromonosporaceae* Krasil'nikov 1938, emend. Zhi et al. 2009 with the type genus *Micromonospora* (Ørskov 1923) is the only member of the order "*Micromonosporales*," class *Actinobacteria* (Stackebrandt et al. 1997; Genilloud 2012). In addition to the genus *Micromonospora*, at present the family includes the following genera: *Actinocatenispora* (Seo and Lee 2009); *Actinoplanes* (Couch 1950; emended by Stackebrandt and Kroppenstedt 1987), *Allocatelliglobosispora* (Lee and Lee 2011), *Asanoa* (Lee and Hah 2002), *Catellatospora* (Asano and Kawamoto 1986; emended by Lee and Hah 2002; emended by Ara et al. 2008a), *Catelliglobosispora* (Ara et al. 2008a), *Catenuloplanes* (Yokota et al. 1993), *Couchioplanes* (Tamura et al. 1994), *Dactylosporangium* (Thiemann et al. 1967), *Hamadaea* (Ara et al. 2008a), *Jishengella* (Xie et al. 2011b), *Krasilnikovia* (Ara and Kudo 2007a), *Longispora* (Matsumoto et al. 2003), *Luedemanella* (Ara and Kudo 2007b), *Phytohabitans* (Inahashi et al. 2010), *Phytomonospora* (Li et al. 2011), *Pilimelia* (Kane 1966), *Planosporangium* (Wiese et al. 2008), *Plantactinospora* (Qin et al. 2009), *Polymorphospora* (Tamura et al. 2006), *Pseudosporangium* (Ara et al. 2008b), *Rugosimonospora* (Monciardini et al. 2009), *Salinispora* (Maldonado et al. 2005a), *Spirilliplanes* (Tamura et al. 1997), *Verrucosispora* (Rheims et al. 1998), and *Virgisporangium* (Tamura et al. 2001).

According to the phylogenetic branching of the 16S rRNA gene tree, the 27 genera represented by the type species form, in general, well-defined clades; however, the genera *Phytomonospora* and *Actinocatenispora* cluster together and form a long branched cluster which is recovered outside the *Micromonosporaceae* main group (*Fig. 28.1*).

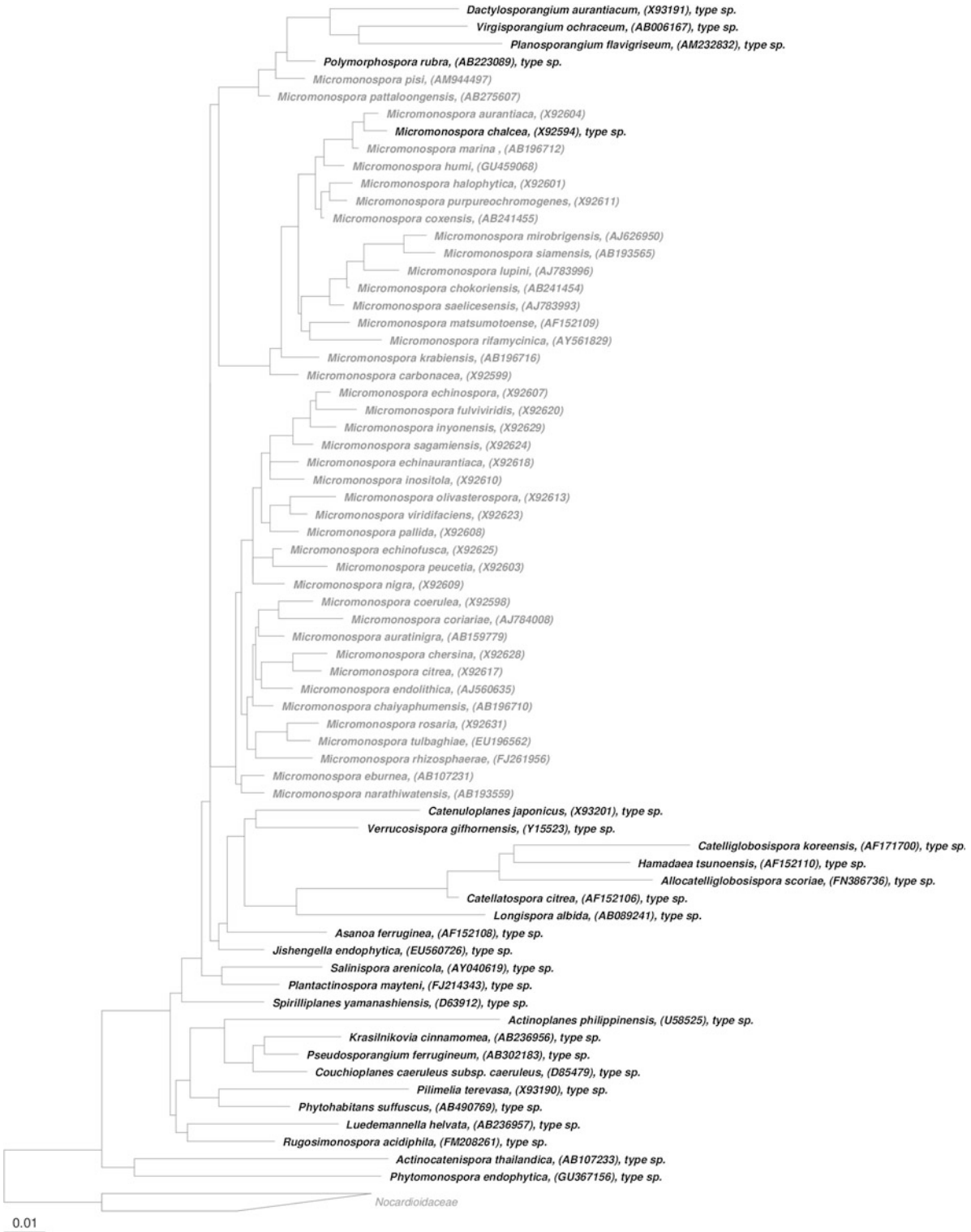

Fig. 28.1

Phylogenetic reconstruction of the family/*Micromonosporaceae*/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 <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

The family *Micromonosporaceae* is moderately related to members of the families *Glycomicetaceae* and *Jiangellaceae*. Interestingly, when representatives of the family *Glycomicetaceae* (the genera *Glycomyces* and *Stackebrandtia*) are included in the same phylogenetic analyses, these taxa show a close relationship with the genus *Phytomonospora* (tree not shown). The three genera share several chemotaxonomic markers including a type II cell wall which contains *meso*-diaminopimelic acid. However, differences in the phospholipid type and menaquinone composition do exist. Before the genus *Phytomospora* was formally described, Ludwig et al. (2012) already pointed the close relationship between the genus *Actinocatenispora* and the members of the family *Glycomycetaceae*. In the previous edition of this volume, the genus *Glycomyces* was included with the "Actinoplanetes" taxa, due to their chemotaxonomic similarities; however, it was made clear that its position in this group was exceptional (Vobis 2006).

The largest group in ❯ *Fig. 28.1* corresponds to the *Micromonospora* species which share 16S rRNA gene sequence similarity values of 95.8–99.5 %. Clearly, the genus *Micromonospora* is polyphyletic. The type strains divide in two clades which in turn form smaller subclusters; the relative positions of the species are generally conserved in the overall topology of other phylogenetic trees constructed with different methods (Carro et al. 2012; Xie et al. 2012b). In the case of the species *Micromonospora pisi* and *Micromonospora pattaloongensis*, the type strains form separate lines of descent which relate to Pol*ymorphospora rubra*. The relationship of these strains was pointed out in recent studies including an MLSA analysis of the genus *Micromonospora* based on 5 genes: 16S rRNA, gyrB, rpoB, atpD, and recA where *P. rubra* was also included (Carro et al. 2012). The main differences between the genera *Micromonospora* and *Polymorphospora* are found in the whole cell-wall sugar composition and fatty acid type. However, García et al. (2010) reported different whole-cell sugar and fatty acid compositions when *P. rubra* TT 97-42^T was reanalyzed in a comparative study that also included *M. pisi* and *M. pattaloongensis*. Further investigation will be necessary to determine whether *P. rubra* TT 97-42^T is sufficiently different to warrant representing a genus distinct from *Micromonospora*.

The *Actinoplanes* species form the second largest group in the family tree. The genus does not represent a monophyletic taxon and also forms several clades (not shown). The species *A. globisporus* has the deepest branch and shows a similarity of 94.1 % with the species *Actinoplanes auranticolor* and up to 96.9 % with the rest of the species. Nevertheless, the phylogenetic position of *A. globosisporus* within the *Actinoplanes* clade is supported by different tree making algorithms. The genera *Krasilnikovia*, *Pseudosporangium*, and *Couchioplanes* are the nearest relatives.

The remaining members of the family (with 1–12 species at the time of writing) all form independent coherent clusters albeit not always monophyletic. Nevertheless, the distribution of genus-specific properties and signature nucleotides that define the genera correlate with the phylogenetic distinctness of each genus. Furthermore, it should be noted that 11 of the current taxa represented on the tree are monospecific genera; therefore, it is expected that the topology of the current tree will change as new representative species of these genera are added.

Molecular Analyses

DNA-DNA Hybridization Studies

DNA-DNA hybridization (DDH) studies have been carried out in many descriptions of new taxa included in the family *Micromonosporaceae*. In general, where studies have been carried out, DNA-DNA reassociation values are rather low compared to their corresponding 16S rRNA gene sequence similarity values.

In the genus *Micromonospora*, the highest DNA-DNA reassociation value, 61.3 %, has been reported between *M. inyonensis* and *M. sagamiensis;* these strains share a 16S rRNA sequence similarity value of 99.4 % (Kroppenstedt et al. 2005). Similar results were also observed between *M. chersina* and *M. endolithica* (33.5 %; Hirsch et al. 2004), *M. aurantiaca* and *M. chalcea* (44 %; Kasai et al. 2000), *M. echinospora* and *M. sagamiensis* (52 %), *M. citrea* and *M. echinofusca* (53 %), *M. echinaurantiaca* and *M. viridifaciens* (53 %) (Kroppenstedt et al. 2005). In all cases, 16S rRNA sequence similarities are >99.1 %.

Recently, Carro et al. (2012) reported a good correlation between DDH and MLSA studies. These authors propose that genomic species within the genus *Micromonospora* could be defined as groups of strains that share >98.5 % similarity in the concatenated nucleotide sequences of gyrB, recA, atpD, rpoB, and 16S rRNA genes.

Many descriptions of *Actinoplanes* species have also included DDH studies to determine the genomic status of the taxa described. In general, DNA-DNA hybridization values are moderately low; however, unlike *Micromonospora* species, the correlation between DDH values and 16S rRNA gene sequence similarities is not clear. The DNA-DNA reassociation value reported for the species *Actinoplanes auranticolor* and *Actinoplanes lobatus* is 41.5 % (Wink et al. 2006), while *Actinoplanes toevensis* and *Actinoplanes tereljensis* have a DDH value of 42.2 % (Ara et al. 2010). These two strain pairs have a 16S rRNA gene sequence similarity >99 %. Interestingly, *A. toevensis* shares a higher DNA-DNA hybridization value (46.7 %) with *A. durhamensis* while these species share a sequence similarity of 97.0 % (Ara et al. 2010). The latter species also appears to be closely related with *A. tereljensis* (56.3 %, Ara et al. 2010).

Unfortunately, DDH studies have not been carried out for various *Actinoplanes* strains that show a 16S rRNA gene sequence similarity higher than 99.5 %. This is the case for *A. campanulatus* and *A. capillaceus*, (16S sequence similarity 99.8 %), or *A. auranticolor* and *A. lobatus* (99.7 %). Likewise, *Actinoplanes humidus* and *Actinoplanes consettensis* (100 % sequence similarity) have not been included

in DNA-DNA hybridization studies to confirm that they are indeed different genomic species. Although the two representative type strains are distinguishable by the reduction of nitrate (Goodfellow et al. 1990), DNA-DNA hybridization studies may be required to confirm that they are genomically defined species.

Six of the twelve *Dactylosporangium* species descriptions have included DNA-DNA comparative studies. The overall 16S rRNA gene sequence similarity between all species ranges from 97.1 % to 99.7 %. Moderately high DNA-DNA values were reported for the species *Dactylosporangium luteum*, *Dactylosporangium luridum*, and *Dactylosporangium salmoneum* and their respective closest phylogenetic neighbors (Kim et al. 2010). Specifically, *Dactylosporangium luteum* was compared against *D. aurantiacum*, *D. luridum*, *D. matsuzakiense*, *D. salmoneum*, and *D. vinaceum*; values reported ranged from 42 % to 53 %. *Dactylosporangium luridum* was tested against *D. aurantiacum* (59 %), *D. matsuzakiense* (61 %), *D. salmoneum* (60 %), and *D. vinaceum* (61 %). DDH values ranging from 52 % to 60 % were reported between *Dactylosporangium salmoneum* and *D. aurantiacum*, *D. matsuzakiense* and *D. vinaceum*. Levels of DNA-DNA relatedness between the above-mentioned strains were established by measuring the divergence between the thermal denaturation midpoints of homologous and heterologous DNA following the procedure developed by Gonzalez and Saiz-Jiménez (2005).

The initial renaturation method (De Ley et al. 1970; Huß et al. 1983) was used to obtain DNA relatedness values between *Dactylosporangium darangshiense* and its closest relatives *D. fulvum* (12.2–14.8 %) and *D. roseum* (2.5–3.6 %); the type strains share 16S rRNA gene sequence similarities of 99.0–99.1 % (Seo and Lee 2010).

Dactylosporangium maewongense was compared against the species *D. aurantiacum*, *D. fulvum, D. matsuzakiense, D. roseum, D. thailandense*, and *D. vinaceum* showing homology values between 9.6 % and 32 % (Chiaraphongphon et al. 2010). DDH studies are also available for *Dactylosporangium tropicum* and its neighboring species *D. matsuzakiense*, *D. salmoneum*, and *D. vinaceum* with values ranging from 20.6 to 38.8 (Thawai et al. 2011). In both studies, the microplate method of Ezaki et al. (1989) was used.

Although *Dactylosporangium roseum* and *D. fulvum* share the highest 16S rRNA gene sequence similarity (99.7 %), DNA-DNA hybridization studies have not been carried out between the corresponding type strains of these species. These microorganisms described by Shomura et al. (1985, 1986) can be differentiated by several phenotypic features including the production of coremia by *D. fulvum*, the reverse color of the substrate mycelium which is pink in *D. roseum* and yellow in *D. fulvum*. In addition, the strains show different reactions to gelatin liquefaction, starch hydrolysis, and tolerance to NaCl (Shomura et al. 1986).

Yokota et al. (1993) performed DNA-DNA hybridization studies for 13 actinomycete strains and proposed to divide them into six DNA-DNA homology groups. These genomic groups were also supported by quantitative fatty acid data; however, no other phenotypic characteristics that differentiated the six DNA homology groups were found at that time (Yokota et al. 1993). Therefore, these authors refrained from describing six species in the new genus *Catenuloplanes* and only proposed one, *Catenuloplanes japonicus*; Tamura et al. (1995) later completed the studies on these strains and proposed five additional species: *Catenuloplanes niger, Catenuloplanes indicus, Catenuloplanes atrovinosus, Catenuloplanes castaneus*, and *Catenuloplanes nepalensis*. The overall DDH values among the six species range from 24 % to 56 %. DNA-DNA relatedness studies were applied to support the reclassification of "*Planopolyspora crispa*" in the genus *Catenuloplanes* as *Catenuloplanes crispus*. The values as determined by the method of Ezaki et al. (1989) between *Catenuloplanes crispus* and the remaining six species ranged from 28 % to 59 % (Kudo et al. 1999).

All five *Catellatospora* species have been defined using DNA-DNA homology studies; the values reported are 16–58 % (Lee et al. 2000; Ara and Kudo 2006). The 16S rRNA gene sequence similarity between the species is 95–99.7 %.

Asanoa ferruginea and *Asanoa ishikariensis* which were formerly classified in the genus *Catellatospora* share a 16S rRNA gene sequence similarity of 99.2 %; however, a DNA-DNA hybridization value of 41 % clearly differentiated between the two species (Lee and Hah 2002). In addition, the recently described species *Asanoa siamensis* which shares a 16S rRNA gene sequence similarities of 98.5–99.5 % with *Asanoa hainanensis* (99.5 %), *Asanoa iriomotensis* (99.0 %), *Asanoa ishikariensis* (98.9 %),and *Asanoa ferruginea* (98.5 %) share low DDH values with these species (19–31 %) (Niemhom et al. 2012).

Most of the corresponding species in the genera *Actinocatenispora*, *Couchioplanes*, *Luedemannella*, *Planosporangium*, *Plantactinospora*, *Rugosimonospora*, *Salinispora*, *Verrucosispora*, and *Virgisporangium* have been defined on the basis of DNA-DNA hybridization studies. Specific values between strain *Actinocatenispora rupis* CS5-AC17^T and the other two species were 6.1–7.1 % (*A. thailandica* DSM 44816^T) and 21.5–27.6 % (*A. sera* NRRL B-24477^T) (Seo and Lee 2009). The two subspecies of *Couchiplanes caeruleus* show a value of 52 % (Tamura et al. 1994). A DDH value of 50 % was reported for the type strains *Luedemannella helvata* and *L. flava* (Ara and Kudo 2007b). The level of DNA-DNA relatedness between the strains *Planosporangium mesophilum* YIM 48875^T and *P. flavigriseum* YIM 46034^T is 45.5 %. The type strain *Plantactinospora mayteni* YIM 111 61359^T exhibits values of 42.7 % and 24.7 % with *P. siamensis* CM2-8^T (formerly classified as *Actinaurispora siamensis*) and *P. endophytica* YIM 68255^T respectively. *P. mayteni* YIM 61359^T and *A. siamensis* CM2-8^T share 97.8 % 16S rRNA gene sequence similarity (Zhu et al. 2011). Overall genome similarity between *Rugosimonospora acidiphila* Delta1^T and *R. afrinaca* Delta3^T was reported to be <10 % (Monciardini et al. 2009). The two obligate marine *Salinispora* species, *S. arenicola* and *S. tropica*, have a DNA-DNA relatedness value of 44.9 % (Maldonado et al. 2005a). The four species assigned to the genus *Verrucosispora* exhibit DDH values above 35 %.

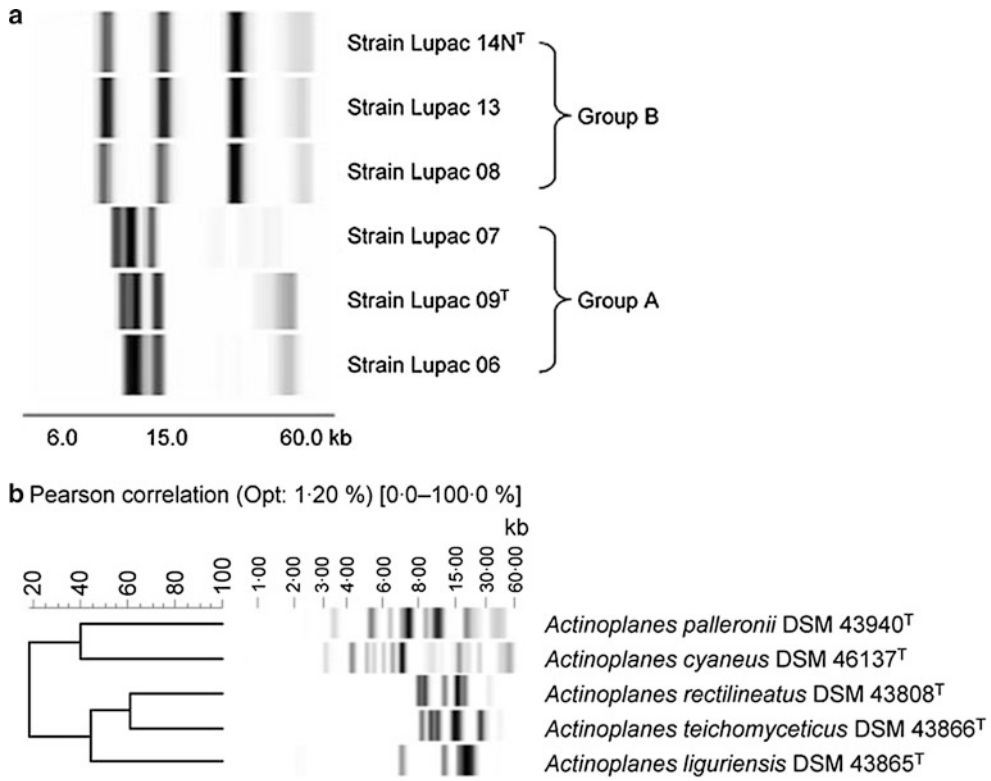

Fig. 28.2
(a) Riboprint patterns of *Micromonospora lupini* (Group B) and *Micromonospora saelicesensis* (Group A) generated with the restriction enzyme PvuII. (b) Diversity of normalized PvuII ribotype patterns found within several members of the genus *Actinoplanes* and their phylogenetic neighbors

Namely, *V. gifhornensis* shows values of 38.2 %, 42 %, and 53 % with *V. lutea*, *V. sediminis*, and *V. maris*, respectively. DNA-DNA relatedness between *V. lutea* and *V. sediminis* is reported to be 53 % (Dai et al. 2010; Goodfellow et al. 2012). 16S rRNA gene sequence similarities between the four type strains are 98.7–99.7 %. The description of the species *Virgisporangium ochraceum* (3 strains) and *V. aurantiacum* (1 strain) included overall genomic comparative studies for the four strains. The DDH values between the three strains classified as *V. ochraceum* ranged from 40 % to 60 %, 16S rRNA gene sequence similarities between these strains is 99.7–99.9 %. The values between the three *V. ochraceum* strains YU655-43^T, YU793-41, and YU794-41 and *V. aurantiacum* YU438-5^T was 12–20 % (Tamura et al. 2001). The comparison of *Virgisporangium aliadipatigenens* IR20-55^T against *V. ochraceum* NBRC 16418^T and *V. aurantiacum* NBRC 16421^T yielded values of 6–21 % (Otoguro et al. 2010).

In the case of *Longispora albida* and *Longispora fulva*, DDH studies were not carried out based on the argument that a low 16S rRNA gene sequence similarity (96.4 %) was shared by the two type strains. Since the 16S rRNA gene sequence similarities between the three species (and two subspecies) of the genus *Pilimelia* is 96.1–98.6 %, DNA-DNA hybridization studies have not been carried out. Given that at this point the genera *Allocatelliglobosispora*, *Catelliglobosispora*, *Hamadaea*, *Jishengella*, *Krasilnikovia*, *Phytohabitans*, *Phytomonospora*, *Polymorphospora*, *Pseudosporangium*, and *Spirilliplanes* contain single species descriptions, no DDH studies have been performed.

Riboprinting and Ribotyping

Only a few members of the family *Micromonosporaceae* have been included in riboprinting or ribotyping analyses. As mentioned by Stakcebrandt and Schumann (see chapter family *Cellulomonadaceae*), the automated riboprinting RiboPrint® system works with entire rrn operons and flanking DNA regions, while the ribotype approach works with PCR amplified 16S rRNA genes which are then subjected to restrictions and one-dimensional gel electrophoresis.

Several taxonomic studies have applied riboprinting for the characterization of members of the family *Micromonosporaceae*, mainly for the characterization of *Micromonospora* and *Actinoplanes* strains (Kroppenstedt et al. 2005; Wink et al. 2006; Trujillo et al. 2007). In all studies, *PvuII* was used as the restriction enzyme for cutting the genomic DNA. In the *Micromonospora* studies, riboprint profiles were obtained from six strains to demonstrate that they belonged to two different species (Fig. 28.2a) (Trujillo et al. 2007), while Kroppenstedt

Table 28.1
Full genome sequences in the family *Micromonosporaceae*

Strain	Chromosome	Size (Mb)	GC%	Genes	Proteins	rRNAs	tRNAs
Micromonospora aurantiaca ATCC 27029^T	Circular	7.03	72.8	6361	6.222	9	52
Micromonospora lupini Lupac 08	ND	7.32	71.9	7.158	7.054	10	77
Micromonospora sp. L5	Circular	6.96	72.8	6.326	6.150	9	53
Actinoplanes missouriensis 431	Circular	8.77	70.8	8.203	8124	18	58
Actinoplanes sp. SE50/110	Circular	9.24	71.3	8.385	8.247	18	97
Salinispora arenicola CNS-205	Circular	5.79	69.5	5.172	4.917	9	52
Salinispora tropica CNB-440	Circular	5.18	69.5	4.654	4.536	9	50
Verrucosispora maris AB-18-032	Circular	6.73	70.96	6.096	6.009	9	51

ND not determined

et al. (2005) produced distinct patterns for 23 *Micromonospora* type strains analyzed. In a similar way, specific profiles were obtained for each one of the *Actinoplanes* species analyzed (*Fig. 28.2b*) (Wink et al. 2006).

MALDI-TOF

Rapid identification of bacteria can be performed by whole-cell matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) methodology. The identification process is based on fingerprinting analyses of ribosomal proteins and other abundant basic proteins. Ribosomal proteins represent about 20 % of the protein mass and about 3 % of the total cellular mass, and as they are specific to individual species, they are ideal to be used as biomarkers (Liyanage and Lay 2006; Uhlik et al. 2011). So far, this technique has only been applied to analyze eight *Micromonospora* type strains (Kroppenstedt et al. 2005). In this study, the strains were analyzed over a mass (m = z) range of 2,000–20,000 and compared to new isolated strains. The number of peaks of significant intensity ranged between 14 (*Micromonospora olivasterospora* DSM 43868^T) and 43 (*M. echinaurantiaca* DSM 43904^T). MALDI-TOF mass spectroscopy of whole cells confirmed the discrete taxonomic entity of each of the test strains and type strains of described species. Mass similarity values for repeated analyses of individual strains ranged above 70 %. In contrast, mass similarities determined between strains were significantly lower, ranging from 0 % to 42 %. The full potential of the MALDI-TOF spectrometric method for identification and authenticity of *Micromonospora* species, and other members of the family *Micromonosporaceae*, still needs to be investigated.

Genome Comparison

The chromosome topology of *Micromonosporaceae* was first determined by Pulse Field Gel Electrophoresis (PFGE) for the strains *Actinoplanes philippinensis* DSM 43019^T and *Micromonospora chalcea* DSM 43026^T. Both strains were shown to contain chromosomes with genome sizes of 8.8 and 7.7 Mb, respectively, and both chromosomes were suggested to be linear (Redenbach et al. 2000). This preliminary information has been gradually enriched after the completion of full genome sequences from a reduced number of strains of the family *Micromonosporaceae*. At present, genome comparison is currently possible for several strains of the genera *Actinoplanes*, *Micromonospora*, *Salinispora*, and *Verrucosispora*. These genome sequences have shown that members of the family present large circular chromosomes ranging from 5.1 to 9.2 Mb (*Table 28.1*). First annotations have revealed that extended regions in these genomes are devoted to secondary metabolism, and to adaptation and colonization of the environment. The first *Micromonospora* genome to be publicly available corresponds to the type strain *Micromonospora aurantiaca* ATCC 27029^T, containing a circular chromosome of 7.03 Mb with 72.8 % GC content. The genome presents 6.361 protein-coding genes, 9 rRNA genes, and 52 tRNA genes, and 6.222 proteins have been predicted, including proteins related to secondary metabolism and several hydrolytic enzymes such as xylanases and amylases that may play a role in their life cycle and the colonization in the environment (Genbank accession no. CP002162.1). The second *Micromonospora* full genome sequence published corresponds to the strain *Micromonospora lupini* Lupac 08, a strain isolated from the root nodules of *Lupinus angustifolius*, with 7.32 Mb and a GC content of 71.96 % (Alonso-Vega et al. 2012). The annotation of this sequence has revealed 7,054 protein-coding genes, 10 rRNA genes and 77 tRNA genes (GenBank accession no. CAIE01000001). Preliminary data obtained from the genome sequence of *M. lupini* Lupac 08 indicate, as in the case of *M. aurantiaca*, that it contains a significant number of genes involved in secondary metabolism as well as genes encoding hydrolytic enzymes such as cellulases, amylases, xylanases, and pectinases. A third *Micromonospora* full genome sequence corresponding to the non-characterized strain of *Micromonospora* sp. L5 isolated from the roots of *Casuarina equisetifolia* in Mexico has been recently made available. The circular genome with 6.96 Mb and 72.8 % GC content has

been shown to contain 6,326 genes coding for 6,150 proteins, 9 rRNAs, and 53 tRNAs (GenBank accession no. CP002399).

The genomes of members of the genus *Actinoplanes* have only been studied for two industrial strains, the strain *Actinoplanes missouriensis* 431 (ATCC 14538, NBRC 102363) (Yamamura et al. 2012), an industrial strain producing xylose isomerase and glucose isomerase, two enzymes used in the production of high-fructose corn syrup (Yamamura et al. 2012) and the strain *Actinoplanes* sp. SE50/110, the producer of the α-glucosidase inhibitor acarbose (Schwientek et al. 2012). The genome of *A. missouriensis* 431 is a circular chromosome of 8.77 Mb with 70.8 % GC content that contains 8.203 genes coding for 8,124 proteins, 18 rRNAs, and 58 tRNAs. The genome sequence of the strain *Actinoplanes* sp. SE50/110 has been recently published (GenBank Accession No. CP003170) as a circular chromosome of 9.24 MB with 71.3 % GC content coding for 8,247 proteins, 18 rRNAs, and 97 tRNAs. Two additional *Actinoplanes* species are currently being sequenced although no completed data are still available: the strain *Actinoplanes friuliensis* DSM 7358^T, the producer strain of the lipopeptide antibiotics friulimicins, and the type strain *Actinoplanes globisporus* DSM 43857^T.

In the case of the obligate marine genus *Salinispora*, full genome sequences are available for both formally described species, Salinispora arenicola CNS-205 (GenBank accession No. CP000850) and Salinispora tropica CNB-440^T (GenBank accession No. CP000667). The genome sequence of both strains revealed circular chromosomes of 5.79 and 5.18 Mb, respectively, with a GC content of 69.5 %. The difference in the genome sizes is translated in the number of 5,172 and 4,654 genes coding for 4,917 and 4,536 predicted proteins. In the case of *S. tropica* CNB-440^T, the producer of the proteasome inhibitor salinosporamide A, almost 9.9 % of its genome is dedicated to natural product assembly with as many as 17 secondary metabolic biosynthesis gene clusters predicted to be involved in siderophore, melanin, polyketide, non-ribosomal peptide, terpenoid, and aminocyclitol production (Udwary et al. 2007). A comparative genomic analysis has revealed a large paralogous family of genes encoding polymorphic membrane proteins of the type V autotransporters, proposed to represent an adaptation to life in low nutrient environments (Penn et al. 2009). More recently, the presence of candidate marine adaptation genes has been proposed in both *Salinispora* genome sequences, *S. arenicola* CNS-205 and *S. tropica* CNB-440^T (Penn and Jensen 2012). In the case of the proposed third species "*Salinispora pacifica*" CNT-133, the partial full genome sequence has revealed so far a smaller genome (4.38 Mb) of similar GC content (69.8 %).

The strain *Verrucosispora maris* AB-18-032^T was isolated from a deep marine sediment sample collected from the Sea of Japan and produces the abyssomicins, natural-product inhibitors of the *para*-aminobenzoic acid biosynthetic pathway, and the antitumoral proximicin A, a furan analogue of netropsin. This strain is the first member of this genus to be sequenced (Roh et al. 2011). It has been shown to contain a circular chromosome of 6.67 Mb with a GC content of 70.9 % and a circular plasmid of 0.58 Mb with a GC content of 70.3 %. The chromosome has 5,947 protein-coding sequences, 51 tRNA genes, and 9 rRNA genes, whereas the plasmid contains 55 coding genes.

Additional full genome sequencing projects are currently in progress as part of a large international full genome sequence project, for the type species *Longispora albida* DSM 44784^T and Catelliglobosispora koreensis DSM 44566^T.

Current genome analysis has been limited to a reduced number of strains but has provided the first insights into the enormous metabolic diversity, adaptation capabilities, and biosynthetic potential of the members of this family that are still largely underexplored. The generation of new genome sequences, an activity currently in expansion, will open new avenues to explore the topology and organization of these genomes across different members of the family *Micromonosporaceae*.

Phages

Several *Micromonospora*-specific actinophages have been reported, including the lytic phages φUW21 and φUW51 (Kikuchi and Perlman 1977, 1978), the temperate phage MPφWR-1 (Tilley et al. 1990), and phages with undetermined infection cycles and specificities (Caso et al. 1990). Several other lytic *Micromonospora* phages have been used to screen for the presence of restriction enzymes (Meyertons et al. 1987). Phage pMLP1 was found to be present in "*Micromonospora carbonacea* var. *africana*" ATCC 39149 as a replicative form as well as an integrative form and plasmid derivatives containing the site-specific *att*/*int* functions of pMLP1 were found to be able to integrate genes into the chromosome (Alexander et al. 2003). None of the *Micromonospora* phages, however, have been developed into a gene cloning vector. Li et al. (2004) described a temperate phage, φHAU8, that is capable of infecting and transfecting *Micromonospora* sp. strain 40027, a producer of fortimicin A, which exhibits potent, broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria both in vitro and in vivo (Ma et al. 1986). These authors developed φHAU8 into a phasmid that functions as a λ-cosmid vector in *Escherichia coli* and as a phage in *Micromonospora* sp. strain 40027. A *Micromonospora*-specific phage isolated from soils of Tropical Rain Forests of Northern Queensland was reported to cross inter-genus boundaries and lysed several *Actinoplanes* and *Couchioplanes* strains (Kurtböke et al. 1998).

Crystalline phage particles have been detected in the cytoplasm of the substrate hyphae of a *Dactylosporangium thailandense* strain; the phage showed poor lytic activity and did not infect other actinomycetes (Higgins and Lechevalier 1969).

Phages infecting the industrially important *Actinoplanes* strain SN223 were isolated from soil samples collected at the shores of inland waters in Germany. The genome sizes range from 53 to 58 kb. Preliminary analyses revealed G+C contents

comparable with the G/C bias of the host. Electron microscopy of three selected viruses displayed no obvious morphological differences, the phage heads being icosahedral and their tails non-contractible. Two of the phages (φAsp2, φAsp3.1) characterized in more detail are capable of provoking putative pseudolysogenic growth of the host bacterium. The carrier state for φAsp2, in which cells are tightly packed with viruses, was demonstrated by electron microscopy. The latter phage is apparently widely distributed, as it was isolated from regions which are distantly located, i.e., more than 600 km apart from each other (Jarling et al. 2004a). Phage φAsp2 has a circularly permutated chromosome that consists of 58,638 bp; its G/C-bias of 70.39 % resembles the host's G+C content (71–73 % within the genus) (Jarling et al. 2004b).

Phenotypic Analyses

Since the last edition of *The Prokaryotes*, most taxa included in the chapter "*Actinoplanes*, and related genera" have been formally grouped in the family *Micromonosporaceae*. At present, the family includes 27 genera, of which the type genus *Micromonospora* is the largest with 48 validly published species at the time of writing. The main morphologic and chemotaxonomic features of members in the family *Micromonosporaceae* are listed in ❯ *Table 28.2*.

Micromonospora Ørskov 1923, 321[AL] Emend. Kasai et al. 2000

Mi.cro.mo.no.spo'ra. Gr. adj. *mikros*, small; Gr. adj. *monos*, single, solitary; Gr. fem. n. *spora*, a seed and in biology a spore; N.L. fem. n. *Micromonospora*, small, single-spored (organism).

Typically, Micromonosporae colonies on agar media are light orange, orange, red, brown, or purple. In many old cultures a green-black, brown-black, or black mucous mass of spores becomes apparent (❯ *Fig. 28.3c*). Good to moderate growth is observed on several ISP media including ISP 3, ISP 4, ISP 5, and ISP 6 agars (Shirling and Gottlieb 1966). Abundant growth is usually obtained on ISP 2, modified Bennett's (Wakisaka et al. 1982), and SA1 agars (Trujillo et al. 2005). Colonies are raised and folded with areas of different colors, and soluble pigments may also be formed (3A). The upper mycelial layers may burst open (❯ *Fig. 28.3b*, *d*) Well-developed, branched, substrate mycelium (0.2–06 µm diameter) is produced while aerial mycelium is usually absent or scanty.

The formation of single spores is the main morphological characteristic of the genus *Micromonospora*; however, spores are often produced in dense clusters on the surface or completely embedded in the substrate (❯ *Fig. 28.3e*). The spores are nonmotile, and spherical to oval in shape, with a diameter of 0.7–1.5 µm. The spore surface ornamentation of Micromonosporae may be smooth, warty, or blunt-spiny as in the case of *M. saelicesesis*, *M. rosaria*, and *M. echinospora* respectively.

Compared to *Streptomyces*, *Micromonospora* spores are quite resistant. Viable spores of *Micromonospora* were recorded from sediments deposited at least 100 years before (Cross and Attwell 1974). Populations of *Micromonospora* species, accompanied by other actinomycetes, have also been frequently found in streams, rivers and lakes (Rowbotham and Cross 1977; Al-Diwany and Cross 1978; de Menezes et al. 2008). Their spores are hydrophilic and wettable and can easily be removed from soil by the passage of water (Ruddick and Williams 1972). The spores withstand ultrasonication, moist heat treatment (20 min at 60 °C), and dry heat up to 75 °C, and they are resistant to various chemical solutions such as acetone (Kawamoto et al. 1982), dimethylformamide, formamide, *tert*-butyl alcohol, and phenol (Hayakawa et al. 1991b). However, they are sensitive to acidic pH (Kawamoto 1989).

Micromonosporae stain Gram-positive, are non-acid fast, chemo-organotrophic and usually grow between 20 °C and 45 °C. NaCl tolerance ranges from 1.5 % to 5 % (w/v). *Micromonospora* strains do not grow below pH 5.0 or above pH 9.5.Most strains are aerobic, but some may grow under microaerophilic conditions (Goodfellow and Williams 1983). Although strictly anaerobic *Micromonospora* strains isolated from the intestinal tract of termites (Hungate 1946) and from the rumen of sheep (Maluszy'nska and Janota-Bassalik 1974) have also been reported, these isolates need to be properly identified to clarify their affiliation to the genus *Micromonospora*.

Micromonospora species are well known for their ability to degrade complex polysaccharides such as cellulose, chitin, and lignin (McCarthy and Broda 1984; Kawamoto 1989; Jendrossek et al. 1997). In particular, cellulose is frequently utilized as substrate (Jensen 1930; Sandrak 1977; Kawamoto 1989). *Micromonospora* strains isolated from fresh water lakes in the United Kingdom were reported to degrade cellulose (de Menezes et al. 2008). Furthermore, these *Micromonospora* populations were also shown to be important members of the active bacterial population in these freshwater lakes, particularly colonizing cellulosic substrates at the lake sediment (de Menezes et al. 2012). The genome sequence of *Micromonospora lupini* Lupac 08 isolated from a nitrogen-fixing nodule of the legume *Lupinus angustifolius* was recently sequenced (Alonso-Vega et al. 2012). Information derived from the genomic data indicated that this strain contains a significant number of genes encoding hydrolytic enzymes such as cellulases, amylases, xylanases, and pectinases that may have a role in the colonization process. Cellulose and xylan degradation was recently confirmed in the laboratory (Trujillo, unpublished results). Selected phenotypic characteristics of the genus *Micromonospora* are given in ❯ *Table 28.3*.

Carbohydrates, amino acids, and organic acids are used as carbon sources by Micromonosporae strains. However, various authors have reported that carbohydrate utilization patterns are affected by the basal medium and this may be an explanation for discrepancies between results when studies are performed on different media (Kawamoto 1989). Carro (2009) carried out carbon source utilization studies for 35 *Micromonospora* type

Table 28.2
Morphological and chemotaxonomic characteristics of genera of *Micromonosporaceae*

Characteristic	*Micromonospora*	*Actinocatenispora*	*Actinoplanes*	*Allocatelliglobosispora*	*Asanoa*	*Catellatospora*	*Catelliglobosispora*	*Catenuloplanes*	*Couchioplanes*	*Dactylosporangium*	*Hamadaea*	*Jishengella*	*Krasilnikovia*	*Longispora*
Aerial hyphae	–	+	–	–	–	–	–	–	+	–	–	–	–	–
Single spores	+	–	–	–	–	–	–	–	–	+	–	+	–	–
Sporangia	–	–	+	–	–	–	–	–	–	+	–	–	–	–
Spore chains	–	+	–	+	+	+	+	+	+	–	+	–	+	+
Motile spores	–	–	+	–	–	–	–	+	+	+	–	–	–	–
NaCl requirements	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diagnostic Diaminoacid(s)	*m*-DAP	*m*-DAP	*m*-DAP	3-OH-DAP	*m*-DAP	3-OH-DAP	*m*- and 3-OH-DAP	L-Lys	L-Lys	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP
Whole-organism sugars	Ara, Xyl	Gal, Xyl, Ara, Glu, Man, Rib	Ara, Xyl	Glu, Rha, Rib, Xyl, Ara, Gal, Man	Ara, Xyl, Gal	Ara, Xyl, Gal	Rha, Man, Xyl, Gal, Glu	Xyl	Ara, Xyl, Gal	Ara, Xyl	Xyl, Man, Rib, Ara, Rha, Glu	Xyl, Gal, Ara, Rib, Glu	Gal, Man, Xyl, Ara, Rib, Glu	Ara, Xyl, Gal
Fatty acid type[a]	3b	3b	2d	3b	2d	3b	3b	2c	2c	3b	3b	3a	2d	2d
Major menaquinones (MK-)	MK-10(H_4, H_6) 9(H_4, H_6)	MK-9(H_4, H_6)	MK-9(H_4) 10(H_4)	MK-10(H_4, H_6) 9(H_4)	MK-10(H_6, H_8)	MK-10(H_8, H_6) 9(H_4, H_6)	MK-10(H_4)	MK-9(H_8) 10(H_8)	MK-9(H_4)	MK-9(H_4, H_6, H_8)	MK-9(H_6)	MK-9(H_4, H_6, H_8)	MK-9(H_6)	MK-10(H_4, 'H_6)
Phospholipid type[b]	PII	PII	PII	PII	PII	PII	PII	PIII	PII	PII	PII	PII	PII	PII
DNA G+C content (mol%)	71–73	72	72–73	70	71–72	71–72	70	71–73	70–72	72–73	70	71	71	70

Characteristic	*Luedemannella*	*Phytohabitans*	*Phytomonospora*	*Pilimelia*	*Planosporangium*	*Plantactinospora*	*Polymorphospora*	*Pseudosporangium*	*Rugosimonospora*	*Salinispora*	*Spirilliplanes*	*Verrucosispora*	*Virgisporangium*
Aerial hyphae	–	–	–	–	–	+	–	+	–	–	+	–	–
Sporangia	+	–	–	+	+	–	–	–	–	–	–	–	+
Spore chains	–	+	–	–	–	–	+	+	–	–	+	–	–
Single spores	–	–	+	–	+	+	–	–	+	+	–	+	–
Motile spores	–	–	–	+	+/–	–	–	–	–	–	+	–	+
NaCl requirements	–	–	–	–	–	–	–	–	–	+	–	–	–
Diagnostic Diaminoacid(s)	*m*-DAP	*m*-DAP, L-Lys	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*- and 3-OH-DAP	3-OH-DAP	*m*-DAP	*m*-DAP	*m*-DAP	*m*-DAP
Whole-organism sugars	Xyl, Gal, Man, Rha, Rib, Ara	Gal, Glu, Man, Rib, Xyl	Man, Rib, Gal, Glu	Ara, Xyl	Ara, Xyl	Ara, Xyl, Gal, Glu	Xyl	Ara, Gal, Glu, Man, Xyl	Ara, Gal, Xyl	Ara, Xyl, Gal	Xyl, Gal	Man, Xyl	Ara, Gal, Man, Rha, Xyl
Fatty acid type[a]	2d	2d	2d	2d	3b	2d	2a	2d	2c	3a	2d	2b	2d
Major menaquinones (MK-)	MK-9(H_4, H_6)	MK-9(H_6), 10(H_4, H_6)	MK-10(H_2, H_4, H_6), 8(H_2), 9(H_2)	MK-9(H_2, H_4)	MK-9(H_4), 10(H_4)	MK-10($H_{4,6,8}$)	MK-10(H_4, H_6) 9(H_4, H_6)	MK-9(H_6)	MK-9(H_6, H_8)	MK-10(H_4)	MK-10(H_4)	MK-9(H_4)	MK-10(H_4, H_6, H_8)
Phospholipid type[b]	II	II	PIII	II	PII	PII	PII	PII	PII	PII	PII	PII	PII
DNA G+C content (mol%)	71	73	70	ND	71	70	71	73	72–73	70–73	69	70	71

Data from Ørskov (1923), Couch (1950), Kane (1966), Thiemann et al. (1967), Asano and Kawamoto (1986), Yokota et al. (1993), Rheims et al. (1998), Kudo et al. (1999), Tamura et al. (1994, 1997, 2001, 2006), Lee and Hah (2002), Matsumoto et al. (2003), Maldonado et al. (2005a), Thawai et al. (2006, 2010), Ara and Kudo (2006, 2007a, b), Ara et al. (2008a, b), Wiese et al. (2008), Monciardini et al. (2009), Qin et al. (2009), Lee and Lee (2011), Inahashi et al. (2010), Xie et al. (2011b)

+ present/positive, – absent/negative

m-DAP *meso*-diaminopimelic acid, l-*Lys* L-Lysine

Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose

[a]According to Kroppenstedt (1985)

[b]According to Lechevalier et al. (1981)

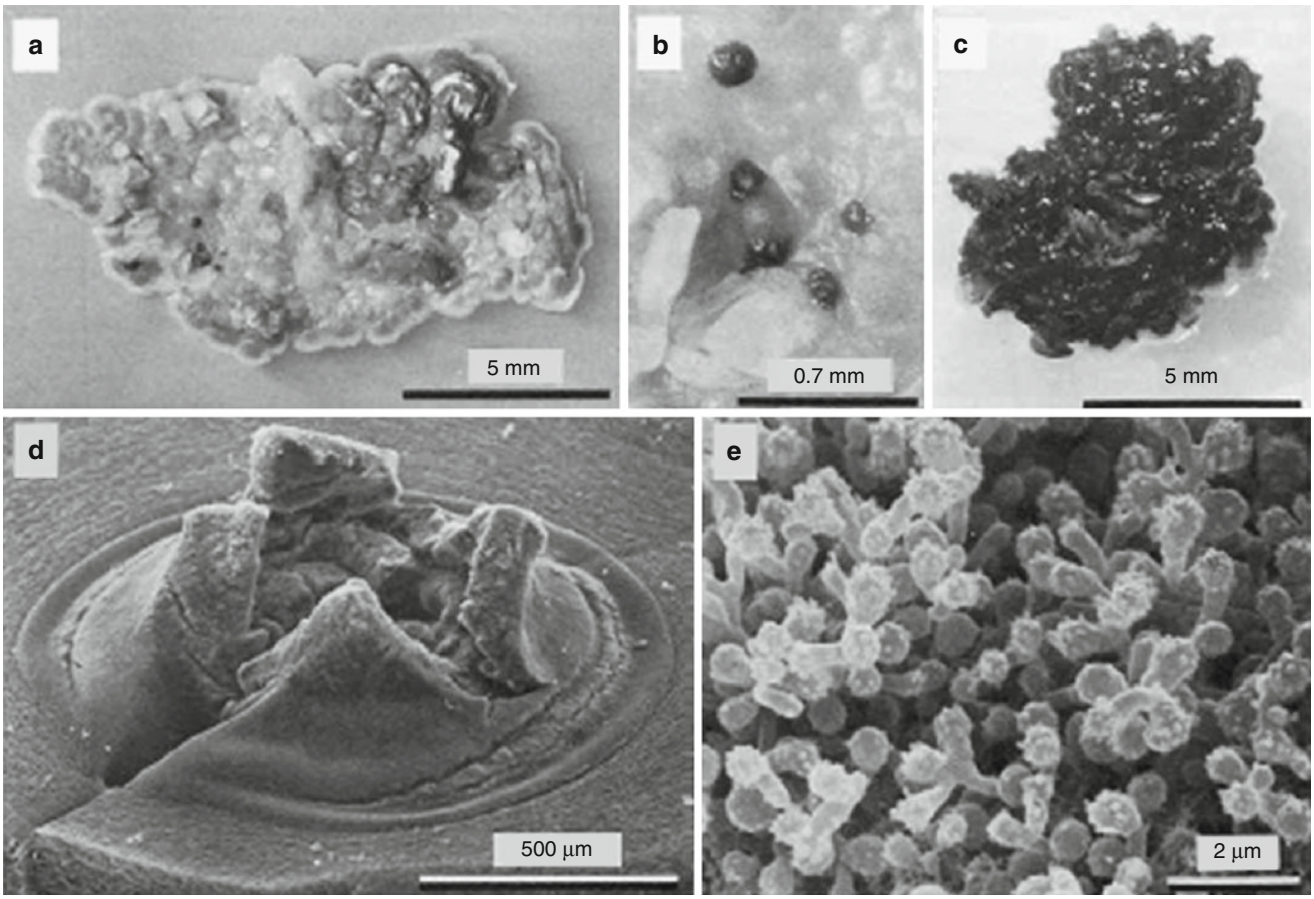

Fig. 28.3

Morphological features of the genus *Micromonospora*. (a) Raised and folded colony with areas of different colors (DM). (b) Clusters of dark spore masses (DM). (c) Colony completely covered by a mucoid, black mass of spores (DM). (d) Crosswise-burst colony exposing the spore-forming substrate mycelium (SEM). (e) Cluster of spores formed on short side-branches of substrate hyphae; surface of the spores is covered with blunt spines (SEM). *DM* dissecting microscope, *SEM* scanning electron microscope

strains using Stevenson's carbon-free medium (Stevenson 1967) as basal medium. These results are consolidated in *Table 28.4*. As expected, all strains utilized glucose, while 80–85 % of the strains utilized arabinose, cellobiose, galactose, mannose, and starch. Arginine, fructose, maltose, raffinose, and saccharose were used by 70–75 % of the strains. On the other hand, none of the strains were able to utilize galacturonic acid, glutaric acid, propionic acid, histidine, *meso*-erythritol, sorbitol, tyrosine, trehalose, xylitol, and xylose. Discrepancies between Carro's results and those published previously for carbohydrate assimilation were observed, confirming earlier observations on the effect of the basal medium on these substrates (Kawamoto et al. 1983; Kawamoto 1989; Genilloud 2012). Inorganic ammonium salts and acidic and basic amino acids are better nitrogen sources than nitrate salts (Kawamoto 1989).

According to the classification scheme of Lechevalier and Lechevalier (1970a), the genus *Micromonospora* is characterized by a cell-wall type II. Glycine and *meso*-diaminopimelic acid (A_2pm) and/or 3-hydroxy-diaminopimelic acid are the characteristic amino acids of the peptidoglycan. The primary structure of the peptidoglycan was described by Kawamoto et al. (1981); glycine, rather than L-alanine, is linked to muramic acid and *meso*-diaminopimelic acid or its hydroxylated derivative is directly cross-linked to the D-alanine of an adjacent peptide subunit. Muramic acid is *N*-glycosylated. The presence of 3-OH-DAP is only found in certain species including *M. carbonacea*, *M. echinospora*, *M. halophytica*, *M. inositola*, *M. matsumotoense*, *M. olivasterospora*, and *M. rosaria*. Deviations from the typical wall chemotype II is found in some *Micromonospora* species, which also contain LL-diaminopimelic acid (Kawamoto et al. 1981). The characteristic whole-cell sugar pattern of *Micromonospora* strains is pattern D, as defined by Lechevalier and Lechevalier (1970b). Xylose and arabinose are the characteristic sugars in *Micromonospora* species; however, the latter sugar is not reported for the species *M. mirobrigensis*, *M. echinofusca*, *M. fulviviridis*, *M. sagamiensis*, or *M. viridifaciens* (Kroppenstedt et al. 2005; Trujillo et al. 2005). In addition, glucose, galactose, mannose, and rhamnose can also be found. Characteristic phospholipids include

Table 28.3

Selected phenotypic characteristics of *Micromonospora* species

Characteristic	*M. aurantiaca*	*M. auratinigra*	*M. carbonacea*	*M. chalcea*	*M. chersina*	*M. chokoriensis*	*M. citrea*	*M. coerulea*	*M. coriariae*	*M. coxensis*	*M. cremea*	*M. chaiyapumensis*	*M. eburnea*	*M. echinaurantiaca*	*M. echinofusca*	*M. echinospora*
Substrate mycelium	Yellow-orange	Bright orange	Orange-Black	Red-orange	Light orange-yellow	Light to dark brown	Yellow-orange	Blue-green	Orange	Cinnamon brown	Cream to orange	Orange, gray-black	Yellow-orange	Light yellow-orange	Orange-brown	Dark brown to purple
Diffusible pigments	–	Brown	–	Light yellow	Yellow	–	Yellow-orange	–	–	–	–	Brown	Pale yellow	–	–	–
Maximum NaCl tolerance (w/v)	4	2	3	5	3	3	3	1.5	1	3	1	3	4	3	3	3
Temperature growth range	12–45	25–30	27–37	27–45	18–49	20–37	12–45	24–41	12–37	15–37	10–37	25–30	25–45	12–45	4–45	27–37
Nitrate reduction	+	–	+	v	v	nd	nd	–	+	nd	v	nd	+	nd	nd	v
Catalase	+	+	–	+	+	+	+	–	+	+	v	nd	+	+	+	–
Oxidase	–	–	+	–	+	+	+	+	+	+	v	nd	+	+	+	+
Degradation of																
Starch	+	+	+	+	+	–	+	+	+	+	v	+	+	+	+	+
Arbutin	+	–	+	+	–	+	+	+	+	+	–	nd	+	+	+	+
Casein	+	+	+	+	+	+	+	+	+	+	v	nd	+	+	+	+
Esculin	+	+	+	+	+	+	–	+	+	+	+	nd	+	+	+	+
Gelatin	+	–	+	–	+	+	+	–	+	+	v	+	+	+	–	+
Tween 20	+	+	+	+	+	+	+	+	+	+	v	nd	+	+	+	+
Tween 80	–	–	–	–	–	–	–	–	–	–	+	nd	–	–	–	–
Tyrosine	+	–	+	+	+	–	+	+	–	–	v	+	+	+	+	–
Urea	–	–	–	–	–	–	+	–	+	+	–	nd	+	+	+	–
Xylan	+	+	+	+	+	–	–	–	+	+	v	nd	+	+	+	+

Table 28.3 (continued)

Characteristic	*M. endolithica*	*M. equina*	*M. fulviviridis*	*M. haikouensis*	*M. halophytica*	*M. humi*	*M. inositola*	*M. inyonensis*	*M. krabiensis*	*M. lupini*	*M. marina*	*M. matsumotoense*	*M. mirobrigensis*	*M. narathiwatensis*	*M. nigra*	*M. olivasterospora*
Substrate mycelium	Orange-olive	Light to deep orange	Light yellow-orange	Orange-yellow	Orange-brown	Sepia-brown	Bright orange	Yellow olive to red-brown	Orange to black	Light orange	Brown-orange	Red to brown-orange	Orange	Yellow-white to gray	Orange-olive	Light brown to orange
Diffusible pigments	–	–	–	–	Red-brown	–	–	Brown yellow	Melanoid	–	Pale yellow	Red-brown	–	Pale yellow	–	Olive green
Maximum NaCl tolerance (w/v)	2.5	2	1	3	4	5	1.5	nd	3	2	7	2	3	4	4	3
Temperature growth range (°C)	8–39	20–37	20–45	nd	18–40	nd	25–40	nd	25–40	20–37	25–30	4–45	20–37	25–30	18–40	28–38
Nitrate reduction	–	w	nd	+	+	–	–	nd	nd	–	–	+	–	+	+	+
Catalase	+	nd	+	nd	+	nd	+	nd	nd	+	nd	+	+	nd	+	+
Oxidase	–	nd	+	nd	–	nd	+	nd	nd	+	nd	+	–	nd	+	+
Degradation of								nd								
Starch	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+
Arbutin	–	+	–	nd	+	nd	+	nd	nd	+	nd	–	+	nd	+	+
Casein	+	+	+	nd	+	nd	+	nd	nd	+	nd	+	+	nd	+	+
Esculin	+	+	+	+	+	nd	+	nd	nd	+	nd	+	+	nd	+	+
Gelatin	–	+	+	–	+	+	+	nd	+	+	+	+	+	+	+	+
Tween 20	+	nd	+	nd	+	nd	–	nd	nd	+	nd	+	+	nd	+	+
Tween 80	–	+	–	nd	–	nd	–	nd	nd	–	nd	–	–	nd	–	–
Tyrosine	–	+	+	nd	+	–	+	nd	–	+	+	+	+	nd	+	+
Urea	–	w	–	nd	–	nd	–	nd	nd	–	nd	–	–	nd	+	–
Xylan	+	w	+	nd	+	nd	–	nd	nd	+	nd	+	+	nd	+	–

Characteristic	*M. pallida*	*M. pattaloongensis*	*M. peucetia*	*M. pisi*	*M. purpureochromogenes*	*M. rhizosphaerae*	*M. rifamycinica*	*M. rosaria*	*M. saelicesensis*	*M. sagamiensis*	*M. sediminicola*	*M. siamensis*	*M. tulbaghiae*	*M. viridifaciens*	*M. yangpuensis*	*M. zamorensis*
Substrate mycelium	Light ivory brown	Yellow-white to pale orange	Deep orange to green	Beige to pale yellow	Dark brown	Brilliant orange	Orange to brown	Orange-brown	Orange	Coral red	Deep brown	Vivid orange	Yellow-brown	Light yellow to brown	Apricot-orange	Bright orange
Diffusible pigments	–	Yellow	–	–	Dark brown	Yellow	–	Wine red	Orange-brown	–	Deep brown	Pale yellow	–	–	Brown	Orange
Maximum NaCl tolerance (%, w/v)	3	3	3	1	1.5	2	3	2	2	1	4	5	5	5	3	3
Temperature growth range	20–37	25–30	4–37	20–37	25–37	28–40	20–37	35–40	20–37	25–45	20–40	20–40	4–37	20–45	10–45	10–37
Nitrate reduction	+	+	nd	nd	v	+	+	–	–	–	–	–	–	–	+	–
Catalase	–	+	+	+	+	+	+	+	+	+	nd	+	nd	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	nd	+	nd	–	–	+
Degradation of																
Starch	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Arbutin	+	+	+	+	+	nd	+	+	+	+	nd	+	nd	–		w
Casein	+	+	+	+	–	nd	+	+	+	+	nd	+	+	+	+	+
Esculin	+	+	+	+	+	nd	+	+	+	+	nd	+	nd	+	+	+
Gelatin	+	+	+	+	+	–	+	+	+	–	+	–	+	+	–	+
Tween 20	+	+	+	–	+	nd	+	–	+	+	nd	+	nd	+	+	–
Tween 80	–	–	–	–	–	nd	–	–	–	–	nd	–	+	–	+	+
Tyrosine	–	–	+	–	+	nd	+	+	+	–	nd	–	nd	+	nd	–
Urea	–	–	–	–	–	nd	–	–	+	–	nd	–	nd	–	nd	–
Xylan	+	–	+	+	–	nd	+	+	+	+	nd	–	nd	–	nd	+

Data from Kroppenstedt et al. (2005), Carro (2009), Thawai et al. (2007), Jongrungruangchok et al. (2008a, b), Kirby and Meyers (2010), Tanasupawat et al. (2010), Xie et al. (2012b), Songsumanus et al. (2011), Wang et al. (2011), Supong et al. (2012), Everest and Meyers (2012), Zhang et al. (2012)

+ positive, – negative, *v* variable, *nd* not determined

Table 28.4
Carbon source profiles of *Micromonospora* type strains

	Alanine	L – Arabinose	Arginine	Cellobiose	*meso* – Erythritol	Fructose	Galactose	Galacturonic acid	Gluconate	Glutaric acid	Glucose	Histidine	Lysine	Maltose	Mannose	Melezitose	Melibiose	Proline	Propionic acid	Quinic acid	Raffinose	Rhamnose	Saccharose	Salicine	Serine	Sorbitol	Sorbose	Starch	Tyrosine	Trehalose	Valine	Xylitol	Xylose
M. aurantiaca DSM 43813^T	–	+	+	+	–	+	+	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	+	–	+	–	–	+	–	–	–	–	–
M. auratinigra DSM 44815^T	–	+	+	+	–	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	+	+	+	+	+	–	–	+	–	–	–	–	–
M. carbonacea DSM 43168^T	–	+	–	+	–	+	+	–	–	–	+	–	–	+	+	+	+	–	+	–	+	–	+	+	–	–	+	+	–	–	–	–	–
M. chalcea DMS 43026^T	–	–	+	–	–	–	+	–	–	–	+	–	–	+	+	+	–	+	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–
M. chersina DSM 44151^T	–	+	–	+	–	+	+	–	–	–	+	–	+	+	+	+	+	+	–	–	+	–	+	–	+	–	–	+	–	–	+	–	–
M. chokoriensis JCM 13247^T	–	+	–	+	–	+	+	–	–	–	+	–	–	+	+	+	+	+	–	–	+	–	+	+	–	–	–	+	–	–	–	–	–
M. citrea DSM 43903^T	–	+	+	+	–	–	+	–	–	–	+	–	–	+	+	+	–	+	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–
M. coerulea DSM 43143^T	–	+	+	+	–	–	+	–	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
M. coriariae DSM 44875^T	–	+	+	+	–	+	+	–	–	–	+	–	–	+	+	–	–	+	–	–	+	+	+	+	+	–	–	+	–	–	–	–	–
M. coxensis JCM 13248^T	–	+	+	+	–	+	+	–	–	–	+	–	–	+	+	+	+	+	+	–	+	–	+	+	–	–	+	+	–	–	–	–	–
M. cremea CR30^T	+	+	+	–	+	+	–	–	–	–	+	–	+	+	+	+	–	+	–	–	+	+	+	–	+	–	–	+	–	+	+	–	–
M. chaiyapumensis MC5-1$^{T\,a}$	nd	+	nd	nd	nd	+	+	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	w	–	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	+
M. eburnea DSM 44814^T	–	–	–	–	–	+	+	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–
M. echinaurantiaca DSM 43904^T	–	+	+	+	–	+	+	–	–	–	+	–	–	+	+	–	+	+	–	–	+	–	+	–	–	–	–	+	–	–	–	–	–
M. echinofusca DSM 43913^T	–	–	+	+	–	+	+	–	–	–	+	–	+	+	+	+	–	+	+	–	+	–	+	+	+	–	–	+	–	+	–	–	–
M. echinospora DSM 43816^T	–	+	–	+	–	+	+	–	–	–	+	–	–	–	–	+	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	–
M. endolithica DSM 44398^T	–	+	+	–	–	–	–	–	+	–	+	–	+	+	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–
M. equina Y22$^{T\,a}$	nd	w	nd	–	nd	w	nd	nd	nd	nd	+	nd	nd	+	nd	–	w	nd	nd	nd	nd	w	nd	w	nd	nd	nd	+	nd	nd	+	nd	nd
M. fulviviridis DSM 43906^T	–	+	+	+	–	+	–	–	–	–	+	–	+	+	+	+	+	+	–	–	+	–	+	+	+	–	+	+	–	+	+	–	–
M. haikouensis 232617$^{T\,a}$	nd	–	nd	nd	nd	–	+	nd	nd	nd	+	nd	nd	nd	–	nd	+	nd	nd	nd	+	+	nd	+	nd	nd	–	nd	+	nd	nd	–	–
M. halophytica DSM 43171^T	–	–	+	+	–	+	+	–	–	–	+	–	–	+	+	+	+	+	–	–	+	–	+	–	+	–	–	+	–	–	–	–	–
M. humi DSM PO402$^{T\,a}$	nd	+	nd	–	nd	–	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	–	nd	nd	nd	–	–	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd
M. inositola DSM 43819^T	–	+	+	+	–	+	+	–	–	–	+	–	–	+	+	–	+	–	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–

M. inyonensis DSM 46123^{T} [a]	nd	w	nd	nd	nd	w	nd	nd	nd	nd	w	nd	nd	nd	nd	nd	nd	nd	nd	nd	w	w	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	w
M. krabiensis MA-2^{T} [a]	nd	+	nd	nd	nd	nd	+	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	+	w	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	+
*M. lupini*14N^{T}	–	+	–	+	–	+	+	–	–	–	+	–	–	+	+	+	–	–	–	–	+	–	+	+	–	–	+	+	–	–	–	–	–	–	–
M. marina JSM1-1^{T} [a]	nd	+	nd	nd	nd	+	+	nd	nd	nd	+	nd	nd	nd	nd	nd	–	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
M. matsumotoense IMSNU22003^{T}	–	+	+	+	–	+	+	–	–	–	+	–	–	–	+	+	+	+	–	–	+	–	+	–	–	–	+	+	–	–	–	–	–	–	–
M. mirobrigensis WA201^{T}	–	+	+	+	–	–	–	–	–	–	+	–	–	+	+	+	+	+	–	–	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–
M. narathiwatensis BTG4-1^{T} [a]	nd	w	nd	+	nd	–	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	+	–	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	+
M. nigra DSM 43818^{T}	–	+	–	+	–	–	–	–	–	–	+	–	+	–	+	+	+	+	–	–	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–
M. olivasterospora DSM 43868^{T}	–	+	–	+	–	+	+	–	–	–	+	–	+	+	+	+	+	+	–	–	+	–	+	–	+	–	–	+	–	–	+	–	–	–	–
M. pallida DSM 43817^{T}	–	+	–	+	–	+	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–
M. pattaloongensis JCM 12394^{T}	–	+	+	+	–	+	+	–	–	–	+	–	–	+	+	+	–	+	–	–	+	–	+	+	+	–	–	+	–	–	+	–	–	–	–
M. peucetia DSM 43363^{T}	–	+	+	+	–	+	–	–	–	–	+	–	–	–	+	–	–	+	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–
M. pisi GUI 15^{T}	–	–	–	–	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	+
M. purpureochromogenes DSM 43821^{T}	–	+	+	+	–	+	+	–	–	–	+	–	+	+	+	–	+	+	–	–	+	–	–	–	+	–	+	+	–	–	–	–	–	–	–
M. rhizosphaerae 211018^{T} [a]	nd	nd	nd	nd	nd	+	w	nd	nd	nd	+	nd	nd	–	nd	–	–	nd	nd	nd	–	–	nd	–	nd	nd	nd	nd	nd	nd	nd	nd	nd	w
M. rifamycinica DSM 44983^{T}	–	–	+	+	–	+	+	–	–	–	+	–	–	+	–	+	+	–	–	–	+	–	+	+	–	–	+	+	–	–	–	–	–	–	–
M. rosaria DSM 803^{T}	–	+	+	+	–	+	+	–	–	–	+	–	–	+	+	–	+	–	–	–	+	–	+	–	+	–	–	+	–	–	–	–	–	–	–
M. saelicesensis Lupac 09^{T}	+	+	+	+	–	+	+	–	–	–	+	–	–	+	+	+	+	+	–	–	+	–	+	+	+	–	+	+	–	–	+	–	–	–	–
M. sagamiensis 43912^{T}	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
M. sediminicola SH2-13^{T} [a]	nd	–	nd	+	nd	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+
M. siamensis JCM 12769^{T}	–	+	+	–	–	+	+	–	–	–	+	–	–	–	–	–	–	+	+	–	+	+	+	+	+	–	–	+	–	–	+	–	–	–	–
M. tulbaghiae TVU1^{T} [a]	nd	–	nd	+	nd	+	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	–	nd	nd	nd	–	+	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	–
M. viridifaciens DSM 43903^{T} [a]		w				w					w										w	w													w
M. yangpuensis FXJ6.011^{T} [a]	+	–	nd	–	nd	–	nd	nd	nd	nd	nd	nd	nd	–	nd	nd	nd	w	nd	nd	+	–	nd	–	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	+
M. zamorensis CR38^{T}	+	+	+	+	–	+	–	–	+	–	+	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	–	–		

All data from Carro (2009), except where indicated

+ positive, – negative, *w* weak, *nd* not determined

[a]Data from Kroppenstedt et al. (2005), Thawai et al. (2007), Jongrungruangchok et al. (2008a, b), Kirby and Meyers (2010), Tanasupawat et al. (2010), Xie et al. (2012b), Songsumanus et al. (2011), Wang et al. (2011), Supong et al. (2012), Everest and Meyers (2012), Zhang et al. (2012)

diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides, a phospholipid type PII (Lechevalier et al. 1977). The main fatty acids are *iso*- and *anteiso*-branched fatty acids. Saturated and unsaturated fatty acids present in *Micromonospora* strains include anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$, and iso-$C_{17:0}$ acids. Unsaturated or 10-methyl fatty acids may be found in certain strains, but not mycolic acids nor cyclic fatty acids (Kroppenstedt and Kutzner 1976; Dassain et al. 1983). The menaquinone composition of members of the genus *Micromonospora* is complex and heterogeneous; strains can be divided into three groups based on the predominant menaquinone. The two major groups encompass species that have menaquinones with either nine (MK-9) or ten isoprene units (MK-10); the exception, *M. pallida*, has menaquinones with 12 units (MK-12) (Collins et al. 1984; Kawamoto 1989; Tomita et al. 1992; Hirsch et al. 2004, Thawai et al. 2004b, 2005b; Kroppenstedt et al. 2005; Trujillo et al. 2005).

Actinocatenispora Thawai et al. 2006, Emend. Seo and Lee 2009

Ac.ti.no.ca.te.ni.spo′ra. Gr. n. *aktis -inos*, ray; L. n. *catena*, chain; Gr. fem. n. *spora*, seed, and in biology a spore; N.L. fem. n. *Actinocatenispora*, spore chain-producing ray (fungus).

Actinocatenispora strains produce well-developed substrate mycelia on ISP 2 agar, oatmeal agar (ISP 3), and peptone-yeast extract-iron agar (Thawai et al. 2006; Matsumoto et al. 2007; Seo and Lee 2009). The color of the substrate mycelium ranges from pale yellow to orange. White aerial mycelium may also be produced by the species *A. rupis* (Seo and Lee 2009). Spore chains which may consist of up to 20 spores are borne on the aerial mycelium or produced directly from the vegetative mycelium. The spores are cylindrical (0.3–0.4 × 0.5–1.0 μm in size), smooth, and nonmotile. The genus currently harbors three species, *A. thailandica*, the type species, *A. rupis* and *A. sera*.

All Actinocatenisporae strains stain Gram-positive, are aerobic, and usually grow best between 18 °C and 37 °C. Only *A. rupis* grows at 42 °C. They are heterotrophic microorganisms capable of using a diverse array of sugars as carbon sources; however, important differences are found in their assimilation profiles and only D-*glucose* is reported to be used by all species (*Table 28.5*) (Thawai et al. 2006; Matsumoto et al. 2007; Seo and Lee 2009). *Actinocatenispora rupis* and *A. sera* are also capable of metabolizing adonitol, while differences between the three species are found in the assimilation of D-arabinose, glycerol, cellobiose, dextran, galactose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, L-rhamnose, salicin, xylitol, and xylose.

Various chemotaxonomic markers have been defined in the genus *Actinocatenispora*. The whole-cell sugars arabinose and xylose have been detected in all strains, while the presence of galactose, glucose, mannose, rhamnose, and ribose varies. The cell wall contains glutamic acid, glycine, alanine, and *meso*-diaminopimelic acid. The fatty acid profile includes saturated, unsaturated, and branched fatty acids of which i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$, ai-$C_{17:0}$ are the major components (type 3b, Kroppenstedt 1985). The predominant menaquinone is MK-9(H_4), but MK-9 (H_6) and MK-9(H_8) are present in some strains (Seo and Lee 2009). The diagnostic polar lipid in all *Actinocatenispora* strains is phosphatidylethanolamine, but the overall profiles may also contain diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, phosphatidylglycerol, and several unidentified ninhydrin-negative phospholipids (Thawai et al. 2006; Matsumoto et al. 2007; Seo and Lee 2009). Selected phenotypic and chemotaxonomic characters are given in *Table 28.5*.

Actinoplanes Couch 1950, 244[AL] Emend. Stackebrandt and Kroppenstedt 1988

Ac.ti.no.pla'nes. Gr. n. *aktis -inos*, ray, beam; Gr. masc. n. *planes*, a wanderer, roamer; N.L. masc. n. *Actinoplanes* literally, a ray wanderer; intended to signify an actinomycete with swimming spores.

The genus *Actinoplanes* is the second largest group in the family *Micromonosporaceae* and currently holds 30 validly published species. Strains grow well on various complex media such as peptone-Czapek agar (Bland and Couch 1981), Bennett's agar (Waksman 1961), and various ISP media (Shirling and Gottlieb 1966). Actipolanetes form compact colonies up to about 3 cm in diameter after 4–6 weeks of incubation (*Fig. 28.4a*, *c*). These colonies are usually elevated and convoluted, and frequently have protuberances in the center (*Fig. 28.4a*). *Actinoplanes* strains are typically orange to bright orange due to the production of carotenoid pigments (Szaniszlo 1968), but colonies may also be black, brown, cream, green, purple, red, rusty brown, or yellow (Parenti and Coronelli 1979; Vobis 1987; Palleroni 1989).

Actinoplanes species produce a branched and septated substrate mycelium (0.2–1.0 μm in diameter). Although fragmentation of the substrate mycelium is not a common feature, it has been observed in the species *Actinoplanes couchii* when cultured on DSMZ medium 65 (Kämpfer et al. 2007). Aerial mycelium is usually absent or only rudimentarily developed. The unusual presence of aerial hyphae is characteristic for *A. couchii*, *A. ferrugineus*, *A. linguriensis*, *A. rectilineatus*, and *A. teichomyceticus* (Palleroni 1989; Wink et al. 2006). Colonies can be covered with a whitish bloom, if abundant sporangia are produced on the surface of the substrate mycelium (*Fig. 28.4b*, *d*). In general, sporangia develop directly on the surface of a colony (*Fig. 28.4d–f*). In *A. minutisporangius*, they are also submerged (Ruan et al. 1986). Frequently, the sporangia arise terminally from "palisade" hyphae, which are thicker in diameter and vertically oriented (Bland and Couch 1981). Inside the sporangia, the spores are arranged in coils (*Fig. 28.4g*); but in *A. rectilineatus*, they run in parallel rows (Lechevalier and Lechevalier 1975). If the

Table 28.5
Selected phenotypic characteristics of *Actinocatenispora* species

Characteristic	*A. thailandica*	*A. rupis*	*A. sera*
Growth on			
ISP 2	+++	+++	+++
ISP 3	+++	++	nd
ISP 4	+++	+	nd
ISP 6	+++	++	+++
ISP 7	+++	+	+
Colony color	Yellow to orange	Pale to yellow	nd
Spore chains	<6 spores	>10 spores	>20 spores
NaCl tolerance (w/v)	7 %	<5 %	4 %
Nitrate reduction	+	−	+
Utilization of			
L-Arabinose	−	+	−
Cellobiose	+	+	−
D-Mannitol	+	+	−
Raffinose	+	−	−
Trehalose	+	+	−
Major menaquinones	MK-9(H_4) and MK-9(H_6)	MK-9(H_4)	MK-9(H_4)
Major polar lipids[a]	PE, DPG, PI, PIM, PG, and unidentified ninhydrin-negative phospholipids	PE, PI, PG	PE
Whole-cell sugars[b]	Glu, Gal, Xyl, Ara, Man and Rib	Glu, Rha, Rib, Ara and Xyl	Ara, Gal and Xyl
Major fatty acids	iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and iso-$C_{17:0}$	iso-$C_{16:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:1}$	ai-$C_{17:0}$, i-$C_{16:0}$, i-$C_{17:0}$ and i-$C_{15:0}$
DNA G+C content (mol%)	72	74.3	72

Data obtained from Thawai et al. (2006), Matsumoto et al. (2007), Seo and Lee (2009)
+ positive, − negative, *nd* not determined
PE phosphatidylethanolamine, *DPG* diphosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PG* phosphatidylglycerol
Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose

sporangial envelopes are very thin and transient, individual sporangia may be attached (*Fig. 28.4e*). The shape of the sporangia ranges from globose, subglobose, oval, umbelliform, cylindrical, or lobate to irregular. The average size of a sporangium is from 4 to 25 µm in diameter. Extreme dimensions of sporangia exist in *A. minutisporangius*, with 2 µm as a minimum (Ruan et al. 1986) and 47 µm as a maximum (G. Vobis, unpublished observations).

The spores of *Actinoplanes* are globose or subglobose to short bacilliform and possess a tuft of flagella. *Actinoplanes missouriensis* NBRC 102363 flagellated spores were recently characterized (Uchida et al. 2011). The average swimming speed of the motile spores was 135 µm/s and belongs to the fastest group among bacterial swimming species so far studied. The number of flagella in this strain ranged from 10 to 19 on an average. The flagella grow from one side of the spore rather than around it, as seen for peritrichous flagella, indicating that the spore has polar flagella despite its round cell body (Uchida et al. 2011). Although it is not common for cocci to retain polar flagella in eubacterial species, this organization has been observed in archaeal species, such as *Methanococcus voltae*, in which more than 80 flagella grow from one side of the cell body (Bardy et al. 2002).

Spores of *Actinoplanes brasiliensis* retain their motility for more than one day in liquid mineral medium with glucose (Palleroni 1983). The zoospores of *Actinoplanes* exhibit chemotactic properties. In *A. brasiliensis*, Palleroni (1976) found bromide and chloride ions acting as attractants at a relatively high concentration (0.1 M). Addition of methionine stimulated this chemotactic effect, suggesting that protein methylation may be involved (Palleroni 1983). Not all species of *Actinoplanes* are attracted by halides. Spores of *A. missouriensis* were attracted to fungal conidia, chlamydospores, sclerotia, and to exudates (Arora 1986). Several sugars had the same function. An extract of cattle horn meal can be more attractive to the spores than chloride ions (G. Vobis, unpublished observations). Phototactic effects could not be observed, but an apparent microaerophilic behavior was seen in *A. brasiliensis* (Palleroni 1976). In baiting experiments simulating an aquatic microhabitat, pollen or hair is exposed to the surface of water. The zoospores, once released from the submerged sporangia, are able to swim to the surface, fasten to the natural substrates,

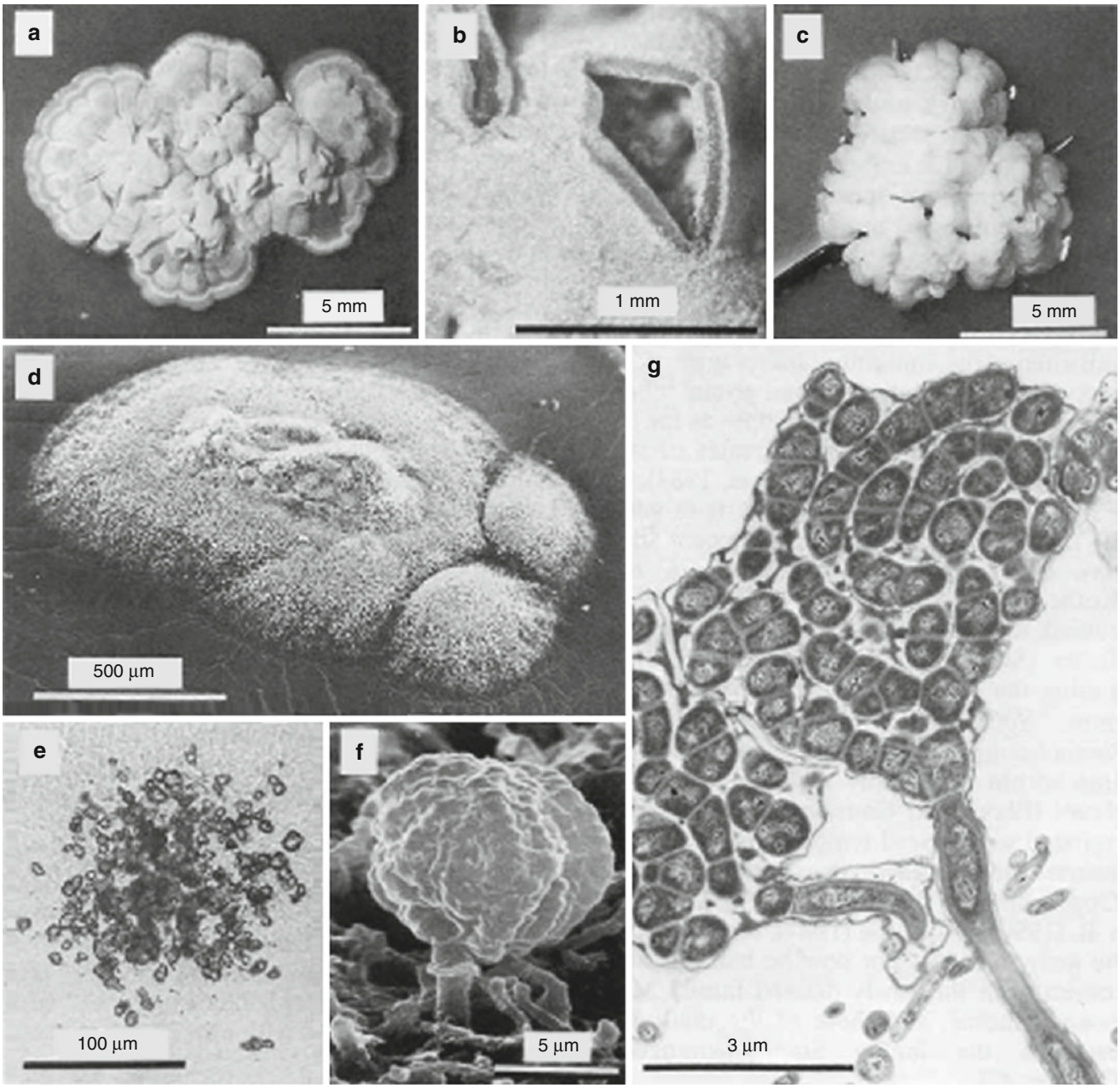

Fig. 28.4
Features of the genus *Actinoplanes*. (a) Colony with rough surface; marginal areas divided into radial and concentric sections, dissecting microscope (*DM*). (b) Burst substrate mycelium covered with a mass of sporangia (DM). (c) Elevated colony with squamules; smooth surface without sporangia (DM). (d) Flat colony with abundant sporangia visible on the substrate mycelium, scanning electron microscope (*SEM*). (e) Irregularly shaped sporangia on agar medium, light microscope (*LM*). (f) Globose sporangium at the tip of a palisade hypha (SEM). (g) Section of a sporangium with coiled chains of spores (*TEM*) (G from Kothe 1987; with permission). *DM* dissecting microscope, *LM* light microscope, *SEM* scanning electron microscope, *TEM* transmission electron microscope

germinate, and colonize them within several days (Couch 1963; Vobis 1984). This may be a result of aerotactic and chemotactic behavior of the spores (Cross 1986). Although the chemotactic response is used effectively in the isolation method of Palleroni (1980), the exact physiological explanation is not yet known.

Green, yellow, or brown soluble pigments are produced by several *Actinoplanes* species. In addition, a cherry-red pigment is characteristic of *Actinoplanes italicus* (Beretta 1973) and a soluble blue pigment, found in *Actinoplanes cyaneus*, has been identified as a celocomycin-actinorodine molecule (Terekhova et al. 1977). Melanoid pigments are also produced by *Actinoplanes digitatis* (Couch 1963).

Actinoplanetes stain Gram-positive, are non-acid fast and aerobic with an oxidative metabolism. The growth temperature

ranges from 10 °C to 35 °C, but the species *A. deccanensis* can grow at 45 °C (Parenti and Coronelli 1979). No growth is observed below 4 °C or above 50 °C. The pH growth range is 6.0–8.0, but several species have been reported to grow in acid (pH 4.0) or alkaline conditions (pH 10.0) (Kothe 1987). Physiological tests such as degradation, hydrolysis, coagulation, peptonization, or liquefaction of various compounds have been carried out for many *Actinoplanes* species (Table 28.6). Thus, casein, chitin, gelatin, lecithin, DNA, and RNA are decomposed by most species. The plant polymer pectin is decomposed by most species, but contradictory results have been reported for the degradation of cellulose (Schäfer 1973; Goodfellow et al. 1990; Solans and Vobis 2003). In addition, hemicellulose can be degraded and lignocellulose can be used as a substrate by several strains (Solans and Vobis 2003). Enzymatic activities for the species *A. liguriensis, A. palleroni, A. rectilenatus, A. regularis, and A. teichomyceticus* have been determined using the API test systems API 20E and API ZYM (Wink et al. 2006). Furthermore, the species *A. couchii*, *A. italicus*, and *A. rectilienatus* have been tested for enzymatic activities based on chromogenic substrates (Kämpfer et al. 2007; Vobis et al. 2012).

Actinoplanes species are capable of assimilating a large variety of substrates as carbon sources including carbohydrates, amino acids, and organic acids. Unfortunately, not all type strains have been tested for the same compounds and it is not easy to make a comparison of their assimilation profiles (Table 28.6). Nevertheless, glucose is utilized by all species, although apparently *A. cyaneus* has never been tested (Vobis and Kämpfer 2012). L-arabinose and D-fructose are used by most species, but contradicting results are reported for *A. liguriensis* (Palleroni 1989; Wink et al. 2006), *A. digitatis* (Vobis and Kothe 1989), and *A. palleroni* (Goodfellow et al. 1990; Wink et al. 2006). In a similar way, D-galactose, D-maltose, D-mannose, D-rhamnose, and D-xylose are used by most species tested (Table 28.6).

According to the classification scheme of Lechevalier and Lechevalier (1970b), the members of the genus *Actinoplanes* are characterized by cell-wall chemotype II. Glycine and *meso*-diaminopimelic acid (A2pm) and/or 3-hydroxy-diaminopimelic acid are the amino acids characteristic of the peptidoglycan. The primary structure of the peptidoglycan was described by Kawamoto et al. (1981). Glycine, rather than L-alanine, is linked to muramic acid, and *meso*-diaminopimelic acid or its hydroxylated derivative is directly cross-linked to the D-alanine of an adjacent peptide subunit: muramic acid is *N*-glycosylated. The *N*-glycolyl muramic acid is a characteristic unique to the genera with cell-wall type II and is found in many *Actinoplanes* and "*Ampullariella*" species (Stackebrandt and Kroppenstedt 1987). It can be easily identified by a colorimetric method (Uchida and Aida 1977).

Actinoplanes species have wall chemotype II and the sugar pattern D (Lechevalier and Lechevalier 1970a) whereby the pentoses xylose and arabinose are the characteristic sugars. Other sugars that may be present are arabinose, galactose, glucose, and mannose. Galactose has not been detected in *Actinoplanes regularis*, *Actinoplanes campanulatus*, or *Actinoplanes digitatis* (Stackebrandt and Kroppenstedt 1987). The phospholipid profile corresponds to type II (Lechevalier et al. 1977; 1981) and is characterized by the presence of phosphatidylethanolamine as diagnostic phospholipid; nevertheless, this phospholipid is not present in *A. regularis* (Stackebrandt and Kroppenstedt 1987). Other lipids that may be present include phosphatidylinositol, phosphatidylinositol mannoside, and phosphatidylglycerol. A hydrophilic glycolipid and other uncharacterized lipids and glycolipids have also been reported for the species *A. couchii* (Kämpfer et al. 2007) and *A. humidus* (Goodfellow et al. 1990).

The genus *Actinoplanes* contains menaquinones with 9–10 tetrahydrogenated isoprene side chains, MK-9(H_4) and MK-10(H_4), and fit into the type 3b of the classification scheme of Kroppenstedt (1985). Minor amounts of MK-9(H_2) and MK-9(H_6) are found in the species *A. auranticolor*, *A. couchii*, *A. derwentensis*, *A. humidus*, *A. palleroni*, and *A. utahensis* (Stackebrandt and Kroppenstedt 1987; Goodfellow et al. 1990; Kämpfer et al. 2007). Minor proportions of MK-9(H_8) can also be found (Goodfellow et al. 1990b; Kämpfer et al. 2007). Smaller amounts of MK-10(H_2) and traces of MK-10(H_6) are characteristic for *Actinoplanes couchii* (Kämpfer et al. 2007). The additional presence of MK-7(H_4, H_6, H_8) and MK-8(H_2, H_4, H_6, H_8) has been reported in various species including *A. consettensis*, *A. derwentensis*, *A. humidus*, *A. lobatus*, and *A. palleroni* where they may occur in minor amounts (Goodfellow et al. 1990).

Complex mixtures of straight-chain, branched-chain, and unsaturated fatty acids are found in *Actinoplanes* species (Goodfellow et al. 1990; Sun et al. 2009; Ara et al. 2010). Although the amount varies significantly between the strains analyzed, in general, the major fatty acids (>10 %) include $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso- $C_{17:0}$, $C_{17:0}\omega9c$, $C_{17:1}\omega8c$ (Stackebrandt and Kroppenstedt 1987; Matsumoto et al. 2000; Wink et al. 2006; Kämpfer et al. 2007; Sun et al. 2009; Ara et al. 2010). No fatty acid data are available for *Actinoplanes cyaneus* and *Actinoplanes digitatis*.

Asanoa Lee and Hah 2002 Emend. Xu et al. 2011

As.a.no′a. N.L. fem. n. *Asanoa*, named after Kozo Asano, the Japanese microbiologist who made the original description of the genus *Catellatospora*.

The genus *Asanoa* was proposed to accommodate two strains previously classified in the genus *Catellatospora* and subsequently reclassified as *Asanoa ferruginea* and *Asanoa ishikariensis* (Lee and Hah 2002). Since then, three additional species have been described, namely, *Asanoa iriomotensis*, *Asanoa hainanensis*, and *Asanoa siamensis* (Tamura and Sakane 2005; Xu et al. 2011; Niemhom et al. 2012). Colony color on ISP 3 agar may be orange-red, bright orange, yellow-green, and olive. Members of this taxon

Table 28.6
Selected phenotypic characteristics of the genus *Actinoplanes*

Characteristic	*A. philippinensis*	*A. auranticolor*	*A. brasiliensis*	*A. campanulatus*	*A. capillaceus*	*A. consettensis*	*A. couchii*	*A. cyaneus*	*A. deccanensis*	*A. derwentensis*	*A. digitatis*	*A. durhamensis*	*A. ferrugineus*	*A. globisporus*	*A. humidus*
Colony color	Apricot-orange	Apricot-orange	Orange	Coral red; coral pink	Pink-yellow	Yellow-brown	Yellow-orange	Blue	Orange	Orange-dark orange	Pink to red-cinnamon	Light to dark orange	Rusty brown	Cream to light orange	Yellow-orange-brown
Soluble pigments	Brown	Yellow-amber	–	Yellow, green, brown	–	nd	Red-brown	Blue	–	–	Yellow, green, brown	Melanoid	Brown	–	Dark
Shape of sporangia	Globose to oval	Very irregular, lobed	Irregular to umbelliform	Bell shape, irregular, pyriform	Bell shape	Globose	Globose to oval	Spherical, globose	Globose	Globose	Digitate, cylindrical	Globose	Globose to irregular	Irregular	Spherical
Spore arrangement	Coils, globose	Irregular; rods	Coils; subglobose	Parallel rows	nd	Irregular	nd	nd	Coils; globose	Irregular	Parallel rows	Irregular	Coils; globose	Coils; globose	Irregular
Temperature growth range (°C)	nd	nd	nd	nd	nd	4–30	nd	nd	26–42	4–30	nd	4–30	nd	nd	4–30
pH growth range	4–8	6–8	nd	6–8	nd	6–8	nd	nd	nd	v	6–8	v	4–10	nd	6–8
NaCl tolerance (%, w/v)	2	0	nd	3	nd	v	nd	nd	nd	v	2	2	nd	nd	1
Nitrate reduction	+	–	+	+	+	–	nd	nd	+	v	+	v	+	–	+
H_2S production	+	+	–	–	nd	v	nd	nd	–	v	–	–	–	–	v
Melanin production	–	+	–	–	–	nd	nd	nd	+	nd	+	+	+	–	nd
Milk coagulation	–	–nd	–	+	v	nd	nd	nd	–	nd	+	nd	nd	–	nd
Milk peptonization	–	nd	–	+	v	nd	nd	nd	–	nd	+	nd	nd	–	nd
Assimilation of															
L-Arabinose	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+
Cellobiose	–	–	nd	–	nd	–	nd	nd	nd	–	–	–	nd	nd	–
Cellulose	+	–	+	nd	nd	nd	nd	nd	–	nd	nd	nd	–	–	nd
D-Fructose	+	+	+	–	+	+	+	nd	–	+	+	+	w	+	+

D-Galactose	+	+	nd	+	+	+	+	nd	nd	+	+	+	nd	+	+
D-Glucose	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+
Inositol	+	+	+	–	–	–	+	nd	nd	+	+	v	–	+	v
D-Lactose	+	+	nd	–	nd	+	nd	nd	+	+	+	+	+	+	+
D-Maltose	+	+	nd	+	+	+	+	nd	nd	+	+	+	nd	+	+
D-Mannitol	+	+	+	+	+	+	nd	nd	–	+	–	+	+	+	+
D-Mannose	+	+	nd	+	+	+	+	nd	+	+	+	+	+		+
Melezitose	+	+	–	–	nd	v	nd	nd	v	nd	–	+	–	nd	v
Raffinose	v	+	–	–	–	+	nd	nd	–	v	v	+	–	–	v
L-Rhamnose	+	+	+	+	+	+	+	nd	+	v	+	+	–	+	v
D-Ribose	+	+	–	+	–	–	–	nd		–	v	v	–	–	v
D-Sorbitol	+	+	+	+	nd	v	–	nd	nd	+	–	v	–	–	+
Sucrose	+	+	+	+	w	nd	+	nd	+	nd	+	nd	–	+	nd
D-Xylose	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+
Degradation of:															
Casein	+	+	+	+	+	+	+	nd	–	+	v	+	+	+	+
Cellulose	v	–	nd	–	v	–	nd	nd	nd	–	+–	–	nd	nd	–
Chitin	+	–	nd	+	nd	v	nd	nd	nd	v	+	v	nd	nd	v
DNA	v	+	nd	+	nd	v	nd	nd	nd	v	+	v	nd	nd	+
Elastin	+	–	nd	–	nd	+	nd	nd	nd	+	v	v	nd	nd	v
Gelatin	+	v	+	v	–	+	nd	nd	+	+	–	+	+	–	+
Guanine	–	–	nd	–	–	–	nd	nd	nd	–	–	–	nd	nd	–
Hypoxanthine	–	–	nd	–	–	–	+	nd	nd	–	+	–	nd	nd	–
Lecithin	+	–	nd	+	nd	+	nd	nd	nd	v	–	+	nd	nd	+
Pectin	+	+	nd	–	nd	v	nd	nd	nd	v	+	–	nd	nd	v
RNA	+	+	nd	+	nd	+	nd	nd	nd	v	+	v	nd	nd	+
Starch	+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+
Tyrosine	+	–	–	–	nd	v	+	nd	nd	v	v	v	+	nd	v
Xanthine	–	–	nd	–	–	–	+	nd	nd	–	–	–	nd	nd	–

Table 28.6 (continued)

Characteristic	*A. iantinogenes*	*A. italicus*	*A. linguriensis*	*A. lobatus*	*A. missourensis*	*A. octamycinicus*	*A. palleroni*	*A. rectilineatus*	*A. regularis*	*A. sichuanensis*	*A. teichomyceticus*	*A. tereljensis*	*A. toevensis*	*A. utahensis*	*A. xinjangensis*
Colony color	Red-purple	Cherry-red	Yellow-orange	Coral red-brick red	Orange	Red-orange	Yellow-brown	Orange	Orange, red, brown	Red-orange	Orange	Gray-brown, red-brown	Yellow to orange-brown	Brown-orange	White to orange
Soluble pigments	–	Cherry-red	Yellow, red	Yellow-green	–	–	Melanoid	–	Yellow, green, brown	–	–	Pink-brown	Pink-brown	–	–
Shape of sporangia	Globose	Globose to oval	Globose to oval	Lobed, irregular, cylindrical	Globose, subglobose	Globose	Spherical	Cylindrical	Cylindrical	Spherical	Globose to oval	Irregular	Globose to oval	Irregular	Spherical to oval
Spore arrangement	nd	Coils; globose to oval	nd	Parallel rows	Coils: globose	nd	nd	Long rows	Parallel rows	nd	Coils; globose	nd	nd	Coils; globose	Coils
Temperature growth range	15–37	nd	nd	nd	nd	15–37	4–30	10–37	nd	10–37	nd	nd	nd	nd	10–37
pH growth range	4–8	6–8	nd	6–8	6–8	6–9	8	4–10	6–8	6.5–10.5	nd	6–9	6–11	v	6.5–8.5
NaCl tolerance (%, w/v)	2	1	nd	0	2	1	2	nd	0	4	nd	3	2	0	7
Nitrate reduction	+	v	–	+	+	+	v	–	+	+	+	+	–	+	+
H_2S production	nd	v	–	nd	–	nd	–	–	–	–	v	nd	nd	+	+
Melanin production	nd	+	–	–	–	nd	nd	+	–	nd	+	nd	nd	+	+
Milk coagulation	nd	–	–	+	–	nd	nd	+	+	nd	–	nd	nd	–	nd
Milk peptonization	nd	+	–	+	–	nd	nd	+	–	nd	+	nd	nd	–	nd
Assimilation of															
L-Arabinose	+	+	v	+	+	+	v	+	+	nd	+	nd	nd	+	nd
Cellobiose	+	–	nd	–	–	+	–	nd	–	–	nd	nd	nd	–	–
Cellulose	nd	–	–	nd	–	nd	nd	nd	–	–	nd	nd	nd	–	–
D-Fructose	+	+	nd	+	+	+	+	+	+	+	+	nd	nd	+	+
D-Galactose	+	+	nd	+	+	+	v	+	–	+	nd	nd	nd	+	–

D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	nd	+	v	–	–	nd	v	v	–	–	–	nd	nd	–	–
D-Lactose	+	+	–	+	+	+	v	+	–	–	w	w	+	–	–
D-Maltose	+	+	nd	+	+	+	+	+	+	+	nd	+	+	+	–
D-Mannitol	+	+	–	v	+	+	v	+	–	–	+	–	–	+	–
D-Mannose	+	+	+	+	+	+	+	+	+	–	+	nd	nd	+	+
Melezitose	nd	+	nd	–	–	nd	v	nd	+	nd	nd	nd	nd	–	nd
Raffinose	nd	–	–	v	–	nd	d	v	–	–	–	nd	nd	–	–
L-Rhamnose	+	+	+	v	+	+	v	+	+	–	–	nd	nd	+	–
D-Ribose	nd	+	nd	+	–	+	–	–	+	+	nd	–	–	+	–
D-Sorbitol	+	v	nd	+	v	+	+	–	+	+	nd	nd	nd	v	+
Sucrose	+	+	–	+	+	+	+	v	+	+	+	nd	nd	+	+
D-Xylose	+	+	–	v	+	+	v	+	v	+	–	+	+	+	+
Degradation of:															
Casein	nd	+	+	+	+	nd	+	+	+	–	+	w	+	+	–
Cellulose	nd	nd	nd	–	–	nd	–	nd	v	nd	nd	nd	nd	–	nd
Chitin	nd	+	nd	+	+	nd	v	nd	+	nd	nd	nd	nd	+	nd
DNA	nd	+	nd	+	+	nd	–	nd	+	nd	nd	nd	nd	+	nd
Elastin	nd	–	nd	+	+	nd	–	nd	–	–	nd	nd	nd	+	–
Gelatin	+	+	v	+	v	+	+	+	+	+	+	–	+	+	+
Guanine	nd	–	nd	–	–	nd	–	nd	–	–	nd	nd	nd	–	–
Hypoxanthine	nd	v	nd	–	–	nd	–	+	–	–	nd	–	–	–	–
Lecithin	nd	+	nd	+	+	nd	+	nd	–	nd	nd	nd	nd	+	nd
Pectin	nd	+	nd	+	+	nd	v	nd	–	nd	nd	nd	nd	+	nd
RNA	nd	+	nd	+	+	nd	v	nd	+	nd	nd	nd	nd	+	nd
Starch	nd	+	+	+	+	nd	+	+	+	+	+	–	+	+	–
Tyrosine	nd	+	–	v	+	nd	v	+	–	+	–	w	w	v	–
Xanthine	nd	v	nd	–	–	nd	–	+	–	–	nd	–	–	–	–

Data from Thiemann (1969), Schäfer (1973), Kothe (1987), Palleroni et al. (1989), Vobis and Kothe (1989), Goodfellow et al. (1990), Matsumoto et al. (2000), Kämpfer et al. (2007), Wink et al. (2006), Tamura et al. (2011), Vobis et al. (2012)

+ positive, – negative, *v* variable, *w* weak, *nd* not determined

produce branched non-fragmented vegetative hyphae (0.3–0.4 µm in diameter), but a true aerial mycelium is not formed. Spore chains are borne on the tip of short sporophores arising directly from the agar surface; tap-water agar, glycerol/calcium malate agar, or HV agar are the recommended media to induce sporulation (Tamura and Sakane 2005; Xu et al. 2011). *Asanoa siamensis* produces a strong red-orange soluble pigment on ISP 2 agar (Niemhom et al. 2012).

Asanoa species stain Gram-positive, are nonmotile and aerobic. Best growth occurs between 20 °C and 30 °C but not below 15 or above 40 °C. Nitrate is reduced to nitrite by *A. hainanensis*, weakly reduced by *A. siamensis* but not reduced by any of the remaining species. The pH range for growth of *Asanoa* strains ranges from 6 to 9, but *A. siamensis* can grow at pH 12 (Niemhom et al. 2012). All members of the taxon are heterotrophic with an oxidative metabolism.

All *Asanoa* strains assimilate D-galactose, D-glucose, melibiose, and L-rhamnose. Utilization of the following substrates varies among the five species: D-arabinose, L-arabinose, D-cellobiose, dextran, dulcitol, D-fructose, D-lactose, maltose, D-mannose, mannitol, methyl α-D-glucoside, D-raffinose, D-ribose, salicin, starch, sucrose, D-trehalose, and D-xylose. Inulin, L-sorbose, D-melezitose, methanol, meso-erythritol, D-sorbitol, and D-xylitol do not appear to serve as carbon sources; however, not all species have been tested (Table 28.7).

The chemotaxonomic characteristics of *Asanoa* strains include the presence of *meso*-diaminopimelic acid and 3-hydroxy-diaminopimelic acid as diaminoacids. The acyl type of the cell wall is glycolyl. The whole-cell sugars found in all *Asanoa* species are glucose, mannose, and xylose, while the presence of arabinose, galactose, rhamnose, and ribose is variable. The predominant menaquinones in *A. ferruginea*, *A. ishikariensis*, and *A. iriomotensis* are MK-10(H_6, H_8). In the case of *A. hainanensis* the presence of MK-9(H_4, H_6, H_8) was reported (Xu et al. 2011). Except for *Asanoa hainanensis* which contains a phospholipid type IV with phosphatidylethanolamine, phosphatidylinositol mannosides, and phospholipids of unknown structure containing glucosamine, all species exhibit a phospholipid type PII pattern comprised of diphosphatidylinositol mannoside, diphosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine. An unknown phospholipid is also reported for *Asanoa ferruginea* and *A. ishikariensis* (Lee and Hah 2002). The fatty acid profile is characterized by significant amounts of anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$, $C_{17:0}$, and iso-$C_{16:0}$ (Table 28.7).

Catellatospora Asano and Kawamoto 1986 Emended. Lee and Hah 2002 Emended. Ara et al. 2008a

Ca.tell.a.to.spo′ra. L. n. *catella*, a small chain; Gr. fem. n. *spora*, a seed and in biology a spore; N.L. fem. n. *Catellatospora*, (organism forming) small chain of spores.

The genus *Catellatospora* was described by Asano and Kawamoto (1986) for aerobic, Gram-staining positive, mesophilic strains that produce short chains of nonmotile spores borne directly on the substrate mycelium. There are currently five recognized species in the genus, namely, *C. citrea* the type species, *C. bangladeshensis*, *C. chokoriensis*, *C. coxensis*, and *C. methionotrophica*. *Catellatospora* strains produce well-developed branched, non-fragmenting, vegetative hyphae (0.3–0.6 µm in diameter). Straight to flexuous chains composed of about 5–30 spores are borne on the substrate mycelium, singly or in clusters. Colonies may be waxy and leathery and lack aerial mycelium (Fig. 28.5). Catellatosporae grow well on complex media such as ISP 2, ISP 3 agar (Shirling and Gottlieb 1966), Bennett's (Waksman 1950), and Hickey-Tresner agar (Hickey and Tresner 1952). Colonies show different shades of yellow depending on the culture medium while diffusible pigments have not been observed. All strains are positive for catalase.

Catellatospora strains are able to assimilate a wide range of carbohydrates as carbon sources. All species use D-galactose, glucose, lactose, sucrose, trehalose, and D-xylose. Most species utilize glycerol, maltose, L-rhamnose, and salicin (Asano and Kawamoto 1986; Ara and Kudo 2006). Other carbon sources utilized are given in Table 28.8.

Catellatospora citrea has been studied for its capacity to produce several enzymes. The type strain showed good activity for esterase lipase (C8), leucine arylamidase, trypsin, chymotrypsin, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase. It is resistant to novobiocin (20 µg/ml) and crystal violet (0.0001 %, w/v) (Asano and Kawamoto 1986). As reported by Asano and Kawamoto (1988), most of the metabolic characteristics of *C. citrea* and *C. methionotrophica* are similar except for the absolute requirement for methionine by the latter species. In addition, *C. methionotrophica* is sensitive to novobiocin (50 µg/ml).

The five *Catellatospora* species show a very homogeneous chemotaxonomic profile. All species include *meso*-diaminopimelic and 3-hydroxy-diaminopimelic acids and a glycosylated peptidoglycan in their cell walls. Their fatty acid profile includes saturated straight-chain, saturated branched, and unsaturated fatty acids, the major components are iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{17:0}$, and $C_{17:1}\omega8c$. The polar lipid pattern of all strains includes phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, and phopstidylinositol mannosides except for *C. citrea* where phosphatidylglycerol is present but not diphosphatidylglycerol. The major menaquinone is MK-9 (H_4) but MK-9(H_2, H_6, H_8) are also found. *Catellatospora citrea* also contains MK-8 (H_4, H_6, H_8). The major differences in the chemotaxonomic profiles of *Catellatospora* strains are found in the whole-cell sugars present. Arabinose and xylose are reported for all species. Galactose, ribose, mannose, and glucose are found in all species except *C. citrea*, and rhamnose has been detected only in *C. bangladeshensis*, *C. chokoriensis*, and *C. coxensis* (Asano and Kawamoto 1988; Lee and hah 2002; Ara and Kudo 2006).

Table 28.7

Selected phenotypic characteristics of *Asanoa* species

	A. ferruginea	*A. ishikariensis*	*A. iriomotensis*	*A. hainanensis*	*A. siamensis*
Colony color on ISP 3	Brilliant orange	Green-yellow	Olive	Red-orange	Orange-yellow
NaCl tolerance	2 %	%2	3 %	3 %	2 %
Nitrate reduction	–	–	–	+	w
Production of urease	–	+	–	–	nd
Milk coagulation	–	+	–	+	–
Gelatin liquefaction	–	–	+	+	–
Assimilation of:					
D-arabinose	+	+	nd	nd	nd
L-arabinose	+	+	w	w	+
Dextran	+	+	nd	w	nd
D-cellobiose	+	+	nd	w	+
D-fructose	+	+	nd	+	+
D-galactose	+	+	+	w	+
D-glucose	+	+	+	+	+
D-lactose	+	+	–	+	+
maltose	+	+	+	nd	w
D-mannose	+	+	+	nd	+
Melibiose	+	+	+	w	+
Methyl α-D-glucoside	+	+	+	nd	nd
D-raffinose	+	+	+	nd	w
L-rhamnose	+	+	+	w	+
D-ribose	+	+	nd	+	nd
Salicin	+	–	nd	nd	nd
Starch	+	–	nd	w	nd
Sucrose	+	+	nd	w	+
D-trehalose	+	+	nd	w	nd
D-xylose	+	+	nd	+	+
Adonitol	+	+	–	–	nd
Gluconate	–	+	nd	nd	nd
Inulin	–	–	nd	nd	nd
D-melezitose	–	–	nd	–	nd
L-sorbose	–	–	nd	–	nd
Dulcitol	–	+	–	+	nd
Butanol	–	–	nd	nd	nd
meso-Erythritol	–	–	nd	–	nd
Ethanol	–	–	nd	nd	nd
Glycerol	–	–	nd	w	w
meso-Inositol	–	–	nd	+	nd
2-Propanol	–	–	nd	nd	nd
D-sorbitol	–	–	–	–	nd
D-xylitol	–	–	nd	nd	nd
Mannitol	nd	nd	+	+	+
L-erythritol	nd	nd	–	nd	nd
L-inositol	nd	nd	–	nd	nd
α-ketoglutaric acid	nd	nd	nd	w	nd
Malonic acid	nd	nd	nd	w	nd

Table 28.7 (continued)

	A. ferruginea	*A. ishikariensis*	*A. iriomotensis*	*A. hainanensis*	*A. siamensis*
Whole-cell sugars	Glu, Gal, Rha, Rib, Man, Xyl, Ara	Glu, Gal, Rha, Rib, Man, Xyl, Ara	Glu, Gal, Man, Xyl	Glu, Rib, Man, Xyl	Glu, Rha, Rib, Man, Xyl
Diaminopimelic acid isomer	3-OH	3-OH	*meso*	*meso*	3-OH
Phospholipid pattern	DPG, PG, PE, PI, PIMs, unknown phospholipids	DPG, PG, PE, PI, PIMs, unknown phospholipids	PE, phospholipids including unidentified glucosamine	PE, PIMs, phospholipids of unknown structure containing glucosamine	DPG, PG, PE, PI, three unknown phospholipids, GL, four lipids
Major fatty acids	anteiso-$C_{15:0}$, $C_{17:0}$, iso-$C_{16:0}$, $C_{17:1}$, iso-$C_{15:0}$,	anteiso-$C_{15:0}$, $C_{17:1}$, iso-$C_{15:0}$, $C_{17:0}$	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$, $C_{17:0}$, iso-$C_{16:0}$.	iso-$C_{16:0}$, $C_{17:0}$, anteiso-$C_{15:0}$, iso-$C_{15:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, $C_{17:0}$, iso-$C_{16:0}$
Menaquinones	MK-10(H_6, H_8)	MK-10(H_6, H_8)	MK-10(H_6, H_8)	MK-9(H_4, H_6, H_8)	MK-10(H_6, H_8), MK-9(H_6, H_8)
DNA G+C mol%	71.5	71.1	69	70.3	72.3

Data from Lee and Hah (2002); Tamura and Sakane (2005); Xu et al. (2011); Niemhom et al. (2012)
+ positive, − negative, *w* weak, *nd* not determined
3-OH, 3-hydroxy-diaminopimelic acid; *m*-DAP, *meso*-diaminopimelic acid
Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose
DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PIMs* phosphatidylinositol mannosides, *GL* glycolipids

Catenuloplanes Yokota et al. 1993 Emend. Kudo et al. 1999

Ca.te.nul.o.plan′es. L. fem. n. *catenula*, a short chain; Gr. masc. n. *planes*, a wanderer; N.L. masc. n. *Catenuloplanes*, a short chain wanderer; intended to signify a motile short chain.

The genus *Catenuloplanes* was proposed by Yokota et al. (1993) to accommodate strains of arthrospore-bearing actinobacteria isolated from soils in Japan, India and Nepal. This taxon currently harbors seven recognized species (Table 28.9). *Catenuloplanes* strains stain Gram-positive, are non-acid fast, and are strict aerobic filamentous microorganisms. A non-fragmenting light yellow to orange substrate mycelium is formed on different complex media, but the formation of a monopodial or dichotomously branched aerial mycelium is scant or absent (Yokota et al. 1993). If produced, spores chains are aggregated into clusters resembling flowers or sporochia, which are compact and flat at the center of the colony but filamentous toward the edge. The short chains on the aerial mycelium are arranged in spirals of one to two turns (hooked or less frequently flexuous). The spore chains may be covered by outer sheaths (Petrolini et al. 1993; Kudo et al. 1999). The spores are rod shaped, straight, or curved (0.6–0.8 by 2–4 μm), have a smooth surface, and motile by peritrichous flagella. Strains show good growth on yeast extract-malt extract agar, inorganic salts-starch agar, starch agar Czapek-sucrose agar, and glucose-asparagine agar (Shirling and Gottlieb 1966). Several diffusible pigments are produced on some of these media (Table 28.9). The optimal temperature range for growth of all species is 21–28 °C.

Members of the genus *Catenuloplanes* have an oxidative metabolism. The degradation of several chemical organic compounds has been determined for all strains; a selection of these tests is presented in Table 28.9. All strains decompose casein, aesculin, and urea, while the degradation of elastin and testosterone is useful for differentiating between the seven species. In a similar way, *Catenuloplanes* strains are able to use a wide range of carbohydrates and organic acids as carbon sources (Table 28.9). All species use L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, L-rhamnose, salicin, starch, sucrose, D-trehalose, and D-xylose. In addition, all strains produce acid from L-arabinose, D-fructose, D-galactose, D-glucose, *myo*-inositol, L-rhamnose, sucrose, and D-xylose. The organic acids fumarate, L-malate, and succinate serve as carbon sources for all strains, unlike benzoate, citrate, mucate, oxalate, and L-tartrate which are not assimilated by any of the present known species. Other physiological characteristics include the coagulation and peptonization of milk, liquefaction of gelatin, and sensitivity to novobiocin and kanamycin (20 μg/ml). On the other hand, hydrogen sulfide is not produced, and nitrate is not reduced to nitrite.

From the chemotaxonomic point of view, *Catenuloplanes* species are a highly homogeneous taxon. The cell walls are of type VI according to the classification of Lechevalier and Lechevalier (1970a) and contain glutamate, serine, glycine,

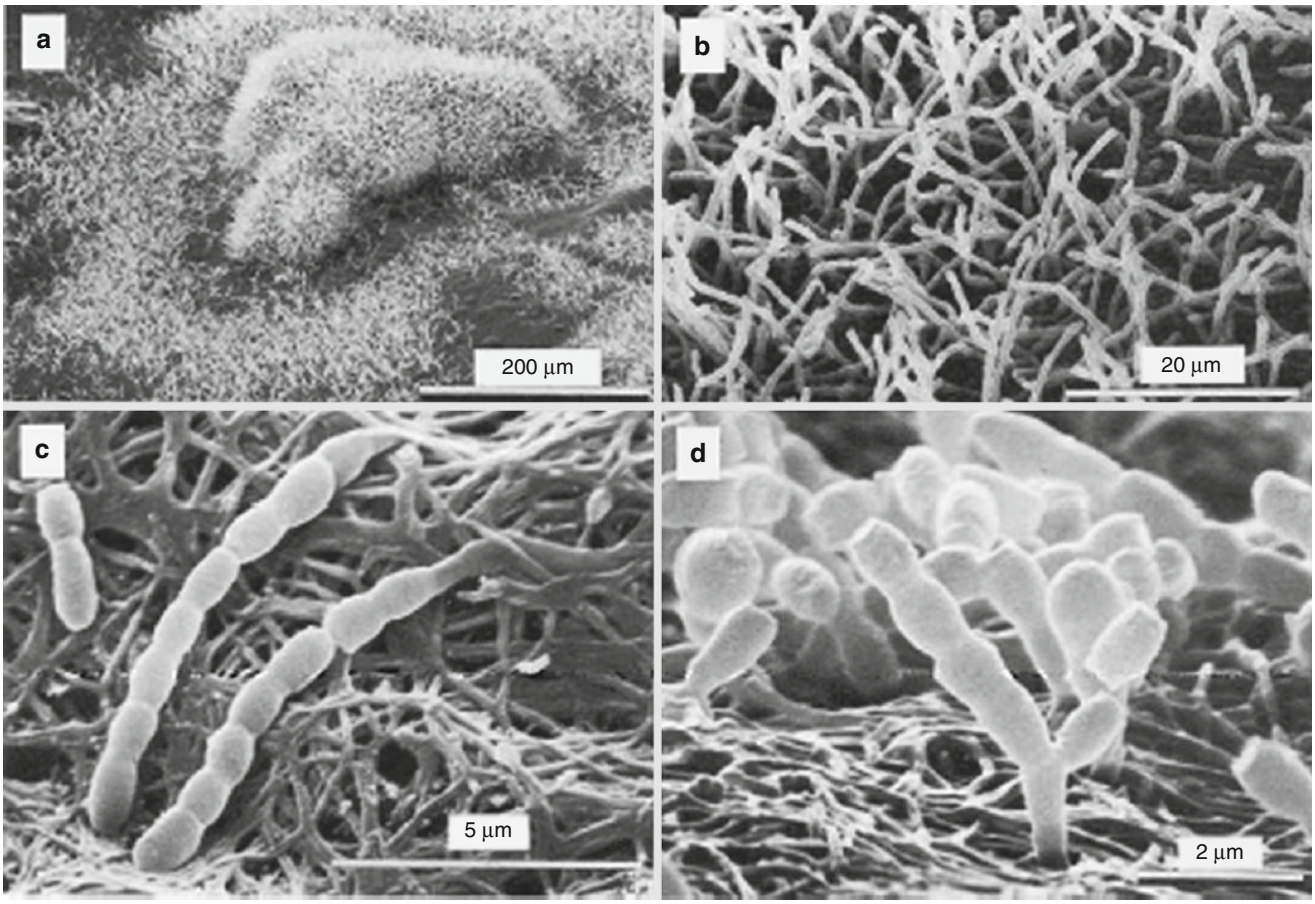

Fig. 28.5
Morphological features of the genus *Catellatospora*. (a) A sporulating colony. (b) Spore-forming hyphae on the surface of agar medium. (c) Short spore chains emerging from substrate hyphae. (d) Branched spore chains on rudimentary sporophores containing cylindrical to ovoid spores. (All SEMs). *SEM* scanning electron microscope

alanine, and lysine in addition to small amounts of ornithine. The configuration of the amino acids present suggests that the peptidoglycan is of type Aα3 (Schleifer and Kandler 1972; Yokota et al. 1993). The whole cell-wall sugars present in all strains are xylose, mannose, ribose, and glucose, while the sugars rhamnose, arabinose, and galactose have only been detected in some species (*Table 28.9*). The cellular fatty acid composition of Catenuloplanetes shows a mixture of saturated and branched fatty acids which include hexadecanoic acid ($C_{16:0}$), octadecanoic acid ($C_{18:0}$), octadecenoic acid ($C_{18:1}$), and 14-methylhexadecanoic acids (anteiso-$C_{17:0}$) as major components. Hexadecenoic acid ($C_{16:1}$), heptadecanoic acid ($C_{17:0}$), and 14-methylpentadecanoic acid (iso-$_{16:0}$) may also be present in smaller amounts (Kudo et al. 1999; Yokota et al. 1993). The polar lipid profile has been determined for the type strains *C. japonicus* N381-16^T, *C. crispus* JCM 9312^T, and *C. niger* N406-14^T, all strains contain phosphatidylcholine as the diagnostic phospholipid (Yokota et al. 1993; Kudo et al. 1999) which corresponds to the type III (Lechevalier et al. 1977). Other phospholipids found in these strains are given in *Table 28.9*. All species contain MK-10 and MK-11 (H_2, H_4, H_6) as part of their menaquinone profile; in addition, the presence of MK-10 with different degrees of hydrogenation (H_2, H_4, H_6 and H_8) varies among the strains.

Dactylosporangium Thiemann et al. 1967

Dac.ty.lo.spo.ran′gi.um. Gr. n. *daktulos*, finger; Gr. n. *spora*, a seed, and in biology a spore; Gr. neut. n. *angeion* (Latin transliteration *angium*), vessel; N.L. neut. n. *Dactylosporangium* an organism with finger-shaped, spore-containing vessels (sporangia).

The genus *Dactylosporangium* proposed by Thiemann et al. (1967) currently harbors 12 validly described species (*Table 28.10*). All *Dactylosporangium* strains stain Gram-positive, are non-acid fast, and produce motile zoospores. The non-septate substrate mycelium (0.5–1.0 µm in diameter) is irregularly branched and does not separate into fragments either in solid or liquid cultures. A true aerial mycelium is not produced; however, some species such as *D. fulvum* produce a rudimentary aerial mycelium on ISP 3 and ISP 4 agar (Shomura et al. 1986).

Table 28.8
Selected phenotyic characters of *Catellatospora* species

	C. citrea	*C. bangladesenshis*	*C. chokoriensis*	*C. coxensis*	*C. methionotrophica*
Colony color	Bright yellow	Light to bright yellow	Light to bright yellow	Light to bright yellow	Light to bright yellow
Spore arrangement	Short chains arise singly or in tufts	Short chains arise singly or in tufts	Short chains	Short chains arise singly or in tufts	Short straight chains
Growth at:					
Temperature	15–30	25–30	15–30	20–30	20–30
pH	6–9	6.8–7.2	6–9	6–9	6.8–7.2
NaCl 1 % (w/v)	−	−	+	−	−
Carbon sources utilized:					
Adonitol	−	+	w	−	−
L-Arabinose	+	+	+		+
Erythritol	−	−	−	−	w
D-Fructose	+	−	−	w	−
D-Galactose	+	+	+	+	+
D-Glucose	+	+	+	+	+
Glycerol	+	+	−	+	w
myo-Inositol	+	−	−	−	w
Lactose	+	+	+	+	+
Maltose	nd	+	+		+
D-Mannitol	+	+	−	−	−
D-Mannose	+	+	+		+
α-D-(+)-Melibiose	+	+	+	+	−
Methyl α-D-glucoside	+	−	−	w	−
D-(+)-Raffinose	−	−	−	−	w
L-Rhamnose	+	+	−	+	+
D-Ribose	+	−	−	+	−
Salicin	+	+	+	−	+
Sucrose	+	+	+	+	+
Trehalose	+	+	+	+	+
D-Xylose	+	+	+	+	+
Whole-cell sugars	Xyl, Ara	Ara, Xyl, Gal, Rha, Rib, Man, Glu	Ara, Xyl, Gal, Rha, Rib, Man, Glu	Ara, Xyl, Gal, Rha, Rib, Man, Glu	Ara, Xyl, Gal, Rib, Man, Glu
Diaminopimelic acid isomer	*meso*- and 3-OH-	*meso*- and 3-OH-	*meso*- and 3-OH-	*meso*- and 3-OH-	*meso*- and 3-OH-
Phospholipid pattern	PE, PG, PI and PIM	PE, DPG, PI and PIM	PE, DPG, PI and PIM	PE, DPG, PI and PIM	PE, DPG, PG, PI and PIM
Major fatty acids	iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{17:0}$, $C_{17:1}\omega8c$	iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{17:1}\omega8c$	iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{17:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{17:0}$	iso-$C_{15:0}$, $C_{17:1}\omega8c$, iso-$C_{17:0}$
Menaquinones	MK-8(H_4, H_6, H_8), MK-9(H_4, H_6, H_8)	MK-9(H_2, H_4, H_6)	MK-9(H_2, H_4, H_6)	MK-9(H_2, H_4, H_6)	MK-9(H_2, H_4, H_6)
DNA G+C mol%	71.5	71	71	71	71

Data from Asano and Kawamoto (1986); Lee et al. (2000); Lee and Hah (2002); Ara and Kudo (2006)
+ positive, − negative, *w* weak, *nd* not determined
3-OH 3-hydroxy-diaminopimelic acid; *m*-DAP *meso*-diaminopimelic acid
Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose
DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside

Table 28.9

Selected phenotypic features of *Catenuloplanes* species

	C. japonicus	*C. atrovinosus*	*C. castaneus*	*C. crispus*	*C. indicus*	*C. nepalensis*	*C. niger*
Colony color	Pale orange to orange	Light pink	Light tan to orange	Yellow to brown	Light brown	Light to bright orange	Light tan to orange
Diffusible pigments	Pale brown, black (ISP 5)	Black (ISP 6, 7)	Pale brown (Bennett)	Yellow (ISP 5)	Yellow (ISP 5)	-	Black (ISP 6, 7)
Tolerance to NaCl 2 % (w/v)	–	+	–	–	+	–	–
Degradation of:							
Adenine	–	–	–	–	–	–	–
Aesculin	+	+	+	+	+	+	+
Casein	+	+	+	+	+	+	+
DNA	–	–	–	–	–	–	–
Elastin	+	w	–	+	+	–	–
Hypoxanthine	+	+	+	+	+	+	+
Testosterone	+	+	–	+	+	–	+
Tyrosine	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+
Xanthine	+	+	+	+	+	+	+
Assimilation of:							
Adonitol	–	–	–	–	–	–	–
L-Arabinose	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+
Dulcitol	–	–	–	–	–	–	–
i-Erythritol	–	–	–	–	–	–	–
D-Fructose	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+
myo-Inositol	+	+	+	+	+	+	+
D-Lactose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
D-Melezitose	–	–	–	–	–	–	–
D-Mannitol	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+
Methyl-α-D-glucoside	+	–	–	–	–	–	+
D-Raffinose	–	–	–	–	–	–	–
L-Rhamnose	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+
D-Ribose	–	–	–	–	–	–	–
Starch	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+
Whole-cell sugars	Rib, Xyl, Man, Rha, Ara, Glu, Gal	Xyl, Glu, Rib, Man, Gal	Rib, Xyl, Man, Glu, Ara, Gal	Xyl, Man, Glu, Rib, Ara, Gal	Xyl, Man, Glu, Rib, Ara, Gal	Xyl, Man, Rib, Ara, Glu, Gal	Xyl, Glu, Rib, Man, Gal

Table 28.9 (continued)

	C. japonicus	*C. atrovinosus*	*C. castaneus*	*C. crispus*	*C. indicus*	*C. nepalensis*	*C. niger*
Menaquinone composition	MK-10; MK-10(H_2, H_4, H_6, H_8); MK-11(H_2, H_4, H_6)	MK-10; MK-10(H_2, H_4, H_6, H_8); MK-11(H_2, H_4, H_6)	MK-10; MK-10(H_2, H_4, H_6); MK-11(H_2, H_4, H_6)	MK-10; MK-10(H_2, H_4, H_6, H_8); MK-11(H_2, H_4, H_6)	MK-10; MK-10(H_2, H_4, H_6); MK-11(H_2, H_4, H_6)	MK-10; MK-10(H_2, H_4); MK-11(H_2, H_4, H_6)	MK-10; MK-10(H_2, H_4, H_6, H_8); MK-11(H_2, H_4, H_6)
Major fatty acids	$C_{16:0}$, $C_{18:1}$, $C_{18:0}$, and anteiso-$C_{17:0}$	anteiso-$C_{17:0}$, $C_{18:1}$, iso-$C_{16:0}$, iso-$C_{17:0}$, $C_{16:0}$	$C_{16:0}$, $C_{18:1}$, $C_{16:1}$, anteiso-$C_{17:0}$	anteiso-$C_{17:0}$, $C_{18:1}$, iso-$C_{16:0}$, $C_{16:0}$, $C_{17:0}$	$C_{18:1}$, anteiso-$C_{17:0}$, $C_{16:0}$, $C_{17:0}$	$C_{18:1}$, $C_{16:0}$, $C_{18:0}$, $C_{17:0}$	$C_{18:1}$, anteiso-$C_{17:0}$, $C_{16:0}$, $C_{18:0}$, iso-$C_{17:0}$
Polar lipids	DPG, PG, PI, PC and PE	nd	nd	nd	nd	nd	DPG, PG, PI, PC and PE
Cell-wall type	VI	VI	VI	VI	VI	VI	VI
DNA G+C mol%	71	72.2–72.7	72–72.4	70	71.3–71.9	71.1	72.2

Data from Yokota et al. (1993), Tamura et al. (1995), Kudo et al. (1999)
+ positive, − negative, *w* weak, *nd* not determined
Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha*, rhamnose, *Rib* ribose, *Xyl* xylose
DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PC* phosphatidylcholine

Two completely different types of spores can be formed in *Dactylosporangium* strains: motile spores inside sporangia and nonmotile spores or globose bodies, which are borne singly on the substrate hyphae. The sporangia sit directly on the substrate hyphae, either singly or in bundles (Fig. 28.6a, b). They are finger-shaped or clafiform, containing only one row of no more than four spores (Fig. 28.6c). The sporangiospores (0.4–1.3 × 0.5–1.8 µm) have an oblong, ellipsoidal, ovoid, or slightly pyriform shape and are sometimes still connected while swimming by means of a polarly inserted tuft of flagella. The nonmotile, globose bodies (1.7–2.8 µm in diameter) exhibit a typical phase-brightness (Fig. 28.6d). They arise terminally on short side-branches of the substrate hyphae (Fig. 28.6e). Amorphous material can be deposited outside the spore wall (Fig. 28.6f). The cytoplasm includes crystalline proteins and structured bodies (Sharples and Williams 1974). The formation of globose bodies has been observed in most species except in *D. matsukiense*, *D. roseum*, *D. thailandense*, and *D. vinaceum*. The development of sporangia depends on the agar media used to cultivate *Dactylosporangiaceae* strains; its production can be promoted by soil agar, calcium malate agar, and inorganic salts-starch agar (Thiemann et al. 1967; Shomura et al. 1986).

Colonies of *Dactylosporangium* are compact, tough, and somewhat leathery. The substrate mycelium color ranges from pale yellow-orange to deep orange or wine color (Table 28.10). Abundant to moderate growth is obtained for most species on the following media: inorganic salts-starch agar, oatmeal agar (ISP 3), tyrosine agar, tryptone-yeast extract agar, and yeast extract-malt extract agar. On the other hand, poor to moderate growth is reported for glycerol-asparagine agar and peptone-yeast extract-iron agar (Kim et al. 2010). Soluble pigments are produced by several species when grown on various of the above media: For instance, on oatmeal agar, *D. vinaceum* produces a wine to purple black pigment, *D. thailandense*, a light brown to brown, and *D. matsuzakiense*, a light brownish pink pigment.

Dactylosporangium strains are strict aerobic and mesophilic. Their optimum growth temperature range is found between 25 °C and 37 °C; however, the species *D. aurantiacum*, *D. luteum*, *D. matsuzakiense*, and *D. thailandense* are reported to grow at 15 °C (Kim et al. 2010). Best growth develops at pH 6.0–7.0; however, *D. ludirum*, *D. matsuzakiense*, *D. roseum*, *D. salmoneum*, and *D. thailandense* are able to grow at pH 4. On the other hand, *D. luteum*, *D. fulvum*, *D. salmoneum*, and *D. thailandense* grow at pH 10.

The importance of using the same basal medium for carbon utilization studies has been pointed out by different authors for members of the family *Micromonosporaceae* (Kawamoto et al. 1983; Kawamoto 1989; Genilloud 2012). Kim et al. (2010) were successful using the same basal medium to study nine of the twelve *Dactylosporangium* species. In the case of the remaining species, *D. darangshiense*, *D. maewongense*, and *D. tropicum*, ISP 9 agar (Shirling and Gottlieb 1966) was used to test the carbon substrates. All species assimilate glucose, and 75–85 % of the strains utilize cellobiose, D-mannitol, raffinose, starch, trehalose, and D-xylose. Several organic acids have also been included in carbon source tests. In this case, the species *D. luridum*, *D. fulvum*, *D. salmoneum*, and *D. thailandense* showed the ability to use most of the substrates tested. Species carbon assimilation profiles for tested substrates are given in Table 28.10.

The ability to degrade various polymers has been studied for many *Dactylosporangium* strains. Most species degrade aesculin and xylan; other degradation activities for a range of substrates are presented in Table 28.10. In addition, most strains are catalase positive, produce hydrogen sulfide, reduce nitrate to nitrite, and tolerate NaCl up to 3 %. Antibiotic resistance (µg/ml) has been recorded for most species for ciprofloxacin (2), clindamycin (8), and lincomycin (8) (Kim et al. 2010). In general, Dactylosporangiae are sensitive to streptomycin (4 µg/ml) except for *D. fulvum*.

Table 28.10

Selected phenotypic characteristics of *Dactylosporangium* species

Characteristic	*D. aurantiacum*	*D. darangshiense*	*D. fulvum*	*D. luridum*	*D. luteum*	*D. maewongense*	*D. matsuzakiense*	*D. roseum*	*D. salmoneum*	*D. thailandense*	*D. tropicum*	*D. vinaceum*
Substrate mycelium color												
Orange	+	–	–	–	–	–	+	–	+	–	–	–
Orange-brown	–	–	–	+	–	–	–	–	–	+	+	–
Orange-yellow	–	+	–	–	+	+	–	–	–	–	–	–
Light yellow	–	–	–	–	–	–	–	–	–	–	–	–
Yellow-brown	–	–	+	–	–	–	–	–	–	–	–	–
Rose	–	–	–	–	–	–	–	+	–	–	–	–
Reddish-wine	–	–	–	–	–	–	–	–	–	–	–	+
Micromorphology												
Globose bodies	+	+	+	+	+	+	–	–	+	–	+	–
Formation of coremia	–	–	+	–	–	–	–	–	–	–	–	–
Degradation of:												
Arbutin	+		+	–	+	nd	+	+	+	+	nd	+
Casein	–	–	–	–	–	nd	–	+	+	–	nd	+
DNA	+	–	+	–	–	nd	–	–	+	–	nd	–
Elastin	+	nd	–	–	–	nd	–	+	–	–	nd	–
Gelatin	+	–	+	–	–	+	–	+	+	+	–	+
RNA	–	nd	–	–	–	nd	–	–	+	–	nd	–
Starch	–	–	–	+	+	+	+	–	+	+	+	+
Tween 40	–	nd	–	–	–	nd	+	+	+	+	nd	+
Tween 60	+	nd	+	+	–	nd	+	+	+	+	nd	+
Tween 80	–	nd	–	–	–	nd	+	–	+	+	nd	–
Assimilation of:												
Adonitol	+	–	+	–	+	nd	–	–	+	–	nd	–
L-Arabinose	–	+	–	–	+	–	–	–	+	–	+	–
D-Arabitol	–	nd	–	–	+	nd	–	–		–	nd	–
Cellobiose	+	+	+	–	+	+	–	+	+	+	–	–

Table 28.10 (continued)

Characteristic	*D. aurantiacum*	*D. darangshiense*	*D. fulvum*	*D. luridum*	*D. luteum*	*D. maewongense*	*D. matsuzakiense*	*D. roseum*	*D. salmoneum*	*D. thailandense*	*D. tropicum*	*D. vinaceum*
Dextrin	+	+	+	–	+	nd	–	–	+	–	nd	–
D-Fructose	–	+	–	–	+	–	–	–	+	+	–	–
D-Galactose	+	–	+	–	+	w	–	–	+	+	+	–
Glycerol	+	+	+	–	+	+	–	–	+	–	+	–
myo-Inositol	–	–	–	–	–	nd	–	–	+	+	nd	–
Inulin	+	nd	–	–	+	nd	–	+	+	+	nd	+
Lactose	–	+	+	–	+	–	–	–	+	+	–	–
Maltose	–	+	–	–	+	nd	–	+	+	+	nd	–
D-Mannitol	–	+	+	–	+	+	–	–	+	+	+	–
D-Mannose	–	+	–	–	+	nd	–	+	+	+	nd	–
D-Melibiose	+	+	–	–	+	–	–	–	+	–	–	–
Methyl-α-D-glucoside	+	–	+	–	+	nd	–	–	+	+	nd	–
Raffinose	+	+	+	–	+	+	–	+	+	+	w	–
L-Rhamnose	+	+	–	–	+	w	–	–	+	+	–	–
Salicin	+	+	+	–	–	+	–	–	+	–	nd	–
Starch	+	nd	+	+	+	nd	+	–	+	+	nd	+
Trehalose	+	+	+	+	+	nd	–	–	+	+	nd	–
D-xylose	+	+	+	+	+	+	–	–	+	+	–	–
Citric acid	–	–	–	–	+	nd	–	–	+	+	nd	–
(+)-L-Lactic acid	–	nd	+	–	+	nd	–	–	–	+	nd	–
Malic acid	–	–	+	–	+	nd	–	+	+	+	nd	–
Propionic acid	–	nd	+	–	+	nd	–	+	+	–	nd	–
Pyruvic acid	+	nd	+	–	+	nd	+	+	+	+	nd	–
(+)-L-Tartaric acid	–	–	+	–	+	nd	–	–	+	+	nd	–

Biochemical tests												
Allantoin hydrolysis	−	nd	−	−	−	nd	−	+	−	+	nd	−
Milk peptonization	−	nd	−	−	−	+	−	−	−	−	+	+
Nitrate reduction	+	−	−	+	+	+	−	+	+	−	+	+
Production of H_2S	+	−	+	+	−	−	+	+	+	+	nd	−
Urease production	+	−	+	−	−	nd	−	−	−	+	nd	+
Chemotaxonomic markers												
Whole-cell sugars	Xyl, Ara	Man, Xyl, Rha, Gal[a], Ara[a], Rib[a]	Xyl, Ara	Ara, Gal, Glu, Man, Xyl	Ara, Gal, Glu, Man, Xyl	Glc, Xyl, Rha, Rib, Ara	Xyl, Glc, Man, Gal, Glu, Ara	Xyl, Ara	Ara, Gal, Glu, Man, Xyl	Xyl, Ara	Gal, Glc, Man, Rha, Rib, Xyl	Xyl, Ara
Diaminopimelic acid isomer	nd	*meso*	3-OH, *meso*	3-OH, *meso*	3-OH, *meso*	3-OH, *meso*	3-OH	3-OH, *meso*	3-OH, *meso*	nd	*meso*-	3-OH
Phospholipid pattern	DPG, PE, PG, PI, PIM, PL	DPG, PE, PI	DPG, PE, PG, PI	DPG, PE, PG, PI, PL	DPG, PE, PG, PI, PL	DPG, PG, lysyl-PG, PE	DPG, PE, PG, PI, PL	DPG, PE, PG, PI	DPG, PE, PG, PI, PL	DPG, PE, PG, PI, PIM, PL	DPG, PG, PI, PE	DPG, PE, PG, PI, PL
Major fatty acids	iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{14:0}$	iso-$C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$, iso- $C_{17:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{18:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$,	iso-$C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{14:0}$	iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{16:1}$G, anteiso-$C_{17:0}$	iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{18:1}\omega c$, 10-Methyl $C_{17:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso- $C_{17:0}$	iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{16:1}$G, anteiso-$C_{17:0}$	iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$	iso-$C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$
Menaquinones	MK-9(H_8), MK-9(H_6), MK-9(H_4)	MK-9(H_8), MK-9(H_6)	MK-9(H_8), MK-9(H_6)	MK-9(H_8), MK-9(H_6), MK-9(H_4)	MK-9(H_8), MK-9(H_6), MK-9(H_4), MK-9(H_2)	MK-9(H_8), MK-9(H_6)	MK-9(H_8), MK-9(H_6)	MK-9(H_8), MK-9(H_6)	MK-9(H_8), MK-9(H_6), MK-9(H_4)	MK-9(H_8), MK-9(H_6)	MK-9(H_8), MK-9(H_6)	MK-9(H_8), MK-9(H_6)
DNA G+C mol%	73	69.8	72.2	70	74	73.2	68.5	69.8	73	71	72	71.7

Data from Thiemann et al. (1967), Shomura et al. (1980, 1983, 1985), Shomura et al. (1986), Chiaraphongphon et al. (2010), Kim et al. (2010), Seo and Lee (2010), Thawai et al. (2011)

+ positive, − negative, *w* weak, *nd* not determined

3-OH, 3-hydroxy-diaminopimelic acid; *m*-DAP, *meso*-diaminopimelic acid

Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose

DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PL* polar lipid

[a]Trace amounts

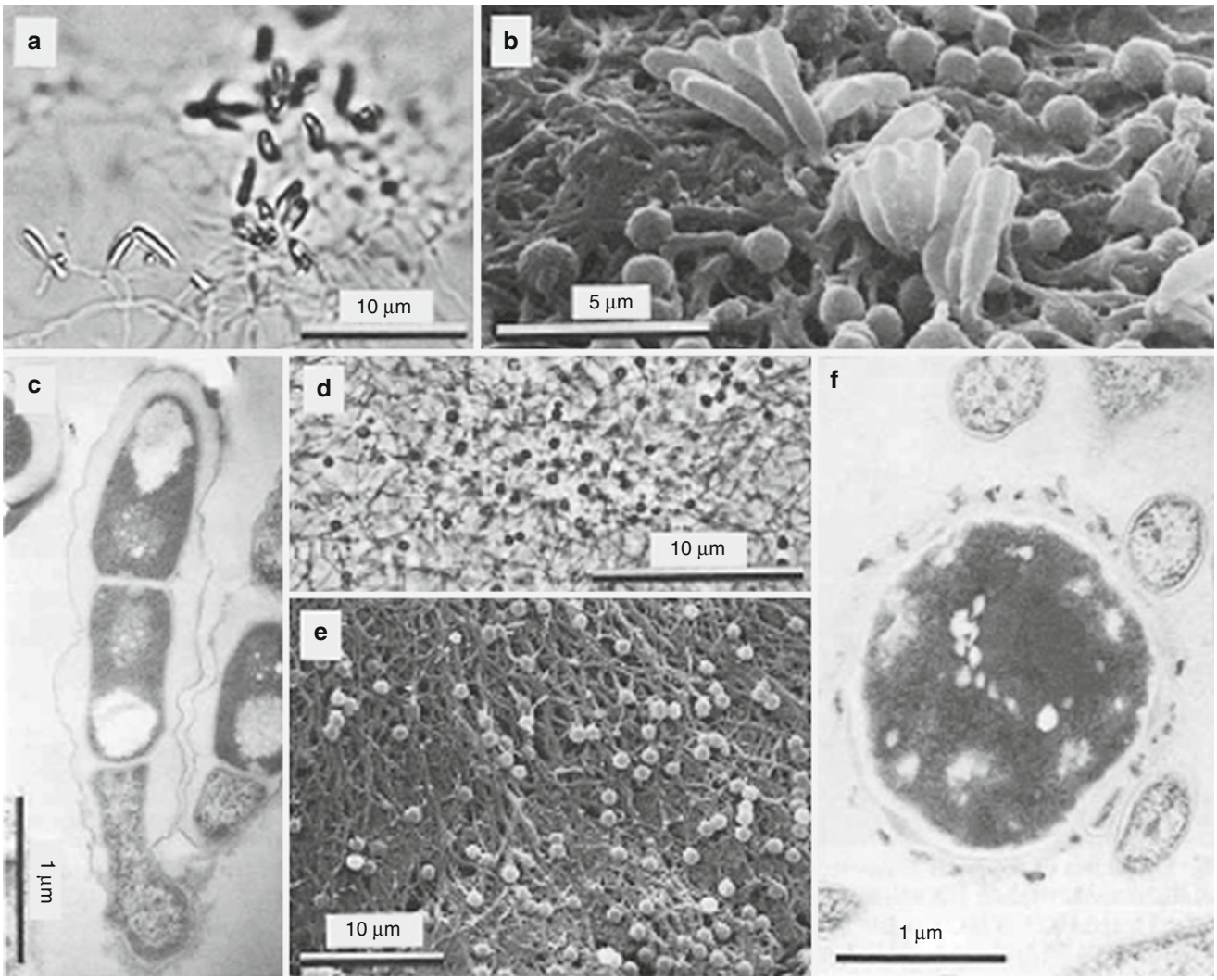

Fig. 28.6
Morphological features of the genus *Dactylosporangium*. (a) Finger-like sporangia on agar medium (LM). (b) Bundles of sporangia and globose spores (SEM). (c) Section of a sporangium with two spores containing reserve material; sporangial envelope is thin and wavy (TEM). (d) Refractile globose spores dispersed in substrate mycelium (PC). (e) Globose spores on the surface of substrate mycelium (SEM). (f) Section of a globose spore with paracrystalline inclusion bodies and perispherical deposits (TEM) (c–f from Vobis 1987; with permission). *LM* light microscope, *PC* phase contrast, *SEM* scanning electron microscope, *TEM* transmission electron microscope

Several chemotaxonomic markers have been determined for all *Dactylosporangium* type strains representing the 12 species. They contain the diaminopimelic acid isomers 3-hydroxy- (3-OH) and *meso*- in their cell-wall peptidoglycan except for *D. darangshiense*, *D. tropicum*, and *D. matsuzakiense* which contain *meso*- and 3-OH respectively. All species also contain the diagnostic whole-cell sugars rhamnose and xylose apart from *D. tropicum* which only contain xylose. Other sugars present may include galactose, glucose, mannose, rhamnose, and ribose (*Table 28.10*). Thus, the chemical composition of the cell walls conforms to chemotype II and sugar pattern D (Lechevalier and Lechevalier 1970a). The fatty acid composition of *Dactylosporangium* strains includes a complex mixture of saturated, unsaturated, branched, and methyl-branched fatty acids (Kim et al. 2010; Seo and Lee 2010). Major components (≥5 %) are iso-$C_{16:0}$, iso-$C_{15:0}$, and anteiso-$C_{17:0}$. The composition of the isoprenoid quinones is characterized by the possession of menaquinones with nine isoprene units (MK-9), whereas isoprenologues with ten units (MK-10) are absent. MK-9(H_2) and MK-9(H_4) are present in minor amounts, and MK-9(H_6) and MK-9 occupy predominant positions (Collins et al. 1984; Goodfellow et al. 1990; Ruan et al. 1998; Kim et al. 2010). The presence of MK-9(H_4), MK-9(H_6) and MK-9(H_8) indicates that *Dactylosporangium* belongs to the menaquinone type 4b of the classification scheme of Kroppenstedt (1985).

Longispora Matsumoto et al. 2003 Emend. Shiratori-Takano et al. 2011

Lon.gi.spo′ra. L. adj. *longus*, long; Gr. fem. n. *spora*, a seed and in biology a spore; N.L. fem. n. *Longispora*, long spore.

The genus *Longispora* with the species *Longispora albida* and *Longispora fulva* was proposed by Matsumoto et al. (2003). The two strains included in this taxon stain Gram-positive, are non-acid fast, aerobic, and nonmotile. Good growth is observed on yeast extract-malt extract agar, oatmeal agar, and nutrient agar; however, *L. albida* shows better growth when agar is substituted for gellan gum (Matsumoto et al. 2003). The colony color ranges from light yellow to gray-greenish olive and have a leathery texture. A branched, non-fragmenting substrate mycelium is produced. The aerial hyphae carry long spore chains (>20 spores) that arise from the tip of short sporophores that branch from the substrate hyphae. The spores are cylindrical (0.4–0.5 × 1.0–1.4 μm), nonmotile, and have a smooth surface.

Longispora strains are strict aerobic and mesophilic, the two existing types strains do not grow above 40 °C; their tolerance to NaCl is 1.5 % (w/v) and is variable with respect to nitrate reduction activities. The strains showed a low activity profile when tested for their potential to degrade several polymers including plant components such as cellulose, starch, and xylan (Table 28.11). Similarly, the ability of the two species to assimilate various substrates as carbon sources was low (Matsumoto et al. 2003; Shiratori-Takano et al. 2011). Only glucose was used by both type strains, while *myo*-inositol, sucrose, and xylose are assimilated by *L. fulva* (Table 28.11).

Chemotaxonomic studies have been included in the characterization of *L. albida* and *L. fluva*. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid, glycine, and alanine and conforms to type A1γ (Schleifer and Kandler 1972). The whole-cell sugars present in both strains are galactose and xylose, while arabinose, rhamnose, galactose, mannose, and ribose are variable. The main menaquinones are MK-10(H_4) and MK-10(H_6), but MK-10(H_8) is also found in both strains in minor amounts. The diagnostic phospholipid of the genus is phosphatidylethanolamine. The predominant cellular fatty acid components are heptadecenoic ($C_{17:1}$), 14-methylpentadecanoic (i-$C_{16:0}$), and octadecenoic ($C_{18:1}$) acids. A summary of their chemotaxonomic properties is given in Table 28.11.

Table 28.11
Selected phenotypic characteristics of *Longispora* species

Characteristic	*L. albida*	*L. fulva*
Colony color on ISP 2	Yellowish-white	Light grayish-olive
Spore chains	>20 spores	>20 spores
Growth on:		
ISP 2	Good	Good
ISP 4	–	Good
ISP 6	Moderate	–
Maximum NaCl tolerance	1.5 %	1.2 %
Nitrate reduction	+	–
Milk coagulation and peptonization	+	nd
Temperature growth range	12–37 °C	10–33 °C
Degradation of:		
Casein	nd	+
Cellulose	–	–
Chitin	nd	–
Gelatin	nd	–
Starch	–	+
Xylan	nd	–
Utilization of:		
L-Arabinose	–	–
Fructose	–	–
Glucose	+	+
myo-Inositol	–	+
D-Mannitol	–	–
Melibiose	–	–
Raffinose	–	–
Rhamnose	–	–
Sucrose	–	+
Xylose	–	+
Diaminopimelic acid isomer	*m-DAP*	*m*-DAP
Whole-cell sugars	Ara, Gal, Xyl	Xyl, Rha, Gal, Man[a], Rib[a]
Phospholipid pattern	PE	DPG, PE, OH-PE, PI, unknown lipids and glycolipids
Major fatty acids	$C_{17:1}$, i-$C_{16:0}$, $C_{18:1}$	i-$C_{16:0}$, 10-methyl-$C_{17:0}$, iso-$C_{17:1}\omega 9c$
Menaquinones	MK-10(H_4), MK-10(H_6), MK-10(H_8)	MK-10(H_4), MK-10(H_6), MK-10(H_8)
DNA G+C mol%	70.7	70

Data from: Matsumoto et al. (2003); Shiratori-Takano et al. (2011)
+ positive, – negative, *nd* not determined
m-DAP *meso*-diaminopimelic acid
Ara arabinose, *Gal* galactose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose
DPG diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *OH-PE*, hydroxyphosphatidylethanolamine
[a]Trace amounts

Luedemannella Ara and Kudo 2007b

Lue.de.mann.ella. N.L. fem. dim. n. *Luedemannella*, referring to G. M. Luedemann, a Russian actinomycetologist who contributed to the taxonomy of the family *Micromonosporaceae*.

The genus *Luedemannella* with two species, *Luedemanella helvata* and *L. flava* groups microorganisms which stain Gram-positive, are non-acid fast and aerobic. Good growth is observed on several complex media including Bennett's and ISP 2 agars, on these media, the color of the colonies ranges from

light yellow to orange (Ara and Kudo 2007b). A non-fragmenting, branched substrate mycelium is produced by *Luedemannella* strains, but the presence of aerial mycelium has not been observed. Nonmotile spores (0.2–0.4 µm) are produced in spherical-shaped sporangia (3.0–5.0 µm) which hold several spores. The shape of the spores is spherical to oval with a smooth surface (Ara and Kudo 2007b).

Luedemannella strains are strictly aerobic and mesophilic microorganisms, their temperature range for growth is 20–30 °C; however *L. helvata* is capable of growing at 37 °C; the pH range for growth is 5–9. In addition, *L. helvata* can grow in the presence of 2 % NaCl. Ara and Kudo (2007b) tested the ability of *L. helvata* and *L. flava* to use a large variety of carbohydrates as carbon sources. In general, *L. flava* was capable of utilizing more substrates although for some of them, a weak reaction was recorded (see *Table 28.12*). Both species utilize L-arabinose, glucose, lactose, maltose, sucrose, trehalose, and xylose.

The chemotaxonomic characteristics of the genus *Luedemannella* conform to those found within the family *Micromonosporaceae* (Ara and Kudo 2007b). The cell-wall peptidoglycan contains *meso*-diaminopimelic acid and glucose, mannose, xylose, galactose, mannose, rhamnose, ribose, and arabinose as the whole-cell sugars, indicating a whole-cell sugar pattern D (Lechevalier and Lechevalier 1970a). The menaquinone composition includes MK-9(H_6), and MK-9(H_4) as predominant menaquinones and small amounts of MK-9(H_2) and MK-9(H_8). Branched iso- and anteiso-, saturated, unsaturated, and branched fatty acids are the main components (fatty acid type 2d, Kroppenstedt 1985). The polar lipid profile comprises phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides. A summary of these characteristics is found in *Table 28.12*.

Table 28.12
Selected phenotypic characteristics of *Luedemannella* species

Characteristic	*L. helvata*	*L. flava*
Growth on:		
Bennett's	Good	Good
Yeast extract-malt extract (ISP 2)	Good	Good
Oatmeal (ISP 3)	Moderate	Good
Hickey-Tresner	Moderate	Good
Yeast extract-starch	Moderate	Good
Colony color	Light yellow to light orange	Cream yellow to wheat yellow
Motile spores	–	–
Growth on NaCl (2 % w/v)	+	–
Temperature growth range (°C)	20–37	20–30
Utilization of:		
Adonitol	–	–
L-Arabinose	+	+
Erythritol	w	–
D-Fructose	–	w
D-Galactose	w	+
D-Glucose	+	+
α-Methyl-D-glucoside	–	w
Glycerol	–	w
myo-Inositol	–	w
Lactose	+	+
Maltose	+	+
D-Mannitol	–	w
D-Mannose	–	+
α-D(+)-Melibiose	–	+
D-Raffinose	+	–
D-Ribose	–	w
L-Rhamnose	–	+
Salicin	w	+
Sucrose	+	+
Trehalose	+	+
D-Xylose	+	+
Diaminopimelic acid isomer	*meso*	*meso*
Whole-cell sugars	Glu, Xyl, Gal, Man, Rha, Rib, Ara	Glu, Xyl,Man, Gal, Rib, Ara, Rha
Phospholipid pattern	DPG, PE, PG, PI, PIMs	DPG, PE, PG, PI, PIMs
Major fatty acids	a-$C_{17:0}$, a-$C_{15:0}$, i-$C_{16:0}$, i-$C_{15:0}$	a-$C_{17:0}$, a-$C_{15:0}$, i-$C_{15:0}$, $C_{17:0}$, i-$C_{16:0}$
Menaquinones	MK-9(H_6), MK-9(H_4), MK-9(H_2), MK-9(H_8)	MK-9(H_6), MK-9(H_8)
DNA G+C mol%	71	71

Data from Ara and Kudo (2007b)

+ positive, – negative, *w* weak, *nd* not determined

m-DAP *meso*-diaminopimelic acid

Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose

DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PIMs* phosphatidylinositol mannosides

Pilimelia Kane 1966 344[AL]

Pi.li.mel'i.a. L. n. *pilus*, a hair; Gr. fem. n. *melia*, *Melia*, a nymph loved by the river god *Inachus*; N.L. fem. n. *Pilimelia*, an aquatic organism growing on hair substrate.

The current species in the genus *Pilimelia* are *P. terevasa*, *P. anulata*, and *P. columellifera* with two subspecies. *Pilimelia* strains form small, compact colonies, which are about 5 mm in diameter after 4 weeks of incubation. Growth is supported by keratinic substances like hair (*Fig. 28.7a*) and cattle horn meal (Vobis 1984). Growth is successful only on complex media that include 50 % diluted skim milk agar (Gordon and Smith 1955), casamino acids-peptone-Czapek agar and nutrient-sugar agar (Henssen and Schäfer 1971), peptone-yeast extract-iron agar (Shirling and Gottlieb 1966), oatmeal-yeast extract agar (Vobis et al. 1986), and yeast extract-starch agar (Emerson 1958).

Pilimelia strains produce branched, septate, substrate hyphae (0.2–0.8 µm in diameter), but aerial mycelium is not developed. The sporangia are developed directly on the surface

of agar medium or on natural substrates (Fig. 28.7b). They are globose, ovoid, pyriform, campanulate, or cylindrical and approximately 10–15 µm in size. In some strains, each sporangium contains up to a thousand spores (Fig. 28.7f), which are rod-like to reniform. The spores can vary from 0.3 to 1.5 µm in length, and are equipped with a laterally inserted tuft of flagella. Nonmotile spores are also produced. Nutrient-poor media with addition of natural keratinic substances promote the production of sporangia. If sporangia are dipped into water, numerous flagellated spores are released, leaving behind the sporangial envelope (Vobis 1984). The wind may act as a vehicle for spore transport over long distances. On reaching natural keratinic substances, e.g., hair of mammals, they colonize the new substrate to produce mycelium and sporangia within 14 d (Vobis 1989a). The life cycle of *Pilimelia* can be considered as "aeroaquatic" (Vobis 1987).

The three *Pilimelia* species show differential morphological and colonial characters. *P. terevasa* has spherical to campanulate sporangia with parallel rows of abundantly branches spore chains. The colonies have a soft consistency and are yellow to yellow-gray. *Pilimelia anulata* has cylindrical sporangia (Fig. 28.7e), the top segment of the sporangiophore is expanded to form a small ring-like structure (Fig. 28.7d). The mycelium has a yellowish color and is soft and pasty. In contrast, the colonies of *P. columellifera* are very solid and spherical to pyriform sporangia are produced, with the spore chains inside arranged in swirls (Vobis 1984). The sporangiophores are unseptate and reach into the lumen of the sporangium, where they are visible as small columns. The substrate mycelium of *P. columellifera* is either golden-yellow to orange or colorless to pale brownish in the subspecies *pallida* (Vobis et al. 1986).

All species of *Pilimelia* are aerobic, mesophilic, and stain Gram-positive. Growth at 10–35 °C has been reported, although growth was also observed at 42 °C (Schäfer 1973). The optimum pH for growth is 7.0; however, strains can also grow between pH 5.0 and 7.8. The physiology of *Pilimelia* strains is not well understood, carbon source substrates have not been clearly identified as strains studied neither utilized the various carbon sources tested (Vobis 1986), nor individual or combinations of purified amino acids (Kane Hanton 1974). Four *Pilimelia* strains isolated from rhizosphere of *Discaria trinervis* were not able to degrade plant polymers such as starch, cellulose, hemicellulose, pectin, or lignin when tested in the laboratory (Solans and Vobis 2003) (Table 28.13). Pilimeliae are able to colonize keratinic substrates like hair of mammalia or snake skin (Karling 1954; Gaertner 1955; Tribe and Abu El-Souod 1979). Although they can aggressively attack the scleroproteins of animals, they are not known as dermatophytes.

The diaminoacid *meso*-diaminopimelic acid is present in the cell-wall peptidoglycan of *Pilimelia* strains, where glycine is also found. Arabinose and xylose are the whole-cell sugars present in all strains, conforming to the sugar pattern D as many other genera in the family *Micromonosporaceae*. Phosphatidylethanolamine and phosphatidylcholine are the characteristic polar lipids in the genus, corresponding to the type II (Lechevalier et al. 1977). The main menaquinones found in *Pilimelia* strains are MK-9(H_2) and MK-9(H_4). As for the fatty acid profile, these microorganisms contain high amounts of i-$C_{15:0}$, i-$C_{15:1}$, and $C_{17:1}$. A summary of the chemotaxonomic features is given in Table 28.13.

Planosporangium Wiese et al. 2008

Pla.no.spo.ran′gi.um. Gr. n. *planes*, a wanderer; N.L. neut. n. *sporangium*, sporangium, spore case; N.L. neut. n. *Planosporangium*, wandering sporangium, referring to the production of sporangia with motile spores.

The genus *Planosporangium* with the species *P. flavigriseum* and *P. mesophilum* accommodates aerobic actinobacteria with branched hyphae, that stain Gram-positive, and have motile or nonmotile spores. *Planosporangium* strains grow well on ISP 2 and ISP 3 agars; however, only the type strain of *P. flavigriseum* shows good growth on potato dextrose-agar (PDA), but it does not grow on nutrient agar (Wiese et al. 2008; Cao et al. 2011). The texture of the colonies is tough and wrinkled. The substrate mycelium (0.6–0.7 µm) is extensively branched with pale gray, orange-yellow, orange or pale gray-olive color, depending on media and culture conditions but diffusible pigments are not formed. The surface of spores is smooth. White aerial mycelium is formed after three (*P. mesophilum*) and nine (*P. flavigrseum*) weeks of growth. Globose spores (0.5–1.5 µm) arising from the tips of short sporophores have been observed in both *Planosporangium* species. In addition, *P. flavigresum* produces motile spores inside finger-like sporangia. Each sporangium contains a single row of three or more straight or slightly curved rod-shaped and motile spores with a single flagellum (Wiese et al. 2008).

As indicated by the species name, *P. mesophilum* grows within the range of 28–30 °C. *P. flavogriseum* grows at 28 °C; however, growth studies at other temperatures have not been carried out. A low enzymatic activity profile was reported for both type strains when tested against several substrates including starch, gelatin, urea, and others (see Table 28.14). Common carbon sources utilized by both species include glucose, maltose, mannose, and D-xylose. A variable result was obtained with other sugar substrates tested; in general, *P. flavogriseum* shows a wider assimilation profile than *P. mesophilum* (Table 28.14).

The cell-wall peptidoglycan of *Planosporangium* strains contains *meso*-diaminopimelic acid. On the other hand, the composition of the cell-wall sugars is quite different in both species. Arabinose and xylose are reported for *P. flavigriseum*, while mannose, ribose, glucose, and galactose were detected in *P. mesophilum*. Phosphatidylethanolamine is the major phospholipid found in both type strains, and the menaquinone MK-9(H_4) is also a common component. Differences in the

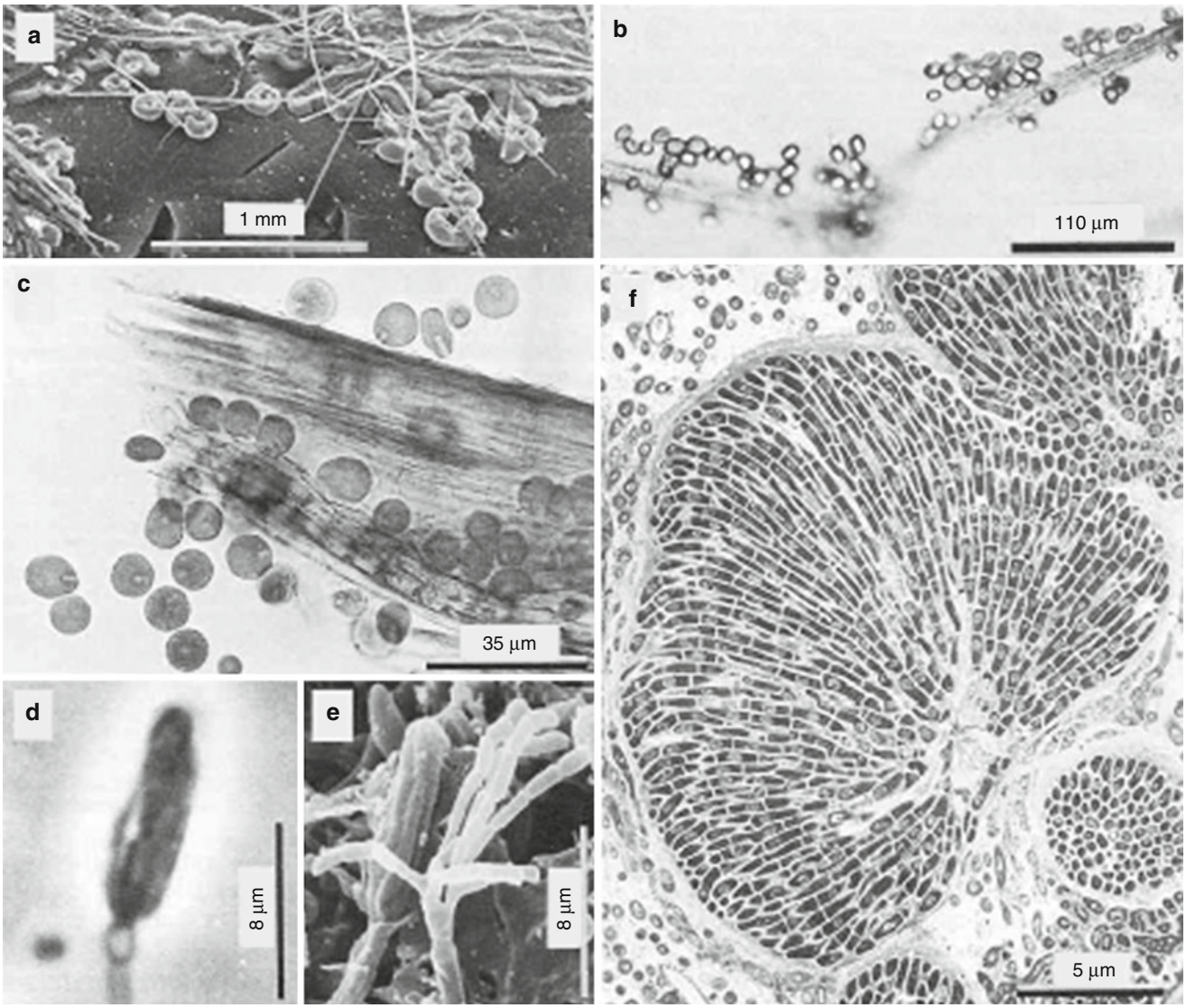

Fig. 28.7

Morphological features of the genus *Pilimelia*. (a) Compact, small colonies on agar medium; hairs added as natural substrate (SEM). (b) Bundles of sporangia formed on hair (LM). (c) Globose to pyriform sporangia with internal columella; structure of the colonized part of the hair was destroyed (LM). (d) Cylindrical sporangium with an annulus at the base (PC). (e) Penicillate conidiophore with bacilliform conidia; the sporangium behind it has parallel-arranged sporogeneous hyphae (SEM). (f) Section of a campanulate sporangium with branched spore chains (TEM) (c, d from Vobis et al. 1986; e from Vobis 1987; F from Vobis 1984; with permission). *LM* light microscope, *PC* phase contrast, *SEM* scanning electron microscope, *TEM* transmission electron microscope

overall menaquinone composition are given in *Table 28.14*. The major fatty acids are anteiso- and iso-branched such as i-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, and $C_{17:1}\omega 8c$.

Plantactinospora Qin et al. 2009

Plan.tac.ti.no.spo′ra. L. n. *planta*, a plant; Gr. n. *actis actinos*, a ray; Gr. fem. n. *spora*, a seed, and in biology a spore; N.L. fem. n. *Plantactinospora*, pertaining to a spore-forming actinomycete isolated from plant tissues.

The genus *Plantactinospora* harbors three species, *P. mayteni*, the type species (Qin et al. 2009), *P. endophytica*, and *P. siamensis* which was previously classified as *Actinaurispora siamensis* (Zhu et al. 2012). *Plantactinospora* strains stain Gram-positive and are non-acid fast. Good growth of all strains is obtained on ISP 2 agar; in this medium, the color of the colonies is orange-yellow to red-brown and a light yellow pigment is produced by *P. siamensis*. White aerial mycelium is sparsely produced. Other media tested for growth can be found in the description of the different species (Qin et al. 2009; Thawai et al. 2010; Zhu et al. 2012). The growth of the colonies is raised and folded.

Table 28.13
Selected phenotypic characteristics of the genus *Pilimelia*

Characteristic	*P. terevasa*	*P. anulata*	*P. columellifera* subsp. *columellifera*	*P. columellifera* subsp. *pallida*
Colony color	Lemon-yellow, yellow-gray	Lemon-yellow, yellow-gray	Golden-yellow, orange	Colorless to pale brown
Sporangia shape	Spherical, flabelliform, campanulate	Cylindrical	Spherical, pyriform	Spherical, pyriform
Spore arrangement	Parallel rows	Parallel rows	Swirl-like	Swirl-like
Sporangiophore	Septate	Septate, annulate	Columnella	Columnella
Temperature growth range (°C)	10–35	15–35	15–35	10–30
Nitrate reduction	–	–	+	–
Casein peptonization	+	+	+	+
Gelatin liquefaction	–	–	+	+
Tyrosine hydrolysis	+	+	–	–
Starch hydrolysis	–	–	–	–
Diaminopimelic acid isomer	*meso*	*meso*	*meso*	*meso*
Whole-cell sugars	Xyl, Ara	Xyl, Ara	Xyl, Ara	Xyl, Ara
Phospholipid pattern	PE, PC	PE, PC	PE, PC	PE, PC
Major fatty acids	i-$C_{15:0}$, i-$C_{15:1}$, $C_{17:1}$	i-$C_{15:0}$, i-$C_{15:1}$, $C_{17:1}$	i-$C_{15:0}$, i-$C_{15:1}$, $C_{17:1}$	i-$C_{15:0}$, i-$C_{15:1}$, $C_{17:1}$
Menaquinones	MK-9(H_2), MK-9(H_4)	MK-9(H_2), MK-9(H_4)	MK-9(H_2), MK-9(H_4)	MK-9(H_2), MK-9(H_4)
DNA G+C mol%	nd	nd	nd	nd

Data from Vobis et al. (1986); Vobis (2006); Vobis et al. (2012)
+ positive, – negative, *w* weak, *nd* not determined
m-DAP *meso*-diaminopimelic acid
Ara arabinose, *Xyl* xylose
PE phosphatidylethanolamine, *PC* phosphatidylcholine

The cells form extensively branched substrate mycelia (0.16–0.23 µm in diameter), which carry smooth or rough surfaced, nonmotile spores (0.63–1.10 µm); these are borne singly or in clusters.

Plantactinospora strains are strict aerobic, and mesophilic. The temperature growth range is 4–45 °C with an optimum of 20–37 °C. The pH range for growth of *P. endophytica* is 5–10, while that of the other two species is more restricted and it ranges from 5 to 8. Tolerance to NaCl is found within the range 2–3 %. The production of catalase and oxidase, nitrate reduction, and production of hydrogen sulfide is variable. Differences between the three species are also found in their ability to degrade several substrates including Tween 20, Tween 40, and Tween 80 (Table 28.15). Cellobiose, D-fructose, glucose, L-rhamnose, and D-xylose are used by all strains as a source of carbon and energy. Additionally, *P. mayteni* and *P. siamensis* utilize D-galactose, inositol, lactose, and mannitol, while *P. endophytica* does not use these substrates as carbon and energy sources. Other physiological results can be found in Table 28.15.

The chemotaxonomic profiles of all three *Plantactinospora* type strains have been determined. In general, their profiles are highly homogeneous, but small differences are found at the level of polar lipids and menaquinone composition. All strains contain *meso*-DAP in their cell-wall peptidoglycan and the whole-cell sugar pattern is D with arabinose, galactose, glucose, mannose, xylose, and rhamnose. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$, and anteiso-$C_{15:0}$. All strains contain a phospholipid type II pattern with phosphatidylethanolamine as the diagnostic polar lipid; however, several unknown lipids are reported for *P. mayteni* and *P. endophytica*. The menaquinone composition includes a complex mixture of MK-9 and MK-10 menaquinones with different degrees of hydrogenation. The composition of each species is given in Table 28.15.

Rugosimonospora Monciardini et al. 2009

Ru.go′si.mo.no.spo′ra. L. adj. *rugosus*, rugose, wrinkled; Gr. adj. *monos*, single; Gr. fem. n. *spora*, a seed and, in bacteriology, a spore; N.L. fem. n. *Rugosimonospora*, a bacterium forming single, rugose spores.

Table 28.14
Selected phenotypic characteristics of *Planosporangium* species

Characteristic	*P. flavigriseum*	*P. mesophilum*
Growth on:		
ISP 2	Good	Good
ISP 3	Good	Good
Nutrient agar	–	Poor
Potato dextrose-agar (PDA)	Good	Poor
Production of aerial mycelium	GYM agar	Several media except PDA
Colony color on ISP 2	Orange-yellow to light yellow	Light orange to light salmon-pink
Spore motility	+	–
NaCl tolerance (%, w/v)	3	2
Temperature growth range (°C)	nd	28–30
Gelatin liquefaction	–	–
Milk coagulation and peptonization	–	–
Starch hydrolysis	–	–
Nitrate reduction	–	–
Production of H_2H	–	–
Urea hydrolysis	nd	–
Utilization of:		
Arabinose	+	–
Cellobiose	+	–
D-Fructose	–	+
Galactitol	+	–
D-Galactose	+	w
Glucose	+	w
Inositol	–	w
Lactose	–	+
Maltose	+	+
Mannitol	+	–
Mannose	+	+
Raffinose	+	w
L-Rhamnose	+	–
D-Ribose	+	+
Sorbitol	+	–
Sucrose	+	–
D-xylose	+	+
Sodium acetate	+	–
DL-Methionine	–	w
Diaminopimelic acid isomer	*meso*	*meso*
Whole-cell sugars	Ara, Xyl	Man, Rib, Glu, Gal
Phospholipid pattern	DPG, PE, PIM	PE
Major fatty acids	i-$C_{15:0}$, i-$C_{16:0}$	ai-$C_{17:0}$, i-$C_{16:0}$, $C_{17:1}\omega 8c$
Menaquinones	MK-9(H_4), MK-10(H_4)	MK-9(H_4), MK-9(H_6), MK-9(H_8)
DNA G+C mol%	71.4	71.6

Data from Wiese et al. (2008), Cao et al. (2011)
+ positive, – negative, *w* weak, *nd* not determined
m-DAP *meso*-diaminopimelic acid
Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rib* ribose, *Xyl* xylose
DPG diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PIM* phosphatidylinositol mannoside

The genus *Rugosimonospora* is represented by the species *R. acidophila* and *R. africana* (Monciardini et al. 2009), and the two type strains were isolated from soil samples collected in Italy and Cameroon, respectively. The microorganisms stain Gram-positive, are non-acid fast and aerobic. Good to abundant growth is observed on several ISP media especially on ISP 2 and ISP 7 agars. The color of the colonies on the above media ranges from colorless to yellow to orange, and green sporulation spots may be observed. Pink to red-brown soluble pigments are produced on tyrosine-containing media. Extensive, non-fragmenting substrate mycelium is formed and single spores arise from the hyphae. The spores are spherical (0.7–0.8 μm in diameter), nonmotile, and have a rugose surface (Table 28.16).

Rugosimonospora strains have and oxidative metabolism. They are mesophilic bacteria that grow between 15 °C and 37 °C with an optimum of 22–28 °C. Best growth is obtained at slightly acidic pH values of 5–6. *Rugosimonospora africana* is tolerant to 1 % NaCl but not *R. acidophila*. Both species hydrolyze casein, gelatin, starch, and tyrosine but do not reduce nitrate. Glycerol, mannose, and xylose are readily used as carbon sources by both *Rugosimonospora* species, while L-arabinose, fructose, inositol, raffinose, and rhamnose are weakly used (Table 28.16) (Monciardini et al. 2009).

The following chemotaxonomic markers are found in both *Rugosimonospora* species. The diaminopimelic acid isomer present in the peptidoglycan is 3-hydroxy-diaminopimelic acid. The whole-cell sugars are galactose, arabinose, and xylose. Differences in fatty acid, menaquinone, and polar lipid composition were reported by Monciardini et al. (2009) as follows: Polar lipids detected in *R. acidiphila* were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, and an unknown phospholipid, while *R. africana* contains several unknown phospholipids in addition to the above-mentioned phospholipids. The common menaquinones found in

Table 28.15
Selected phenotypic characteristics of *Plantactinospora* species

Characteristic	*P. mayteni*	*P. endophytica*	*P. siamensis*
Growth on:			
ISP 2	Good	Good	Good
ISP 3	Good	Good	nd
Nutrient Agar	Poor	Good	nd
Colony color	Orange-yellow, pale yellow to black	Orange-red to brown	Red-orange
Aerial mycelium	white, scant	–	–
Spore shape/surface	single-clusters/smooth	single/smooth	Single/rough
Motility	–	–	–
Temperature growth range (°C)	4–45	10–45	20–37
NaCl tolerance (%, w/v)	3	3	2
Catalase	–	w	–
Oxidase	+	–	–
Nitrate reduction	+	–	–
Production of H_2S	+	–	–
Degradation of:			
Adenine	+	nd	nd
Cellulose	nd	+	nd
Hypoxanthine	+	nd	nd
Starch	+	nd	–
Tween 20	–	+	+
Tween 40	+	+	–
Tween 80	–	+	–
Xanthine	+	nd	nd
Utilization as carbon sources			
D-Arabinose	+	–	–
Dulcitol	+	–	–
D-Galactose	+	–	+
Inositol	+	–	+
Lactose	+	–	+
Mannitol	+	–	+
D-Mannose	+	–	–
Raffinose	+	–	–
D-Ribose	+	–	–
Sorbitol	+	–	–
Trehalose	+	–	–
Xylitol	+	–	–

Table 28.15 (continued)

Characteristic	*P. mayteni*	*P. endophytica*	*P. siamensis*
Utilization as nitrogen sources			
L-Arginine	+	+	–
L-Lysine	–	+	+
Hypoxanthine	+	–	–
L-Hydroxyproline	–	+	–
L-Ornithine	+	+	–
L-Serine	–	+	+
L-Valine	+	+	–
Xanthine	+	–	–
Diaminopimelic acid isomer	*meso*	*meso*	*meso*
Whole-cell sugars	Ara, Gal, Glu, Man, Xyl, Rha	Ara, Gal, Glu, Man, Xyl, Rha	Ara, Gal, Glu, Man, Xyl, Rha
Phospholipid pattern	PE, DPG, PI and several unknown phospholipids	PE, DPG, PI and several unknown lipids	PE, DPG, PI, PIMs
Major fatty acids	anteiso-$C_{17:0}$, anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{17:1}\omega 8c$ and iso-$C_{17:0}$	anteiso-$C_{17:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$
Menaquinones	MK-10(H_6), MK-10(H_8), MK-10(H_4), MK-10(H_2)	MK-10(H_6), MK-10(H_8), MK-9(H_6), MK-10(H_4)	MK-9(H_6), MK-10(H_6), MK-9(H_8), MK-9(H_4), MK-10(H_4), MK-9(H_2)
DNA G+C mol%	69.7	73	72.6

Data from Qin et al. (2009), Thawai et al. (2010), Zhu et al. (2011)
+ positive, – negative, *nd* not determined
m-DAP *meso*-diaminopimelic acid
Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha* rhamnose, *Xyl* xylose
DPG diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PIMs* phosphatidylinositol mannosides

both *Rugosimonospora* species are MK-9(H_8) and MK-9(H_6); in addition, *R. acidiphila* contains MK-9(H_4). The most striking difference is found in the fatty acid profiles of both species. While both strains contain iso-$C_{16:0}$ and anteiso-$C_{17:0}$ in high amounts (>17 %), cyclohexyl $C_{17:0}$ is reported as the major component of *R. acidiphila* (>25 %). The presence of cyclohexyl $C_{17:0}$ was always detected as the major component found under different culture conditions, while it was never detected in *R. africana* (Monciardini et al. 2009). Cyclohexyl $C_{17:0}$ has been reported in major amounts in

Table 28.16
Selected phenotypic characteristics of *Rugosimonospora* species

Characteristic	*R. acidiphila*	*R. africana*
Growth on:		
ISP 2	Good	Abundant
ISP 3	Good	Good
ISP 4	Moderate	Moderate
ISP 5	Moderate	Good
ISP 7	Good	Abundant
Diffusible pigments (ISP 7)	Red-brown	Pink
Colony color	Colorless to yellow-orange	Colorless to yellow-orange
Motile spores	–	–
Spore shape/surface	Spherical/rugose	Spherical/rugose
Temperature growth range (°C)	17–32	15–37
NaCl tolerance (%, w/v)	0	1
Nitrate reduction	–	–
Production of H_2S	+	+
Casein hydrolysis	+	+
Gelatin hydrolysis	+	+
Starch hydrolysis	+	+
Tyrosine hydrolysis	+	+
Carbon sources		
L-Arabinose	w	w
Fructose	w	w
Glycerol	+	+
Inositol	w	w
Mannose	+	+
Raffinose	w	w
Rhamnose	w	w
Xylose	+	+
Sensitivity to lysozyme (<10 μg/ml)	+	+
Diaminopimelic acid isomer	OH-DAP	OH-DAP
Whole-cell sugars	Gal, Ara, Xyl	Gal, Ara, Xyl
Phospholipid pattern	PG, DPG, PI, PE, methyl-PE and an unknown aminolipid	PG, DPG, PI, PE, methyl-PE and unknown phospholipids
Major fatty acids	cyclohexyl $C_{17:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	ai-$C_{17:0}$, i-$C_{16:0}$,
Menaquinones	MK-9(H_8), MK-9(H_4), MK-9(H_6)	MK-9(H_8), MK-9(H_6)
DNA G+C mol%	72.7	71.9

Data from Monciardini et al. (2009)
+ positive, – negative, *w* weak, *nd* not determined
OH-DAP hydroxy-diaminopimelic acid
Ara arabinose, *Gal* galactose, *Glu* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose
DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *methyl-PE* methyl phosphatidylethanolamine

some representatives of the family *Microbacteriaceae* (Suzuki and Komagata 1983; Aizawa et al. 2007; Qiu et al. 2007; Vaz-Moreira et al. 2008), but not in the family *Micromonosporaceae*.

Salinispora Maldonado et al. 2005a

Sa.li.ni.spo′ra. L. adj. *salinus*, saline; Gr. fem. n. *spora*, a seed and, in bacteriology, a spore; N.L. fem. n. *salinispora*, a spore-forming bacterium originating from a saline habitat, indicating the marine habitat of the organism.

The genus *Salinispora* encompasses a group of microorganisms isolated from tropical and subtropical marine sediments (Jensen et al. 1991). The genus currently harbors two species, *S. tropica* and *S. arenicola*. Salinisporae stain Gram-positive, are non-acid fast and nonmotile. *Salinispora* strains grow well on complex media such as ISP 2 and M4 (Jensen et al. 1991); however, media must be prepared with seawater or be supplemented with sodium. Mincer et al. (2002) reported that *Salinispora* strains did not grow when sodium salts were replaced with equimolar concentrations of potassium salts, suggesting a specific sodium ion requirement. Nevertheless, growth is reported at low sodium concentrations (5 mM) if the medium is supplemented with sufficient concentrations of the appropriate non-sodium salts (Kim et al. 2005; Tsueng and Lam 2008a). Cells may lyse if the osmotic strength of the growth medium is not sufficiently high.

Colonies are tough, leathery, and adhere to the agar surface. The color of the colonies ranges from pale to bright orange to brown. Dark brown to black, bright orange, or pink diffusible pigments are frequently produced. These actinobacteria form extensively branched, non-fragmenting substrate hyphae (0.25–0.5 μm in diameter) that carry single or clusters of smooth-surfaced spores (0.8–3.8 μm), which may be sessile or borne on short sporophores. Aerial mycelium is not produced.

Table 28.17
Selected phenotypic characteristics of *Salinispora* species

Characteristic	*S. arenicola*	*S. tropica*
Growth on:		
ISP 2	Good	Good
Bennett's	Good	Good
Colony color	Orange to brown	Orange to brown
Motile spores	–	–
Spore arrangement/ surface	Single or clusters// smooth	Single or clusters// smooth
Temperature growth range (°C)	10–30	10–30
Sea water/sodium requirement	25–50 %	+
Resistance to rifampicin (25 μg/ml)	+	–
Degradation of:		
Arbutin	+	+
Casein	+	+
Cellulose	–	–
Chitin	–	–
Elastin	+	+
Gelatin	+	+
Starch	+	+
Tributyrin	–	–
Xylan	–	–
Carbon sources		
Arbutin	+	nd
Cellobiose	+	+
D-Fructose	–	–
Galactose	+	–
Inulin	–	+
α-Lactose	+	+
D-Mannose	–	–
D-Melezitose	+	+
D-Ribose	–	–
L-Sorbose	–	–
D-Salicin	+	–
Starch	+	+
D-Xylose	–	–
Diaminopimelic acid isomer	*meso*	*meso*
Whole-cell sugars	Ara, Gal, Xyl	Ara, Gal, Xyl
Phospholipid pattern	DPG, PE, PG, PI	DPG, PE, PG, PI
Major fatty acids	iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{18:0}$, $C_{17:0}$, 10-methyl-$C_{18:0}$	iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{18:0}$, $C_{17:0}$, 10-methyl-$C_{18:0}$
Menaquinones	MK-9(H_4)	MK-9(H_4)
DNA G+C mol%	70–73	70–73

Data from Maldonado et al. (2005a)
+ positive, –negative, *w* weak, *nd* not determined
meso-DAP *meso*-diaminopimelic acid, *Ara* arabinose, *Gal* galactose, *Xyl* xylose, *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol

Salinispora strains are strict aerobic, chemo-organotrophic, and mesophilic; good growth is observed at 10–30 °C and pH 7–12. Both species degrade arbutin, casein, elastin, gelatin, and starch but not cellulose, chitin, tributyrin, or xylan (Table 28.17). The following carbon sources are assimilated by both species: cellobiose, α-lactose, melezitose, and starch. Fructose, mannose, ribose, sorbose, or xylose is not utilized. Additional carbon sources are given in Table 28.7. *Salinispora arenicola* is resistant to rifampicin (25 μg/ml), but *S. tropica* is sensitive.

The diaminopimelic isomer found in the cell-wall peptidoglycan of *Salinispora* strains is *meso*-diaminopimelic acid and the whole-cell sugars are arabinose, galactose, and xylose. The phospholipid profile corresponds to type II (Lechevalier et al. 1977, 1981) and is characterized by the presence of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol. The major menaquinone detected in both *Salinispora* species is MK-9(H_4). The fatty acid composition includes a complex mixture of saturated, iso-, and anteiso-fatty acids such as iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{18:0}$, $C_{17:0}$, 10-methyl-$C_{18:0}$.

Salinisporae are important producers of secondary metabolites with potential pharmacological application (see section "Application" in this chapter).

Verrucosispora Rheims et al. 1998

Ver.ru'co.si.spo.ra. L. fem. n. *verruca* wart; Gr. n. *spora* a seed; M.L. fem. n. *Verrucosispora* an organism with warty spores).

The genus *Verrucosispora* currently comprises four species, *V. gifhornensis* the type species (Rheims et al. 1998), *V. lutea* (Liao et al. 2009), *V. maris* (Goodfellow et al. 2012), and *V. sediminis* (Dai et al. 2010), the type strains of which have been isolated from a peat bog, a mangrove sediment, and deep-sea sediments, respectively. Cells stain Gram-positive, are non-acid fast and nonmotile. Morphologically, the genus is characterized by branching hyphae which form a well-developed substrate mycelium, absent or sparse aerial mycelium is

produced and spores with smooth, warty, or hairy surfaces which are borne singly, in pairs, or clusters. All *Verrucosispora* strains show good growth on ISP 1, ISP 2, ISP 3, and ISP 4 agar media, and only moderate to poor growth on ISP 5 and ISP 6 agars. Light orange, orange, or even dark brown-orange are the usual colony colors of *Verrucosispora* strains. An orange diffusible pigment is produced by *V. gifhornensis* on several ISP media (Rheims et al. 1998).

Verrucosisporae are aerobic microorganisms that grow at 20–45 °C, at pH values of 3.5–10, and have an oxidative metabolism. Variation is found in their ability to reduce nitrate, to produce hydrogen sulfide, and the degradation of various polymers including aesculin, arbutin, and elastin (Table 28.18). *Verrucosispora lutea* and *V. sediminis* are able to grow in media supplemented with 5 % NaCl (w/v).

A diverse variety of carbohydrates have been tested as carbon and energy sources for all *Verrucosispora* type strains, assimilation results for each one of the four species are given in Table 28.18. As expected, all strains utilize glucose. In addition, *V. maris* is able to use organic acids and alcohols as carbon sources (Goodfellow et al. 2012). Furthermore, L-histidine is used as a carbon and nitrogen source by all species except *V. lutea*. Several amino acids, including L-alanine, L-phenylalanine, L-arginine, L-glutamic acid, L-histidine, L-methionine, L-serine, and L-valine, have also been tested as nitrogen sources (Table 28.18).

The chemotaxonomic characteristics of the genus *Verrucosispora* coincide with others found within the family *Micromonosporaceae*. The presence of *meso*-diaminopimelic acid is the diaminoacid isomer found in the peptidoglycan. The whole-cell sugar hydrolysates of *V. gifhornis* and *V. maris* contain mannose and xylose, but *V. lutea* has xylose and glucose, while *V. sediminis* has glucose, mannose, glucose, and glucosamine. Phosphatidylethanolamine is the diagnostic phospholipid (type II, Lechevalier et al. 1977, 1981); in addition, diphosphatidylglycerol and phosphatidylinositol mannoside are also found in all strains. Differences in the polar lipid profile of the four type strains are given in Table 28.18. The main menaquinone detected in *Verrucosisporae* strains is MK-9(H_4), but minor amounts of MK-9(H_6), MK-10(H_4), MK-9(H_2), and MK-9(H_{10}) may also be found (Table 28.18). The fatty acids i-$C_{16:0}$, i-$C_{15:0}$, and ai-$C_{17:0}$ are the main components of the species *V. gifhornesis*, *V. lutea*, and *V. sediminis*. The fatty acid profile of the type strain of *V. maris* has not been determined.

Virgisporangium Corrig. Tamura et al. 2001 Emend. Otoguro et al. 2010

Vir.go.spo.ran′gi.um. L. n. *virga*, a slender green branch, rod; N.L. neut. n. *sporangium* (from Gr. n. *spora*, a seed and in biology a spore; Gr. n. *angeion*, vessel), sporangium (spore-containing vessel); N.L. neut. n. *Virgosporangium*, an organism with rod-shaped sporangia (spore-containing vessels).

The genus *Virgisporangium* includes the species *V. ochraceum*, *V. aurantiacum*, and V. *aliadipatigenens*, isolated from different soil samples collected in Japan (Tamura et al. 2001; Otoguro et al. 2010). These bacteria stain Gram-positive, are non-acid fast and aerobic. Moderate to good growth is obtained on ISP 2, ISP 3, and ISP 7 agar media. The color of the vegetative mycelium is yellow to orange, and soluble pigments are produced by all species on ISP 7 agar. *V. ochraceum* and *V. aliadipatigenens* produce a light brown pigment, while *V. aurantiacum* produces a red-orange to brown pigment. *Virgisporangium* strains produce branching, non-fragmented hyphae, and slender sporangia are formed on short sporangiophores on the substrate mycelium. Each sporangium typically contains a single row of six or more spores which are oval or short rods (0.6–0.9 by 0.8–1.5 μm) and are motile.

Virgisporangium strains grow at 15–37 °C and are heterotrophic microorganisms with an oxidative metabolism. Nitrate is reduced by all species; however, it is strain variable in the case of *V. aliadipatigenens*. The latter species is also able to assimilate calcium malate. Starch is degraded by all strains while gelatin is not.

Several carbon sources are used by all *Virgisporangium* species and include L-arabinose, D-fructose, glucose, glycerol, maltose, mannose, and xylose. Other carbon sources utilized are found in Table 28.19. Several enzymatic activities have been determined using the API ZYM system (Otoguro et al. 2010). All species produce alkaline phosphatase; other activities are given in Table 28.19.

The cell-wall of *Virgisporangium* strains contains glutamic acid, glycine, alanine, and the diagnostic diaminoacid 3-hydroxy-diaminopimelic acid. The whole-cell sugars 3-*O*-methylmannose, rhamnose, mannose, glucose, arabinose, xylose, and galactose are found in *V. ochraceum* and *V. aurantiacum*, but the sugars rhamnose and arabinose are lacking in *V. aliadipatigenens*. The phospholipid type of *Virgisporangium*, like many other genera in the family *Micromonosporaceae*, is type II (Lechevalier et al. 1981) with phosphatidylethanolamine as the diagnostic phospholipid. The major menaquinones are MK-10(H_4) and MK-10(H_6). The major fatty acids are iso-$C_{16:0}$, and anteiso-$C_{17:0}$ or *cis* 9-$C_{17:1}$ and $C_{17:0}$.

Monospecific Genera

The family *Micromonosporaceae* contains 11 monospecific genera which are listed below. Selected morphological characteristics are summarized in Table 28.20.

Allocatelliglobosispora Lee and Lee 2011

Al.lo.ca.tel′li.glo.bo.si.spo′ra. Gr. adj. *allos*, another, the other; N.L. fem. n. *Catelliglobosispora*, a bacterial generic name; N.L. fem. n. *Allocatelliglobosispora*, the other *Catelliglobosispora*, an organism that is phylogenetically close to *Catelliglobosispora* but chemotaxonomically distinct.

Table 28.18

Selected phenotypic characteristics of *Verrucosispora* species

Characteristic	*V. gifhornensis*	*V. lutea*	*V. maris*	*V. sediminis*
Growth on:				
ISP 1	Abundant	Abundant	Abundant	Abundant
ISP 2	Abundant	Abundant	Abundant	Abundant
ISP 3	Moderate	Abundant	Abundant	Abundant
ISP 4	Moderate	Abundant	Abundant	Abundant
ISP 5	Moderate	Poor	Moderate	Moderate
ISP 6	Poor	Poor	Poor	Abundant
ISP 7	Moderate	Abundant	Poor	Moderate
Colony color	Light orange to orange	Light orange to dark brown	Light orange to dark brown-orange	Light to dark orange
Aerial mycelium	–	Scant	–	Scant
Motile spores	–	–	–	–
Spore arrangement	Single, pairs, clusters	Single, pairs, clusters	Single, clusters	Single, pairs
Spore ornamentation	Smooth, warty, hairy	Smooth	Warty	Warty
Temperature growth range (°C)	20–40	20–45	20–37	20–45
pH growth range	6.5–8.2	6–8	5–10	3.5–10
Nitrate reduction	–	+	–	+
Production of H_2S	–	+	–	+
Growth in 5 % NaCl (w/v)	–	+	–	+
Degradation of				
Aesculin	+	–	–	–
Arbutin	+	–	+	–
Elastin	–	+	–	–
Guanine	+	–	+	+
Starch	+	–	+	+
Urea	–	+	–	+
Xylan	–	–	–	+
Carbon sources				
Adonitol	–	+	+	+
Amygdalin	+	–	+	–
L-Arabinose	+	+	–	–
D-Arabitol	–	+	+	+
Cellobiose	–	+	–	+
Dulcitol	+	–	+	–
Erythritol	+	–	+	–
L-Fucose	–	+	+	+
Galactose	–	+	–	+
Glycerol	–	+	+	+
Lactose	–	+	+	+
Maltitriose	+	–	+	–
Mannitol	–	+	+	+
Melibiose	–	+	+	+
Raffinose	+	–	+	+
Rhamnose	–	+	–	+
Ribose	–	+	–	+

Table 28.18 (continued)

Characteristic	*V. gifhornensis*	*V. lutea*	*V. maris*	*V. sediminis*
Sorbitol	–	+	–	+
Turanose	+	–	+	–
Nitrogen sources				
L-alanine	–	–	+	+
L-phenylalanine	–	–	+	+
L-arginine	–	+	+	+
L-glutamic acid	+	–	–	+
L-histidine	+	+	+	–
L-methionine	+	–	+	–
L-serine	+	–	–	–
L-valine	+	–	+	+
Diaminopimelic acid isomer	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP
Whole-cell sugars	Man, Xyl	Xyl, Glu	Man Xyl	GlcN, Glu, Man
Phospholipid pattern	PE, DPG, PIM, PS, PL	PE, DPG, PIM, PI, PL	DPG, PE, PG, PIM, PS, unknown glyco- and phospholipids	PE, DPG, PIM, PI, PL
Major fatty acids	i-$C_{16:0}$, i-$C_{15:0}$, ai-$C_{17:0}$	i-$C_{16:0}$, i-$C_{15:0}$	nd	$C_{17:0}$, i-$C_{16:0}$, i-$C_{15:0}$
Menaquinones	MK-9(H_4), MK-9(H_6), MK-10(H_4), MK-9(H_2)	MK-9(H_4), MK-9(H_6), MK-9(H_2), MK-9(H_{10})	MK-9(H_4), MK-9(H_2), MK-9(H_6)	MK-9(H_4), MK-9(H_2)
DNA G+C mol%	70	69.3	70.9	66.8

Data from Theims al. (1998), Liao et al. (2009), Dai et al. (2010), Goodfellow et al. (2012)
+ positive, – negative, *w* weak, *nd* not determined
meso-DAP *meso*-diaminopimelic acid
Glu glucose *GluN* glucosamine, *Man* mannose, *Xyl* xylose
DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PS* phosphatidylserine, *PL* polar lipid

Catelliglobosispora **Ara et al. 2008a**
Ca.tel.li.glo′bo.si.spo′ra. L. n. *catella*, small chain; L. adj. *globosus*, spherical; Gr. fem. n. *spora*, a seed and in biology a spore; N.L. fem. n. *Catelliglobosispora*, (organism forming) small chain and spherical spores.

Couchioplanes **Tamura et al. 1994**
Couch′i.o.pla.nes. N.L. masc. n. *Couchius*, a personal name, referring to J.N. Couch (1896–1986), a mycologist who contributed to the taxonomy of the family *Actinoplanaceae*; Gr. masc. n. *planes*, a wanderer; N.L. masc. n. *Couchioplanes*, a wanderer organism of the family *Actinoplanaceae* named after J.N. Couch.

Hamadaea **Ara et al. 2008a**
Ha.ma.da.e′a. N.L. fem. n. *Hamadaea*, named after Masa Hamada, the Japanese microbiologist who made a tremendous contribution to actinomycete research.

Jishengella **Xie et al. 2011b**
Ji.sh.eng.ell′a. N.L. fem. n. *Jishengella*, named after Jisheng Ruan, the Chinese microbiologist.

Krasilnikovia **Ara and Kudo 2007a**
kra.sil.ni.kov.ia. N.L. adj. Krasilnikovia, referring to N. A. Krasil'nikov, a Russian actinomycetologist who contributed to the taxonomy of the family *Micromonosporaceae*.

Phytohabitans **Inahashi et al. 2010**
Phy.to.ha′bi.tans. Gr. n. *phyton* plant; L. part. adj. *habitans* inhabiting; N. L. part. adj. used as a masc. n. *Phytohabitans* plant-inhabiting, isolated from plants.

Phytomonospora **Li et al. 2011**
Phy.to.mo.no.spo′ra. Gr. n. *phuton*, a plant; Gr. adj. *monos*, single, solitary; Gr. fem. n. *spora*, a seed, and in biology a spore; N.L. fem. n. *Phytomonospora*, pertaining to a spore-forming actinomycete originally isolated from plant tissues.

Polymorphospora **Tamura et al. 2006**
Po.ly.mor.pho.spo′ra. Gr. adj. *polymorphos* multiform; N.L. fem. n. *spora* a spore; N.L. fem. n. *Polymorphospora* polymorphic spore.

Table 28.19

Selected phenotypic characteristics of *Virgisporangium* species

Characteristic	*V. ochraceum*	*V. aurantiacum*	*V. aliadipatigenens*
Growth on:			
ISP 2	Good	Moderate	Poor
ISP 3	Moderate	Good	Good
ISP 4	Poor	Moderate	Good
ISP 5	Poor	Moderate	Good
ISP 6	Moderate	Moderate	Poor
ISP 7	Moderate	Moderate	Good
Colony color	Yellow-orange	Yellow-orange	Yellow-orange
Soluble pigments (ISP 7)	Light brown	Reddish-orange to brown	Light brown
Motile spores	+	+	+
Sporangia	Single/clusters	Single/clusters	Single/clusters
Spore shape	Oval/short rods	Oval/short rods	Oval/short rods
Temperature growth range (°C)	15–37	15–30	20–37
Nitrate reduction	+	+	v
Milk peptonization	+	+	-
Growth in 2 % NaCl (w/v)	+	+	-
Assimilation of calcium malate	−	−	v
Degradation of:			
Gelatin	−	−	−
Starch	+	+	+
Carbon sources			
L-Arabinose	+	+	v
D-Fructose	+	+	v
Galactose	nd	nd	+
Glucose	+	+	+
Glycerol	+	+	v
Inositol	+	−	−
Lactose	−	+	−
Maltose	+	+	v
Mannose	+	+	+
D-Mannitol	+	+	−
Melibiose	+	+	−
D-Sorbitol	+	−	−
Sucrose	nd	nd	+
Xylose	+	+	v
API ZYM:			
Acid phosphatase	−	w	+
Alkaline phosphatase	+	+	+
Chymotrypsin	+	+	−
Esterase (C4)	w	w	−
α-Galactosidase	−	w	+
N-Acetyl-β-glucosaminidase	+	+	−
Trypsin	+	+	v
Diaminopimelic acid isomer	3-OH	3-OH	3-OH
Whole-cell sugars	Man, 3-*O*-methyl-Man, Rha, Glu, Ara, Xyl, Gal	Man, 3-*O*-methyl-Man, Rha, Glu, Ara, Xyl, Gal	Glu, Man, Gal, Xyl, 3-*O*-methyl-Man

Table 28.19 (continued)

Characteristic	*V. ochraceum*	*V. aurantiacum*	*V. aliadipatigenens*
Diagnostic phospholipid	PE	PE	PE
Major fatty acids	i-$C_{16:0}$, ai-$C_{17:0}$, *cis* 9-$C_{18:1}$, *cis* 9-$C_{17:1}$	i-$C_{16:0}$, ai-$C_{17:0}$, *cis* 9-$C_{18:1}$, i-$C_{15:0}$	*cis* 9-$C_{17:1}$, $C_{17:0}$, i-$C_{16:0}$
Menaquinones	MK-10(H_4), MK-10(H_6), MK-10(H_8)	MK-10(H_8), MK-10(H_4), MK-10(H_6)	MK-10(H_4), MK-10(H_6), MK-10(H_8)
DNA G+C mol%	71	71	71.7

Data from Tamura et al. 2001; Otoguro et al. 2010
+ positive, − negative, *w* weak, *v* variable, *nd* not determined
3-OH 3-hydroxy-diaminopimelic acid
Ara arabinose, *Gal* galactose, *Gluc* glucose, *Man* mannose, *Rha* rhamnose, *Xyl* xylose, *3-O-methyl-Man* 3-*O*-methyl mannose
DPG diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol

***Pseudosporangium* Ara et al. 2008b**

Pseu.do.spo.ran′gi.um. Gr. adj. *pseudes* false; Gr. n. *spora* seed; Gr. neut. n. *angeion* a vessel; N.L. neut. n. *Pseudosporangium* false sporangium.

***Spirilliplanes* Tamura et al. 1997**

Spi.ril.li.plan′es. N.L. dim. neut. n. *spirillum*, a small spiral; Gr. masc. n. *planes*, a wanderer; N.L. fem. (*sic*) n. *Spirilliplanes*, an organism with wandering cells, in spirals.

The genus *Couchioplanes* was first described by Tamura in 1994 to accommodate a group of actinomycetes exhibiting motile arthrospores that included two strains producing characteristic blue mycelia and previously described as *Actinoplanes caeruleus* and "*Actinoplanes azureus*" as well as a new soil isolate. All these organisms form spore chains and aerial mycelia aggregating into clusters resembling sporangia and they are characterized by the presence of L-Lysine instead of *meso*-diaminopimelic acid in the cell wall, clearly differing from previously described members of other genera with motile arthrospores. The three strains were included in the new species *Couchioplanes caeruleus*, where two subspecies were identified, *Couchioplanes caeruleus* subsp. *caeruleus* IFO 13939^T and *Couchioplanes caeruleus* subsp. *azureus* IFO 13993^T on the basis of their differences in the production of soluble pigments, growth in the presence of 2 % NaCl, and the utilization of rhamnose and mannitol as carbon sources (Tamura et al. 1994).

A second taxon that produces zoospores is the genus *Spirilliplanes* described by Tamura in 1997 and clearly distinguished from other motile actinomycetes in the wall chemotype, as well as menaquinone and fatty acid content. In this genus, zoospores are produced in chains of very narrow and coiled sporogeneous hyphae that aggregate and often appear to be sporangium-like structures, but are not true sporangia and spores are not enclosed in a sporangial wall. The genus differs from other actinomycetes genera with a chemotype II wall (glycine and meso-diaminopimelic acid) and is clearly distinguishable from the genera that produce motile spores by the absence of arabinose as whole-cell sugar. The type strain is *Spirilliplanes yamanashiensis* IFO 15828^T.

The genera *Catelliglobosispora* and *Hamadaea* were proposed to accommodate two species originally classified as *Catellatospora koreensis* and *Catellatospora tsunoense* (Ara et al. 2008a). The strains were shown to form by phylogenetic analysis a distinct clade within the family *Micromonosporaceae*, also supported by differences in the chemotaxonomic markers when compared to other members of the genus *Catellatospora*. The type species *Catelliglobosispora koreensis* DSM 44566^T produces straight, short chains of nonmotile spores borne directly on the substrate mycelium and abundant globose bodies, similar to those observed in the species *Hamadaea tsunoensis* and *Allocatelliglobosispora scoriae*, but not observed in other *Catellatospora* species. The three strains can be clearly distinguished by their chemotaxonomic composition (Ara et al. 2008a). The type strain *Hamadaea tsunoensis* DSM 44101^T produces well-developed branched mycelium and can be distinguished physiologically from *Catelliglobosispora koreensis* by the differential utilization of carbon sources and other growth requirements (Ara et al. 2008a). The genus *Allocatelliglobosispora* was established to accommodate a strain isolated from volcanic ashes collected in the Republic of Korea (Lee and Lee 2011). The type species *Allocatelliglobosispora scoriae* DSM 45362^T develops short chains of nonmotile spores singly or in clusters, together with globose bodies. Phylogenetic analysis based on 16S rRNA genes sequences relate remotely this genus to the genera *Catellatospora*, *Catelliglobosispor*a and *Hamadea*.

The genus *Polymorphospora* was described by Tamura et al. (2006) to accommodate new isolates of the family *Micromonosporaceae* obtained from Japanese soils surrounding mangrove roots and characterized by a strong reddish pigmentation of the mycelium and the formation of short and nonmotile spore chains. These strains were shown to form a distinct monophyletic clade and could be distinguished from other *Micromonosporaceae* by their chemotaxonomic and morphological characteristics. The type species is *Polymorphospora rubra* DSM 44947^T.

Three different genera associated to endophytic actinomycetes have been described within the family

Table 28.20
Morphological characteristics of monospecific genera

Genus	Sporulation	Spore surface	Spore motility	Sporangia	Substrate mycelium color	Soluble pigment
Allocatelliglobosispora	*Short spore chains from substrate mycelium*	nd	No	Globose bodies	Light brown to brown	Brown on ISP3 and oatmeal nitrate agar
Catelliglobosispora	*Strait short spore chains from substrate mycelium*	Smooth	No	Globose bodies	Cream to light yellow	No
Couchioplanes	*Spore chains in branched aerial mycelia*	Smooth	Yes	Pseudosporangia	Yellowish orange to blue	Yellow to pale brownish on peptone-yeast extract-iron agar
Hamadaea	*Strait short spore chains from substrate mycelium*	Smooth	No	Globose bodies	Pale yellow to bright marigold	No
Jishengella	Single spores on substrate mycelium	Warty	No	No	Vivid orange to dull orange	No
Krasilnikovia	*Long chains, coiled and aggregated from substrate mycelium*	Smooth	No	Globose pseudosporangia on substrate mycelium	Light yellow to cinnamon	No
Phytohabitans	*Long chains, > 10 spores*	Smooth	No	No	Pale orange to pale brown	No
Phytomonospora	Single spores on substrate mycelium	Smooth	No	No	Light yellow to yellowish brown	No
Polymorphospora	Short spore chains	nd	No	No	Red to reddish-orange	No
Pseudosporangium	*Spore chains*	Smooth	No	Pseudosporangia	Rusty to clove brown	No
Spirilliplanes	Spore chains in spirals; aggregated coiled sporogeneous hyphae	Smooth	Yes	No	Yellow to orange	Brownish pigment in tyrosine agar

Data for reference genera from Tamura et al. (1994, 1997, 2006), Ara and Kudo (2007), Ara et al. (2008a, b), Inahashi et al. (2010), Lee and Lee (2011), Xie et al. (2011b)
nd not determined

Micromonosporaceae: the genera *Phytohabitans*, *Phytomonospora*, and *Jishengella*. The genus *Phytohabitans* was described by Inahashi et al. (2010) for a new endophytic actinomycete isolated from the roots of a variety of orchid collected in Japan and producing long chains of nonmotile spores. The type strain *Phytohabitans sulfuscus* DSM 45306^T showed sequence similarity with the genera *Catenuloplanes* and *Asanoa* in the phylogenetic analysis, but could be distinguished by the different menaquinone composition, the characteristic whole-cell sugars, and the fatty acid and phospholipid profile (Inahashi et al. 2010). The genus *Jishengella* was described for another endophytic actinomycete isolated from the roots of *Acanthus illicifolius* in China, producing extensively branched substrate mycelium, carrying uneven warty-surfaced and nonmotile spores born singly on the substrate mycelium, and with similar morphology to strains of the genus *Verrucosispora* (Xie et al. 2012a). The type strain is *Jishengella endophytica* DSM 45430^T. The genus *Phytomonospora* is the most recently described monospecific genus for a novel endophytic actinomycete isolated from the roots of *Artemisia annua* L. in Yunnan, China. The strain *Phytomonospora endophytica* DSM 45386^T produces single nonmotile spores borne directly on the vegetative mycelium and can be distinguished from other single-spored *Micromonosporaceae* by its distinct phylogenetic position and its physiological and chemotaxonomic characteristics, especially the predominant menaquinone, characteristic whole-cell sugars, and fatty acids and polar lipid profiles (Li et al. 2011).

Two genera have been described for isolates forming irregular pseudosporangia on substrate mycelia. The genera *Krasilnikovia* and *Pseudosporangium* were described respectively by Ara and Kudo (2007a) and Ara et al. (2008b), to accommodate two strains isolated from sandy soils from Bangladesh. Both strains form spherical pseudosporangial structures that develop singly and are formed directly from aggregated spore chains on the substrate mycelia. Spores in spore chains are nonmotile and present an oval or reniform morphology in the case of *Krasilnikovia*. The type species, *Krasilnikovia cinnamoneae* JCM 13252^T, is clearly distinguishable on the basis of morphological, physiological, chemotaxonomic, and phylogenetic analyses from the closest genera *Couchioplanes* and *Actinoplanes*. The genus *Pseudosporangium* produces many small, nonmotile, spherical, smooth-surfaced spores in chains and forms irregular pseudosporangia on the sparse aerial mycelium by the aggregation of spore chains and individual spores. The type strain is *Pseudosporangium ferrugineum* is JCM14710^T.

Isolation, Enrichment, and Maintenance Procedures

Isolation and Enrichment

Without applying selective procedures, it is difficult to isolate members of family *Micromonosporaceae* from soil or other natural substrates. Although they are mesophilic and aerobic organisms, the growth rate of their colonies is often very slow, and on routine actinomycete isolation plates, the fast-growing streptomycetes can overrun them before they have developed conspicuous mycelia. Special pretreatments of the freshly collected samples enhance the number of *Micromonosporaceae* strains and reduce the non-desirable concomitant microorganisms, these methods include heating soil suspensions at 70 °C for 10–30 min (Rowbotham and Cross 1977; Sandrak 1977), 50 °C for 1 h (Monciardini et al. 2009) and dry heating of soil samples at 120 °C for 60 min (Shearer 1987). The pretreatment of soil suspensions with ammonia or dry heating for 1 h subsequent treatment with chlorine for 10–30 min (Burman et al. 1969; Willoughby 1969a) or with 1.5 % (w/v) phenol have been shown to be highly effective isolation methods (Hayakawa et al. 1991b; Wang et al. 2011). Dilution and heat shock treatments and stamping methods have been effective for the isolation of *Micromonospora* and *Salinispora* strains from sediments (Mincer et al. 2002; Magarvey et al. 2004).

The addition of humic acid activates the germination of spores (Hayakawa and Nonomura 1987a), and media with low nutrient concentration favor the growth of *Micromonospora* (Rowbotham and Cross 1977). To avoid growth of fungi on the isolation plates, cycloheximide (50 μg/ml) and/or nystatin (100 units/ml) can be added to the isolation media (Rowbotham and Cross 1977). The selection effect can be enhanced by the addition of antibiotics like gentamicin (Ivanitskaia et al. 1978); nalidixic acid; novobiocin (Sveshnikova et al. 1976); and tunicamycin (Wakisaka et al. 1982; Nonomura and Hayakawa 1988). Other chemicals such as the antifungal reagent Benlate (Matsumoto et al. 2007) or potassium bichromate (Xu et al. 2011) have been employed for the isolation of novel *Micromonosporaceae* strains.

Useful isolation media include Actinomycete isolation agar, pH 8.2 (Difco), arginine-glycerol-salts agar (Hunter et al. 1984), arginine-vitamin agar, cellulose-asparagine agar (Goodfellow and Haynes 1984), colloidal chitin agar (Hsu and Lockwood 1975), glucose–asparagine–vitamin agar (Takahashi et al. 1996), humic acid-vitamins agar (Hayakawa and Nonomura 1987a), Kodoka's cellulose benzoate agar (Sandrak 1977), M3 agar (Rowbotham and Cross 1977), mannitol-rifampicin agar (Kämpfer et al. 2007), raffinose–histidine medium (Williams et al. 1984), yeast extract-mannitol agar (Vincent 1970), starch-casein-nitrate agar supplemented with B vitamins (Shearer 1987), and 1/10 ATCC 172 agar (Xie et al. 2012b).

The differing behavior of the spores permits the use of two isolation methods: (1) direct isolation on selective agar media for genera having nonmotile spores and (2) techniques using spore motility to specifically enrich the sporangiate members. Obviously, strains belonging to one group can appear also on the isolation plates intended for the others.

The isolation plates and the enrichment cultures are usually incubated at 22–28 °C. Because of the very slow growth rate of *Micromonosporaceae* strains, the incubation time has to be extended up to 10 weeks in some cases (Goodfellow and Haynes 1984). The use of a dissecting microscope is recommended to select the colonies grown on the isolation plates. The mycelia can be picked up and transferred with toothpicks or with a thin metal needle.

In agreement with the wide distribution of *Micromonosporaceae* strains in nature, samples from various habitats have proved to be favorable sources of inoculum: soil, sediment, mud, water, plant material, and invertebrates. The above-mentioned isolation methods are applied according to the characteristics of different samples.

Isolation from Water

The freshly collected water samples should be stored at 4 °C until processed. If necessary, the spores can be concentrated from a relatively large volume of water either by the membrane filtration technique (Burman et al. 1969) or by centrifugation (Okami and Okazaki 1978). To reduce the number of the concomitant vegetative bacterial cells, a pretreatment either with mild heating or chemical substances is recommended. For the heat treatment procedure, 2 ml of the water sample is placed in a glass tube which is sealed and heated in a water bath. Various periods of incubation and temperatures have been used: 6 min at 55 °C (Rowbotham and Cross 1977), 10 min at 70 °C (Cross 1981a), or 60 min at 44 °C (Burman et al. 1969). An alternative pretreatment with chlorine was suggested by

Burman et al. (1969) and Willoughby (1969b): The samples are first treated with 4 mg/l ammonia, followed by 2 mg/l chlorine (added as 1 ml of a hypochlorite solution containing 200 mg/l of available chlorine). Samples are allowed to stand for 10–30 min; then the chlorine is neutralized with sodium thiosulfate. The correct amount has to be calculated from titration of a blank sample.

After brief mixing of the pretreated samples (either heat or chlorine), spreading can be carried out immediately with 0.2 ml of the sample on each agar plate (Rowbotham and Cross 1977). If necessary, dilutions can be made, either with sterile buffer (0.5 M KH_2PO_4 adjusted with NaOH to pH 7.2; Hsu and Lockwood 1975) or with quarter-strength Ringer's solution containing gelatin (0.01 % w/v; pH 7.0; Rowbotham and Cross 1977). The inoculated plates are incubated at 28 °C or 30 °C for 3–4 weeks. Direct plating of water samples on isolation agar led to the discovery of *Micromonospora mirobrigensis* WA201^T: 100 µl of pond water was plated directly on soil extract agar (SEA) at pH 6.5. SEA plates were incubated for 2 weeks at 28 °C in the dark (Trujillo et al. 2005). A significant number of Micromonospora strains isolated from the water column, sediment, and cellulose baits placed in freshwater lakes were recovered (de Menezes et al. 2008).

Isolation from Soil and Sediments from Freshwater and Marine Habitats

Soil-Dilution-Plate Techniques Soil samples, marine sediments, or mud from lakes and rivers are air-dried at room temperature and then ground in a mortar (Shearer 1987). About 1 g of the sample is added to 10 ml of saline solution. The suspension is mixed (vortex mixer) for 1 min and diluted in series with a sterile salt solution. The salt solution proposed by Wakisaka et al. (1982) contains 0.01 % $MgSO_4 \cdot 7H_2O$ and 0.002 % Tween, from which air is eliminated by use of a vacuum desiccator for about 30 min. Instead of a salt solution, sterile water can also be used for suspension and serial dilutions (Hayakawa and Nonomura 1987a). Ultrasonic processing (Matsumoto et al. 2007) or Ribolisation with the FastPrep-Instrument for 2 s at a speed of 4.0 m/s (Xie et al. 2012b) is helpful for dispersing of soil particles. Petri dishes are prepared one day before plating and incubated at 37 °C overnight to eliminate films of moisture on the agar surface (Shearer 1987). An inoculum (0.1 ml) of the proper dilution is placed on each plate and spread with a sterile glass rod. Plates are incubated at 28–30 °C for 4–5 weeks.

Cellulose-decomposing micromonosporae can be isolated from soil adjacent to the roots of wheat and maize according to the method of Sandrak (1977). One milliliter of soil suspension is mixed with 0.67-g sterile cellulose powder (as used for thin-layer chromatography) and 2 ml of liquid Kadota's benzoate medium (Sandrak 1977). The mixture is spread on plates with Kadota's benzoate agar. The cellulose layer is allowed to dry before the plates are incubated for 25–30 days at 28 °C (Cross 1981b). For *Micromonospora* species from marine sediments, Goodfellow and Haynes (1984) incubated the isolation plates at 18 °C for 10 weeks (duplicates at 4 °C for 6 months).

Heat or chlorine treatment, as described for water samples, can also be used with soil and sediment dilutions (Cross 1981b). An alkaline pretreatment method is suggested by Wakisaka et al. (1982): One milliliter of the diluent is mixed with 9 ml of 0.01 N NaOH. After standing for 5–10 min, the mixture is neutralized with 0.1 N HCl to pH 6–7 (with cooling) before serial dilution and plating. Nonomura and Hayakawa (1988) treated the soil-water suspension with 1.5 % phenol at 30 °C for 30 min.

The routine plating technique has been successfully applied for the isolation of strains of the genera *Allocatelliglobosispora, Catellatospora, Dactylosporangium, Krasilnikovia, Longispora, Luedemannella Micromonospora* and *Verrucosispora.*

Dry Heat Technique A procedure which involved dry heating of soil samples at extreme temperatures was originally developed by Nonomura and Ohara (1969) for the isolation of *Microbispora* and *Streptosporangium* species. Shearer (1987) demonstrated that this technique is also useful for the isolation of *Dactylosporangium* and *Micromonospora* strains. The samples are first air-dried at room temperature and ground in a mortar. Then they are heated in a drying oven at 120 °C for 60 min. One gram of the heat-treated soil is added to 10 ml of saline solution and then processed as described for the routine dilution-plating technique. Inoculated plates are incubated at 28 °C for 4–5 weeks. Arginine-vitamin agar, humic acid-vitamins agar (Hayakawa and Nonomura 1987a), and starch-casein-nitrate agar with B vitamins are used as selective media (Shearer 1987). *Spirilliplanes yashamaniensis* was isolated by this pretreatment (Tamura et al. 1997).

Stamping Technique The stamp technique was used successfully in the study of actinomycete populations of salt marsh ecosystems (Hunter et al. 1984). Depending on the moisture content, the samples of soil or mud are air-dried in Petri dishes for several days at room temperature. Two methods of further pretreatment were suggested by Hunter-Cevera et al. (1986): (1) the dried samples are ground with a pestle in a mortar and heated for 2 h at 60–65 °C, and (2) dried samples are mixed with powdered chitin (1: 1) and incubated for 2–3 weeks at 26 °C.

The pretreated and ground samples are stamped onto the isolation plates using the following procedure: A small circular sponge (Dispo culture plug, 16 mm; Scientific Products) is pressed into the powdered sample and removed. The excess small crumbs are shaken off. A stack of a dozen plates with various different alternating selective media is then inoculated by successively "stamping" (lightly touching) the sponge to the agar surface 10 times in a circle around the perimeter and three times in the middle of each plate (Hunter et al. 1984). Continuously stamping with the same plug yields the desired dilution effect. Plates are incubated at 26–28 °C for 2 weeks. Arginine-glycerol salts agar, starch-casein-nitrate agar, and thin pablum agar were recommended as the selective media for the isolation of *Micromonospora* strains (Hunter-Cevera et al. 1986).

The stamping method was used by Mincer et al. (2002) for the isolation of Salinisporae strains. In this case, 10 ml of wet

sediment was aseptically placed into a sterile aluminum dish, dried (ca. 24 h) in a laminar flow hood, ground lightly with a pestle, pressed into a sterile foam plug (14 mm in diameter), and inoculated onto agar media by stamping eight or nine times in a circular fashion, giving a serial dilution effect.

Isolation from Plant Material

Surface sterilization should be carried out before isolation from plant material. A special wash technique was employed by Willoughby (1968, 1969a) for the investigation of actinomycete populations on decomposing leaf litter. The leaves are collected at a fairly early stage of the decomposition. Small pieces of approximately 3 cm^2 are cut out, and each piece is transferred to a 100-ml conical flask containing 25 ml of sterile-filtered lake water. After 2 min of agitation on a rotary shaker, small aliquots of the leaf-washing liquid are either incorporated into molten agar (0.5 ml/plate) or spread onto the surface of agar, 0.2 ml for each plate, using a right-angled glass rod. The plates are incubated at 25 °C for 3–5 weeks (Willoughby 1968). The most successful isolation medium for strains of *Actinoplanes* and *Micromonospora* was colloidal chitin agar with cycloheximide as the antifungal agent. Ethanol and sodium hypochlorite are commonly used as sterilization agents. Mercury chloride (2.5 % w/v) has been used for root nodule surface sterilization (Trujillo et al. 2007). After sterilization, the nodules are homogenized using a glass rod and plated onto yeast-mannitol agar.

Endophytic *Micromonospora* strains have been recovered from surface-sterilized wheat roots. The roots were excised and subjected to a three-step surface sterilization procedure: a 60-s wash in 99 % ethanol, followed by a 6-min wash in 3.125 % NaOCl, a 30-s wash in 99 % ethanol, and a final rinse in sterile reverse osmosis-treated (RO) water. The surface-sterilized roots were then aseptically sectioned into 1-cm fragments and distributed onto the isolation media, followed by incubation at 27 °C for up to 4 weeks (Coombs and Franco 2003). Surface-sterilized nitrogen-fixing legume nodules have been shown to be extremely rich reservoirs for the isolation of novel *Micromonospora* strains (Trujillo et al. 2006, 2007, 2010; García et al. 2010; Carro et al. 2012). Several *Plantactinospora* strains have also been isolated from plant and root materials (Qin et al. 2009; Zhu et al. 2011).

Special Isolation Methods Using Motile Spores

The following very selective isolation methods are used when dormant sporangia are present in the substrate to be tested. The sporangia can release actively swimming spores when submerged in water. The individual spores must be motile for many hours and must show positive chemotaxis to specific chemical substances. Once fastened to a natural or cultural substrate, they must be able to germinate and form new mycelia and, for use of the baiting technique, produce a new generation of sporangia.

Baiting Technique The baiting technique is the classical isolation method for *Actinoplanes*, which made possible the first discovery of actinomycetes with motile spores (Couch 1949). Although other powerful techniques are available, baiting is still the only way to isolate keratinophilic *Pilimelia* strains.

The sample (0.5–1.0 g) is placed in a small, sterilized Petri dish (3 or 4 cm in diameter) or in a chamber of a multi-well microtiter plate, which is then half flooded with sterile demineralized water. After cautiously stirring, the particles settle to the bottom. Natural baits are exposed singly or in combination on the surface of the water: pollen of *Pinus*, *Liquidambar*, or *Sparganium*, boiled *Paspalum* grass leaves, hair of mammalian (human, dog, deer, cattle, white mice, etc.), or bits of snake skin (Couch 1949, 1954; Karling 1954; Gaertner 1955; Kane 1966; Schäfer 1973; Tribe and Abu El-Souod 1979; Makkar and Cross 1982; Hayakawa et al. 1991d). A ring of parafilm can be used to ensure the baits do not stick to the wall of the Petri dish (Hayakawa 2003). The baits must be presterilized, depending on their consistency, either chemically with ethanol or propylene dioxide or by autoclaving (Gaertner 1955; Schäfer 1973; Makkar and Cross 1982). The baiting enrichment cultures are closed and stored undisturbed at room temperature for several weeks. The water level can be regulated by addition of sterile distilled water. The examination for actinoplanetes can begin after one week with a dissecting microscope using 100× magnification and horizontal lighting (Bland and Couch 1981). Further examination after 3–4 weeks is recommended for keratinophilic organisms (Schäfer 1973).

Sporangia of actinoplanetes are recognizable as glistening beads on the air-exposed sides of the baits. Such baits are then removed carefully from the water and transferred to a 3 % agar plate (Bland and Couch 1981). Individual sporangia are separated from the bait and rolled several centimeters over the surface of agar, using a thin-pointed tungsten needle, which has a tip curved like a hockey stick. In this way, contaminants are removed from the sporangial surface. Cleaned sporangia can be transferred either directly or together with a small, cut-out agar block onto a Petri dish with suitable agar medium. Media for isolation from pollen and grass leaves include Czapek sucrose agar, peptone-Czapek agar (Bland and Couch 1981), half-concentrated casamino acids-peptone-Czapek agar (Schäfer 1973), or Emerson's yeast extract-starch agar (Emerson 1958). Sporangia from keratinic baits should be transferred to highly diluted skim milk-cattle horn-meal agar (Vobis 1984).

The colonies originating from the individual sporangia are visible with the naked eye after 1–4 weeks of incubation and can partly be used as the inoculum for the new strain on slant cultures. The other part of the mycelium can be transferred onto sporulation agar for morphological identification.

Dehydration-Rehydration This technique utilizes the ability of the sporangia to withstand desiccation and to release motile spores when they are subsequently in contact with water. Besides soil samples, it is also applicable to leaf litter, decaying plant material from aquatic habitats, organic debris, etc. (Makkar and Cross 1982).

The samples are dried at 28–30 °C for 7 days. For rehydration, 0.5 g of soil or corresponding substrate is mixed with 50 ml of sterile tap water in a 150-ml beaker or Erlenmeyer flask, which is covered with sterile aluminum foil (Vettermann and Prauser 1979; Shearer 1987). The suspension is incubated at 20–30 °C for about 1 h. During the first 30 min, the vessel can be shaken at irregular intervals. After that, the particles should be permitted to settle. From the supernatant, 0.5–1.0 ml are removed with a sterile pipette and spread onto agar plates (Shearer 1987). If it is necessary, dilutions can be prepared from the inoculation fluid (Makkar and Cross 1982).

Rehydration and Centrifugation Method An enrichment method incorporating differential centrifugation after rehydration was developed by Hayakawa et al. (2000). Samples are rehydrated with 10 mM phosphate buffer containing 10 % soil extract at 30 °C for 90 min. The liquid enriched with zoospores is centrifuged at 1,500×*g* for 20 min. Portions of the supernatant are then plated on humic acid-vitamin agar supplemented with nalidixic acid and trimethoprim or cycloheximide. The centrifugation procedure specifically eliminates strains of *Streptomyces* and other nonmotile actinomycetes (Hayakawa 2003). Using this method, several *Actinoplanes* strains have been obtained (Ara et al. 2010; Yamamura et al. 2012).

Chemotactic Method The spores of *Actinoplanes* exhibit an apparently microaerophilic reaction and are attracted to chloride and bromide ions (Palleroni 1976). Therefore, a chemotactic method can be used to isolate these strains. An essential part of this technique is a simple isolation chamber, a sterilizable plastic block (80 × 40 × 12 mm) with two circular holes (9-mm deep and 24 mm in diameter) whose centers are 32 mm apart. They are connected by a channel that is 2-mm wide and 3-mm deep (Palleroni 1980). One gram of a soil sample is divided into two equal parts and then placed in each compartment. Sterile water is added nearly to the rim and stirred cautiously. After incubation for 1 h at 30 °C, the spores are released from the sporangia and move freely in the water. Using a sterilized tweezer, a sterile 1-µl glass capillary about 32-mm long is filled with 0.01 M phosphate buffer (pH 7.0) containing 0.01 M KCl and placed in the channel. The capillary must be submerged, connecting the two suspensions. After incubation at 30 °C for 1 h more, the attracted spores are concentrated in the lumen of the capillary, which is then removed and washed from the outside with a jet of sterile water. The contents of the capillary are blown into 1-ml sterile water or buffer. Portions of the dilution are taken with a sterile pipette and spread onto carefully dried agar plates. The plates are then incubated at 28 °C. Starch-casein sulfate agar is recommended as the isolation medium (Palleroni 1980). Although colonies can be selected after 4 days, slowly growing actinomycetes may only be detectable after 3 weeks.

An alternative chemotactic method which employed the capillary technique of Palleroni (1980), but using g-collidine or vanillin (100 mM) as chemoattractants, (Hayakawa et al. 1991c) was used for the isolation of *Dactylosporangium* and *Virgisporangium*, using humic acid agar (Tamura et al. 2001; Hayakawa 2003).

Moist Incubation Technique This method is suitable for the direct detection of actinoplanetes on natural substrate. Although the ability to produce motile spores obviously plays no role, *Actinoplanes* strains can be readily enriched (Willoughby 1968). Portions of decaying leaves or other biological substrates, freshly collected from the field, are washed with sterile water to remove adhering detritus. They are placed in prepared Petri dishes, the bottoms of which have been covered with very moist filter paper or layers of cellulose before autoclaving. The Petri dishes, working as moist chambers, are sealed and incubated for about 4 weeks at 25 °C. Examination with both dissecting and light microscopes is necessary to identify the sporangia of the actinoplanetes (Willoughby 1969a).

Enrichment and Isolation Procedures of *Dactylosporangium*

More recently, Hayakawa (2003) summarized his own valuable experiences of isolating rare actinomycetes. To isolate selectively high numbers of *Dactylosporangium* strains from soil, combinations of several techniques are recommended. At first, the soil samples are dried slowly at room temperature for a week, sieved, and ground slightly in a mortar (Nonomura and Ohara 1969). After that, the samples can be pretreated physically with dry heat (120 °C) for 1 h, followed by treatment with the chemical germicide BC (0.01 or 0.03 %), exposed for 30 min at 30 °C (Hayakawa et al. 1991a). Especially in the latter case, the globose bodies (aleuriospores) function as the surviving units.

The enrichment procedures profit by the release of zoospores from the sporangia in an aqueous environment. One possibility is an improved chemotactic method employing the capillary technique of Palleroni (1980), but using g-collidine or vanillin (100 mM) as chemoattractant instead of the traditional 0.01 M KCl (Hayakawa et al. 1991c). A further enrichment method named "rehydration and centrifugation" was developed by Hayakawa and colleagues (2000). The samples are flooded with 10 mM phosphate buffer containing 1 % soil extract at 30 °C for 90 min. The fluid is centrifuged at 1,500×*g* for 20 min and the supernatant, containing actively swimming zoospores, is used for plating. The isolation medium used was humic acid-vitamin agar (Hayakawa and Nonomura 1987a, 1987b) supplemented with nalidixic acid (20 mg/l) (Nonomura and Hayakawa 1988; Hayakawa et al. 1991a; 1995). An alternative source for isolation of *Dactylosporangium* was demonstrated by Okazaki (2003) using leaves as the substrate. Freshly picked leaves were cut into several pieces, rinsed with sterile water, and soaked in 70 % ethanol for 1 min. They are then washed once more with sterile water, soaked in 1 % NaClO for 3 min, and rinsed again with sterile water. After these treatments, the leaf pieces were incubated on 0.8 % water agar for several weeks. A culture-independent, nested PCR procedure based on

genus-specific oligonucleotide primers was used to detect the presence of members of the genus *Dactylosporangium* in several environmental samples. This information was used to develop a reliable strategy for the selective isolation and characterization of dactylosporangiae using several selective media supplemented with antifungal antibiotics and either gentamicin or oxytetracycline (Kim et al. 2011).

Maintenance procedures Sporulated cultures can be maintained for months on agar slopes or sealed plates at 4 °C. Long-term storage can be achieved by lyophilization, by liquid drying, or by maintaining spores or liquid seed cultures in 10–15 % glycerol at −80 °C (Wellington and Williams 1978). Lyophilization of spores or hyphal suspensions in 10 % skim milk + 1 % monosodium glutamate and L-drying in 0.01 M potassium phosphate buffer (pH 7.0) containing 3 % monosodium glutamate (Sakane and Kuroshima 1997) are also recommended for long-term preservation.

Ecology

Habitat

Members of family *Micromonosporaceae* are widely distributed in soil and aquatic environments. Different kinds of wet soils or sediments are their main habitats and include peat swamps (Thawai et al. 2004, 2005a, b; Songsumanus et al. 2011) and soils close to waterfalls (Ara and Kudo 2006; Kämpfer et al. 2007). They also appear in different plant rhizospheres (Tamura et al. 2005; Wang et al. 2011; Xu et al. 2011), and cliff soils (Seo and Lee 2009). There is evidence that mangrove environments also contain high populations of members of the family *Micromonosporaceae* (Eccleston et al. 2008; Hong et al. 2009). This is well illustrated by the isolation of *Asanoa* (Tamura and Sakane 2005; Xu et al. 2011), *Micromonospora* (Huang et al. 2008; Thawai et al. 2008; Wang et al. 2011; Songsumanus et al. 2012; Xie et al. 2012a), *Verrucosispora* (Liao et al. 2009; Xi et al. 2011; Xie et al. 2012b), *Polymorphospora* (Tamura et al. 2006), and *Jishengella* (Xie et al. 2011b). It is also believed that marine environments contain large populations of *Micromonospora* spp. (Maldonado et al. 2005b; Eccleston et al. 2008). Furthermore, uncultured actinomycetes related to the genera *Actinoplanes, Micromonospora*, and *Salinispora* were revealed by a DGGE analysis of 16S rRNA gene fragments from river water samples (Yoshida et al. 2008). Plant materials, especially nitrogen-fixing nodules (Trujillo et al. 2006, 2007, 2010; Carro et al. 2012) and roots, are also common habitats for *Micromonosporaceae* strains (Qin et al. 2009; Inahashi et al. 2010; Kirby and Meyers 2010; Li et al. 2011; Xie et al. 2012b; Zhu et al. 2012).

Specific primers to detect members of the family *Micromonosporaceae* including the genera *Dactylosporangium* (Monciardini et al. 2002; Kim et al. 2011), *Micromonospora* (Qiu et al. 2008), and *Verrucosispora* (Xie et al. 2012a) have been designed and employed in culture-independent surveys for diverse environmental samples. A new PCR primer system for the selective identification of actinobacteria was recently developed and used for the analysis of several environmental samples and building materials (Schäfer et al. 2010). In this study, the genera *Actinocatenispora* (2.1 %), *Dactylosporangium* (13.5 %), *Micromonospora* (1.0 %), *Polymorphospora* (18.8 %), and *Verrucosispora* (1.0 %) were detected from a compost sample clone library, while a sequence of *Actinoplanes*, representing 1 % of the total clone library was detected in plaster. In addition, the genera *Actinoplanes Pilimelia* and *Polymorphospora* were found in a bioaerosol of a compost plant. A pair of family-specific PCR primers for *Micromonosporaceae* was designed and employed for the clone library analysis of 16 soil samples collected at 4 mangrove sites in China. The results revealed that one third of the reported genera including *Actinoplanes, Asanoa, Jishengella, Krasilnikovia, Micromonospora, Plantactinospora, Polymorphospora, Pseudosporangium, Rugosimonospora*, and *Verrucosispora* belonged to the target family; of these, the genera *Micromonospora, Rugosimonospora, Plantactinospora*, and *Verrucosispora* were detected in all four sampling sites, suggesting their wide distribution (K Hong's group, unpublished data).

A BLAST search to detect other habitats where members of the family *Micromonosporaceae* have been detected using both dependent and independent culture methods includes anthracene-contaminated soils, coal beds, banana plantations, ants, urban aerosols, marine sponges, semi-arid lead-zinc mines, and permafrost soils (Brodie et al. 2006, 2007; unpublished sequence accession numbers: DQ125928; DQ129567; EF612364; GU002091; HQ336732; HQ864100; JF417727; JQ427735; JX1333365;). These examples reflect the wide distribution of *Micromonosporaceae* strains in many diverse habitats.

In the following paragraphs, we describe in more detail the ecology of the genera *Micromonospora, Actinoplanes, Dactylosporangium, Pilimelia, Salinispora, and Verrucosispora*, given their importance in several biological processes and application within the family. The habitat of members of monospecific genera where only the type strain is available must be considered tentative.

Micromonospora Micromonosporae have been isolated from diverse habitats including soil, water, and marine sediments (Kawamoto 1989; Zhao et al. 2004; Maldonado et al. 2005b; de Menezes et al. 2008). Micromonosporas are very common in alkaline and neutral soils (Jensen 1930; Vobis 2006), and although many strains have been shown to be sensitive to acid pH (Kawamoto 1989), they have been isolated from acid soils (Zenova et al. 2004). However, their predominant incidence seems to be in aquatic ecosystems, including both freshwater and marine habitats (Cross 1981a; Goodfellow and Haynes 1984). Since they decompose chitin, cellulose, and lignin of lake sediments, they might play an important role in lacustrine ecology (Erikson 1941).

The presence of *Micromonospora* in lake systems has been shown by investigations in many countries and was comprehensively reviewed by Cross (1981a, b).

Besides streptomycetes and nocardioforms, micromonosporas were the predominant actinomycetes in the bottom sediments of Blelham Tarn, UK, with numbers increasing from littoral to profundal mud samples (Willoughby 1969b). This dominance was even more striking in deeper mud layers, as could be shown in studies of other lakes of the English Lake District (Johnston and Cross 1976; de Menezes et al. 2008). Similar observations were made by Fernandez (1984) at a thermal lake, Lake Héviz, in Hungary. Compared with the surface of the mud, the number of micromonosporas increased twofold at a depth of 20 cm, whereas the number of streptomycetes decreased significantly in the same layer. Under those conditions, the spores of *Micromonospora* seem to be more resistant than the propagules of *Streptomyces* and nocardioform actinomycetes. This could be confirmed in investigations on the longevity of actinomycete spores in deep mud cores. Viable spores of *Micromonospora* were recorded from sediments deposited at least 100 years before (Cross and Attwell 1974). Their tolerance to low oxygen tensions suggests that micromonosporae may grow under the microaerophilic conditions found in alluvial soils, floodplain meadows, and wet soils of river ecosystems (Goodfellow and Williams 1983; Vobis 2006; Zenova and Zviagintsev 2002), plant litter, lichens, roots, and organic soil horizons (Zenova et al. 1994; González et al. 2005).

Micromonospora species have been isolated from many different marine habitats, ranging from coastal regions to deep-sea sediments. Abundant micromonosporas were found in salt marsh ecosystems in New Jersey (USA), with seasonal fluctuations in quantity (Hunter et al. 1981). In a study at the San Francisco Bay (USA) National Wildlife Refuge, Hunter et al. (1984) showed that micromonosporas occur more frequently in rhizospheric soils of seashore plants than in mud samples obtained from plant-free areas. Watson and Williams (1974) studied the actinomycetes in a coastal sand belt near Formby, Lancashire (UK). In sea water and beach strand, the *Micromonospora* strains predominated. They grew well on freshwater media and most of them tolerated seawater salinity. Okazaki and Okami (1972) isolated micromonosporaes from littoral muds and from samples collected in shallow sea areas of the Pacific Ocean, occurring more frequently at the bottom than in the sea water. Relative numbers of *Micromonospora* species increased with the depth, while an opposite trend was seen for *Streptomyces* species during investigation on rare actinomycetes of shallow water sediments of the Trondheim fjord, Norway (Bredholdt et al. 2007). Weyland (1969, 1981) found that the micromonosporas predominated in the deep-sea sediments, and his results were confirmed by Goodfellow and Haynes (1984). Furthermore, some Micromonosporae were recovered from marine sediments of over 3,000 m depth (K Hong's group, unpublished data), or detected by culture-independent methods (Stach et al. 2003). Some authors suggest that the actinomycetes are a part of the indigenous marine microflora, able to grow in seawater and its sediments (Okami and Okazaki 1978; Weyland 1981; Jensen et al. 1991; Takizawa et al. 1993; Mincer et al. 2002; Magarvey et al. 2004). Active mycelial growth was demonstrated in sand particles (Jensen et al. 2005). Using cellulose baits, de Menezes et al. (2008) demonstrated that the proportion of hyphal fragments to spores of Micromonosporae was higher in the baits closer to the surface and decreased with depth, and inferred that the organisms spores are germinated when the growth condition is available and not likely as washed in from the surrounding soil.

Micromonosporae strains have also been isolated from different plant materials. They have been described as colonizing the roots of *Casuarina* and *Triticum* species (Coombs and Franco 2003; Valdés et al. 2005). A *Micromonospora* isolate was also described from surface-sterilized wheat root tissue and culture-independent methods have identified clones of endophytic actinobacteria most closely related to *M. endolithica* and *M. peucetia* in wheat roots, suggesting the presence of a large diversity of *Micromonospora* species in plants (Conn and Franco 2004). Surface-sterilized nitrogen-fixing legume root nodules of *Lupinus angistifolius*, *Pisum sativum*, and other legumes have been shown to be extremely rich reservoirs for the isolation of novel *Micromonospora* strains (Trujillo et al. 2007, 2010; García et al. 2010; Carro et al. 2012). A collection of >2,500 strains is kept in our laboratory (Trujillo, unpublished results).

Actinoplanes Actinoplanetes are widely distributed in soil throughout the world (Couch 1963; Schäfer 1973; Parenti and Coronelli 1979). They occur in all types of soil, arid desert areas (Makkar and Cross 1982), sand dune systems close to seashores (Palleroni 1976), and subtropical and tropical regions. Schäfer (1973) isolated *Actinoplanes* strains from 56 % of soil samples investigated; Nonomura and Takagi (1977) isolated strains from 75 % soil samples collected from Japan; and Vobis (1987) isolated strains from 65 % of Argentina samples.

In general, the sporangiate actinoplanetes can be considered as normal inhabitants of soil and leaf litter (Cross 1981b), although they can also be isolated directly from lake or river water (Willoughby 1969b, 1971). A frequent drying and wetting of the substrates increases their occurrence. Favored habitats are edges of ponds, drainage ditches, and barnyards (Shearer 1987). Sediments of rivers are also a good source for the isolation of *Actinoplanes* strains (Goodfellow et al. 1990). In a large-scale investigation of the distribution of the actinoplanetes in soil in Japan, Nonomura and Takagi (1977) demonstrated a correlation between their abundance, the type of soil, its pH value, and the content of organic matter. Relatively few actinoplanetes occurred in soils with pH 4.0–5.0 and abundant organic matter content. Their number increased with lower humus content and a pH value between 6.4 and 7.2. Soils with a permanent high content of water (e.g., paddy rice fields) have no advantage compared with cultivated fields, which are dry for longer periods.

Strains of *Actinoplanes* can also colonize plant or animal debris (Cross 1981b; Makkar and Cross 1982). They occur frequently on twigs submerged in streams (Willoughby 1971), muddy dead leaves that are caught and dried on branches of overhanging trees (Cross 1981b), and on allochthonous leaf litter cast up on the shores of lakes (Willoughby 1969b).

The function of actinoplanetes in soil ecosystems is not well known. With a behavior of typical saprophytic microorganisms, abilities to degrade any kind of biological material may be possible. Chitin has been used as a carbon source for the isolation of strains by Makkar and Cross (1982). However, chitin is not easily degraded and growth may be very slow; thus, in some cases, the aim for using a chitin medium for isolation is to inhibit or decrease the growth of other microorganisms (Willoughby 1968). Degradation tests using chitin from insects and fungi (Schäfer 1973) did not agree with the positive results of Goodfellow et al. (1990) who used the basal medium of Gordon (1967) supplemented with 0.5 % (w/v) chitin.

Since *Actinoplanes* strains exhibit good growth on xylose and arabinose, it is possible that they play a role in decomposing plant origin sugars (Parenti and Coronelli 1979). A study of saprophytic actinomycetes associated with the root system of the actinorhizal plant *Discaria* revealed that all 27 isolated *Actinoplanes* strains could degrade starch, cellulose, and pectin; nine of the isolates were capable of decomposing hemicellulose and/or colonized preferably the thin sections of dead wood (Solans and Vobis 2003). The most active *Actinoplanes* strains, BCRU-ME 3, promoted *Frankia* symbiosis of *Discaria trinervis* and *Sinorhizobium meliloti*//*Medicago sativa* symbiosis (Solans 2007; Solans et al. 2009).

Dactylosporangium Members of the genus *Dactylosporangium* are distributed worldwide. They have been found in diverse types of soil, including cultivated fields, forest, and pasture soils (Hayakawa et al. 1991c; Vobis 2006). These include soil samples from Italy (Thiemann 1970a), Japan (Shomura et al. 1983a, b, 1985, 1986), Thailand (Thiemann 1970a, b; Chiaraphongphon et al. 2010), and UK (Kim et al. 2010). Other strains have been found from soil of an uncultivated field of grass in Colombia (Shearer 1987), and from soil samples collected in tropical and subtropical regions in Yunnan, China (Xu et al. 1996). Isolates have also been reported from soil samples collected in Zambia (Africa), Lüneburger Heide (Germany), and the National park Taman Negawa (Malaysia) (G. Vobis and J.M. Wink, unpublished results).

Thiemann et al. (1967) isolated 33 strains of *Dactylosporangium* from different soil samples collected in Thailand, Brazil, and Argentina. In another study, 140 *Dactylosporangium* isolates were obtained from 454 soil samples collected from various parts of the world (Thiemann 1970a). The soil types included sandy as well as loamy soils. No correlation could be established between the type of soil, its pH (4.0–9.0), and the incidence of *Dactylosporangium*. Hayakawa and Nonomura (1987a) obtained isolates from soil samples from vegetable and corn fields with pH ranging from 5.4 to 6.1, collected in different Prefectures (Nagano, Mie, Gunma, and Iwate) in Japan. Field soils seem to be the most fruitful sources for isolating *Dactylosporangium* strains, together with other diverse rare actinomycete taxa, but they were also isolated frequently from mountainous forest soils. Field, mountain grass-land, and rice paddy with soil of pH 6.0–7.0, organic matter content <5 %, and immature brown humic acid <0.8 (D log K) are the characteristic soil habitats of *Dactylosporangium* (Hayakawa 2003).

Only a few sources other than soil were successfully tested as natural substrates inhabited by *Dactylosporangium* or simply utilized as intermediate locations by its resistant structures like the globose bodies. Johnston and Cross (1976) isolated strains from the surface muds of two lakes of the English Lake District in Great Britain. *Dactylosporangium* strains were also found on plant debris (Lechevalier 1981). Leaf litter in marsh water in New Jersey (USA) was used as substrate to isolate the antibiotic dactylocycline-producing strain SC 14051. More recently, Okazaki (2003) reported an antibiotic-producing strain, *Dactylosporangium aurantiacum*, isolated from fresh plant leaves of *Cucubalus* sp.

A culture-independent, nested PCR procedure based on genus-specific oligonucleotide primers detected the presence of members of the genus *Dactylosporangium* in 14 out of 21 diverse environmental samples. This information was then used to isolate Dactylosporangiae using a selective isolation procedure. Presumptive *Dactylosporanigum* strains were isolated from 13 of the 14 environmental samples found to contain members of the genus *Dactylosporangium* in the culture-independent studies. It is interesting that many of the isolates were recovered from acidic soils, notably those isolated from the hay meadow soil (pH 5.5) and from the agricultural soils from Kuala Lumpur (pH 5.7) and Srinagar (pH 5.5) (Kim et al. 2011).

Pilimelia These strains are able to colonize keratinic substrates like hair of mammalia or snake skin (Karling 1954; Gaertner 1955; Tribe and Abu El-Souod 1979). Although they can aggressively attack the scleroproteins of animals, they are not known as dermatophytes. They are distributed worldwide and occur statistically in about one of every five soil samples (Schäfer 1973; Vobis et al. 1986). Using the baiting technique, Karling (1954) had found them in soil cultures first from New York City (USA), and later from various parts of the Amazon Valley in Brazil, as well as from Indiana, Iowa, Louisiana, New Jersey, and Virginia (USA). Sixteen percent of soil samples from the African continent were positive for the presence of *Pilimelia* (Gaertner 1955). Schäfer (1973) discovered sporangia of this genus in enrichment cultures in 22 % (96 out of 427) soil samples collected from different geographical regions of the world. Strains presumptively identified as *Pilimelia terevasa* and *Pilimelia columellifera* have been reported to be widely distributed in diverse soils from England (Tribe and Abu El-Souod 1979). Thirty four *Pilimelia*-like isolates could be recovered from 8 out of 32 soil samples from the arid environment of the Mojave Desert along the California–Nevada border using the baiting technique (Garrity et al. 1996). Although it appears that *Pilimelia* may be abundant in nature, they are very difficult to isolate and cultivate (Schäfer 1973; Vobis et al. 1986).

Salinispora Over 1,000 *Salinispora* strains have been isolated from sediments collected from the subtropical Atlantic,

the Red Sea, and the Sea of Cortez, suggesting a pan-tropical distribution. The maximum depth of successful isolation of this genus is 1,100 m (Mincer et al. 2002). Though the genus is broadly distributed in tropical and subtropical sediments (Jensen and Mafnas 2006), the three *Salinispora* species known (including "*S. pacifica*" not validly published) show distinct biogeographical patterns. *S. tropica* appears to have a restricted distribution and it is limited to the Caribbean Sea. Salinispora *arenicola* has the broadest distribution and has been recovered from all sites in which the genus has been reported. "*S. pacifica*" has an intermediate distribution, and is yet to be cultured from the Caribbean (Jensen and Mafnas 2006). A culture-independent method was used to detect *Salinispora* in environmental DNA from different depths of marine sediments collected in Bahamas in different years. No new species level diversity was detected, and 97 % of the 105 strains examined by restriction fragment length polymorphism belonged to one phylotype (*S. arenicola*) (Mincer et al. 2002). *Salinisporae* have also been reported from a marine sponge (Kim et al. 2005).

This genus has been claimed to be an obligate marine actinomycete, and all strains tested required seawater indicating a high level of marine adaptation (Mincer et al. 2002; Maldonado et al. 2005b). Although the comparative genomic analysis explained their adaptation in marine environments (Penn and Jensen 2012), good growth of *S. tropica* strains CNB440, CNB476, and NPS21184 was detected in both agar and liquid media containing the potassium-chloride-based salt formulation with sodium concentration of 5.0 mM (Tsueng and Lam 2008a, b; 2010).

Verrucosispora The type species of *Verrucosispora* was first isolated from a samples taken from a peat bog near Gifhorn, Lower Saxony, Germany (10°33′E, 52°30′N). Samples were taken from a depth of 20–40 cm after removal of the top peat layer. Later, two strains isolated from marine sediments were found to produce novel compounds and showed promising antibacterial and anticancer activities (Bister et al. 2004; Fiedler et al. 2008); this finding promoted the interest of looking for more *Verrucosisporae* from marine environments (Maldonado et al. 2009). Thus, Verrucosisporae appear to be natural inhabitants of marine environments, deep-sea sediments, and mangrove samples. They also occur in surface-sterilized roots of the mangrove plant *Acanthus illicifolius* (K Hong's group, unpublished data).

Pathogenicity: Clinical Relevance

At present, it appears that none of the members classified in the family *Micromonosporaceae* have been reported as pathogens or have any clinical relevance. Although *Pilimelia* strains can aggressively attack the scleroproteins of animals, they are not known as dermatophytes.

Application

Many members of the *Micromonosporaceae* produce useful secondary metabolites and enzymes. They have important applications in industry, biotechnology, and agriculture. For example, gentamicin (Weinstein et al. 1963a, b; Kumar et al. 2008) and vitamin B 12 (Florent and Ninet 1979) is produced by *Micromonospora* strains. *Actinoplanes* sp. SE50/110 is known as the wild type producer of the alpha-glucosidase inhibitor acarbose, a potent drug used worldwide in the treatment of type-2 diabetes mellitus (Wehmeier and Piepersberg 2004; Schwientek et al. 2012). Applications which have been established already or might be used commercially in the future are described below.

Antibiotics

In 2012, the number of known antibiotic compounds produced by all the actinomycetes together amounted to about 13,700 (Bérdy 2012), and the proportion produced by members of the family *Micromonosporaceae* (mainly *Micromonospora*, *Actinoplanes*, and *Dactylosporangium*) increased from less than 1 % in 1966 up to 10 % in 2005 (Lechevalier and Lechevalier 1967; Bérdy 2005). As can be seen in *Table 28.21*, genera in the family *Micronomosporaceae* cover nearly all of the chemical groups of antibiotics that are produced by the genus *Streptomyces*. But they complement one another, only the β-lactam antibiotics seem to be absent.

Micromonospora Among the antibiotics produced by the family *Micromonosporaceae*, those of *Micromonospora* occupy the most important commercial position (Crueger and Crueger 1982). An intensive screening of *Micromonospora* species as sources for new antibiotics began in 1963 with the discovery of gentamicin (Weinstein et al. 1963a). Over 740 different antibiotics have been described (Bérdy 2005). Aminoglycosides of *Micromonospora* show antibiotic effect against both Gram-positive and Gram-negative bacteria and have been introduced into clinical practice. The gentamicins C1, C1a, and C2 are produced by *M. purpurea* and *M. echinospora* and exhibit excellent activity against *Staphylococcus aureus* and species of *Pseudomonas* and *Proteus*. Because of their nephrotoxicity, they are used in human therapy only for severe infections (Wagman and Weinstein 1980; Crueger and Crueger 1982; Kumar et al. 2008). Sisomycin and fortimicins, derived from *M. inyonensis* and *M. olivoasterospora*, respectively, have a similar spectrum of effectivity as gentamicin and can be used against gentamicin-resistant organisms (Wagman and Weinstein 1980).

The range of chemical structures produced by micromonosporae is quite large (*Table 28.21*). Examples include the aminoglycosides, represented by gentamicin, sisomicin, and verdamicin; the antibiotics G-52, G-418, and JI-20; mannosidostreptomycin; kanamycin; neomycin B (antibiotic 460); sagamicin (gentamicin C2b);

Table 28.21

Antibiotics groups produced by the genera of the *Micromonosporaceae* and the *Streptomyces*

Actinomycete	AG	ML	AML	BLA	PEP	GP	ANC	TC	NUC	POL	QN
Actinoplanes	+				+	+	+		+	+	+
Dactylosporangium	+	+			+			+	+	+	
Micromonospora	+	+	+		+		+		+		+
Salinispora		+			+						+
Verrucosispora		+			+				+		
Streptomyces	+	+	+	+	+	+	+	+	+	+	+

Table modified from Vobis (2006)

AG aminoglycoside, *ML* macrolide, *AML* ansamacrolide, *BLA* β-lactam, *PEP* peptide, *GP* glycopeptides, *ANC* anthracycline, *TC* tetracycline, *NUC* nucleotide, *POL* polyene, *QN* quinine, + production

paromamine fortimicins; and antibiotics 66–40 and SF 1854 (Nara et al. 1977; Wagman and Weinstein 1980). The macrolides comprise megalomycins, rosaramicin, juvenimicins, the M-4365 complex, erythromycins, and antibiotic XK 41-B-2. Examples for ansamacrolides (ansamycins) are halomicins, rifamycins, and compound 32656. Bottromycin, microsporonin, the 70591 complex, and actinomycin should be cited as representatives of the peptide antibiotics. Other miscellaneous antibiotics isolated from *Micromonospora* species include the oligosaccharides everninomycin and antlermicin, the nucleosides PA-1322 and XK-101–2, and the quinone PA-2046 (Wagman and Weinstein 1980).

Other compounds include arisostatins A and B (Furumai et al. 2000); ziracin oligosaccharides (Chu et al. 2002); anthraquinones lupacidins A, B, and C (Igarashi et al. 2007, 2011b); maklamicin (Igarashi et al. 2011a); and lipopeptide FW523-3 (Xie et al. 2011a). Most of the above compounds produced by micromonosporae showed antimicrobial and anticancer cell activities.

Actinoplanes More than 248 antibiotics are known from *Actinoplanes* species (Bérdy 2005), including chemical groups of peptides, glycopeptides, anthracyclines, nucleosides, polyenes, and quinones (Okami and Hotta 1988; Vobis 1992) (*Table 28.21*). The polypeptides generally exhibit activity against Gram-positive bacteria, such as the acidic peptide 41.012, an agent active against mycobacteria (Celmer et al. 1977). The antibiotics A-10947, A-7413, taitomycin, and gardimicin are sulfur-containing polypeptides; the latter two are also active against anaerobic bacteria (Parenti and Coronelli 1979; Yaginuma et al. 1979). The cyclic polypeptides A/287 and mycoplanecin show growth-promoting and antituberculosis effects, respectively (Hamill and Stark 1974; Nakajima et al. 1983).

The proline antimetabolite L-acetidine-2-carboxylic acid was isolated from *A. ferrugineus*. This amino acid has not been found in any other prokaryote and has only been found in eukaryotes (Palleroni 1979). *A. teichomyceticus* produces the glycopeptide teicoplanin (formerly called teichomycin A2), which is composed of six factors. It belongs to the vancomycin family (Malabarba et al. 1984). The same strain also produces a phosphorus-containing glycolipid (teichomycin A1). Both carbohydrate antibiotics are active against Gram-positive bacteria (Parenti et al. 1978). Some amino-acid derivatives are of clinical relevance, e.g., teicoplanin, a glycopeptide from *A. teichomyceticus* ATCC 31121^T (Bardone et al. 1978), actaplanin, a glycopeptide from *A. missouriensis* ATCC 23342 (Debono et al. 1984), and ramoplanin, a glycolipodepsipeptide from *Actinoplanes* sp. ATCC 33076 (Ciabatti and Cavalleri 1989).

Some metabolites belonging to various other chemical groups are also found in strains of *Actinoplanes*. The polycyclic xanthones actinoplanone A and B were found to be potent cytotoxins in in vitro assays with Hela cells (Kobayashi et al. 1988). Chuangxinmycin, composed of a unique bicyclic system formed of an indole nucleus fused to a thiopyran residue, is clinically effective in cases of septicemia and urinary and biliary infections caused by *Escherichia coli* (Parenti and Coronelli 1979). The antibiotic A/15104 Y is a chlorophenol derivative, active against bacteria and fungi. It represents the first example of a halogenated pyrrole from actinomycetes; the other biological sources have been sponges and pseudomonads (Cavalleri et al. 1978). A well-studied example of the antifungal polyenic macrolides is the antibiotic 67–121 (Sch16656). It is a complex of four polyene heptaenes produced by *A. caeruleus* (Horan and Brodsky 1986). It is also produced by *A. azureus*, a strain which also produces the plauracins (Parenti and Coronelli 1979). Purpuromycin is a naphthoquinone antibiotic of the rubromycin type, effective against bacteria and fungi (Coronelli et al. 1974). The neplanecins are nucleosides produced by another *A. regularis* strain. They are antitumor antibiotics with additional activities against phytopathogenic fungi (Yaginuma et al. 1981). Viriplanin is an anthracyclic antibiotic isolated from *A. regularis*, which shows activities against herpes simplex viruses (Hütter et al. 1986). Integramycin is an alkaloid that inhibits HIV-1 integrase produced by

Actinoplanes sp. ATCC202188 (Singh et al. 2002). A cyclic peptide, philipimycin, with strong antibacterial activities against Gram-positive bacteria is produced by *A. philippinensis* MA7347 (Zhang et al. 2008).

Dactylosporangium Pyridomycin is an antimycobacterial antibiotic produced by the type strain of *Dactylosporangium fulvum* SF 2113^T (Shomura et al. 1986). At present, more than 58 antibiotics are known from *Dactylosporangium* strains (Bérdy 2005), belonging to several chemical divisions (Table 28.21). As with *Micromonospora*, the major antibiotics seem to be aminoglycosides. Dactimicin is produced by *D. matsuzakiense* and *D. vinaceum* (Shomura et al. 1980, 1983b). It is a member of the pseudosaccharide group of antibiotics, is active against a wide variety of bacteria, including resistant strains with aminoglycoside-modifying enzymes (Omoto et al. 1987). The closely related aminoglycosides gentamicin, sisomicin, fortimicin, and antibiotic G-367 were also isolated from products of *D. thailandense* strain G-367. Aminoglycosides of the fortimicin antibiotic group are produced by *D. matsuzakiense* ATCC 31570^T (Dairi et al. 1992), and by the *Dactylosporangium* strain G 308. Another carbohydrate antibiotic is known from *D. roseum*, namely, the orthosomycine complex SF-2107 (Shomura et al. 1985). All the above-mentioned antibiotics are generally active against Gram-positive and Gram-negative bacteria.

Capreomycin, a polypeptide compound previously known from *Streptomyces capreolus*, was also isolated from "*D. variesporium*." It is of primary interest for its use as an antituberculosis agent (Tomita et al. 1977). The tetracycline antibiotic compound Sch34164 (Patel et al. 1987) and the macrolide tiacumicin (Hochlowski et al. 1986) have been isolated from other *Dactylosporangium* species. Dactylocyclines A and B, produced by strain SC 14051, have tested positive against tetracycline-resistant bacteria (Tymiak et al. 1993). Tiacumicins, a complex of 18-membered macrolide antibiotics, are metabolites produced by strain AB718C-41, described as *D. aurantiacum* subsp. "hamendensis" (Theriault et al. 1987). Tiacumicins B and C present positive results against diarrhea-associated bacterium *Clostridium difficile* in vitro and in vivo tests (Swanson et al. 1991). Strain SF-2253 produces L-threo-b–hydroxyaspartic acid, an antibiotic useful against a wide spectrum of microorganisms. This amino acid is also an inhibitor of glutamate uptake, frequently used in neurological studies (Alexander et al. 1997). All these examples suggest that the capacity for producing antibiotic metabolites in this genus may be very large.

Salinispora Salinisporae are best known for their production of secondary metabolites including the highly selective proteasome inhibitor salinosporamide A (Feling et al. 2003), which is currently undergoing clinical trials for the treatment of cancer (Fenical et al. 2009). Members of the genus *Salinispora* have proven to be a particularly rich source of new chemical structures. Many distinct structural types have been characterized from this genus, including the hybrid PKS-NRPS salinosporamides A, B, and C (Feling et al. 2003; Williams et al. 2005); the modified enediyne sporolide polyketides A and B (Buchanan et al. 2005; McGlinchey et al. 2008); the novel polyene macrolactam salinilactam A (Udwary et al. 2007); the compounds known as arenicolides (Williams et al. 2007); the depsipeptide arenamides (Asolkar et al. 2009, 2010); the polyketide rifamycin, the cyclic heptapeptide cyclomarin A, the indolocarbazole staurosporine (Fenical and Jensen 2006) and their derivatives saliniketal (Williams et al. 2007) and cyclomarazine (Schultz et al. 2008) from *S. arenicola* CNS-205. In addition, the antitumor antibiotic lomaivitacin (He et al. 2001), the cyanosporasides A and B (Oh et al. 2006), and four new compounds of new polyketides, salinipyrones A and B and pacificanones A and B, are produced by "*S. pacifica*" strains CNS103 and CNS-237, respectively. The 16S rRNA gene sequence of the two "*S. pacifica*" strains differs by only three nucleotide positions, but the chemical screening by LC-MS analysis indicated that their secondary metabolite profiles are very different.

Based on the analysis of two genome sequences of *S. tropica* CNB-440 and *S. arenicola* CNS-205, secondary metabolism is the major functionally annotated class of metabolic genes that differentiates the two species (Penn et al. 2009, 2012). This is supported by the observation that secondary metabolite production occurs in species-specific patterns with *Salinispora arenicola* strains producing rifamycins and staurosporines while *Salinispora tropica* strains produce salinosporamides and sporolides (Jensen et al. 2007).

Verrucosispora *Verrucosispora* is another genus from marine environment that has attracted considerable interest as they produce new bioactive compounds, exemplified by the discovery of the polycyclic polyketides, abyssomicins A to H from *V. maris* AB-18-032 (Bister et al. 2004; Keller et al. 2007; Goodfellow et al. 2012), the aminofuran antibiotics, proximicins A to C from *Verrucosispora* strain MG-37 (Fiedler et al. 2008), and the diterpenes, gifhornenolones A and B from *V. gifhornensis* (Shirai et al. 2010). Abyssomicin C is active against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-intermediate/resistant *Staphylococcus aureus* (VRSA) by inhibiting the *para*-aminobenzoic acid pathway that therefore inhibits folic acid biosynthesis at an early stage (Riedlinger et al. 2004). Proximicins show strong cytostatic effect on various human tumor cell lines (Fiedler et al. 2008). The whole genome sequence of *V. maris* AB-18-032^T revealed 23 biosynthetic gene clusters that encode the known or predicted secondary metabolites (Roh et al. 2011).

Inhibitors of α-Glucosidase as Pharmaceutical Drugs

In the course of screening for inhibitors of amylases and other mammalian intestinal carbohydrate-splitting enzymes, strains of *Actinoplanes* exhibited higher amounts of activity than did those of *Streptomyces* and *Streptosporangium*

(Frommer et al. 1979). Applied orally together with food carbohydrates like starch and other oligosaccharides, these glycoside hydrolase inhibitors slow down oligosaccharide decomposition and reduce or avoid postprandial hyperglycemia and hyperinsulinemia of type IV. Therefore, they may be useful to treat metabolic illnesses such as diabetes mellitus, adipositas, and hyperlipoproteinemia (Truscheit et al. 1981; Creutzfeldt 1988).

Pseudo-oligosaccharides with an essential core consisting of an unsaturated cyclitol and 4-amino-4, 6-dideoxyglucose are the most important group of α-glucosidase inhibitors. In culture filtrates of *Actinoplanes* strain SE 50, a very effective pseudotetrasaccharide with the generic name "acarbose" was found. This low-molecular-weight compound is stabile to acid, alkali, and heat treatment and exhibits pronounced inhibition of sucrase, maltase, and amylase (Truscheit et al. 1981). Since 1990, the large-scale fermentation has been performed in industrial production of acarbose (Wehmeier and Piepersberg 2004). The complete genome sequence of *Actinoplanes* sp. SE50/110 was just published recently. The 9.2 Mb genome consists of one circular chromosome. Besides the already published acarbose biosynthetic gene cluster sequence, several new gene clusters of non-ribosomal peptide, polyketide, and their hybrids were identified (Schwientek et al. 2012).

Enzymes

Xylose isomerase can be obtained from strains of *Actinoplanes* and *Micromonospora* (Crueger and Crueger 1982; Peczyńska-Czoch and Mordarski 1988). The glucose isomerase converts D-glucose into D-fructose and is used commercially in the starch industry to obtain high-fructose corn syrup (Aunstrup et al. 1979). Starting from about 95 % glucose syrup, a twice sweeter fructose syrup is produced which is usually composed of 53 % of D-glucose, 42 % of D-fructose, and 5 % of oligosaccharides (Crueger and Crueger 1982).

Actinoplanes missouriensis strain ATCC 14538 produces an intracellular, soluble glucose isomerase with a molecular weight of about 80,000 Da. The optimal pH of the enzyme is 7.0 at temperatures between 60 °C and 65 °C. A requirement for cobalt ions for optimal activity is eliminated if the proper amount of magnesium ions is used (Gong et al. 1980). The xylose isomerase of *Ampullariella* strain ATCC 31354 (transferred later to *Actinoplanes*) exhibits superior thermostability and activity over a wide range of conditions. However, the strain itself is difficult to use as a production organism, which makes it desirable to clone and express its enzyme in a more convenient microorganism (Saari et al. 1987).

An extracellular enzyme system capable of lysing cells of various yeast species was produced by *Micromonospora chalcea* when grown on a defined medium containing laminarin as the sole carbon source. β (1 → 3) glucanase and protease were the most prominent hydrolytic activities present in the culture supernatants. The system also displayed weak chitinase and β (1 → 6) glucanase activities while devoid of mannanase activity (Gacto et al. 2000).

The genome analysis results showed that *Micromonospora* strains L5 and Lupac 08, isolated from *C. equisetifolia* and *Lupinus angustifolius* nitrogen-fixing nodules, respectively, have cellulase and xylanase, pectin and chitin activities (Hirsch and Valdés 2010; Alonso-Vega et al. 2012).

Application in Agriculture

Reports of hyperparasitism by actinoplanetes on parasitic *Peronosporales* and *Saprolegniales* demonstrate a possible biological control of serious diseases of economic plants (Lechevalier 1988). The oospores of pink rot-causing *Phytophthora megasperma* var. *sojae* or f. sp. *glycinea* can be parasitized by certain strains of *Actinoplanes* and *Micromonospora* (Sneh et al. 1977). The hyphae of *Actinoplanes missouriensis* penetrate the walls of the oogonia and the oospores without forming appressoria or haustoria or changing the morphological and internal structures of the oospores (Sutherland et al. 1984). In greenhouse experiments, the root rot of soybeans caused by *Phytophthora* could be reduced by *Actinoplanes missouriensis*, *A. utahensis*, and *Micromonospora* sp. (Filinow and Lockwood 1985). A number of studies have already implicated *Micromonospora* and other actinomycetes as biocontrol agents in the protection of carrot (El-Tarabily et al. 1997), wheat (Coombs and Franco 2003) Chinese cabbage (Lee et al. 2008), and cucumber (El-Tarabily et al. 2009).

Conn et al. (2008) demonstrated that *Micromonospora* and other endophytic actinobacteria were able to suppress a number of pathogens, both in vitro and *in planta*, through the activation of key genes in the systemic acquired resistance or the jasmonate/ethylene pathways in *Arabidopsis thaliana*. Antibiotic and cellulase production is another way to inhibit plant pathogens (Shomura et al. 1983a; El-Tarabily et al. 1996; Ismet et al. 2004). An isolate from mangrove rhizosphere soil in West Malaysia, *Micromonospora* sp. M39, produces metabolites including 2, 3-dihydroxybenzoic acid, phenylacetic acid, and the antibiotics cervinomycin A1 and A2 that are effective against the rice blast pathogen *Pyricularia oryza* MPO 292 (Ismet et al. 2004). Large *Micromonospora* populations have been isolated from nitrogen-fixing nodules of legume and non-legume plants (Valdés et al. 2005; Trujillo et al. 2010). These studies suggest that the genus *Micromonospora* maintains a close interaction with these plants.

Furthermore, the presence of this microorganism does not appear to be restricted to a single legume plant species, and has been isolated from at least 20 different legume plant species (Trujillo et al. 2006, 2007, 2010; García et al. 2010; Carro et al. 2012). The potential ecological role played by this actinobacterium in both actinorhizal and legume root nodules is yet to be unraveled.

The acidic substance SF 2185 is an antibiotic against plant pathogens, particularly the causal organisms of cucumber downy mildew and rice blast. The producing strain SF-2185 was identified as *D. aurantiacum* subsp. "gifuense"

(Matsumoto et al. 1985). *D. aurantiacum* strain SANK 61299 produces the plant growth inhibitors streptol, A-79197-2 (disaccharide of streptol), and A-79197-3 (trisaccharide of streptol), which inhibit the germination of *Brassica rapa*. The depsipeptide antibiotics plauracin A 17002 and A2315 belong to the virginiamycin group, composed of a mixture of macrocyclic lactones and depsipeptides. They can be used for growth promotion in chicken, swine, and ruminants (Hamill and Stark 1975; Parenti and Coronelli 1979). "*D. salmoneum*" produces the polyether antibiotic compound 44,161, which is useful for the control of coccidiosis in poultry and improving feed efficiency in ruminants (Celmer et al. 1978). The compound 44161 is identical to nigerimicin, which is also known as a herbicidal agent (Heisey and Putnam 1990). Members of *Dactylosporangium* produce the streptol moieties that are important for herbicidal activity (Kizuka et al. 2002).

Degradation and Remediation

The production of hydrolytic enzymes allows *Micromonospora* species to play an active role in the degradation of organic matter in their natural habitats. Most *Micromonospora* species probably degrade biopolymers (Erikson 1941), and they can even attack lignin complexes (McCarthy and Broda 1984). Many of the salt marsh isolates of Hunter et al. (1981) were active in the decomposition of chitin and cellulose. In particular, cellulose is frequently utilized as substrate (Jensen 1930; Sandrak 1977; Kawamoto 1989; de Menezes et al. 2008). The cellulose studied from "*Micromonospora melanosporea*" was found to be more heat stable than those of the fungus *Trichoderma*, but less stable than the enzymes of thermophilic actinomycetes. The principal sugar released by *Micromonospora* cellulase from ball-milled bagasse and filter paper was cellobiose (Van Zyl 1985). *Micromonospora* strains isolated from the water column, sediment, and cellulose baits placed in freshwater lakes were shown to be able to degrade cellulose in lake water without any addition of nutrients (de Menezes et al. 2008, 2012). *Micromonospora* together with other three genera were able to efficiently degrade rice straw pieces in minimal medium, causing significant weight loss between 50 % and 61 %. Application of this knowledge may improve the management of waste rice straw (Abdulla and El-Shatoury 2007).

Several members of the family *Micromonosporaceae* (e.g., *Actinoplanes*, *Micromonospora*, and *Dactylosporangium*) were found to degrade rubber and use it as a sole carbon source. An endo-cleavage mechanism of degradation was indicated by the molecular mass reduction (Jendrossek et al. 1997; Rose and Steinbüchel 2005). *Micromonospora aurantiaca* strain W2b was found to be a weaker rubber decomposer that did not grow adhesively but formed clear zones around its colonies after cultivation on natural rubber dispersed in mineral agar (Linos et al. 2000). This activity may also have potential biotechnological applications.

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29 The Family *Mycobacteriaceae*

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Abstract
In this chapter, the medically-important species of the family of *Mycobacteriacea*, with a single genus, *Mycobacterium*, will be briefly discussed.

The family of *Mycobacteriaceae* contains the single genus *Mycobacterium* with over 150 species (Fig. 29.1). They are broadly distributed in various natural environments, and with several notable exceptions, they do not appear in parasitic or disease associations. This genus also includes *Mycobacerium tuberculosis*, currently responsible for serious bacterial infections of humans in many parts of the world, with millions of deaths annually. Other *Mycobacterium* species can cause a variety of infections as well; for example, worldwide, there are over quarter million new cases of leprosy, a debilitating disease caused by *Mycobacterium leprae*. A number of mycobacterial saprophytes have been shown to cause opportunistic infections in immunocompromised individuals, and many are pathogens of animals.

Mycobacteriaceae are obligate aerobic organisms; they do not take up stain well in the Gram-staining procedure but are considered Gram-positive based on the structure and composition of their cell envelope. They are acid fast when the Ziehl-Neelsen stain is applied and appear as rods in chains or as individual cells. Growth requirements vary from simple media containing carbon, nitrogen, and energy sources to more substantially supplemented media, primarily with molecules involved in iron acquisition or certain vitamins. Some extreme examples such as *Mycobacterium leprae* cannot be cultured on laboratory media at all. Most other species can utilize sugars, simple alcohols, and acids as well as complex organic molecules such as aromatic or aliphatic hydrocarbons. They possess a full electron transport chain, and under hypoxic conditions, electron acceptors other than oxygen can be used, most commonly nitrate or fumarate. The various *Mycobacterium* species exhibit a great variation of growth rates, with doubling times ranging from a few hours to days. Although they can grow on simple media, their growth improves significantly when rich sources of lipids and fatty acids are included, and egg yolk is usually included in media to stimulate growth. These growth differences are used occasionally to categorize mycobacteria into fast- and slow-growing groups, and this division is in general supported by phylogenetic clustering based on molecular markers such as 16S rRNA genes or whole genome sequence comparisons. Overall, pathogenic *Mycobacteriaceae* grow slowly on most laboratory media while the nonpathogenic strains belong to the fast growth group (Cook et al. 2009).

One of the key features of the physiology of *Mycobacterium* species is the presence of a thick, relatively impermeable cell wall consisting of various lipids attached to peptidoglycan, the most prominent of which are differentially substituted forms of mycolic acid. The mycolic acids are highly hydrophobic because of their abundance and the size of the fatty acid chains ranging between 60 and 100 carbons in length. It is this structure that renders mycobacteria largely impermeable, and although they are Gram-positive, the outer layer of the cell wall encloses a compartment analogous to the periplasm of Gram-negative bacteria.

Owing to its medical importance, the most extensively studied *Mycobacterium* species is the obligate human pathogen *M. tuberculosis*, the causative agent of the disease tuberculosis. The bacteria are transmitted from infected individuals by aerosol in droplets following sneezing or coughing. Once inhaled, the mycobacteria reach the alveoli of the lung and are phagocytized by alveolar macrophages frequently resulting in their elimination. However, in a fraction of infected individuals, *M. tuberculosis* are not killed by the macrophages, and instead, they replicate within these immune cells. The infected macrophages are often killed; the bacteria are released and infect other macrophages. *M. tuberculosis* can spread within the lung and cause pulmonary tuberculosis. The bacteria can also enter the blood stream through damaged vessels and disseminate to various organs where they can replicate and cause tuberculosis-like diseases in bones and joints, kidneys, and intestinal tract and reinfect the lung. In the majority of infected individuals, the bacteria are restricted to a small area of the lung; the infected cells are walled off and form a small granuloma (an aggregate of cells and fibrous material). This is called the latent infection, and the majority of the *M. tuberculosis* cells are confined, and some even lose viability. However, in ca. 10 % of individuals with latent tuberculosis, the dormant bacteria can be reactivated often years after the initial exposure and lead to active tuberculosis.

Several other *Mycobacterium* species are phylogenetically closely related to *M. tuberculosis*; they are responsible for primary human infections or they can be transmitted from infected animals. Together, these are often referred to as "*M. tuberculosis* complex" and include, in addition to *M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microti*, *Mycobacterium caprae*, and *Mycobacterium pinipedii*. The genetic and therefore physiological closeness of this group is highlighted by the efficacy of a vaccine derived from *M. bovis* (*M. bovis* BCG) in protecting against human infections by *M. tuberculosis*.

M. africanum causes tuberculosis exclusively in West Africa or in emigrants from this region of the world. It is a true opportunistic pathogen capable of infecting individuals with compromised defenses including the elderly, those with an

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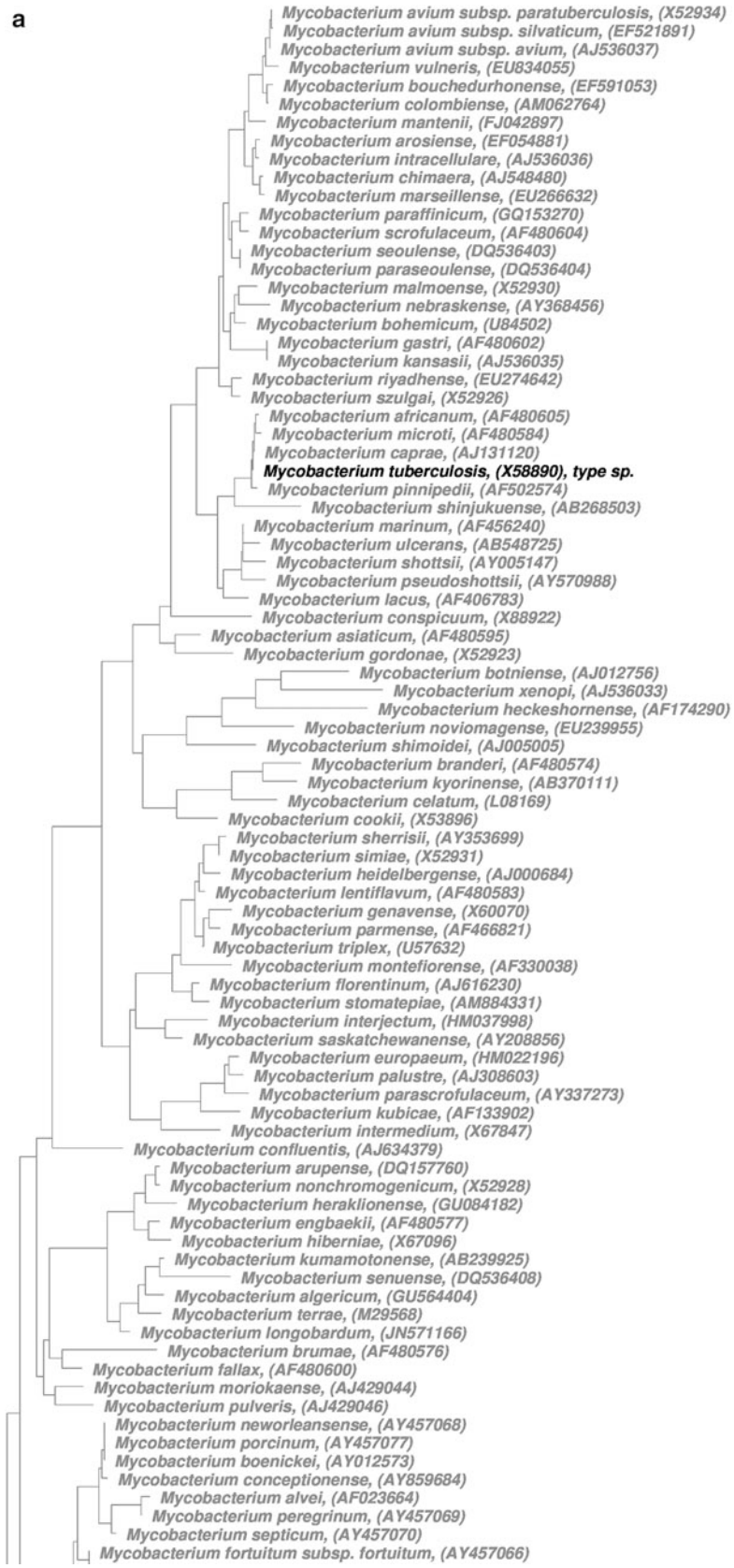

Fig. 29.1 (Continued)

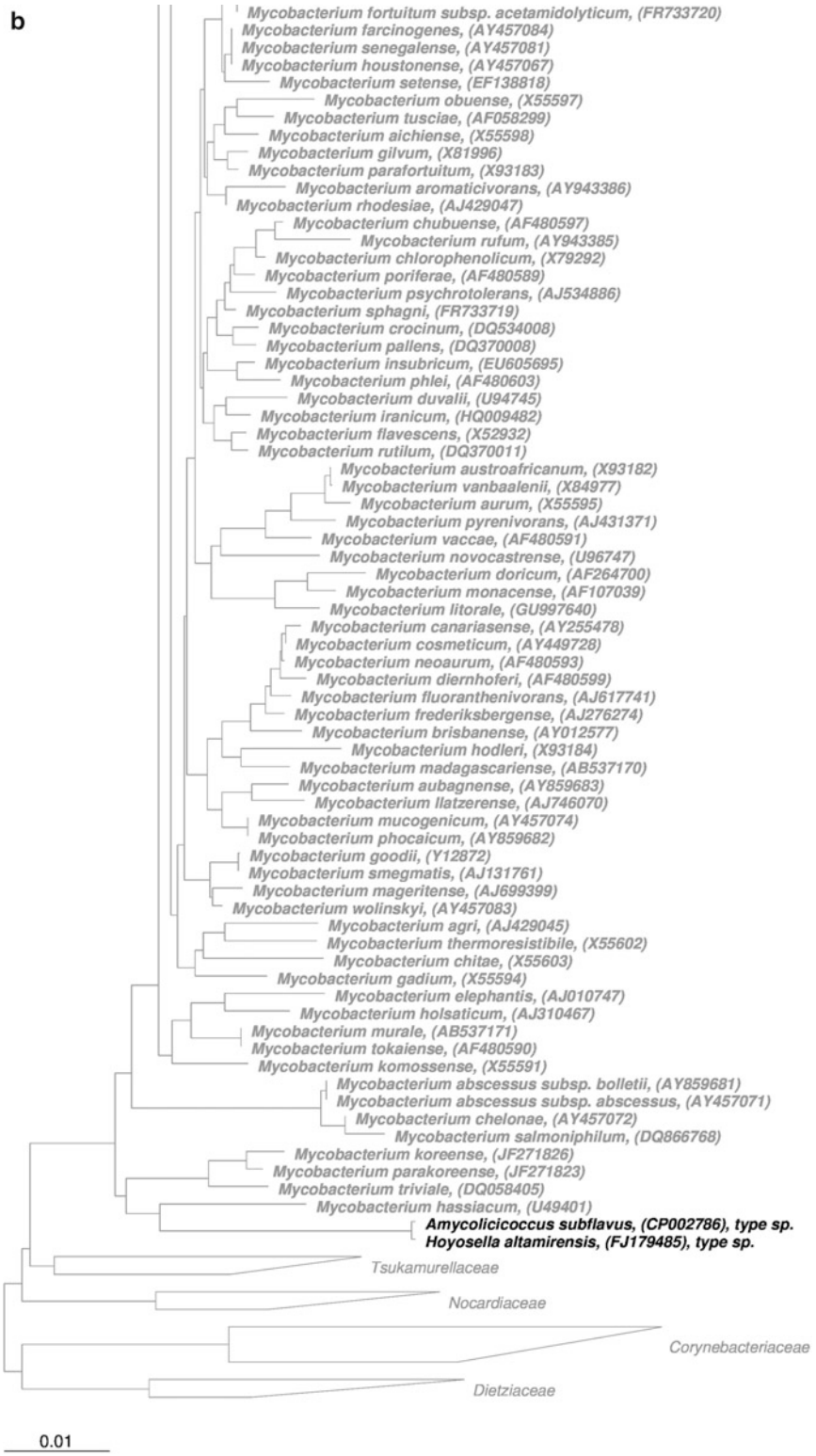

Fig. 29.1

Phylogenetic reconstruction of the family *Mycobacteriaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. (a and b) Overlap in order to follow through the whole topology. 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

HIV infection, or suffering from malnutrition. Patients with an *M. africanum* infection present the classical pulmonary and extrapulmonary symptoms of tuberculosis. *M. bovis* can infect domestic animals (cattle, goats, and pigs) and occasionally wild animals such as bison, deer, and elk. Cattle are the most common animal hosts for *M. bovis*, where it causes a disease called bovine tuberculosis. Animals with active diseases usually transmit the bacteria between each other by aerosol; on the other hand, zoonotic transmission to humans can take place by any one of several routes including inhalation and consumption of or direct contact with the flesh or milk of the infected animals. Bovine tuberculosis is similar to tuberculosis in humans with a major lung involvement and potential dissemination into organs. In humans, *M. bovis* infection by the respiratory route follows a similar course as the disease caused by *M. tuberculosis* including an ability to establish a latent form of the disease. Ingestion of infected animal products usually leads to a disease that remains localized in the intestinal tract and lymphatic tissues. Small wild rodents and farm animals are the primary reservoirs of *M. microti*, and they can occasionally infect humans. One common route is via cats that serve as intermediary hosts. The symptoms in humans are those of classical pulmonary tuberculosis. Similar disease is caused by the opportunistic pathogen *M. canettii*; it is endemic in Eastern Africa, and the mode of transmission remains unknown. *M. pinnipedii* has been isolated from seals, where it caused tuberculosis-like lung lesions. Transmission to animal handlers, but without symptoms of the disease, was detected using serological methods,

Mycobacterium leprae is responsible for leprosy (Hansen's disease), an infection of the skin, the subcutaneous tissue with a major involvement of the peripheral nervous system, leading to the loss of sensation, while the infection of the eyes can result in blindness (Britton and Lockwood 2004). Nerve damage can result in disfigurement and loss of limbs. Leprosy is a chronic infection with a long latent period of several years. The slow progression of the disease makes it difficult to trace the mode of transmission. Air droplets from patients with the disease are the most likely sources of new infections. *M. leprae* is one of the few *Mycobacterium* species that has not been cultured in any laboratory medium necessitating passage via animal hosts, usually in footpads of mice or in armadillos. The doubling time in these animals is approximately 2 weeks. For the laboratory identification of the infecting organisms, direct microscopic observations of samples from the skin or various lesions showing acid-fast rod-shaped bacteria, together with the classical symptoms of leprosy, provide the definitive evidence for the confirmation of leprosy. A new form of leprosy was recently identified in Central America; it is caused by *Mycobacterium lepromatosis*, a species closely related to *M. leprae*. Since neither organism can be cultured and appears identical microscopically, they can be differentiated from each other only by polymerase chain reaction–based species-specific assays of DNA obtained from infected lesions.

The remaining *Mycobacterium* species with a disease-causing potential are frequently called nontuberculous mycobacteria. These mycobacteria represent a heterogeneous group of broadly distributed environmental organisms, and a substantial number have been implicated in infections of immunocompromised individuals (Esteban et al. 2012). Four closely related organisms referred to as *M. avium* complex are commonly found in fresh- and saltwater and occasionally in animals (Rindi and Garzelli 2014). *Mycobacterium avium* Subsp. *hominissuis* causes lung and soft tissue infections in immunodeficient individuals such as those with AIDS; the *Mycobacterium avium* Subsp. *avium* infects chickens, where it can cause avian tuberculosis; *Mycobacterium avium* Subsp. *Paratuberculosis* causes in ruminants Johne's disease, a chronic progressive enteritis, and *Mycobacterium avium* Subsp. *silvaticum* is responsible for a tuberculosis-like disease in pigeons. In humans, they can cause initially a respiratory disease and subsequently a disseminated infection most commonly in patients with HIV infections. There is little information regarding the mode of acquisition of these organisms; however, person-to-person transmission is very unlikely.

Sporadic infections of humans by *Mycobacterium kansassi*, *Mycobacterium malmoense*, *Mycobacterium simiae*, *Mycobacterium szulgai*, and *Mycobacterium xenopi* can be responsible for a respiratory disease in both immunocompetent and immunocompromised individuals. *Mycobacterium abscessus*, *Mycobacterium chelonae*, *Mycobacterium haemophilum*, *Mycobacterium ulcerans*, and *Mycobacterium marinum* cause skin and soft tissue infections.

The availability of the genome sequences of a large number of *Mycobacterium* species greatly increased our understanding of genetic changes that took place during the evolution and environmental adaptation of this group of organisms (Behr 2013). Moreover, comparing sequence variations including the location of single-nucleotide changes in the same genes in different species and strains within individual species allows for a reconstruction of the evolutionary history of various mycobacteria. It is now recognized that the progenitor of current virulent *M. tuberculosis* originated from environmental mycobacteria, most likely a strain of *M. canettii* in East Africa (Supply et al. 2013). Its genome and that of early *M. tuberculosis* ancestors was assembled through multiple horizontal gene transfer events. Following adaptation to latency, it reemerged, and at the time of animal domestication, it adapted to these new hosts as *M. bovis*. On its evolutionary path towards becoming a specialized pathogen with a limited host range, *M. tuberculosis* has undergone a significant genome reduction leading to a highly conserved genetic makeup within 4 megabase chromosomes with ca. 4,000 genes and a relatively low level of genetic variation at the nucleotide level. In contrast, other mycobacteria like *M. marinum* have assembled large mosaic genomes providing them an ability to survive in a broad range of environments including human hosts, where they can produce a tuberculosis-like disease.

A more dramatic reductive genome occurred in *M. leprae* with a massive loss of genes through the deletion or accumulation of inactivating mutations giving rise to pseudogenes (Singh and Cole 2011). Nearly half of *M. leprae* genes are nonfunctional due to mutations, and some are completely

or partially deleted. Another remnant of active reduction of the genome is the presence of large numbers of related insertion sequences and repetitive genetic elements dispersed in the chromosome; these can facilitate the deletion of large blocks of DNA or a rearrangement of groups of genes by homologous or site-specific recombination. The direct consequence of these massive genetic changes is greatly restricted metabolic capabilities and the confinement of this organism to a limited number of hosts.

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30 The Family *Nakamurellaceae*

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Abstract
Nakamurellaceae, a family within the order "*Frankiales*," originally comprised the three monospecific genera, *Nakamurella*, *Humicoccus*, and *Saxeibacter*. Very recently, with the description of a novel taxon (*Nakamurella panacisegetis*) within *Nakamurellaceae*, the genera *Humicoccus* and *Saxeibacter* were united with the genus *Nakamurella* on the basis of high levels of 16S rRNA and *rpoB* gene sequence similarity, together with a robust phylogenetic relationship, and the overall chemotaxonomic similarity. Consequently, *Nakamurellaceae* is a monogeneric family and contains four species, *Nakamurella multipartita*, *Nakamurella flavida* (formerly *Humicoccus flavidus*), *Nakamurella lactea* (formerly *Saxeibacter lacteus*), and *N. panacisegetis*. Members of the family *Nakamurellaceae* are mainly found in soil, but they have been isolated from activated sludge, a rock, glacier sediment, and a plant root as well. This chapter is a modified and updated version of previous family descriptions (Tao et al. Int J Syst Evol Microbiol 54: 999–1000, 2004; Zhi et al. Int J Syst Evol Microbiol 59:589–608, 2009).

Taxonomy: Historical and Current

Short Description of the Family

Na.ka.mu.rel.la'ce.ae. N.L. fem. n. *Nakamurella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Nakamurellaceae* the *Nakamurella* family. The description is an emended version of the descriptions given by Tao et al. (2004) and Zhi et al. (2009).

Phylogenetically a member of the order "*Frankiales*" (Normand and Benson 2012), class *Actinobacteria*, phylum Actinobacteria. The pattern of 16S rRNA signatures consists of nucleotides at positions 195 (G), 196 (C), 841 (C), 952:1229 (C-G), 955:1225 (A-U), 986:1219 (A-U), 1059:1198 (U-A), and 1308:1329 (U-A) (Stackebrandt et al. 1997; Zhi et al. 2009). The family contains only the type genus *Nakamurella* (Tao et al. 2004; emended by Kim et al. 2012).

The family description is based upon the phylogenetic position and the presence of 16S rRNA sequence signatures. Phenotypic properties that differentiate *Nakamurellaceae* from the nearest neighboring families are summarized in ❯ *Table 30.1*.

Phylogenetic Structure of the Family and Its Genera

According to the phylogenetic branching of actinobacterial type strains in the RAxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010; Release LTPs108), the family is moderately related to the families *Cryptosporangiaceae*, *Sporichthyaceae*, and *Geodermatophilaceae* of the order "*Frankiales*." The reduced tree (❯ *Fig. 30.1*) also shows that the family *Nakamurellaceae* is closely related to the three families, the latter being a sister clade. However, *Nakamurellaceae* is clearly distinguished from the neighboring families in that it contains a menaquinone with eight isoprene units as its major quinone component and does form neither hyphae nor spores (❯ *Table 30.1*). Until now, a couple of studies have indicated that the six families of the order "*Frankiales*" do not form a coherent phylogenetic lineage, and the taxonomy of this order needs to be emended (Normand 2006; Gao and Gupta 2012; Ludwig et al. 2012). According to the publication by Zhi et al. (2009), the

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Table 30.1

Phenotypic properties that differentiate the family *Nakamurellaceae* from the three neighboring families of the order "*Frankiales*" (Fig. 30.1)

Characteristic	*Nakamurellaceae*[a]	*Cryptosporangiaceae*[b, c, d]	*Sporichthyaceae*[e, f]	*Geodermatophilaceae*[g, h, i, j]
Morphology	Cocci, short rods	Substrate and aerial mycelia	Short aerial hyphae; no substrate mycelium	Thallus consisting of cuboid to oval cells; multilocular sporangia; rudimentary hyphae; no aerial mycelium[g, j] or Cocci, rods, vibrios, pairs, tetrads, clusters[i] or Rods, cocci[h]
Spore/bud formation	–	Sporangiospores[b, c] or –(fragmentation of aerial hyphae)[d]	Coccoid to rod-shaped spores	Zoospores[g, j] or Buds[h, i]
Motility	–	+/–	+	+/–
Cell-wall diamino acid[k]	*meso*-DAP	*meso*-DAP	LL-DAP	*meso*-DAP
Diagnostic polar lipids[k]	DPG, PE, PI, APL, PGL	PE[b] or DPG, PE, PI, PS[d]	DPG, PG, PI, PL	DPG, PE, PI, PIM[g, j] or DPG, PE, PG, PI[h, i]
Major menaquinones	MK-8 (H_4)	MK-9(H_6), MK-9(H_8), MK-9(H_4)	MK-9(H_8), MK-9(H_6), MK-8(H_6)	MK-9 (H_4)
DNA G + C content (mol%)	68–74	65–76	70–71	68–75

Symbols and abbreviations: +, present; –, absent; +/–, variable

Data taken from:

[a]Kim et al. 2012

[b]Tamura et al. 1998

[c]Tamura and Hatano 2001

[d]Carlsohn et al. 2008

[e]Rainey et al. 1993

[f]Tamura et al. 1999

[g]Luedemann and Fonseca 1989

[h]Mevs et al. 2000

[i]Urzì et al. 2004

[j]Zhang et al. 2011

[k]*DAP* diaminopimelic acid, *APL* unidentified aminophospholipid, *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PGL* unidentified phosphoglycolipid, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PL* unidentified phospholipid, *PS* phosphatidylserine

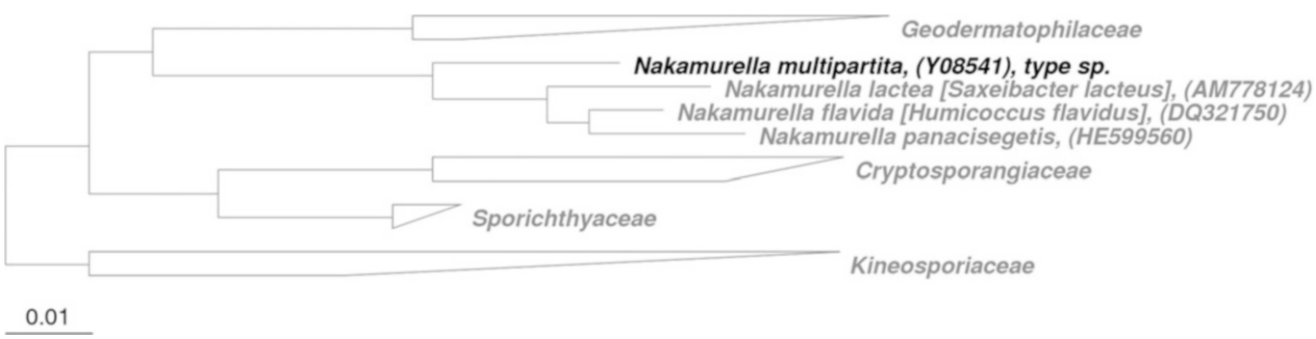

Fig. 30.1

Maximum-likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the family *Nakamurellaceae*. 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 1 % sequence divergence

families *Frankiaceae* (including the type of the order) and *Acidothermaceae* branched independently of the families *Nakamurellaceae*, *Cryptosporangiaceae*, *Sporichthyaceae*, and *Geodermatophilaceae* in the maximum-parsimony tree and furthermore, *Nakamurellaceae* branched independently of the other families of the order "*Frankiales*" in the maximum-likelihood tree. Therefore, the affiliation of the family *Nakamurellaceae* into the order "*Frankiales*" remains uncertain.

The family *Microsphaeraceae* was created by Stackebrandt et al. (1997) to accommodate a single genus and species, *Microsphaera multipartita* (Yoshimi et al. 1996), on the basis of phylogenetic position and the presence of a unique set of 16S rRNA sequence signatures. Later, the name *Microsphaera* was proven to be illegitimate because of precedence of the fungal genus *Microsphaera* (Wallr.) (Léveillé 1851), and accordingly, the genus and family names were replaced by *Nakamurella* and *Nakamurellaceae*, respectively (Tao et al. 2004). Originally, *Nakamurella multipartita* (formerly *Microsphaera multipartita*) was a strictly aerobic, Gram-positive, nonmotile, non-spore-forming, coccus-shaped actinobacterium (Yoshimi et al. 1996). The chemotaxonomic markers characteristic of the genus *Nakamurella* were MK-8(H_4) as the predominant menaquinone; $C_{15:0}$ iso, $C_{16:0}$ iso, and $C_{18:1}$ as the major fatty acids; and *meso*-diaminopimelic acid in the cell-wall peptidoglycan (Yoshimi et al. 1996). Later, the genus *Humicoccus* was described in *Nakamurellaceae*, this was distinguished from the genus *Nakamurella* by the differences in predominant menaquinones, major fatty acids, and whole-cell sugars (Yoon et al. 2007), and more recently, the genus *Saxeibacter* was reported to be another deep-rooted member of this group and characterized by some unique features with respect to cell morphology, colony pigmentation, and fatty acid and polar lipid profiles (Lee et al. 2008). Subsequently, the description of *Nakamurellaceae* was emended due to the newly described genera and the availability of their 16S rRNA gene sequences (Zhi et al. 2009).

Very recently, the taxonomic reevaluation of the genera *Nakamurella*, *Humicoccus*, and *Saxeibacter* was carried out during the description of a novel taxon that occupies an intermediate position within the family *Nakamurellaceae* (Kim et al. 2012). It revealed that the three genera of *Nakamurellaceae* are related to each other at the genus level, considering high levels of 16S rRNA and *rpoB* gene sequence similarity and the overall chemotaxonomic similarity. Accordingly, the genera *Humicoccus* and *Saxeibacter* were united with the genus *Nakamurella* having a priority, and as such, the novel taxon was assigned to the genus *Nakamurella* with the name *Nakamurella panacisegetis* (Kim et al. 2012). At that time, the description of the genus *Nakamurella* was emended, but the emendation of the family was not done. Currently, *Nakamurellaceae* is a monogeneric family containing four species, *N. multipartita*, *Nakamurella flavida* (formerly *Humicoccus flavidus*), *Nakamurella lactea* (formerly *Saxeibacter lacteus*), and *N. panacisegetis*, which form a robust phylogenetic relationship, irrespective of algorithms applied to the set of 16S rRNA gene sequences (Kim et al. 2012).

Molecular Analyses

The phylogenetic dendrogram (Fig. 30.1) of *Nakamurellaceae* species indicates the presence of one sister clade. The clade is composed of *N. flavida* and *N. panacisegetis*. *N. lactea* branches more deeply, and the outermost species is *N. multipartita*. According to the maximum-likelihood tree showing relationships between all representative members of the order "*Frankiales*" (Kim et al. 2012), *N. flavida* and *N. lactea* form a sister clade that shares 97.4 % 16S rRNA gene sequence similarity with *N. panacisegetis* branching more deeply (~97.0 % similarity). *N. multipartita* also occupies the outermost position and shows ~96.4 % similarity to the other *Nakamurella* species. Next, the type strains of *Nakamurellaceae* species show ~94.2 % similarity to *Sporichthya* type strains and ~93.8 % similarity to *Blastococcus* type strains. The dendrogram based on complete *rpoB* gene sequences also shows a robust phylogenetic relationship between *Nakamurellaceae* species, in which *N. panacisegetis* and *N. flavida* form a deep-rooted clade and *N. lactea* branches most deeply (Kim et al. unpublished). *rpoB* gene sequence similarity between the type strains of *Nakamurellaceae* species is 85.8–89.2 % (Kim et al. 2012), which is slightly higher than the 85.5 % cutoff value for genus delineation (Adékambi et al. 2008).

DNA-DNA hybridization (DDH) study on *Nakamurellaceae* has been performed only by Kim et al. (2012). During the description of the genus *Humicoccus*, *N. flavida* (*H. flavidus*) strain DS-52^T and the type strain of *N. multipartita* shared 96.5 % 16S rRNA gene sequence similarity, which was below the 97 % threshold value indicative of separate genomospecies (Stackebrandt and Goebel 1994). Therefore, no DDH study was done. During the description of the genus *Saxeibacter*, *N. lactea* (*S. lacteus*) strain DLS-10^T shared 97.1 % 16S rRNA gene sequence similarity with the type strain of *N. flavida* (*H. flavidus*). However, due to the significant phenotypic differences between them, DDH study was not done. According to the results of Kim et al. (2012), *N. panacisegetis* P4-7^T shows 8–15 % relatedness to *N. multipartita* KCTC 19567^T, *N. flavida* KCTC 19127^T, and *N. lactea* KCTC 19285^T, and *N. flavida* KCTC 19127^T shows 11–26 % relatedness to *N. multipartita* KCTC 19567^T and *N. lactea* KCTC 19285^T.

The only study including a DNA fingerprinting method was that of Kim et al. (2012), in which random amplified polymorphic DNA (RAPD) analysis was performed using the Ready-To-Go RAPD Analysis Beads (GE Healthcare), and genomic fingerprints of the type strains of *Nakamurellaceae* species generated with two RAPD analysis primers confirmed their separate species status. Neither riboprinting nor MALDI-TOF analysis is available for any member of the family *Nakamurellaceae*.

Genome Analysis

Nakamurella multipartita DSM 44233^T is the only strain of the family *Nakamurellaceae* for which the full genome sequence has been generated (INSDC ID CP001737) (Tice et al. 2010).

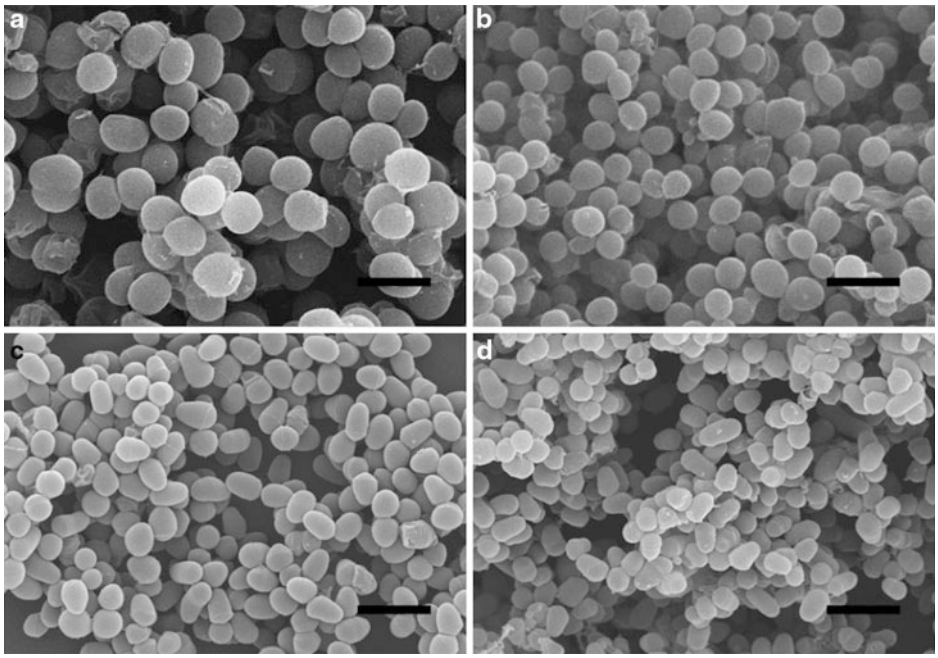

Fig. 30.2
Scanning electron micrographs of cells of *N. multipartita* KCTC 19567^T (a), *N. flavida* KCTC 19127^T (b), *N. lactea* KCTC 19285^T (c), and *N. panacisegetis* P4-7^T (d). Bars, 2 μm.

The single replicon genome, a part of the *Genomic Encyclopedia of Bacteria and Archaea* project, is 6,060,298-bp long with a G + C content of 70.92 mol%. This DNA G + C value is higher or slightly higher than those determined by the HPLC method; 67.5 mol% from Yoshimi et al. (1996) and 70.2 mol% from Kim et al. (2012). Extrachromosomal elements were absent. Besides 175 pseudogenes, 5,471 genes have been predicted of which 5,415 were protein-coding genes, and 56 were RNA genes. The majority of the genes (66.5 %) were assigned a putative function, 67.14 % of the genes were assigned to clusters of orthologous groups (COGs), while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COG functional categories indicates that the highest number of genes is involved in transcription (400; 9.1 %), followed by genes coding for carbohydrate transport and metabolism (341; 8.3 %) and amino acid transport and metabolism (334; 8.1 %). A detailed listing of COG categories is given by Tice et al. (2010).

The sequences of the two 16S rRNA gene copies in the genome of strain DSM 44233^T are identical but differ by three nucleotides (one N and two gaps) from the published 16S rRNA gene sequence of the same strain (Y-104^T) deposited as D50066. The former sequence was used as a reference by Kim et al. (2012), and the latter was used at the time of the original description of *N. multipartita* (Yoshimi et al. 1996). The sequence Y08541 of *N. multipartita* JCM 9343^T has been used in the Living Tree Project that is the basis for the tree shown in *Fig. 30.1* and other publications (Yoon et al. 2007; Lee et al. 2008).

Recently, a genome-based study on phylogenetic framework and molecular signatures for the main clades of the phylum Actinobacteria has been performed (Gao and Gupta 2012). The phylogenetic tree based upon concatenated sequences for 35 conserved proteins indicates that *N. multipartita*, a part of the order "*Frankiales*," forms an outgroup of the "*Pseudonocardiales*" clade, and a clade consisting of *N. multipartita* and "*Pseudonocardiales*" species is also strongly supported (100 % bootstrap value). However, other "*Frankiales*" species (i.e., *Frankia alni*, unclassified *Frankia* spp., *Acidothermus cellulolyticus*, and *Geodermatophilus obscurus*) do not branch with *N. multipartita*, which is in agreement with the 16S rRNA gene-based phylogeny shown in the maximum-likelihood tree by Zhi et al. (2009).

Phenotypic Analyses

The main features of members of *Nakamurellaceae* are listed in *Table 30.1*.

Nakamurella Tao, Yue, Chen and Chen 2004, 999^{VP}, Emend. Kim, Lee and Lee 2012, 294

Na.ka.mu.rel'la. M.L. dimin. ending *-ella*; N.L. fem. n. *Nakamurella* to honor the Japanese microbiologist Professor Kazunori Nakamura

Cells are strictly aerobic, Gram-positive, nonmotile, non-spore-forming, cocci or short rods (*Fig. 30.2*). A cell-wall-like structure occurs in the middle of cells (*Fig. 30.3*). Colonies on nutrient agar are 0.5–1.0 mm in diameter (within 4 days), circular, convex, smooth, and translucent to opaque. Catalase-positive. Oxidase-negative. The optimum growth temperature

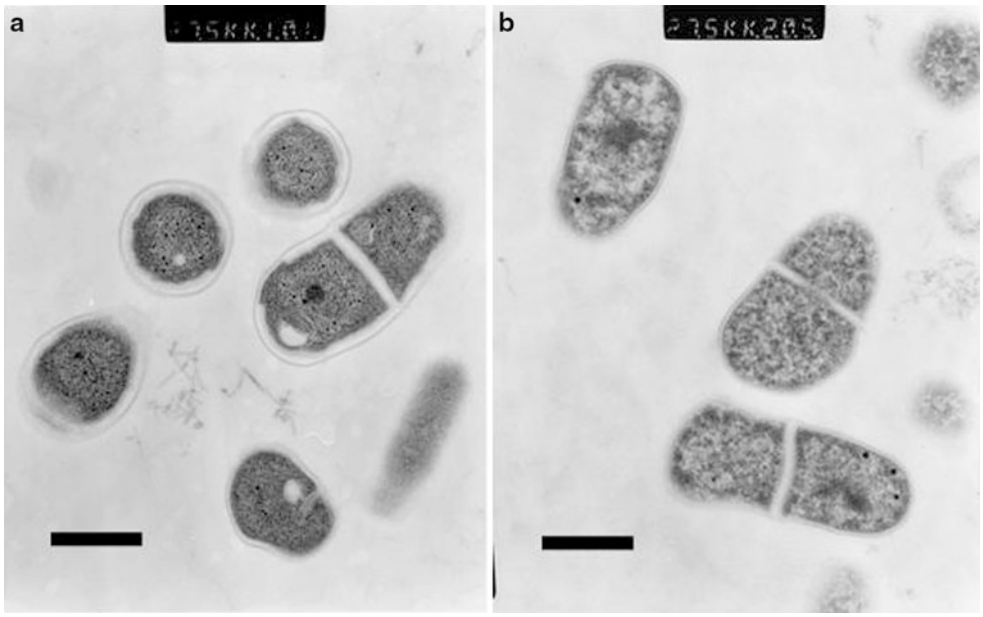

Fig. 30.3
Transmission electron micrographs of thin sections of *N. lactea* KCTC 19285^T (a) and *N. panacisegetis* P4-7^T (b) showing a cell-wall-like structure in the middle of cells. Bars, 0.5 μm

is 25 °C. The optimum pH is 6.0–7.0. NaCl tolerance range is variable. Indole and H_2S are not produced. The methyl red and Voges-Proskauer tests are negative. Hydrolyzes aesculin and gelatin, but not casein, hypoxanthine, Tweens 20 and 80, tyrosine, or xanthine. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and phenylalanine deaminase. On egg yolk agar, lecithinase activity is absent. Utilizes D-arabitol, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, D-maltose, D-mannitol, D-mannose, L-rhamnose, salicin, starch, sucrose, D-trehalose, and D-turanose as sole sources of carbon and energy, but not L-arginine, citrate, itaconate, L-lysine, malonate, or suberate. Acid is not produced from dulcitol, D-galactose, lactose, D-mannitol, L-rhamnose, salicin, or D-sorbitol. According to API ZYM test results, esterase lipase (C8), leucine arylamidase, acid phosphatase, and α-glucosidase are present, but alkaline phosphatase, lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase, and α-fucosidase are absent. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The whole-cell sugars are glucose, mannose, ribose, and rhamnose; additional sugars, galactose, and xylose, may be present. The acyl type of the peptidoglycan is acetyl. The major quinone is MK-8(H_4). The major fatty acids are branched-chain saturated ($C_{15:0}$ iso, $C_{16:0}$ iso, $C_{15:0}$ ante and $C_{17:0}$ ante), and straight-chain saturated ($C_{16:0}$ and $C_{17:0}$) or unsaturated ($C_{18:1}$ ω9c). Mycolic acids are not present. The diagnostic polar lipids are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), an unidentified aminophospholipid (APL), an unidentified phosphoglycolipid (PGL1), and an unidentified lipid (UL1). Additional phenotypic characteristics are listed in *Table 30.2*. The DNA G + C content is 67.5–74.3 mol%.

The type species is *Nakamurella multipartita*.

Isolation, Enrichment, and Maintenance Procedures

Isolation and Enrichment

N. multipartita Y-104^T was isolated from activated sludge acclimated with sugar-containing synthetic wastewater under aerobic conditions (Yoshimi et al. 1996). Activated sludge was cultured aerobically in a fed batch reactor system with synthetic wastewater containing (per liter) glucose 0.75 g, peptone 0.03 g, yeast extract 0.03, $(NH_4)_2SO_4$ 0.1 g, and KH_2PO_4 0.07 g. After 90 days acclimation, strain Y-104^T was isolated from the sludge by the dilution plate method with GPY agar containing (per liter) glucose 1.0 g, peptone 0.5 g, yeast extract 0.5 g, KH_2PO_4 0.1 g, $(NH_4)_2SO_4$ 0.1 g, and $MgSO_4 \cdot 7H_2O$ 0.1 g (pH 7.0) incubated at 25 °C for 20 days.

N. flavida (*H. flavidus*) DS-52^T was isolated from soil samples collected in Dokdo, Korea, using the standard dilution plating technique at 25 °C on tenfold diluted nutrient agar (Difco) (Yoon et al. 2007).

N. lactea (*S. lacteus*) DLS-10^T was isolated from a rock sample collected from the peak of Darangshi Oreum (a small mountain 300 m above sea level) in Jeju, Korea (Lee et al. 2008). Rock samples (1 g) were ground to powder using a pestle and suspended in 10-ml sterilized, distilled water. Aliquots of serial dilutions of the sample were transferred to starch-casein agar containing (per liter) soluble starch 10 g, casein 0.3 g, KNO_3 2 g, NaCl 2 g, KH_2PO_4 2 g, $CaCO_3$ 0.02 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, and agar 18 g (pH 7.2). Subsequently, strain DLS-10^T was isolated after 7 days incubation at 30 °C.

N. multipartita P4-7^T was isolated from soil of a ginseng field located in Geumsan County, Korea (Kim et al. 2012).

Table 30.2

Phenotypic characteristics that differentiate the type strains of *Nakamurella* species (Data taken from Kim et al. (2012) unless indicated)

Characteristic	*N. multipartita* KCTC 19567^T	*N. flavida* KCTC 19127^T	*N. lactea* KCTC 19285^T	*N. panacisegetis* P4-7^T
Cell shape	Cocci	Cocci	Short rods	Short rods
Cell size (μm)	0.8–3.0[a]	0.6–1.2[b]	0.4–0.7 × 0.9–1.0[c]	0.6–0.9 × 0.8–1.2
Colony color	white at the early stage of growth, after about 2 weeks of incubation, the colonies become cream colored[a]	Light yellow[b]	Cream[c]	Cream
Range for growth				
Temperature (°C)	10–35[a]	4–32[b]	4–37[c]	5–37
pH	5.0–9.0[a]	5.0–8.5[b]	5.1–9.1[c]	5.0–8.0
NaCl (%)	0–6[a]	0–4[b]	0–3[c]	0–2
Nitrate reduction	w	–	+	+
Urease	+	+	–	+
Hydrolysis of				
CM-Cellulose	–	+	–	–
DNA	–	+	+	–
Starch	+	+	–	–
Carbon sources utilized				
N-Acetylglucosamine	+	–	+	+
L-Alanine	+	–	–	+
D-Amygdalin	–	+	+	–
L-Aspartate	–	–	+	–
L-Fucose	–	–	+	–
Gluconate	–	+	+	+
L-Glutamate	+	–	+	–
L-Glutamine	+	–	+	–
L-Histidine	+	–	+	–
Inositol	+	+	–	–
Lactate	+	–	–	+
Malate	–	+	–	+
D-Melibiose	+	–	–	+
L-Proline	–	–	+	+
Propionate	+	–	–	+
Pyruvate	+	–	+	–
D-Raffinose	+	+	–	–
D-Ribose	–	+	+	+
L-Serine	–	–	–	+
D-Sorbitol	+	+	–	+
D-Xylose	+	+	–	+
Acid production from				
D-Glucose	+	–	–	–
D-Maltose	+	–	–	–
D-Mannose	+	–	–	–
D-Raffinose	–	+	–	–
Sucrose	–	+	–	–
Glycerol	+	–	–	–

Table 30.2 (continued)

Characteristic	*N. multipartita* KCTC 19567^T	*N. flavida* KCTC 19127^T	*N. lactea* KCTC 19285^T	*N. panacisegetis* P4-7^T
Enzyme activity[d]				
Esterase (C4)	+	+	–	–
Valine arylamidase	–	–	+	–
Cystine arylamidase	–	–	+	–
Naphthol-AS-BI-phosphohydrolase	–	+	–	–
α-Galactosidase	–	–	–	+
β-Galactosidase	–	–	+	+
β-Glucosidase	+	+	–	+
N-Acetyl-β-glucosaminidase	–	–	+	–
α-Mannosidase	–	–	+	–
Whole-cell sugars[e]	Glc, Man (trace), Xyl, Rib, Rha (trace)	Gal, Glc, Man, Xyl, Rib, Rha	Glc, Man (trace), Rib, Rha	Glc, Man, Xyl, Rib, Rha
Polar lipids[e]	DPG, PE, PI, APL, PGLs, ULs	DPG, PE, PI, APL, PGLs, UL	DPG, PE, PI, APL, PGLs, GL, UL	DPG, PE, PI, APL, PGLs, GLs, UL
Menaquinones (In %)	MK-8(H_4) (98 %), MK-7(H_4) (2 %)	MK-8(H_4) (84 %), MK-8(H_2) (9 %), MK-9(H_4) (7 %)	MK-8(H_4) (89 %), MK-9(H_4) (7 %), MK-7(H_4) (4 %)	MK-8(H_4) (97 %), MK-7(H_4) (3 %)
Major fatty acids (>10 % of total)	$C_{16:0}$ iso, $C_{18:1}$ ω9c, $C_{15:0}$ iso	$C_{15:0}$ ante, $C_{17:0}$, $C_{15:0}$ iso	$C_{15:0}$ ante, $C_{16:0}$, $C_{17:0}$ ante, $C_{16:0}$ iso	$C_{16:0}$ iso, $C_{15:0}$ ante, $C_{17:0}$ ante
DNA G + C content (mol%)	67.5[a]–70.9[f]	72.6[b]–74.3	70.4–74.3[C]	69.9

+, positive; w, weakly positive; −, negative
Data taken from:
[a]Yoshimi et al. 1996
[b]Yoon et al. 2007
[c]Lee et al. 2008
[d]Data from API ZYM tests
[e]*Gal* galactose, *Glc* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose, *Xyl* xylose, *APL* unidentified aminophospholipid, *DPG* diphosphatidylglycerol, *GL* unidentified glycolipid, *PE* phosphatidylethanolamine, *PGL* unidentified phosphoglycolipid, *PI* phosphatidylinositol, *UL* unidentified lipid
[f]Tice et al. 2010

A soil sample collected from a ginseng field was diluted serially in sterile distilled water, and samples of each serial dilution were spread on one-tenth-strength nutrient agar. Subsequently, strain P4-7^T was isolated after 4 weeks incubation at 25 °C.

Maintenance

Members of this family do not require special procedures for maintenance and medium- and long-term storage. Generally, strains are maintained on isolation medium as agar slants at room temperature or at 4 °C for a week. *N. multipartita* may be maintained for a month on GPY agar slants.

Medium-term maintenance is in 20 % (v/v) glycerol suspensions at −20 °C or at −70 °C. Long-term preservation is by lyophilization or in liquid nitrogen.

Ecology

The habitat of all *Nakamurellaceae* species is only known for the type strain. As for other members of the order "*Frankiales*," soil appears to be the natural environment for two species to thrive: one type strain was isolated soil of Dokdo (the easternmost island of Korea) and the other strain from soil of a ginseng field. On the other hand, the isolation source activated sludge or a mountain rock may indicate random niche occupation of water or soil-origin organisms. The publications or sequence

depositions on *Nakamurella*-related strains, on which intensive taxonomic studies have yet to be done, may provide more diverse isolation information. Antarctic bacterium 3C6, closely related to *N. panacisegetis* (99 % 16S rRNA gene sequence similarity), was isolated from the glacier sediment of Antarctica (García-Echauri et al. 2011). *Saxeibacter* sp. R-36686 and *Frankineae* bacterium MI-1.2 V7, moderately related to *N. lactea* (98 % and 97 % similarity, respectively), and Actinobacterium R-36375, related to *N. flavida* (95 % similarity), were isolated from soil samples of Antarctica and the Subantarctic island (Peeters et al. 2011; Sanyika et al. 2012). *Actinomycetales* bacterium Gsoil 972 (DNA accession no. AB245399) isolated from soil of a ginseng field was closely related to *N. lactea* (almost 100 % similarity). *Nakamurella* sp. I10A-02501 (JX273663) and *Nakamurella* sp. I10A-02502 (JX273664) isolated from a plant root were related to *N. multipartita* (96 % similarity for each). The NCBI taxonomy browser lists several additional bacterial clones affiliated to *Nakamurellaceae*, e.g., from biofilm reactor, USA (EF125941), freshwater sediment, Chile (EF632902), coal bed, China (JF417732), permafrost and meadow soil, China (JQ978619, HQ645167 and HQ864131), rice phyllosphere, Philippines (HE589887 and HE589890), midgut homogenate of humus-feeding larva, Germany (AJ576407), and giant panda feces, China (JF920386).

Pathogenicity: Clinical Relevance

Until now, no clinical studies on the pathogenicity of *Nakamurellaceae* species have been reported and no strains have been isolated from the clinical specimen. However, according to the studies on diversity and dynamics of bacterial community in indoor environments (Rintala et al. 2008; Täubel et al. 2009) and on temporal shifts in the skin microbiome (Kong et al. 2012), six bacterial clones were found to be affiliated to *Nakamurellaceae* in the former two studies and 22 bacterial clones for the latter. This means that some members of *Nakamurellaceae* are closely associated with human environments.

N. multipartita is sensitive by agar diffusion method to amikacin (30 μg), bacitracin (10 U), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), erythromycin (15 μg), kanamycin (30 μg), neomycin (30 μg), rifampin (5 μg), streptomycin (10 μg), tetracycline (30 μg), trimethoprim (5 μg) and vancomycin (30 μg) but resistant to ampicillin (10 μg), aztreonam (30 μg), carbenicillin (100 μg), ceftazidime (30 μg), cephalothin (30 μg), gentamicin (10 μg), nalidixic acid (30 μg), novobiocin (30 μg), oxacillin (1 μg), penicillin (10 U), and polymyxin B (300 U) (Kim et al. 2012).

N. flavida is sensitive to amikacin (30 μg), ampicillin (10 μg), bacitracin (10 U), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), novobiocin (30 μg), polymyxin B (300 U), rifampin (5 μg), streptomycin (10 μg), tetracycline (30 μg), trimethoprim (5 μg) and vancomycin (30 μg) but resistant to aztreonam (30 μg), carbenicillin (100 μg), ceftazidime (30 μg), cephalothin (30 μg), nalidixic acid (30 μg), oxacillin (1 μg), and penicillin (10 U) (Kim et al. 2012).

N. lactea is sensitive to amikacin (30 μg), ampicillin (10 μg), bacitracin (10 U), carbenicillin (100 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), polymyxin B (300 U), streptomycin (10 μg) and vancomycin (30 μg) but resistant to aztreonam (30 μg), ceftazidime (30 μg), cephalothin (30 μg), nalidixic acid (30 μg), oxacillin (1 μg), novobiocin (30 μg), penicillin (10 U), rifampin (5 μg), tetracycline (30 μg), and trimethoprim (5 μg) (Kim et al. 2012).

N. panacisegetis is sensitive to amikacin (30 μg), bacitracin (10 U), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), novobiocin (30 μg), polymyxin B (300 U), rifampin (5 μg), streptomycin (10 μg), tetracycline (30 μg) and vancomycin (30 μg) but resistant to ampicillin (10 μg), aztreonam (30 μg), carbenicillin (100 μg), ceftazidime (30 μg), cephalothin (30 μg), nalidixic acid (30 μg), oxacillin (1 μg), penicillin (10 U), and trimethoprim (5 μg) (Kim et al. 2012).

Application

The only evidence that any strain of the family may be involved in application originates from a study by Yoshimi et al. (1996) with *N. multipartita* Y-104^T. This strain can take up a number of sugars and accumulate large amounts of polysaccharide in its cells in the absence of nitrogen and phosphate sources. This ability may play an important role in the competition among various bacteria to take up limiting substrates in activated sludge processes and the rapid decrease of reserve polysaccharide levels in activated sludge treating carbohydrate wastes.

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31 The Class *Nitriliruptoria*

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Abstract
The *Nitriliruptoria* line of descent is one of the deeply branching actinobacterial lineages, containing the orders *Nitriliruptorales* and *Euzebyales*. Each of them is defined by a single family, genus, and species, respectively: within *Nitriliruptoraceae*, it is the genus *Nitriliruptor* with *Nitriliruptor alkaliphilus* (Sorokin et al. 2009) and within *Euzebyaceae*, it is *Euzebya* with *Euzebya tangerina* (Kurahashi et al. 2010). The higher classification as a subclass *Nitriliruptoridae*, and recently as a class *Nitriliruptoria*, followed the description of *Euzebya tangerina* and the notion that *Nitriliruptorales* and *Euzebyales* are phylogenetic neighbors, sharing a common origin. *Nitriliruptor alkaliphilus* has been isolated from soda lake sediments of the Kulunda Steppe (Altai, Russia). The Gram-positive type strain ANL-iso2^T is the only known alkaliphilic bacterium to degrade isobutyronitrile [iBN, (CH3)2CHCN] and utilizes it as a sole source of energy, carbon, and nitrogen. Biodegradation is via the nitrile hydratase/amidase system. *Euzebya tangerina* was isolated from abdominal epidermidis of a sea cucumber, *Holothuria edulis*. In addition to the type strains, the lineage contains several unclassified isolates and hitherto uncultured strains from sources different to those of the type strains.

Taxonomy: Historical and Current

Short Description of Higher Taxa

The descriptions of order and family (Sorokin et al. 2009), and subclass (Kurahashi et al. 2010) and class (Ludwig et al. 2012), and is based solely on one and two strains, respectively, and they likely need to be emended with more taxa described. The definition of the class *Nitriliruptoria* is based solely on its phylogenetic position. Morphological, cultural, and chemotaxonomic properties differentiating the two genera of this class from each other and from neighboring genera are shown in ❯ *Table 31.2*.

Nitriliruptoridae Kurahashi, Fukunaga, Sakiyama, Harayama, Yokota 2010, 2318^{VP}

Ni.tri.li.rup.to.ri'da.e. N.L. masc. n. *Nitriliruptor* type genus of the type order of the subclass; suff. -idae, ending to denote a subclass; N.L. fem. pl. n. Nitriliruptoridae the *Nitriliruptor* subclass.

The description is the same as that for the genus *Nitriliruptor*. Separation of these organisms into a class is justified by their distinct lineage within the phylum Actinobacteria based on 16S rRNA gene sequences. The pattern of 16S rRNA gene sequence signature nucleotides of members of the class is as for the family *Nitriliruptoraceae*. The class contains the type order *Nitriliruptorales* and the order *Euzebyales*.

Nitriliruptorales Sorokin, van Pelt, Tourova, Evtushenko 2009, 252^{VP}

Ni.tri.li.rup.to.ra'les N.L.masc.n. *Nitriliruptor* type genus of the family -ales ending to denote an order; N.L. fem. pl. n. *Nitriliruptorales* the order of the type genus *Nitriliruptor*. The description is the same as that for the genus *Nitriliruptoraceae*. The pattern of 16S rRNA gene sequence signature nucleotides of members of the subclass is as for the type family *Nitriliruptoraceae*. *Nitriliruptor* is the type genus.

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_197,

Euzebyales Kurahashi, Fukunaga, Sakiyama, Harayama, Yokota 2010, 2318[VP]

Eu.ze.by'a.les. N.L. fem. n. *Euzebya* type genus of the family; -ales, ending to denote an order; N.L. fem. pl. n. *Euzebyales* the order of the genus *Euzebya*. The description is the same as that for the genus *Euzebyaceae*. The pattern of 16S rRNA gene sequence signature nucleotides of members of the order is as for the family *Euzebyaceae*. *Euzebya* is the type genus.

Nitriliruptoraceae Sorokin, van Pelt, Tourova, Evtushenko 2009, 251[VP]

Ni.tri.li.rup.to.ra'ce.ae. N.L. masc. n. *Nitriliruptor* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Nitriliruptoraceae* the family of the genus *Nitriliruptor*.

The description is the same as that for the genus Nitriliruptor. The pattern of 16S rRNA gene signature nucleotides and nucleotide pairs of members of the family consists of: 47–396 (G–C), 232 (S), 241–285 (G–C), 291–309 (U–A), 294–303 (A–U), 295–302 (G–C), 361 (A), 443–491 (G–C), 890 (C), 1165–1171 (U–A), 1311–1326 (A–U). The type genus is *Nitriliruptor*.

Euzebyaceae Kurahashi, Fukunaga, Sakiyama, Harayama, Yokota 2010, 2318[VP]

Eu.ze.by.a.ce'ae. N.L. fem. n. *Euzebya* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. Euzebyaceae the family of the genus *Euzebya*.

The description is the same as that for the genus *Euzebya*. The pattern of 16S rRNA gene sequence signature nucleotides and nucleotide pairs of members of the family consists of 101 (G), 211 (C), 346 (G), 427 (U), 579 : 762 (U–C), 589 : 650 (U–A), 612 : 628 (U–A), 614 : 626 (A–U), 841 : 845 (A–U), 986 : 1219 (A–U), 1002 : 1038 (G–U), 1031 (G), 1075 : 1082 (C–G). The type genus *Euzebya*.

Phylogenetic Structure of the Families and Their Genera

The previous higher classification as a subclass *Nitriliruptoridae* followed the description of *Euzebya tangerina* and the notion that *Nitriliruptorales* and *Euzebyales* are phylogenetic neighbors, sharing a common origin. More recently, with the publication of *Bergey's Manual of Systematic Bacteriology*, Vol. 5: The Actinobacteria (Ludwig et al. 2012) and the elevation of the order *Actinomycetales* to the class level (*Actinobacteria*), the subclass "Nitriliruptoridae" was consequently also elevated to the rank of a class, together with other deeply rooting lineages such as "Acidimicrobiia," "Coriobacteriia," "Rubrobacteria," and "Thermoleophili." As depicted in ❯ *Fig. 31.1* the *Nitriliruptor* lineage is phylogenetically remotely related to a clade embracing *Acidimicrobiaceae*, *Iamia*, *Ilumatobacter*, and *Candidatus* "Microthrix parvicella" (not shown) as well as other deeply rooting actinobacterial classes. This is in accordance with the topology depicted by Kurahashi et al. (2010).

The type strain of *Nitriliruptor alkaliphilus* is phylogenetically related to several as-yet uncultured actinobacteria. The number of such taxa has increased significantly since the original description of *N. alkaliphilus*. More than 50 records with at least 91 % and higher BLAST similarity values are recorded when the 16S rRNA gene sequence of *N. alkaliphilus* (EF422408) is run against the public database. Clone sequences from the top scoring entries (93–95 %) have been retrieved from soils contaminated with anthracene (Castro-Silva et al., unpublished [e.g., HM438005], ultramafic rocks in the Leka ophiolite complex (Central Norway) (JN003088), soil from the Mars Desert Research Station (Utah, USA) (HQ910264), highly alkaline saline soil from Lake Texcoco (Mexico) (Valenzuela-Encinas et al. 2008), soda lake sediment from Lake Xiarinur (Inner Mongolia) (GU083674). Especially the latter two alkaline sites as well as the locations mentioned by Sorokin et al. (2007) allows the conclusion that members related to *Nitriliruptor alkaliphilus* thrive in alkaline environments, e.g., asphalt seep and pits (Kim and Crowley 2007), Mono Lake (Humayoun et al. 2003), or Wadi an Natrun, Egypt (Mesbah et al. 2007).

A similar situation is seen with the type strain of *Euzebya*. Since the original description of the type species in 2010, dozens of novel environmental clone sequences deposited in public nucleic acid databases are found to be related, remotely though, to *E. tangerina* above BLAST cutoff values of 91 %. Interestingly and not expected from the significant differences in metabolism described for *N. alkaliphilus* and *E. tangerina*, several clone sequences found in the same environment, such as the highly alkaline saline soil from Lake Texcoco (Mexico) (Serna et al., unpublished) and soils contaminated with anthracene (Castro-Silva et al., unpublished [e.g., JQ426201], Dendooven and Vazquez-Nunez, unpublished [e.g., HM438033]), were related to both species. Horath and Bachhofen (2009) detected remotely related *Euzebya* clone sequences in dolomite rocks in the central Alps.

Though the two type strains have been isolated from very different environments, the frequent isolation from the same of genomic material from strains moderately related to these two taxa indicates that at the higher taxonomic rank, species may share more genomic similarities than described for the two type strains.

Genome Comparison

The genome of *Nitriliruptor alkaliphilus* was recently sequenced (Otten, L. G. and Muyzer, G. unpublished) and annotated using the RAST annotation server (Aziz et al. 2008). Although the sequencing did not result in the assembly of a single contig, the total amount of 5,262,836 sequenced base-pairs within the 1825 contigs is very similar to the expected value of 5.2 Mbps, as measured by Pulse Field Electrophoresis. Furthermore, the

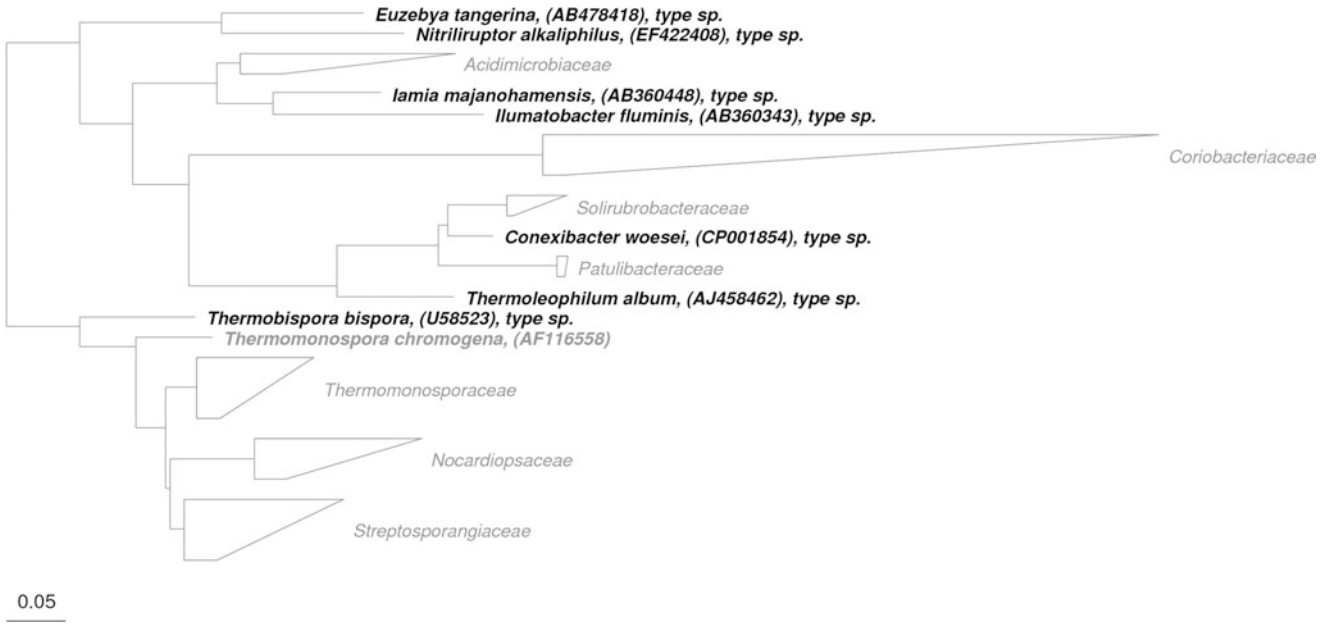

Fig. 31.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all type strains present in the LTP_106 (Yarza et al. 2010). The tree was reconstructed by using a subset of sequences representative of closely and moderately related taxa 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 1 % sequence divergence

amount of annotated genes is quite similar to the amount assigned in the genome of *Thermomonospora curvata* DSM 43183, which is an Actinobacterium with a similar total genome size (5.56 Mbps). This means that although the contigs were not merged to one genome read, most genes will be present in the annotation. Since this genome is the only genome sequenced in this order, it cannot be compared within the order. Therefore, we compared genome features to related organisms in the *Actinobacteria* class, namely, *Thermomonospora curvata* DSM 43183 (Chertkov et al. 2011), *Rhodococcus jostii* RHA1 (McLeod et al. 2006), and *Acidothermus cellulolyticus* 11B (Barabote et al. 2009) (*Table 31.1*).

The most important difference between *N. alkaliphilus* and the other organisms is the amount of genes coding for membrane transport systems (5 % of the total annotated genes compared to 1.5 % on average in the others). There are at least 2 full multi-subunit cation antiporters, probably Na+ / H+ antiporters (subunits A to G) and an MhnB-related cluster. All ion pumps are situated in very close clusters next to each other. The abundance of so many ion transporters is easily explained by the fact that this organism was isolated from an alkaline soda lake, where there is a lot of salt and pH stress (Sorokin et al. 2007).

Furthermore, there are relatively many genes related to fatty acid, lipid, and isoprenoid syntheses. Most of these genes are part of the fatty acid biosynthesis system FASII or fatty acid metabolism cluster. There are however no genes annotated in the "glycerolipid and glycerophospholipid metabolism" subsystem. The cell wall does however contain glycerol phosphates and phosphodiesters (Sorokin et al. 2009). It is therefore likely that several genes have been mis-annotated, but it also might point to the fact that this organism is using a somewhat different enzyme system to prepare its cell wall. May be the cell wall components are slightly different from other bacteria, to sustain growth in such a hostile environment.

Strangely enough, the amount of genes in the stress response subsystem is relatively low. This might mean that this organism has either different stress response systems than comparable organisms, or it saves energy by not responding to a lot of different stresses, which could have evolved in this environment because of the energetic benefits.

The amount of genes producing carbohydrates seems to be elevated compared to the other organisms, but the amount is similar to genes in the amino acids and derivatives cluster, like in *R. jostii* and *A. cellulolyticus*, which means that this elevation is probably not meaningful. On the other hand, genes in the virulence, disease, and defense and miscellaneous category are much lower. We expect this to stem from the fact that the genome was not assembled to one contig and from the different genetic makeup of these enzymes, thereby missing some genes in the annotation or placing it in a subsystem (73 % of the annotated genes are not in a subsystem). This latter reason is also valid for RNA metabolism, since there are potentially 51 RNAs, which is less than *T. curvata* (68) and *R. jostii* (64), but similar to *A. cellulolyticus* (49), so one would expect the same amount of genes for the production of RNA. The subsystems protein

Table 31.1

Genome comparison of the type strain *Nitriliruptor alkaliphilus* ANL-Iso2 and related organisms. Number (#) and percentages (%) of genes per subsystem are given for all 4 genomes. Relatively high percentages or low percentages for *N. alkaliphilis* are highlighted bold and *italic* respectively (see text)

Subsystem	*Nitriliruptor alkaliphilus* ANL-iso2		*Thermomonospora curvata* DSM 43183		*Rhodococcus jostii* RHA1		*Acidothermus cellulolyticus* 11B	
	#	%	#	%	#	%	#	%
Cofactors, vitamins, prosthetic groups, pigments	167	8.5	247	11.4	404	10.3	221	12.6
Cell wall and capsule	62	3.2	49	2.3	70	1.8	65	3.7
Virulence, disease, and defense	27	**1.4**	51	2.3	78	2.0	36	2.1
Potassium metabolism	14	0.7	17	0.8	24	0.6	0	0.0
Photosynthesis	0	0.0	0	0.0	0	0.0	0	0.0
Miscellaneous	76	**3.9**	150	6.9	257	6.5	115	6.6
Phages, prophages, transposable elements, plasmids	0	0.0	1	0.0	10	0.3	1	0.1
Membrane transport	98	**5.0**	29	1.3	26	0.7	27	1.5
Iron acquisition and metabolism	0	0.0	5	0.2	17	0.4	3	0.2
RNA metabolism	52	**2.7**	94	4.3	132	3.4	92	5.3
Nucleosides and nucleotides	66	3.4	91	4.2	132	3.4	66	3.8
Protein metabolism	135	6.9	220	10.1	222	5.6	184	10.5
Cell division and cell cycle	14	0.7	28	1.3	25	0.6	25	1.4
Motility and chemotaxis	0	0.0	0	0.0	2	0.1	64	3.7
Regulation and cell signaling	29	1.5	46	2.1	57	1.5	38	2.2
Secondary metabolism	0	0.0	14	0.6	15	0.4	0	0.0
DNA metabolism	63	3.2	129	5.9	117	3.0	91	5.2
Fatty acids, lipids, and isoprenoids	133	**6.8**	118	5.4	240	6.1	84	4.8
Nitrogen metabolism	14	0.7	14	0.6	51	1.3	20	1.1
Dormancy and sporulation	5	0.3	4	0.2	3	0.1	2	0.1
Respiration	123	6.3	122	5.6	217	5.5	76	4.3
Stress response	67	**3.4**	115	5.3	184	4.7	51	2.9
Metabolism of aromatic compounds	42	2.1	32	1.5	168	4.3	4	0.2
Amino acids and derivatives	367	18.7	316	14.5	708	18.0	224	12.8
Sulfur metabolism	14	0.7	32	1.5	78	2.0	6	0.3
Phosphorus metabolism	36	1.8	34	1.6	47	1.2	32	1.8
Carbohydrates	354	**18.1**	214	9.9	647	16.5	222	12.7
Total	1,958	100.0	2,172	100.0	3,931	100.0	1,749	100.0

metabolism and cofactors, vitamins, prosthetic groups, and pigments are both slightly decreased, which might mean that there are less enzymes with cofactors available, which could have a broader substrate range, or again the difference in gene makeup of this organism prevents enzymes from being annotated correctly by a computer program. To explore these differences in gene amounts, annotation and assignment to certain subsystems should be manually checked. Also, the whole organism or separate genes can be tested for a lot of different enzymatic steps.

There is a putative cobalt uptake protein, but no uptake proteins for nickel are annotated. This cobalt uptake protein is totally different from other organisms, which could point to another function than supplying cobalt for cobalamin-dependent enzymes. One of the options is that this cobalt uptake might be important for the NHase found in this organism. The organism was isolated by using isobutyronitrile as the carbon, energy, and nitrogen source (Sorokin et al. 2009). NHase and amidase activity was established (Sorokin et al. 2007), and the NHase genes were found in the usual setting, together with an amidase and a chaperone. From the sequence, it is clear that the NHase is a cobalt enzyme, which was already expected from the substrate specificity and stability of the enzyme. Furthermore, there are 3 nitrilases assigned, which

together with the NHase-amidase cluster can account for the excellent growth on so many different nitriles as sole carbon and nitrogen source.

Phenotypic Analyses

Nitriliruptor alkaliphilus and *Euzebya tangerina* are Gram-positive, catalase- and oxidase-positive, nonmotile rods, sharing the same peptidoglycan type (A1γ) and a similar DNA base composition. They slightly differ from each other in fatty acid composition and in the composition of whole cell sugars (Table 31.2). Most significantly, *N. alkaliphilus* ANL-iso2^T differs from *E. tangerina* F10^T in its alkaliphily and in the metabolism of a wide range of nitriles. It should, however, be noted that strain F10^T has not been investigated in this respect and the observation that as yet-uncultured strains of both species thrive in the same and in similar environments may indicate that the metabolism of the species *E. tangerina* may be more versatile than described.

Nitriliruptor Sorokin et al. 2009, 251^{VP}

Ni.tri.li.rup'tor. N.L. n. *nitrilum* nitrile, nitrile group; L. masc. n. *ruptor* breaker; N.L. masc. n. *nitriliruptor* nitrile-breaker.

In addition to the properties listed in Table 31.2: utilizes short-chain organic acids, amides, and aliphatic nitriles as energy and carbon source. Alkaliphilic and moderately salt-tolerant. The type species is *Nitriliruptor alkaliphilus*.

In addition to the properties given for the genus, the description of the type strain description is based on that given by Sorokin et al. (2009): Cells are 0.4 × 1.5–3.0 μm in size, occurring singly or in pairs under most conditions. When grown on glucose and yeast extract, chain formation was observed. Colonies are colorless, flat, and spreading. Able to grow on propionitrile (C_3), butyronitrile (C_4), isobutyronitrile (C_4), valeronitrile (C_5), and capronitrile (C_6) as carbon and energy source. No growth on acetonitrile (C_2). Growth on nitriles is biphasic, with fast initial hydrolysis of nitriles to the corresponding amides, carboxylic acids, and ammonia and slow further utilization of these products, resulting in biomass growth: Products of isobutyronitrile conversion are isobutyroamide, isobutyrate, and ammonium. Able to metabolize, without growth, a large spectrum of aliphatic and some aromatic nitriles via the nitrile hydratase/amidase enzyme system. Growth on acetate, propionate, pyruvate, butyrate, isobutyrate, valerate, succinate, malate, citrate and fumarate, glucose, maltose, fructose, arabinose, mannose, sucrose, α-trehalose, melezitose, inositol, cellobiose and glycerol and complex organic substrates such as yeast extract and peptone. Gelatin is hydrolyzed while starch, casein, cellulose, chitin, pectin, and xylane are not hydrolyzed. The presence of an anionic carbohydrate-containing polymer(s) in the cell wall is indicated by the presence of glycerol phosphates, phosphodiesters, and an unidentified amino sugar.

The type strain ANL-iso2^T (=DSM 45188^T = NCCB 100119^T = UNIQEM U239^T) was isolated from soda lake sediments of the Kulunda Steppe (Altai, Russia).

Euzebya Kurahashi et al. 2010, 2318^{VP}

Eu.ze'by.a. N.L. fem. n. *Euzebya* named for Jean Paul Marie Euzeby, a French microbiologist.

Properties given for the genus are indicated in Table 31.2. Phosphatidylglycerol is the polar lipid; contains in addition several unidentified glycolipids and glycophospholipids.

The type species is *Euzebya tangerina*.

In addition to the properties given for the genus, the description of the type strain description is based on that given by Kurahashi et al. (2010): Cells are approximately 0.6–0.8 × 1.5–6.0 μm. Colonies on MA plates are pulvinate with entire edge, tangerine in color, nearly opaque, displaying a hard texture after 5 weeks of incubation at 25 °C. Reduction of nitrate to nitrite and of nitrite to N_2. Positive for hydrolysis of gelatin and urea, production of acetoin, and assimilation of L-arabinose and melibiose. Negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, utilization of citrate, production of H_2S and indole, assimilation of amygdalin, D-glucose, inositol, D-mannose, rhamnose, sucrose and sorbitol.

The type strain is F10^T (=NBRC 105439^T = KCTC 19736^T), isolated from the ventral epidermis of the sea cucumber *Holothuria edulis* at Aka Island, Okinawa, Japan.

Isolation, Enrichment, and Maintenance Procedures

Nitriliruptor alkaliphilus ANL-iso2^T was isolated from an iBN enrichment of a mixed sediment sample, consisting of 10 samples from Kulunda Steppe (southwestern Siberia, Altai, Russia). The mineral medium used for enrichment and maintenance (Sorokin et al. 2007) is the same used by the German Collection for Microorganisms and Cell Cultures for routine maintenance (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium1105.pdf).

For enrichment (Sorokin et al. 2007), liquid culture was incubated on a rotary shaker at 100 rpm and 28 °C. At ammonia concentration of 2 mM, the culture was transferred into a new medium at a 1:100 dilution and after several transfers, the culture from a maximal positive dilution (10^{-11}) was plated onto solid medium, either by surface spreading or by the agar-shake technique. After 30 days incubation in closed jars, separate colonies were placed into 5-mL liquid medium with appropriate substrates at a 5–20 mM concentration in 30-mL serum bottles closed with rubber septa.

In the case of the solid medium, iBN is added after the medium is cooled down to 50 °C. Growth of pure cultures is performed in 250-mL closed serum bottles with 50-mL liquid on a rotary shaker at 100–150 rpm and 30 °C.

Table 31.2

Major cultural and chemotaxonomic properties differentiating the type strains of type species of the classes *Nitriliruptoria* and related taxa. All type strains are oxidase Gram-positive and non-endospore-forming rods (no data are available for oxidase reaction by strain YM22-133^T)

Properties	*Nitriliruptor alkaliphilus* ANL-iso2^{T1}	*Euzebya tangerina* F10^{T2}	*Acidimicrobium ferrooxidans* ICP^{T3}	*Iamia majanohamensis* F-12^{T4}	*Ilumatobacter fluminis* YM22-133^{T5}
Motility	Nonmotile	Non motile	Motile[f]	Nonmotile	Nonmotile
pH range of growth (optimum)	Obligate alkaliphilic, 8.3–10.6	7–9	2	6–9 (7)	7–11
Catalase	+	+	–	+	n.d.
Salt tolerance (M NaCl)	0.1–2.0	0.1–2.0	n.d.	n.d.	Growth on Marine Agar 2216. No growth in the absence of NaCl
°C temperature range of growth (optimum)	Mesophilic (32)	15–35 (20–28)	25–50 (45–50)	15–40 (24–30)	26–31
Metabolism	Strictly aerobic, chemoorganotrophic; able to grow with C_3-C_6 aliphatic nitriles	Aerobic, chemoorganotrophic	Aerobic, autotrophic growth on ferrous iron; heterotrophic growth on yeast extract	Aerobic, chemoorganotrophic	Aerobic
Diagnostic amino acid in peptidoglycan (type)	meso-DAP (A1γ)	meso-DAP (A1γ) (acyl type)	meso-DAP (A1γ)	meso-DAP (A1γ) (glycolyl type)	LL-DAP-Gly (A3γ) (glycolyl type)
Major Fatty acid	$C_{16:0}$, iso-$C_{14:0}$, iso-$C_{16:0}$, $C_{16:1}\omega7$	$C_{16:1}\omega7c$, $C_{16:0}$, $C_{17:1}\omega8c$	$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$	$C_{17:0}$, $C_{17:1}\omega8c$, $C_{15:0}$, $C_{16:0}$	iso-$C_{16:0}$, $C_{17:1}\omega9c$, iso-$C_{17:0}$, iso$C_{16:1}$, $C_{17:1}\omega8c$, $C_{18:1}\omega7c$, iso-$C_{18:1}$, anteiso-$C_{17:0}$
Major menaquinone	n.d.	MK9(H4)	MK9(H8)[d]	MK9(H6), MK9(H4), MK9(H8)	MK9(H8)
Cell wall sugars[g]	glu, gal, gly	rham, gal	n.d.	rham, man, ara, gal, xyl	n.d.
Mol% G+C of DNA	70.8	68.3	67.0–69	74.4	68.0

n.d. not determined
[a]Sorokin et al. (2009)
[b]Kurahashi et al. (2010)
[c]Clark and Norris (1996), Normand (2006)
[d]Kurahashi et al. (2009)
[e]Matsumoto et al. (2009)
[f]According to Clark and Norris (1996), cells are motile during heterotrophic growth on yeast extract
[g]glu, glucose; gal, galactose; gly, glycerol; rham, rhamnose; ara, arabinose; man, mannose; xyl, xylose

Long-term storage in the DSMZ occurs as vacuum-dried culture.

Euzebya tangerina F10^T was isolated from the abdominal epidermis of a sea cucumber, *Holothuria edulis* (Kurahashi et al. 2010) which had been collected off the coast of Aka Island, Okinawa prefecture, Japan, at a depth of 6 m. The holothurian specimen was washed several times with sterile sea water, the epidermis homogenized and diluted serially to a ratio of 1:10 in sterile sea water (Kurahashi and Yokota 2004). A 0.1-mL aliquot of the dilution was spread onto SN medium (http://www-cyanosite.bio.purdue.edu/media/table/SN.html) and incubated at 25 °C for about 5 weeks. Subcultivation of strain F10^T was carried out on marine agar 2216 (MA; Becton Dickinson) at 20 °C. Stock cultures can be

maintained in marine broth 2216 (Becton Dickinson) with 5 % DMSO at −80 °C. The strain can be lyophilized. Rehydration medium is per liter: 5-g peptone, 3-g yeast extract, 1-g $MgSO_4 \cdot 7H_2O$, pH 7.0

Application

Biocatalysis for Added-Value Products

Nitriles are organic compounds widely used in industry as intermediates and building blocks in organic synthesis, as well as organic solvents. Nitrile-hydrolyzing enzymes are used as a green alternative to the heterogeneous copper catalyst process performed at temperatures over 100 °C. They can be used to produce a wide spectrum of higher value carboxylic amides and acids from the nitrile intermediates under relatively mild conditions (van Pelt et al. 2008b; Prasad and Bhalla 2010). The ability of *Nitriliruptor alkaliphilus* ANL-iso2^T to metabolize a wide range of nitriles offers a great potential for organic synthesis, especially as this actinobacterium is the first alkaliphilic and moderately salt-tolerant representative of nitrile-degrading microorganisms (Sorokin et al. 2009). In addition to hydrolysis of C3 to C6 nitriles, resting cells pre-grown in the presence if iBN, were able to metabolize a much wider range of industrially important nitriles, suggesting that this strain offers a useful potential for the sustainable production of chemicals with high commercial value.

The cobalt-containing nitrile hydratase of strain ANL-iso2^T has been purified, and its activity and enantioselectivity tested on a wide range of chiral and achiral nitriles (van Pelt et al. 2011). As compared to the activity of the hydratase against hexane-nitrile, the enzyme is active against 2-chloro-2-phenylacetonitrile (69 % as compared to hexane-nitrile), benzonitrile (60 %), and phenylpropionitrile (28 %), but converted only the latter chemical enantioselectively, with preference for the (S)-enantiomer. Although little activity was detected for 2-(4-nitrophenyl) propane-nitrile (7 μmol min^{-1} mg^{-1}) and naproxen-nitrile (17 μmol min^{-1} mg^{-1}), very high enantioselectivity was seen, resulting in the latter case in the amide precursor of the active enantiomer of the pain reliever naproxen.

Although high activity (and enantioselectivity) is important for industrial use of biocatalysts, the enzyme also has to be stable under harsh conditions. The nitrile hydratase of strain ANL-iso2^T was immobilized as a cross-linked enzyme aggregate (CLEA) and appeared to be highly stable under various conditions (van Pelt et al. 2008a). The CLEA could be reused in more than 35 cycles without losing any activity, which makes it a very good candidate for commercial use. Furthermore, the enzyme was shown to work very well in a bi-enzymatic cascade reaction in order to make the amide directly from the aldehyde and cyanide (van Pelt et al. 2009). Even at a pH of 4.5, which is necessary to keep the hydrocyanation reaction enantioselective, the enzyme was still active and the substrate was fully converted by both enzymes. In summary, the nitrile hydratase of *Nitriliruptor alkaliphilus* ANL-iso2^T is a very versatile and stable biocatalyst, which can be used for the hydration of various nitriles under a broad range of pHs, even though it was isolated from an alkaliphilic host.

Bioremediation

As indicated by Sorokin (2009), the enzymic makeup of *Nitriliruptor alkaliphilus* ANL-iso2^T to metabolize a wide range of nitriles also offers great capability for environmental biotechnology. This strain, either alone or as part of a consortium, is able to metabolize such a wide range of industrially important nitriles, that it could be useful for the bioremediation of polluted environments. While iBN hydrolysis of strain ANL-iso2^T to isobutyroamide, which is the first product of iBN hydrolysis in the nitrile hydratase/amidase pathway, is accompanied with little biomass growth, isobutyroamide is rapidly scavenged in a haloalkaliphilic consortium by a novel member of the gamma-proteobacterial genus *Marinospirillum*, *strain* ANL-isoa (Sorokin et al. 2007), also isolated from the same lake sediments. A consortium like this could be used to purify high pH waste streams from nitrile-manufacturing sites, although also neutral pH waste streams can be resolved as the nitrile hydratase is >90 % active between pH 7 and 11.5, and still has useful activities at pH 4–11.5.

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32 The Family *Nocardiaceae*

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Abstract
The family *Nocardiaceae*, a member of the order *Corynebacteriales*, encompasses eight phylogenetically closely related genera, that can be distinguished using a combination of chemotaxonomic, morphological, and physiological criteria. The genus "*Prescottella*" and, its constituent species, "*Prescotella equi*" (formerly *Rhodococcus equi*) have still to be validated though their taxonomic integrity is supported by genotypic and phenotypic data, including results from comparative genomic analyses. *Nocardiaceae* strains are widely distributed in aquatic and terrestrial habitats, notably soil and as constituents of foam and mixed liquors in activated sludge wastewater treatment plants. In general, members of the family are considered to have a saprophytic lifestyle though it is becoming increasingly evident that they should be seen as opportunistic pathogens when isolated from clinical material. Some *Nocardia* species are causal agents of two serious diseases, nocardiosis and actinomycetoma. These and other infections are probably underreported due to the lack of reliable selective isolation and identification procedures. "*Prescottella equi*" is a facultative intracellular pathogen that causes severe suppurative bronchopneumonia in foals, while *Rhodococcus fascians* is a soil-borne pathogen that induces the formation of differentiated galls in many herbaceous plants. The application of comparative genomic, genetic, and molecular biological studies show that *Gordonia*, *Nocardia*, and *Rhodococcus* strains exhibit remarkable metabolic diversity that can be exploited for a broad range of biotechnological purposes. It is also evident that these genera are grossly underspeciated.

Taxonomy: Historical and Current

Short Description of the Family

No. car. di. a. ce' a.e. N.L. fem. n. *Nocardia* type genus of the family, suff. *–aceae* ending to denote a family N.L. fem. pl. n. *Nocardiaceae* the *Nocardia* family.

The family *Nocardiaceae* is a member of the order *Corynebacteriales* (Goodfellow and Jones 2012) in the phylum Actinobacteria. The genus *Nocardia* (Trevisan 1889) is the type genus of the family which at the time of writing includes eight phylogenetically closely related genera (http://www.bacterio.cict.fr) that can be distinguished using a combination of chemotaxonomic, morphological, and phylogenetic characteristics.

Members of the family *Nocardiaceae* stain Gram-positive to Gram-variable and are typically acid-alcohol fast to

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Fig. 32.1

Phylogenetic reconstruction of the family *Nocardiaceae* 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). Scale bar indicates estimated sequence divergence

partially acid-alcohol-fast at some stage of the growth cycle. They are aerobic, nonmotile, mesophilic, chemo-organotrophs with an oxidative metabolism. Some strains form an extensively branched substrate mycelium that fragments into coccoid- and rod-shaped elements, others are coccoid or rod-like or have a rod-coccus/rod-coccus-mycelium growth cycle. Colonies may be smooth to rough, convex, irregular and show a range of colors, including beige, brown, buff, orange, pink, red, and yellow. The wall peptidoglycan contains *meso*-diaminopimelic acid and is of the A1γ type. Whole-organism hydrolysates are rich in arabinose and galactose, with variable amounts of other sugars. Muramic acid moieties are *N*-glycolated. Phosphatidylethanolamine is the diagnostic phospholipid though diphosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol mannosides are also found as major components. Cells contain major proportions of straight-chain, saturated, unsaturated, and 10-methyloctadecanoic (tuberculostearic) fatty acids though the latter is only found in trace amounts in some strains. Mycolic acids have 30–77 carbon atoms and up to six double bonds. The fatty acids released on pyrolysis gas chromatography of mycolic acid methyl esters have 12–20 carbon atoms. Menaquinone composition is variable. A family-specific pattern of 16S rRNA gene sequence signatures is found at positions 250 (U), 316:337 (C–G), 418:425 (C–G), 580:761 (U–A), 559–639 (C–G), 662–743 (C–G), 987–1218 (G–C), and 1000–1040 (A–U). The DNA G+C content ranges from 63 to 73 mol%.

Microorganisms classified in the family *Nocardiaceae* have been isolated from diverse habitats, notably soil, coniferous litter, herbivorous dung, freshwater and marine sediments, and wastewater systems (Goodfellow and Williams 1983; Colquhoun et al. 1998; Seviour and Nielsen 2010; Golinska et al. 2013). Some species cause diseases in animals, including humans, a few are plant pathogens (Goethals et al. 2001; Brown-Elliott et al. 2006; Fahal 2006; Goodfellow and Maldonado 2012; van de Sande 2014; van de Sande et al. 2014a, b).

Molecular Analyses

Phylogenetic Structure of the Family and Its Genera

As currently defined, the family *Nocardiaceae* (Castellani and Chalmers 1919), emended by Zhi et al. (2009) with the type genus *Nocardia* (Trevisan 1889) is one of eight genera classified in the order *Corynebacteriales*, class *Actinobacteria* (Stackebrandt et al. 1997). In addition to the genus *Nocardia*, the family currently includes the following taxa: *Gordonia* (Tsukamura 1971) emended by Stackebrandt et al. (1988); *Millisia* (Soddell et al. 2006a); "*Prescottella*" (Jones et al. 2013a). *Rhodococcus* (Zopf 1891) emended Goodfellow et al. (1998a); *Skermania* (Chun et al. 1997), *Smaragdicoccus* (Adachi et al. 2007), and *Williamsia* (Kämpfer et al. 1999). The inferred phylogenetic relationships between these taxa are shown in *Fig. 32.1*.

Until recently, membership of the family *Nocardiaceae* was restricted to the genera *Nocardia* and *Rhodococcus* (Rainey et al. 1995; Stackebrandt et al. 1997; Garrity et al. 2005), but was later extended to include the genera *Gordonia*, *Millisia*, *Skermania*, and *Williamsia* mainly on the basis of taxon-specific signatures in 16S rRNA genes (Zhi et al. 2009). This classification is recognized here albeit with the addition of the genera "*Prescottella*"

(Jones et al. 2013a) and *Smaragdicoccous* (Adachi et al. 2007). A case can be made for the continued recognition of the family *Gordoniaceae* (Stackebrandt et al. 1997) to include not only the type genus but also the genera *Millisia*, *Skermania*, and *Williamsia*, as these taxa have been found to form a branch in the *Corynebacteriales* 16S rRNA gene tree (Goodfellow et al. 2012). Consequently, the current assignment of genera to the family *Nocardiaceae*, like earlier ones, should be seen as a staging post to improved classifications in the future.

The genus *Nocardia* currently contains 84 validly published species which form the largest clade in the *Nocardiaceae* 16S rRNA gene tree (Fig. 32.2). This well-defined monophyletic clade is most closely related to the genera "*Prescottella*," *Rhodococcus*, and *Smaragdicoccus*. It is apparent from the tree that *Nocardia* species fall into several multimembered subclades, one of which includes *N. asteroides*, the type species, and *N. abscessus*, *N. asiatica*, *N. cyriacigeorgica*, *N. farcinica*, *N. higoensis*, *N. neocaldonensis*, *N. puris*, *N. shimofusensis*, and *N. thailandica*, all but two of which were isolated from clinical material. Similarly, a subclade toward the foot of the tree encompasses *N. aciditolerans*, *N. africana*, *N. aobensis*, *N. cerradoensis*, *N. elegans*, *N. kruczakiae*, *N. mikami*, *N. vaccinii*, *N. veterana*, and *N. vermiculata*. This taxon includes the two most closely related *Nocardia* species, *N. kruczakiae* and *N. veterana*, the type strains of which share a 16S rRNA gene sequence similarity of 99.8 % (Conville et al. 2006). The importance of using high quality 16S rRNA gene sequences to distinguish between closely related *Nocardia* species was underlined by Roth et al. (2003).

The key causal agents of the diseases actinomycetoma and nocardiosis, namely *N. asteroides*, *N. cyriacigeorgica*, *N. brasiliensis*, *N. nova*, and *N. otitidiscaviarum*, are scattered throughout the nocardial tree though the final member of this group, *N. brasiliensis*, belongs to the *N. asteroides* subclade. Similarly, the fish pathogens, *N. salmonicida* and *N. seriola*, are in different subclades as is *N. crassostreae*, the oyster pathogen. *Nocardia jiangxiensis* and *N. miyunensis* form a distinct phyletic line which is well separated from the third aciditolerant species, *N. aciditolerans*, an organism that is common in spruce litter (pH 3.8–4.9; Golinska et al. 2013).

There is evidence that the Sec A1 protein, which is involved in the export of proteins across bacterial membranes (Schmidt and Kiser 1999), may provide a reliable way of distinguishing between members of closely related *Nocardia* species. Thus, sequence analysis and alignment of a 468 bp region of the *Sec A1* gene gives higher resolution between *Nocardia* species than corresponding 16S rRNA gene sequence data (Conville et al. 2006). These workers showed that the sequence similarity of type and reference nocardiae to their closest phylogenetic neighbors was in the range 85.0–98.7 % for the *Sec A1* gene compared with 94.4–99.8 % for corresponding 16S rRNA gene sequences. The type strains of *N. kruczakiae* and *N. veterana*, for instance, gave sequence diversities with the *Sec A1* and 16S rRNA genes of 91.9 % and 99.8 %, respectively.

The genus *Gordonia* (formerly "*Gordona*") was proposed by Tsukamura (1971), became a synonym of the genus *Rhodococcus* (Goodfellow and Alderson 1977), and was revived by Stackebrandt et al. (1988) based on 16S rRNA sequence data. At present, the genus comprises 32 validly published species which form a distinct lineage in the 16S rRNA gene tree (Fig. 32.3). It can be seen from the tree that *Gordonia* species fall into a number of loosely defined subclades with many short branches though *G. kroppenstedtii* forms a distinct independent branch at the periphery of the tree. However, greater resolution is found between *Gordonia* species based on *gyr B* gene sequence analysis (Shen et al. 2006a). Shen and his colleagues also found that gordoniae have much lower interspecies *gyr B* substitution rates than *Corynebacterium*, *Nocardia*, and *Rhodococcus* strains.

The phylogenetic relationships between validly published *Rhodococcus* species are shown in Fig. 32.4. The species cluster into several lineages, a result which is in good agreement with previous studies (Rainey et al. 1995; McMinn et al. 2000; Jones and Goodfellow 2012); the taxonomic status of some of these lineages is supported by 16S rRNA and high bootstrap values and 16S rRNA gene signatures (Goodfellow et al. 1998a; Gürtler et al. 2004; Zhao et al. 2012; Jones et al. 2013b). Nevertheless, the taxonomic implications of rhodococcal lineages remain unclear though it has been mooted that some of them, notably the *R. erythropolis* and *R. rhodochrous* subclades, might merit generic status (Goodfellow et al. 1998a; Gürtler et al. 2004; McMinn et al. 2004, Jones and Goodfellow 2012). It is also interesting that *R. cerastii*, *R. fascians*, *R. kyotensis*, and *R. yunnanensis* form an independent lineage loosely associated with the *R. erythropolis* subclade. Similarly, *R. rhodnii* forms a distinct branch in the rhodococcal tree just beyond the periphery of the *R. rhodochrous* subclade. Finally, *R. corynebacteroides*, *R. kroppenstedtii*, *R. triatomae*, and *R. trifolii* form a somewhat diffuse subclade; *R. canchipurensis* also belongs to this taxon (Nimaichand et al. 2013).

The genus *Williamsia*, the remaining multimembered taxon, forms a monophyletic clade within the evolutionary radiation encompassed by the family *Nocardiaceae* (Fig. 32.5). The eight validly published species fall into two subclades that encompass strains with short branches. The two most closely related strains, *W. marianensis* and *W. muralis*, share a 99.5 % 16S rRNA gene similarity, but were isolated from markedly different environments, namely, sediment collected from the Challenger Deep of the Mariana Trench and from non-water-damaged building material, respectively (Kämpfer et al. 1999; Pathom-aree et al. 2006).

The remaining genera classified in the family *Nocardiaceae* are monospecific. Three of them, *Millisia brevis*, *Skermania piniformis*, and *Smaragdicoccus niigatensis*, form markedly independent lineages (Fig. 32.6). The fourth, "*Prescottella equi*," is the causal agent of equine pneumonia and has had a tortuous taxonomic history. Until recently, this organism sat uneasily within the genus *Rhodococcus*, as *R. equi*, as it was not clear from 16S rRNA gene sequence analyses whether it should be seen as a genus in its own right (Ruimy et al. 1995; McMinn et al. 2000; Gürtler et al. 2004; Gürtler and Seviour 2010) or as a taxon more closely related to the genus *Nocardia* than to other members of the genus *Rhodococcus* (Ruimy et al. 1994, 1995;

Fig. 32.2

Phylogenetic reconstruction of the genus *Nocardia* 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). Scale bar indicates estimated sequence divergence

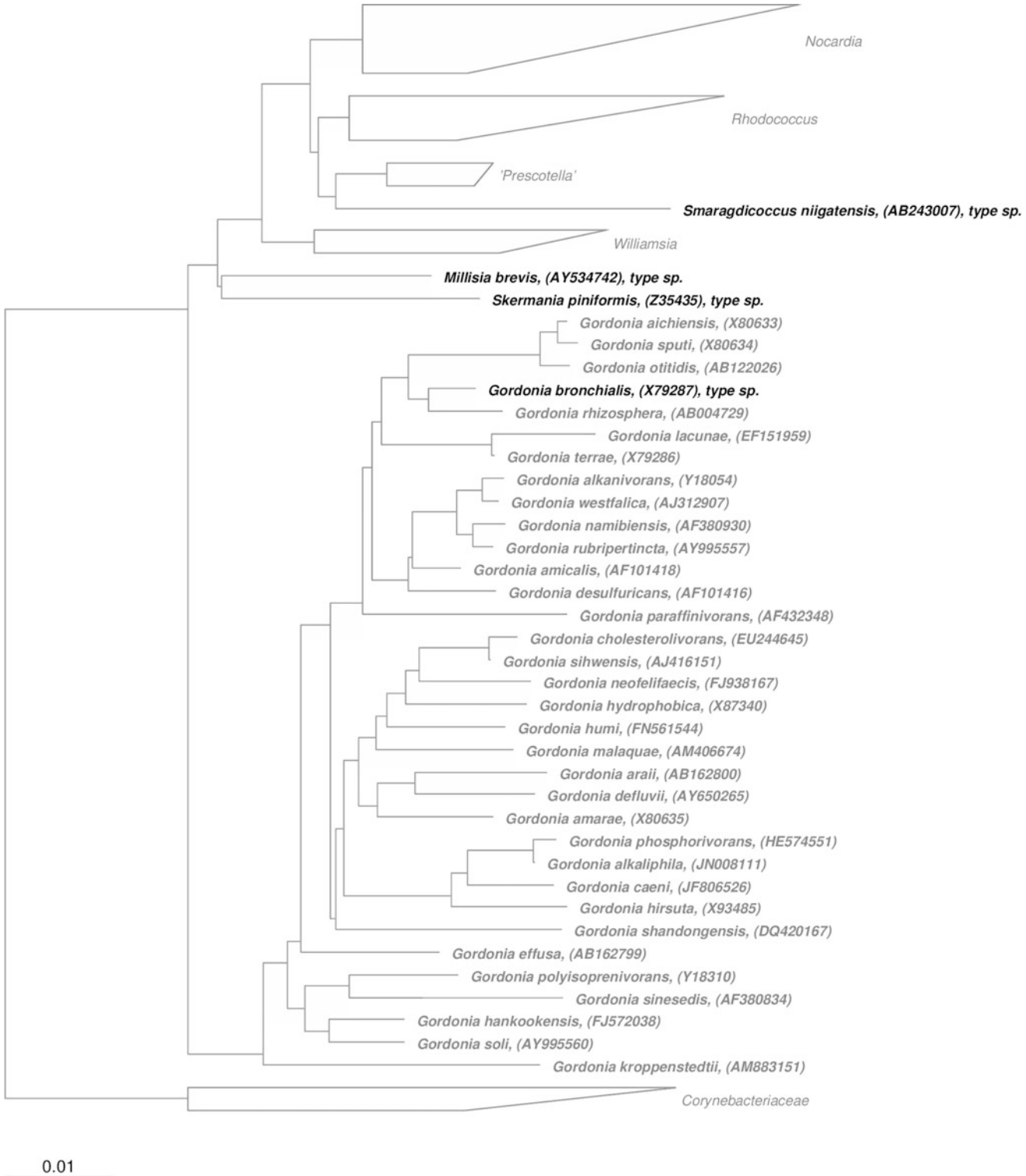

Fig. 32.3

Phylogenetic reconstruction of the genus *Gordonia* 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). Scale bar indicates estimated sequence divergence

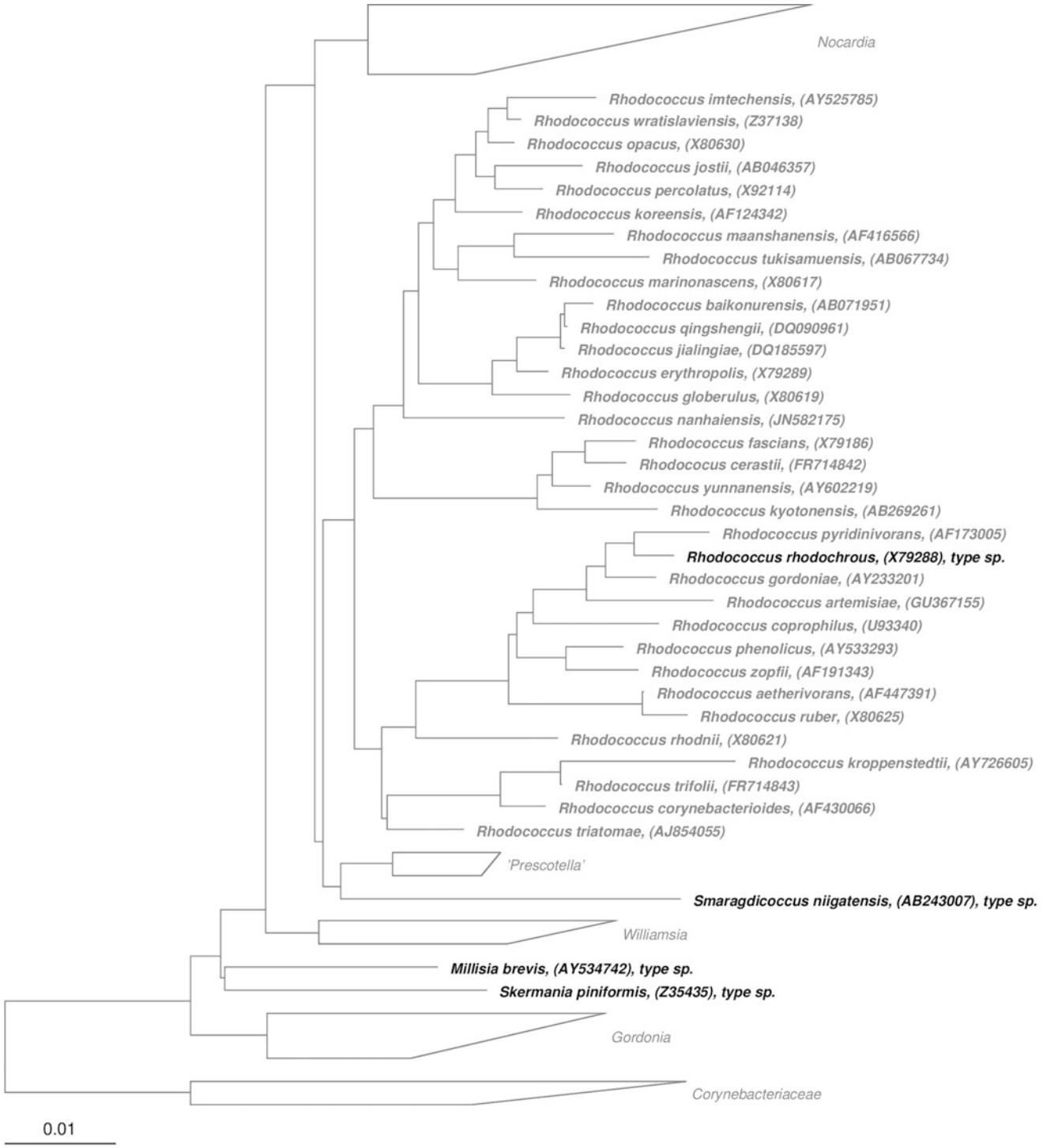

Fig. 32.4

Phylogenetic reconstruction of the genus *Rhodococcus* 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). Scale bar indicates estimated sequence divergence

Goodfellow et al. 1998a; Matsuyama et al. 2003; Yoon et al. 2000a). This Gordonian knot was severed when Jones et al. (2013b) proposed that *R. equi* be reclassified as "*Prescottia equi*." This binomial was subsequently replaced by "*Prescotella equi*" when the name *Prescottia* was found to be illegitimate as it had previously been used for a plant genus belonging to the family *Orchidaceae* (Jones et al. 2013a). The genus name "*Prescottella*" and its constituent species "*P. equi*"

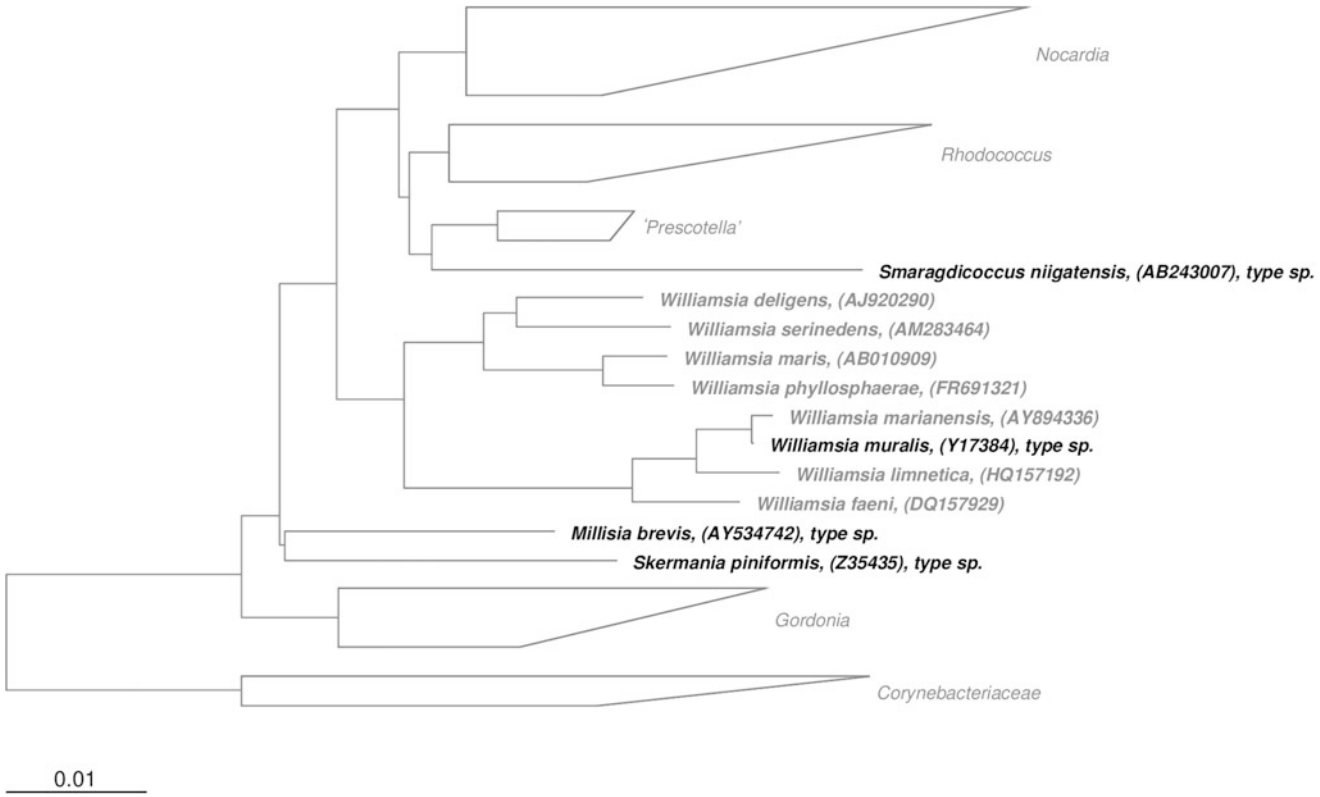

Fig. 32.5
Phylogenetic reconstruction of the genus *Williamsia* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence dataset and alignment were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Scale bar indicates estimated sequence divergence

have still to be validated, but will be used throughout this chapter as comparative genomic analyses show that members of this taxon are closely related to one another, but distantly related to the remaining *Rhodococcus* strains and to *N. brasiliensis* (Sangal et al. 2014). The taxonomic status of *R. kunmingensis* needs to be clarified as this organism forms a loose branch in the *Nocardiaceae* gene tree with *P. equi* (*Fig. 32.1*), as found by Jones et al. (2013b). *Smaragdicoccus niigatensis* is loosely associated with this lineage.

Molecular Analyses

DNA:DNA Hybridization Studies

It is laborious and time-consuming to establish DNA:DNA hybridization (DDH) values; hence, where possible, it is advisable to avoid doing DDH measurements. Stackebrandt and Goebel (1994) recommended that if two strains shared 16S rRNA gene sequence similarities at or below a 97 % threshold, it was not necessary to undertake DDH determinations to prove that they belonged to the same species. This threshold was subsequently raised to 98.7–99 % based, as before, on an empirical dataset compiled from the taxonomic literature (Stackebrandt and Ebers 2006). Using real-world 16S rRNA gene sequence and DDH data, Meier-Kolthoff et al. (2013) have concluded, using a regression model, that a threshold of 99 % is a reasonable cut-off point at which DDH experiments are no longer required to show that strains belong to the same species. This means that many of the DDH experiments carried out on members of the family *Nocardiaceae* could have been avoided without a significant risk of strains being misclassified.

DNA:DNA hybridization assays have been used extensively to delineate *Gordonia*, *Nocardia*, *Rhodococcus*, and *Williamsia* species. In general, such studies have shown that DNA:DNA relatedness values are low when compared with corresponding 16S rRNA gene similarities. This has proved to be particularly so in studies designed to clarify relationships between closely related nocardiae. The type strains of *N. kruczakiae* and *N. veterana*, for instance, share a 16S rRNA gene similarity of 99.8 % and a DNA:DNA homology of 55 ± 8.5 (Conville and Witebsky 2005), a relatedness value below the universally recognized 70 % cut-off point for the delineation of bacterial species (Wayne et al. 1987). In contrast, DDH studies showed that representatives of aciditolerant nocardiae which shared 16S rRNA gene similarity values between 99.4 % and 99.8 % belonged to the same genomic species (Golinska et al. 2013). These results suggest that the relationship between

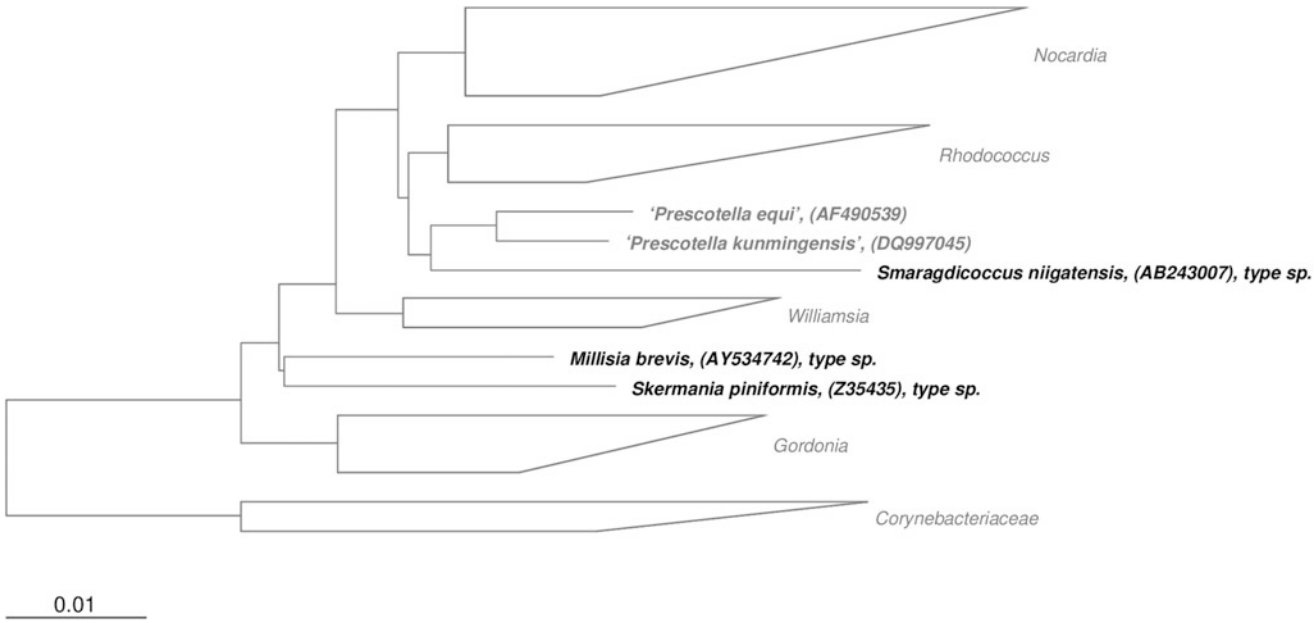

Fig. 32.6

Phylogenetic reconstruction of the genera '*Prescotella*', *Smaragdicoccus, Millisia* and *Skermania* 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). Scale bar indicates estimated sequence divergence

N. cummidelens and *N. soli* needs to revisited, given their position in the *Nocardia* tree (*Fig. 32.2*).

DDH assays have been especially useful in clarifying relationships between clinically significant isolates assigned to the *N. asteroides* complex, as exemplified in the recognition of *N. abscessus* (Yassin et al. 2000), *N. anaemiae* (Kageyama et al. 2005a), *N. araoensis* (Kageyama et al. 2004a), *N. concava* (Kageyama et al. 2005b) *N. cyriacigeorgica* (Yassin et al. 2001a), *N. exalbica* (Iida et al. 2006), *N. higoensis* (Kageyama et al. 2004b), and *N. niigatensis* (Kageyama et al. 2004c). Similarly, DHH studies provided valuable data for the classification of strains assigned to the *N. transvalensis* 16S rRNA gene clade as *N. blacklockiae* and *N. wallacei* (Conville et al. 2008) and for the establishment of *N. mexicana* (Rodrigues-Nava et al. 2004) and *N. pseudobrasiliensis* (Ruimy et al. 1996) for strains previously classified as *N. brasiliensis*. DDH studies have also been pivotal in the classification of nocardiae isolated from diverse habitats, as seen by the recognition of *N. artemsiae* (Zhao et al. 2012), *N. endophytica* (Xing et al. 2011), *N. goodfellowii* (Sazak et al. 2012), *N. iowensis* (Lamm et al. 2009), *N. jiangxiensis* (Cui et al. 2005), *N. rhamnosiphila* (Everest et al. 2011), *N. takedensis* (Yamamura et al. 2005), and *N. xishanensis* (Zhang et al. 2004).

It is evident from DDH studies that all *Gordonia* species form well-delineated genomic species (Goodfellow et al. 2012), including those which have high 16S rRNA gene similarity values. The type strains of *G. aichiensis* and *S. sputi*, for instance, share a 16S rRNA gene similarity of 99.7 %, but have a DNA:DNA relatedness value of only 38–40 % 3(Klatte et al. 1994b; Goodfellow et al. 1998a). Similarly, relatively high DDH similarities have been recorded between *G. alkaliphila* and *G. hirsuta* (Cha and Cha 2013), *G. caeni* and *G. hirsuta* (Srinivasan et al. 2012), *G. humi* and *G. amarae* (Kämpfer et al. 2011a), and *G. neofelifaecis* (Liu et al. 2011a) and *G. sihwensis* (Kim et al. 2003). In addition, DNA:DNA relatedness data provided strong support for the proposal that *G. nitida* (Yoon et al. 2000a) be recognized as a later synonym of *G. alkalivorans* (Arenskőtter et al. 2005).

Some *Rhodococcus* species, notably ones classified in the *R. erythropolis* subclade, share high 16S rRNA gene sequence similarities, but relatively low DNA:DNA relatedness values (Zhang et al. 2005; Ghosh et al. 2006; Xu et al. 2007; Li et al. 2008; Wang et al. 2010; Kämpfer et al. 2013b). The type strain of *R. jialingiae*, for example, is closely related to the type strains of *R. baikonurensis* (99.8 % 16S rRNA gene sequence similarity), *R. erythropolis* (99.1 %), *R. globerula* (98.3 %), and *R. quinshengii* (98.8 %), but the corresponding DDH values are low at 19.3 %, 18.6 %, 10.6 %, and 27.7 %, respectively (Wang et al. 2010). Similar results have been reported for species assigned to the *R. rhodochrous* subclade (Goodfellow et al. 2004; Jones et al. 2004; Zhao et al. 2012) and to the heterogeneous subclade that encompasses *R. canchipurensis*, *R. corynebacteroides*, *R. kroppenstedtii*, *R. triatomae*, and *R. trifolii* (Mayilraj et al. 2006; Kämpfer et al. 2013b; Nimaichand et al. 2013); the type strains of *R. aetherivorans* and *R. ruber*, for example, have a 16S rRNA gene sequence similarity of 99.6 % and 46 % DNA relatedness (Goodfellow et al. 2004). The type strains of *R. corynebacteroides* and *R. kroppenstedtii* show 62 % DNA:DNA relatedness (Mayilraj et al. 2006), a value previously

incorrectly attributed to *R. kroppenstedtii* and "*P. equi*" (Jones and Goodfellow 2012). It is also clear from DNA:DNA pairing and associated data that the *Rhodococcus* strain RHA1 (McLeod et al. 2006) is a *bona fide* member of the species, *R. jostii* (Jones et al. 2013c).

DNA:DNA relatedness studies were not carried out between the type strains of *W. deligens*, *W. faeni*, and *W. serinedens* and their nearest phylogenetic nerighbors, given low 16S rRNA gene sequence similarities (Yassin and Hupfer 2006; Yassin et al. 2007; Jones et al. 2010). Indeed, several of the DDH assays that were carried out between the type strains of novel *Williamsia* species could have been safely omitted, given the 16S rRNA gene sequence threshold recommended by Meier-Kolthoff et al. 2013). The type strains of the two most closely related species, *W. marianensis* and *W. maris*, share a 99.5 % 16S rRNA gene sequence similarity and 11 % DNA:DNA relatedness (Pathom-aree et al. 2006). Similarly, *W. limnetica* DSM 45521^T, a relatively close relative of *W. marianensis* DSM 44944^T and *W. muralis* DSM 44343^T, shares DDH values with the latter of 18.8 % and 22.4 %, respectively (Sazak and Sahin 2012). Further, the type strain of *W. phyllosphaerae* has a DDH similarity of 29.4 % with *W. maris* DSM 44693^T, its phylogenetic neighbor (Kämpfer et al. 2011b); the corresponding value between the type strains of *W. maris* and *W. muralis* was recorded as 23 % (Stach et al. 2004).

"*Prescotella equi*" NCTC 1621^T shares low levels of DNA:DNA similarity with representative strains of *G. rubripertincta* (8–10 %), *R. coprophilus* (10–16 %), *R. rhodochrous* (7–18 %), and *R. ruber* (9–20 %) (Mordarski et al. 1980, 1981). In contrast, a somewhat higher DNA:DNA relatedness value, 34 ± 10 %, was reported between the type strains of *R. kunmingensis* and "*P. equi*" which form a loose clade in the *Nocardiaceae* 16S rRNA gene tree (➔ *Fig. 32.1*). The type strains of *M. brevis*, *S. piniformis*, and *S. niigatensis*, the only species classified in the genera *Millisia*, *Skermania* and *Smaragdicoccus* respectively, have such low levels of 16S rRNA gene similarities to their nearest neighbors, namely, species of *Gordonia* (93.7–95.7 %), *Nocardia*, and *Rhodococcus* (94.5–95.7), and *Nocardia* (94.5–95.0 %), respectively, that it was not necessary to carry out DNA:DNA relatedness studies.

Phages

Our scant knowledge about *Nocardiaceae* phages is surprising, given their potential as biocontrol agents (Petrovski et al. 2011a, b) and as key drivers of bacterial niche adaptation and evolution (Chanchaya et al. 2003; Comeau and Krisch 2005). Bradley and his colleagues studied the growth and characteristics of nocardiophages (Bradley and Ritzi 1967; Brownell et al. 1967) and showed that they could be used to distinguish between actinomycete genera (Bradley and Anderson 1958; Bradley et al. 1961). Following Bradley's lead, extensive phage host range studies were undertaken by Prauser and coworkers (Prauser and Falta 1968; Prauser and Momirova 1970; Prauser 1976, 1981) who found that actinophages, including those active against *Nocardia* and related organisms (nocardiophages), only showed activity on hosts with the same wall chemotype (as described by Lechevalier and Lechevalier 1970). The close relationship between the genera *Nocardia* and *Rhodococcus* was underpinned by actinophage susceptibility patterns (Prauser 1981), a result underlined and extended in more recent studies (Thomas et al. 2002; Petrovski et al. 2011a, d).

Phages that infect *Nocardia* species have received little attention though ones active against *N. asteroides* (Andrzejewski and Pietkiewicz 1972; Pulverer et al. 1975; Andrzejewski et al. 1978; Petrovski et al. 2011b), *N. brasiliensis* (Pulverer et al. 1974; Petrovski et al. 2013a), *N. carnea* (Williams et al. 1980; Petrovski et al. 2013a), *N. nova* (Petrovski et al. 2013b), and *N. otitidiscaviarum* (Prauser 1981) have been reported. A phage isolated from a *N. asteroides* strain was found to have a non-contractible tail and a head, the shape of an icosahedron (Andrzejewski and Müller 1975). Putative prophages have been detected in the whole-genome sequence of *N. farcinica* IMF 10152 (Ishikawa et al. 2004).

Phages targeting *Rhodococcus* strains have received more attention than those propagated in *Nocardia* species. In early studies, phages active against rhodococci were used extensively as genetic markers in mating experiments involving strains labeled "*N. canicruria*" and "*N. erythropolis*." Some of these phages, such as φC and φEC, were examined in growth and characterization experiments (Brownell et al. 1967, 1980; Brownell and Denniston 1984; Brownell and Crockett 1971). However, while a limited number of *Rhodococcus* phages have been characterized, relatively little was known until recently about *Rhodococcus* phage genomics, as exemplified by studies on *Rhodococcus* bacteriophages RGL3 and RER2 (Petrovski et al. 2011a, 2013a).

Phages RGL3 and RER2, which lyse *Rhodococcus* and *Nocardia* species, have novel genomes of 46.5 and 48.0 kb, respectively, and share a modular genome organization, as seen in other sequenced *Siphoviridae* phage genomes (Petrovski et al. 2013a). Interestingly the genomes of these phages do not share any similarity with other *Nocardia* or *Rhodococcus* phages, but are related to *Mycobacterium* phages. Other polyvalent phages active against rhodococci were isolated from four different activated sludge plants and one of them, phage RRH1, was shown to have the smallest recorded *Siphoviridae* genome (14.2 kb) with only 20 genes (Petrovski et al. 2011d).

"*Prescotella equi*" phages isolated from soil have been the subject of characterization, genomic and functional analyses. Summer et al. (2011) sequenced the genomes of four such phages and found that they had features characteristic of mycobacteriophages, including the prevalent long-tailed morphology and the presence of genes encoding Lys B-like mycolate-hydrolyzing lysis proteins. Twenty-seven "*P. equi*" phages were isolated from geographically separated locations by Salifu et al. (2013a) who divided them into 16 distinct groups based on host range, genomic restriction patterns, and virion protein profiles. Most of the phages were *Siphoviridae*, but one

group belonged to the *Myoviridae*, the first nonmycobacterial actinomycete phage assigned to this family. A comprehensive genomic and proteomic analysis of one of the phages assigned to this unique group had a circular chromosome and an average GC content (67.7 mol%) similar to that of its host, "*P. equi*" NCIMB 10027 (Salifu et al. 2013b). Petrovski et al. (2013b) isolated a novel *Siphoviridae* phage, REQ1, with a genome size of 51.3 bp. The lytic ability of this phage was assessed against 65 different actinobacterial strains (Petrovski et al. 2011c), but it was only active on a single strain, "*P. equi*" Requ 28.

There are grounds for believing that "*P. equi*" phage may be of value in reducing "*P. equi*" populations in environments where foals are susceptible to infection (Summer et al. 2011; Petrovski et al. 2013b; Salifu et al. 2013a). In a similar vein, Shibayama and Dabbs (2011) found that phage YF1, which they propagated on "*P. equi*" ATCC 14887, encoded multiple inhibitory products which might contribute to antibacterial drug discovery by providing new antibacterial targets.

Gordonia is the only one of the remaining genera classified in the family *Nocardiaceae* that contains strains used to propagate phage. Polyvalent *Gordonia* phage GTE2, a member of the family *Siphovirdiae*, has a characteristic icosahedral head encompassing a double-stranded DNA linear genome (45.5 kb) with 10 bp 3′-protruding ends, and is a candidate for the biocontrol of *Gordonia*—*Rhodococcus*—and *Nocardia*—stabilized foams in activated sludge plants (Petrovski et al. 2011a). Petrovski et al. (2012) described two phages, GRU1 and GTE5, originally isolated from activated sludge by Thomas et al. (2002) that targeted *G. rubripertincta* and *G. terrae*, respectively. These phages were shown to be closely related members of the family *Siphoviridae*, possessed similar-sized icosahedral heads that encompassed double-stranded DNA (~65 kb) though only phage GRU1 propagated in a strain of *N. nova*. The two phages reduced or eliminated foam formation by their host cells under laboratory conditions and hence quality as prospective components of cocktails of lytic phages designed to reduce populations of foam generating mycolic acid–containing actinomycetes below the threshold required for stable foam formation in activated sludge plants (Thomas et al. 2002; Withey et al. 2005; Petrovski et al. 2011c). Similarly, *Tsukamurella* phage TPA2, an important member of the activated sludge phage metapopulation, targets a wide range of *Tsukamurella* species and hence may be useful for the biocontrol of *Tsukamurella* stabilized foams (Petrovski et al. 2011e).

Plasmids

Plasmids have been detected in pathogenic strains of *Nocardia* (Provost et al. 1996; Ishikawa et al. 2004), including *N. asteroides* (Kasweck et al. 1981, 1982; Kasweck and Little 1982), in *Gordonia* strains (Brőker et al. 2004; Indest et al. 2010), in "*P. equi*" (Vásquez-Boland et al. 2010; Giguere et al. 1999, 2011), but are especially common in *Rhodococcus* species (Gürtler et al. 2004; Larkin et al. 2010).

Provost and his colleagues (1996) found a statistically significant correlation between the localization of cutaneous infections and the virulence of plasmid-bearing nocardiae, but were not able to relate the presence of plasmids to specific phenotypic traits. Two circular plasmids, pNF1 and pNF2, were detected in a *N. farcinica* strain (Ishikawa et al. 2004). Xia et al. (2006) sequenced a small circular plasmid, PXT 107, from *Nocardia* strain 107 and showed that it consisted of 4335 base pairs (bp) and encoded a replication palindromic (Rep) protein and six hypothetic proteins. They also found that the *E. coli–Nocardia* shuttle vector pHA Q22, which includes the rep gene of PXT 107, propagated in *Nocardia*, but not in *Streptomyces*. Descriptions of other native plasmids in *Nocardia* include pN 1100 (Liu et al. 2000), p C1 (Shen et al. 2006c), and PYS1, a cryptic, broad-host range plasmid from *N. aobensis* IFM 10795 (Shibayama et al. 2011). *Nocardia*-derived plasmids are being used increasingly as cloning vectors, as outlined by Luo et al. (2013a). A *Rhodococcus*, *Gordonia–E. coli* shuttle vector (Kalscheuer et al. 1999) was found to replicate in *Nocardia* (Luo et al. 2013b).

Plasmids have been isolated and characterized from a few *Gordonia* strains, including the type strain of *G. bronchialis* (see NCBI Genome Database). A native 101-kbp megaplasmid, pkB1, isolated from *G. westfalicia* KbI, encodes genes essential for cadmium resistance and rubber degradation (Brőker et al. 2004). Subsequently, Brőker and his colleagues (2008) constructed mobilized *E.coli–Gordonia* shuttle vectors based on the origin of replication of megaplasmid pkBI and showed that it could be transferred from the *G. westfalicia* strain to related bacteria such as *G. polyisoprenivorans*. Indest et al. (2010) characterized megaplasmid pGKT2 from *Gordonia* sp. strain KTR9 that contains flavodoxin reductase genes (XpIB) which are involved in the degradation of hexahydro-1,3,5-trinitro-1,3,5 triazine (Hexogen); these genes were upregulated nearly fourfold when the strain was grown in the presence of Hexogen, but were repressed by various inorganic nitrogen sources.

The many different types of plasmids found in *Rhodococcus* strains range from small cryptic, closed circular plasmids to large linear ones (Larkin et al. 1998, 2005, 2010; van der Geize and Dijkhuizen 2004; Matsui et al. 2007; Letek et al. 2008). Many rhodococcal cells contain circular and linear plasmids, as exemplified by *R. rhodochrous* strain B-276 which has four circular cryptic and four linear megapasmids (Saeki 1998; Saeki et al. 1999). Similarly, *R. erythropolis* PR4, an alkane-degrading strain, contains one linear and two circular plasmids (Sekine et al. 2006). The innumerable small circular plasmids that have been detailed in rhodococci, include plasmids pKA 22 (4,969 bp) and pKTL1 (100 bp) that encode haloalkane-degrading genes (Kulakova et al. 1995), and a 150 bp plasmid in *R. erythropolis* strain IGTS 8 which encodes genes involved in desulfurization of organosulfur compounds (Denis-Larose et al. 1997). Other circular plasmids code for diphenyl metabolism (Masai et al. 1997), chloroalkane degradation (Kulakova et al. 1995), 2-methylalanine metabolism (Schreiner et al. 1991), and propene degradation (Matsui et al. 2007). Some cryptic plasmids have mobilization functions (Yang et al. 2007a).

A distinctive feature of many studies on rhodococci is that large linear plasmids are associated with the presence of catabolic genes. Thus, *R. jostii* strain RHA1 (Jones et al. 2013c), the most effective degrader of polychlorinated biphenyls, contains three large linear plasmids, pRHL1 (1,100 kb), pRHL2 (450 kb), and pRHL3 (330 kb) (McLeod et al. 2006). Other large linear rhodococcal plasmids encode genes for the catabolism of alkylbenzene (Kim et al. 2002), naphthalene (Uz et al. 2000; Kulakov et al. 2005), toluene (O'Brien et al. 2002; Priefert et al. 2004), trichloroethene (Saeki et al. 1999), and chloroaromatic compounds (Konig et al. 2004). Such plasmids are often conjugative and have conjugative genes and functions (Yang et al. 2007b).

Other functions coded by linear plasmids include hydrogen autotrophy in *R. opacus* (Kalkus et al. 1990, 1993), plant virulence genes in *R. fascians* (Crespi et al. 1992; Depuydt et al. 2009a, b), and isopropyl metabolism in *R. erythropolis* (Masai et al. 1995). Sequences and genome annotations have been generated for some linear plasmids, including pBD2 from *R. erythropolis* (Strecker et al. 2003), pREC1 and p REL2 from *R. erythropolis* PR4 (Sekine et al. 2006), and pRHL3 from *R. jostii* strain RHA1 (Warren et al. 2004). Rhodococcal plasmids have been used as vectors for the expression of recombinant proteins (Nakashima and Tamura 2004a, b) and for the development of shuttle vectors (Kostichka et al. 2008; Lessard et al. 2004; Mangan et al. 2005).

Plasmids associated with pathogenicity determinants in "*P. equi*" have been characterized extensively (Prescott 1991; Takai et al. 1991a; Tkachuk-Saad and Prescott 1991; Ribeiro et al. 2005; Vásquez-Boland et al. 2010). These large, circular plasmids encode *vap* genes that are involved in the pathogenicity of horses (*vap A*) and pigs (*vap B*). Several variants of the *vap A* plasmid have been determined based on restriction fragment length polymorphisms (Takao et al. 1999; Ribeiro et al. 2005). A simple PCR assay is available to distinguish between *vap A* and *vap B* plasmid types (Oldfield et al. 2004). There is evidence that vap genes evolved in plasmid-encoded pathogenicity islands (Letek et al. 2008). These workers also noted that "*P. equi*" virulence factors are representative of a new family of actinobacterial plasmids that combine plasmid-specific insertions with a conserved backbone structure linked to plasmid maintenance and transfer.

Molecular Identification and Typing

Reliable methods are available to assign unknown *Nocardiaceae* isolates to the genus level (Goodfellow and Jones 2012), but accurate identification to species remains problematic even for clinically significant strains. The difficulties associated with accurate identification of unknown nocardiae based on biochemical, chemical, morphological, and physiological criteria led to the introduction of molecular diagnostic tools, notably to distinguish between species of pathogenic *Nocardia* (Laurent et al. 1996; Isik and Goodfellow 2002; Gürtler et al. 2004; Goodfellow and Maldonado 2012; van de Sande et al. 2014a, b). PCR-restriction pattern analysis of a 441-bp fragment of the 65-kDA host shock protein (*hsp 65*) was used to differentiate between individual *Nocardia* species (Steingrübe et al. 1995, 1997; Wilson et al. 1998; Rodrigues-Nava et al. 2006). Sequence-based approaches such as those based on 16S rRNA gene polymorphisms have also been used for this purpose (Mellmann et al. 2003; Cloud et al. 2004; Patel et al. 2004; Conville et al. 2006). Molecular methods can also be used to directly detect nocardiae in clinical material (Couble et al. 2005; Marchandin et al. 2006).

Approaches such as those outlined above, while of value, have led to the misidentification of both clinically and environmentally significant nocardiae when used to distinguish between a broad range of *Nocardia* species (Conville et al. 2000; Pottumarthy et al. 2003; Rodrigues-Nova et al. 2006). This problem is compounded by the fact that the genus *Nocardia* is underspeciated (Wang et al. 1999; Orchard and Goodfellow 1980; Roth et al. 2003) and includes important pathogenic species, such as *N. asteroides*, *N. farcinica*, and *N. nova*, that are heterogeneous (Goodfellow and Maldonado 2012). Consequently, for accurate identification, unknown nocardiae need to be compared with their nearest phylogenetic neighbors using appropriate diagnostic procedures.

Molecular-based approaches have been used to distinguish between *Gordonia* and *Rhodococcus* species. Clinically significant gordoniae have been assigned to species based on PCR-restriction enzyme analysis of the 439-bp Telenti fragment of the 65 *hsp* gene (Patel et al. 2004), while primers targeted at species-specific 16S rRNA gene signatures have been shown to distinguish between some *Rhodococcus* species (Bell et al. 1999). Primers have also been used to detect a unique 700-bp fragment of "*P. equi*" chromosomal DNA (Arriaga et al. 2002). This organism can also be detected using a PCR assay that targets the choE gene (Ladrón et al. 2003).

At one time, serological and skin testing for cutaneous hypersensitivity were used for early diagnosis of nocardial infections in animals and humans (Pier et al. 1968; Pier and Fichtner 1971, 1981; Magnusson 1976; Angeles and Sugar 1987; Boiron and Provost 1990a; Boiron and Stynen 1992; Boiron et al. 1993). In general, these approaches have fallen from grace, partly because of their low sensitivity and lack of specificity, but also due to the introduction of diagnostic molecular procedures. However, the enzyme-linked immunosorbent assay (ELISA) introduced for the serological diagnosis of *N. brasiliensis* was useful in cases of mycetoma where the identification of the causal agent in culture was not possible (Salinas-Carmona et al. 1993).

Typing methods have been used to establish the infection source and mode of transmission of clinically significant *Nocardiaceae* species, especially with respect to hospital-acquired nocardial infections (Schaal and Lee 1992; McNeil and Brown 1994; Brown-Elliott et al. 2006). Thus, an outbreak of nocardiosis among immunocompromised patients in a renal unit was attributed to a specific *N. asteroides* serotype (Stevens et al. 1981). Clinically relevant *N. asteroides* strains have been

Table 32.1

Comparison of the general features of the complete genomes of strains classified in the family *Nocardiaceae*

Strains	Chromosomal topology	No. of plasmids (topology)	Total genome size (Mb)	Average GC%	No. of genes	No. of proteins	rrn operons	tRNA genes
Gordonia bronchialis Strain 3410^T	Circular	1 (Circular)	5.29	67.1	4,984	4,696	2	49
Nocardia brasiliensis Strain HUJEG-1	Circular	–	9.44	68.0	8,474	8,414	3	51
Nocardia cyriacigeorgica Strain GUH-2	Circular	–	6.19	68.4	5,560	5,477	3	49
Nocardia farcinica Strain IFM 10152	Circular	2 (Circular)	6.29	70.7	5,998	5,934	3	53
"*Prescotella equi*" ATCC 33707	Circular	–	5.26	68.8	5,105	5,030	5	52
Rhodococcus erythropolis Strain PR4	Circular	1 (Linear), 2 (Circular)	6.90	62.3	6,511	6,437	5	54
Rhodoccous jostii Strain RHA1	Linear	3 (Linear)	9.70	67.0	9,221	9,145	4	50
Rhodococcus opacus Strain B4	Linear	2 (Linear), 3 (Circular)	8.83	67.6	8,259	8,197	4	49

Note:
1. The virulence plasmid of *P. equi* ATCC 33707 was not included
2. The details were obtained from NCBI Genome Database (http://www.ncbi.nlm.nih.gov/genome)

typed using plasmid (Jonsson et al. 1986), restriction fragment length polymorphisms (Patterson et al. 1992), pulsed field gel electrophoresis, and randomly amplified polymorphic DNA PCR (Louie et al. 1997). Pulsed field gel electrophoresis was used to show that a *N. farcinica* strain was responsible for postoperative wound infections in a hospital surgical ward in Germany (Blümel et al. 1998). Studies on herds of dairy cattle where cases of mastitis were evident showed that the causal *N. asteroides* strains were usually of the same serotype, implying animal-to-animal transmission (Pier and Fichtner 1981).

Molecular epidemiological studies on "*P. equi*" have revealed considerable heterogeneity in the genotype of clinical and fecal strains isolated from foals, horses, and their immediate environment (Soedarmanto et al. 1997; Morton et al. 2001; Cohen et al. 2003; Venner et al. 2007). Bolton and his colleagues (2010) examined *rep*-PCR amplicons, in an automated microfluidics format, to type virulent "*P. equi*" isolates from a single foal and found that the animal was infected with multiple strains of the pathogen. A simple PCR typing system for "*P. equi*" based on three virulent plasmids demonstrated that there was an association between specific plasmid types and animal hosts (Ocampo-Sosa et al. 2007).

Genome Comparisons

Only eight full genome sequences are available for members of the family *Nocardiaceae* (Table 32.1). However, 25 *Nocardia*, 17 *Rhodococcus*, and 1 *Smaragdicoccus niigatensis* draft sequences have been published by the NCBI Genome Database (http://www.ncbi.nlm.nih.gov/genome) with the promise of many more to come (Luo et al. 2013a).

Complete genome sequences are available for three strains of *Nocardia*: *N. brasiliensis* HUJEG-1 (Vera-Cabrera et al. 2012), *N. cyriacigeorgica* (Zoropogui et al. 2012), and *N. farcinica* IFM 10152 (Ishikawa et al. 2004). All three organisms have circular chromosomes though the one for *N. brasiliensis* is considerably larger than those for the other two strains (Table 32.1). The complete genome of *N. farcinica* IFM 10152, an organism that was isolated from the bronchus of a 68-year-old male Japanese patient, consists of a single, circular chromosome (6.01 Mb) with a mean GC content of 70.8 mol% and two plasmids, pNF1 (184,027 bp) and p NF2 (87,093 bp), with mean G+C contents of 67.2 % and 68.4 %, respectively. This chromosome contains many candidate genes for multidrug resistance, secondary metabolism, and virulence, whereas plasmids pNF2 and pNF1 encode 90 and 160 predicted protein-coding genes, respectively. Analysis of paralogous protein families indicates that the organism has the capacity to live as a saprophyte in the soil ecosystem and as a pathogen in animal tissue.

It is also apparent from the complete genomes of the *N. brasiliensis* and *N. cyriacigeorgica* strains that they have the capacity to follow both saprophytic and pathogenic modes of life (Vera-Cabrera et al. 2013; Zoropogui et al. 2012). The *N. brasiliensis* strain has been used widely in antimicrobial and immunological assays (Salinas-Carmona and Rocha-Pizaña 2011;

Salinas-Carmona et al. 2011; Trevino-Villareal et al. 2012). The complete genome of this organism contains many catabolic and lipid biosynthetic genes, orthologs for virulence factors, and gene clusters for the synthesis of bioactive compounds, including antibiotics, polyketides, and terpenes (Vera-Cabrera et al. 2012, 2013). Virulence factors, such as lipases, phosphatases, and proteases, were also found. An *in silico* analysis of the genome sequence indicated that the organism had acquired diverse genes by horizontal gene transfer from other members of the soil ecosystem.

The *N. cyriacigeorgica* (formerly *N. asteroides*) strain was isolated from a fatal human infection (Beaman and Maslam 1977) and subsequently used extensively for studying nocardial infection (Beaman 1981; Beaman and Beaman 1994, 2000). The whole genome of this organism includes candidate genes similar to those found in the complete genomes of *M. tuberculosis* H37Rv^T and *N. farcinica* IFM 10152 (Cole et al. 1998; Ishikawa et al. 2004). The genes included 6 complete *myc* loci which code for mammalian cell entry, a 385-kDa—antigen protein family and 19 lipoproteins (Zoropogui et al. 2012). In addition, two superoxide dismutases and three catalase genes were found to be similar to genes involved in macrophage resistance in *Nocardia* (Beaman et al. 1985).

A complete genome is available for *G. bronchialis* strain 3410^T (Ivanova et al. 2010) and a draft one for *G. neofelifaeces* NRRL B-51395^T (Ge et al. 2011). The general features of the genome of the *G. bronchialis* strain are shown in *Table 32.1*. Nearly 67 % of the protein-coding genes of this organism were given a predicted function, 5.5 % for lipid transport and metabolism, and 4.2 % for metabolite biosynthesis, transport, and metabolism. The *G. neofelifaecis* strain was isolated from the feces of a clouded leopard (*Neofilis nebulosa*) and shown to transform cholesterol to androsta-1,4-diene-3,17-dione by side chain cleavage (Liu et al. 2011a, b). The draft genome (4.25 Mb) includes 46 genes for tRNA, 5 rRNA loci, and, as predicted, several gene clusters for cholesterol degradation.

Complete genome sequences have been generated for *R. jostii* strain RHA 1 (McLeod et al. 2006), *R. erythropolis* strain PR4, and *R. opacus* strain B4 though published reports on the latter two organisms are awaited. However, all three organisms have similar genomic features though the *R. erythropolis* has a circular chromosome (*Table 32.1*). The *R. jostii* strain, an isolate from lindane-contaminated soil (Seto et al. 1995), is particularly well known for its ability to transform polychlorinated biphenyls. This organism has a large genome (9.70 Mb) composed of a linear chromosome and three linear plasmids. The genome contains many catabolic genes which squares with the remarkable catabolic versatility of the strain. The predicted protein-coding genes are not only rich in ligases and oxygenases, but also in nonribosomal peptide synthase genes that are indicative of an extensive secondary metabolism. The organism was considered to have evolved to simultaneously metabolize plant-derived compounds in oxygen-rich habitats.

Draft genomes are available for several industrially significant rhodococci, as exemplified by *R. erythropolis* sp. strain Xp (Tao et al. 2011), *R. pyridinivorans* strain AK37 (Kriszt et al. 2012), *Rhodococcus* sp. strain R04 (Yang et al. 2011), and *Rhodococcus* sp. strain P14 (Zhang et al. 2012). The genome of the *R. erythropolis* strain (7.2 Mb), a soil isolate shown to be effective in deep desulfurization of petroleum soils (Yu et al. 2006), contained several genes considered relevant to the degradation of dibenzothiophene. Similarly, the genome sequence of *R. pyridinivorans* strain AK37, a pyridine-degrading bacterium isolated from industrial wastewater in Korea (Yoon et al. 2000c), contained genes that encoded for at least six different pathways for monocyclic aromatic hydrocarbon degradation; key enzymes for alkane and biphenyl degradation were also identified. *Rhodococcus* strain R04, an isolate from contaminated soil, degrades polychlorinated biphenyls (PGB) through both ring cleavage and dechlorination, and has a large genome (9.12 Mb). The genome contains many genes that are potentially involved in xenobiotic metabolism, including four extradiol dioxygenase genes (*bpHC*) and two hydrolase genes (*bhpD*) involved in PCB degradation. Finally, the draft genome sequence of *Rhodococcus* sp. strain P14, an isolate from oil-contaminated sediment that mineralizes polycyclic hydrocarbons with three to five rings, contained nearly 1,000 genes predicted to be involved in biodegradation of xenobiotics.

To date, the smallest complete genome found in *Nocardiaceae* strains is that of "*P. equi*" 103S, a protolypic clinical isolate (Letek et al. 2010). It can be seen from *Table 32.1* that the 5.0 Mb "*P. equi*" genome is significantly smaller than those of the three *Rhodococcus* strains; it lacks extensive catabolic and secondary metabolism genes and displays unique adaptations for host colonization. Letek and his colleagues provided evidence that the relatively small size of the "*P. equi*" genome was not a consequence of reductive evolution, as in the *M. leprae* genome (Cole et al. 2001), but was due to gene expansion in the rhodococcal strains.

Phenotypic Markers

Since the last edition of *The Prokaryotes*, the family *Nocardiaceae* has expanded to include the genera *Gordonia*, *Millisia*, "*Prescotella*," *Skermania*, *Smaragdicoccus*, and *Williamsia*. *Nocardia*, the type genus, encompasses 84 validly published species, including the type species *N. asteroides*. The key chemical and morphological properties of the genera classified in the family *Nocardiaceae* are shown in *Table 32.2*.

Nocardia Trevisan 1889^{AL}

No. card' ia. N.L. fem. n. *Nocardia* named after Edmond Nocard (1850–1903), a French veterinarian who first isolated members of this taxon.

Nocardia is the oldest name in current use for an aerobic actinobacterial genus. Strains grow well on most standard media, as exemplified by brain-heart infusion (Difco), glycerol-asparagine (ISP 5; Shirling and Gőttlieb 1996), glucose-yeast extract (Gordon and Mihm 1962), modified

Table 32.2
Morphological and chemotaxonomic characteristics of genera classified in the family *Nocardiaceae*

Characteristic	*Nocardia*	*Gordonia*	*Millisia*	*"Prescottella"*	*Rhodococcus*	*Skermania*	*Smaragdicoccus*	*Williamsia*
Cell morphology	Mycelia fragment into rods and cocci	Short rods and cocci occur singly, in pairs, as V shapes or as short rods	Rudimentary right-angled branching	Rods and cocci/traces of branching	Rods to extensive substrate mycelium, the latter fragments into rods and cocci	Extensive substrate mycelium that does not fragment into undisturbed culture	Coccoid cells	Cocci or thin irregular rods occur singly or in clusters
Aerial hyphae	Sparse to abundant	Absent	Absent	Absent	Visible microscopically in some strains	Visible microscopically	Absent	Absent
Acid-fastness	Partially acid-fast	Partially acid-alcohol fast	Acid-alcohol fast	Acid-alcohol fast	Partially acid-fast	Not acid-fast	ND	Not acid-fast
Growth of visible colonies (days)	1–5	1–3	1–3	1–2	1–3	9–21	3–4	1–4
Fatty acid composition	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, (T)	S, U, T
Major menaquinone(s) (MK-)	−8 (H_4, ω cycl)	−9 (H_2)	−8 (H_2)	−8 (H_2)	−8 (H_2)	−8 (H_4, ω cycl)	SQA-8 (H_4 ω cycl) and SQB (H_4 w cycl)	−8 (H_2)
Mycolic acids:								
No. of carbons	46–64	46–70	44–52	28–50	30–54	58–64	43–49	50–56
No. of double bonds	0–4	1–6	ND	0–4	0–4	2–6	ND	ND
Fatty acids released on pyrolysis	12–18	16–18	ND	12–16	12–16	16–20	ND	ND
DNF G+C content (mol.%)	63–72	63–69	64.7	69–72	63–73	67.5	63.7	64–65

Data from: Goodfellow and Jones (2012) and Jones et al. (2013b)
Examples of abbreviations: MK-8 (H_4, ω cyclo), hexahydrogenated menaquinone with eight isoprene units where the two end units are cyclized; SQA and SQB, smaragdiquinones A and B
Abbreviations: *ND* not determined, *S* saturated, *T* tuberculostearic acid, (*T*) trace amounts of tuberculostearic acid, *U* unsaturated

Bennett's (Jones 1949), modified Sauton's (Mordarska et al. 1972), Sabouraud glucose and yeast extract-malt extract (ISP 2; Shirling and Gottlieb 1966) agars, as well as on Middlebrook media (Lorian 1968) and Mueller-Hinton 11 medium supplemented with glucose (Kageyama et al. 2004a, b, c, d, e, f, g, h). Media should be incubated at 25–37 °C for up to 3 weeks. Aciditolerant nocardiae belonging to *N. aciditolerans*, *N. jiangxiensis*, and *N. miyunensis* grow well on media adjusted to pH 5.5 (Cui et al. 2005; Golinska et al. 2013). Colonies may be smooth to granular and irregular, wrinkled, or heaped. Most strains synthesize carotenoid-like pigments which import various shades of brown, orange, pink, red, or yellow colors to colonies growing on solid culture media. Brown or yellowish diffusible pigments may be produced.

The only constant morphological characteristic of nocardiae is their capacity to produce filamentous branched cells which fragment into irregular coccoid and rod-like elements. Aerial hyphae are almost invariable formed though at times they may only be visible microscopically. The growth and stability of aerial and substrate mycelia are influenced by cultivation conditions (Locci 1976; Williams et al. 1976; Beaman and Beaman 1994). Developmental morphological studies undertaken by Locci (1976) on *N. asteroides* and *N. farcinica* strains demonstrated the value of scanning electron microscopy in elucidating the intricate growth cycles of these organisms.

Short-to-long chains of well-to-poorly differentiated spores may be detected occasionally on aerial hyphae and, more rarely, on aerial and substrate mycelia. Short chains of spores are formed on the aerial mycelia of *N. aobensis* (Kageyama et al. 2004d), *N. artemisiae* (Zhao et al. 2011), *N. asiatica* (Kageyama et al. 2004e), *N. callitridis* (Kaewkla and Franco 2010), *N. inohanensis* (Kageyama et al. 2004c), *N. niigatensis* (Kageyama et al. 2004c), and *N. takedensis* (Yamamura et al. 2005). *Nocardia beijingensis* and *N. brevicatena* form spores on aerial and substrate mycelia (Lechevalier et al. 1961; Wang et al. 2001).

Nocardiae are Gram-positive to Gram-variable and are acid-alcohol-fast positive at some stages of the growth cycle. Most strains grow between 20 °C and 40 °C, optimally ~28 °C, and in pH range 5–10, optimally ~pH 7.0. However, some species, such as *N. acidivorans*, *N. harenae*, *J. jejunensis*, and *S. spelucae*, grow at 10 °C and others, like *N. arthritidis*, *N. brasiliensis*, *N. ignorata*, *N. jinanensis*, *N. kruczakiae*, and *N. otitidiscaviarum*, grow well at 45 °C. All members of the genus are heterotrophic with an oxidative metabolism. A generation time of 5.5 h has been reported for *N. asteroides* and *N. brasiliensis* strains (Beadles et al. 1980). Some strains reach stationary phase within 3–7 days, others grow more slowly.

Nocardiae do not have specific growth requirements. Most strains grow on media containing casein, meat or yeast extract, and simple nitrogen sources, including amino acids and nitrate. They degrade complex polysaccharides and can assimilate diverse carbon compounds, including glucose, acetate, and propionate (Goodfellow 1971; Kämpfer et al. 2004; Golinska et al. 2013; Luo et al. 2013a). However, additional comparative data are needed before their biochemical, degradative, and carbon assimilation profiles are known as representatives of many species of *Nocardia*, including type strains, have still to be examined (Goodfellow and Maldonado 2012). This problem is compounded by the fact that many properties attributed to nocardiae in the early literature (Arai et al. 1988; Cain 1981; Peczyńska-Czoch and Mordarski 1988; Finnerty 1992; Tárnok 1976) were acquired from strains now classified in the genera *Amycolatopsis*, *Gordonia*, and *Rhodococcus* (Goodfellow and Jones 2012; Goodfellow and Maldonado 2013; Goodfellow et al. 2012; Tan and Goodfellow 2012). Biochemical and degradative profiles and sole carbon utilization patterns of *Nocardia* species are shown in *Table 32.3*. However, recent studies show that authenticated representatives of the genus exhibit a high degree of metabolic activity (Goodfellow and Maldonado 2012; Luo et al. 2013a), as shown by their ability to synthesize novel bioactive products (Schneider et al. 2009; El-Gendy et al. 2008; Lamm et al. 2009) and degrade complex organic compounds (Berekaa 2006; Le et al. 2010).

The cell envelopes of nocardiae contain a pronounced peptidoglycan layer, sugars and lipids, notably mycolic acids (Michel and Bordet 1976; Minnikin 1982, 1993). Ester bonds connect mycolic acids to an arabinogalactan layer which is attached to the peptidoglycan. The outer mycolic acid layer is analogous to the outer membrane of Gram-negative bacteria. The latter contain channel-forming proteins, the porins, that allow the passage of solutes through the outer membrane. Little is known about the molecular basis of nocardial cell wall permeability though porins have been detected in the mycolic acid layers of *N. asteroides* and *N. farcinica* strains (Riess et al. 1998, 1999).

Nocardia species have cell walls that contain major amounts of *meso*-diaminopimelic acid, arabinose, and galactose (Michel and Bordet 1976; Goodfellow et al. 1999), that is, they have a wall chemotype IV sensu Lechevalier and Lechevalier (1970), an A1γ peptidoglycan (Schleifer and Kandler 1972) and *N*-glycolyl muramic acid (Uchida and Aida 1979a, b; Uchida and Seino 1997; Uchida et al. 1999). They contain diphosphatidylglycerol, phophatidylethanolamine (taxonomically significant nitrogenous phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides as major polar lipids and thereby have a phospholipid type II pattern (Lechevalier et al. 1977, 1981) albeit with a variable distribution of phosphatidylglycerol (Minnikin et al. 1977; Kämpfer et al. 2004). The predominant respiratory quinone is hexahydrogenated menaquinone with eight isoprene units in which the two terminal ones are cyclized (Collins et al. 1987; Howarth et al. 1986); minor components detected in some species include MK-8(H_2), MK-8(H_4), and MK-9 (Rodrigues-Nava et al. 2004, 2007; Yamamura et al. 2007; Ezeoka et al. 2013; Sazak et al. 2012). Nocardiae contain characteristic lipid soluble iron-binding compounds, the nocobactins (Ratledge and Patel 1976), while the G+C content of their genomic DNA falls within the range 63–73 mol%. Nocardiae typically contain major amounts of straight-chain saturated, unsaturated, and tuberculostearic fatty acids, that is, they have a type II fatty acid profile sensu Kroppenstedt (1985). Most strains have qualitatively similar fatty acid profiles though some closely related species can be distinguished by

Table 32.3
Selected phenotypic properties of *Nocardia* species

Characteristic	*N. asteroides*	*N. abscessus*	*N. acidivorans*	*N. aciditolerans*	*N. africana*	*N. alba*	*N. altamiriensis*	*N. amikacinitolerans*	*N. amamiensis*	*N. anaemiae*	*N. aobiensis*	*N.araoensis*	*N. artemiensis*	*N. arthritidis*	*N. asiatica*	*N. beijingiensis*	*N. blacklockiae*	*N. brasiliensis*	*N. brevicatena*	*N. cashijiensis*	*N. callitridis*	*N. carnea*	*N. cerradoensis*	*N. concave*	*N. coubleae*	*N. crassostreae*	*N. cummidelens*	*N. cyriacigeorgica*	
Biochemical tests:																													
Allantoin	+	+	nd	–	+	nd	nd	nd	+	nd	nd	nd	nd	nd	+	+	nd	+	–	+	nd	+	+	nd	nd	–	+	–	
Esculin	+	–	+	–	–	nd	nd	–	–	nd	nd	–	nd	+	+	+	+	+	+	–	–	+	+	+	nd	+	+	+	
Nitrate	+	+	nd	+	+	+	+	nd	+	nd	nd	–	–	–	+	+	nd	+	–	+	nd	+	+	nd	nd	–	+	+	
Urea	+	+	nd	+	–	nd	+	+	+	+	+	+	+	–	+	+	nd	+	–	+	nd	–	+	+	nd	–	+	+	
Degradation of:																													
Adenine	–	–	nd	–	–	–	–	–	–	–	–	–	nd	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	
Arbutin	+	+	nd	–	–	nd	nd	nd	–	nd	+	–	nd	+	+	+	nd	+	+	–	–	–	nd	+	–	nd	+	nd	
Casein	–	–	nd	–	+	–	nd	–	–	–	–	–	nd	–	–	–	–	+	–	–	nd	–	–	–	–	–	–	–	
Elastin	–	–	nd	–	–	nd	nd	nd	–	nd	nd	nd	nd	–	–	–	nd	+	–	–	nd	–	–	nd	nd	–	–	–	
Hypoxanthine	–	–	nd	–	–	–	+	–	–	+	–	–	nd	–	–	–	+	+	–	–	–	–	–	+	–	–	–	–	
Tyrosine	–	–	nd	–	–	–	+	–	–	nd	–	–	nd	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	
Uric acid	–	–	nd	–	–	–	+	nd	+	+	+	–	nd	nd	+	–	+	–	–	+	nd	–	–	nd	–	–	–	+	
Xanthine	–	–	nd	–	–	–	nd	–	–	nd	–	–	nd	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	
Sole carbon sources:																													
Adonitol	–	–	nd	–	nd	–	nd	nd	–	–	–	–	nd	–	–	–	+	–	–	–	nd	–	–	nd	nd	nd	nd	–	
L-Arabinose	–	–	+	+	–	–	–	+	–	+	–	–	–	–	–	+	–	–	–	–	nd	+	+	+	–	–	–	–	
Cellobiose	–	–	–	–	–	nd	nd	+	–	+	nd	–	+	–	–	+	–	–	–	+	–	–	–	nd	nd	nd	nd	–	
Meso-Erythritol	–	–	nd	+	–	–	nd	–	–	–	–	–	nd	–	–	+	+	–	–	–	nd	–	+	–	nd	nd	nd	–	
Fructose	+	nd	–	+	–	nd	+	+	+	nd	nd	+	+	+	–	+	+	+	nd	+	+	nd	+	+	+	nd	+		
Galactose	–	–	+	+	–	–	+	nd	–	+	–	–	+	–	–	+	–	+	–	+	+	+	–	+	+	–	–	–	
Inositol	–	–	nd	+	–		nd	+	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	nd	nd	–	–	
Maltose	–	+	–	–	–	+	nd	+	+	–	–	–	+	–	+	+	–	+	–	+	–	–	+	–	+	–	–	–	
Mannitol	–	–	+	+	–	+	+	–	–	nd	nd	–	+	+	+	+	+	+	–	–	–	–	–	+	+	–	–	–	
Mannose	–	nd	+	+	–	+	nd	–	+	–	–	–	+	–	–	+	+	–	–	+	+	+	+	–	+	nd	+	–	
Raffinose	–	–		+	–	–	–	nd	–	nd	nd	nd	–	–	+	+	–	–	+	+	+	–	–	+	–	nd	–	–	

Characteristic	*N. elegans*	*N. endophytica*	*N. exalbida*	*N. farcinica*	*N. flavorosea*	*N. fluminea*	*N. gambensis*	*N. goodfellowii*	*N. grenadensis*	*N. harenae*	*N. higoensis*	*N. ignorata*	*N. inohanensis*	*N. iowensis*	*N. jejunensis*	*N. jiangxiensis*	*N. jinanensis*	*N. kruczakiae*	*N. lijiangensis*	*N. mexicana*	*N. mikamii*	*N. miyunensis*	*N. neocaledonensis*	*N. niigatensis*	*N. ninae*	*N. niwae*	*N. nova*		
L-Rhamnose	–	+	–	–	–	+	–	+	–	–	–	–	+	–	+	+	–	–	+	+	nd	–	+	–	–	–	–	–	
Sorbitol	–	–	–	+	–	–	–	–	–	+	–	–	–	–	+	+	+	–	–	–	nd	+	+	–	–	–	+	–	
Sucrose	+	+	–	–	–	+	–	+	–	nd	nd	+	+	–	+	+	–	+	–	+	+	–	+	–	+	+	–	+	
Trehalose	+	+	–	+	–	nd	nd	+	+	nd	nd	+	nd	–	+	+	+	+	+	+	+	+	+	nd	nd	+	nd	–	
Xylose	–	–	nd	+	–	+	nd	+	–	nd	–	–	+	–	+	+	–	–	–	+	+	–	+	nd	nd	–	+	–	
Biochemical tests:																													
Allantoin	nd	nd	+	+	–	+	nd	–	+	–	+	+	nd	nd	+	+	–	nd	nd	–	nd	+	+	nd	nd	+	+		
Esculin	+	nd	+	+	–	+	+	+	+	+	nd	+	nd	+	+	+	+	+	–	+	–	+	+	nd	nd	–	–		
Nitrate	nd	–	nd	+	+	–	+	+	nd	–	nd	nd	nd	nd	+	+	nd	nd	+	+	+	nd	+	nd	nd	+	nd		
Urea	+	nd	+	+	+	–	+	+	+	+	nd	+	+	+	+	–	nd	nd	+	nd	+	nd	+	+	+	+	+		
Degradation of:																													
Adenine	–	nd	–	–	–	–	–	–	+	–	–	–	–	nd	–	+	nd	–	–	+	–	–	+	–	+	–	–		
Arbutin	nd	nd	–	+	–	+	+	+	nd	nd	nd	nd	–	nd	nd	+	nd	nd	nd	nd	nd	–	nd	–	–	+	+		
Casein	–	nd	+	–	–	–	+	–	+	–	–	–	–	–	+	–	nd	–	nd	–	–	+	–	–	–	–	–		
Elastin	–	nd	–	–	–	–	nd	nd	nd	–	nd	–	nd	nd	–	–	–	nd	nd	nd	nd	–	–	nd	nd	–	–		
Hypoxanthine	–	+	–	–	–	–	–	+	–	–	–	–	+	+	+	–	nd	–	+	+	–	–	–	+	+	–	–		
Tyrosine	–	nd	–	–	–	+	+	nd	+	–	–	–	–	+	+	–	nd	–	+	–	–	–	+	–	–	–	–		
Uric acid	nd	nd	+	–	+	–	+	+	nd	nd	nd	–	+	+	+	–	–	nd	nd	+	nd	nd	–	nd	–	–	–		
Xanthine	–	nd	–	–	–	–	–	–	–	–	–	–	–	+	–	–	nd	–	+	–	–	–	–	–	+	–	–		
Sole carbon sources:																													
Adonitol	–	nd	nd	–	–	–	–	+	–	–	nd	–	–	–	–	–	nd	–	+	+	–	–	–	nd	nd	–	–		
L-Arabinose	–	nd	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	+	–	nd	+	–	–		
Cellobiose	–	+	nd	–	–	–	+	–	+	+	nd	–	nd	nd	–	+	+	–	+	nd	–	+	–	nd	nd	–	–		
Meso-Erythritol	–	nd	–	–	+	+	–	nd	–	–	–	–	nd	–	nd	+	nd	–	+	nd	–	+	nd	nd	nd	–	–		
Fructose	–	–	nd	–	–	–	+	+	+	+	nd	+	nd	+	+	+	+	–	+	+	+	+	+	nd	nd	–	nd		
Galactose	–	–	–	–	–	–	+	+	+	nd	–	–	–	–	–	+	+	–	+	+	+	+	–	nd	+	+	+		

Table 32.3 (continued)

Characteristic	*N. elegans*	*N. endophytica*	*N. exalbida*	*N. farcinica*	*N. flavorosea*	*N. fluminea*	*N. gambensis*	*N. goodfellowii*	*N. grenadensis*	*N. harenae*	*N. higoensis*	*N. ignorata*	*N. inohanensis*	*N. iowensis*	*N. jejunensis*	*N. jiangxiensis*	*N. jinanensis*	*N. kruczakiae*	*N. lijiangensis*	*N. mexicana*	*N. mikamii*	*N. miyunensis*	*N. neocaledonensis*	*N. nigatensis*	*N. ninae*	*N. niwae*	*N. nova*	
Inositol	−	−	−	−	−	+	−	−	+	−	−	−	+	+	+	+	−	−	+	+	−	+	−	pd	pd	−	−	
Maltose	−	+	pd	−	−	pd	pd	+	+	+	−	+	−	+	−	+	pd	+	+	−	−	+	+	pd	+	−	−	
Mannitol	−	pd	pd	−	−	−	−	+	+	+	pd	+	pd	−	−	+	+	−	+	+	−	−	+	−	−	−	−	
Mannose	−	+	−	+	−	pd	+	+	+	−	−	+	+	pd	−	+	+	−	+	+	−	+	+	−	−	−	−	
Raffinose	−	+	pd	−	−	pd	−	pd	−	−	pd	−	pd	pd	−	+	+	−	+	−	−	+	+	pd	−	−	−	
L-Rhamnose	−	pd	−	+	−	+	−	−	−	−	−	−	−	−	−	+	+	−	−	+	−	+	+	−	−	+	−	
Sorbitol	−	pd	−	−	−	−	pd	+	pd	−	−	−	+	pd	+	+	+	−	−	+	−	+	−	−	−	+	−	
Sucrose	+	−	pd	+	−	+	−	−	−	+	pd	+	−	−	+	+	pd	−	+	pd	−	+	−	−	pd	+	−	
Trehalose	+	pd	pd	+	−	pd	+	pd	+	+	pd	+	−	+	−	+	−	−	−	pd	+	+	+	−	pd	−	−	
Xylose	−	pd	pd	−	−	+	−	−	+	+	pd	−	pd	−	−	+	−	−	+	+	−	+	−	pd	pd	−	−	
Biochemical tests:																												
Allantoin	+	+	+	+	+	−	pd	−	−	+	+	pd	+	+	+	+	pd	+	+	pd	+	+	−	pd	−	+	+	
Esculin	+	−	−	−	−	+	pd	+	+	+	+	pd	+	+	+	+	+	+	+	pd	+	+	−	pd	+	+	+	
Nitrate	+	+	+	−	−	−	pd	pd	−	+	+	pd	−	+	pd	+	−	−	pd	pd	−	+	+	pd	−	+	pd	
Urea	+	+	nd	nd	+	+	pd	+	−	+	−	+	pd	−	+	+	−	+	−	−	−	+	+	−	+	−	+	
Degradation of:																												
Adenine	−	−	−	−	−	+	nd	−	−	−	−	−	−	−	nd	−	−	−	−	−	−	−	−	−	−	−	−	
Arbutin	+	+	+	−	+	−	nd	−	−	+	+	nd	nd	+	nd	+	+	+	−	nd	+	+	−	nd	nd	nd	+	
	N. otitidiscaviarum	*N. paucivorans*	*N. pigrifrangens*	*N. pneumoniae*	*N. polyresistans*	*N. pseudobrasiliensis*	*N. pseudovaccinii*	*N. puris*	*N. rhamnosphila*	*N. salmonicida*	*N. seriola*	*N. shimofusensis*	*N. sienata*	*N. soli*	*N. speluncae*	*N. takadensis*	*N. tenerifensis*	*N. terpenica*	*N. testacea*	*N. thailandica*	*N. transvalensis*	*N. uniformis*	*N. vaccinii*	*N. vermiculata*	*N. veterana*	*N. vinacea*	*N. wallacea*	*N. xinshiensis*	*N. yamamensis*

Casein	−	−	−	−	+	+	nd	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−
Elastin	−	−	−	−	+	+	nd	−		−	−	nd	−	−	−	−	nd	nd	+	−	+	+	−	nd	−	−	nd	−	nd
Hypoxanthine	+	−	−	−	−	+	nd	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	−	−	−	+	+	−	+
Tyrosine	−	−	−	−	−	+	nd	−	−	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−	−	−	−
Uric acid	+	−	+	−	+	+	nd	+	nd	−	−	nd	−	−	+	+	+	−	−	−	+	+	−	nd	+	+	+	+	+
Xanthine	+	−	−	−	+	−	nd	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Sole carbon sources:																													
Adonitol	−	−	−	−	+	−	−	−	−	−	−	nd	−	nd	−	−	−	+	−	nd	+	−	−	nd	nd	+	−	−	−
L-Arabinose	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−
Cellobiose	−	−	+	nd	+	−	−	−	−	−	nd	nd	−	nd	−	−	nd	nd	+	nd	−	−	−	nd	−	−	−	+	nd
Meso-Erythritol	−	−	+	−	nd	−	−	−	nd	−	−	−	−	nd	−	−	nd	−	−	nd	+	nd	−	−	−	−	−	−	−
Fructose	−	nd	nd	nd	+	+	+	nd	−	+	nd	nd	nd	+	−	−	−	nd	−	−	+	+	+	nd	−	−	−	+	nd
Galactose	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	+	+	+	+	nd	+	−	+	−	−	+	+	+	−
Inositol	−	−	nd	−	+	+	+	+	−	−	−	−	−	−	−	−	+	+	−	−	−	+	−	−	+	+	+	+	+
Maltose	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	nd	−	−	−	−	+	−	−	−	−	−	+	+	−
Mannitol	+	−	−	−	+	−	−	+	−	+	−	nd	−	−	+	−	+	+	−	nd	+	−	+	nd	−	+	−	−	nd
Mannose	nd	nd	+	−	+	−	−	nd	−	nd	−	−	+	+	−	+	−	−	−	−	+	+	+	−	−	−	−	+	−
Raffinose	−	−	−	nd	−	−	−	−	nd	−	nd	nd	nd	−	nd	−	nd	nd	nd	nd	−	−	−	nd	−	nd	−	−	nd
L-Rhamnose	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	+	−	+	−	+	−	+	−	−	+	−
Sorbitol	−	−	−	−	+	+	−	+	nd	+	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	+	−
Sucrose	−	−	+	nd	+	−	−	+	−	−	−	nd	nd	+	+	+	−	−	nd	nd	+	−	−	nd	−	−	+	+	nd
Trehalose	+	+	+	nd	+	+	+	−	+	+	−	nd	nd	nd	+	−	+	nd	−	nd	+	−	+	nd	−	nd	+	+	nd
Xylose	−	−	+	−	−	−	−	−	−	−	nd	nd	+	+	−	−	nd	−	+	−	−	−	+	nd	−	nd	nd	−	nd

Data from: Hamid et al. (2001), Yassin et al. (2001a, b), Zhang et al. (2003), Li et al. (2004), Kageyama et al. (2004b), Xu et al. (2005), le Roes and Meyers (2006), Seo and Lee (2006), Seo et al. (2006), Hoshino et al. 2007), Kämpfer et al. (2007), Laurent et al. (2007), Rodriguez-Nava et al. (2007), Conville et al. (2008), Jurado et al. (2008), Lamm et al. (2009), Sun et al. (2009), Jannat-Khah et al. (2010), Everest et al. (2011), Moser et al. (2011), Xing et al. (2011), Zhao et al. (2011), Goodfellow and Maldonado (2012), Kämpfer et al. (2012), Sazak et al. (2012), Ezeoke et al. (2013), and Golinska et al. 2013)
Symbols: + positive, − negative, *nd* not determined

qualitative differences in fatty acid composition (Kageyama et al. 2004a, b, c; Kämpfer et al. 2007; Lamm et al. 2009; Kaewkla and Franco 2010).

The most characteristic components of the walls of nocardiae are the mycolic acids (Minnikin and Goodfellow 1976, 1980; Minnikin 1982, 1993). Mycolic acids with between 44 and 64 carbon atoms and up to 4 double bonds have been detected in several species, including *N. amikacinitolerans* (Ezeoke et al. 2011), *N. asteroides* (Bordet et al. 1965), *N. brasiliensis* (Lanéelle and Asselineau 1970), *N. farcinica* (Yano et al. 1990), *N. niwae* (Moser et al. 2011), *N. otitidis-caviarum* (Alshamaony et al. 1976), and *N. seriolae* (Kudo et al. 1988). *Nocardia asteroides* can adapt to temperature changes by altering wall mycolic acid composition (Tomiyasu 1982). The fatty acid esters released on pyrolysis gas chromatography of mycolic acid methyl esters contain 12–18 carbon atoms (Minnikin and Goodfellow 1976, 1980). Two lipid spots, one corresponding to mycolic acids (Rf value ~ 0.47) and the other to nonhydroxylated fatty acids (Rf value ~ 0.9), are usually detected by one-dimensional thin-layer-chromatography of nocardial whole-organism hydrolysates (Minnikin et al. 1975; Yassin and Brenner 2005; Yassin et al. 2001b). However, a second mycolic acid has been reported in some species, as exemplified by *N. abscessus* (Yassin et al. 2000), *N. asiatica* (Kageyama et al. 2004e), *N. beijingensis* (Wang et al. 2001), and *N. exalbida* (Iida et al. 2006).

Gordonia (Tsukamura 1971) Stackebrandt, Smida and Collins 1988, 345[VP]

Gor.do' ni. a. N.L. fem. n. *Gordonia*, named after Ruth E. Gordon, a celebrated bacterial systematist.

Gordonia strains grow well on standard media used to cultivate actinomycetes, such as modified Bennett's (Jones 1949), modified Sauton's (Mordarska et al. 1972), R2A (Oxoid), and tryptone-soy (Oxoid) agars. Colony morphology ranges from circular, convex, shiny, and smooth colonies with entire edges to rough, matt and folded ones with irregular edges. Most colonies are 2–10 mm in diameter, have a soft texture, and may be cream, beige, yellow or tan through to apricot, orange, pink, or red. Diffusible pigments are not produced. The type strains of *G. alkanivorans* and *G. westfalica* form smooth and rough colonies (Kummer et al. 1999; Linos et al. 2002). Glycosylated peptidoglycolipids influence the colony morphology of *G. hydrophobica* (Moorman et al. 1997).

Gordoniae tend to form short rods and cocci which may occur singly, in pairs, in small groups, as V-shaped elements, or as short chains. Some species have a typical rod-coccus life cycle, whereby cells in early growth phase are rods and those in exponential phase are cocci (Kummer et al. 1999). Other species form elementary branched hyphae which fragment into rod and coccoid-like forms, as shown by *G. alkanivorans* (Kummer et al. 1999), *G. lacunae* (Le Roes et al. 2008), *G. polyisoprenivorans* (Linos et al. 1999), and *G. soli* (Shen et al. 2006b). *Gordonia amarae* strains form moderately branching, substrate hyphae that grow into agar media and do not fragment in undisturbed culture (Lechevalier and Lechevalier 1974). *Gordonia defluvii* shows acute-angled and right-angled branching (Soddell et al. 2006b) which is reminiscent of the right-angled branching pattern of *G. amarae* (Lechevalier and Lechevalier 1974; Klatte et al. 1994) and the "pine-tree-like" morphology of *S. piniformis* (Blackall et al. 1989; Chun et al. 1997). *Gordonia amarae* and *G. defluvii* strains form microscopically visible aerial hyphae (Lechevalier and Lechevalier 1974; Soddell et al. 2006b).

Gordonia strains are Gram-positive to Gram-variable and usually partially acid-alcohol-fast. In general, they grow at 10–37 °C, optimally ~ 28 °C, but do not grow at either 5 °C or 45 °C. The pH growth range is 5–11, and optimally ~ pH 7.0. *Gordonia alkaliphila* grows from pH 6 to 11 and optimally at pH 9 (Cha and Cha 2013). Gordoniae grow well in the absence of NaCl, but can grow in the presence of 4 %, w/v NaCl. All members of the genus are heterotrophic with an oxidative metabolism.

Gordonia species do not have any specific growth requirements, but do show remarkable metabolic activity (Arenskőtter et al. 2004). They are biochemically active, degrade complex polysaccharides, and use diverse compounds as sole carbon sources (*Tables 32.4* and *32.5*). Currently, a broad comparison of their phenotypic features is not possible as the type strains of several species have not been examined for such properties. However, it is known that gordoniae can cleave diverse 7-amino-4-methylcoumarin and 4-methylumbelliferone conjugated fluorogenic substrates (Goodfellow et al. 1991). Enzymatic profiles of some species, including *G. alkanivorans* (Cha and Cha 2013), *G. soli* (Shen et al. 2006b), and *G. caeni* (Srinivasan et al. 2012), have been determined using API-ZYM kits.

The metabolic activity of *Gordonia* strains rivals that of rhodococci, as witnessed by their ability to break down environmental pollutants and xenobiotics, and by their capacity to transform or synthesize organic compounds of biotechnological interest (Drzyzga 2012). Thus, cholesterol is metabolized by *G. cholesterolivorans* (Drzyzga et al. 2009, 2011), nitriles by *G. namibiensis* (Brandão et al. 2001), phenol by *G. kroppenstedtii* (Kim et al. 2009), hydrocarbons such as liquid paraffin by *G. paraffinivorans* (Xue et al. 2003), natural rubber substrates by *G. polyisoprenivorans* (Linos et al. 1999) and *G. westfalica* (Linos et al. 2002), and pyrene by a *Gordonia* strain (Xu et al. 2011). Xenobiotic compounds that are metabolized include alkanes by *G. alkanivorans* (Kummer et al. 1999), dibenzothiophene by *G. amicalis* (Kim et al. 2000), and benzothiophene by *G. desulfuricans* (Kim et al. 1999). Other unusual compounds metabolized by gordoniae include butyl benzyl phthalates (Chaterjee and Dutta 2003), fluroanthene (Britto et al. 2000) and hazardous nitro-compounds, such as hexahydro-1,3,5-trinitro-1,3,5-triazine (Hexogen), an explosive that is difficult to degrade (Gorontzy et al. 1994; Thompson et al. 2005). Specific anabolic capabilities include the ability of *G. alkanivorans* to produce exopolysaccharides (Ta-Chen et al. 2008) and *Gordonia* strains to synthesize biosurfactants (Franzetti et al. 2009) and steroids (Schneider et al. 2008).

Table 32.4

Selected phenotypic properties of *Gordonia* species

Characteristic	*G. bronchialis*	*G. aichiensis*	*G. alkanivorans*	*G. amarae*	*G. amicalis*	*G. defluvii*	*G. desulfuricans*	*G. hankoonensis*	*G. hirsuta*	*G. hydrophobica*	*G. lacunae*	*G. namibiensis*	*G. paraffinivorans*	*G. polyisoprenivorans*	*G. rhizosphaera*	*G. rubripetincta*	*G. sinisedis*	*G. soli*	*G. sputi*	*G. terrae*	*G. westfalica*
Biochemical tests:																					
Allantoin hydrolysis	–	–	–	+	–	–	–	nd	–	+	–	+	+	–	–	–	–	nd	–	–	nd
Arbutin hydrolysis	–	+	–	+	–	nd	–	nd	–	+	+	–	–	–	–	–	–	–	+	+	–
Esculin hydrolysis	–	–	–	+	–	–	–	+	–	+	+	+	+	–	–	+	–	–	+	+	–
Nitrate reduction	+	+	+	–	–	+	+	+	+	+	+	–	–	–	–	–	+	–	+	+	+
Urea hydrolysis	+	+	+	+	–	–	+	–	–	–	+	+	+	+	–	+	+	+	+	+	+
Degradation of:																					
Adenine	+	nd	–	+	–	–	–	nd	–	+	–	–	nd	+	–	–	+	nd	+	+	–
Hypoxanthine	–	–	–	+	–	–	+	–	–	+	–	+	–	–	–	+	+	–	+	+	–
Starch	+	–	+	+	+	–	+	–	+	–	–	–	–	+	+	+	+	+	+	+	+
Tributyrin	–	–	+	–	–	+	–	nd	–	–		+	nd	+	–	+	+	+	–	–	–
Tween 80	–	+	+	–	–	+	+	+	+	+	+	–	+	+	–	–	+	+	–	–	+
Tyrosine	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Uric acid	+	+	–	+	–	+	+	nd	–	+	nd	+	–	+	+	+	+	–	+	+	–
Xanthine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
Growth on sole carbon sources:																					
Arbutin	–	–	–	+	+	+	+	nd	+	+	nd	–	nd	–	+	–	–	nd	–	–	nd
Cellobiose	–	–	–	–	–	–	–	nd	+	–	nd	+	–	–	+	–	–	nd	–	–	nd
Glycerol	+	+	+	+	+	+	+	Nd	+	+	Nd	–	+	–	–	+	–	Nd	+	+	nd
N-acetyl-D-glucosamine	–	–	+	+	–	–	–	nd	+	+	nd	+	nd	+	+	+	+	nd	–	–	nd
Betaine	–	–	–	–	–	nd	–	nd	–	–	nd	–	nd	–	+	–	–	nd	+	+	nd
Propan-1-ol	–	–	–	+	–	nd	+	nd	–	+	nd	+	nd	–	+	–	+	nd	–	+	nd
Sodium adipate	–	+	–	–	+	nd	+	nd	–	+	nd	+	nd	+	+	–	–	nd	+	+	nd
Sodium fumarate	+	–	+	–	+	nd	–	nd	–	–	nd	+	nd	–	+	+	+	nd	–	+	nd
Sodium oxalate	–	–	–	–	–	nd	–	nd	–	–	nd	–	nd	–	–	–	–	nd	+	+	nd

Modified from Goodfellow et al. (2012) with additional data from Park et al. (2009)

Symbols: + positive, – negative

Table 32.5
Carbon assimilation profiles of the type strains of *Gordonia* species

Characteristic	*G. bronchialis*	*G. alkanivorans*	*G. amarae*	*G. amicalis*	*G. caeni*	*G. cholesterolivorans*	*G. desulfuricans*	*G. effusa*	*G. hirsuta*	*G. humi*	*G. hydrophobica*	*G. malaquae*	*G. rubripertincta*	*G. shandongensis*	*g. sihwensis*	*G. terrae*	*G. westfalica*
3-Hydroxybenzoate	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–
2-α–Ketoglutarate	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Acetate	+	–	–	w	w	w	–	–	–	+	w	+	–	–	+	–	–
Caprate	+	–	–	w	–	–	–	–	–	–	+	–	–	–	–	–	–
Citrate	+	–	–	–	–	+	+	w	w	+	–	–	+	w	–	+	w
Gluconate	+	+	w	w	+	–	+	w	–	w	+	+	+	+	–	w	+
DL-Lactate	–	w	–	–	–	–	+	w	w	–	+	–	–	w	–	w	w
L-Malate	+	+	–	–	+	+	+	+	–	+	+	w	w	w	+	+	+
Propionate	+	–	–	+	–	w	–	–	–	+	+	–	w	–	w	–	–
D-Valerate	–	–	w	w	–	w	w	w	–	+	+	–	w	–	+	–	–
L-Arabinose	–	w	–	–	+	–	+	w	–	w	+	+	w	+	–	+	–
L-Fucose	+	+	–	–	+	–	–	–	–	–	w	–	–	+	–	–	–
D-Glucose	+	w	+	+	+	w	+	w	–	w	+	+	w	+	+	+	+
Maltose	–	w	w	–	+	w	+	w	–	+	–	+	–	+	–	+	+
D-Mannose	–	w	–	–	+	–	+	w	–	w	+	+	+	+	+	w	+
Melibiose	–	w	–	–	+	–	w	–	–	–	–	–	–	–	–	w	–
D-Ribose	–	+	w	–	+	–	+	+	+	–	–	–	+	w	–	+	w
Sucrose	+	+	+	–	+	+	+	+	+	–	–	–	+	+	–	+	+
Myo-Inositol	–	+	w	–	+	–	+	+	+	–	–	–	+	w	–	+	w
D-Mannitol	+	w	w	+	+	–	+	w	–	+	+	+	+	+	w	+	w
D-Sorbitol	+	–	+	+	+	+	+	+	w	–	w	–	+	+	–	–	+
L-Histidine	+	–	–	+	–	w	w	–	–	+	+	–	–	–	–	–	–
N-acetyl-D-glucosamine	+	w	–	+	+	–	+	w	+	+	–	+	–	+	+	+	w
Salicin	+	w	+	+	+	+	+	+	+	–	–	–	+	+	–	w	w
Glycogen	–	w	w	–	w	w	–	+	w	–	+	+	+	w	w	w	w

Adapted from Srinivarsan et al. (2013)
+ positive, *w* weak positive, – negative

Whole-cell hydrolysates of gordoniae contain *meso*-diaminopimelic acid as the sole diamino acid in the peptidoglycan and arabinose and galactose as characteristic whole-cell sugars (wall chemotype IV, according to Lechevalier and Lechevalier 1970). They have a A1γ peptidoglycan (Schleifer and Kandler 1972), *N*-glycolyl muramic acid moieties (Uchida and Aida 1979a; Uchida and Seino 1997; Srinivasan et al. 2012), and typically contain diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides as major components (Minnikin et al. 1977), which equates to a phospholipid pattern type II after Lechevalier et al. (1977, 1981); additional components detected in some species include unidentified aminophospholipids, glycolipids, phosphoglycolipids, and phospholipids (Xue et al. 2003; Cha and Cha 2013). Dihydrogenated menaquinones with nine isoprene units (MK-9 [H_2]) are the predominant isoprenologue (Alshamaony et al. 1976; Collins et al. 1977, 1985); minor components, such as MK-7 (H_2) and MK-8 (H_2), have been detected in some species (Kummer et al. 1999; Kämpfer et al. 2011a, 2013a). *Gordonia effusa* is unusual as it contains major amounts of MK-9 [H_4) and MK-9 [H_8) with a smaller proportion of MK-9 [H_4) (Kageyama et al. 2006).

In general, *Gordonia* species contain similar mixtures of straight-chain saturated, unsaturated, and tuberculostearic acids (type II fatty acid pattern sensu Kroppenstedt (1985) though species-specific patterns and intraspecific differences have been reported (Klatte et al. 1994; Kim et al. 2003; Drzyzga et al. 2009; Liu et al. 2011). The mycolic acids of gordoniae have 46–70 carbon atoms with up to 4 double bonds (Alshamaony et al. 1976; Kageyama et al. 2006); the fatty acids released on pyrolysis gas chromatography of mycolic acid methyl esters have 12–16 carbon atoms (Minnikin and Goodfellow 1976, 1980). *Gordonia amarae* strains isolated from activated sludge foam contain shorter chain and more fully saturated mycolic acids than those derived from analyses of *Gordonia* type strains (Stratton et al. 1999). One-dimensional TLC of gordonial whole-organism hydrolysates yields two lipid spots, one corresponding to mycolic acids (Rf value ~ 0.5) and the other to monohydroxylated fatty acids (Rf ~ 0.9). A lipoglycan structurally related to mycobacterial lipoarabinomannan has been detected in *G. rubripertincta* (sic) (Flaherty and Sutcliffe 1999).

Rhodococcus (Zopf 1891) emend. Goodfellow, Alderson and Chun 1998a

Rho. do. coc' cus. Gr. N. rhodon, the rose; N.L. masc. n. coccus (from Gr. masc. n. kokkos, grain, seed) coccus; N.L. masc. n. *Rhodococcus*, a red coccus.

Rhodococcus strains grow well on media such as glucose-yeast extract (Gordon and Mihm 1962), trypticase soy (Oxoid) and yeast extract-malt extract (ISP 2, Shirling and Gottlieb 1966) agars and on modified Bennett's agar supplemented with thiamine (Mordarska et al. 1972). *Rhodococcus marinonascens* requires seawater for growth, grows well at 5 °C and optimally at about 20 °C (Helmke and Weyland 1984). Colonies may be rough, smooth, or mucoid and pigmented buff; cream, orange, red, or yellow; and most have a soft texture. Colony pigmentation may be enhanced by exposure to light (Rowbotham and Cross 1977a).

Rhodococci show a remarkable cellular heterogeneity, but do not exhibit any distinctive morphological features other than the ability of some species to form hyphae that fragment into rods and/or cocci (Locci 1976, 1981; Williams et al. 1976; Helmke and Weyland 1984; Locci and Sharples 1984; Apajalahti et al. 1986). The growth cycle starts with a coccus or short rod stage, and this is usually followed by a series of more or less complex morphological developments. Cocci may differentiate into short rods, branched filaments, or in the most differentiated form into extensively branched hyphae. The next generation of cocci and short rods is formed by fragmentation of the rods, filaments, and branched hyphae. The timing of the fragmentation process is influenced by the growth conditions (Williams et al. 1976). *Rhodococci* do not usually form aerial hyphae; an exception is *R. coprophilus* which produces feeble aerial hyphae (Locci and Sharples 1984). The time taken to complete the growth cycle ranges from 24 h in relatively undifferentiated forms, such as *R. erythropolis*, to several days for those like *R. coprophilus* which show pronounced morphological differentiation (Locci et al. 1982).

Rhodococcus strains are Gram-positive to Gram-variable and are usually partially acid-alcohol-fast at some stage of the growth cycle. In general, they grow from 15 °C to 40 °C, optimally ~ 30 °C, from pH 5.0 to 10.0 with an optimum ~ pH 7.0; they also grow in the presence of 5 %, w/v NaCl. *Rhodococcus cerastii* and *R. trifolii* grow at 50 °C (Kämpfer et al. 2013b). In contrast, *R. marinonascens* does not grow at temperatures above 28 °C (Helmke and Weyland 1984). *Rhodococci* are heterotrophic, with an oxidative type of metabolism.

Rhodococci use a broad range of compounds as sole carbon compounds, as shown by species assigned or associated with the *R. corynebacteroides*, *R. erythropolis*, and *R. rhodochrous* 16S rRNA clades (*Tables 32.6–32.8*). More to the point, they show an astonishing catabolic versatility that has been the subject both of broadly based reviews (Bell et al. 1998, Gürtler et al. 2004, van der Geize and Dijkhuizen 2004; De Carvalho and da Fonseca 2005; Larkin et al. 2006; Alvarez 2010a; Yam et al. 2010), as well as to ones that concentrate on their capacity to produce industrially important enzymes (Bunch 1998; Beard and Page 1998; Hughes et al. 1998; Martínková et al. 2010; Ma 2010) or to turnover environmental pollutants, including xenobiotics (Warhurst and Fewson 1994; Larkin et al. 2005; Kuyukina and Ivshina 2010). Their metabolic versatility is mainly due to the presence of large linear plasmids and multiple homologs of enzymes in catabolic pathways (van der Geize and Dijkhuizen 2004; Larkin et al. 2005, 2010; McLeod et al. 2006).

The metabolic versatility of *Rhodococcus* strains can be illustrated by the ability of *R. aetherivorans* to metabolize methyl t-butyl ether (Goodfellow et al. 2004), *R. bakonurensis* to break down diesel oil (Li et al. 2006), *R. erythropolis* to desulphuranize

Table 32.6
Selected phenotypic properties of *Rhodococcus* species classified in the *Rhodococcus corynebacteroides* 16S rRNA subclade

Characteristic	*R. corynebacteroides*	*R. canchpurensis*	*R. kroppenstedtii*	*R. triatomae*	*R. trifolii*
Biochemical tests:					
Esculin	–	nd	–	–	+
Growth on sole carbon sources:					
Adonitol	+	nd	–	–	–
L-Arabinose	–	nd	+	–	–
Galactose	+	–	–	–	–
Inostiol	+	–	+	–	+
Maltose	+	–	+	–	+
Mannitol	+	–	+	–	+
Mannose	+	+	–	nd	–
Rhamnose	–	–	+	–	+
Salicin	–	nd	–	nd	+
Sorbitol	+	nd	+	–	–
Trehalose	+	–	+	–	–
Xylose	+	+	+	–	–
Xylose	+	+	+	–	–

Data from: Yassin (2005), Yassin and Schaal (2005), Mayilraj et al. (2006), Kämpfer et al. (2013b) and Nimaichand et al. (2013)
Symbols: + positive, – negative, *nd* not determined

benzothiophene and dibenzothiophene (Oldfield et al. 1997, 1998), *R. koreensis* to utilize 2,4-dinitrophenol (Yoon et al. 2000a), and *R. zopfii* to degrade natural estrogens (Yoshimoto et al. 2004) while *R. qinghengii* metabolizes carbendiazine, a commonly used benzimidazole fungicide (Xu et al. 2007) which damages the liver and endocrine system and has mutagenic and teratogenic effects on animals at low concentrations (Mazellier et al. 2003). In addition, alkyl ether (Kim et al. 2007), dibenzofuran (Aly et al. 2008), methyl-*s*-trizones (Fujii et al. 2007), nitrophenol (Ghosh et al. 2007), polychlorinated bipheyl (PCB) (Leigh et al. 2006), and xylene (Taki et al. 2007) are degraded by strains provisionally classified by Gűrtler and Seviour (2010) as *R. ruber*, *R. pyridinivorans*, *R. jostii*, *R. imtechensis*, *R.wratislaviensis*, and *R. opacus*, respectively. Several genetic tools have been developed to realize the metabolic potential of *Rhodococci* (Finnerty 1992; Larkin et al. 1998), including *E. coli–Rhodococcus* shuttle vectors (Shao et al. 1995; De Mot et al. 1997; Mangan et al. 2005; Matsui et al. 2006, 2007). In addition, cloned and characterized amidase and nitrile hydratase genes from *Rhodococci* have been expressed in *E. coli* (Ikehata et al. 1989; Hashimoto et al. 1991; Kobayashi et al. 1991).

Rhodococci have an A1γ peptidoglycan in which the muramic acid residues of the glycan strands are *N*-glycolated (Schleifer and Kandler 1972; Uchida and Seino 1997). Cells typically contain diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant phospholipid), phosphatidylinositol, and phosphatidylinositol mannosides as major phospholipids (type II pattern after Lechevalier et al. 1977, 1981); fatty acids rich in straight-chain saturated, monounsaturated, and branched-chain components (fatty acid type 1b after Kroppenstedt 1985); and dihydrogenated menaquinones with eight isoprene units as the predominant isoprenologue (Collins et al. 1985). Mycolic acids have 28–54 carbons with up to 4 double bonds, while fatty acids released on pyrolysis gas chromatography of mycolic acid methyl esters have 12–16 carbon acids (Alshamaony et al. 1976); Minnikin and Goodfellow 1980; Sutcliffe 1998). Selective ion monitoring gas-chromatography-mass spectrometric analysis gives a much more detailed profile of mycolic acid composition than gas-chromatography-mass spectrometry, as exemplified by a *R. rhodochrous* strain in which the mycolic acids were assigned to 60 subgroups based on α- and β-mycolic acid chain lengths and degrees of unsaturation (Stratton et al. 1999). These workers also noted that mycolic acid composition was sensitive to growth conditions.

The most recent model of the cell wall organization of *Rhodococci* is that proposed by Sutcliffe et al. (2010). The rhodococcal cell wall is dominated by mycolic acids that are covalently linked to the peptidoglycan-arabinogalactan complex and which form an outer permeability barrier. In line with this, channel-forming proteins have been extracted from whole-cells of *R. corynebacteroides* and *R. erythropolis* strains (Lichtinger et al. 2000, Riess and Benz 2000). Other non-covalently associated components found in cell envelopes of *Rhodococci* include free lipids, lipoproteins, and polysaccharides (Sutcliffe et al. 2010). A lipoarabinomannan (LAM)-like lipoglycan of a *R. ruber* strain was shown to have a truncated structure in which the lipomannan core was directly substituted with *t*-arabinofuranose residues (Gibson et al. 2003). LAM-like

Table 32.7

Selected phenotypic properties of *Rhodococcus* species classified in or associated with the *Rhodococcus erythropolis* 16S rRNA gene clade

Characteristic	*R. erythropolis*	*R. baikonensis*	*R. cerastii*	*R. fascians*	*R. globerulus*	*R. imtechensis*	*R. iostii*	*R. koreensis*	*R. kyotonensis*	*R. maanshanensis*	*R. marinonascens*	*R. namhaensis*	*R. opacus*	*R. percolatus*	*R. quinshengii*	*R. tukisamuensis*	*R. wratislaviensis*	*R. yunnanensis*
Biochemical tests:																		
Aesculin hydrolysis	+	+	+	+	+	nd	–	–	+	+	+	nd	–	–	nd	+	+	+
Urea hydrolysis	+	+	nd	+	+	+	–	+	+	+	–	–	+	+	+	–	+	–
Decomposition of:																		
Tween 80	+	+	nd	+	+	+	–	+	+	+	nd	–	+	+	+	+	–	+
Growth on sole carbon sources:																		
L-Arabinose	–	–	+	+	–	–	+	+	–	–	–	–	–	–	nd	nd	+	+
Arabitol	–	–	nd	+	+	–	+	+	–	–	–	nd	+	+	nd	–	+	+
Cellulose	–	nd	–	–	–	–	+	–	–	–	–	–	+	–	–	+	–	–
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	–	–	+	+	–	+	+	+	+	+	–	–	+	+	–	+	–	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
Glycerol	+	nd	nd	+	+	nd	+	+	+	+	+	–	+	+	+	+	+	nd
Inostiol	–	–	+	–	–	+	+	+	+	–	+	–	+	+	–	–	+	–
Inulin	+	–	nd	+	+	nd	+	+	–	–	+	nd	+	+	–	+	+	nd
Lactose	–	–	nd	–	–	+	+	+	–	–	–	–	+	–	–	–	–	+
Maltose	+	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+
Mannitol	+	–	+	+	+	+	+	+	+	–	–	–	+	+	–	–	+	+
Mannose	+	+	–	+	+	nd	+	+	–	+	+	–	+	+	+	+	+	+
Melezitose	–	–	nd	–	–	–	+	+	–	+	–	nd	+	+	nd	+	–	nd
Melibiose	–	–	+	–	–	–	+	+	–	+	–	nd	+	+	–	+	+	–
Raffinose	–	–	nd	–	–	–	+	+	–	–	–	nd	+	+	–	+	–	nd
Rhamnose	–	nd	–	–	–	+	–	+	+	–	–	–	–	–	–	+	+	–
Ribose	+	+	+	+	+	–	+	+	+	+	–	–	+	+	+	+	–	+
Sorbitol	+	–	+	+	+	+	–	+	+	–	+	–	+	+	–	–	+	+
Sucrose	+	–	+	+	+	+	–	+	+	+	+	nd	+	+	+	+	+	+
Trehalose	+	–	+	+	+	+	+	+	–	–	–	–	+	+	–	+	+	+
D-Xylose	–	–	–	+	–	–	+	+	–	–	–	nd	+	+	nd	–	+	nd
L-Xylose	–	–	nd	+	+	–	+	+	–	–	+	nd	–	+	nd	–	+	+

Data from: Jones and Goodfellow (2012), Li et al. (2012), Jones et al. (2013), Kämpfer et al. (2013)

+ positive, – negative, *nd* not determined

Table 32.8

Selected phenotypic properties of species classified in or associated with the *Rhodococcus rhodochrous* 16S rRNA gene clade

Characteristic	*R. rhodochrous*	*R. aetherivorans*	*R. artemsiae*	*R. canchipurensis*	*R.coprophilus*	*R. corynebacteroides*	*R. gordoniae*	*R. kroppenstedtii*	*R. phenolicus*	*R. pyrinidovorans*	*R. rhodnii*	*R. ruber*	*R. triatomae*	*R. trifolii*	*R. zopfii*
Biochemical tests:															
Aesculin hydrolysis	+	–	nd	nd		–	–	–	–	+	–	–	–	+	+
Urea hydrolysis	+	–	–	–	–	–	–	–	–	+	+	+	–	nd	+
Decomposition of:															
Starch	–	–	–	+	+	–	+	–	–	–	–	+	nd	nd	+
Tween 80	+	nd	–	+	+	nd	nd	+	+	+	–	+	+	nd	+
Tyrosine	+	nd	nd	–	–	–	+	–	+	+	+	+	–	nd	+
Growth on sole carbon sources:															
Cellobiose	–	+	+	–	–	–	+	–	–	+	–	–	–	–	+
Fructose	+	+	+	+	+	+	+	+	–	+	+	+	nd	+	+
Galactose	–	+	+	–	–	–	+	–	–	+	–	–	–	–	+
Glucose	+	+	+	nd	+	+	+	+	–	–	+	+	+	+	+
Inositol	–	–	–	–	–	+	–	+	nd	–	–	–	–	+	–
Maltose	+	+	+	–	+	–	+		–	–	–	+	–	+	+
Mannitol	+	+	+	–	–	+	nd	+	–	+	+	+	–	+	–
Melezitose	–	+	nd	nd	+	–	+	nd	nd	+	–	–	–	nd	+
Rhamnose	–	nd	+	–	–	–	nd	+	nd	–	–	–	–	+	–
Ribose	+	+	+	nd	+	–	+	–	–	+	–	+	nd	–	+
Salicin	+	+	nd	nd	–	+	+	–	nd	+	–	–	nd	+	+
Sorbitol	+	+	+	nd	–	+	+	+	–	+	+	+	–	–	–
Sucrose	+	+	+	nd	+	+	+	+	nd	+	+	+	–	+	–
Trehalose	+	+	nd	–	+	+	+	–	+	–	+	+	–	–	–
Citrate	+	nd	–	nd	–	+	nd	+	nd	+	+	+	+	+	–
Gluconate	+	–	nd	nd	+	+	–	+	nd	–	+	+	–	–	+
m-Hydroxybenzoic acid	+	+	nd	nd	+	–	+	nd	nd	–	–	+	–	nd	+
p-Hydroxybenzoic acid	+	+	nd	nd	–	–	+	+	nd	+	+	+	–	nd	+
Pyruvate	+	+	nd	nd	+	+	+	nd	+	+	–	+	nd	nd	+
Succinate	+	+	nd	+	–	+	+	nd	+	+	+	+	nd	nd	+
Growth on sole carbon and nitrogen source:															
Acetamide	+	+	nd	nd	–	–	+	nd	+	+	+	+	+	nd	+

Data from: Jones and Goodfellow (2012), Zhao et al. (2012), Kämpfer et al. (2013), Nimaichand et al. (2013)

+ positive, – negative, *nd* not determined

lipoglycans have been detected in the type strain of *R. rhodnii* (Flaherty et al. 1996). In addition, the cell walls of *R. corynebacteroides*, *R. erythropolis*, *R. rhodochrous*, and *R. ruber* and *R. wratislaviensis* are a rich source of structurally diverse lipids, including glycolipids, lipopeptides, and glycolipopeptides (Sutcliffe et al. 2010).

Williamsia et al. 1999, Kämpfer, Andersson, Rainey, Kroppenstedt and Salkinoja-Salonen 1999[VP]

Wil. li. am' si. a. N.L. fem n. Williamsia named to honor Stanley Thomas Williams (1937–2004), a British microbiologist, for his numerous contributions to the taxonomy and ecology of actinomycetes.

The genus *Williamsia* currently encompasses eight validly published species which grow well on complex media such as Brain-Heart Infusion (BBL), glucose-yeast extract (Gordon and Mihm 1962), modified Bennett's (Jones 1949), nutrient (Bacto), R2A (BBL), and yeast extract-malt extrat (Shirling and Gottlieb 1966) agars. Colonies tend to be smooth, entire, and slightly convex, 1–3 mm in diameter and have a soft texture. Some strains produce yellow colonies but in the main orange to red colonies are formed.

Williamsia strains are Gram-positive, non-acid-alcohol-fast, nonmotile actinomycetes which form short rods and/or coccoid elements. Most strains grow from 20 °C to 37 °C, optimally ∼ 28 °C, and at or around pH 7.0. However, *W. faeni* and *W. maris* grow at 10–30 °C, but not at 37 °C (Stach et al. 2004; Jones et al. 2010). The cellular morphology of *W. muralis* is unusual as transmission electron microscopy reveals that the surface of cells is covered by fimbrial-like structures (Kämpfer et al. 1999). Similar structures have been detected in *R. percolatus* (Briglia et al. 1996). All members of the genus are heterotrophic with an oxidative metabolism. A broad range of compounds are used as sole carbon sources (*Table 32.9*).

Williamsiae typically contain *meso*-diaminopimelic acid, arabinose, and galactose in whole-organism hydrolysates (wall chemotype IV after Lechevalier and Lechevalier 1970); major amounts of straight-chain saturated, unsaturated, and tuberculostearic acids; phosphatidylethanolamine (taxonomically significant component), diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol as major components (phospholipid type II sensu Lechevalier et al. 1977, 1981) and mycolic acids with 50–58 carbon atoms (Kämpfer et al. 1999; Sazak and Sahin 2012). The fatty acid methyl esters released on pyrolysis gas chromatography of mycolic acid methyl esters of the type strain of *W. serinedens* have 16 and 18 carbon atoms (Yassin et al. 2007).

Williamsia strains show considerable variation in cellular fatty acid profiles and to a lesser degree in isoprenoid quinone composition. Most contain major amounts of palmitic ($C_{16:0}$), hexadecenoic ($C_{16:1}$), oleic ($C_{18:1}$), and tuberculostearic (10-methyl $C_{18:0}$) acids (Pathom-aree et al. 2006; Jones et al. 2010), that is, they have a fatty acid type 1b pattern sensu Kroppenstedt (1985). *Williamsia muralis* has a similar fatty acid profile albeit with minor amounts of $C_{16:1}$ *cis*9 (Kämpfer et al. 1999). In addition, *W. deligens* contains a minor amount of $C_{16:1}$, but shows a significant presence (40 %) of C_{20} saturated and unsaturated fatty acids (Yassin and Hupfer 2006). All but one of the *Williamsia* type strains contain dihydrogenated menaquinones either as the sole isoprenologue, as in the case of *W. faeni*, *W. marianensis*, or with minor amounts of MK-7 (H_2) and/or MK-8 (H_2), as with *W. serinedens* and *W. phyllosphaerae*. The exception, *W. limnetica*, has MK-8 (H_2) as the major component albeit with sizeable proportions of MK-7 (H_2) and MK-9 (H_2) (Sazak and Sahin 2012).

Monospecific Genera

Millisia Soddell, Stainsby, Eales, Kroppenstedt, Seviour and Goodfellow 2006a[VP]

Mil. li.' si.a. N.L. fem. n. named after Nancy F. Mills, a celebrated microbiologist who promoted wastewater microbiology in Australia.

Key features of the four monospecific genera classified in the family *Nocardiaceae* are considered here.

The history of the genus *Millisia* began when Soddell and Seviour (1994) isolated two unusual actinomycetes from an Australian wastewater treatment plant that showed rudimentary right-angled branching and salmon-pink filamentous colonies. Additional studies based on numerical taxonomic and 16S rRNA gene sequence data indicated that the isolates might merit generic status (Soddell and Seviour 1998). Subsequent work undertaken by Soddell et al. (2006a) underpinned this point as they were able to show that the two isolates could be distinguished from members of other mycolic acid-containing taxa using a combination of genotypic and phenotypic data. Soddell and his colleagues considered that the type species, *Millisia brevis*, was most closed related to the genera *Gordonia* and *Skermania*.

It is now apparent that the genus *Millisia* forms a deep branch in the *Nocardiaceae* 16S rRNA gene tree in which it shows a loose association with the genus *Skermania* (*Fig. 32.1*). Soddell and his colleagues (2006a) showed that the type strain of *M. brevis* can be distinguished from representatives of the genus *Skermania* and other mycolic acid-containing genera as it synthesized a high proportion of oleic acid ($C_{18:1}$; 26.3 %) and a low proportion of tuberculostearic acid (3 %). Further, thin-layer chromatographic analysis showed that the *M. brevis* type strain gave a mycolic acid spot with a similar Rf value to those of representatives of the genera *Gordonia* and *Rhodococcus*. Additionally, Curie-point pyrolysis mass spectrometric analysis showed that the type strain of *M. brevis* was clearly separated from representatives of other mycolic acid–containing genera. An actinomycete associated with a deep-water marine invertebrate has been found to share an identical partial 16S rRNA gene sequence with *M. brevis* strain J81^T (Sfanos et al. 2005).

Table 32.9
Selected phenotypic properties of *Williamsia* species

Characteristic	*W. muralis*	*W. deligens*	*w. faeni*	*w. limnetica*	*W. marianensis*	*W. maris*	*W. phyllosphaerae*	*W. serinedens*
Growth at (°C):								
4	–	–	+	–	+	–	nd	–
10	–	–	+	+	+	+	nd	+
37	+	+	–	+	+	+	nd	–
45	+	–	–	–	–	–	nd	–
Degradation of:								
Adenine	–	–	–	+	–	–	nd	–
Casein	–	–	–	–	+	–	nd	–
Gelatin	–	–	+	–	–	–	nd	+
Hypoxanthine	–	–	–	–	+	–	nd	–
Growth on sole carbon sources:								
Adonitol	+	–	+	–	–	–	–	+
L-Arabinose	–	–	+	nd	+	–	–	+
D-Cellobiose	–	–	+	+	–	–	–	–
D-Galactose	–	–	+	+	–	–	–	+
myo-Inositol	+	–	+	–	–	–	–	–
Maltose	–	+	+	+	–	–	–	+
D-Mannitol	–	+	+	nd	+	–	+	+
D-Melezitose	–	–	nd	+	–	–	nd	–
D-Melibiose	+	–	+	+	–	–	–	+
D-Raffinose	–	–	+	–	–	nd	nd	–
α-L-Rhamnose	+	–	+	nd	–	+	–	–
D-Sorbitol	+	+	+	–	+	–	+	+
D-Sucrose	+	+	+	nd	+	–	nd	+
D-Trehalose	–	+	+	nd	+	–	+	+
D-Xylose	–	+	+	–	+	+	+	+

Data from: Kämpfer et al. (1990, 2011), Stach et al. (2004), Jones et al. (2004, 2010), Pathom-aree et al. (2006), Yassin and Hupfer (2006) and Sazak and Sahin (2012)
Symbols: + positive, – negative, *nd* not determined

"*Prescottella*" Jones, Sutcliffe and Goodfellow 2013a

Pres. cot. tel'la. N.L. fem dim. n. *Prescotella*, named after John Prescott to celebrate his many contributions to unraveling the pathogenicity of *Rhodococcus equi*.

The genus "*Prescottella*" with the species "*Prescottella equi*" was proposed by Jones et al. (2013a) to replace the illegitimate name "*Prescottia*" Jones et al. (2013b) and the illegitimate combination *Prescottia equi* (Jones et al. 2013b). Following an extensive polyphasic taxonomy study, these names had been proposed to provide a new home for an old pathogen, namely, *R. equi* (formerly *Corynebacterium equi*). This organism, an important pathogen of foals, had been a somewhat atypical member of each of these taxa (Barton and Hughes 1980; Rainey et al. 1995; Gürtler and Seviour 2010).

"*Prescottella equi*" strains are Gram-positive, acid-alcohol fast, pleomorphic actinomycetes. In smears, cells tend to show clumping or palisade configurations or L- or V-shaped elements though a rod-coccus life cycle may be exhibited. Smears from liquid cultures may exhibit branching filaments with swollen ends. The organism grows well from 5 °C to 40 °C, optimally ~ 30 °C, on standard media used to cultivate gordoniae and rhodococci. Smooth, shiny, orange to red colonies with entire margins are formed on glucose-yeast extract agar. Some strains produce copious amounts of slime which may fall onto the covers of inverted Petri dishes during incubation. Strains of "*P. equi*" are metabolically active; they use a broad range of sole carbon sources, degrade complex polysaccharides, and show considerable enzymatic activity (Jones et al. 2013b).

The chemotaxonomic properties of "*P. equi*" are similar to those of members of the genus *Rhodococcus* (Barton et al. 1989;

Jones et al. 2013b). Strains have whole-organism hydrolysates rich in *meso*-diaminopimelic acid, arabinose, and galactose (wall chemotype IV according to Lechevalier and Lechevalier; 1970), an A1γ peptidoglycan (Schleifer and Kandler 1972) and *N*-glycolated muramic acid moieties (Uchida and Seino 1997). They typically contain dihydrogenated menaquinones with eight isoprene units as the predominant isoprenologue (Collins et al. 1977; Barton et al. 1989) and have qualitatively similar fatty acids composed of straight-chain saturated, monounsaturated, and 10-methyl-branched fatty acids (Collins et al. 1982a; Barton et al. 1989), significant numbers of strains synthesize the carbon 16 version of 10-methyloctadecanoic acid (Jones et al. 2013b). Polar lipid patterns contain major amounts of diphosphatidylglycerol and phosphatidylethanolamine (taxonomically significant component), less intensive spots corresponding to phosphatidylglycerol, phosphatidylinositol, and mono- and diacylated phosphatidylinositol diamannosides, characteristic polar glycolipids, and a less polar trehalose mycolate (Barton et al. 1989). This polar lipid pattern is distinctive as it serves to distinguish "*P. equi*" from *Corynebacterium* and *Rhodococcus* strains (Minnikin et al. 1978, 1980). The mycolic acids of "*R. equi*" have 28–50 carbon atoms and up to four double bonds, the fatty acids released on pyrolysis gas chromatography of mycolic acid methyl esters have 12–16 carbon atoms (Collins et al. 1982b; Barton et al. 1989).

Skermania, Chun, Blackall, Kang, Hah and Goodfellow 1997, 129[VP]

Sker. man. i.a. N.L. n. Skermania named after Victor Bruce Darlington Skerman (1921–1993), a celebrated Australian bacterial systematist.

The genus *Skermania* was proposed by Chun et al. (1999) for actinomycetes that had initially been described as pine-tree-like Organisms due to their distinctive tree-like micromorphology (Blackall et al. 1988). Subsequently, somewhat tentatively, Blackall and her colleagues (1989) assigned members of this taxon to the genus *Nocardia*, as *Nocardia pinensis*, mainly on chemotaxonomic and biological evidence while highlighting that they showed atypical nocardial features, such as the presence of unusual mycolic acids, a characteristic antibiotic sensitivity pattern, and a relatively slow growth rate. *Skermania piniformis* forms a deep-rooted branch in the *Nocardiaceae* 16S rRNA gene tree showing a loose, albeit distant relationship to *Millisia brevis* (Fig. 32.1), another organism isolated from activated sludge foam from sewage treatment plants. There is evidence that the genus may be underspeciated (Soddell and Seviour 1994).

Skermaniae grow on glycerol-asparagine agar (ISP 5; Shirling and Gottlieb 1966), SMAS medium (BBL) enriched with 1 % (v/v) horse serum (Soddell and Seviour 1994), as well as on tryptone-yeast extract-glucose (TYG), yeast extract, and yeast extract-glucose agars (Blackall et al. 1989; Soddell and Seviour 1998). Like mycobacteria, they grow best on media containing glycerol as a carbon source and asparagine as a nitrogen source. TYG agar supports the growth of orange, opaque, circular colonies that are 1–2 mm in diameter (Blackall et al. 1989). Colonies have a pasty texture, are difficult to emulsify and culture, and while they appear dry and friable macroscopically, they are moist and shiny when examined microscopically. Whole colonies tend to remain intact when taken from the surface of agar media. Macroscopically visible colonies are evident in slightly turbid TRG broth. On solid TRG medium, strains may take 10–21 days to produce colonies that are about 1–2 mm in diameter with growth rates being variable (Blackall et al. 1989).

Skermania strains produce an extensive substrate mycelium that does not fragment in undisturbed culture. Secondary branching is rare or absent. The branch angles of skermaniae are acute, not right-angled as in *G. amarae*. Branches near the apex are shorter than those further away, while the septate mycelia taper after branch points. Short branched and unbranched aerial hyphae are visible microscopically. Phase bright spherical regions can be detected at terminal and intercalary positions in microcolonies.

Skermaniae are Gram-positive and non-acid-fast. Filaments from activated sludge plants and from culture media contain intracellular sudanophilic and polyphosphate inclusions. The maximum and minimum growth temperatures are 31 °C and 15 °C, respectively. Growth is not enhanced in an atmosphere containing 5 % CO_2. Eales et al. (2006) found that skermaniae did not behave as strict aerobes in foam, as expected from pure culture studies (Chun et al. 1997). Indeed, they are facultative aerobes capable of anaerobic nitrate respiration as substrates are taken up under anoxic (NO_3) conditions. Eales and her colleagues also pointed out that an ability to take up a substrate in the absence of oxygen does not necessarily mean that an organism can grow in the absence of oxygen. The metabolic activity of *S. piniformis* strains is evident from their ability to cleave diverse 7-amino-4-methylcoumarin and 4-methylumbelliferone conjugated substrates (Chun et al. 1997) and their capacity to grow on diverse compounds as sole carbon sources (Soddell and Seviour 1996). These workers also found that skermaniae produced more biomass and grow faster on hydrophilic substrates, like oils, than on hydrophobic substrates, such as glucose.

Skermaniae show a range of chemotaxonomic properties found in other genera classified in the family *Nocardiaceae* (Blackall et al. 1989). They have a wall chemotype IV, an A1γ peptidoglycan, *N*-glycolated muramic acid residues, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as major phospholipids, predominant amounts of hexahydrogenated menaquinones with eight isoprene units with the last two cyclized (MK-8 [H_4, ω cycl]), large proportions of straight-chain saturated, unsaturated, and 10-methyloctadecanoic fatty acids and mycolic acids with 58–64 carbon atoms, 2–6 double bonds, and monounsaturated side chains, while fatty acids released on pyrolysis gas chromatography of mycolic acid methyl esters

have 16–20 carbon atoms. *Skermania* strains are especially rich in hexadecanoic ($C_{16:0}$), hexadecanoic ($C_{16:1}$), octadecanoic, and 10-methyloctadecanoic acids.

Smaradicoccus Adachi, Katsula, Matsunda, Peng, Misawa, Shizuri, Kroppenstedt, Yokota and Kasai 2007[VP]

Sma. rag. di'coc'cus. L.n. smaragdus malachite, N.L. masc. n. coccus (from Gr. masc. n. kokkus) grain; N.L. masc. n. *Smaragdicoccus malachite* (−colored) coccus.

The type strain of the type species of the genus *Smaragdicoccus* was isolated from a petroleum-contaminated soil by enrichment culture (Adachi et al. 2007). *Smaragdicoccus niigatensis* forms a deep-rooted branch in the *Nocardiaceae* 16S rRNA gene tree and shows a loose association with "*P. equi*" (Fig. 32.1). The type and only strain is Gram-positive, nonmotile, forms coccoid cells (0.86-µm in diameter), but not spores. On W-medium (Peng et al. 2003), malachite green-like, round colonies, 1–3 mm in diameter, are formed after 7 days at 25 °C in a hexadecane-saturated atmosphere. The organism also grows at 4–37 °C, but not at 45 °C, on 1/10 trypticase soy agar (Difco). It grows at 30 °C on yeast extract-malt extract and peptone-yeast extract-iron agars (ISP media 2 and 6; Shirling and Gottlieb 1966). Fructose glucose, hexadecane, and sodium-*n*-butyrate are utilized after 14 days at 30 °C, and sucrose following a month's incubation; better growth was observed when the W-medium was supplemented with 0.1 % than with 1.0 % (w/v) of the carbon source. Esterase (C4), esterase lipase (C8), lipase (C4), leucine arylamidase, and naphthol-AS-BI phosphohydrolase activities have been detected using API ZYM kits.

Smaragdicoccus niigatensis exhibits several chemical markers typical of the family *Nocardiaceae*. It has a wall chemotype IV, an A1γ peptidoglycan, *N*-glycolated muramic acid, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannoside as major phospholipids and straight-chain saturated and unsaturated fatty acids though 10-methyl-branched components, if present, are in trace amounts. The mycolic acids have 43–49 carbon atoms. In contrast, *S. niigatensis* has an unusual menaquinone profile that consists of two components, namely, MK-8 (H_4, ω-methylenecyclyl) and MK-8 (H_4, diacyl). The earlier component has the same UV and MS/MS profiles as MK-8 (H_4 cycl) which is characteristic of *Nocardia* (Howarth et al. 1986; Collins et al. 1987) and *Skermania* (Blackall et al. 1989).

Isolation, Enrichment, and Maintenance Procedures

Selective Isolation and Enrichment

It is difficult to isolate *Nocardiaceae* strains from clinical and environmental samples by using standard methods as isolation plates tend to be overrun by fast-growing bacteria. Consequently, as with other actinomycetes, a plethora of selective isolation procedures have been recommended for the isolation of *Nocardia* and related mycolic acid–containing genera from samples taken from clinical and environmental sources (Goodfellow 2010; Tiwari and Gupta 2012a). In general, the most effective isolation strategies are those designed to select specific fractions of *Nocardiaceae* communities based on their biological properties and distribution patterns in aquatic and terrestrial ecosystems (Rowbotham and Cross 1977b; Seviour et al. 2010; Golinska et al. 2012). Nevertheless, most procedures used to isolate *Nocardia* and related genera involve the detachment of vegetative cells and mycelial fragments (propagules) bound to particulate matter, chemical and/or physical pretreatment of substrates, choice of selective media, fine-tuning of cultural conditions, and finally the recognition of target organisms using taxon-specific identification procedures.

Physicochemical bonds between propagules and particulate matter influence the recovery of actinomycetes from samples taken from natural habitats. Standard methods used to detach propagules from organic matter, sediment, and soil particles, such as shaking in water and weak buffers (e.g., 1/4 strength Ringer's solution) although widely used, are not particularly effective (Hopkins et al. 1991a). In contrast, the dispersion and differential centrifugation (DDC) technique, a multistage procedure introduced by Hopkins et al. (1991b), combines several physicochemical treatments shown to be effective in increasing the yield of actinomycetes from natural habitats when compared with reciprocal shaking methods (MacNaughton and O'Donnell 1994; Atalan et al. 2000; Sembiring et al. 2000; Maldonado et al. 2005). Soil suspensions prepared using the DDC procedure when plated onto an acidified medium led to the isolation of the type strains of *N. jiangxiensis* and *N. myunensis* (Cui et al. 2005).

Several pretreatment regimes have been used to selectively isolate *Nocardia*, *Rhodococcus*, and related strains from environmental samples. Propagules of these and other actinomycetes tend to be more resistant to desiccation and heat than cells of vegetative bacteria; hence, simply heating or drying soil or sediment samples reduces the number of Gram-negative bacteria able to grow on selective isolation plates (Labeda and Shearer 1991; Goodfellow 2010). The type strains of *N. acidivorans* and *N. tenerifensis*, for instance, were isolated from soil samples that had been held at 100 °C prior to plating-out soil suspensions onto isolation media (Kämpfer et al. 2004, 2007). Similarly, a *N. rhamnosiphila* strain was isolated from a soil sample that had been heated at 100 °C for an hour before inoculating soil dilutions onto Czapek Solution agar (Everest et al. 2011).

Several *Nocardia* species have been isolated from soil, water, and deteriorating rubber rings following pretreatment of 10^{-1} preparations at 55 °C for 6 min (Orchard and Goodfellow 1974; Orchard et al. 1977; Maldonado et al. 2000). This heat pretreatment regime also led to the isolation of the type strain of *G. sinesedis* from a soil sample (Maldonado et al. 2003) and to

the recovery of many *R. coprophilus* strains from aquatic and terrestrial environmental samples (Rowbotham and Cross 1977b). Sucrose gradient centrifugation has been used to selectively isolate *Nocardia* spp., including *N. takedensis*, from soil (Yamamura et al. 2003, 2005).

Nocardiaceae strains are generally isolated by plating pretreated or non-pretreated suspensions of clinical or environmental samples onto nutritionally rich empirically formulated media. The lack of accepted, taxon-specific isolation procedures helps explain why so little is known about the occurrence, distribution, numbers, and activities of constituent members of the family *Nocardiaceae* in natural habitats. The somewhat haphazard approaches to the isolation of members of the family also go someway to explain why so many *Nocardiaceae* species are based on descriptions of single isolates.

Nocardia. Early procedures used to selectively isolate nocardiae were based on their ability to grow on hydrocarbons as sole carbon sources (Cross et al. 1976; Tárnok 1976; Nesterenko et al. 1978a), as exemplified by the paraffin baiting technique (Sőhngen 1913). Modifications of this technique have been used occasionally to isolate *Nocardia* strains from clinical material (Ashdown 1990) and soil (Portaels 1976; Schaal and Bickenbach 1978; Khan et al. 1997; Rodriguez-Nava et al. 2007). However, it is now general practice to isolate nocardiae from either pretreated or non-pretreated substrates by plating-out serial dilutions onto either general purpose or selective media supplemented with antifungal antibiotics (e.g., actidione and/or nystatin at 50 μg mL^{-1}). Inoculated plates are incubated at 20–37 °C under aerobic conditions.

High numbers of nocardiae have been isolated, using the heat pretreatment regime described above, by plating-out serial dilutions of substrates onto Diagnostic Sensitivity Agar supplemented with various combinations of tetracycline and antifungal antibiotics (Orchard and Goodfellow 1974; Orchard et al. 1977; Maldonado et al. 2000). Similarly, many aciditolerant nocardiae have been isolated by inoculating serial dilutions of litter and acidic soil from a spruce forest onto starch-casein medium with agar or gellan gum as gelling agents; the media were supplemented with antifungal antibiotics and adjusted to pH5.5 with IN HC l (Golinska et al. 2012). In each case, *Nocardia* colonies growing on the isolation media were recognized by their cream colored substrate mycelia and abundant, translucent white aerial hyphae. Numerous nocardiae have been detected by plating dilutions of sediment, soil, and water samples onto chitin, glycerol-asparagine, and starch-casein agars (Jiang and Xu 1996; Xu et al. 1996).

Single and small numbers of nocardial colonies have been isolated from soil and sand using general purpose media with and without antibacterial antibiotics, as exemplified by the isolation of *N. acidivorans* on mannitol-rifampicin agar (Kämpfer et al. 2007), *N. altamirensis* on tryptone-soy agar (Jurado et al. 2008), *N. beijingensis* on glucose-asparagine agar (Wang et al. 2001), *N. caishijiensis* on Bennett's agar (Zhang et al. 2003), *N. cerradoensis* on R5 agar (Alburquerque-Barros et al. 2003), *N. goodfellowii* and *N. thraciensis* on Gauze's No. 2 agar supplemented with nalidixic acid (Sazak et al. 2012), *N. grenadensis* on soil extract agar (Kämpfer et al. 2012), *N. jinanensis* on modified Sauton's agar (Sun et al. 2009), *N. lijiangensis* on HV agar (Xue et al. 2003), and *N. neocaledonensis* on oatmeal agar (Saintpierre-Bonaccio et al. 2004). In addition, nocardiae have been isolated on nutrient-rich media inoculated with surface-sterilized plant material, as shown by the growth of *N. artemisiae* and *N. endophytica* on sodium propionate-asparagine-salts agar (Xing et al. 2011; Zhao et al. 2011) and *N. callitridis* on VL10 agar supplemented with nalidixic acid and a cocktail of amino acids (Kaewkla and Franco 2010).

Standard nutrient-rich media used in clinical settings have led to the isolation of novel pathogenic nocardiae from clinical specimens, as witnessed by the recovery of *N. ninae* on blood agar Laurent et al. (2007) and *N. anaemiae*, *N. aobensis*, *N. arthritidis*, and *N. vermiculata* (Kageyama et al. 2004f) on Muller-Hinton 11 medium supplemented with glucose and glycerol (Kageyama et al. 2004a, e, 2005a). Other media formulations recommended for the isolation of clinically significant nocardiae include brain-heart infusion agar (Schaal 1977), Sabouraud's glucose agar supplemented with chloramphenicol (Ajello and Roberts 1981), and paraffin agar (Shawar et al. 1990). Pathogenic nocardiae have been isolated from clinical material using robust selective isolation procedures. Hamid et al. (2001), for instance, isolated strains of a new pathogen, *N. africana*, by treating sputa of patients with pulmonary disease using the digestion-decontamination procedure of Roberts et al. (1991) and then plating-out the digested products onto Lowenstein Jensen slopes.

Gordonia. In general, the approaches used to isolate gordoniae depend upon whether the target organisms are of clinical, biotechnological, or environmental interest. Gordoniae, including *G. amarae* and *G. defluvii*, are common in foaming-activated sludge plants where they present a possible health hazard (de los Reyes et al. 1998a, b; Seviour and Nielsen 2010). Large numbers of putatively novel *Gordonia* spp. have been isolated by inoculating dilutions of activated sludge foam onto glucose-yeast extract agar and incubating plates at 30 °C for 14 days (Goodfellow et al. 1996, 1998b). Gordoniae are easy to recognize on isolation plates as they present as large, rough, grayish-pink dry colonies. Suitable media for the isolation of the ubiquitous *G. amarae* include Czapek's agar supplemented with yeast extract (Higgins and Lechevalier 1969) and glycerol agar (Gordon and Smith 1953). Similarly, *G. caeni* was isolated from a sludge sample taken from a sewage disposal plant by plating dilutions onto R2A agar (Srinivasan et al. 2012). In contrast, *G. defluvii* strains have been recovered from activated sludge foam by micromanipulation, as described by Soddell and Seviour (1998).

Enrichment culture is the favored approach to isolate gordoniae of biotechnological interest from environmental samples, as exemplified by the selective isolation of *G. amicalis* (Kim et al. 2000), *G. cholesterolivorans* (Drzyzga et al. 2009), *G. desulfuricans* (Kim et al. 1999), *G. kroppenstedtii* (Kim et al. 2009), *G. namibiensis* (Brandão et al. 2001), *G. neofelisfaecis* (Liu et al. 2011), and *G. paraffinivorans* (Xue et al. 2003).

In contrast, soil suspensions inoculated onto nutrient-rich media led to the isolation of *G. lacunae* (le Roes et al. 2008) and *G. shandongensis* (Luo et al. 2007).

Although most gordoniae have been isolated from environmental sources, they are being reported increasingly in clinical settings where they should be seen as potential opportunistic human pathogens (Lal et al. 2010; Johnson et al. 2011). The methods used to isolate clinically significant gordoniae are rarely given in case reports though it seems likely that standard procedures will apply. *Gordonia otitidis*, for example, was isolated on blood agar plates that had been incubated at 37 °C for 7 days (Iida et al. 2004). Clinically significant gordoniae have been isolated using procedures designed to detect pathogenic mycobacteria (Tsukamura 1971; Gil-Sande et al. 2006).

Rhodococcus. *Rhodococci* have been isolated from diverse habitats by enrichment culture and by plating pretreated and non-pretreated substrates onto nutrient-rich media such as Czapek's agar (Higgins and Lechevalier 1969), glycerol agar (Gordon and Smith 1953), and glycerol-asparagine agar (Shirling and Gottlieb 1966). In contrast, taxonomically novel *Rhodococci* were isolated from Pacific Ocean sediments by Colquhoun et al. (1998) using a nutritionally poor medium, M3 agar, formulated by Rowbotham and Cross (1977b). *Rhodococci* from activated sludge have been isolated by micromanipulation (Soddell et al. 1992).

Specific fractions of rhodococcal communities present in aquatic and terrestrial habitats have been isolated by plating suspensions of environmental samples onto diverse rich nutrient media. Thus, *R. canchipurensis* was isolated from a limestone deposit on starch-casein agar (Nimaichand et al. 2013), *R. imtechensis* from pesticide-contaminated soil using tryptic soy agar (Ghosh et al. 2006), *R. jostii* from human skeletal remains using peptone-yeast extract agar (Takeuchi et al. 2002), and *R. maanshanensis* from soil using modified Sauton's agar (Zhang et al. 2002). In contrast, use of a mineral salts medium supplemented with alkanes was used to isolate "*R. luteus*" (a subjective synonym of *R. fascians*; Klatte et al. 1994) from soil and the skin of carp (Nesterenko et al. 1982). The obligate marine species, *R. marinonascens*, was isolated by plating dilutions of marine sediments onto rich media supplemented with seawater and incubating for up to 12 weeks (Weyland 1969; Helmke and Weyland 1984). The endophyte *R. artemisiae* was isolated by inoculating extensively pretreated plant suspensions onto propionate-asparagine-salt agar supplemented with sodium chloride and nalidixic acid (Zhao et al. 2012). The type strains of *R. cerastii* and *R. trifolii* were obtained from the leaf surface of *Trifolium repens* after growth for 14 days on M125 medium (Kämpfer et al. 2013b).

Rhodococci of potential biotechnological value have been isolated from environmental samples using selective enrichment procedures, as exemplified by the recovery of the *R. aetherivorans* from methyl *t*-butyl enrichments of petrochemical sludge from a chemical effluent plant (Salanitro et al. 1994; Goodfellow et al. 2004). Similarly, *R. erythropolis* was isolated from the discharge of an industrial site polluted with haloalkanes using a defined growth medium supplemented with 1-chlorobutane as the sole source of carbon and nitrogen (Sallis et al. 1990). Selective enrichment of industrial wastewaters using mineral salts media supplemented with 2,4-dinitrophenol, and pyridine led to the isolation of *R. koreensis* (Yoon et al. 2000b) and *R. pyridinivorans* (Yoon et al. 2000c), respectively. Novel and presumptively novel *Rhodococcus* spp. have been isolated from contaminated soil (Xu et al. 2007).

"***Prescottella***." Several selective media have been used to isolate "*P. equi*" from clinical and environmental sources (Makrai et al. 2005; Muscatello et al. 2007). One such formulation, NANAT medium, which consists of a tryptone-soy broth, yeast extract agar base supplemented with nalidixic acid, novobiocin, and potassium tellurite, has been used to good effect (Woolcock et al. 1979; Mutimer and Woolcock 1980; Muscatello et al. 2007). "*Prescotella equi*" has also been isolated from soil using TANT broth, a selective enrichment medium that contains actidione, nalidixic acid, penicillin, and potassium tellurite, prior to subculturing onto Tinsdale (Oxoid) agar containing actidione and M3 agar supplemented with potassium tellurite (Barton and Hughes 1981). Colonies of "*P. equi*" are evident on these selective media after 4 to 5 days incubation at 30 °C. Another selective isolation medium, based on a Mueller-Hinton base supplemented with ceftazdine and novobiocin, was developed by von Graevenitz and Punter-Streit (1995). A modified version of this medium gave significantly higher yields of "*P. equi*" from soil compared with NANAT agar (Muscatello et al. 2007).

Skermania. A micromanipulatory technique (Skerman 1968) has been used extensively to isolate skermaniae from activated sludge foam and mixed liquors of wastewater treatment plants in Australia (Blackall et al. 1989; Soddell and Seviour 1998; Seviour and Nielsen 2010). The first successful isolation of *S. piniformis* was achieved by Blackhall and her colleagues who used a Skerman micromanipulator to pick up and transfer acute-angled, branching filaments from activated sludge samples onto either yeast extract-glucose agar or tryptone-yeast extract agar plates which were incubated at several temperatures. Visible colonies of *S. piniformis* (1–2 mm in diameter) grew on the isolation media within 10–21 days.

Smaragdicoccus and Williamsia. Better procedures are needed to establish the numbers, distribution, and diversity of *Smaragdicoccus* and *Williamsia* strains in natural habitats. The sole strain of *S. niigatensis* was isolated from petroleum-contaminated soil by enrichment culture using hexadecane as the sole carbon source (Adachi et al. 2007). Single representatives of *Williamsia* species have been isolated from several habitats using standard isolation media with and without antibacterial antibiotics. *Williamsia maris* and *W. marianensis* were isolated from marine sediments using M3 agar and raffinose-histidine agar supplemented with antifungal antibiotics (Stach et al. 2004; Pathom-aree et al. 2006) and *W. limnetica* from a freshwater sediment on starch-casein agar containing antifungal antibiotics and rifampicin (Sazak and Sahin 2012). Similarly, the type strain of *W. faeni* was isolated

from suspensions of a hay meadow soil plated onto Gauze medium 2 supplemented with antifungal antibiotics, nalidixic acid, and novobiocin and incubated at 30 °C for 21 days (Jones et al. 2010).

Maintenance Procedures

Standard methods can be used for short- and long-term storage. Short-term preservation of cultures can be achieved by serial transfer onto media such as modified Bennett's (Jones 1949) and glucose-yeast extract (Gordon and Mihm 1962) agar slopes with storage between transfers at 4 °C. Lyophilization, preservation in liquid nitrogen, and frozen glycerol suspensions can be used for long-term storage. For lyophilization, propagules are suspended in a suitable fluid such as glucose serum or skimmed milk plus glucose (each at 7.5 %, v/v). For preservation in liquid nitrogen, organisms are grown on a suitable medium in small tubes and when sufficient growth is visible, the tubes are sealed with cotton wool plugs dipped in liquid paraffin wax, and then stored in a liquid nitrogen vessel. Glycerol suspensions are prepared by scraping growth from inoculated agar plates and making heavy suspensions in aqueous glycerol (3 mL) held in small vials which are stored at −20 °C (Wellington and Williams 1978). Frozen glycerol suspensions can be used as a long-term means of preservation and as a quick source of inoculum. Working inocula are prepared by thawing suspensions at room temperature and, after use, they are promptly frozen and stored again at −20 °C.

Ecology

Members of the genera *Nocardia*, *Gordonia* and *Rhodococcus* are common in aquatic and terrestrial habitats where they are considered to have a role in organic matter turnover and in the degradation of xenobiotics (Cross et al. 1976; Goodfellow and Williams 1983; Larkin et al. 2010; Luo et al. 2013a). The most comprehensive ecological studies on these and other mycolic acid–containing actinomycetes, notably skermaniae, have been focused on their presence, abundance, and activities in activated sludge wastewater treatment plants (Soddell and Seviour 1990; de los Reyes et al. 2002; Seviour et al. 2008; Seviour and Nielsen 2010). However, nocardiae are best known as causal agents of mycetoma and nocardiosis (McNeil and Brown 1994; Goodfellow 1992,1996; 1998; Brown-Elliott et al. 2006; Goodfellow and Maldonado 2012; van de Sande et al. 2013) though gordoniae and *Rhodococci* are being recognized increasingly as opportunistic pathogens of humans (Jones and Goodfellow 2012; Goodfellow et al. 2012; Drzyzga 2012). It is also becoming increasingly apparent that "*P. equi*" is an agent of human disease (Jones and Goodfellow 2012) though this organism is best known as a serious pathogen of foals (Barton and Hughes 1980; Prescott 1991; Vásquez-Boland et al. 2010).

The ecology, pathogenicity, and biotechnological applications of the genera *Nocardia*, *Gordonia*, *Rhodococcus*, and "*Prescotella*" will be considered further. However, little more can be said about the genus *Williamsia* as so few strains have been assigned to this taxon and even less about the genera *Millisia* and *Smaragdicoccus* as these taxa are currently represented by two and a single strain, respectively.

Nocardia. Nocardiae are widely distributed in diverse natural habitats (Cross et al. 1976; Goodfellow and Williams 1983; Goodfellow and Maldonado 2012). Some strains have been found to form endophytic associations with plants (Kaewkla and Franco 2011; Xing et al. 2011; Zhao et al. 2011) and others form mutualistic associations with blood-sucking insects (Cross et al. 1976; Xu et al. 1996; Yamamura et al. 2003b). However, their primary habitat seems to be soil as large populations (up to 1.4×10^5 colony forming units per gram of soil) have been reported for temperate and tropical soils (Orchard et al. 1977; Xu et al. 1996). In addition, many nocardiae have been detected in litter and soil from a spruce forest in County Durham, UK (Golinska et al. 2012), from sediment and water samples from freshwater lakes in Yunnan Province in Southwest China (Jiang and Xu 1985, 1996), from soil amended with either sewage effluent or dried sludge (Orchard et al. 1977; Orchard 1979, 1981), and from oil-contaminated soil (Khan et al. 1997).

Relatively little is known about the functional roles of nocardiae in natural habitats though there is evidence that they are involved in organic matter turnover (Orchard 1979, 1981). They have been implicated in the biodeterioration of natural rubber joints in water and sewage pipes (Hutchinson et al. 1979; Hookey 1984) and in foam formation in activated sludge plants (Soddell and Seviour 1990; Seviour and Nielsen 2010) though it would seem that their role in this latter respect has been overplayed (Soddell et al. 1992; Stainsby et al. 2002). The unfolding metabolic activity of nocardiae isolated from environmental sources is in sync with a saprophytic lifestyle (Goodfellow and Maldonado 2012; Luo et al. 2013a), as shown by the ability of *N. nova* and *N. takedensis* strains to degrade natural rubber and microbial gutta percha (Warneke et al. 2007; Luo et al. 2013b).

Improved nocardial systematics has been the motive force behind the rapid increase in the number of *Nocardia* species isolated from environmental sources (Goodfellow and Maldonado 2012). Novel species recovered from aquatic and littoral habitats include *N. grenadensis* from Caribbean Sea sand (Kämpfer et al. 2012), *N. harenae* from beach sand (Seo and Lee 2006), and *N. takedensis* from activated sludge scum (Yamamura et al. 2005). Similarly, novel taxa derived from terrestrial habitats include *N. aciditolerans* from litter and soil horizons of a spruce forest (Golinska et al. 2012), *N. altamirensis* from a cave soil (Jurado et al. 2008), *N. artemsiae* from a surface-sterilized stem of *Artemesia annua* (Zhao et al. 2011), *N. callitritis* from a surface-sterilized root of a native Australian pine tree (Kaewkla and Franco 2010), *N. endophytica* from a healthy stem of *Jatropha curcas* (Xing et al. 2011), *N. goodfellowii* and *N. thraciensis* from Turkish soils (Sazak and Sahin 2012), and *N. neocaledoniensis* from a brown hypermagnesian ultramafic soil (Saintpierre-Bonaccio et al. 2004). Studies such as these provide a useful insight into the

distribution and species diversity of nocardiae, but are of limited ecological value as species descriptions tend to be based on single isolates.

Gordonia. Interest in the biotechnological potential of gordoniae and improvements in their systematics has led to a substantial increase in the number of validly published *Gordonia* species (Arenskőtter et al. 2004; McLeod et al. 2006; Drzyzga 2012; Goodfellow et al. 2012). Expressions of this taxonomic trend include the recognition of *G. alkaliphila* from a tidal flat sediment (Cha and Cha 2013), *G. caeni* and *G. cholesterolivorans* from sewage sludge (Drzyzga et al. 2009; Srinivasan et al. 2012), *G. defluvii* from activated sludge foam (Soddell et al. 2006b), *G. humi* from soil (Kämpfer et al. 2011a), *G. lacunae* from an estuarine sand sample (le Roes et al. 2008), and *G. neofelisfaecis* from fresh feces of a clouded leopard (Liu et al. 2011a).

Gordoniae are widely distributed in aquatic and terrestrial ecosystems and have been found in especially high numbers in foaming-activated sludge plants across the world (Soddell and Seviour 1990; Goodfellow et al. 1996, 1998b; Lechevalier and Lechevalier 1974; Soddell et al. 1992), but it is now apparent that taxonomically diverse mycolic-acid-containing actinomycetes are implicated in this process (Petrovski et al. 2011c), including *G. defluvii* (Soddell et al. 2006b), *M. brevis* (Soddell et al. 2006a) and *S. piniformis* (Chun et al. 1997). The outer mycolic acid layer of the walls of such actinomycetes is considered to render cells sufficiently hydrophobic that they accumulate on the surfaces of aeration tanks in wastewater treatment plants (Seviour and Nielsen 2010).

Gordonia amarae is used as the model organism to establish relationships between foaming and numbers of mycolic acid-containing actinomycetes in activated sludge plants. To this end, oligonucleotide hybridization studies were used to identify and quantify *G. amarae* and related foam-inducing actinomycetes (de los Reyes et al. 1997, 1998a, b, c, Oerther et al. 1999; Iwahori et al. 2001); a cause-effect relationship was eventually reported between foaming and levels of *G. amarae* cells (de los Reyes and Raskin 2002). Similarly, Davenport et al. (2000) reported a relationship between foaming and the concentration of mycolic acid–containing actinomycetes in a mixed activated sludge reactor using a quantitative fluorescent in situ hybridization (FISH) procedure. However, ecophysiological studies on *G. amarae* cast doubt on the relationship between filament numbers determined using FISH and incidents of foaming (Carr et al. 2006). Indeed, laboratory-based foaming tests carried out on diverse mycolic acid–containing actinomycetes indicated that stable foam formation required three components: air bubbles, surfactants, and hydrophobic cells (Petrovski et al. 2011c). It was also shown that the ability to generate foam depended on a threshold cell number which varied between individual strains and was reduced markedly in the presence of surfactant. These data challenge the concept that a universal threshold is applicable to all mycolic acid–containing actinomycetes involved in foaming. To complicate matters further, differences in substrate utilization patterns of FISH probed *G. amarae* have been established in samples taken from different activated sludge plants (Kragelund et al. 2007). It has been shown that *G. amarae* causes foaming problems when biosurfactants are synthesized in the presence of hexadecane (Pagilla et al. 2002).

The remarkable metabolic versatility shown by gordoniae suggests that they are involved in the degradation of recalcitrant organic compounds in water and soil though ecophysiological studies are needed to determine whether this is so. However, *Gordonia* strains are known for their capacity to survive near-starvation conditions (Warhurst and Fewson 1994) and to metabolize toxic environmental pollutants, as well as natural and synthetic isoprene rubber (Arenskőtter et al. 2004; Drzyzga 2012).

Rhodococcus. Relatively little is known about the numbers, distribution, and roles of *Rhodococci* in natural habitats though they are common in soil and in freshwater and marine sediments (Goodfellow and Williams 1983; Jiang and Xu 1996; Colquhoun et al. 1998) and have been isolated from the gut contents of blood-sucking arthropods with which they form mutualistic associations (Cross et al. 1976; Goodfellow and Aubert 1980; Yassin 2005). An extensive ecological study was carried out on *R. coprophilus* by Rowbotham and Cross (1977b) who found that this organism grew on herbivorous dung and was present in high numbers in grazed pastures, and in streams, rivers, and lake muds that received run-off from dairy farms. Al-Diwany and Cross (1978) found a significant correlation between the numbers of fecal streptococci and *R. coprophilus* in polluted water and suggested that the latter might be used as an indicator of farm animal effluent, a view that was to be shared by others (Mara and Oragui 1981; Oragui and Mara 1985).

Relatively little is known about the ecology of other *Rhodococcus* species though *R. erythropolis*, *R. rhodochrous*, and *R. ruber* seem to be common in soil (Goodfellow and Williams 1983) and in activated sludge foam (Lemmer and Kroppenstedt 1984; Sezgin et al. 1988). *Rhodococcus gordoniae*, *R. intechensis*, and *R. quinshengii* were isolated from contaminated landsites (Jones et al. 2004; Ghosh et al. 2006; Xu et al. 2007), *R. koreensis* and *R. pyridinivorans* from industrial wastewater (Yoon et al. 2000a, c), and *R. coprophilus* from both aquatic and terrestrial habitats (Rowbotham and Cross 1977a, b). Other rhodococcal species have been isolated from more rarified habitats, as exemplified by the isolation of *R. artemisiae* from the medicinal plant *Artemesia annua* (Zhou et al. 2012), *R. baikonurensis* from the MIR space station (Li et al. 2004), *R. cerastii* from *Trfolium repens* (Kämpfer et al. 2013b), *R. josti* from a medieval grave (Takeuchi et al. 2002), and *R. trialomae* from a blood-sucking insect belonging to the genus *Triatomae* (Yassin 2005). *Rhodococci* involved in foam formation in activated sludge plants (Lemmer and Kroppenstedt 1984; Mori et al. 1998; Sezgin et al. 1988; Lemmer et al. 1998; Stainsby et al. 2002) have received little attention from either ecophysiological or taxonomic perspectives. However, representatives of several rhodococcal species produce stable foam under laboratory conditions though on the cessation of aeration, the foam films proved to be unstable (Petrovski et al. 2011c). Strains identified as *R. coprophilus*, *R. erythropolis*, *R. rhodochrous*, and *R. ruber* have been isolated from activated sludge foam

(Lemmer and Kroppenstedt 1984; Sezgin et al. 1988). Davenport et al. (1998) designed two genus-specific 16S rRNA oligonucleotide gene probes and used them to detect *Rhodococci* in samples of activated sludge foam using confocal laser scanning microscopy.

"***Prescotella***". Members of this monospecific genus are best known as facultative intracellular multihost pathogens (Letek et al. 2010; Vásquez-Boland et al. 2010). However, the natural environment of "*P. equi*" is soil, notably ones enriched with fecal material from domestic and wild animals (Barton and Hughes 1980; Prescott 1991; Takai et al. 1986, 2006). It is possible that the organism contributes to foaming in activated sludge plants as under laboratory conditions, two "*P. equi*" strains were found to produce stable foams (Petrovski et al. 2011c). Additional studies are needed to unravel the biology of "*P. equi*" in the environment.

Skermania. Skermaniae are associated with foaming-activated sludge plants in Australia (Blackall et al. 1988, 1989; Seviour et al. 1990; Soddell and Seviour 1994, 1998) and other parts of the world (de los Reyes et al. 2002; Eales et al. 2005; Soddell and Seviour 1990; Soddell et al. 1993). It is not known whether the organism is more widely distributed in the environment as all strains to date have been isolated from foam and mixed liquor systems by micromanipulation of pine-tree-like organisms (PTLOs; alias *S. piniformis*) onto rich nutrient media followed by incubation for up to 3 weeks. There is also evidence that *S. piniformis* has a slow growth rate in activated sludge (de los Reyes et al. 2002).

PTLOs have been the subject of several ecophysiological studies based on the use of in situ techniques, notably fluorescent in situ hybridization/microautoradiography (FISH/MAR) studies (Seviour et al. 2008). Using this latter procedure, Eales et al. (2005) found that PTLOs were highly selective in their substrate utilization in foams as out of eight substrates, they only assimilated oleic acid; this compound was metabolized both aerobically and anaerobically though uptake was low under anoxic conditions with nitrate and nitrite as oxygen accepters. Eales and her colleagues showed that a high proportion of the PTLOs were metabolically inactive in the foam. Most exhibited low respiratory activity as detected using the redox dye CTC and low intensity signals from FISH probing with 16S rRNA targeted probes. They also found a strong correlation between the accumulation of polyhydroxyalkanoates in filaments and the intensity of their fluorescent FISH signals.

Pathogenicity

Members of the family *Nocardiaceae* other than some *Nocardia* species and "*P. equi*" are usually considered to have a saprophytic lifestyle. However, it is becoming increasingly evident that mycolic acid–containing bacteria per se should be seen as potential opportunistic pathogens of humans (Koerner et al. 2009; Drzyzga 2012; Jones and Goodfellow 2012). Consequently, unusual *Nocardiaceae* strains isolated in diagnostic microbiological units should not be summarily dismissed as being of little consequence. However, within the family, pathogens have only been detected, to date, in the genera *Nocardia*, *Gordonia*, *Rhodococcus*, and "*Prescotella*."

Nocardia. Strains belonging to this genus are being reported with increasing frequency from clinical material, notably from specimens taken from immunocompromised patients (Poonwan et al. 1995, 2005; Boiron et al. 1998; Farina et al. 2001, 2007; Pintado et al. 2002; Saubolle and Sussland 2006; Serrano et al. 2007; Cargill et al. 2010). In this context, nocardiae are known best as causal agents of suppurative and granulomatous diseases of humans and animals, notably mycetoma and nocardiosis (Boiron et al. 1993; Beaman and Beaman 1994; McNeil and Brown 1994; Goodfellow 1996, 1998; Brown-Elliott et al. 2006; Goodfellow and Maldonado 2012; Luo et al. 2013a; van de Sande et al. 2013a).

Mycetoma, a major health problem in many tropical and subtropical regions, is caused by aerobic, filamentous actinomycetes (actinomycetoma) and by fungi (eumycetoma). It is an infection of subcutaneous tissue that is characterized by large tumor-like swellings mainly located at the extremities (Schaal and Beaman 1984; Fahal 2004, 2006; Brown-Elliott et al. 2006). The disease is mainly confined to tropical and subtropical areas, in Mexico, Senegal, and Sudan, though its true incidence and prevalence are not known (van de Sande 2013). Such gaps in our knowledge about mycetoma may now be addressed with greater vigor as the disease has been added to the World Health Organisation's list of Tropical Neglected Diseases (van der Sande et al. 2013a, b).

About 60 % of human mycetoma is caused by actinomycetes (Vera-Cabrera et al. 2004), mainly by *N. asteroides*, *N. brasiliensis*, *N. otitidiscaviarium*, and *N. transvalensis* (Buot et al. 1987; Beaman and Beaman 1994; Mirza and Campbell et al. 1996; Lum and Vadmal 2003; Fahal 2004). *Nocardia brasiliensis*, the major causal agent, is responsible for the vast majority of cases in Mexico (Lopez-Martinez et al. 1992). Other *Nocardia* species, such as *N. africana*, *N. mexicana*, and *N. veterana*, have been reported to cause human mycetoma (Hamid et al. 2001; Kano et al. 2002; Rodrigues-Nava et al. 2004). Mycetoma has been simulated in a mouse model (Gonzalez-Ochoa 1973; Zlotnik and Buckley 1980; Salinas-Carmona et al. 2011) that has been used to study host-parasite relationships (Ortiz-Ortiz et al. 1984; Zlotnik 2007).

Nocardiosis, unlike actinomycetoma, has a worldwide distribution. It tends to develop as an opportunistic infection that complicates primary diseases, such as leukemia, lymphoma, and other neoplasms (McNeil and Brown 1994; Brown-Elliott et al. 2006), other predisposing factors include steroid therapy, chronic bronchopulmonary disease, organ transplantations, and acquired immunodeficiency syndrome (Poonwan et al. 1995; Choucino et al. 1996; Gallant and Ko 1996; Patel and Paya 1997; Diego et al. 2005). Immunocompetent hosts can be infected usually after traumatic inoculation into skin (Beaman and Beaman 1994; Maraki et al. 2004). Accurate diagnosis and treatment of the disease still depends on the isolation and identification of the causal organism (Brown-Elliott et al. 2006; Goodfellow and Maldonado 2012). Many in vitro antibiotic

sensitivity assays have been carried out on *Nocardia* strains (Goodfellow and Orchard 1974; Wallace et al. 1983; Boiron and Provost 1988, 1990b; Vera-Cabrera et al. 2004), some of which have influenced antibiotic regimes recommended for treating nocardial infections (Brown-Elliott et al. 2006; Minero et al. 2009; Wilson 2012; van de Sande et al. 2013b).

Clinically human cases of nocardiosis can be described as skin infections (cutaneous, subcutaneous and lymphocutaneous), pulmonary and extrapulmonary, and systemic infections (Schaal and Beaman 1984; Schaal 1998). Cutaneous and lymphocutaneous infections are usually due to *N. asteroides*, *N. brasiliensis*, *N. farcinica*, and *N. transvalensis*, and pulmonary and systemic infections to these taxa together with *N. africana*, *N. mikami*, *N. nivae*, *N. pseudobrasiliensis* and *N. veterana* (Torres et al. 2000; Hamid et al. 2001; Farina et al. 2001, 2007; Conville et al. 2003; Pottumarthy et al. 2003; Jannat-Khah et al. 2010; Moser et al. 2011). In Thailand, the most prevalent agents of nocardiosis were found to be *N. beijingensis*, *N. cyriacigeorgica*, and *N. farcinica* (Poonwan et al. 2005). Nocardiae isolated from abscesses include *N. abscessus* (Yassin et al. 2000), *N. niigatensis* (Kageyama et al. 2004c), *N. nova* (Hamdad et al. 2007), *N. puris* (Yassin et al. 2003), and *N. yamanashensis* (Kageyama et al. 2004c).

Initially, nocardiosis was seen to be a late presenting, community-acquired infection, but it is now known that it is a transmissible disease (Young et al. 1971; Cox and Hughes 1975; Exmelin et al. 1996). Nosocomial infections caused by *N. asteroides* have been reported from liver (Sahathevan et al. 1991) and renal transplant (Houang et al. 1980; Baddour et al. 1986) units. Similarly, *N. farcinica* was found to be responsible for postoperative wound infections in patients undergoing vascular surgery (Schaal 1991). This organism is a particularly serious and versatile pathogen which can cause cerebral, cutaneous, and pulmonary abscesses (Schaal and Lee 1992; Kageyama et al. 2001; Hitti and Wolff 2005; Farina et al. 2007).

Nocardiae cause infections other than mycetoma and nocardiosis. Nocardial infections have been recorded in HIV patients (Kim et al. 1991; Javaly et al. 1992; Poonwan et al. 1995) and, more specifically, as the cause of brain abscesses (Barnaud et al. 2005; Hashimoto et al. 2008), endocarditis (Watson et al. 2001; Cargill et al. 2010), keratitis (Lalitha et al. 2006, 2012), and pleural emphysema (Maraki et al. 2006). It can be concluded that nocardiae are not only widespread in natural habitats but also in clinical settings where they can cause serious, sometimes fatal infections (Martinez et al. 2008; Minero et al. 2009). The recent dramatic increases in the reported frequency in nocardial infections can be attributed to the widespread use of immunosuppressive drugs and to raised clinical and microbiological awareness (Goodfellow 1996, 1998; Poonwan et al. 2005; Brown-Elliott et al. 2006). Mice have been used extensively as the experimental animal to study *Nocardia*-host interactions (Beaman 1973, 1984, 1992, 1993; Beaman and Ogata 1993; Beaman and Beaman 1994, 1998, 2000).

Nocardia strains cause infections in domesticated and wild animals (Beaman and Sugar 1983; Beaman and Beaman 1994; Ramos-Vara et al. 2007). Mastitis can be a serious problem in dairy animals, notably cows (Bushnell et al. 1979; Battig et al. 1990; Stark and Anderson 1990; Da Costa et al. 1999). *Nocardia farcinica* was implicated in an epizootic infection of cattle in Canada (Manninen et al. 1993); this organism is a significant cause of mastitis of goats in Sudan (Maldonado et al. 2004). The most frequently identified nocardial pathogens of animals are *N. asteroides*, *N. brasiliensis*, and *N. otitidiscaviarum*. In addition, *N. africana* has been isolated from a feline mycetoma (Hattori et al. 2003), *N. crassastreae* causes nocardiosis in Pacific oysters (Friedman et al. 1998), and *N. salmonicida* and *N. seriola* are fish pathogens (Kudo et al. 1988; Isik et al. 1999).

The mechanisms involved in nocardial pathogenicity and host immunity to nocardial infections have been extensively studied by Blaine Beaman and his colleagues (Beaman and Moring 1988; Beaman and Beaman 1994, 1998, 2000) who found that virulent strains of *N. asteroides* appeared to be influenced by several interrelating factors, such as the stage in the growth cycle, capacity to inhibit phagosome-lysozyme fusion, to resist oxidative killing mechanisms of phagocytes, and to alter enzymes within phagocytes. However, the factors involved in host resistance to nocardial infections are complex and remain poorly understood. The role of L-forms in nocardial infections also requires further study (Beaman 1992).

Gordonia. Most gordoniae have been isolated from environmental sources, but some have been associated with human infections in immunocompromised and immunocompetent patients from around the time the genus was validly published in 1988 (Richet et al. 1991; Riegel et al. 1991; Drancourt et al. 1997). Gordonial infections are on the increase mainly in immunocompromised patients with underlying health problems, such as those with pulmonary conditions (Drzyzga 2012; Goodfellow et al. 2012). In many cases, infections occur after major surgery and are associated with medical devices, such as heart machines and catheters (Jannat-Khan et al. 2009; Drzyzga 2012). Gordoniae have also been reported to cause abscesses (Drancourt et al. 1994; Werno et al. 2005), cutaneous infections (Zardawi et al. 2004), endocarditis (Lesens et al. 2000; Verma et al. 2006), mycetoma of the hand (Bakker et al. 2004), septicaemia (Kempf et al. 2004), but they are most frequently associated with catheter-related bacteremia (Pham et al. 2004; Blaschke et al. 2007; Renvoice et al. 2009; Lal et al. 2010).

A better understanding of the incidence, epidemiology, and clinical significance of *Gordonia* strains is needed. These aims should be fostered by improvements in gordonial systematics, as exemplified by the recognition of new pathogenic species. *Gordonia aria* was proposed for an organism isolated from the sputum of a patient with bacterial pneumonia (Kageyama et al. 2006), *G. effusa* from a patient with kidney malfunction (Kageyama et al. 2006), and *G. otitidis* from patients with bronchitis and external otitis (Iida et al. 2005). To date, gordoniae have not been associated with infections of animals, apart from a case of mesenteric lymphadenitis in pigs (Tsukamura et al. 1988).

"***Prescotella***." "*Prescotella equi*" is a facultative intracellular pathogen that is best known as a cause of severe suppurative

bronchopneumonia in foals (Barton and Hughes 1980; Prescott 1991; Vásquez-Boland et al. 2010). The prevalence and fatalities of "*P. equi*" pneumonia are high (Takai et al. 1995; Prescott 1991). Infections of animals other than horses are rare though a broad range of domesticated and wild animals are known to have been infected. An increasing number of "*P. equi*" infections are being recorded in cats and dogs (Patel 2002; Takai et al. 2003) and goats (Tkachuk-Saad et al. 1998; Kabongo et al. 2005). The typical expression of "*P. equi*" pneumonia is as a chronic suppurative bronchopneumonia with extensive abscess formation and associated lymphadenitis (Barton and Hughes 1980; Prescott 1991). Foals are typically infected within the first 5 months of birth probably due to the relative immaturity of the immune system in individual foals (Hooper-McGrevy et al. 2001; Chaffin et al. 2004; Darrah et al. 2004). Nearly all strains isolated from infected foals have the virulent 30–90 kB plasmid which encodes the 15–17 kDA protein, Vap A (Takai et al. 1991a, b, 2003). Loss of this plasmid leads to an inability to cause disease in foals (Hondalus and Mosser 1994; Giguere et al. 1999). The variant plasmid encoding Vap B has been identified in non-equine animal hosts (Oldfield et al. 2004; Letek et al. 2010). Strains which lack Vap A and B proteins are relatively common in non-equine isolates (Meijer and Prescott 2004).

The mechanisms involved in "*P. equi*" pathogenicity will only be touched upon here as they have been considered in detail elsewhere (Prescott 1991; Meijer and Prescott 1994; Vásquez-Boland et al. 2010). Once access to the host has been gained, cells of "*Prescotella equi*" are engulfed by macrophages in the lungs. Central to the pathogenicity of the organism is its ability to survive and replicate in alveolar macrophages (Hondalus and Mosser 1994). A key survival mechanism is the active manipulation of the endosomal pathway of the host (Fernandez-Mora et al. 2005; Toyooka et al. 2005). Intracellular proliferation of the pathogen eventually causes the death of macrophages and extensive damage to lung tissue characterized by cavitation and granuloma formation (Luhrmann et al. 2004; Meijer and Prescott 2004). "*Prescottella equi*" is increasingly being seen as a pathogen of immunocompromised humans, particularly those with AIDS (McNeil and Brown 1994; Kedlaya et al. 2001; Weinstock and Brown 2002). The clinical presentation of infections in humans reflects the immune condition of the host and the virulence of the pathogen. Infections range from localized to fatal systemic infections (Nasser and Bizri 2001; Kohl and Tillmanns 2002; Kamboj et al. 2008). The first fatal case of a "*P. equi*" infection in a previously healthy individual was reported by Gabriels et al. (2006).

Rhodococcus. *Rhodococci* are common in the environment but have been rarely implicated as human pathogens, mainly as agents of pulmonary disease in severely immunocompromised individuals (Osoagbaka 1989; McNeil and Brown 1994). *Rhodococcus globerulus* and *R. ruber* have been considered agents of keratitis (Cuello et al. 2002; Lalitha et al. 2006, 2012) and a *Rhodococcus* strain as the cause of osteomyelitis in a healthy immunologically normal young girl (Broughton et al. 1981). There is evidence that *Rhodococci* can cause mycetoma (Severo et al. 1987). It seems likely that the clinical presentation of rhodococcal infections will be influenced by the immune state of the host and the virulence of the pathogen, but at present, there is little information on either of these matters. In contrast, *R. fascians* is a well-known pathogen of numerous plants (Crespi et al. 1992; Veereke et al. 2005; Putnam and Miller 2007).

Rhodococcus fascians is a soil-borne pathogen that induces the formation of differentiated galls in many herbaceous plants (Putnam and Miller 2007). Galls consist of proliferating masses of meristematic tissue covered by very short hypertrophied shoots (shoot hyperplasia) that appear at the crown of infected plants (de O Manes et al. 2001; Goethals et al. 2001). These symptoms are triggered by cytokinins secreted by the colonizing bacteria, these induce a signaling cascade leading to activation of mitotic cell division, prevention of endoreplication, and ectopic expression of meristem-specific KNOX genes (Crespi et al. 1992, 1994; Depuydt et al. 2008, 2009a). The establishment of the disease in *Arabidopsis thaliana* has been studied extensively, notably by using an integrated genomics approach (Depuydt et al. 2009b). The severity of the disease induced by the pathogen is influenced by the route of infection and by the age and type of the plant (Vereeke et al. 2000).

The virulence determinants for the *R. fascians*-plant interaction are located on a linear plasmid, pFiD 188 (Vereecke 1997; Crespi et al. 1992, 1994; Stange et al. 1996; Temmerman et al. 2000). Two bacterial loci, *atl* and *fas*, are needed for complete virulence (Cornelis et al. 2002). The *alt* operon is involved in the biosynthesis of regulatory compounds that are necessary to induce *alt* and *fas* gene expression and are induced during the interaction with the plant (Maes et al. 2001). The *fas* operon encodes proteins, including an isopentenyl transferase which is involved in the biosynthesis of a compound that shows cytokinin activity (Crespi et al. 1992, 1994; Temmerman et al. 2000). It is thought that novel compounds produced by the pathogen disrupt plant hormone balances, eventually leading to disease (Vereeke et al. 2000). Temmerman et al. (2000) isolated an AraC-type regulatory gene, *fasR*, located on the linear plasmid pFi D188, and found that it was required for pathogenicity and *fas* gene expression.

Applications

It is apparent from earlier sections and from recent reviews of the literature that members of the family *Nocardiaceae*, especially *Gordonia*, *Nocardia*, and *Rhodococcus* strains, show extraordinary metabolic diversity that can be exploited for biotechnological applications (Larkin et al. 1998, 2005, 2006; Drzyzga 2012; Luo et al. 2013a), especially given the availability of toolkits for genetic engineering (McLeod et al. 2005; Larkin et al. 2010; Luo et al. 2013a). The increasing interest that is being shown in *Nocardiaceae* strains as a source of commercially significant primary and secondary metabolites and as agents of biodegradation and bioremediation reflects these developments (Drzyzga 2012; Luo et al. 2013a). Applications that have been

established already or which might be commercially useful in the future are considered below.

Biodegradation and Bioremediation

Nocardiaceae strains are common in natural ecosystems where, given their metabolic prowess, they can be expected to have an integral role in the turnover or organic matter and in the degradation of xenobiotic compounds. Indeed, given the ability of gordoniae, nocardiae and rhodococci to grow in polluted habitats makes them serious candidates for the bioremediation of industrially polluted sites and for the management of agricultural and urban waste (Warhurst and Fewson 2010; Larkin et al. 2005, 2010; Kuyukina and Ivshina 2010; Drzyzga 2012; Luo et al. 2013a). *Rhodococci*, for instance, are especially effective at degrading aromatic and aliphatic amides and nitriles and hence are of potential value for waste cleanup where these chemicals are produced (Bunch 1998; Heald et al. 2001; Martínková et al. 2010).

Enzymes

Presently the most important bioactive compounds synthesized by members of the family *Nocardiaceae*, notably by *Rhodococcus* strains, are enzymes (Tarnók 1976; Peczynska-Czoch and Mordarski 1988; Warhurst and Fewson 1994; Alvarez 2010b). Such enzymes are of current or potential use in several areas of biotechnology, such as clinical chemistry, medicinal therapy, and in the manufacturing of fine and bulk chemicals. In general, the value of *Nocardiaceae* strains in the commercial production of enzymes is enhanced by their high yield, cost efficiency, and susceptibility to genetic manipulation.

Nocardia. Members of this taxon are attracting considerable interest in academic and industrial circles as the extent of their metabolic versatility begins to unfold (Goodfellow and Maldonado 2012; Luo et al. 2013a, b). Some strains, notably *N. iowensis* NRRL 5646^T (Lamm et al. 2009), synthesize an extraordinary diversity of biotransforming enzymes that convert natural and synthetic compounds into valuable products such as novel carnosic acid derivatives (Hosny et al. 2002), flavanoids (Maatooq and Rosazza 2005), vanillic acid (Dhar et al. 2007), and 4-vinylphenol (Lee and Rosazza 2004). This organism contains the first nitric oxide synthase system characterized in prokaryotes (Chen and Rosazza 1994, 1995).

Gordonia. The number of compounds of biotechnological significance synthesized by *Gordonia* species is on the rise as the extent of their metabolic capabilities is realized (Arenskőtter et al. 2004; Goodfellow et al. 2012; Drzyzga 2012). Gordoniae are, for instance, a potential source of carotenoid pigments (De Miguel et al. 2000, 2001; Takaichi et al. 2008), novel steroids (Schneider et al. 2008; Liu et al. 2011b) and biosurfactants, including gordonan (Kondo et al. 2000; Drzyzga et al. 2009; Franzetti et al. 2009).

Gordoniae are also of interest given the ability of some strains to remove sulfur from hydrocarbons, notably from fossil fuels (Ma 2010). Most work on biological desulfurization systems use dibenzothiophene (DBT) or benzothiophene as model compounds (Gilbert et al. 1998; Kim et al. 1999, 2000; Matsui et al. 2001a; Santos et al. 2006). The pathway specifically involved in cleaving C-S bonds during metabolic desulfurization has been dubbed the "4S" pathway (Gallagher et al. 1993; Kilbane 2006), as four different molecules are formed during DBT desulfurization (Ma 2010). Sulfur removal mediated by bacteria is seen as a potential alternative to the deep hydrodesulfurized processes used in oil refineries to produce low sulfur fuel oils. *Gordonia* sp. strain CYKS1, the first member of the genus shown to be capable of dibenzothiophene desulfurization (Rhee et al. 1998), was used as a desulfurization catalyst in a two stage process developed by Chang et al. (2001) for the treatment of model and diesel oils, a process that was subsequently optimized (Choi et al. 2003).

Rhodococcus. Rhodococcal enzymes have received the most attention, notably as biocatalysts in industrial processes (Martínková et al. 2010; Ma 2010). In particular, *Rhodococci* have a remarkable capacity to synthesize enzymes that transform nitrile compounds (Beard and Page 1998; Hughes et al. 1998; Martínková et al. 2010). The enzymes involved in this process are the nitrilases, which catalyze the hydration of nitriles to amides (Bunch 1998). Many rhodococcal enzymes are able to convert synthetic nitriles into amides and acids have been found and some of these conversions have been developed commercially. Such biotransformations are an attractive alternative to chemically catalyzed reactions as they proceed at moderate temperatures, at near neutral pH, and yield highly pure products without the formation of undesirable by-products (Beard and Page 1998; Hughes et al. 1998; Martínková et al. 2010). The first successful biocatalyst process involving the production of a commodity chemical was the nitrile hydrolase catalyzed process for the production of acrylamide developed by the Nitto Chemical Industrial Company in collaboration with Kyoto University (Kobayashi et al. 1992).

Currently, the production of acrylamide is carried out by the Mitsubishi Rayon Company using immobilized cells of *R. rhodochrous* strain J1 (Martínková et al. 2010). The versatile nitrile hydratase synthesized by this strain is induced when the culture medium is supplemented with cobalt and crotonamide (Nagasawa et al. 1988, 1993; Nagasawa and Yamada 1990; Yamada and Kobayashi 1996); approximately 30,000 t of acrylamide are produced annually using this process. The same biocatalyst is used for the production of nicotinamide by Lonza AG (Shaw et al. 2003). Nitrilases have been used to manufacture fine chemicals on a small scale, such as 6-hydroxypicolinic acid (Lonza AG; Liese et al. 2000) and (R)-mandelic acid and (R)-3-chloromandelic acid (Mitsubishu Rayon Co., Brady et al. 2004). The application of nitrile biocatalysts for other industrial applications is promising, given encouraging results from laboratory scale experiments (Brady et al. 2004; Martínková et al. 2010).

Rhodococci have been examined extensively to determine their suitability for large-scale removal of sulfur from fossil fuels (Ma 2010). Most attention has been focused on *R. erythropolis* IGTS8, notably on the enzymes and genes involved in the DBT desulfurization pathway (Kilbane and Jackowzki 1992; Oldfield et al. 1997, 1998; Santos et al. 2007). This organism was isolated by Kilbane and Bielaga (1990) and used by Energy Biosystems Corporation in the development of their industrial microbial desulfurization strategy. The "4" pathway for sulfur removal mentioned earlier was first reported in *R. erythropolis* IGTS8 (Gallagher et al. 1993). Several mutants of this strain have been genetically engineered to increase its ability to effect the desulfurization process (Hirasawa et al. 2001; Matsui et al. 2001b; Holland et al. 2003). The influence of operational conditions during batch culture of strain IGTS8 was examined by Del Olmo et al. (2005) who proposed a kinetic model to describe the development of its desulfurization ability during growth. Extensive desulfurization of diesel fuels was achieved by Zhang et al. (2007) using a *R. erythropolis* strain isolated from oil-contaminated soil.

Rhodococcus strains are a potential source of carotenoid pigments (Tao et al. 2004, 2006) and novel bioflocculants and biosurfactants (Lang and Philp 1998; Kuyukina and ivshina (2010). Carotenoids synthesized by *R. erythropolis* have been characterized as 4-keto-γ-carotene and γ-carotene, respectively (Tao et al. 2004). In an additional study, the β-carotene gene (crtU) from *Brevibacterium linens* was expressed in *R. erythropolis* to produce chlorobactene, an asymmetrical aryl carotenoid which has a similar structure to the food colorant isorenieratene (Tao et al. 2006); the former has potential as a colorant that gives a different shade of color than isorenieratene and its hydroxyl derivatives (Dufossé et al. 2001). Rhodococcal biosurfactants are attractive for certain applications due to their biodegradability, low toxicity, and nonionic properties, as well as their pH and temperature stability (Ristau and Wagner 1983; Rapp and Gabriel-Jűrgens 2003). Particular attention has been paid to trehalose mycolates, notably those from *R. erythropolis* and *R. wratislaviensis* strains (Kim et al. 1990; Tuleva et al. 2008; Marques et al. 2009).

Secondary Metabolites

New drugs, notably antibiotics, are urgently needed to control the spread of antibiotic-resistant pathogens and to treat life-threatening diseases such as cancer (Donadio et al. 2010; Goodfellow and Fiedler 2010; Cragg and Newman 2013). Actinomycetes, notably streptomycetes, remain the richest source of novel antibiotics though relatively few of the nearly 14,000 known antibiotic compounds produced by actinomycetes have been derived from members of the family *Nocardiaceae* (Bérdy 2012). However, evidence from whole-genome sequences indicates that *Nocardiaceae* strains have the capacity to produce secondary metabolites (Ishikawa et al. 2004; McLeod et al. 2006; Vera-Cabrera et al. 2013). It is also known that some members of the family *Nocardiaceae*, notably *Nocardia* strains, are a source of novel secondary metabolites (Goodfellow and Maldonado 2012; Tiwari and Gupta 2012b; Luo et al. 2013a).

Nocardia. Several clinically significant *Nocardia* species have been found to produce novel antibiotics (Goodfellow and Maldonado 2012; Luo et al. 2013a). *Nocardia brasiliensis* strains are a particularly rich source of bioactive compounds, as exemplified by the discovery of brasilibactin A, a novel cytotoxic compound (Tsuda et al. 2005), brasilicardins A-D, new immunosuppressive terpenoid antibiotics (Shigemori et al. 1998; Komaki et al. 1989; Komatsu et al. 2004), brasilidine A, a cytotoxic indole alkaloid (Kobayashi et al. 1997), brasilinolides A and B, immunosuppressive macrolides (Shigemori et al. 1996; Komaki et al. 1998; Mikami et al. 2000), benzoquinones A-D, novel benz[a] anthroquinones (Nemoto et al. 1997; Tsuda et al. 1996, 1999) and nocarasins A-C, antibacterial benzenoid antibiotics (Tsuda et al. 1999).

Nocardia strains isolated from natural habitats have also been shown to synthesize novel bioactive compounds, as exemplified by nocadicin A, the first naturally occurring monocyclic β-lactam, from *N. uniformis* subsp. *tsuyamanesis* (Aoki et al. 1976) and tubelactomicin A, a 16-membered macrolide antibiotic isolated from a *Nocardia* strain (Igarashi et al. 2000) that was subsequently identified as *N. vinacea* (Kinoshita et al. 2001). Nocardiae exhibit other interesting biological activities, as shown with respect to the anthraqinone chrysophanol, the first secondary metabolic known to be synthesized in an organism-specific way, namely, through different folding mechanisms (Bringmann et al. 1996). These workers showed that *Nocardia* strain Acta 1057, an isolate from a hay meadow soil, produced chrysophanol through folding mode S, whereas eukaryotes (fungi, insects and plants) used folding mechanism F. A third folding mode, S′, was later found in *Streptomyces* strain AK671 (Bringmann et al. 2009).

New bioactive compounds derived from other clinically significant nocardiae include asterobactin, a siderophore with antitumor activity from *N. asteroides* (Nemoto et al. 2002), a cytotoxic macrolide from *N. otitidiscaviarum* (Mikami et al. 1990), immunosuppressive terpenoids from *N. terpenica* (Tanaka et al. 1997a; Hoshino et al. 2007), nocardiocyclines A and B, anthracyclines from *N. pseudobrasiliensis* (Tanaka et al. 1997b), and transvalecin A, a thiozolidine type antibiotic from *N. transvalensis* (Hoshino et al. 2004a, b). Additional novel compounds produced by *Nocardia* strains include the nocardichelins, siderophores with antitumor activity (Schneider et al. 2007), and the nocanthicins, thiazole peptide antibiotics that inhibit gram-positive bacteria (Leet et al. 2003; Li et al. 2003).

Gordonia. Bendigoles A ∼ C are the first secondary metabolites and first sterols to be isolated from a *Gordoniai strain* (Schneider et al. 2008). They were detected in a culture filtrate extract of "*G. australis*" Acta 2299 by HPLC-diode array analysis and characterized as novel steroids by mass spectrometry and NMR experiments; the strain was isolated from activated sludge foam collected from a Biological Nutrient Removal Plant, near

Bendigo, Victoria, Australia. Bendigole C showed binding affinity to human progesterone and bendigoles A∼C weak androgenic activities.

Rhodococcus. In general, *Rhodococci* have not been seen as a potential source of secondary metabolites though natural products have been detected in crude extracts of *Rhodococcus* strains by low resolution mass spectrometry (Geniloud et al. 2011). It has been suggested that *Rhodococcus* sp. strain RO4, a polychlorinated-biphenyl degrader, may be a source of bioactive steroid compounds (Yang et al. 2011). The discovery that *R. jostii* RHA1 contains 24 nonribosomal peptide synthase genes and seven polyketide synthase genes (McLeod et al. 2008) indicates that *Rhodococci* should not be overlooked in the search for novel bioactive compounds.

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33 The Family *Nocardioidaceae*

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Abstract
Nocardioidaceae, a family within the order *Propionibacteriales* in the Propionibacterinae, embraces the genera *Nocardioides, Marmoricola, Aeromicrobium, Kribbella, Actinopolymorpha, Thermasporomyces*, and *Flindersiella*. Recent analysis of 16S rRNA gene sequences (using different clustering algorithms) shows that the genera which currently belong to the family *Nocardioidaceae* form at least two separate phylogenetic clusters. The two clusters are approximately at the same distance from each other and also from the family *Propionibacteriaceae*. The grouping is strengthened also by morphological and chemotaxonomic features. They are Gram-positive bacteria. Young cultures within the family show different morphologies, ranging from branching vegetative hyphae and pleomorph shapes to irregular rods and coccoid cells. Chemoorganotrophic organisms, having respiratory type of metabolism, are mostly catalase positive. Their cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine as diagnostic amino acid (peptidoglycan A3γ). Their predominant menaquinone component is with partially saturated side chain, consisting 8, 9, or 10 isoprene units. Mycolic acids are absent. Members of the family occur in various environments (soils, aquatic environments, plants, etc.); no medically relevant strains or species have been described within the family till now. Some members of the family are active in degradation of recalcitrant chemicals. This contribution is a modified and updated version of previous family descriptions (Nesterenko et al. 1985, 1990; Stackebrandt et al. 1997; Zhi et al. 2009).

Taxonomy, Historical and Current

Short Description of the Family

No.car.di.o.i.da.ce.a.e. N.L. masc. n. *Nocardioides* type genus of the family; suff.—*aceae* ending to denote a family; N.L. fem. pl. n. *Nocardioidaceae* the *Nocardioides* family.

This description is an emended version given by Zhi et al. (2009).

The family *Nocardioidaceae* originally was described by Nesterenko et al. (1985, 1990) (emended by Stackebrandt et al. 1997 and Zhi et al. 2009). At the beginning the family involved the genus *Nocardioides* (Prauser 1976) with the species *N. albus* (Prauser 1976), *N. luteus* (Prauser 1984a), and *N. simplex* (O'Donnell et al. 1982) and some other related organisms with LL-DAP in their cell wall: *Pimelobacter jensenii* (Suzuki and Komagata 1983) and *Arthrobacter tumescens* (Conn and Dimmick 1947; Jensen 1934). *A. tumescens* later was considered to reclassify as *Pimelobacter tumescens* (Suzuki and Komagata 1983). In the same publication, the authors suggested to rename *P. jensenii* as *Nocardioides jensenii* based on many physiological and chemotaxonomic properties. But the name was not validated, so the genus *Pimelobacter* was kept with three species: *P. simplex*, *P. jensenii*, and *P. tumescens*. In the meanwhile O'Donnell et al. (1982) proposed to reclassify *Arthrobacter simplex* (Lochhead 1957) as *Nocardioides simplex*. (Therefore, in 1985, the species *N. simplex* belonged to this genus).

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Based on taxonomic reconsiderations (including 16S rDNA analysis), *N. albus*, *N. luteus, P. simplex*, and *P. jensenii* were shown to form a common phylogenetic group; meanwhile *P. tumescens* represented a separate line of descent (Collins et al. 1989). Chemotaxonomic data and also phage typing of Prauser (1976; 1984) confirmed the abovementioned data; therefore, *P. jensenii* was transferred to the genus *Nocardioides*, and *P. tumescens* was transferred to a newly developed genus as *Terrabacter tumescens* (Stackebrandt et al. 1997). Simultaneously, Collins and Stackebrandt (1989) described the species *Nocardioides fastidiosa* (which was transferred to the genus *Aeromicrobium* by Tamura and Yokota in 1994). In 1994, *N. plantarum* was also described (Collins et al. 1994). Thus, when Stackebrandt et al. (1997) proposed the emended description of the family *Nocardioidaceae*, it contained the genus *Nocardioides* (with the following five species: *N. albus, N. luteus, N. simplex, N. jensenii*, and *N. plantarum*) and the genus *Aeromicrobium* (with species *A. erythreum* and *A. fastidiosum*).

Genera *Kribbella*, *Marmoricola, Hongia*, *Actinopolymorpha*, *Thermasporomyces*, and *Flindersiella* were added to the family between 1999 and 2011 based on polyphasic studies (Vandamme et al. 1996; Park et al. 1999; Urzí et al. 2000; Lee et al. 2000; Wang et al. 2001; Stackebrandt and Schumann 2006; Yabe et al. 2011; Kaewkla and Franco 2011b). When the taxonomic status of *Hongia koreensis* was reclassified (Sohn et al. 2003) this taxon was transferred to the genus *Kribbella* (Park et al. 1999) as *K. koreensis*.

The genus *Jiangella* (Song et al. 2005) was also assigned to belong to the family *Nocardioidaceae*, but recently it has been transferred to a separated family *Jiangellaceae* in the suborder Jiangellineae (Tang et al. 2011). Two other novel genera *Thermasporomyces* (Yabe et al. 2011) and *Flindersiella* (Kaewkla and Franco 2011b) were also added to this family.

At this time the family *Nocardioidaceae* phylogenetically belongs to the order *Actinomycetales*, in the suborder Propionibacterinae, and contains the genera *Nocardioides* (Nesterenko et al. 1990; Stackebrandt et al. 1997; Zhi et al. 2009), *Aeromicrobium* (Miller et al. 1991), *Kribbella* (Park et al. 1999), *Marmoricola* (Urzí et al. 2000), *Actinopolymorpha* (Wang et al. 2001), *Thermasporomyces* (Yabe et al. 2011), and *Flindersiella* (Kaewkla and Franco 2011b). The type genus of the family is *Nocardioides.* Recently, the suborder Propionibacterinae has been elevated to order rank (Patrick and McDowell 2012).

Gram-staining positive (in old cultures Gram variable staining can also be observed at some genera). Cultures show different morphologies, ranging from branching vegetative hyphae and pleomorph shapes to irregular rods and coccoid cells. Clusters of irregular cells can also be observed at some taxa. In some genera even a morphogenetic rod-coccus cell cycle can be observed, and rods can be motile. Aerial hyphae are produced by members of several genera. Vegetative and aerial hyphae can undergo different degrees of fragmentation resulting to rodlike or coccoid cells (depending also on the organism and growth conditions).

Bacteria are chemoorganotrophic, having a respiratory type of metabolism. Their cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine as diagnostic amino acid (peptidoglycan A3γ). Their predominant menaquinone component is with partially saturated side chain, consisting 8, 9, or 10 isoprene units. Cellular fatty acids and their polar lipid profile differ among genera. Mycolic acids are absent.

The set of 16S rRNA gene sequence signature nucleotides are at positions 328 (C), 407:435 (A-U), 451 (G), 453 (C), 819 (U), 825:875 (G-C), 827 (U), 828 (A), 832:854 (G-G), 833–853 (U-C), and 844 (C).

Phylogenetic Structure of the Family and Its Genera

Recent analysis of 16S rRNA gene sequences (using different clustering algorithms) shows that the genera comprising the current family *Nocardioidaceae* form at least two separate phylogenetic clusters. The two clusters are approximately at the same distance from each other and also from the family *Propionibacteriaceae* (Fig. 33.1a). The grouping is strengthened also by morphological and chemotaxonomic features of the genera involved.

The first cluster of the family includes the genera *Nocardioides, Aeromicrobium*, and *Marmoricola*. Their morphology is relatively simple; motile rods can be often observed. One predominant menaquinone occurs in their profile (with tetra-hydrogenated side chain of 8 or 9 isoprene units), and significant amounts of straight-chain saturated and unsaturated fatty acids can be found in their cells.

The second cluster contains the genera *Kribbella, Actinopolymorpha, Flindersiella*, and *Thermasporomyces*. In these genera more complex cell morphology can be observed and the lack of motile cells is characteristic. Their menaquinone profile is more complex involving longer and more saturated side chains. Their fatty acid profiles are dominated by iso- and anteiso-branched fatty acids and contain only a minor amount of straight-chain components.

Moreover, the two clusters have different genome sizes based on the two available complete genome sequences: the genome size of *Nocardioides* sp. JS614 is 4.99 Mb (Coleman et al. 2011) and that of *Kribbella flavida* DSM 17836 7.58 Mb (Pukall et al. 2010).

Within the family the 16S rRNA gene sequence clustering still clearly defines the main lines, and except of genera *Marmoricola* and *Nocardioides*, the allocation of a novel strain into a certain genus is still achievable based on it. In the case of the latter two genera, the position of some species is doubtful: *Marmoricola bigeumensis* groups together with *Nocardioides mesophilus* and *Nocardioides iriomotensis* (Fig. 33.1b). The affiliation of *M. bigeumensis* in the genus *Marmoricola* is based mainly upon on the 16S rRNA gene clustering (Dastager et al. 2008b) though the type strain has a higher binary similarity (97 %) to *Nocardioides jensenii* than to the type species (*M. aurantiacus*) of the genus *Marmoricola*.

The type species *Marmoricola aurantiacus* and *Nocardioides jensenii* share a 96.4 % 16S rRNA gene sequence similarity. The treeing algorithm places these two organisms to a second lineage containing other species of *Nocardioides*. Based on 16S rRNA gene sequence similarity, several secondary-structure-forming nucleotides that are missing in *Nocardioides* species and the unique combination of phylogenetic characteristics and chemotaxonomic properties resulted in the proposal of the new genus *Marmoricola* (Urzí et al. 2000) with the type species *M. aurantiacus*.

Therefore, allocation of a new species into the genus *Marmoricola* or *Nocardioides* is mainly based upon phylogenetic evidence combined with genus-specific chemotaxonomic properties.

The position of members of the type genus *Nocardioides* (Prauser 1976) should also be reconsidered. At present, the genus contains phylogenetically distant species (the most unrelated species show only 92–93 % 16S rRNA gene sequence similarity to one another) which is often lower than the threshold values delineating many phylogenetically well-defined genera within actinomycete families. Moreover species of the genus *Nocardioides* often show phenotypically different characteristics.

The differences between some *Nocardioides* species in the 16S-23S ITS region and RNase P gene sequence also show low similarity values (often at the level of different genera) (Yoon et al. 1998; Yoon and Park 2000).

The genus *Aeromicrobium* was established by Miller et al. (1991) with the type species *Aeromicrobium erythreum*. Subsequently, *Nocardioides fastidiosa* was transferred to the genus as *Aeromicrobium fastidiosum* (Tamura and Yokota 1994). The description of the genus was emended by Yoon et al. (2005a). Comparative analysis of 16S rRNA gene sequences (◉ *Fig. 33.1a*) shows that the genus *Aeromicrobium* is phylogenetically related to members of the genera *Marmoricola* and *Nocardioides* within the radiation of the family *Nocardioidaceae* (Stackebrandt et al. 1997; Urzí et al. 2000; Yoon et al. 2005a).

The genus *Kribbella* was established by Park et al. (1999) as a result of the reclassification of two strains, "*Nocardioides fulvus*" IFO 14399 (Ruan and Zhang 1979) and *Nocardioides* sp. ATCC 39419 (Matson and Bush 1989), as the type strains of *K. flavida* and *K. sandramycini*, respectively. The type species of the genus *Hongia* (*H. koreensis* described by Lee et al. 2000) was transferred to the genus *Kribbella* by Sohn et al. (2003) as *K. koreensis*. The 17 validly described *Kribbella* species form a monophyletic cluster in the phylogenetic tree (◉ *Fig. 33.1c*) based upon 16S rRNA sequence analysis.

The genus *Actinopolymorpha* was placed in the family *Nocardioidaceae* on the basis of 16S rRNA gene sequence analysis: the type strain of *A. singaporensis* carries all family-specific nucleotide signatures except for a G-C pair at nucleotide positions 370:379 and an A-U pair at position 602:636 instead of a G-U pair. On the other hand, *A. singaporensis* strain IM 7744^{T} possesses a number of properties that distinguish it from all other genera of the family (Wang et al. 2001) and shares low levels of 16S rDNA sequence similarities with the other members.

Thermasporomyces and *Flindersiella* are with type species of *T. composti* (Yabe et al. 2011) and *F. endophytica* (Kaewkla and Franco 2011b), respectively (◉ *Fig. 33.1 b* and *c*).

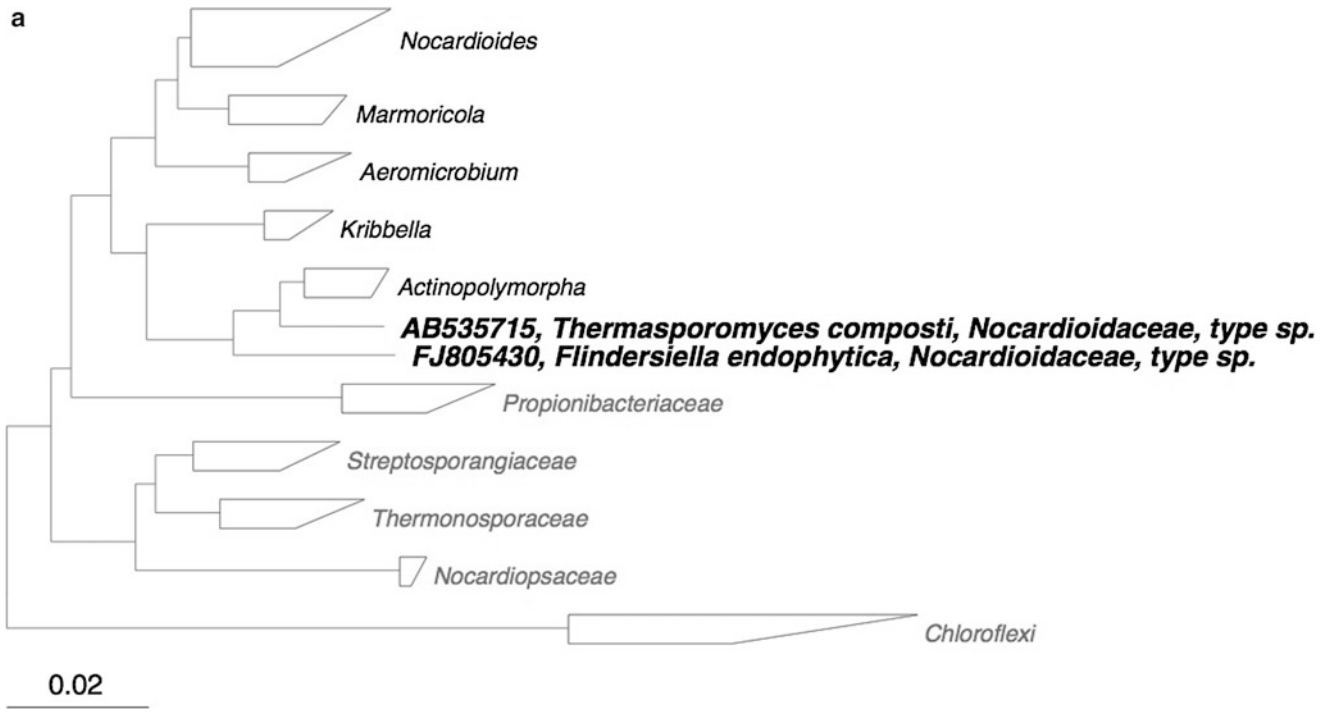

Fig. 33.1 (Continued)

Fig. 33.1 (Continued)

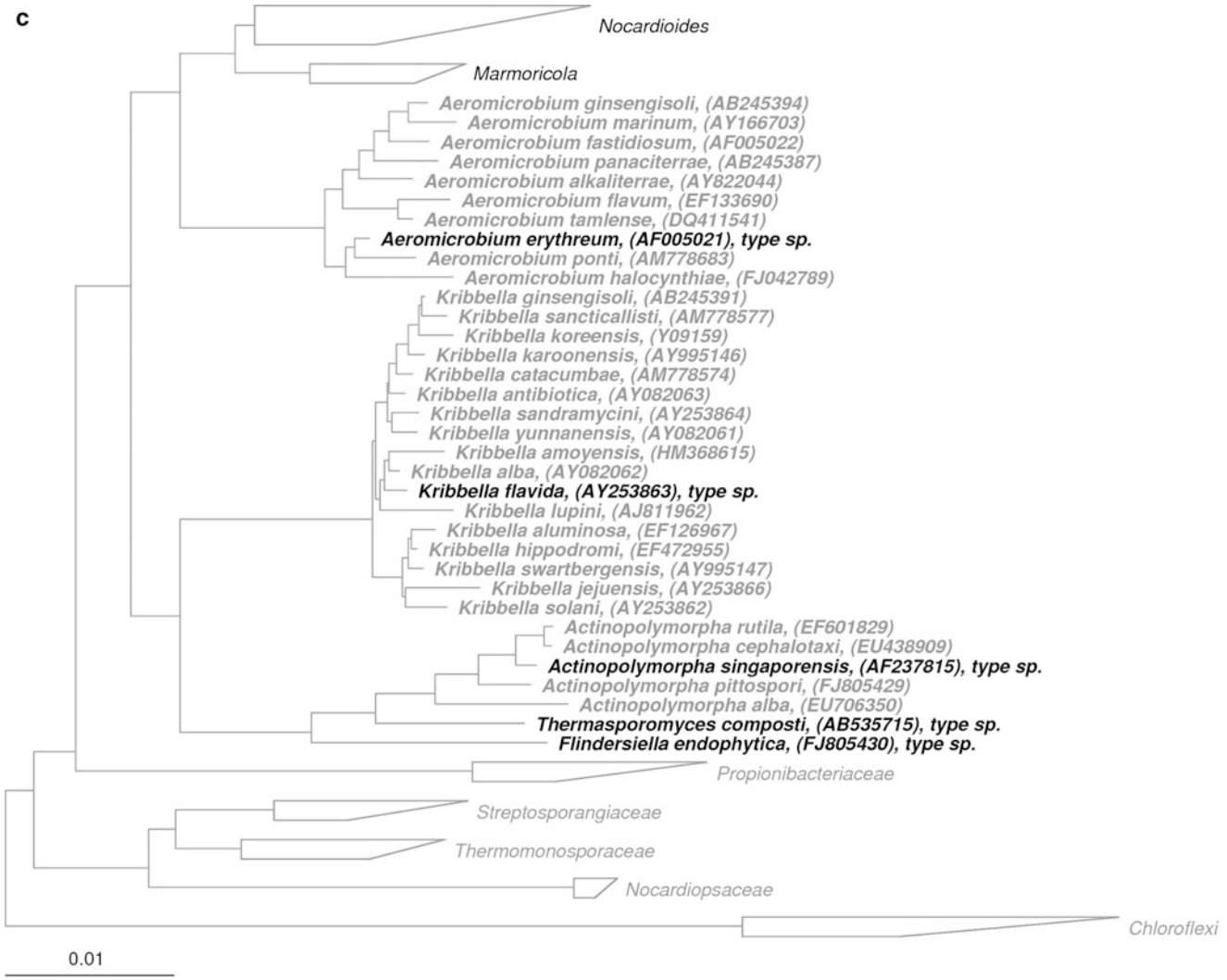

Fig. 33.1

(a–c) Phylogenetic reconstruction of the family *Nocardioidaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. Most 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) except yet not validly published species between question marks, these sequences were imported from the SILVA database (Quast et al. 2013). 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 50 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence

Molecular Analyses

DNA-DNA Hybridization Studies

DNA-DNA relatedness was measured between several species within the genera *Nocardioides* (Yoon et al. 1999, 2004, 2005b, 2006b, 2009, 2010; Schippers et al 2005; Park et al. 2008; Lee et al. 2008; 2011; Kubota et al. 2005; Dastager et al. 2009; Zhang et al. 2009; Cui et al. 2009; Yamamura et al. 2011; Alias-Villegas et al. 2012; Liu et al. 2012; Qin et al. 2012), *Aeromicrobium* (Bruns et al. 2003; Yoon et al. 2005a; Lee and Kim 2007; Cui et al. 2007; Kim et al. 2010), *Kribbella* (Li et al. 2004, 2006; Song et al. 2004; Trujillo et al. 2006; Carlsohn et al. 2007; Urzí et al. 2008; Cui et al. 2010; Xu et al. 2012), *Marmoricola* (Lee and Lee 2010; Lee et al. 2011b), and *Actinopolymorpha* (Wang et al. 2008; Cao et al. 2009; Yuan et al. 2010; Kaewkla and Franco 2011a).

All these results show that within the family *Nocardioidaceae*, the DNA-DNA relatedness values (DDH) are below the threshold value of 70 % recommended for definition of bacterial species (Wayne et al. 1987) and the values between the strains of the same species are much above this.

Riboprinting, Ribotyping, and MALDI-TOF MS

Only some members of the genus *Nocardioides* were studied by the automated RiboPrint system (three different strains of *N. hungaricus*, *N. hankookensis*, *N. pyridinolyticus*, and *N. aquiterrae*). These strains were analyzed also by MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometric approach. Both methods demonstrated that the strains of the same species are grouped well together, while other species could be well separated showing that within the genera most probably both methods support the result of 16S rRNA gene sequencing (Tóth et al. 2011).

Within the genus *Kribbella*, MALDI-TOF analysis was performed with the type strains of *K. flavida, K. karoonensis, K. koreensis, K. catacumbae* and *K. sancticallisti*, and the results were in agreement with the results of RiboPrint and 16S rRNA gene sequence analysis (Urzí et al. 2008).

RNase P Gene, ITS Multilocus Sequence Analysis

Comparative sequence analysis of ribonuclease P (RNase P) genes was studied by Yoon and Park (2000) with LL-DAP-containing actinomycetes, among them are some members of the genus *Nocardioides* (*N. luteus* KCTC 9575^T, *N. flavus* IFO 14396^T, *N. fulvus* JCM 3335^T, *N. simplex* KCTC 9106^T, *N. nitrophenolicus* KCTC 0457BP^T, *N. plantarum NCIMB* 12834^T, *N. pyridinolyticus* KCTC 0074BP^T, and *N. jensenii* KCTC 9134^T). The mean RNase P gene similarity among the studied strains is approximately 76.6 ± 12.5 %. The topologies of the resultant trees (using different treeing methods) are divergent when compared with the levels of 16S rRNA gene similarities. *N. albus* and *N. luteus* are phylogenetic neighbors with nucleotide similarity of 94 %. The clustering of *N. simplex* and *N. nitrophenolicus* is supported though by low bootstrap values when neighbor-joining and maximum-likelihood methods are used. *N. jensenii*, *N. plantarum*, and *N. pyridinolyticus* form phylogenetic lineages distinct from each species. It is important to mention that the sequence similarity values between more distant *Nocardioides* species are often close to the values between *Nocardioides* species and strains of *Kribbella* or *Aeromicrobium*.

Yoon et al (1998) studied the 16S-23S ITS regions of several *Nocardioides* species. The size of 16S-23S ITS regions varies from 328 (*N. nitrophenolicus*) to 539 bp (*N. albus* group). In few *N. albus* strains (DSM 43874, JCM 5851, JCM 5862) and in *N. simplex* NCIMB 12919, two types of ITS sequences were found (differing in size from 1 to 12 bp). Most probably in these cases, at least two rRNA operons exist in the genome of the strains. The nucleotide similarity of the ITS region between the studied strains ranges from 48.4 % to 84.8 %.

For the multilocus sequence analysis (MLSA) of 16 validly described *Kribbella* species, the following genes were selected: *gyrB* gene (DNA gyrase B subunit), *rpoB* gene (RNA polymerase beta subunit), *recA* gene (recombinase A), *atpD* gene (ATP synthase F1 beta subunit), and *relA* gene (encoding ppGpp synthetase) (Curtis and Meyers 2012). In a previous study, Kirby et al. (2010) demonstrated that the *gyrB*-based genetic distances between *Kribbella*-type strains ranged from 0.0164 to 0.1495, supporting the use of the 0.014 genetic distance value as the threshold for species delineation within this genus. The five-gene concatenated sequence analysis of 4,099 nt revealed that most *Kribbella*-type strains can be distinguished by a genetic distance of >0.04. The MLSA-based tree resulted in a marked improvement in the resolution compared to the 16S rRNA gene tree. By using MLSA, a better understanding of the genus *Kribbella* has been established, and the use of the genes *gyrB, rpoB, recA, relA*, and *atpD* proved to be useful for exploring the phylogenetic relationships within the genus and for determining the closest phylogenetic relatives (Curtis and Meyers 2012).

Genome Comparison

The complete genome of *Nocardioides* sp. strain JS614 has been sequenced (Coleman et al. 2011). Based on the results, the genome (5.3 Mb) consists of a single circular 4,985,871-bp chromosome and one 307,814-bp plasmid (pNOCA01). The average G+C content of the chromosome is 71.65 %, and that of the plasmid is 68.01 %. The chromosome contains 4,645 putative protein-coding genes, 46 tRNAs, and two rRNA operons. The plasmid contains 256 protein-coding genes. In total approximately 69 % of the genome had function predictions.

The genome of the type strain of *Kribbella flavida* IFO 14399^T, DSM 17836 (Park et al. 1999), is 7,579,488 bp long and contains 7,086 protein-coding genes, including 60 RNA genes, and the mol% G+C of DNA is 70.6 % (Pukall et al. 2010). The latter is in agreement with the species description value (Park et al. 1999). The highest number of genes associated with general COG functional categories is found for transcription and carbohydrate transport and metabolism (762 and 636, respectively), followed by amino acid transport and metabolism (397) and energy production and conversion (308).

Phages

Many bacteriophages have been described which multiply in *N. albus*, *N. luteus*, *N. simplex*, and *N. jensenii* but not in any other actinomycete strains (Prauser 1976; 1984; Miller et al. 1991; Williams et al. 1980). The phage sensitivity of *Arthrobacter simplex* and *Arthrobacter jensenii* was rational for the reclassification of the genus *Nocardioides* (Prauser 1976; 1984; Kurtboke and Williams 1991). The following phages cause true lysis and/or clearing effects on strains of all *Nocardioides* ssp. X6 (DSM 49135), X10 (DSM 49104), X24 (DSM 49105). Phages of the following subset cause true lysis and/or clearing effects on strains of the *Nocardioides* sensu stricto species *N. albus*, *N. luteus*, "*N. fulvus*," and "*N. flavus*" X1 (DSM 49101) and X3 (DSM 49102).

Phenotypic Analyses

The main features of members of *Nocardioidaceae* are listed in Table 33.1.

Young cultures within the family show different morphologies, ranging from branching vegetative hyphae and pleomorphic shapes to irregular rods and coccoid cells. Aerial hyphae are produced by members of several genera. Vegetative and aerial hyphae can undergo different degrees of fragmentation resulting in rod-like or coccoid cells (depending also on the organism and growth conditions). Clusters of irregular cells can also be observed in some taxa. Members of the genera *Aeromicrobium* and *Nocardioides* often show a rod-coccus morphogenetic cell cycle, and rods can be motile. Members of the genus *Marmoricola* form coccoid cells. Members of the family are Gram positive (in old cultures Gram variable staining can be also observed at some genera) and are non-acid-fast.

They are chemoorganotrophic, mostly catalase-positive bacteria, having a respiratory type of metabolism. Their cell-wall peptidoglycan contains LL-diaminopimelic acid and glycine as diagnostic amino acid (peptidoglycan A3γ). Their predominant menaquinone components contain partially saturated side chains, consisting 8, 9, or 10 isoprene units. Cellular fatty acids and their polar lipid profile differ among genera (see Table 33.1). Mycolic acids are absent.

Members of the family occur in various environments (see Table 33.2–33.6); no medically relevant strains or species have been described within the family until now.

Nocardioides Prauser (1976)

No.car.di.o.i'des. N.L. fem. n. *Nocardia* name of a genus; L. suff. *-oides* (from Gr. suff. *-eides* from Gr. n. *-eidos*, as form, shape, figure) resembling, similar; N.L. masc. n. *Nocardioides Nocardia*-like, referring to the similarity of life cycles of the type species of this genus and *Nocardia*.

The type strain of *Nocardioides albus* 895-50^T was isolated from soil (Prauser 1976).

In young cultures cells of the genus are often irregular short rods, in some species branched vegetative hyphae can also be observed (*N. albus*, Prauser 1976; *N. luteus*, Prauser 1984). Morphogenetic rod-coccus cycle often occurs in different species. The morphogenetic cycle usually starts with coccoid cells or short rods which develop into rods or short filaments. If mycelia occur, the vegetative and aerial hyphae also undergo various degree of fragmentation. Rod-shaped bacteria may be motile. Colonies can be whitish, creamy, or yellow, rarely orange.

Strains are catalase-positive oxidase variable and grow under aerobic conditions. *Nocardioides* species are chemoorganotrophs and utilize a wide range of carbon and nitrogen sources (see Table 33.2). They may adapt to oligotrophic conditions. Mattes et al. (2005) found that *Nocardioides* sp. JS614 is able to grow even chemolitotrophically.

The majority of *Nocardioides* strains show esterase lipase (C8) activity but are negative for β-glucuronidase, α-fucosidase, and α-mannosidase in API ZYM (bioMérieux) test. Negative test results are observed for H_2S production except for *N. panacisoli* (Cho et al. 2010). The results for Voges-Proskauer test vary between species and experiments. Many species degrade DNA, degradation of cellulose is uncommon, and only few organisms can degrade chitin (Prauser 1976; Tóth et al. 2008) or xylan (Park et al. 2008).

An important characteristic of the *Nocardioides* species is their capability to degrade complex, unusual substrates, including common toxic environmental pollutants and aromatic chemicals (see at applied studies).

Nocardioides species are mostly mesophilic (grow well between 25 °C and 30 °C), but some species grow also at psychrophilic conditions (show growth even at 4 °C). The reported optimal temperature for growth varies among species. Most species of this genus are neutrophilic, but some grow at initial pH values of 5 and/or 12 (see Table 33.2). Most of them are non-halophilic but salt-requiring organisms (mainly isolated from marine and marine-related environments) can also occur (see Table 33.2). These species need usually 0.5–6 % NaCl for their growth.

The cell-wall teichoic acids are present. The predominant menaquinone is MK-8(H_4) containing a tetra-hydrogenated side chain with eight isoprene units. Cellular fatty acids are complex mixtures of saturated and monounsaturated, straight-chain, and iso-, anteiso-, and methyl-branched components; usually 14-methyl pentadecanoic acid (iso C16:0) dominates (see Table 33.2). Polar lipid profile contains different combination of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, rarely phosphatidylcholine, methyl-phosphatidylinositol, or phosphatidylethanolamine. The polyamine patterns usually contain cadaverine as the predominant component, occasionally with putrescine, spermine, or spermidine (Busse and Schumann 1999; Yamada and Komagata 1972).

The antibiotic sensitivity was studied only on few species within the genus *Nocardioides* (*N. aquiterrae, N. aquaticus, N. caricicola, N. daedukensis, N. dokdonensis, N. fonticola, N. hankookensis, N. insulae, N. pyridinolyticus*, and *N. terrigena*). Except *N. pyridinolyticus*, strains of these species are sensitive for chloramphenicol and streptomycin in different concentrations (Song et al. 2011).

DNA G+C content is 66.6–74.8 mol%.

Marmoricola Urzi et al. (2000), Emend Dastager (2008), Emend Lee and Lee (2010)

Mar.mo.ri'co.la. L. neutr. n. *marmor* marble; L. masc. suffix *–cola* inhabitant of; *Marmoricola* inhabitant of marble.

The type strain *Marmoricola aurantiacus* BC 361^T was isolated from marble (Urzí et al. 2000).

Cells are spherical, occurring singly, in pairs, in tetrads, or in small clusters. No rod-coccus morphogenetic cell cycle occurs.

Table 33.1
Differential characteristics of the genera within the family *Nocardioidaceae*

	Nocardioides	*Marmoricola*	*Actinopolymorpha*	*Aeromicrobium*	*Kribbella*	*Flindersiella*	*Thermosporomyces*
Morphology	Rods, cocci, hyphae	Coccoid cells	Polymorph to hyphae	Rods, cocci	Hyphae, rods, coccoid cells	Hyphae, rods	Hyphae, rods
Gram staining	Gram positive to variable	Positive	Positive	Positive	Positive	Positive	Positive
Catalase	v	v	+	v	+[a]	+	ND
Oxidase	v	–	+[a]	v	v	ND	ND
Motility	v		–	–	–	–	–
Predominant cellular fatty acid(s)[b]	$iC_{16:0}$	$C_{18:1w9c;}C_{16:0}$	$iC_{15:0}$; $iC_{16:0}$	$C_{18:1\omega9c;}$10-Me $C_{18:0}$	ai-$C_{15:0;}$i-$C_{16:0;}$	i-$C_{16:0}$; ai-$C_{17:0}$; ai-$C_{15:0}$	i-$C_{15:0}$; ai-$C_{15:0}$; i-$C_{17:0}$; ai-$C_{17:0}$
Major polar lipids[b]	Different combination of DPG, PG, PI, rarely PC, MePI or PE	PI,PG,DPG	PIM, PI, DPG,PG	DPG, PG	DPG, PC, PG, PI	DPG, PG, PI	PG, DPG
Major menaquinone	MK-8(H_4)	MK-8(H_4)	MK9(H_6), MK9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-10(H_6)	MK-9(H_4)
DNA G+C content (mol%)	66.6–74.8	71–72.9	66.6–69.6	65.5–75.9	66.3–71.3	68.8	69.2

v variable among species
[a]At not all species determined
[b]Present in most species was determined

Table 33.2

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

Nocardioides	*N. simplex*[a,b,c,d]	*N. albus*[a,d]	*N. luteus*[a,d,e]	*N. jensenii*[e,f,g]	*N. plantarum*[h]
Morphology	Irregular rods, cocci	Rods, cocci, hyphae	Rods, cocci, hyphae	Rod-coccus cycle	Irregular short rods, cocci
Aerial mycelium	–	+	+	–	–
Gram staining	+	+	+	+	+
Isolation/habitat	Soil	Soil	Soil	Soil	Herbage
Catalase	+	+	+	+	+
Oxidase	+	–	–	–	–
Motility	+	–	–	–	–
Nitrate reduction	–	–	–	+	–
Hydrolysis of					
Aesculin	+	w	+	–	w
Urea	–	–	–	+	–
Gelatin	+	+	+	+	+
Starch	w	+	+	–	–
Casein	+	+	+	+	+
Cellulose	–	–	–	–	–
Tween 80	+	+	+	+	+
Utilization of					
D-Arabinose	–	+	+	–	–
Cellobiose	–	+	+	–	+
D-Fructose	–	+	+	–	+
D-Galactose	–	+	–	–	–
D-Glucose	+	+	+	+	+ ©
Glycerol	–	w	–	w	+
D-Lactose	–	–	–	–	–
D-Mannitol	–	+	+	–	–
D-Mannose	–	+	+	–	–
Maltose	–	+	+	+	+
D-Raffinose	–	–	–	–	–
L-Rhamnose	–	+	–	+	+
D-Ribose	–	–	–	–	–
Sucrose	+	–	–	+	+
Trehalose	+			+	+
D-Xylose	–	+	+	–	+
Enzyme reaction					
Alkaline phosphatase	+	+	v	w	–
Esterase (C4)	–	+	+	w	+
Lipase (C14)	–	–	–	–	w
Valine arylamidase	w	–	–	v	w
Cystine arylamidase	w	–	–	–	+
Trypsin	+	+	+	w	–
Acid phosphatase	w	–	–	v	w
Naphthol-AS-BI-phophohydrolase	–	w	v	v	+
α-Galactosidase	–	–	–	–	–
β-Galactosidase	–	v	v	–	–

Table 33.2 (continued)

Nocardioides	*N. simplex*[a,b,c,d]	*N. albus*[a,d]	*N. luteus*[a,d,e]	*N. jensenii*[e,f,g]	*N. plantarum*[h]
α-Glucosidase	+	+	v	v	+
β-Glucosidase	w	w	–	–	+
α-Mannosidase	–	–	+	–	–
Growth temperature	10–37 °C	18–37 °C	15–37 °C	20–37 °C	5–30 °C
pH range	ND	ND	ND	6–8.5	ND
NaCl tolerance	ND	ND	ND	0–7 %	0–4 %
Predominant cellular fatty acid(s)	$iC_{16:0}$ $C_{17:1\omega6c}$	$iC_{16:0}$ $C_{17:1\omega6c}$ $C_{17:0}$	$iC_{16:0}$ $C_{17:1\omega6c}$	$iC_{16:0}$ $iC_{16:1}H$	$C_{18:1}$, $aC_{17:0}$, $iC_{16:0}$
Major polar lipids	ND	ND	ND	DPG, PG, PI, OHPG	ND
DNA G+C content	72–74 %	67 %	74.6–74.8 %	68.8 % ©	69 %

Nocardioides	*N. pyridinolyticus*[i]	*N. nitrophenolicus*[j]	*N. aquiterrae*[k]	*N. aquaticus*[l]
Morphology	Rod-coccus cycle	Rod-coccus cycle	Rod	Cocci, short rod
Aerial mycelium	–	–	–	–
Gram staining	+/variable	+/variable	+/variable	+
Isolation/habitat	Oxic zone of an oil shale column	Industrial waste water	Groundwater	Lake water
Catalase	+	+	+	+
Oxidase	–	+	+	–
Motility	+	+	+	–
Nitrate reduction	+	–	+	+
Hydrolysis of				
Aesculin	+	w	+	+
Urea	–	+	–	–
Gelatin	+	+	+	+
Starch	+	+	–	w
Casein	+	+	+	+
Cellulose	–	–	–	–
Tween 80	–	+	+	+
Utilization of				
D-Arabinose	–	–	–	–
Cellobiose	+	–	+	–
D-Fructose	+	+	+	+
D-Galactose	+	–	+	–
D-Glucose	+	+	+	+
Glycerol	–	–	–	w
D-Lactose	–	–	–	–
D-Mannitol	–	–	+	+
D-Mannose	+	– (w)	–	–
Maltose	+	–	+	w
D-Raffinose	–	–	w	–
L-Rhamnose	+	+	w	+
D-Ribose	+	+	–	–
Sucrose	+	+	+	+
Trehalose	+	+	+	w
D-Xylose	+	+	+	w
Enzyme reaction				
Alkaline phosphatase	+	+	–	v
Esterase (C4)	–	–	–	w

Table 33.2 (continued)

Nocardioides	*N. pyridinolyticus*[i]	*N. nitrophenolicus*[j]	*N. aquiterrae*[k]	*N. aquaticus*[l]
Lipase (C14)	–	–	–	–
Valine arylamidase	w	+	–	w
Cystine arylamidase	–	w	w	v
Trypsin	+	+	+	w
Acid phosphatase	+	+	+	+
Naphthol-AS-BI-phophohydrolase	+	+	+	–
α-Galactosidase	–	–	–	–
β-Galactosidase	–	–	–	–
α-Glucosidase	+	+	+	+
β-Glucosidase	–	w	w	–
α-Mannosidase	–	–	–	–
Growth temperature	20–40 °C	15–40 °C	15–42 °C	3–43.5 °C
pH range	5–9	6–10	6–7	5.5–9.5
NaCl tolerance				0–15 %
Predominant cellular fatty acid(s)	$iC_{16:0}$; $aC_{17:0}$	$iC_{16:0}$ $C_{18:0}$ $C_{17:1\omega6c}$	$iC_{16:0}$	$C_{18:1}$, $aC_{17:0}$
Major polar lipids	ND	ND	ND	PI, PG, DPG
DNA G+C content	72.5 %	71.4 %	73 %	69 %
Nocardioides	*N. ganghwensis*[m]	*N. aestuari*[n]	*N. oleivorans*[o]	*N. aromaticivorans*[p]
Morphology	Rods	Rods	Irregular rods	Rods
Aerial mycelium	–	–	–	–
Gram staining	+	+	+	+
Isolation/habitat	Sediment of getbol	Sediment of getbol	Crude oil	River water, sediment, soil
Catalase	+	+	+	+
Oxidase	–	–	ND	–
Motility	–	–	–	–
Nitrate reduction	+	–	–	–
Hydrolysis of				
Aesculin	w	w	+	+
Urea	–	ND	ND	+
Gelatin	+	+	–	ND
Starch	+	–	–	–
Casein	+	+	+	+
Cellulose	–	–	–	ND
Tween 80	+	+	–	–
Utilization of				
D-Arabinose	+	–	–	+ or w
Cellobiose	+	+	+	+
D-Fructose	+	+	+	+
D-Galactose	+	+	+	–
D-Glucose	+	+	+	+
Glycerol	+	w	–	w
D-Lactose	+	w	–	–
D-Mannitol	+	+	–	+ or w
D-Mannose	+	–	+	w
Maltose	ND	ND	+	+ or w

Table 33.2 (continued)

Nocardioides	*N. ganghwensis*[m]	*N. aestuari*[n]	*N. oleivorans*[o]	*N. aromaticivorans*[p]
D-Raffinose	+	w	–	–
L-Rhamnose	–	–	+	w
D-Ribose	–	–	–	+
Sucrose	+	+	+	+
Trehalose	+	ND	+	+ or w
D-Xylose	+	+	–	+
Enzyme reaction				
Alkaline phosphatase	+	w	+	+
Esterase (C4)	v	+	w	–
Lipase (C14)	–	–	–	–
Valine arylamidase	+	+	+	–
Cystine arylamidase	w	–	w	–
Trypsin	–	+	–	–
Acid phosphatase	w	w	–	–
Naphthol-AS-BI-phophohydrolase	–	w	–	–
α-Galactosidase	+	–	–	–
β-Galactosidase	+	+	+	–
α-Glucosidase	+	+	+	+
β-Glucosidase	–	–	–	–
α-Mannosidase	–	–	–	ND
Growth temperature	10–40 °C	20–35 °C	30 °C	22–40 °C
pH range	6–10	6–10	ND	5–8
NaCl tolerance	0–8 %	0–8 %	0–2 %	2 %
Predominant cellular fatty acid(s)	$iC_{16:0}$; $C_{17:1\omega8c}$	$iC_{16:0}$; $iC_{16:1}$ H	$C_{18:1\omega9c}$; $iC_{16:0}$; $C_{18:0}$	$iC_{16:0}$; $iC_{17:0}$; $C_{18:1}$
Major polar lipids	ND	ND	ND	ND
DNA G+C content	72 %	70 %	ND	72–72.4 %

Nocardioides	*N. kribbensis*[q]	*N. dubius*[r]	*N. alkalitolerans*[s]	*N. lentus*[t]	*N. kongjuensis*[u]
Morphology	Short rods, cocci	Rod-coccus cycle	Rod-coccus cycle	Rod-coccus cycle	Rod-coccus cycle
Aerial mycelium	–	–	–	–	–
Gram staining	+/v	+	+/variable	+/variable	+
Isolation/habitat	Alkaline soil	Alkaline soil	Alkaline soil	Alkaline soil	Soil
Catalase	+	+	+	+	+
Oxidase	+	+	+	ND	+
Motility	–	+	–	–	–
Nitrate reduction	+	–	+	+	–
Hydrolysis of					
Aesculin	+	+	–	–	–
Urea	–	–	–	–	–
Gelatin	+	+	v(+)	+	+
Starch	–	–	–	–	–
Casein	+	+	+	+	+
Cellulose	ND	ND	ND	ND	ND
Tween 80	+	–	+	+	+
Utilization of					
D-Arabinose	– ©	–	+	w	–

Table 33.2 (continued)

Nocardioides	*N. kribbensis*[q]	*N. dubius*[r]	*N. alkalitolerans*[s]	*N. lentus*[t]	*N. kongjuensis*[u]
Cellobiose	+	–	+	+	–
D-Fructose	–	–	–	–	–
D-Galactose	+	–	–	+	–
D-Glucose	+	+	–	+	+
Glycerol	ND	ND	ND	ND	ND
D-Lactose	–	–	–	v(+)	ND
D-Mannitol	+	–	–	+	ND
D-Mannose	–	– ©	–	–	w
Maltose	+	–	v (–)	+	–
D-Raffinose	+	ND	+	+	ND
L-Rhamnose	+	–	–	+	ND
D-Ribose	+	–	–	–	–
Sucrose	+	+ ©	–	+	+
Trehalose	+	–	+	+	+
D-Xylose	–	–	–	v(+)	–
Enzyme reaction					
Alkaline phosphatase	+	+	+	+	+
Esterase (C4)	+	+	+	+	–
Lipase (C14)	–	–	+	–	–
Valine arylamidase	–	+ ©	–	–	–
Cystine arylamidase	–	–	–	–	–
Trypsin	–	+ ©	–	–	–
Acid phosphatase	+	+	+	+	+
Naphthol-AS-BI-phophohydrolase	+ ©	+	w	+	w
α-Galactosidase	–	–	–	–	–
β-Galactosidase	w	–	–	–	–
α-Glucosidase	+	+	+	–	+
β-Glucosidase	–	+	–	+	–
α-Mannosidase	–	–	–	–	–
Growth temperature	4–35 °C	10–37 °C	4–40 °C	4–34 °C	10–40 °C
pH range	6–11	6–10.5	5.5–12	6.5–9.5	5.5–8
NaCl tolerance	0–3 %	0–5 %	0–5 %	0–5 %	0–5 %
Predominant cellular fatty acid(s)	$iC_{16:0}$	$iC_{16:0}$	$iC_{16:0}$; $10MeC_{18:0}$; $C_{18:1\omega9C}$	$iC_{16:0}$	$iC_{16:0}$; $10MeC_{18:0}$; $C_{17:1\omega6c}$
Major polar lipids	ND	DPG; PG; PE; PI	ND	ND	ND
DNA G+C content	73–74 %	70.6 %	72.4–73.6 %	74.6–74.8 %	72.1 %
Nocardioides	*N. insulae*[v]	*N. furvisabuli*[w]	*N. marinus*[x]	*N. panacihumi*[y]	*N. terrigena*[z]
Morphology	Rods, cocci	Rods	Rods, cocci	Rod-coccus cycle	Rod-coccus cycle
Aerial mycelium	–	–	–	–	–
Gram staining	+	+	+	+	+/variable
Isolation/habitat	Soil	Black sand	Seawater	Ginseng field	Soil
Catalase	+	+	w	ND	+
Oxidase	ND	ND	–	ND	ND
Motility	–	+	–	–	–
Nitrate reduction	+	+	–	w	+

Table 33.2 (continued)

Nocardioides	*N. insulae*[v]	*N. furvisabuli*[w]	*N. marinus*[x]	*N. panacihumi*[y]	*N. terrigena*[z]
Hydrolysis of					
Aesculin	–	–	+	+	–
Urea			–	–	–
Gelatin	+	–	–	+	+
Starch	–	+	–	–	w
Casein	+	+	+	+	+
Cellulose		–		–	
Tween 80	+	–	+	ND	w
Utilization of					
D-Arabinose	–	+	–	–	–
Cellobiose	+	+	ND	–	+
D-Fructose	–	+	+	+	+
D-Galactose	+	+	+	–	+
D-Glucose	+	+	+	+	+
Glycerol	ND	+	–	–	–
D-Lactose	ND	+	ND	–	+
D-Mannitol	ND	+	+	–	+
D-Mannose	w	+	–	–	–
Maltose	w	+		+	+
D-Raffinose	ND	+	–	+	
L-Rhamnose	ND	–	+	+	+
D-Ribose	ND	ND	–	–	–
Sucrose	–	–	+	+	+
Trehalose	+	+	+	–	+
D-Xylose	w	+	+	+	+
Enzyme reaction					
Alkaline phosphatase	+	+	+	+	+
Esterase (C4)	+	–	w	+	+
Lipase (C14)	–	–	–	–	–
Valine arylamidase	–	w	w	w	–
Cystine arylamidase	–	–	–	w	–
Trypsin	–	–	+	+	–
Acid phosphatase	+	–	w	+	–
Naphthol-AS-BI-phophohydrolase	–	–	–	–	+
α-Galactosidase	–	w	–	–	–
β-Galactosidase	–	+	–	–	+
α-Glucosidase	–	–	+	+	+
β-Glucosidase	–	–	–	+	–
α-Mannosidase		–	–	+	–
Growth temperature	10–34 °C	4–37 °C	10–40 °C	15–30 °C	4–35 °C
pH range	6.5–8.5	5.1–10.1	6–9	5–8	ND
NaCl tolerance	0–3 %	0–6 %	0.5–8 %	0–1 %	0–3 %
Predominant cellular fatty acid(s)	$iC_{16:0}$; $aC_{17:0}$	$iC_{16:0}$; $C_{18:1\omega9C}$	$iC_{16:0}$	$iC_{16:0}$	$iC_{16:0}$; $C_{17:1\omega8c}$; $C_{17:0}$
Major polar lipids	ND	PC, PG, PI	ND	ND	ND
DNA G+C content	71.1 %	69.1 %	72.9 %	73 %	71.5 %

Table 33.2 (continued)

Nocardioides	*N. exalbidus*[aa]	*N. marinisabuli*[ab]	*N. dokdonensis*[ac]	*N. daphniae*[ad]
Morphology	Irregular rods	Rods	Rods	Rods
Aerial mycelium	–	–	–	–
Gram staining	+	+	+	+
Isolation/habitat	Lichen	Beach sand	Sand sediment	Water flea
Catalase	+	+	+	+
Oxidase	–	–	–	–
Motility	–	–	–	–
Nitrate reduction	– ©	–	+	+
Hydrolysis of				
Aesculin	–	+	–	+
Urea	–	–	+	–
Gelatin	+	–	–	+
Starch	–	w	–	
Casein	ND	+	–	w
Cellulose	ND	ND	ND	ND
Tween 80	ND	–	+	–
Utilization of				
D-Arabinose	–	–	–	ND
Cellobiose	–	–	–	–
D-Fructose	+	+	+	+
D-Galactose	–	+	–	–
D-Glucose	+	–	–	+
Glycerol	–	–	+	–
D-Lactose	–	–	–	–
D-Mannitol	+	– ©	+	–
D-Mannose	–	–	+	+
Maltose	–	– ©	–	–
D-Raffinose	–	–	–	–
L-Rhamnose	+	–	–	–
D-Ribose	–	–	+	+
Sucrose	+	–	–	–
Trehalose	–	+	+	+
D-Xylose	–	+	w	–
Enzyme reaction				
Alkaline phosphatase	+	+	+	w
Esterase (C4)	w	–	+	ND
Lipase (C14)	–	–	–	w
Valine arylamidase	+	–	w	w
Cystine arylamidase	w	–	–	–
Trypsin	–	–	+	–
Acid phosphatase	w	–	+	w
Naphthol-AS-BI-phophohydrolase	+	–	+	w
α-Galactosidase	+	–	–	ND
β-Galactosidase	+	–	–	–
α-Glucosidase	+	+	+	–

Table 33.2 (continued)

Nocardioides	*N. exalbidus*[aa]	*N. marinisabuli*[ab]	*N. dokdonensis*[ac]	*N. daphniae*[ad]
β-Glucosidase	w	–	–	–
α-Mannosidase	–	–	–	ND
Growth temperature	15–35 °C	4–40 °C	4–30 °C	4–38 °C
pH range	6–9	5.1–12.1	5–10	5.5–10.5
NaCl tolerance	ND	0–8 %	0–7 %	0–5 %
Predominant cellular fatty acid(s)	$iC_{16:0}$; $C_{18:0}$	$iC_{16:0}$	$iC_{16:0}$; $C_{18:1\omega9C}$; $C_{18:0}$	$iC_{16:0}$; $C_{18:1\omega9C}$;
Major polar lipids	DPG, PI	DPG, PI	ND	PG, DPG
DNA G+C content	74 %	73.1	69.1 %	69.9 %
Nocardioides	*N. hwasunensis*[ae]	*N. islandiensis*[af]	*N. dilutes*[ag]	*N. tritolerans*[ah]
Morphology	Rods	Rods	Cocci, rods	Rods
Aerial mycelium	–	–	–	–
Gram staining	+	+	+	+
Isolation/habitat	Water	Farming soil	Soil	Soil
Catalase	+	–	ND	ND
Oxidase	–	–	ND	ND
Motility	–	–	+ ©	+
Nitrate reduction	–	–	+	+
Hydrolysis of				
Aesculin	–	–	–	–
Urea	–	–	–	–
Gelatin	v	–	–	–
Starch	+	+	–	+
Casein	–	+ ©	+	+
Cellulose	ND	ND	–	–
Tween 80	ND	+	+	+
Utilization of				
D-Arabinose	v(–)	–	ND	–
Cellobiose	+	+	+	+
D-Fructose	+	–	–	–
D-Galactose	+	–	+	+
D-Glucose	+	+	+	+
Glycerol	–	ND	ND	ND
D-Lactose	ND	+	+	+
D-Mannitol	+	–	ND	+
D-Mannose	+	– ©	+	+
Maltose	+	+	–	–
D-Raffinose	–	–	+	+
L-Rhamnose	+	–	+	+
D-Ribose	–	–	–	–
Sucrose	+	+	+	ND
Trehalose	+	+	ND	ND
D-Xylose	+	+	+	+
Enzyme reaction				
Alkaline phosphatase	+	+	–	+
Esterase (C4)	w	–	+	+
Lipase (C14)	–	–	+	–

Table 33.2 (continued)

Nocardioides	*N. hwasunensis*[ae]	*N. islandiensis*[af]	*N. dilutes*[ag]	*N. tritolerans*[ah]
Valine arylamidase	+	–	+	+
Cystine arylamidase	–	–		+
Trypsin	–	–	–	–
Acid phosphatase	ND	+	+	+
Naphthol-AS-BI-phophohydrolase	–	+	+	+
α-Galactosidase	–	–	–	–
β-Galactosidase	–	–	+	–
α-Glucosidase	w	– ©	+	+
β-Glucosidase	–	–	+	–
α-Mannosidase	–	–	–	–
Growth temperature	4–37 °C	opt. 28 °C	opt. 26–28 °C	20–40 °C
pH range	5.1–9.1	5–12	opt 7–8	6–11
NaCl tolerance	0–4 %	0–7 %		0–7 %
Predominant cellular fatty acid(s)	$iC_{16:0}$	$C_{18:1\omega7C}$;$C_{16:0}$	$iC_{16:0}$; $C_{18:1\omega9C}$;	$iC_{16:0}$; $C_{17:1\omega8c}$;
Major polar lipids	PG, DPG, PI	ND	PG, DPG	PG, DPG
DNA G+C content	71.1–72.2 %	71.4	71.8 %	67.6 %
Nocardioides	*N. halotolerans*[ai]	*N. koreensis*[aj]	*N. bigeumensis*[ak]	*N. agariphilus*[al]
Morphology	Cocci, rods	Irregular rods	Cocci to short rods	Cocci to short rods
Aerial mycelium	–	–	–	–
Gram staining	+	+	+	+
Isolation/habitat	Agricultural soil	Soil	Soil	Soil
Catalase	–	–	–	–
Oxidase	–	–	–	–
Motility	– ©	+	+	+
Nitrate reduction	+	–	–	+
Hydrolysis of				
Aesculin	–	–	–	–
Urea	+	ND	–	ND
Gelatin	–	–	–	–
Starch	– ©	+	–	–
Casein	–	ND	–	ND
Cellulose	–	–	–	–
Tween 80	– ©	+	+	+
Utilization of				
D-Arabinose	ND	ND	ND	ND
Cellobiose	ND	–	ND	ND
D-Fructose	–	–	–	–
D-Galactose	+	–	–	–
D-Glucose	+	+	ND	ND
Glycerol	ND	ND	ND	ND
D-Lactose	+	+	+	–
D-Mannitol	+	–	–	–
D-Mannose	+	+	+	–
Maltose	–	–	–	–

Table 33.2 (continued)

Nocardioides	*N. halotolerans*[ai]	*N. koreensis*[aj]	*N. bigeumensis*[ak]	*N. agariphilus*[al]
D-Raffinose	+	–	–	+
L-Rhamnose	+	–	–	–
D-Ribose	–	–	–	–
Sucrose	ND	+	ND	ND
Trehalose	+	–	ND	ND
D-Xylose	+	ND	ND	ND
Enzyme reaction				
Alkaline phosphatase	+	+	+	+
Esterase (C4)	+	+	+	+
Lipase (C14)	–	ND	ND	ND
Valine arylamidase	–	–	–	–
Cystine arylamidase	+	–	–	+
Trypsin	–	–	–	–
Acid phosphatase	–	ND	ND	ND
Naphthol-AS-BI-phophohydrolase	+	ND	ND	ND
α-Galactosidase	–	ND	ND	ND
β-Galactosidase	+	–	–	–
α-Glucosidase	+	ND	ND	ND
β-Glucosidase	+	+	+	+
α-Mannosidase	–	ND	ND	ND
Growth temperature	opt. 28–30 °C	27–37 °C	20–35 °C	25–37 °C
pH range	opt. 7–8	7–8	ND	ND
NaCl tolerance	0–10 %	0–5 %	0–1 %	0–1 %
Predominant cellular fatty acid(s)	$iC_{16:0}$	$iC_{16:0}$	$iC_{16:0;}$ $iC_{15:0;}$	$iC_{16:0;}$ $C_{17:1\omega8c;}$
Major polar lipids	PG, DPG	PG, DPG	PG, DPG	PG, DPG
DNA G+C content	69.7 %	69.9 %	69.3 %	69.4 %

Nocardioides	*N. salarius*[am]	*N. fonticola*[an]	*N. hankookensis*[ao]	*N. basaltis*[ap]	*N. sediminis*[aq]
Morphology	Rods	Rods	Rods	Rods	Short rods
Aerial mycelium	–	–	–	–	–
Gram staining	+	+	+/variable	+	+
Isolation/habitat	Zooplankton enriched seawater	Freshwater spring	Soil	Black beach	Sediment
Catalase	+	+	+	+	+
Oxidase	–	–	+	–	–
Motility	–	–	–	–	+
Nitrate reduction	–	–	–	–	+
Hydrolysis of					
Aesculin	+	+	+	–	–
Urea	–	–	–	–	–
Gelatin	+	+	+	+	+
Starch	w	+	+	–	+
Casein	+	+	+	–	+
Cellulose	ND	ND	ND	ND	ND
Tween 80	+	+	w	–	+

Table 33.2 (continued)

Nocardioides	*N. salarius*[am]	*N. fonticola*[an]	*N. hankookensis*[ao]	*N. basaltis*[ap]	*N. sediminis*[aq]
Utilization of					
D-Arabinose	–	+	–	–	–
Cellobiose	+	+	+	+	+
D-Fructose	–	+	+	–	+
D-Galactose	–	+	+	–	+
D-Glucose	+	+	+	+	+
Glycerol	–	ND	–	–	–
D-Lactose	–	ND	–	–	+
D-Mannitol	+	+	+	+	+
D-Mannose	–	+	ND	–	–
Maltose	ND	–	+	–	+
D-Raffinose	–	ND	–	–	–
L-Rhamnose	–	ND	+	–	+
D-Ribose	+	ND	–	–	–
Sucrose	+	–	+	+	+
Trehalose	+	–	+	+	+
D-Xylose	+	+	+	–	–
Enzyme reaction					
Alkaline phosphatase	+	+	+	+	+
Esterase (C4)	+	+	+	+	+
Lipase (C14)	–	–	–	–	–
Valine arylamidase	+	–	–	–	–
Cystine arylamidase	+	–	–	w	–
Trypsin	+	–	–	w	–
Acid phosphatase	+	+	+	–	–
Naphthol-AS-BI-phophohydrolase	+	+	+	+	–
α-Galactosidase	–	–	–	–	–
β-Galactosidase	–	+	–	–	–
α-Glucosidase	+	+	–	+	–
β-Glucosidase	–	+	–	–	–
α-Mannosidase	–	–	–	–	–
Growth temperature	10–35 °C	25–37 °C	10–34 °C	10–37 °C	ND
pH range	6–10	5–9	5.5–8	5.5–8	ND
NaCl tolerance	1–10	0.5–1.0 %	0–2 %	1–10 %	ND
Predominant cellular fatty acid(s)	$iC_{16:0}$	$iC_{16:0}$; $C_{17:0}$; $C_{17:1\omega8c}$; $C_{18:1\omega9c}$	$iC_{16:0}$; $C_{17:1\omega8c}$; $C_{18:1\omega9c}$	$iC_{16:0}$; $C_{17:1\omega8c}$	$iC_{16:0}$; $C_{17:1\omega8c}$
Major polar lipids	ND	ND	ND	ND	ND
DNA G+C content	73.3 %	71.8 %	71.3 %	68 %	71.5 %
Nocardioides	*N. terrae*[ar]	*N. humi*[as]	*N. ginsengisoli*[at]	*N. caeni*[au]	*N. ginsengisegetis*[av]
Morphology	Cocci to short rods	Rods	Short rods	Cocci, rods	Short rods
Aerial mycelium	–	–	–	–	–
Gram staining	+	+	+	+	+
Isolation/habitat	Forest soil	Soil	Soil	Domestic wastewater	Soil
Catalase	+	– ©	+	+	+

Table 33.2 (continued)

Nocardioides	*N. terrae*[ar]	*N. humi*[as]	*N. ginsengisoli*[at]	*N. caeni*[au]	*N. ginsengisegetis*[av]
Oxidase	–	+	–	+	+
Motility	–	–	–	–	–
Nitrate reduction	+	–	–	–	+
Hydrolysis of					
Aesculin	+	+	ND	–	+
Urea	–	–	+	–	–
Gelatin	–	–	+	+	+
Starch	–	+ ©	ND	+	–
Casein	+	+	+	+	–
Cellulose	ND	ND	–	ND	–
Tween 80	–	ND	ND	+	–
Utilization of					
D-Arabinose	ND	–	+	–	+
Cellobiose	+	+	ND	+	+
D-Fructose	ND	+	+	–	–
D-Galactose	+	ND	ND	–	–
D-Glucose	+	+	+	–	+
Glycerol	ND	–	ND	–	
D-Lactose	ND	–	ND	–	+
D-Mannitol	+	+	+	–	+
D-Mannose	–	–	–	–	–
Maltose	+	–	+	ND	–
D-Raffinose	ND	–	ND	–	–
L-Rhamnose	+	+	–	+	–
D-Ribose	ND	+ ©	–	–	+
Sucrose	ND	+	+	+	–
Trehalose	ND	+	ND	+	+
D-Xylose	ND	–	+	–	–
Enzyme reaction					
Alkaline phosphatase	+	+	ND	+	+
Esterase (C4)	–	+	ND	–	–
Lipase (C14)	–	–	ND	–	–
Valine arylamidase	–	+	ND	w	–
Cystine arylamidase	–	+	ND	w	–
Trypsin	+	+	ND	–	–
Acid phosphatase	+	+	ND	+	+
Naphthol-AS-BI-phophohydrolase	+	+	ND	w	+
α-Galactosidase	–	–	ND	–	–
β-Galactosidase	+	+	ND	–	+
α-Glucosidase	+	+	ND	+	+
β-Glucosidase	–	+	ND	–	+
α-Mannosidase		+ ©	ND	–	–
Growth temperature	16–24 °C	25–42 °C	15–37 °C	10–35 °C	4–37 °C
pH range	5.5–8.5	5–11	5–8.5	6–9.5	5.5–9.5
NaCl tolerance	0–1 %	ND	0–5 %	0–1 %	0–3 %

Table 33.2 (continued)

Nocardioides	*N. terrae*[ar]	*N. humi*[as]	*N. ginsengisoli*[at]	*N. caeni*[au]	*N. ginsengisegetis*[av]
Predominant cellular fatty acid(s)	$iC_{16:0}$; $10MeC_{17:0}$; $10MeC_{18:0}$	$iC_{16:0}$; $iC_{17:0}$; $C_{18:1\omega9c}$	$iC_{16:0}$	$iC_{16:0}$; $C_{18:1\omega9c}$	$iC_{16:0}$; $C_{18:1\omega9c}$
Major polar lipids	ND	ND	ND	ND	ND
DNA G+C content	71.6 %	71 %	70.2 %	71.5 %	71.6 %

Nocardioides	*N. mesophilus*[aw]	*N. daedukensis*[ax]	*N. panacisoli*[ay]	*N. hungaricus*[az]
Morphology	Short irregular rods from mycelia	Rod-coccus cycle	Rods	Rods
Aerial mycelium	–	–	–	–
Gram staining	+	+/variable	+	+/variable
Isolation/habitat	Soil	Soil	Ginseng field	Drinking water
Catalase	+	+	+	+
Oxidase	–	+	+	–
Motility	+ (Without flagella)	–	–	–
Nitrate reduction	–	ND	+	–
Hydrolysis of				
Aesculin	–	+	ND	+
Urea	–	–	–	–
Gelatin	–	+	+	v
Starch	+	–	–	–
Casein	+	+	–	–
Cellulose	ND	ND	–	
Tween 80	–	+	ND	+
Utilization of				
D-Arabinose	ND	ND	ND	–
Cellobiose	+	–	–	–
D-Fructose	–	–	+	–
D-Galactose	–	–	ND	–
D-Glucose	+	–	+	–
Glycerol	ND	ND	–	–
D-Lactose	–	ND	ND	–
D-Mannitol	+	ND	–	–
D-Mannose	–	–	–	–
Maltose	–	–	+	–
D-Raffinose	+	ND		–
L-Rhamnose	–	ND	–	–
D-Ribose	–	ND	–	–
Sucrose	+	–	+	ND
Trehalose	ND	+	ND	–
D-Xylose	+	–	–	–
Enzyme reaction				
Alkaline phosphatase	+	–	+	+
Esterase (C4)	+	–	–	+
Lipase (C14)	–	–	–	w
Valine arylamidase	–	–	–	v
Cystine arylamidase	–	–	–	–
Trypsin	–	–	+	–

Table 33.2 (continued)

Nocardioides	*N. mesophilus*[aw]	*N. daedukensis*[ax]	*N. panacisoli*[ay]	*N. hungaricus*[az]
Acid phosphatase	+	–	+	+
Naphthol-AS-BI-phophohydrolase	+	w	+	+
α-Galactosidase	–	–	–	–
β-Galactosidase	–	–	–	+
α-Glucosidase	–	–	+	+
β-Glucosidase	–	–	–	+
α-Mannosidase	–	–	–	–
Growth temperature	ND	4–37 °C	10–42 °C	20–37 °C
pH range	ND	6–10	5.5–8.5	5–9
NaCl tolerance	ND	0–9 %	0–2 %	0–2.5 %
Predominant cellular fatty acid(s)	$iC_{16:0}$; $C_{16:1}$	$iC_{16:0}$; $C_{17:1}$; $C_{17:0}$	$iC_{16:0}$; $C_{18:1\omega9c}$	$iC_{16:0}$; $aC_{17:0}$
Major polar lipids	ND	ND	PI, PG	ND
DNA G+C content	68.7 %	68.7 %	73 %	71.4 %
Nocardioides	*N. caricicola*[ba]	*N. iriomotensis*[bb]	*N. ultimimeridianus*[bc]	*N. maradonensis*[bd]
Morphology	Cocci, rods	Cocci, irregular rods	Rods	Rods
Aerial mycelium	–	–	–	–
Gram staining	+	+	+	+
Isolation/habitat	Halophytic plant	Forest soil	Rhizosphere soil	Rhizosphere soil
Catalase	+	ND	+	+
Oxidase	–	ND	–	–
Motility	–	–	–	–
Nitrate reduction	+	+	–	–
Hydrolysis of				
Aesculin	+	+	–	–
Urea	–	ND	–	–
Gelatin	–	+	–	–
Starch	–	+	–	–
Casein	+	+	+	–
Cellulose		ND	–	–
Tween 80	+	w	ND	ND
Utilization of				
D-Arabinose	–	ND	–	–
Cellobiose	ND	ND	+	+
D-Fructose	ND	ND	+	+
D-Galactose	ND	ND	ND	+
D-Glucose	–	ND	+	+
Glycerol	ND	ND	–	–
D-Lactose	ND	ND	–	–
D-Mannitol	ND	ND	–	–
D-Mannose	–	ND	–	–
Maltose	–	ND	–	–
D-Raffinose	ND	ND	+	+
L-Rhamnose	ND	ND	–	–
D-Ribose	ND	ND	–	–

Table 33.2 (continued)

Nocardioides	*N. caricicola*[ba]	*N. iriomotensis*[bb]	*N. ultimimeridianus*[bc]	*N. maradonensis*[bd]
Sucrose	ND	ND	+	+
Trehalose	ND	ND	–	–
D-Xylose	ND	ND	–	–
Enzyme reaction				
Alkaline phosphatase	+	+	–	–
Esterase (C4)	+	+	–	+
Lipase (C14)	–	–	–	–
Valine arylamidase	–	–	+	+
Cystine arylamidase	–	–	+	+
Trypsin	+	–	w	–
Acid phosphatase	+	+	+	+
Naphthol-AS-BI-phophohydrolase	–	+	–	–
α-Galactosidase	–	–	–	–
β-Galactosidase	+	+	–	–
α-Glucosidase	+	+	+	+
β-Glucosidase	+	+	–	–
α-Mannosidase	–	–	–	–
Growth temperature	10–45 °C	12–37 °C	10–42 °C	20–37 °C
pH range	7–9	6–8	5–10.1	5–10
NaCl tolerance	0–0.5 %	0–7	0–1 %	0–1 %
Predominant cellular fatty acid(s)	$iC_{16:0}$; $C_{18:2\omega6,9c}$ $aC_{18:0}$	$iC_{16:0}$; $C_{17:1}$	$C_{16:0}$; $C_{18:1\omega9c}$; $C_{16:0}$2-OH	$C_{16:0}$; $C_{18:1\omega9c}$; $C_{16:0}$2-OH
Major polar lipids	ND	DPG, PG, PI	PG, PI	PG, PI
DNA G+C content	71.7 %	73.7 %	73 %	71.7 %
Nocardioides	*N. daejeonensis*[bd]	*N. ginsengagri*[be]	*N. alpinus*[bf]	*N. perillae*[bg]
Morphology	Rod-coccus cycle	Rods		Rods
Aerial mycelium	–	–	–	–
Gram staining	+	+	+	+
Isolation/habitat	Sewage sludge	Ginseng field soil	Glacier cryoconite	Root
Catalase	+	+	+	–
Oxidase	–	+	–	+
Motility	–	–	–	+
Nitrate reduction	+	–	+	–
Hydrolysis of				
Aesculin	+	ND	–	ND
Urea	–	–	–	–
Gelatin	–	–	–	–
Starch	–	–	–	+
Casein	ND	+	+	–
Cellulose	ND	ND	ND	–
Tween 80	ND	–	+	+
Utilization of				
D-Arabinose	ND	ND	ND	+
Cellobiose	ND	ND	ND	+
D-Fructose	ND	ND	ND	+

Table 33.2 (continued)

Nocardioides	*N. daejeonensis*[bd]	*N. ginsengagri*[be]	*N. alpinus*[bf]	*N. perillae*[bg]
D-Galactose	ND	ND	ND	+
D-Glucose	–	–	+	–
Glycerol	ND	ND	ND	ND
D-Lactose	+	ND	ND	–
D-Mannitol	–	ND	+	–
D-Mannose	–	–	w	+
Maltose	+	–	+	+
D-Raffinose	ND	ND	ND	+
L-Rhamnose	+	ND	ND	–
D-Ribose	–	ND	ND	ND
Sucrose	–	ND	ND	+
Trehalose	ND	ND	ND	+
D-Xylose	ND	ND	ND	
Enzyme reaction				
Alkaline phosphatase	+	–	+	+
Esterase (C4)	w	–	w	+
Lipase (C14)	–	–	+	+
Valine arylamidase	+	+	+	+
Cystine arylamidase	w	–	+	+
Trypsin	w	–	ND	–
Acid phosphatase	+	+	+	+
Naphthol-AS-BI-phophohydrolase	+	–	w	+
α-Galactosidase	–	–	–	–
β-Galactosidase	–	–	–	–
α-Glucosidase	–	+	+	ND
β-Glucosidase	+	–	–	–
α-Mannosidase	–	–	–	ND
Growth temperature	15–37 °C	10–37 °C	1–25 °C	20–37 °C
pH range	5.5–9	5.5–9.5	7–8	6–11
NaCl tolerance	0–10 %	0–1 %	0–3 %	0–3 %
Predominant cellular fatty acid(s)	$iC_{16:0}$; $iC_{17:0}$; $C_{18:1\omega9c}$	$C_{18:1\omega9c}$; $C_{16:0}$; $C_{17:1\omega8c}$	$C_{17:1\omega8c}$; $iC_{16:0}$;	$C_{17:1\omega9c}$; $C_{16:0}$; $C_{18:1\omega9c}$; $C_{17:0}$
Major polar lipids	DPG, PE, PG	PG	DPG, PG, PC, PI	DPG, PG, PI
DNA G+C content	71.2 %	70.3 %	71.9 %	70.4 %

Nocardioides	*N. albertani*[bh]	*N. szechwanensis*[bi]	*N. psychrotolerans*[bi]	*N. lianchengensis*[bj]	*N. panzhihuaensis*[bk]
Morphology	Rods, cocci	Rods	Rods	Rods	Irregular rods
Aerial mycelium	–	–	–	–	+
Gram staining	+	+	+	+	+
Isolation/habitat	Green biofilm	Glacier	Glacier	Soil	Oil-seed plant
Catalase	+	–	+	+	
Oxidase	–	–	–	–	+
Motility	–	–	–	–	–
Nitrate reduction	–	+	+	–	+
Hydrolysis of					
Aesculin	–	–	–	+	+
Urea	–	–	–	+	–

Table 33.2 (continued)

Nocardioides	*N. albertani*[bh]	*N. szechwanensis*[bi]	*N. psychrotolerans*[bi]	*N. lianchengensis*[bj]	*N. panzhihuaensis*[bk]
Gelatin	+	+	+	+	ND
Starch	ND	–	–	w	ND
Casein	ND	–	+	+	+
Cellulose	ND	ND	ND	ND	–
Tween 80	ND	–	–	+	+
Utilization of					
D-Arabinose	+	–	–	–	+
Cellobiose	ND	+	+	–	+
D-Fructose	ND	+	+	+	–
D-Galactose	ND	–	–	+	+
D-Glucose	+	+	–	+	w
Glycerol	ND	–	+	+	ND
D-Lactose	ND	–	–	–	ND
D-Mannitol	+	+	+	+	ND
D-Mannose	+	+	–	+	+
Maltose	+	–	–	–	–
D-Raffinose	ND	–	+	–	ND
L-Rhamnose	–	+	+	+	+
D-Ribose	ND	–	–	+	+
Sucrose	ND	+	–	+	ND
Trehalose	ND	+	+	+	+
D-Xylose	ND	+	–	+	+
Enzyme reaction					
Alkaline phosphatase	+	+	+	+	+
Esterase (C4)	+	+	+	+	+
Lipase (C14)	–	–	–	–	+
Valine arylamidase	+		–	+	+
Cystine arylamidase	+	–	–	–	–
Trypsin	–	–	–	–	–
Acid phosphatase	+	ND	–	w	–
Naphthol-AS-BI-phophohydrolase	+	ND	ND	+	+
α-Galactosidase	–	–	–	–	–
β-Galactosidase	+	–	+	–	+
α-Glucosidase	+	–	–	+	+
β-Glucosidase	+	–	–	+	+
α-Mannosidase	+	–	–	–	–
Growth temperature	10–30 °C	0–35 °C	0–25 °C	10–40 °C	10–23 °C
pH range		6.5–11.0	6.5–9.0	6–9	6–11
NaCl tolerance	0–10 %	0–1 %	0–0–5 %	0–4 %	0–12 %
Predominant cellular fatty acid(s)	$iC_{16:0}$	$C_{17:1w8c}$; $iC_{16:0}$;	$C_{18:1w9c}$; $iC_{16:0}$; Sum feature3; $C_{16:0}$	$iC_{16:0}$; $aC_{17:0}$	$iC_{16:0}$; $C_{18:1w9c}$
Major polar lipids	ND	DPG, PG, PC, PI	DPG, PG, PC, PI	PG, PI, MePI, DPG	DPG, PG, PC
DNA G+C content	69.7 %	67.9 %	67.8 %	71.8 %	70.1 %

Data taken from
[a]Prauser 1976
[b]Suzuki and Komagata 1983
[c]O'Donnell et al. 1982

[d]Prauser 1986
[e]Prauser 1984
[f]Suzuki and Komagata 1983
[g]Collins et al. 1989
[h]Collins et al. 1994
[i]Yoon et al. 1997
[j]Yoon et al. 1999
[k]Yoon et al. 2004
[l]Lawson et al. 2000
[m]Yi and Chun 2004b
[n]Yi and Chun 2004a
[o]Schippers et al. 2005
[p]Kubota et al. 2005
[q]Yoon et al. 2005c
[r]Yoon et al. 2005b
[s]Yoon et al. 2005d
[t]Yoon et al 2006a
[u]Yoon et al. 2006b
[v]Yoon et al. 2007a
[w]Lee 2007b
[x]Choi et al. 2007
[y]An et al. 2007
[z]Yoon et al. 2007b
[aa]Li et al. 2007
[ab]Lee et al. 2007
[ac]Park et al. 2008
[ad]Tóth et al. 2008
[ae]Lee et al. 2008
[af]Dastager et al. 2008a
[ag]Dastager et al. 2008d
[ah]Dastager et al. 2008f
[ai]Dastager et al. 2008e
[aj]Dastager et al. 2008c
[ak]Dastager et al. 2008c
[al]Dastager et al. 2008c
[am]Kim et al. 2008a
[an]Chou et al. 2008
[ao]Yoon et al. 2008
[ap]Kim et al. 2009a
[aq]Dastager et al. 2009
[ar]Zhang et al. 2009
[as]Kim et al. 2009b
[at]Cui et al. 2009
[au]Yoon et al. 2009
[av]Im et al. 2010
[aw]Dastager et al. 2010
[ax]Yoon et al. 2010
[ay]Cho et al. 2010
[az]Tóth et al. 2011
[ba]Song et al. 2011
[bb]Yamamura et al. 2011
[bc]Lee et al. 2011a
[bd]Woo et al. 2012
[be]Lee et al. 2012
[bf]Zhang et al. 2012a
[bg]Du et al. 2012
[bh]Alias-Villegas et al. 2012
[bi]Liu et al. 2012
[bj]Zhang et al. 2012b
[ak]Qin et al. 2012

Symbols and abbreviations: + positive, − negative, *w* weakly positive, *v* variable, *ND* no data available, © conflicting data, *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PC* phosphatidylcholine, *MePI* methyl-phosphatidylinositol, *PE* phosphatidylethanolamine

Table 33.3

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

Characteristic	*A. erythreum*[a]	*A. fastidiosum*[b]	*A. marinum*[c]	*A. alkaliterrae*[d]	*A. tamlense*[e]
Colony color	Beige to amber beige	White	Ivory	Cream	Yellow
Cell morphology	Irregular rods, cocci	Rods, cocci	Rods	Rods, cocci	Irregular rods
Catalase	+	+	+	+	ND
Oxidase	+	+	–	–	ND
Nitrate reduction	–	–	–	–	–
Hydrolysis of					
Aesculin	+	ND	ND	–	+
DNA	w	+	w	+	ND
Elastin	+	–	–	+	ND
Urea	–	–	–	–	–
Gelatin	+	+	ND	+	+
Starch	+	+	–	–	ND
Casein	+	+	–	+	–
Cellulose	+	–	–	–	ND
Tyrosine	w	–	–	–	–
Voges-Proskauer reaction	+	+	+	–	–
Utilization of					
Acetate	+	+	+	–	+
D-Arabinose	+	+	–	ND	–
L-Arabinose	+	+	–	+	–
Cellobiose	–	+	+	+	+
Citrate	+©	+©	–	–	–
D-Fructose	+	+	–	–	+
D-Galactose	+	+	+	+	+
D-Glucose	+	+	–	+	+
Glycerol	+	+	–	ND	+
Lactose	–	–	–	–	+
D-Mannose	–	+	–	–	+
Maltose	–	–	–	+	+
Mannitol	–	–	+	–	–
L-Rhamnose	–	–	–	–	–
Salicin	–	–	–	+	–
L-sorbose	–	–	–	–	–
Succinate	+	–	+	+	+
Sucrose	+	+	–	+	+
Trehalose	+	+	+	+	+
D-xylitol	–	–	–	–	–
D-Xylose	+	+	–	–	–
API ZYM					
Alkaline phosphatase	–	+	–	–	+
Esterase (C4)	+	+	+	+	–
Esterase lipase (C8)	+	+	+	+	w
Lipase (C14)	–	–	–	–	–
Leucin arylamidase	+	+	+	+	+
Valine arylamidase	–	–	–	–	–
Cystine arylamidase	–	–	–	–	–

Table 33.3 (continued)

Characteristic	*A. erythreum*[a]	*A. fastidiosum*[b]	*A. marinum*[c]	*A. alkaliterrae*[d]	*A. tamlense*[e]
Trypsin	–	–	–	–	w
α-chymotrypsin	–	–	–	–	–
Acid phosphatase	+	+	–	+	+
Naphthol-AS-BI-phosphohydrolase	w	w	w	+	w
α-galactosidase	–	–	–	–	–
β-galactosidase	–	–	–	–	–
β-glucuronidase	–	–	–	–	–
α-glucosidase	+	+	–	+	+
β-glucosidase	–	–	–	–	–
N-Acetyl-β-glucosaminidase	–	–	–	–	–
α-mannosidase	–	–	–	–	–
α-fucosidase	–	–	–	–	–
Peptidoglycan composition	Ala, Glu, Gly, LL-DAP	Ala, Glu, Gly, LL-DAP	LL-DAP	LL-DAP	ND
Predominant cellular fatty acids	TBSA; $C_{18:1\omega9c}$; $C_{16:0}$	TBSA; $C_{18:1\omega9c}$; $C_{16:0}$	$C_{18:1\omega9c}$; TBSA; $C_{16:0}$; $C_{16:0}$2-OH	$C_{16:0}$; $C_{16:0}$2-OH; TBSA	$C_{18:1\omega9c}$; $C_{16:0}$; TBSA; $C_{18:0}$; $C_{16:0}$2-OH
Major polar lipids[k]	DPG, PE, PG	DPG, PE, PG	ND	ND	DPG, PG, PI
DNA G+C content	71–73	71–72	70.6	71.5	72.7
Characteristic	***A. panaciterrae***[f]	***A. ponti***[g]	***A. flavum***[h]	***A. ginsengisoli***[i]	***A. halocynthiae***[j]
Colony color	Yellowish white	Yellow	Yellow	White	Light yellowish
Cell morphology	Rods	Rods	Irregular rods	Cocci	Rods
Catalase	+	+	+	–	+
Oxidase	–	–	+	+	–
Nitrate reduction	–	–	+	–	ND
Hydrolysis of					
Aesculin	ND	ND	+	ND	–
DNA	–	+	ND	ND	ND
Elastin	–	+	ND	ND	ND
Urea	–	–	w	–	–
Gelatin	+	+	+	+	ND
Starch	–	–	ND	ND	ND
Casein	–	+	–	ND	ND
Cellulose	–	+	ND	–	ND
Tyrosine	–	+	ND	ND	ND
Voges-Proskauer reaction	–	+	w	ND	+
Utilization of					
Acetate	+	+	+	+	+
D-Arabinose	ND	–	–	+	–
L-Arabinose	–	+	–	+	+
Cellobiose	+	+	–	+	–
Citrate	–	+	ND	–	–
D-Fructose	–	+	+	+	+
D-Galactose	+	+	ND	ND	–
D-Glucose	+	+	+	+	+
Glycerol	+	ND	+	ND	+
Lactose	–	–	ND	ND	ND

Table 33.3 (continued)

Characteristic	*A. panaciterrae*[f]	*A. ponti*[g]	*A. flavum*[h]	*A. ginsengisoli*[i]	*A. halocynthiae*[j]
D-Mannose	+	+	–	+	+
Maltose	+	+	+	+	+
Mannitol	+	–	ND	–	+
L-Rhamnose	–	–	–	+	–
Salicin	+	w	ND	–	–
L-sorbose	–	–	ND	ND	–
Succinate	+	+	ND	+	ND
Sucrose	+	+	+	+	+
Trehalose	+	ND	+	+	+
D-xylitol	–	–	–	ND	ND
D-Xylose	–	+	–	+	+
API ZYM					
Alkaline phosphatase	+	+	–	–	–
Esterase (C4)	w	+	+	+	+
Esterase lipase (C8)	+	+	+	ND	+
Lipase (C14)	–	–	w	ND	–
Leucin arylamidase	+	–	+	ND	+
Valine arylamidase	–	w	–	ND	w
Cystine arylamidase	–	–	–	ND	–
Trypsin	–	–	–	–	–
α-chymotrypsin	–	–	–	ND	–
Acid phosphatase	+	+	–	+	–
Naphthol-AS-BI-phosphohydrolase	+	–	–	+	w
α-galactosidase	–	–	–	ND	–
β-galactosidase	–	–	–	ND	–
β-glucuronidase	–	–	–	ND	–
α-glucosidase	–	–	+	+	w
β-glucosidase	–	–	+	ND	–
N-Acetyl-β-glucosaminidase	–	–	–	ND	–
α-mannosidase	–	–	–	ND	–
α-fucosidase	–	–	–	ND	–
Peptidoglycan composition	LL-DAP	LL-DAP	LL-DAP	ND	LL-DAP
Predominant cellular fatty acids	TBSA; $C_{16:0}$; $C_{16:0}$2-OH; 10Me $C_{17:0}$; 10Me $C_{16:0}$	$C_{18:1\omega9c}$; $C_{16:0}$; $C_{16:0}$2-OH; TBSA	$C_{18:1\omega9c}$; $C_{18:0}$	$C_{16:0}$; TBSA; $C_{18:0}$	$C_{18:1\omega9c}$; $C_{16:0}$; TBSA
Major polar lipids[k]	ND	DPG, PG, PI, UP	ND	ND	ND
DNA G+C content	65.5	74.0	73.3	66.8	75.9

Data taken from
[a]Miller et al. 1991
[b]Tamura and Yokota 1994
[c]Bruns et al. 2003
[d]Yoon et al. 2005a
[e]Lee and Kim 2007
[f]Cui et al. 2007
[g]Lee and Lee 2008
[h]Tang et al. 2008
[i]Kim et al. 2008b
[j]Kim et al. 2010
[k]*DGP* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PE* phosphatidylethanolamine, *UP* unknown phospholipid
Symbols and abbreviations: + positive, – negative, *w* weakly positive, *ND* no data available, © conflicting data

Table 33.4

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

Characteristic	*K. flavida*[a]	*K. sandramycini*[a]	*K. koreensis*[b,c]	*K. antibiotica*[d]
Aerial mycelium	ND	ND	White	Light yellow
Substrate mycelium	ND	ND	Creamy	Yellow white
Melanin production	+	–	+	+
Morphology	Hyphae, rods	Hyphae	Hyphae, rods	Hyphae, rods
Catalase	+	+	+	ND
Oxidase	+	+	–	ND
Nitrate reduction	+	–	–©	–
Hydrolysis of				
Urea	+	+	+	+
Gelatin	–	+	+	+
Starch	–	–/w	–©	+
Casein	+©	+	–©	+
Cellulose	–	–	–	–
Production of				
H_2S	–	–	+©	–
Utilization of				
L-Arabinose	–	w	+	+
D-Fructose	–©	+	+	+
D-Galactose	–	+	+	+
D-Glucose	+	+	+	+
Glycerol	–©	+	+	+
myo-Inositol	+	+	+	+
Inulin	+	+	+	ND
Lactose	–©	+©	+	+
Maltose	+	+	+	+
D-Mannitol	+	+	+	+
D-Mannose	–©	+©	+	+
D-Rhamnose	–©	+	+	+
D-sorbitol	–©	–©	+	+
D-Xylose	–	+	+	+
Sodium acetate	–	–	+	+
Trisodium citrate	+	–	+	+
Predominant cellular fatty acids	ai-$C_{15:0}$; i-$C_{16:0}$; i-$C_{16:1}$	ai-$C_{15:0}$; i-$C_{14:0}$; i-$C_{15:0}$	ai-$C_{15:0}$; i-$C_{16:0}$; i-$C_{15:0}$	ai-$C_{15:0}$; i-$C_{15:0}$
Cell-wall sugars[n]	Man, Glc, Gal	Man, Glc, Gal	Man, Glc, Gal, Rib	Glc, Xyl, Rib, Man
Major polar lipids[o]	PC	PC	DPG, PC, PG, PI	PC, DPG, PG, PI
DNA G+C content (mol%)	70	68.3	71.3	67

Characteristic	*K. solani*[e]	*K. jejuensis*[e]	*K. yunnanensis*[f]	*K. alba*[f]	*K. lupini*[g]
Aerial mycelium	White	White	White	Yellow white	White
Substrate mycelium	Creamy	Creamy	Pale yellow	Pale yellow	Creamy
Melanin production	ND	ND	w	+	ND
Morphology	Hyphae, rods	Hyphae, rods	Hyphae, rods	Hyphae, rods	Hyphae, rods
Catalase	+	+	+	+	+
Oxidase	ND	ND	+	–	+
Nitrate reduction	–	–	–	–	–

Table 33.4 (continued)

Characteristic	*K. solani*[e]	*K. jejuensis*[e]	*K. yunnanensis*[f]	*K. alba*[f]	*K. lupini*[g]
Hydrolysis of					
Urea	−	+	ND	+	−
Gelatin	ND	+	−	+	+
Starch	−	−	+	+	−
Casein	−	−	ND	ND	+
Cellulose	ND	ND	−	−	ND
Production of					
H_2S	ND	ND	−	−	ND
Utilization of					
L-Arabinose	ND	ND	ND	+	+
D-Fructose	ND	ND	+	+	ND
D-Galactose	−	+	−	+	+
D-Glucose	+	+	+	+	+
Glycerol	ND	ND	+	+	ND
myo-Inositol	−	−	+	+	ND
Inulin	−	+	ND	ND	ND
Lactose	+	+	+	+	ND
Maltose	ND	ND	+	+	©
D-Mannitol	−	−	+	+	+
D-Mannose	+	−	+	+	+
D-Rhamnose	+	+	+	+	+
D-sorbitol	ND	ND	+	+	+
D-Xylose	+	+	+	+	ND
Sodium acetate	ND	ND	+	+	ND
Trisodium citrate	ND	ND	+	−	−
Predominant cellular fatty acids	ai-$C_{15:0}$; i-$C_{16:0}$; i-$C_{14:0}$	ai-$C_{15:0}$; i-$C_{16:0}$; i-$C_{15:0}$	ai-$C_{15:0}$; i-$C_{15:0}$; i-$C_{16:0}$	ai-$C_{15:0}$; i-$C_{15:0}$; i-$C_{16:0}$; i-$C_{17:1\omega9c}$	ai-$C_{15:0}$; i-$C_{16:0}$; i-$C_{17:1}$
Cell-wall sugars[n]	Man, Glc, Gal, Rib	Man, Glc, Gal, Rib	Man, Glc, Gal, Rib	Glc, Gal, Rib	Gal
Major polar lipids[o]	DPG, PC, PI	DPG, PC, PI	DPG, PC, PG	DPG, PC, PG, PI	ND
DNA G+C content (mol%)	69	68	68.6	67.9	68

Characteristic	*K. karoonensis*[h]	*K. swartbergensis*[h]	*K. aluminosa*[i]	*K. hippodromi*[j]
Aerial mycelium	Pale cream	White	White	White
Substrate mycelium	Cream-yellow	Cream	Cream yellow	Cream
Melanin production	ND	ND	ND	ND
Morphology	Hyphae, rods	Hyphae, rods	Hyphae, rods, coccoid elements	Hyphae
Catalase	+	+	+	+
Oxidase	+	ND	−	−
Nitrate reduction	+	+	−©	+©
Hydrolysis of				
Urea	+	+	+©	−©
Gelatin	+	ND	ND	+
Starch	+	+	+	w
Casein	+	+	+	+
Cellulose	ND	ND	−	−
Production of				
H_2S	+	+	+	+

Table 33.4 (continued)

Characteristic	*K. karoonensis*[h]	*K. swartbergensis*[h]	*K. aluminosa*[i]	*K. hippodromi*[j]
Utilization of				
L-Arabinose	–	w	+	+
D-Fructose	+	+	+	+
D-Galactose	w	ND	+	+
D-Glucose	+	+	+	+
Glycerol	+	w	+	+
myo-Inositol	+	w	+	+
Inulin	–	–	+	+
Lactose	+	w/+	ND	–
Maltose	+	+	+	+
D-Mannitol	+	w	+	+
D-Mannose	+	w/+	+	+
D-Rhamnose	+	w/+	+	+
D-sorbitol	–	+	ND	ND
D-Xylose	+	+	+	–
Sodium acetate	ND	w	+	w
Trisodium citrate	ND	w	+	w
Predominant cellular fatty acids	ND	ND	ai-$C_{15:0;}$ i-$C_{16:0}$	ND
Cell-wall sugars[n]	ND	ND	ND	ND
Major polar lipids[o]	ND	ND	PC, DPG, PI, UP, UG	ND
DNA G+C content (mol%)	ND	ND	ND	ND
Characteristic	***K. catacumbea***[k]	***K. sancticallisti***[k]	***K. ginsengisoli***[l]	***K. amoyensis***[m]
Aerial mycelium	White	White	ND	White yellow
Substrate mycelium	yellow	cream	ND	yellow
Melanin production	+	w	ND	ND
Morphology	Hyphae	Hyphae	Hyphae	Hyphae, irregular rods, coccoid elements
Catalase	+	+	+	ND
Oxidase	+	+	+	–
Nitrate reduction	–	+	–	+
Hydrolysis of				
Urea	–	–	–	–
Gelatin	+	+	+	+
Starch	–	–	w	+
Casein	+	+	+	+
Cellulose	ND	ND	–	ND
Production of				
H_2S	ND	ND	–	ND
Utilization of				
L-Arabinose	+	+	+	–
D-Fructose	+	+	+	ND
D-Galactose	+	+	+	+
D-Glucose	+	ND	+	+
Glycerol	+	+	+	ND
myo-Inositol	–	–	+	+
Inulin	ND	ND	–	ND

Table 33.4 (continued)

Characteristic	*K. catacumbea*[k]	*K. sancticallisti*[k]	*K. ginsengisoli*[l]	*K. amoyensis*[m]
Lactose	+	+	+	–
Maltose	+	+	+	–
D-Mannitol	+	+	+	+
D-Mannose	+	+	+	+
D-Rhamnose	+	+	+	+
D-sorbitol	w	–	–	+
D-Xylose	+	+	+	+
Sodium acetate	ND	ND	ND	ND
Trisodium citrate	ND	ND	ND	ND
Predominant cellular fatty acids	i-$C_{15:0}$; ai-$C_{15:0}$; i-$C_{17:1\omega9c}$	i-$C_{16:0}$; ai-$C_{15:0}$; i-$C_{15:0}$	ai-$C_{15:0}$; i-$C_{16:0}$	i-$C_{16:0}$; i-$C_{14:0}$; ai-$C_{15:0}$
Cell-wall sugars[n]	ND	ND	Gal, Rib, Xyl	ND
Major polar lipids[o]	ND	ND	DPG, PC, PG, PI	PG, DPG, PC, PI, UP
DNA G+C content (mol%)	ND	ND	66.3	ND

Data taken from
[a]Park et al. 1999
[b]Lee et al. 2000
[c]Sohn et al. 2003
[d]Li et al. 2004
[e]Song et al. 2004
[f]Li et al. 2006
[g]Trujillo et al. 2006
[h]Kirby et al. 2006
[i]Carlsohn et al. 2007
[j]Everest and Meyers 2008
[k]Urzí et al. 2008
[l]Cui et al. 2010
[m]Xu et al. 2012
[n]*Gal* galactose, *Glc* glucose, *Man* mannose, *Rib* ribose, *Xyl* xylose
[o]*PG* phosphatidylglycerol, *DGP* diphosphatidylglycerol, *PI* phosphatidylinositol, *PC* phosphatidylcholine, *UP* unknown phospholipid, *UG* unknown glycolipid
Symbols and abbreviations: + positive, – negative, *w* weakly positive, *ND* no data available, © conflicting data

Nonmotile, and no endospore formation can be observed. Colonies are yellow or orange pigmented. They can become rough and shaped like craters in old cultures.

Marmoricola species are aerobic, catalase-positive, and oxidase-negative organisms. Strains of all species are positive for aesculin hydrolysis and negative for Voges-Proskauer reaction, urease activity, and indole production. Each utilizes glucose, D-mannitol, and methyl-α-D-glucoside as sole source of carbon but not formate, *meso*-inositol, methyl-α-D-mannoside, or L-sorbose. For other detailed data, see *Table 33.5*.

All members of the genus *Marmoricola* are positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, α-glucosidase, and β-glucosidase and negative for lipase (C14), α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and tryptophane deaminase (Lee and Lee 2010).

Most strains are mesophilic and grow at 20–28 °C; *M. korecus* (Lee et al. 2011a) grows even at 4 °C. They grow in neutrophilic or slightly alkaliphilic conditions (pH range of their growth is 5.1–12.1).

Polar lipid profile contains phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol. Their cellular fatty acid profile consists of straight-chain saturated and mono-unsaturated components (C16:0, C18:1ω9c, C17:1ω8c); in case of *M. aurantiacus* and *M. scoriae* also, TBSA is present. The predominant menaquinone is MK-8(H_4).

DNA G+C content is 71–72 mol%.

Aeromicrobium Miller et al. (1991), Emend Yoon et al. (2005)

Aer.o.mi.cro'bi.um. N. L. noun *Aeromicrobium*, aerobic microbe.

The type strain of *Aeromicrobium erythreum* NRRL B-3381^T was isolated from soil collected in the Lajas Valley near Cabo Rojo, Puerto Rico (Miller et al. 1991).

Cells are Gram positive, non-endospore forming, and mostly nonmotile rods. Irregular rods and coccoid forms are also observed. Branching or mycelial form does not occur. Aerobic, and with the exception of *A. ginsengisoli*, strains are

Table 33.5

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

Marmoricola	*M. aurantiacus*[a]	*M. aequoreus*[b]	*M. bigeumensis*[c]	*M. scoriae*[d]	*M. korecus*[e]
Morphology	Coccus (single, pairs, clusters)	Coccus (single, pairs, chains)	Coccus (single, pairs)	Coccus (single, pairs, clusters)	Coccus
Gram staining	+	+	+	+	+
Isolation/habitat	Marble	Beach sediment	Agricultural soil	Volcanic ash	Volcanic ash
Catalase	+	+	+	+	+
Oxidase	–	–	–	–	–
Nitrate reduction	–	+	+	–	–
Hydrolysis of					
Urea	–	–	–	–	ND
Gelatin	–	+	–	+	+
Starch	–	–	+	–	–
Casein	–	+	–	+	+
Xanthine	–	–	+	–	–
Utilization of					
Acetate	+	+	+	+	–
D-Arabinose	– ©	–	–	+	–
Citrate	+	+	–	–	–
Dextran	+	+	+	–	–
D-Fructose	+	+	–	+	–
D-Galactose	+	+	–	+	–
Glycerol	+	+	–	–	–
Lactose	+	+	+	–	–
D-Mannose	+	+	+	–	+
Maltose	+	+	+	+	–
D-Raffinose	ND	–	–	+	–
L-Rhamnose	+	–	+	+	–
L-Ribose	–	+	–	+	–
Sucrose	+	+	–	+	–
Trehalose	+	+	–	+	–
XD-Xylose	+	+	–	+	+
Enzyme activity					
Acid phosphatase	+	–	+	+	+
α-Chymotripsin	–	w	+	-	–
Cystine arylamidase	–	w	+	w	w
Esterase (C4)	w	w	+	–	–
Trypsin	–	–	+	w	w
Valine arylamidase	w	+	–	+	+
Growth temperature	18–28 °C	10–37 °C	20–37 °C	10–37 °C	4/37 °C
pH range	5.1–8.7	5.1–12.1	6.0–12.0	6.1–12.1	5.1–12.1
NaCl tolerance	0.5–2 %	0–5 %	0–7 %	0–3 %	0–2 %
Predominant cellular fatty acids	$C_{16:0}$, $C_{18:1\omega9c}$, $C_{16:1}$, TBSA	$C_{16:0}$, $C_{18:1\omega9c}$	$iC_{16:0}$	$C_{16:0}$, $C_{18:1\omega9c}$, TBSA	$C_{16:0}$, $C_{17:1\omega8c}$, $C_{18:1\omega9c}$,
Major polar lipids[f]	PI, PG, DPG	PI, PG, DPG	PI, PG, DPG	PI, PG, DPG, PC	PI, PG, DPG, PC
DNA G+C content	72 %	72.4 %	72.9 %	72 %	71 %

Data taken from

[a]Urzí et al. 2000

[b]Lee 2007a

[c]Dastager et al. 2008b

[d]Lee and Lee 2010

[e]Lee et al. 2011b

[f]*DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PC* phosphatidylcholine

Symbols and abbreviations: + positive, –, negative, *w* weakly positive, *ND* no data available, © conflicting data

Table 33.6
Comparison of selected characteristics of members of the genus *Kribbella*

Actinopolymorpha	*A. singaporensis*[a]	*A. rutila*[b]	*A. alba*[c]	*A. cephalotaxi*[d]	*A. pittospori*[e]
Morphology	Polymorph to hyphae	Polymorph to hyphae	Polymorph to hyphae	Polymorph to hyphae	Polymorph to hyphae
Gram staining	+	+	+	+	+
Isolation/habitat	Soil	Forrest soil	Rhizosphere soil	Rhizosphere soil	Plant leave
Catalase	+	+	+	-	+
Nitrate reduction	+	–	–	+	ND
Hydrolysis of					
Urea	ND	+	+	+	ND
Gelatin	+	+	+	+	+
Cellulose	–	–	–	–	–
Starch	–	–	–	–	ND
Utilization of					
Glucose	+	-	+	+	+
Mannitol	+©	-	-	+	-
Raffinose	–	+	+	+	w
Rhamnose	+	–	–	+	ND
Fructose	+	+	–	+	+
Sucrose	+	+	–	+	–
Inositol	+	–	–	+	–
Sorbitol	+	–	–	+	ND
Galactose	+	–	–	+	+
Arabinose	–	+	+	+	w
Milk coagulation	+	–	–	+	ND
Milk peptonisation	+	–	–	+	ND
Growth temperature	25–37 °C	15–37 °C	10–45 °C	20–28 °C	15–27 °C
pH range	6–10	6–8 ©	6–7 ©	6–8 ©	6–10
NaCl tolerance	8–15 %	5 % ≤	7 % ≤	5 % ≥	1 %
Predominant cellular fatty acids	$iC_{15:0}$; $iC_{16:0;}$ $iC_{16:1\ H}$	$iC_{15:0}$; $iC_{16:0;}$ $iC_{16:1\ H}$	$iC_{15:0}$; $iC_{16:0;}$ $iC_{14:0\ 3OH}$	$iC_{15:0}$; $iC_{16:0;}$ $iC_{16:1\ H}$	$iC_{15:0}$; $iC_{16:0;}$ $aC_{17:0;}$ $iC_{17:0}$
Cell-wall sugars	glu, rha, rib	glu, rha, rib	glu, rha, rib	glu	glu, rib, gal
Major polar lipids[f]	PIM, PI, DPG, PG	PIM, PI, DPG, PG	PIM, PI, DPG, PG	PI, DPG, PG	PIM, PI, DPG, PG
DNA G+C content	69.5	67.7	66.6	69.3	69.6

Data taken from
[a]Wang et al. 2001
[b]Wang et al. 2008
[c]Cao et al. 2009
[d]Yuan et al. 2010
[e]Kaewkla and Franco 2011a
[f]*PIM* phosphatidylinositol mannosides, *PI* phosphatidylinositol, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol
Symbols and abbreviations: + positive, – negative, *w* weakly positive, *ND* no data available, © conflicting data

catalase positive. Except for *A. flavum*, they reduce nitrate to nitrite. All type strains hydrolyze gelatin, utilize trehalose, and produce esterase lipase (C8) but unable to assimilate D-xylitol and negative for cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-Acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. All strains of *Aeromicrobium* species are mesophilic which can grow best at 20–30 °C. *Aeromicrobium* species show growth up to 3–10 % (w/v) NaCl concentration and between pH 5.0 and 8.0. Other selected phenotypic properties are given in ➤ *Table 33.3*.

MK-9(H_4) is the predominant menaquinone. The major components of cellular fatty acids are TBSA (tuberculostearic acid, 10Me-C18:0), C18:1ω9c, and C16:0.

Polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, and phosphatidylethanolamine.

DNA G+C content is 65.5–75.9 mol%.

Kribbella Park et al. (1999)

Kribbella (Kribb.el'la. M. L. dim. fem. ending -ella; M.L. fern. n. *Kribbella* arbitrary name formed from the acronym of the Korea Research Institute of Bioscience and Biotechnology, KRIBB, where taxonomic studies of this taxon were performed).

The type strain of *Kribbella flavida* IFO 14399^T was isolated from soil in China and first described as "*Nocardioides fulvus*" by Ruan and Zhang (1979).

Cultures of *Kribbella* produce both vegetative (substrate) and aerial mycelia on many different media (e.g., ISP media, Czapek's agar, nutrient agar) tested that fragment into rod-shaped elements. Cells are nonmotile. The substrate mycelium appears creamy to pale yellow which is generally extensively branched and can penetrate into the agar medium. The aerial mycelium is white or yellow-white. Diffusible pigment production is not observed. Melanin production is variable among the species and depends on the composition of the cultivation medium. Other selected phenotypic properties are displayed in ❯ *Table 33.4*. All strains are Gram positive, aerobic, and catalase positive, but strains of *K. flavida, K. solani, K. jejuensis, K. koreensis*, and *K. sandramycini* show also moderate or weak anaerobic growth (Kirby et al. 2006; Everest and Meyers 2008). Some species are positive for oxidase (*K. flavida, K. sandramycini, K. yunnanensis, K. lupine, K. karoonensis, K. catacumbae, K. sancticallisti* and *K. ginsengisoli*) and/or reduce nitrate (*K. flavida, K. karoonensis, K. swartbergensis, K. hippodromi, K. sancticallisti*, and *K. amoyensis*). D-fructose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, D-rhamnose, and D-xylose are the most frequently utilized carbon sources. All strains of *Kribbella* species are mesophilic and neutrophilic which can grow best at 20–30 °C on agar media used in the ISP (Shirling and Gottlieb 1966) and at pH values between 6.0 and 8.0. *Kribbella* species are non-halophilic; they show growth up to 2–5 % (w/v) NaCl concentration.

The major menaquinone is MK-9(H_4). Branched-chain fatty acids (e.g., i-C16:0, ai-C15:0, and i-C15:0) are dominant; other fatty acids (e.g., i-C14:0, i-C16:1, i-C17:1, i-C17:1ω9c) occur in lower amounts. The characteristic whole-cell sugars of *Kribbella* species involve ribose, mannose, xylose, galactose, and glucose. Besides phosphatidylcholine present in all species, additional phospholipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. Some representatives of the genus *Kribbella* contain teichulosonic acid (TULA), a novel type of a glycopolymer with a pseudaminic acid derivative, as the main chain component. In addition, a neutral polysaccharide (mannan) can also be present in varying amounts (Shashkov et al. 2009; Tulskaya et al. 2011).

DNA G+C content is 66.3–71.3 mol%.

Actinopolymorpha Wang et. al (2001)

Ac.ti.no.po.ly.mor'pha. Gr. n. *actis, actinos* a ray; Gr. adj. poly many; Gr. n. *morphus* form, shape; M.L. adj. *Actinopolymorpha* actinomycete of many shapes.

The type strain *Actinopolymorpha singaporensis* IM 7744^T was isolated from a soil in the tropical rainforest of Singapore (Wang et al. 2001).

Members of this genus usually show slow growth on most-tested medium. Irregular cells are in *A. cephalotaxi* (Yuan et al. 2010) which appear to divide through apical and lateral budding and remain attached after division. They often keep in short chains or aggregates in the early stages of their growth. Cells later elongate at different degree and can even swell, forming branched hyphae with uneven thickness.

Salt is often required for their growth; *A. singaporensis* (Wang et al. 2001) tolerates 15 % NaCl concentration. Most species are mesophilic and neutrophiles.

All are catalase positive and able to decompose gelatin, utilize glucose as sole carbon source, and produce acid from arabinose, glucose, and trehalose. All strains are negative for production acid from 1,2-propanediol.

In case of the genus *Actinopolymorpha*, only *A. cephalotaxi, A. rutila*, and *A. alba* were tested for their enzymes in detail (API ZYM). All were positive for esterase (C4), α- and β-glucosidases, and α-mannosidase.

Polar lipid profile contains phosphatidylinositol mannosides, phosphatidylinositol, phosphatidylglycerol, and diphosphatidylglycerol. Their cellular fatty acid profile consists of mainly branched-chain saturated (iC16:0, iC15:0) as major components; in case of *A. pittospori* also iC17:0 and aC17:0 occur. The predominant menaquinone is MK-9(H_6) or/and MK-9(H_4).

DNA G+C content is 67.7–69.6 mol%.

Thermasporomyces Yabe et al. (2011)

Thermasporomyces [Ther.ma.spo.ro.my'ces. Gr. n. *therme* heat; Gr. prefix. a not; Gr. n. spora a seed, and in biology a spore; Gr. masc. n. *mukes* mushroom or other fungus; N.L. masc. n. *Thermasporomyces* the heat (−loving) non-spored fungus].

The type strain of *Thermasporomyces composti* I3^T was isolated from mature compost produced by a field-scale composter (Hazaka system; Hazaka Plant Kogyo Co., Ltd), which is used for the treatment of livestock excreta (Yabe et al. 2011).

Strain I3^T grows well on ISP 3 medium and weakly on ISP 2, 4, and 6 media and nutrient agar. No growth occurs on ISP 1 or ISP 5 media. Strain I3^T develops pale-yellow colonies on ISP 3, pale-yellow-orange colonies on ISP 2, and white colonies on other media. No aerial mycelia or diffusible pigments are observed on any of the media. Strain I3^T forms branched hyphae that fragmented into short chains or aggregates. The fragments are coccoid or short rods. Spores are not observed.

Strain I3^T stained is Gram positive and positive for catalase, oxidase, nitrate reduction, gelatin hydrolysis, milk coagulation,

and peptonization. Casein, carboxymethylcellulose, and xylan are hydrolyzed, while starch, xanthine, and L-tyrosine are not. Raffinose, sorbitol, lactose, cellobiose, and D-arabinose are utilized, but not sodium citrate or sodium succinate. Acid is produced from mannose and maltose, but not from L-rhamnose, sorbitol, inositol, or D-galactose. Enzyme activities detected by API ZYM are alkaline phosphatase, C4 and C8 esterases, leucine arylamidase, α-chymotrypsin, naphthol phosphohydrolase, trypsin, α-galactosidase, α-glucosidase, lipase C14, valine arylamidase, N-acetyl-β-glucosaminidase, and α-mannosidase. Activities not detected by API ZYM are β-galactosidase, β-glucosidase, β-glucuronidase, and α-fucosidase. Growth occurs between 35 °C and 62 °C (optimum 50–55 °C), at pH 5.7–10.0 (optimum pH 7.0) and in the presence of up to 5 % (w/v) NaCl.

The major fatty acids are i-C15:0, ai-C15:0, i-C17:0, and ai-C17:0. The major menaquinones are MK-9(H_4), MK-10(H_4), and MK-11(H_4). Cell-wall sugars are rhamnose and arabinose. The polar lipids consist of ninhydrin-positive phosphoglycolipids, phosphatidylglycerol, diphosphatidylglycerol, and an unknown glycolipid.

DNA G+C content is 69.2 mol%.

Flindersiella Kaewkla and Franco (2011)

Flindersiella (Flin.der.si.el'la. N.L. fem. dim. n. *Flindersiella* named after Flinders University, signifying the site of the host tree from which the type strain originated).

The type strain of *Flindersiella endophytica* EUM 378^T was isolated from surface-sterilized root tissue of a *Eucalyptus microcarpa* tree that grows on the campus of Flinders University, Adelaide, South Australia (Kaewkla and Franco 2011b).

Colony morphology of strain EUM 378^T ranges from smooth to rogues. Colonies are white on ISP 4 and yellowish white on ISP 5, Bennett's media, and NA. They are greyish yellow on ISP 3 and ISP 7 and pale yellow on ISP 2 media. Colonies are olive green on HPDA and change to a bluish purple color after 30 days of growth. The type strain produces a greyish-orange pigment on tyrosine agar. Substrate mycelium develops well on most media, but aerial mycelium is rarely formed. Substrate mycelium is branched with irregular thickness and fragments into short chains or aggregates. Spores are tiny rods on short chains that develop from aerial mycelium. White spores are produced on ISP 3, ISP 4, ISP 7, and HPDA media.

The type strain is aerobic, Gram positive, and catalase positive. Decomposition of gelatin and starch is positive. Acid is produced from adonitol, D-arabinose, cellobiose, fructose, galactose, glucose, maltose, mannitol, mannose, *myo*-inositol, ribose, sorbitol, sucrose, trehalose, xylose, and salicin, but not from 1,2 propanediol and dulcitol. Growth occurs between 15 °C and 37 °C, at pH 5.0 and 10.0 and in the presence of up to 5 % (w/v) NaCl. Optimum growth is achieved at a temperature of 27–37 °C, at pH 6 and 10 and in the presence of <3 % (w/v) NaCl.

The predominant menaquinone is MK-10(H_6). The major fatty acids are i-C16:0, ai-C17:0, and ai-C15:0. Diphosphatidylglycerol and phosphatidylglycerol are the major polar lipids.

DNA G+C content is 68.8 mol%.

Isolation, Enrichment, and Maintenance Procedures

Members of the family *Nocardioidaceae* could be isolated from various sources (see *Table 33.2–33.6*).

Most *Nocardioides* strains have been isolated from diverse terrestric and aquatic environments but also communities associated with plants, animals or humans (*Table 33.2*). To isolate *Nocardioides* species, usually no selective media or enrichment procedures are needed; they generally grow well on different complex media like nutrient agar (Difco), TSA agar (Difco), or YM agar (Difco). Selective enrichment was used from polluted environments in case of *N. pyridinolyticus* (Yoon et al. 1997), *N. aromaticivorans* (Kubota et al. 2005), and *N. oleivorans* (Schippers et al. 2005). For cultivation of *Nocardioides* species, media recommended by the International Streptomyces Project (ISP), furthermore glucose-asparagine agar (Lindenbein 1952) or chitin agar (Prauser 1976), are also adequate. However, organic media with low nutrient concentrations are generally advantageous to isolate diverse *Nocardioides* species from natural habitats. For this reason R2A (Difco), twofold or tenfold diluted R2A agar media, tenfold diluted nutrient agar, and oligotrophic medium PYGV (Staley 1968) have been used. For cultivation of *Nocardioides* from marine ecosystems or hypersaline environments, PYGV medium supplemented with seawater (Lawson et al 2000), marine agar (Difco), or other nutrient media supplemented with seawater or salts in adequate concentrations (Lee 2007b; Choi et al. 2007; Park et al. 2008) were used.

As cells or mycelia of these species are often strongly associated with mineral and organic particles, shaking or other mechanical or chemical desorption of the samples should be performed prior to isolation (Herron and Wellington 1990; Futamata et al. 2004). Endophytic representatives of the genus could be isolated after surface-sterilizing the adequate part of the plant (Coombs and Franco 2003; Qin et al. 2012) on nutrient-poor media, like TWYE (tap water-yeast extract) agar (Crawford et al. 1993), YECD (yeast-extract-casein hydrolysate agar) (Coombs and Franco 2003) or on humic acid-vitamin B (HV) agar (Hayakawa and Nonomura 1987). The incubation conditions of the isolation plates vary according to the natural habitat; usually 25–30 °C is adequate but lower (10–15 °C) or higher (40 °C) temperature values can also be used (Iwabuchi et al. 1998; Lawson et al. 2000; Kim et al. 2008a).

Members of the genus *Marmoricola* were isolated from different environments. *M. aurantiacus* was isolated from a marble statue (Urzí et al. 2000) by dilution plating on Bunt and Rovira medium (Bunt and Rovira 1955) but was also able to grow well even on Leudemann medium (Luedemann 1968).

M. aequoreus was isolated from a deep sandy sediment sample (Lee 2007a). The isolation medium (SC-SW) consisted of 1 % soluble starch, 0.03 % casein, 0.2 % KNO_3, 0.2 % NaCl, 0.002 % $CaCO_3$, 1.8 % agar, 0.005 % $MgSO_4 \times 7H_2O$, and 0.001 % $FeSO_4 \times 7H_2O$ in a 60:40 mixture of natural seawater and distilled water. Later, the strain was maintained on yeast-extract/malt agar medium (Shirling and Gottlieb 1966) prepared in a mixture of seawater and distilled water (YE-SW agar). *M. bigeumensis* was isolated and maintained on one-tenth-strength R2A medium (Reasoner and Geldreich 1985). *M. scoriae* and *M. korecus* were isolated from volcanic habitats on starch-casein agar medium (Lee and Lee 2010). All species can be maintained on R2A medium.

Type strains of the genus *Aeromicrobium* were cultivated on several different culture media which involve TYE medium; marine, nutrient, and R2A agar from the Difco Laboratories; SC-SW and YE-SW agar (Lee and Kim 2007); TGY agar (Brim et al. 2003); and A1 + C medium (Kim et al. 2010). Most *Aeromicrobium* species were maintained on the isolation medium.

Members of the genus *Kribbella* are able to grow on a wide range of culture media. Some species (e.g., *K. flavida, K. sandramycini, K. antibiotica, K. yunnanensis*, and *K. alba*) were isolated on ISP 2 or ISP 5 (International *Streptomyces* Project) media. Among the other species, *K. solani* and *K. jejuensis* were cultivated on GYM (DSMZ 65) medium, *K. lupini* on yeast-extract-mannitol agar (Vincent 1970), *K. karoonensis* on PV8 agar (Tormo et al. 2003), *K. swartbergensis* on MC agar (Nonomura and Ohara 1971), *K. hippodromi* on SM1 agar (Tan et al. 2006), and *K. catacumbae* and *K. sancticallisti* on BRII agar (Urzí et al. 2001). In addition, type strains of *K. koreensis* and *K. aluminosa* were cultured on tap water or water agar (Sohn et al. 2003; Carlsohn et al. 2007), while *K. ginsengisoli* cells grew on modified R2A medium (Cui et al. 2010) reflecting their oligotrophic characteristics. In several cases the selectivity of the isolation medium was provided by adding nalidixic acid or nystatin and cycloheximide antibiotics to the basal medium (Lee et al. 2000; Kirby et al. 2006; Carlsohn et al. 2007; Everest and Meyers 2008). Most *Kribbella* species were maintained on ISP 2 agar.

Members of the genus *Actinopolymorpha* are usually slow-growing microorganisms. They were isolated from soils or rhizosphere or as plant endophyte. Initial isolation was usually performed on special media but later on most of them could be cultivated on ISP media. *A. singaporensis* was isolated on ISP2 medium by Wang et al (2001) after the soil suspension was vigorously shaked in LB medium containing 100 μg ml^{-1} penicillin and streptomycin (in order to kill fast-growing bacteria). *A. rutila* was isolated from a forest soil (Wang et al. 2008) and grew well on ISP media (2, 3, 4, 5), on Czapek's agar plates (Pridham and Lyons 1980), on potato agar, and on nutrient agar medium. *A. alba* (Cao et al. 2009) was isolated from a plant rhizosphere using Gauze 1 agar medium (Gauze et al. 1983) later could be maintained as written in case of *A. rutila*. *A. cephalotaxi* is also a plant rhizosphere bacterium, isolated on a selective medium (L^{-1})—5 g starch, 5 g glycerol, 1 g proline, 1 g $(NH_4)_2SO_4$, 1 g NaCl, 2 g $CaCl_2$, 1 g K_2HPO_4, 1 g $MgSO_4 \times 7H_2O$, 50 mg $K_2Cr_2O_7$, 25 mg aztreonam, and 15.0 g agar—and had a final pH of 7.2. The strain was maintained on ISP 2 agar slants. *A. pittospori* was isolated from a leaf sample of a native apricot tree (Kaewkla and Franco 2011a); after 11 weeks of growth, the crushed leaf tissue was placed on VL70 medium containing a mixture of 17 amino acids (Hudson et al. 1989; Schoenborn et al. 2004).

Thermasporomyces composti was isolated from ISP 3 agar supplemented with 20 mg l^{-1} trimethoprim, 10 mg l^{-1} nalidixic acid, and 20 mg l^{-1} kanamycin (Yabe et al. 2011). During the cultivation, agar plates were incubated at 50 °C for 7 days.

Flindersiella endophytica, isolated from the root of Grey Box, an endemic eucalyptus tree, was cultivated on VL70 medium containing a mixture of 17 amino acids and solidified with 0.8 % gellan gum (Hudson et al. 1989; Schoenborn et al. 2004; Song et al. 2005). Before plating, the root samples were sterilized with 70 % ethanol and 6 % hypochlorite for 5 min each, washed thoroughly with sterile water, and then treated with sterile 10 % $NaHCO_3$ for 10 min before being washed with sterile water. Then, the root tissue was crushed in a sterile mortar.

Cultures of members of the family *Nocardioidaceae* can be maintained in 20 % glycerol suspensions at −20 or −80 °C; for long-term preservation, lyophilization or keeping in liquid nitrogen can be adequate.

Ecology

Habitat

The species of the genus *Nocardioides* occur widespread in natural environments and appear to be ubiquitous microorganisms: they are present in soils and aquatic environments, even at low temperature, deep surface ecosystems, and deserts or in oligotrophic habitats (Boivin-Jahns et al. 1995; Groth et al. 1999; Katayama et al. 2007; Rintala et al. 2008; Zhang et al. 2009; Tóth et al. 2011). Representatives of the genus *Nocardioides* can also occur in polluted soils or wastewaters with toxic, even aromatic compounds (Suzuki and Komagata 1983; Lee et al. 1991, 1994; Rajan et al. 1996; Coleman et al. 2002; Hamamura and Arp 2000; Kubota et al. 2005; Schippers et al. 2005). Bacteria of this genus were also found connected with plants—rhizosphere and roots—some have been reported even as plant endophyte (Coombs and Franco 2003; Song et al. 2011; Qin et al. 2012) or in association with lichens (Li et al. 2007). Members of this genus have been detected also in association with vertebrate and invertebrate animals: daphnia, termites, and humans (Fall et al. 2007; Tóth et al. 2008; El-Shatoury et al. 2009).

As other actinomycetes, also the members of the genus *Nocardioides* are most probably involved in the turnover of the organic material in different ecosystems, as they can degrade a wide range of organic substrates. Their ability to metabolize aromatic (even polyaromatic) substances and toxic chemicals suggests (Iizuka and Komagata 1964; Behrend and Heesche-Wagner 1999; Hamamura and Arp 2000; Cho et al. 2000;

Ebert et al. 2001; Hamamura et al. 2001; Schippers et al. 2005; Inoue et al. 2007; Cui et al. 2009; Yoon et al. 2009) that they can play a significant role in natural degradation processes of these compounds.

Members of the genus *Marmoricola* occurs mainly in different environments—soil (Dastager et al. 2008b), sandy sediment (Lee 2007b), and volcanic ash (Lee et al. 2011b; Lee and Lee 2010)—and strains of this genus were isolated also from a marble statue (Urzí et al. 2000). Except of *M. korecus*, they are able to degrade several substrates; most of them are able to grow even alkaline conditions.

Most type strains of *Aeromicrobium* species were isolated both from terrestrial and aquatic habitats. Species of *A. erythreum*, *A. alkaliterrae*, *A. panaciterrae*, and *A. ginsengisoli* were cultivated from different soil samples (Miller et al. 1991; Yoon et al. 2005a; Cui et al. 2007; Kim et al. 2008b) while *A. marinum* and *A. ponti* from seawater (Bruns et al. 2003; Lee and Lee 2008). The type strain of *A. tamlense* originated from dried seaweed collected from Samyang Beach in Jeju Island, Korea (Lee and Kim 2007), while *A. halocynthiae* originate from the siphon tissue of a marine ascidian, *Halocynthia roretzi*, collected off the coast of Gangneung, Korea (Kim et al. 2010). The isolation source of strains of *A. fastidiosum* was herbage (Collins and Stackebrandt 1989). The type strain of *A. flavum* was isolated from an air sample from the campus of Wuhan University, China (Tang et al. 2008).

Most *Kribbella* species (*K. flavida, K. sandramycini, K. koreensis, K. antibiotica, K. jejuensis, K. yunnanensis, K. alba, K. karoonensis, K. swartbergensis, K. hippodromi, K. ginsengisoli*, and *K. amoyensis*) were isolated from different soils (Li et al. 2004, 2006; Song et al. 2004; Kirby et al. 2006; Cui et al. 2010) including a gold-mine cave in Korea (Lee et al. 2000), a racecourse soil in South Africa (Everest and Meyers 2008), and a rhizosphere soil of a pharmaceutical plant (*Typhonium giganteum* Engl.) located in Xiamen City, China (Xu et al. 2012). An acidic and heavy-metal-containing rock surface in a medieval alum slate mine located in Thuringia, Germany was the isolation source of the type strain of *K. aluminosa* (Carlsohn et al. 2007). The type strain of *K. solani* originated from a potato tuber with scab lesions (Song et al. 2004), while *K. lupini* from root nodules of *Lupinus angustifolius* growing near a former uranium mine in Salamanca, Spain (Trujillo et al. 2006). Type strains of two *Kribbella* species (*K. catacumbae* and *K. sancticallisti*) were isolated from whitish-grey patinas in the catacombs of St Callistus in Rome, Italy (Urzí et al. 2008).

Members of the genus *Actinopolymorpha* thrive in soil (Wang et al. 2001) and rhizosphere of plants (Cao et al. 2009; Yuan et al. 2010) or can exist as plant endophytes (Kaewkla and Franco 2011a).

The type strains of *Thermasporomyces* and *Flindersiella* genera were isolated from mature compost produced by a field-scale composter which was used for the treatment of livestock excreta (Yabe et al. 2011) and the surface-sterilized root tissue of an endemic Australian tree, *Eucalyptus microcarpa*, known as Grey Box (Kaewkla and Franco 2011b), respectively.

Pathogenicity, Clinical Relevance

Though the members of the genus *Nocardioides* have been isolated and also associated with human diseases (Harris et al. 2007), they are considered nonpathogenic to humans or other warm-blooded animals.

Application

Bioremediation

An important characteristic of many members of the genus *Nocardioides* is the ability to metabolize recalcitrant and complex compounds, including toxic environmental pollutants, alkanes of various lengths, crude oils, and derivatives (Hamamura and Arp 2000; Iizuka and Komagata 1964; Hamamura et al. 2001; Schippers et al. 2005). Degradation of N-heterocyclic aromatic compound originating from crude oil, creosote, and shale oil was also observed (Inoue et al. 2007). There are reports on the ability of members of this genus to degrade phenols and nitrophenolic compounds (Gundersen and Jensen 1956; Collins et al. 1994; Cui et al. 2009; Yoon et al. 2009). Some species are able to utilize heterocyclic compounds such as dibenzofurans and chloroaromatics, and also dibenzo-p-dioxins (Futamata et al. 2004; Inoue et al. 2007; Kubota et al. 2005; Sukda et al. 2009). Some species are able to degrade different herbicides, like 2,4,5-trichlorophenoxyacetic acid (Ebert et al. 1999, 2001) and atrazine (Mulbry et al. 2002; Vibber et al. 2007). *Nocardioides* sp. PD653 is reported to mineralize hexachlorobenzene, a recalcitrant environmental pollutant (Takagi et al. 2009). *Nocardioides* sp. JS614 is capable of aerobic growth with ethane (known as a plant hormone)and vinyl chloride (Chuang and Mattes 2007; Mattes et al. 2005, 2007; Owens et al. 2009), and Ikunaga et al. (2011) observed also the degradation capacity of a mycotoxin, deoxynivalenol, by *Nocardioides* sp. WSN05-2. Fokina et al. (2003) demonstrated the bioconversion of steroids by *N. simplex* VKM Ac-2033D.

Formation of Added-Value Products

Representatives of the genus *Nocardioides* inhabiting marine shellfish were found to show wide-spectrum antimicrobial effect and antitumor activities (El-Shatoury et al. 2009). Insecticidal activities were also observed in some *Nocardioides* strains (Dellweg et al. 1988). Macrolide antibiotics are produced by some *Nocardioides* strains (Omura et al. 1987). *N. albus* DSM 3176 and DSM 3177 synthesize leucylblasticidin S and a peptidyl nucleoside (Dellweg et al. 1988). A piericidin producer *Nocardioides* strain was detected by Kubota et al. (2003) which compound inhibited cell division of fertilized starfish (*Asterina pectinifera*) eggs. *Nocardioides aromaticivorans* IC177 was described as a carbazole degrader bacterium (Inoue et al. 2006).

Aeromicrobium erythreum (Miller et al. 1991) produces the macrolide antibiotic erythromycin A. As a biologically valuable secondary metabolite, sandramycin antibiotic is produced by *Kribbella sandramycini* (Park et al. 1999).

The type strain of *Aeromicrobium halocynthiae* (Kim et al. 2010) produces taurocholic acid, a bile acid, as a major secondary metabolite.

Enzymes

A *Nocardioides* strain (N106) was isolated by Masson et al. (1995) which was highly active in chitosan degradation. The histamine dehydrogenase (homodimeric enzyme, catalyzes oxidative deamination of histamine) was studied on the genetic level by Fujieda et al. (2004). Gesheva and Vasileva-Tonkova (2012) demonstrated that an Antarctic *Nocardioides* strain (A-1) produces a broad spectrum of hydrolytic enzymes. This strain had also strong antimicrobial effect against several Gram-positive and Gram-negative bacteria.

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34 The Family *Nocardiopsaceae*

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Abstract
The family *Nocardiopsaceae* has been defined on phylogenetic grounds (Rainey et al. 1996). It is one of ten families included in the order *Streptosporangiales* Goodfellow (*Bergey's Manual of Systematic Bacteriology*, vol 5 Part B, Springer, New York, 2012). Morphologically, members of the family are characterized by an extensively branched mycelium, which bear nonmotile spores of different numbers and shapes. Diaminopimelic acid is the diagnostic amino acid of peptidoglycan. Fatty acids and polar lipid patterns are complex. Menaquinones have 9–11 isoprenoid units with varying degrees of hydrogenation. Members are widely distributed in soil, especially saline and hypersaline soils. The four genera described at the time of the latest release of *Bergey's Manual of Systematic Bacteriology*, e.g., *Nocardiopsis* (Hozzein and Trujillo 2012), *Haloactinospora* (Trujillo and Goodfellow 2012), *Streptomonospora* (Cui 2012) and *Thermobifida* (Trujillo and Goodfellow 2012) cover extensively the taxonomic properties, enrichment, ecology and presence of secondary metabolites of the species. This communication will cover additional species and genera described since 2012.

Phylogeny and Taxonomy

In the past few years several additional species of the genus *Nocardiopsis* and *Streptomonospora* and four new genera have been described. The position of many of these taxa is shown in the neighbor-joining tree of *Fig. 34.1*. In contrast to its genus affiliation, Nocardiopsis arabia clusters closely with *Streptomonospora halophila*, already mentioned by Hozzein and Trujillo (2012). As a consequence of its position, this species has been reclassified as *Streptomonosopra arabia* (Zhang et al. 2013) as DNA-DNA similarities were sufficiently low to distinguish two genomospecies. The genus description of the *Streptomonospora* has been emended by Cui et al. (2001), Li et al. (2003) and by Zhang et al. (2013). Based upon genome sequences of *Streptosporangium roseum* DSM 43021^T (Nolan et al. 2010), *Thermobifida fusca* strain YX (Lykidis et al. 2007) and *Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111^T Sun et al. 2010) the isolated phylogenetic position of the order *Streptosporangiales* could be confirmed by molecular signatures (indels) (Gao and Gupta 2012). The genome of an additional species of *Nocardiopsis*, *N. alba*, has been sequenced by Qiao et al. (2012). Taxonomic properties of the novel species of *Nocardiopsis* and *Streptomonospora* are listed in *Tables 34.1* and *34.2*, respectively. The salient features of the novel genera affiliated to the family are shown in *Table 34.3*.

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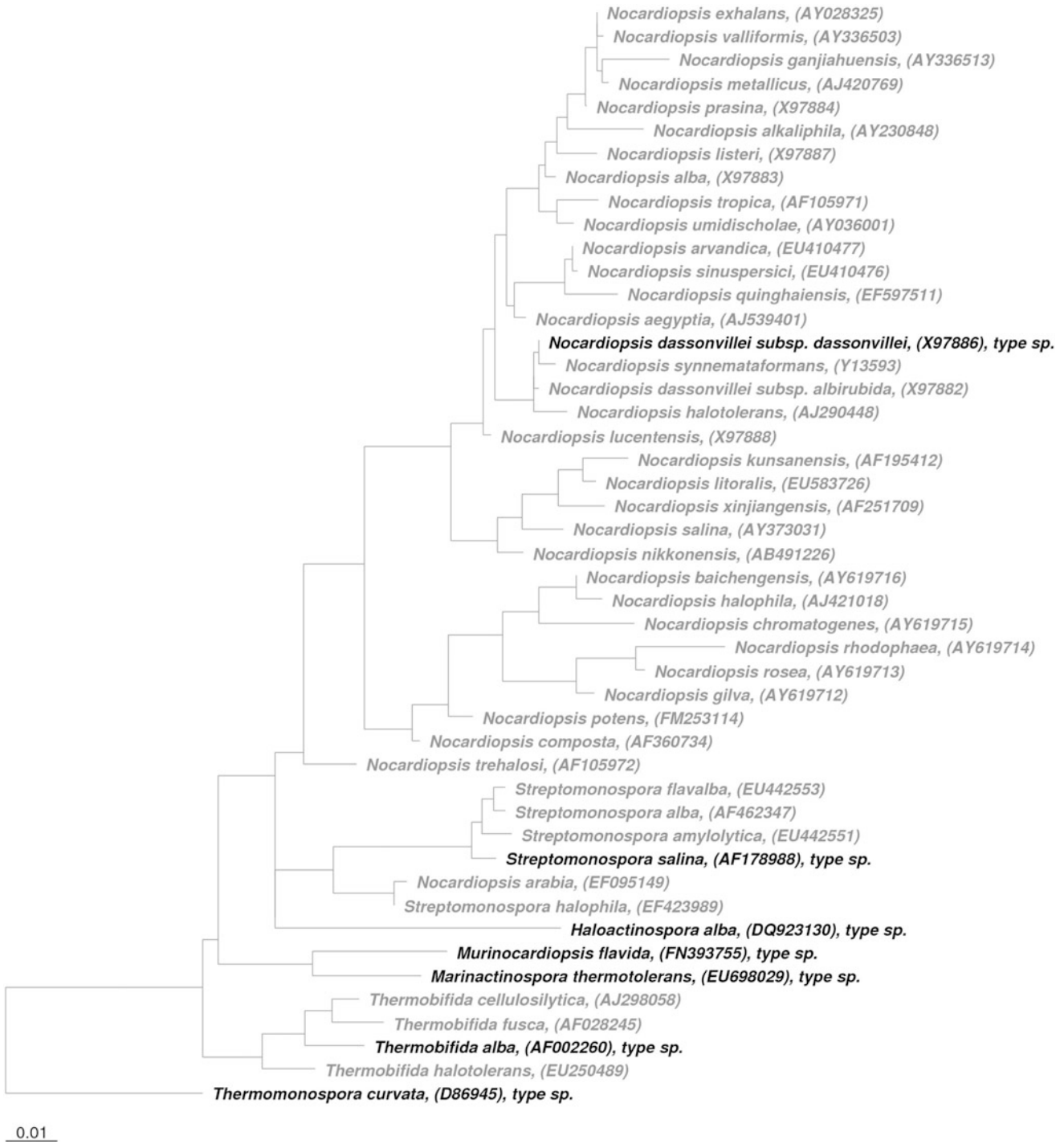

Fig. 34.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the validly described species in the family *Nocardiopsaceae* The tree was reconstructed by using a subset of sequences representative of close relative genera to stabilize the tree topology (Yarza et al. 2010). In addition, a 40 % conservational filter for the whole bacterial domain was used to remove hypervariable positions. The bar indicates 1 % sequence divergence. Sequences of recently described taxa are not included

Table 34.1

Short characterization of recently described *Nocardiopsis* species

Characteristic	*N. arvandica*[a]	*N. coralliicola*[b]	*N. terrae*[c]	*N. sinuspersici*[d]	*N. nikkonensis*[e]	*N. flavescens*[f]	*N. fildesensis*[g]
Type strain	HM7^T	SCSIO 10427^T	YIM 90022^T	HM6^T	1183–22^T	SA6^T	GW9–2^T
Color of[h]							
Aerial mycelium	Light brown	White	White	White, light yellow	White	White	White
Substrate mycelium	Light brown	Yellow-white to pale grey-yellow	Yellow-white to deep brown	Pale or dirty yellow	Pale yellow	Pale yellow to brownish	White to yellow-white
Utilization of							
L-Arabinose	–	nd	–	–	+	w	–
Cellobiose	nd	–	+	nd	–	nd	+
D-Galactose	+	+	–	+	+	+	+
Inositol	–	–	–	–	–	+	w
Lactose	–	–	–	+	–	+	w
Maltose	nd	–	–	nd	–	+	w
Mannitol	–	+	–	+	–	+	+
Melibiose	+	nd	–	+	–	+	+
L-Rhamnose	–	–	–	+	+	+	w
Sucrose	–	–	+	+	+	+	+
Trehalose	nd	nd	+	nd	nd	+	+
D-Xylose	–	–	+	+	+	+	–
Nitrate reduction	nd	+	+	+	–	+	+
Hydrolysis of							
Casein	nd	nd	–	+	nd	+	nd
Tyrosin	nd	nd	nd	nd	nd	+	nd
Tween 80	nd	–	–	nd	+	nd	w
Urea	nd	–	–	–	nd	–	–
Growth in/at							
pH range	5–12	7–10	6–10.5	5–12	6–11	5.5–11	5–11
0 % NaCL	+	+	–	nd	+	+	+
10 % NaCL	+	+	+	+	+	+	+
20 % NaCL	–	–	–	+	w		–
10 °C	–	–	+	–	w	–	–
42 °C	+	+	+	–	–	–	–
45 °C	+	+	+	nd	–	–	–
Chemotaxonomic properties							
Major fatty acids (<10 %)	anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$	iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{17:1}\omega 8c$, $C_{18:1}\omega 9c$	iso-$C_{16:0}$, anteiso-$C_{17:0}$, 10-methyl $C_{18:0}$, 10-methyl $C_{17:0}$	iso-$C_{16:0}$, anteiso-$C_{17:0}$	iso-$C_{16:0}$, anteiso-$C_{17:0}$, 10-methyl $C_{18:0}$	iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{18:0}$	iso-$C_{16:0}$, anteiso-$C_{17:0}$
Major polar lipids	PC, DPG, PE, PI, PG, uGLs	DPG, PME, PE, PG, PC, 3uPL	DPG, PC, PG, PME	PG, PE, PC, PI, uPL	PC, PG	PC, PME, PG, DPG	PG, PC, PME, uPLs
Major menaquinones	9-(H_2), 10-(H_0, H_2,H_4)	10-(H_6, H_8)	9-(H_8), 10-(H_4, H_6, H_8)	10(H_0,H_2),9-(H_0)	10-(H_8, H_{10})	10-(H_2, H_4)	9-(H_4),10-(H_4, H_6, H_8), 7
Mol% D + C of DNA	71.5	69.5	71.5	71.6	72.3	68.6	76.8
Habitat	Sandy river bank, Iran	Coral *Menella praelonga*, China	Saline soil, China	Rhizospheric soil, seashore, Iran	Compost, Japan	Marine sediment, China	Coastal soil, Fildes Peninsula, Antarctica

[a]Hamedi et al. (2011)

[b]Li et al. (2012)

[c]Chen et al. (2010)

[d]Hamedi et al. (2010)

[e]Yamamura et al. (2010)

[f]Fang et al. (2011)

[g]Xu et al. (2014)

[h]Color depending on growth media, e.g., ISP 5 medium

Abbreviations: *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PC* phosphatidylcholine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PME* phosphatidylmethylethanolamine, *U*, unknown, *PGL* phosphoglycolipid, *GL* glycolipid, *PL* phospholipid, *pL* polar lipid

Table 34.2

Short characterization of recently described *Streptomonospora* species. Data of the type species *S. salina* YIM 91355^T is included for comparison (Zhang et al. 2013)

Characteristic	*S. salina*[a,b]	*S. sediminis*[b]	*S. nanhaiensis*[b]	*S. arabica*[b]
Type strain	YIM 91355^T	YIM 11335^T	12A09^T	S186^T
Color of[h]				
Aerial mycelium	White	White	White	Yellowish white
Substrate mycelium	Shades of yellow	Yellow white	Different colors on different media	Greyish-yellow
Spore characteristics	Short spore chains, spores oval to rod-shaped, wrinkled surfacace		Spores chains with wrinkled surface	Single spores with wrinkled surface, short sporophores
Utilization of				
Alanine	–	+	–	+
Arginine	–	+	+	–
Asparticamide	–	+	+	–
Glycine	–	–	–	–
Glutamate	+	+	+	–
Histidine	–	–	+	+
Lysine	–	+	+	–
Methionine	–	–	+	–
Phenylalanine	–	+	+	+
Threonine	–	+	+	+
Tryptophane	–	+	+	–
Tyrosine	–	+	+	–
Valine	–	+	+	–
Nitrate reduction	nd	+	+	–
Hydrolysis of				
Cellulose	–	+	+	+
Starch	+	–	–	–
Gelatine	–	–	–	–
Urea		–	–	–
Growth in/at				
pH range	7 (opt)	6–9	6–9	6–9
NaCL range(% w/v)	15 (opt)	0–20	0–20	0–20
Temperature range	28 (opt)	15–45	10–30	10–40
Chemotaxonomic properties				
Whole cell sugars	glu ,gal, rib, ara, xyl, man	gal	glu	gal, glu
Major fatty acids (<10 %)	nd	iso-$C_{16:0}$, $C_{16:0}$	iso-$C_{16:0}$	iso-$C_{16:0}$, 10-methyl $C_{18:1}$
Polar lipids	(PG, PI, PE.	DPG, PG, PI, PC, PIM, uPGLs, uGL, uPL, upL	DPG, PG, PI, PC, PIM, uPGLs, uGL, uPL, upL	DPG, PG, PI, PC, PIM, PME, uGL, uPL
Major Menaquinones (<5 %)	9-(H_6), 10(H_2, H_4)	10 (H_2,H_4,H_6, H_8), 11-(H_2, H_4, H_6, H_8)	10 (H_4,H_6), 11-(H_4, H_6)	9-(H_4), 0 (H_4,H_6), 11-(H_6)
Mol% G + C of DNA	nd	70.7	74.4	72.3
Habitat	Soil, salt lake, China	Naval area, India	Sediment sample South China Sea, China	Sand-dune soil, Egypt

[a]Cui et al. (2001)

[b]Zhang et al. (2013)

Abbreviations see also legend to *Table 34.1*: *Opt* optimum, *gal* galactose, *glu* glucose, *rib* ribose, *man* mannose, *xyl* xylose, *ara* arabinose

Table 34.3

Short characterization of the four new genera described as members of the family *Nocardiopsaceae*

Characteristic	*Marinactinospora*[a]	*Murinocardiopsis*[b]	*Salinactinospora*[c]	*Spinactinospora*[d]
Type species	*M. thermotolerans*	*M. flavida*	*S. qingdaonensis*	*S. alkalitolerans*
Aerial mycelium	Long spore chains, spores with wrinkled surface	Not formed	Long chains of spores	Long or short chains of ellipsoid and cylindric spores with spiny surface
Substrate mycelium	Branched, without fragmentation	Without fragmentation		Branched, without fragmentation
pH range	nd	nd	7–12	6–10
Temperature range °C	10–55	nd	16–50	16–44
Growth on NaCL (%)	0–5	nd	1–23	1–15
Major fatty acids	10-methyl $C_{18:0}$, iso-$C_{16:0}$, iso-$C_{16:1}$G	iso-C16:0, anteiso-C17:0 and $C_{18:1}\omega 9c$	iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{16:0}$	iso-$C_{16:0}$, anteiso-$C_{17:0}$, $C_{18:0}$
Major Phospholipids	DPG, PC, PG, PIM, PI, uPL	PC, DPG, PG, PI, uLs	DPG, PG, uPLs, uGLs, uLs	DPG, PC, PG, PI
Major menaquinones	11-(H_8, H_{10}), 10-(H_8)	10(H_4), 11(H_4) -12(H_2)	9(H_8), 10(H_2,H_6, H_8)	10(H_6, H_8), 9(H_8)
DNA G + C (%)	72		60.1	71.1
Habitat	Deep sea sediment, South China Sea, China	Mould-contaminated wallpaper, Germany	Salt pond, China	Marine sediment, Yellow Sea, China

[a]Tian et al. (2009)
[b]Kämpfer et al. (2010)
[c]Chang et al. (2011)
[d]Chang et al. (2012)
Abbreviations see legend to *Table 34.1*

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35 The Family *Promicromonosporaceae*

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Abstract
The family *Promicromonosporaceae*, composed of 7 genera and 26 validly named species, is a family of the order *Micrococcales*. Morphologically, most members of the family form a primary mycelium; aerial hyphae are sparse and are formed only on certain media. Individual cells, often fragmentation products of vegetative mycelium, are nonmotile and short rods to coccoid in shape. Chemotaxonomically, members are defined by L-lysine at position 3 of the peptide subunit of the peptidoglycan and a dicarboxylic amino acid in its interpeptide bridge (peptidoglycan type A4α). Members are similar with respect to their polar lipids, major menaquinones, and principal fatty acids. Certain members of *Cellulosimicrobium* have been isolated in the clinical environment and should be considered as opportunistic pathogens. Several species are of industrial significance due to the presence of glucanases, cellulases, xylanases, and mannases. Culture and nonculture studies revealed their presence in diverse environmental samples but rarely in significant numbers.

Taxonomy, Historical and Current

The monogeneric family *Promicromonosporaceae* was established to accommodate *Promicromonospora* because of its distinct phylogenetic position within the suborder *Micrococcineae*, *Actinobacteria*, and the presence of a taxon-specific set of 16S rRNA signature nucleotides (Stackebrandt et al. 1997). In the following years, several new genera were added, either by reclassification of misclassified species [i.e., *Cellulosimicrobium*, by reclassifying *Cellulomonas cellulans* (Stackebrandt and Keddie 1986) as *Cellulosimicrobium cellulans* (Schumann et al. 2001); *Xylanimicrobium*, by transferring *Promicromonospora pachnodae* (Cazemier et al. 2003) as *Xylanimicrobium pachnodae* (Stackebrandt and Schumann 2004); and *Isoptericola*, by reclassifying *Cellulosimicrobium variabile* (Bakalidou et al. 2002) as *Isoptericola variabilis* (Stackebrandt et al. 2004)], or as novel taxa, such as *Myceligenerans* (Cui et al. 2004), *Xylanimonas* (Rivas et al. 2003), and *Xylanibacterium* (Rivas et al. 2004). A molecular emendation of the family was given by Zhi et al. (2009) by revising the set of 16S rRNA gene sequence signature nucleotides, consisting of nucleotides at the following positions: 120 : A; 131–231 : A-G; 196 : U; 342–347 : C-G; 444–490 : A-U; 580–761 : C-G; 602–636 : G-U; 670–736 : A-U; 822–878 : G-C; 823–877 : G-C; 826–874 : C-G; 827: U; 843 : U; 950–1231 : U-A;

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1047–1210 : G-C; 1109 : C; 1145 : G; 1309–1328 : G-C; 1361 ; G; 1383 : C. The type genus is *Promicromonospora* Krasil'nikov, Kalakoutskii and Kirillova 1961, 107^{AL}.

With the second edition of *Bergey's Manual of Systematic Bacteriology*, the class *Actinobacteria* was emended to phylum level (Goodfellow 2012) and the suborder *Micrococcineae* was elevated to the order *Micrococcales* (Busse 2012). Within this order, *Promicromonosporaceae* (Schumann and Stackebrandt 2012a) is 1 of 15 families.

Members of the family resemble each other in most of their chemotaxonomic properties (*Table 35.1*), such as diaminoacid of the peptidoglycan (L-lysine), phospholipid composition (phosphatidylglycerol, diphosphatidylglycerol), major menaquinone (MK-9[H]$_4$), predominant fatty acids (iso- and anteiso-branched), and mol% G+C content of DNA (70–75 mol%). Differences are seen in the structure of the peptidoglycan–interpeptide bridge, where variations occur in the presence of a monocarboxylic amino acid and the nature of the dicarboxylic D-amino acid. By and large, each of the multispecies genera is rather homogeneous with respect to the peptidoglycan structure (*Table 35.1*). Morphologically, the four multispecies genera form a well-developed substrate mycelium that is lacking in the three monospecific genera. Many representatives of the family are capable of degradation of polysaccharides, such as cellulose, chitin, mannan, and xylan.

The phylogenetic structure of the family, as analyzed by the neighbor-joining algorithm, shows the species of the genera *Promicromonospora, Cellulosimicrobium, Isoptericola*, and *Myceligenerans* cluster according to their taxomomic affiliation (*Fig. 35.3b*). The three monospecific genera cluster together as a sister clade of the genus *Isoptericola*. In contrast, according to the maximum-likelihood algorithm (*Fig. 35.3a*), the genus *Myceligenerans* as well as the three monospecific genera branch from within the radiation of *Isoptericola* species. Also, *Promicromonospora flava* does not group with the other members of its genus. Nevertheless, the family appears as a phylogenetically homogeneous entity, well separated from neighboring families—which are, according to the TLP tree (http://www.arb-silva.de/fileadmin/silva_databases/living_tree/LTP_release_106/LTPs106_SSU_tree.pdf), *Rarobacteraceae*, and slightly less close, *Sanguibacteraceae*, *Cellulomonadaceae*, and some other families. This is consistent with the position according to the road map in *Bergey's Manual of Systematic Bacteriology*, second edition (Ludwig et al. 2012).

Molecular Analyses

DNA-DNA Hybridization

DNA-DNA relatedness between selected type strains of the family were mostly performed by the spectroscopic method of Huss et al. (1983) or the colorimetric microdilution plate method of Ezaki et al. (1989) and applied to confirm the species status of the isolates. Some similarity values were moderately high (~40–63 %), such as for *Promicromonospora endophytica* and *P. xylanilytica* (62.6 %, Kaewkla and Franco 2012), *Isoptericola dokdonensis* and *I. jiangsuensis* CLG^T (58.7 %, Wu et al. 2010), *Myceligenerans halotolerans* and *M. xiligouense* (56 %, Wang et al. 2011) and *Cellulosimicrobium cellulans* and *C. funkei* (47 %, Brown et al. 2006). The majority of values, however, were lower than 30 %. At the intraspecies level, data were obtained for *Cellulosimicrobium funkei* (76–79 %, Brown et al. 2006) using the hydroxyapatite method of Brenner et al. (1982).

Phages

Some actinophages that cause true lysis and/or clearing of host cells have been described. P1 (DSM 49141), P2 (DSM 49106), P3 (DSM 49107), and P4 (DSM 43108) are effective against strains of *Promicromonosporas citrea* and *P. sukumoe* (Stackebrandt and Prauser 1991), but other strains of the family have not been tested. *Cellulosimicrobium cellulans* strains are hosts of O5 (DSM 49112; host strain DSM 43881; Stackebrandt and Prauser 1991) and O13 (DSM 49139; host strain DSM 43879^T; Prauser 1986).

RiboPrinting

Both restriction enzymes *Pvu*II and *Pst*I are suited for the generation of RiboPrint patterns for strains of the family *Promicromonosporaceae*. *Pvu*II seems to provide a more complete digestion of the DNA under the conditions used by the RiboPrinter® System because the patterns display less undigested DNA (band at ca. 50 kbp) when compared to the *Pst*I patterns (*Fig. 35.1*). The *Pvu*II- and *Pst*I-generated patterns of all type strains in *Fig. 35.1*, including those of the pairs *Promicromonospora aerolata—P. vindobonensis* and *Cellulosimicrobium funkei—C. cellulans* showing the highest similarities in matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectra and 16S rRNA gene sequences (see below), can easily be differentiated by their band positions.

In many cases, RiboPrint patterns are strain-specific, as demonstrated by an example in the genus *Myceligenerans* (Groth et al. 2005). One of the three strains of *M. crystallogenes* shared the *Pvu*II RiboPrint pattern with the type strain HKI 0369^T, whereas the third isolate HKI 0371 differed from this Ribogroup by displaying an additional strain-specific band. Distinct RiboPrint patterns and the DNA-DNA similarity value of < 15 % support the differentiation of *M. crystallogenes* from its close relative *M. xiligouense* (Groth et al. 2005).

Table 35.1

Differential characteristics of the genera included in *Promicromonosporaceae* (Data are from original genus descriptions and their additions as reported in species descriptions)

Property	*Promicromonospora* 10 species	*Isoptericola* 7 species	*Myceligenerans* 3 species	*Cellulosimicrobium* 3 species	*Xylanimicrobium pachnodae* DSM 12657^T	*Xylanibacterium ulmi* XIL08^T	*Xylanimonas cellulosilytica* XIL07^T
Aerial hyphae	− (+ on some media)	−	− (Sparse on some media)	−	−	−	−
Primary mycelium present	+, fragmenting	+	+	+, fragmenting	Mycelia-like fringes in basal medium	−	−
Morphology of individual cells	Non-motile short V- or Y-shaped rods, or coccoid	Non-motile short rods, V-shaped or coccoid	Irregular non-motile rods and cocci in one species	Motile or non-motile; short rods, or coccoid	Irregular shaped, single or in pairs	Small rods	Coccoid
Fermentation	Rarely	+	w or −	+	+	+	w
Peptidoglycan structure[a]	L-Lys ← L-Ala ← D-Glu	L-Lys ← D-Asp or L-Lys ← D-Glu	L-Lys ← L-Thr ← D-Glu	L-Lys ← D-Ser ← D-Asp, or L-Lys ← L-Thr ← D-Asp	L-Lys ← L-Ser ← D-Glu	L-Lys ← L-Ala ← D-Glu	L-Lys ← D-Asp
Cell wall sugars[a]	Gal, Glc, Rha; Gal, Rha or Gal, Glc	Gal; Gal, Rha, Glc; Rib and/or Xyl may be present	Glc, Man; Gal or Ara may be present	Gal, Rha, Fuc, or Gal	Rha, Gal, Glc	Rha, Fuc, Man, Gal, Ara, Glc	Gal, Rha
Phospholipid composition[a, b]	PG, DPG; PIM and/or PI may be present	PG, DPG, PI	PG, DPG, PI or PIM may be present	PG, DPG (only one species tested)	PG, DPG, PI,	PG, DPG, PI, PIM	PG, DPG, PI, PIM
Major menaquinone partially saturated MK-9	(H_4); (H_8), (H_4) and (H_2) may be present	(H_4); (H_4), (H_2) fully saturated MK-9 occurs in one species	(H_8), (H_6); (H_8) (H_6), (H_4), (H_2); or (H_4)	(H_4)	(H_4)	(H_4), MK-8(H_4)	(H_4), MK-8(H_4)
Predominant fatty acids[a]	ai-$C_{15:0}$, i-$C_{15:0}$; ai-$C_{17:0}$ or i-$C_{16:0}$ may be present	ai-$C_{15:0}$, i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{14:0}$, i-$C_{16:0}$, ai-$C_{17:0}$ i-$C_{14:0}$ and 10-methyl $C_{17:0}$ may be present	ai-$C_{15:0}$, i-$C_{15:0}$; i-$C_{16:0}$ or ai-$C_{17:0}$ may be present	ai-$C_{15:0}$, i-$C_{16:0}$, $C_{16:0}$, i-$C_{15:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, $C_{16:0}$, $C_{14:0}$	ai-$C_{15:0}$, iC$_{15:0}$, $C_{16:0}$, $C_{14:0}$	ai-$C_{15:0}$, i-$C_{15:0}$
DNA mol% G+C	70–72	70–74	72	73–75	70	72	73

Abbreviations: Peptidoglycan—*Asp* aspartic acid, *Lys* lysine, *Ser* serine. Whole cell sugars—*Ara* arabinose, *Gal* galactose, *Rha* rhamnose, *Fuc* fucose, *Man* mannose, *Glc* glucose, *Xyl* xylose. Polar lipids—*PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides

+ positive, − negative, *w* weak

[a]Data from Cazemier et al. (2003), Rivas et al. (2003, 2004), Busse et al. (2003), Schumann et al. (2001), Stackebrandt and Schumann (2004), Cui et al. (2004), Stackebrandt et al. (2004), Zhang et al. (2005), Groth et al. (2005, 2006), and Yoon et al. (2007)

[b]Range of polar lipids may contain one or more additional unknown phospholipids, phosphoglycolipids and/or glycolipids

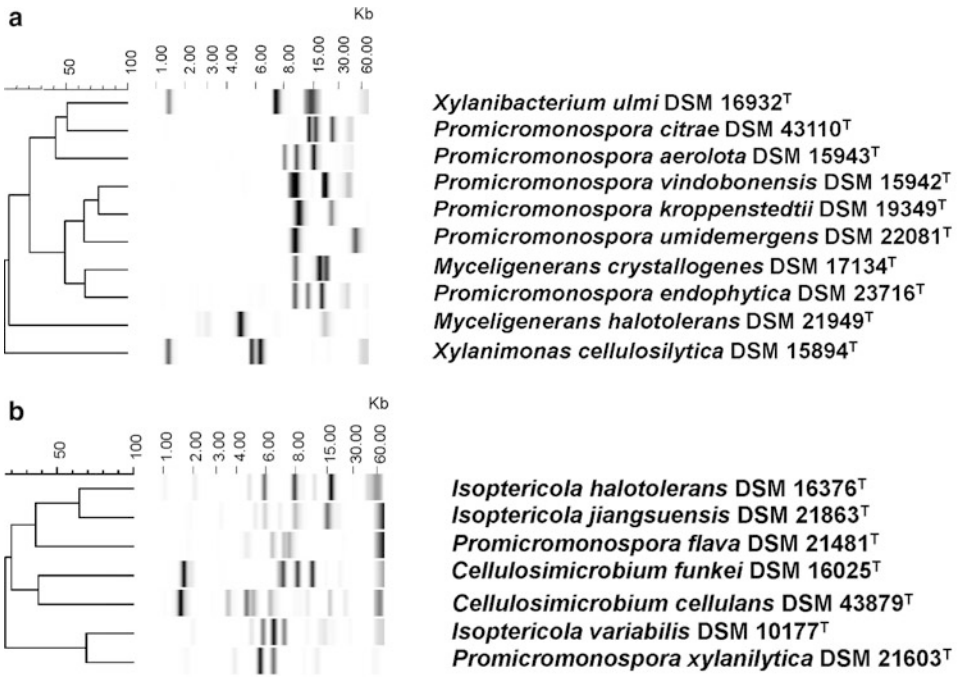

Fig. 35.1

RiboPrint patterns of selected type strains of the family *Promicromonosporaceae* cut by *Pvu*II (a) and *Pst*I (b). The dendrograms were generated with BioNumerics software (Applied Math, Kortrijk, Belgium)

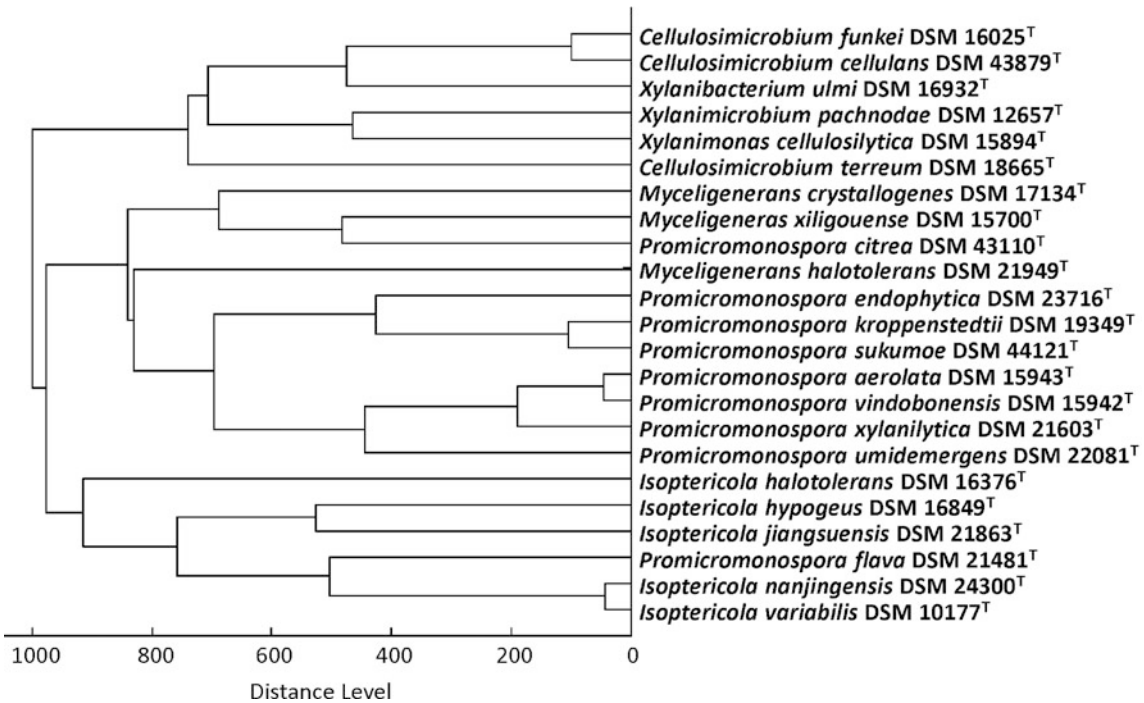

Fig. 35.2

Score-oriented dendrogram generated by the BioTyper software (version 2.0, Bruker Daltonics) showing the similarity of MALDI-TOF mass spectra for cell extracts of type strains of the family *Promicromonosporaceae*

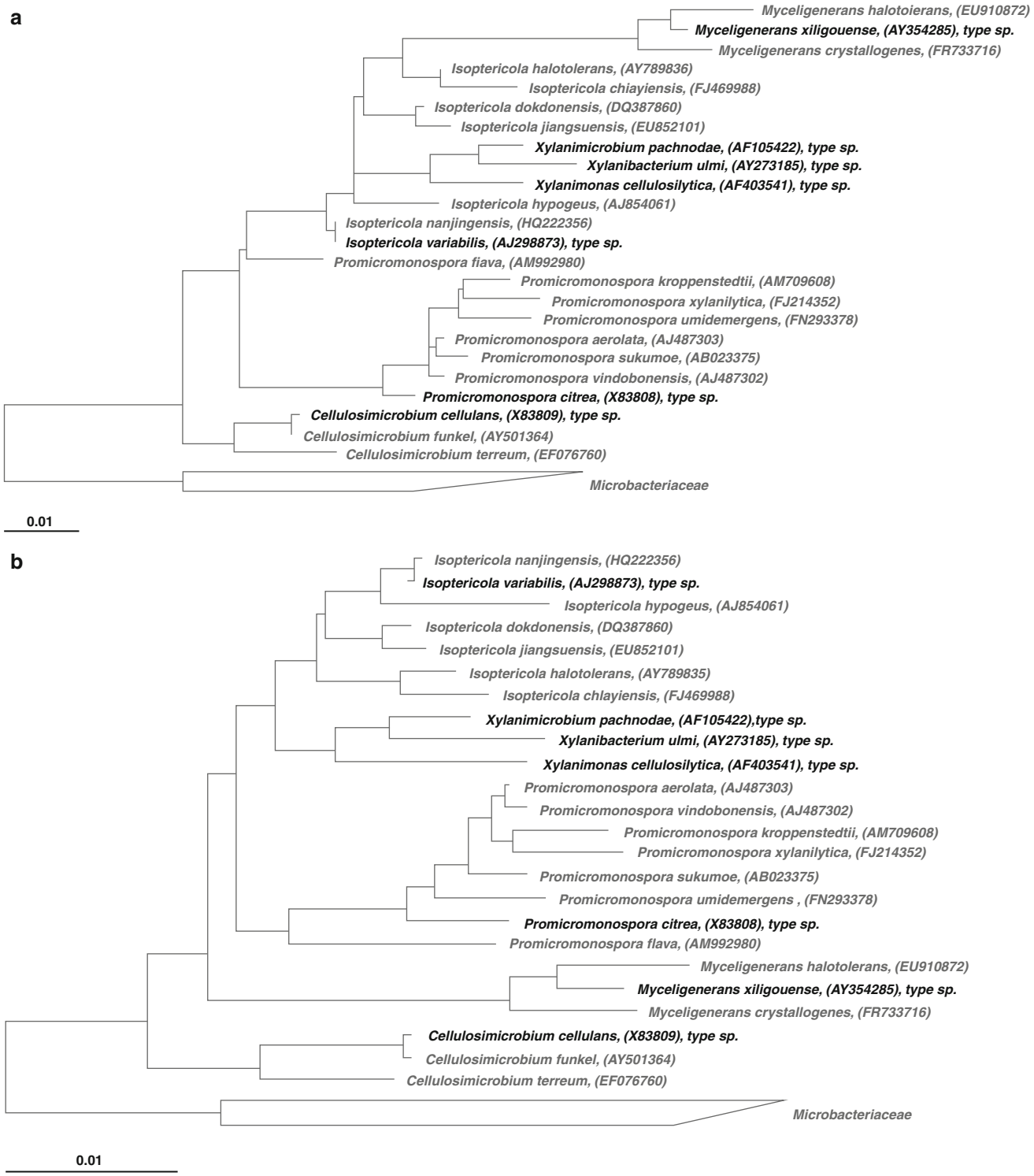

Fig. 35.3

Maximum likelihood based on the randomized axelerated maximum likelihood (RAxML) algorithm (Stamatakis 2006) (a) and Neighbor-Joining (b) genealogy reconstruction of the sequences of all members of the family *Promicromonosporaceae* present in the LTP_106 (Munoz et al. 2011). The trees were reconstructed by using a subset of sequences representative of close relative genera to stabilize the tree topology. In addition, a 60 % conservational filter for the whole bacterial domain was used to remove hypervariable positions. The bar indicates 1 % sequence divergence. Type strain numbers referring to the accession numbers are indicated in *Tables 35.2–35.8*

Table 35.2

Distinguishing phenotypic properties of some *Promicromonospora* type strains[a]

Properties	*P. citrea* ATCC 15908^T Krasil'nikov et al. (1961)	*P. sukumoe* NBRC 14650^T Takahashi et al. (1987)	*P. vindobonensis* V45^T Busse et al. (2003)	*P. aerolata* V54A^T Busse et al. (2003)	*P. kroppenstedtii* RS16^T Alonso-Vega et al. (2008)
API Coryne test					
Nitrate reductase	–	+	+	–	+
Pyrrolidonyl arylamidase	w	w	+	+	–
Urease	w	+	–	–	–
Alkaline phosphatase	–	–	–	–	+
Pyrazinamidase		+	+	+	–
α-Glucosidase	–	–	–	–	+
β-Galactosidase	–	–	–	–	+
N-Acetyl-β-glucosaminidase	–	–	–	–	+
Fermentation of					
Glucose	–	–	–	+	+
Maltose	–	–	–	+	–
Sucrose	–	–	–	+	w
Glycogen	–	–	–	+	–
Xylose	–	–	–	–	+
Acid production from (API 50CH)					
Glycerol, methyl α-glucoside, salicin, sucrose, arbutin, maltose, turanose, amygdalin, melizitose, D-mannose, galactose, D-glucose, trehalose, glycogen	–	–	–	+	nd
D-Fructose	–	–	+	+	nd
Cellobiose	–	+	–	+	nd
Mannitol, *N*-acetylglucosamine	–	–	–	w	nd
Assimilation of					
N-acetylglucosamine	+	+	+	w	+
Acetate	+	+	+	w	–
D-Fructose	+	+	+	+	+
D-Glucose, D-xylose	+	+	+	–	+
D-Maltose	+	+	+	–	+
D-Ribose, sucrose	–	+	w	–	+
D-Sorbitol	–	+	w	–	+
D-Mannose, salicin	–	+	w	–	–
L-Arabinose	+	+	–	–	+
L-Aspartate, L-Histidin	+	+	–	–	nd
Pyruvate	w	+	w	w	+[c]
L-Prolin	w	+	+	w	w[c]
Raffinose	+	–	+	–	–
3-Hydroxybutyrate, DL-lactate, L-malate	+	+	w	w	+[c]
Fumarate	+	+	w	–	+[c]
L-Alanine	w	+	–	–	–[c]
L-Rhamnose	+	–	w	w	–[c]
D-Trehalose[c]	+	+	+	w	+

Table 35.2 (continued)

Properties	*P. citrea* ATCC 15908^T Krasil'nikov et al. (1961)	*P. sukumoe* NBRC 14650^T Takahashi et al. (1987)	*P. vindobonensis* V45^T Busse et al. (2003)	*P. aerolata* V54A^T Busse et al. (2003)	*P. kroppenstedtii* RS16^T Alonso-Vega et al. (2008)
Glutarate	–	–	w	w	–[c]
D-Melibiose, *cis*-aconitate, citrate	–	+	–	–	nd
Propionate	–	–	+	–	–[c]
Cellobiose	+	+	w	+	+
Hydrolysis of					
pNP phenylphosphonate, L-proline pNA, L-alanine pNA, pNP β-D-xylopyranoside, pNP β-D-glucopyranoside	+	+	+	–	+[c]
Esculin	+	+	w	–	+[c]
bis-pNP phosphate	+	+	–	–	+[c]
pNP β-D-galactopyranoside	w	w	–	–	+[c]

All strains are negative for β-glucuronidase; fermentation of ribose, mannitol, and lactose and production of acid (no data available for *P. kroppenstedtii*) from erythrol, D-arabinose, L-arabinose, ribose, xylose, adonitol, methyl-β-xyloside, sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl-α-mannoside, lactose, melibiose, inulin, starch, xylitol, gentobiose, lyxose, tagatose, fucose, arabitol, gluconate, 2-ketogluconate and 5-ketogluconate (API 50CH)
Abbreviations: + positive, *w* weakly positive, –, negative, *nd* no data avialable, *pNA* p-nitrophenyl
[a]Data from Busse et al. (2003) and Alonso-Vega et al. (2008) for *P. kroppenstedtii*
[b]Except for *P. kroppenstedii* (API 50CH), data were obtained according to Kämpfer et al. (1991)
[c]Data from Kämpfer et al. (2010)

MALDI-TOF Mass Spectrometry

MALDI-TOF mass spectrometry (MS) is well suited for the differentiation of species of the family *Promicromonosporaceae*. The pairs of species *Promicromonospora vindobonensis*—*P. aerolata*, *Cellulosimicrobium funkei*—*C. cellulans*, and *Isoptericola nanjingensis*—*I. variabilis* show branching points with the lowest distance levels in the dendrogram based on mass spectra (Fig. 35.2). The species forming these pairs are highly related, as revealed by 16S rRNA gene sequence comparison (Fig. 35.3). However, the high mass spectral similarity of *Promicromonospora kroppenstedtii* and *P. sukumoe* is not supported by a notably high 16S r RNA gene sequence similarity (Fig. 35.3). The MALDI-TOF spectra of *Promicromonospora citrea and P. flava*—the two most deeply branching members of the genus in Fig. 35.3—fall outside of the cluster of the *Promicromonospora* spectra. Spectra of the *Isoptericola* species cluster together, whereas one species each of the genera *Cellulosimicrobium* and *Myceligenerans* (*C. terreum* and *M. halotolerans*) represent a separate lineage in the dendrogram (Fig. 35.2).

Genome Analysis

Full genome sequences are available for *Xylanimonas cellulosilytica* DSM 15894^T (Foster et al. 2010) and the non-type strain *Isoptericola variabilis* 225 (Lucas et al. 2011 unpublished).

The genome of strain DSM 15894^T (accession number CP001821), comprising the main circular chromosome and one 88,604-bp-long plasmid, is 3,831,380 bp with a DNA GC content of 72.5 %. Of the 3,546 genes predicted, 3,485 were protein-coding genes (2,426 with predicted functions) and 61 were RNAs. The number of rRNA operons was 3. In addition, 42 pseudogenes were identified. The majority of genes (68.4 %) were assigned with a putative function, while the remaining genes were annotated as hypothetical proteins. The distribution of 2,403 genes into Clusters of Orthologous Groups functional categories shows that 294 genes (8.4 %) were assigned to carbohydrate transport and metabolism function, whereas 414 genes (6.1 %) were assigned to amino acid transport and metabolism function.

The number of nucleotides of the strain 225 genome (direct submission, accession number NC_015588) is 3,307,740, the number of protein genes is 2,881 (approximately 2,200 are proteins with functional assignments), the number of RNA genes is 60, and the number of rRNA copies is 9.

Phenotypic Analyses

The Family *Promicromonosporaceae* Rainey, Ward-Rainey and Stackebrandt 1997, 484^{VP}, emend. Zhi, Li and Stackebrandt 2009, 598

Pro.mi.cro.mo.no.spo.ra'ce.ae. N.L. fem. n. *Promicromonospora*, type genus of the family; L. suff. *–aceae*, ending to denote a family; N.L. fem. pl. n. *Promicromonosporaceae*, the *Promicromonospora* family.

As of February 2014, the family contained 7 genera and 26 validly named species. The latest edition of *Bergey's Manual of Systematic Bacteriology* (Goodfellow 2012) covers all genera and 16 species. The phylogeneric relationships at the intrafamily and intrageneric levels are depicted in Fig. 35.3, whereas

Table 35.3
Distinguishing phenotypic properties of other *Promicromonospora* type strains[a]

Properties	*P. flava* CC 0387^T Jiang et al. (2009)	*P. umidemergens* 09-Be-007^T Martin et al. (2010b)	*P. thailandica* S7F-02^T Thawai and Kudo (2012)	*P. xylanilytica* YIM 61515^T Qin et al. (2012)	*P. endophytica* EUM 273^T Kaewkla and Franco (2012)
Acid production					
from D-cellobiose	nd		–	+	+
D-Fructose			–	+	+
D-Galactose			–	+	+
D-Mannitol			w	+	+
D-Raffinose			–	+	+
Assimilation of					
D-Fructose	–	+	–	w	nd
D-Glucose	+	+	nd	+	
D-Xylose	–	+	–	nd	
Maltose	–	+	nd	+	
D-Mannose	–	+	nd	+	
Ribose	+	+	nd	–	
Sucrose	+	w	nd	+	
Sorbitol	–	w	nd	–	
Galactose	–	w	+	–	
Lactose	+	+	–	w	
Mannitol	–	–	+	–	
Raffinose	–	+	w	+	
Rhamnose	–	+	–	–	
Sorbose	–	w	nd	–	
Starch	+	+	nd	+	

Abbreviations: + positive, *w* weakly positive, – negative, *nd* no data avialable
[a]Data from type strain descriptions. References are indicated in column headings

Table 35.1 shows the properties defining the genera. The present format lists the salient phenotypic and chemotaxonomic differences of presently validly named species per genus; the original descriptions should be consulted for additional information.

Promicromonospora Krasil'nikov, Kalakoutskii and Kirillova 1961, 107[AL]

pro.mi.cro.mo.no.spo'ra. Gr. pref. *pro* before, primitive; Gr. adj. *mikros* small; Gr. adj. *monos* single, solitary; Gr. fem.n. *spora* a seed, and in microbiology a spore.; N.L.fem.n. *Promicromonospora*, the genus name, was coined to reflect the combination of traits then thought to be characteristic of the actinomycete form genera "*Proactinomyces*" (the tendency of the mycelium to fragment) and *Micromonospora* (the formation of single spores on the substrate mycelium).

Cells are Gram-stain-positive. The members produce branching septate hyphae. The substrate mycelium, which may penetrate into the agar, is well developed and fragments into nonmotile, coccoid, Y-shaped, V-shaped, or curved bacillary elements. If indicated, hyphae are 0.2–0.5 to 0.4–1.0 μm in diameter, whereas fragmented elements are 0.3–0.5 × 0.6–1.5 μm. In some species single-sessile, oval spores are observed on some media (*P. endophytica* [0.5 × 0.6 μm], *P. kroppenstedtii*). No diffusible pigments were produced. Colonies of all type strains were cream to pale yellow, or white to yellowish-white to orange except for *P. thailandica*, whose colonies were pale greenish-yellow. Several type strains degrade xylane and cellobiose.

The chemotaxonomic properties are indicated in *Table 35.1*. Detailed fatty acid patterns were provided by Martin et al. (2010b) and Qin et al. (2012). No study on phenotypic properties compares all type strains; the methods used are often not comparable and the number of tests vary greatly. Although several studies include or concentrate on acid production from carbohydrates, the same selection is not used in all studies. Similarly, the selection of carbohydrates applied in assimilation tests of recently described type strains only allows the comparison of a small dataset. To avoid a large number of undetermined entries, the table of phenotypic properties was split. *Table 35.2* displays the phenotypic reactions for those strains investigated by a large set of reactions, whereas

Table 35.4

Chemotaxonomic properties of type strains of *Isoptericola*. Fatty acids from Huang et al. (2012b)

Characteristic	*I. variabilis* DSM 10177^T Stackebrandt et al. (2004)	*I. hypogeus* DSM 16849^T Groth et al. (2005)	*I. halotolerans* YIM 70177^T Zhang et al. (2005)	*I. dokdonensis* DS-3^T Yoon et al. (2006)	*I. jiangsuensis* CLG^T Wu et al. (2010)	*I. chiayiensis* 06182 M-1^T Tseng et al. (2011)	*I. nanjingensis* H17^T Huang et al. (2012b)
Peptidoglycan variation	L-Lys-D-Asp[a]	L-Lys-D-Glu	L-Lys-D-Asp	L-Lys-D-Asp	L-Lys-D-Asp	L-Lys-L-Ala-L-Glu-D-Asp	L-Lys-D-Asp
Polar lipids	DGP, PG, PI, uPL	DGP, PG, PI, PIM, 2 uPL, uGL	DGP, PG, PI	DGP, PG, PI, 2 uGL	DGP, PG, PI, uPL, 3 uPGL, uL	DGP, PG, PI, 2 uGL, 2 u PGL	DGP, PG,PI, PGL, uPL, uGL, uL
Major menaquinones; MK-	9(H_4)	9(H_2), 9(H_4), 9	9(H_4), 9(H_2)	9(H_4)	n.d.	9(H_4), 9(H_2)	9(H_4)
Cell wall sugars	Gal, Glc, Rha	Gal, Glc, Rha, Man	Gal	Gal, Glc, Rha, Rib	Gal, Rha, Xyl	Gal, Glc, Rha	Gal, Xyl, Man
Major fatty acids (>10 %), [%]	ai-$C_{15:0}$ [53.6], i-$C_{15:0}$ [17.0]	ai-$C_{15:0}$ [44.0], i-$C_{15:0}$ [18.1], i-$C_{16:0}$ [22.6]	ai-$C_{15:0}$ [54.5], $C_{16:0}$ [20.1], ai-$C_{17:0}$ [10.7]	ai-$C_{15:0}$ [58.6], i-$C_{15:0}$ [11.5], ai-$C_{17:0}$ [41.0]	ai-$C_{15:0}$ [60.1], i-$C_{15:0}$ [14.3]	ai-$C_{15:0}$ [55.0], i-$C_{15:0}$ [12.0]	ai-$C_{15:0}$ [48.6], i-$C_{15:0}$ [22.4]
Minor fatty acids (5–10 %)	i-$C_{16:0}$, $C_{14:0}$, $C_{16:0}$	i-$C_{14:0}$	i-$C_{15:0}$	$C_{16:0}$	ai-$C_{17:0}$	$C_{16:0}$, ai-$C_{17:0}$, i-$C_{16:0}$	i-$C_{16:0}$, $C_{14:0}$, $C_{16:0}$
DNA G+C (mol%)	70–72	73.8	72.8	74.1	70.3	72.8	72.4

For abbreviations see the footnote of *Table 35.1*

[a]In the original description (Bakalidou et al. 2002) the murein was described to contain the amino acids lysine, aspartic acid, glutamic acid and alanine in a molar ratio of 1.0:0.9:2.0:1.8. This molar ratio was later corrected to 1:0.9:1:2 of lysine, aspartic acid, glutamic acid, and alanine (Stackebrandt et al. 2004)

Table 35.3 lists those type strains for which a smaller set was determined, either acid production from or assimilation of carbohydrates. For additional reactions, the reader is referred to the original description (shown under the species names in the column headings).

The type species is *Promicromonospora citrea* Krasil'nikov, Kalakoutskii and Kirillova 1961, 107^{AL}. In addition to the validly named species, the literature refers to "*Promicromonospora cymbopogonis*" (Chen et al. unpublished), with a 16S rRNA gene sequence accession number of EU200679.

Isoptericola Stackebrandt, Schumann and Cui, 2002, 686^{VP}

I.sop.te.ri´co.la N.L.n. *Isoptera* order of termites, L. masc. suffix –cola inhabitant; N.L. masc. n. *Isoptericola* inhabitant of termites.

Cells are rod-shaped, V-shaped or coccoid, non-motile (0.8–1.1 × 0.8–4.4 μm), and non-spore-forming. Primary mycelium may be formed, which may fragment into short, irregular, non-motile rods and cocci in the stationary growth phase. An aerial mycelium is absent. The diameters of the colonies are 1–4 mm; they are mostly pale-yellow or orange-yellow. Cells are aerobic to facultative anaerobic, and acid is produced from some carbohydrates. Some strains are cellulolytic and/or xylanolytic. *N*-glycolylmuramic acid, mycolic acids, and hydroxy fatty acids are absent. The acyl type is acetyl. The chemotaxonomic properties are indicated in *Tables 35.1* and *35.4*, whereas the physiological properties are indicated in *Table 35.5*.

The type species is *Isoptericola variabilis* (*Cellulosimicrobium variabile* Bakalidou et al. 2002, 1189^{VP}) Stackebrandt, Schumann, and Cui 2004, 687^{VP}. Strains of the invalidly named species *Promicromonospora yunnanensis* were reclassified as strains of *Cellulosimicrobium cellulans* (Zhang and Liu 2006).

Cellulosimicrobium Schumann, Weiss and Stackebrandt, 2001, 1009^{VP}, emend. Brown, Steigerwalt, Morey, Daneshvar, Romero and McNeil, 2006; emend Yoon, Kang, Schumann, and Oh, 2007

Cel.lu.lo.si.mi.cro'bi.um. N.L. n. *cellulosa* cellulose; Gr. adj. *micros* small; Gr. masc. n. *bios* life; N.L. neut. n. *Cellulosimicrobium* cellulose microbe.

Cells are Gram-positive but very readily decolorized; they are not acid-fast. In young cultures, a primary mycelium is produced; it later fragments (at 1–4 weeks) into irregular, curved, and Y- and club-shaped rods, which may be arranged in V forms. After exhaustion of the medium, the rods are transformed into shorter rods or even spherical cells. Cells, which do not form endospores, are motile or non-motile and chemoorganotrophic. The metabolism

Table 35.5

Distinguishing physiological properties of *Isoptericola* type strains (Data from Huang et al. (2012b). Although some of the data differ from those given in the original species description, the data from Huang et al. (2012b) were used because all type strains were included in the same study, using comparable conditions)

Characteristic	*I. nanjingensis* H17^T	*I. variabilis* DSM 10177^T	*I. hypogeus* DSM 16849^T	*I. jiangsuensis* CLG^T	*I. dokdonensis* DS-3^T	*I. halotolerans* YIM 70177^T	*I. chiayiensis* 06182 M-1^T
Relation to O_2	Aerobic	Facultative anaerobic	Aerobic to microaerophilic	Aerobic	n.d. (anaerobic respiration with nitrate)	Aerobic to microaerophilic	Aerobic
Oxidase	+	+	–	–	+	+	–
Growth at 10 °C	+	–	+	+	+	+	n.d.
Growth at 42 °C	–	+	–	–	–	–	–
Growth with 10 % NaCl (w/v)	+	+	–	+	–	+	+
Nitrate reduction	–	+	+	+	+	–	–
Methyl red	+	+	–	–	–	–	–
Indole	–	+	–	–	+	+	+
Hydrolysis of							
Casein	–	+	+	–	–	–	–
Gelatine	–	+	+	–	+	–	–
Hypoxanthine	+	+	+	–	–	–	–
Tyrosine	–	+	–	–	–	–	–
Urea	–	+	–	–	–	–	–
Xanthine	+	+	+	–	–	–	–
Utilization of							
Amygdalin	+	+	+	+	–	+	–
D-Arabinose	–	+	–	–	–	w	w
D-Arabitol	+	+	w	–	–	–	w
D-Mannitol	+	+	–	–	–	+	–
D-Ribose	+	+	–	+	–	+	w
Gentobiose	+	+	–	+	–	+	+
Inulin	+	–	–	+	–	+	–
Lactose	+	+	–	+	–	–	–
L-Lyxose	+	–	–	–	–	–	–
L-Rhamnose	+	+	–	w	–	–	+
Melibiose	+	–	–	+	–	–	–
Melizitose	+	+	–	+	–	–	–
Methyl-α -D-glucopyranoside	+	+	–	+	–	–	–
Methyl-α -D-mannopyranoside	+	–	–	–	–	–	–
Methyl-β -D-xylopyranoside	+	–	–	–	–	–	–
N-Acetyglucosamine	+	+	–	+	–	–	–
Raffinose	+	+	–	+	–	–	–
Trehalose	+	–	–	+	+	–	w
Turanose	+	+	–	+	+	+	–
API ZYM tests							
Acid phosphatase	–	–	–	+	+	–	–
Alkaline phosphatase	+	+	–	+	+	–	+

Table 35.5 (continued)

Characteristic	*I. nanjingensis* H17^T	*I. variabilis* DSM 10177^T	*I. hypogeus* DSM 16849^T	*I. jiangsuensis* CLG^T	*I. dokdonensis* DS-3^T	*I. halotolerans* YIM 70177^T	*I. chiayiensis* 06182 M-1^T
Esterase (C4)	+	+	–	+	–	w	+
Lipase (C14)	+	+	–	+	–	+	+
Trypsin	–	+	–	+	+	–	+
α-Chymotrypsin	–	+	–	+	+	–	+
α-Galactosidase	+	+	–	+	–	–	–
β-Glucuronidase	+	+	–	–	–	–	–

All taxa are positive for Gram-staining; catalase; hydrolysis of aesculin and starch; utilization of glycerol, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, arbutin, salicin, cellobiose, maltose, sucrose, starch, and glycogen; and the activity of esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α- and β-glucosidase. All taxa are negative for motility and utilization of erythritol, L-xylose, D-adonitol, L-sorbose, dulcitol, inositol, D-sorbitol, xylitol, D-tagatose, D-fucose, L-arabitol, L-fucose, potassium gluconate, and potassium 2- and 5-ketogluconate

– negative, + positive, *n.d.* not determined

is respiratory and the cells are facultatively anaerobic. Acid is produced from several carbohydrates. Cells are catalase positive and cellulolytic. Nitrate reduction is positive or negative. The major menaquinone is MK-9(H_4); other chemotaxonomic and physiological properties differentiating between type strains are indicated in Table 35.6.

C. cellulans forms acetic acid as the main acidic intermediary product of aerobic glucose dissimilation. Anaerobically, the resting cells of this species ferment glucose predominantly to CO_2, acetic acid, and L-lactic acid, whereas ethanol and formic acid are minor end products. Other species were not investigated. Additional properties are indicated in the original descriptions, summarized by Schumann and Stackebrandt (2012b).

The type species is *Cellulosimicrobium cellulans*.

The rational for the reclassification of *Nocardia cellulans*, *Oerskovia xanthineolytica*, and related species first as *Cellulomonas cartae* (Stackebrandt and Kandler 1980), then as *Cellulomonas cellulans* (Stackebrandt and Keddie 1986) and subsequently as *Cellulosimicrobium cellulans*, (Schumann et al. 2001) has been outlined by Stackebrandt et al. (2002) and Schumann and Stackebrandt (2012b).

The morphological cycle of strains of *C. cellulans* (described for *Nocardia cellulans* by Metcalfe and Brown 1957) on nutrient agar (similar on yeast-extract peptone agar) includes the following stages: After 2 days of incubation at 25 °C, the cells consist of branching aseptate filaments that are 30–40 μm in length, often with swellings at intervals. Shorter filaments are present, but few are less than 7.0 μm in length. Fragmentation commences after the fourth day, and the number of short rods (1.5–2.0 × 1.0 μm) increases rapidly. Branched filaments (up to 10 μm in length) are present until the 10th day or later. Hyaline spore-like structures are produced from the seventh day on as slight terminal swellings on the filaments. After 28 days, the colonies consist of very short rods, cocci, and spore-like cells.

In *C. funkei*, rods are short (1 μm) and thin, whereas those of *C. terreum* are 0.4–0.8 × 0.4–2.0 μm. The color of the colonies depends upon the media composition; it ranges from creamy yellow to bright yellow.

Myceligenerans Cui, Schumann, Stackebrandt, Kroppenstedt, Pukall, Xu, Rohde and Jiang 2004, 1292^{VP}, emend. Wang, Tang, Lou, Mao, Jin, Klenk, Zhang and Li 2011, 976^{VP}

My.ce.li.ge.ne.rans. N.L. neut. n. *mycelium*, filamentous cell; L. part. adj. *generans*, producing; N.L. neut. subst. *Myceligenerans*, hyphae-forming microbe.

Cells are Gram-positive. The substrate mycelium is well developed and branched in and on the media. The aerial mycelium is absent or sparse (*M. halotolerans*). The surface of the substrate mycelium bears fragmented cells and spore chains, with one or two non motile spores at the tips of the mycelium. Spores are coccoid to rod-shaped and nonmotile. (The description of *M. halotolerans* does not mention spores but indicates fragmentation of the mycelium.) Mycolic acids are absent. Cells are aerobic to microaerophilic. The peptidoglycan type is A4α, with variation L-Lys ← L-Thr ← D-Glu. If determined, the acyl type is acetyl. Other chemotaxonomic as well as physiological differentiating properties are indicated in Table 35.7.

The type species is *Myceligenerans xiligouense*.

The three monospecific genera *Xylanimicrobium*, *Xylanibacterium*, and *Xylanimonas* are compared together because of their ability to degrade xylan and a high number of common physiological reactions (see Table 35.7). They can be distinguished from each other by several chemotaxonomic properties (see Table 35.1).

Xylanimicrobium Stackebrandt and Schumann, 20041385^{VP}

Xy.la.ni.mi.cro'bi.um. N.L. neut. n. *xylanum* xylan, a polysaccharide; Gr. adj. *mikros* small; Gr. masc. n. bios life; N. L. neut. n. *Xylanimicrobium* xylan-hydrolysing microbe.

White, dry, and wrinkled colonies are formed on plates with basal medium 2 containing glucose. In addition to the properties listed in Tables 35.1 and 35.8, growth occurs

Table 35.6

Phenotypic characteristics that differentiate *Cellulosimicrobium cellulans*, *C. funkei* and *C. terreum* (Data are from Brown et al. (2006) and Yoon et al. (2007))

Properties	*C. cellulans* ATCC 12830^T Schumann et al. 2001	*C. funkei* W6122^T Brown et al. 2006	*C. terreum* DS-61^T Yoon et al. 2007
Color of colonies	Yellow whitish to cream	Pale yellow	Yellow
Motility	–	Motile by one to five polar and/or lateral flagella[a]	–
Peptidoglycan type	L-Lys-D-Ser-D-Asp	n.d.	L-Lys-L-Thr-D-Asp
Polar lipids	n.d.	n.d.	DPG, PG, PI, PL
Main cell-wall sugar	Gal, Rha, Glc, Fuc, Man	Gal	Gal
Major fatty acids, [>10 %]	ai-$C_{15:0}$ [35.7], i-$C_{15:0}$ [16.5], ai-$C_{17:0}$ [14.8], i-$C_{16:0}$ [12.1]	ai-$C_{15:0}$ [44.4], i-$C_{15:0}$ [21.9], ai-$C_{17:0}$ [15.1]	ai-$C_{15:0}$ [52.5], i-$C_{15:0}$ [14.5]
Minor fatty acids, 5–10 %	$C_{16:0}$	$C_{16:0}$, i-$C_{16:0}$	$C_{16:0}$, i-$C_{16:0}$, ai-$C_{17:0}$
DNA mo% G+C	74	74.5	72.9
Nitrate reduction	+	–	+
Hydrolysis of gelatine	+	+	–
Hydrolysis of urea	+	+	+
ß-Galactosidase	+	+	–
ß-Glucodidase	–	w	–
N-Acetyl-ß-glusosaminidase	+	+	–
Acid production from			
Melibiose	+	–	–
L-Arabinose	–	–	+
Resistance to ampicillin	+	+	–

All strains are positive for catalase; hydrolysis of casein, hypoxanthine, and xanthine; acid production from D-glucose and sucrose; utilization of D-glucose, cellobiose, D-mannose, D-xylose, L-arabinose, sucrose, maltose, alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase. All are negative for hydrolysis of tyrosine; acid production from L-rhamnose, D-mannitol, inositol and D-sorbitol; utilization of L-malate, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase

For abbreviations see the footnote of *Table 35.1*

[a]Sottnek et al. (1977)

under aerobic and anaerobic conditions and is accompanied by secretion of xylanases and endoglucanases. Fermentation products on glucose and xylose are formate, lactate, ethanol, and acetate. Optimum growth is at a pH of 7.5 and a temperature of 35 °C. The type species is *Xylanimicrobium pachnodae* Cazemier et al. 2003).

Xylanibacterium Rivas, Trujillo, Schumann, Kroppenstedt, Sánchez, Mateos, Martinez-Molina, Velázquez 2004, 560^{VP}

Xy.la.ni.bac.te'ri.um. N.L. neut. n. *xylanum* xylan, a polysaccharide; N.L. neut. n. *bacterium* from Gr. neut. n. baktron small rod; N.L. neut. n. *Xylanibacterium* xylan-hydrolyzing small rod.

In addition to the properties listed in *Tables 35.1* and *35.8*, other physiological traits are indicated in the original species description of the type species *Xylanibacterium ulmi* (Rivas et al. 2004). The optimal pH for growth is 7. Colonies grown on yeast extract + dextrose (YED) medium are circular convex, white, opaque, and usually 1–3 mm in diameter within 7 days at 28 °C.

Xylanimonas Rivas, Sánchez, Trujillo, Zurdo-Piñeiro, Mateos, Martinez-Molina, Velázquez 2003, 102^{VP}

Xy.lan.i.mo.nas. N.L. n. *xylanum* xylan, a polysaccharide; Gr. n. *monas* a unit; N.L. n. *Xylanimonas* a monad from xylan.

In addition to the properties listed in *Tables 35.1* and *35.8*, other physiological traits are indicated in the original species description of the type species *Xylanimonas cellulosilytica* (Rivas et al. 2003). Optimal growth occurs at a temperature of 30 °C and a pH of 7. Colonies grown on YED medium are circular, convex, white, and opaque and usually 1–3 mm in diameter within 7 days at 28 °C.

Table 35.7

Chemotaxonomic and physiological properties distinguishing type strains of *Myceligenerans* (Data are from Groth et al. (2006) and Wang et al. (2011), excluding variable Biolog GP2 reactions)

Characteristic	*M. xiligouense* XLG9A10.2^T Cui et al. 2004	*M. crystallogenes* DSM 17134^T Groth et al. 2006	*M. halotolerans* XJEEM 11063^T Wang et al. 2011
Pigmentation	Yellow	White to cream	White to cream
Temperature range for growth (°C)	4–50	10–40	10–40
NaCl tolerance (w/v, %)	2–17.5	0–5	0–10
Nitrate reduction	–	+	+
Cell wall sugars	Glc, Man, Gal	Glc, Man, Gal	Glc, Man, Ara
Major menaquinone, MK-	9(H_4), 9(H_6)	9(H_8), 9(H_4), 9(H_6), 9(H_2)	9(H_4).
Polar lipids	DPG, PG, PI, uPL, uGl	DPG, PG, uPL, uGL	DPG, PG, PI, PIM, uPL, uGL
Major fatty acids (%)	ai-$C_{15:0}$, i-$C_{15:0}$[a]	ai-$C_{15:0}$ [54.4], i-$C_{15:0}$ [18.5], i-$C_{16:0}$ [16.5]	ai-$C_{15:0}$ [41.3], i-$C_{15:0}$ [31.4], ai-$C_{17:0}$ [19.1]
Utilization of acetate	+	+	–
Decomposition of			
L-Tyrosine	–	+	–
Urea	–	+	–
Casein	+	+	–
Tween 80	+	+	–
Xylan	+	+	–
Xanthine	–	–	+
Adenine	–	–	+
Biolog GP2 test panel			
Glycogen	+	–	–
Mannan	+	–	–
Arbutin	+	–	+
myo-Inositol	–	–	+
D-Mannose	–	+	+
D-Melibiose	+	–	+
α-Methyl-D-Galactoside	+	–	+
L-Rhamnose	–	–	+
D-Sorbitol	+	–	+
α-Ketovaleric Acid	+	+	–
Lactamide	–	–	+
Pyruvic Acid Methyl Ester	+	+	–
Putrescine	–	–	–
Adenosine	+	–	+
Uridine-5′-Monophosphate	–	–	+
D-Glucose-6-Phosphate	+	–	–
Enzyme assay (API Zym)			
Alkaline phosphatase	+	+	–
Leucine arylamidase	+	+	–
Cystine arylamidase	+	+	–
Acid phosphatase	+	+	–
α-Galactosidase	+	+	–
β-Galactosidase	+	+	–
N-Acetyl-β- glucosamidase	+	+	–
Trypsin	+	+	–

All strains decompose esculin, starch, and gelatin. In the Biolog GP2 test panel, all strains were positive for oxidation of dextrin, Tween 40, maltotriose, D-xylose, pyruvic acid, and thymidin; one of the strains oxidized *N*-acetyl-β-D-mannosamine, xylitol, α- hydroxybutyric acid, β- hydroxybutyric acid, γ-hydroxybutyric acid, ρ-hydroxy-phenylacetic acid, α-ketoglutaric acid, α-methyl-D-mannoside L-fucose, D-galacturonic acid, D-malic acid, L-malic acid, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, adenosine-5′-monophosphate, and thymidine-5′-monophosphate

– Negative, + positive

[a]No percentages were indicated by Cui et al. (2004)

Table 35.8

Physiological properties differentiating type strains of *Xylanimicrobium pachnodae* DSM 12657^T, *Xylanibacterium ulmi* XIL08^T, and *Xylanimonas cellulosilytica* XIL07^T. Data from Cazemir et al. (1999), Rivas et al. (2003, 2004), respectively and the compilation of Stackebrandt and Schumann (2004)

Reactions for	*Xylanimicrobium pachnodae* VPCX2^T Cazemier et al. (2003)	*Xylanibacterium ulmi* XIL08^T Rivas et al. (2004)	*Xylanimonas cellulosilytica* XIL07^T Rivas et al. (2003)
Morphology	Irregular rods, spherical cells in the stationary phase	Small rods	Spherical cells
Catalase	+	–	w
Oxidase	+	+	+
Nitrate reduction	+	–	+
Hydrolysis of gelatin	+	w	+
Urease	–	–	–
N-Acetyl-β-glucosamine	+	+	–
Acetate	+	+	–
Arbutin, L-fucose, salicin, glycogen, D-fucose, rhamnose	–	–	+
Starch	–	n.d.	+
Amygdalin, lactose, maltose, tagatose	+	–	+
Gluconate, 2-ketogluconate, ribose, xylitol	–	+	–
5-Ketogluconate	–	+	+

All strains are xylanolytic and aerobic or facultatively anaerobic. All strains use the following compounds of the API 50CH substrate panel: glycerol, L-arabinose, D-xylose, L-lyxose, galactose, D-fructose, D-mannose, cellobiose, Aesculin, sucrose, trehalose, β-gentiobiose, and D-turanose. None of the strains use erythritol, D-arabinose, D-arabitol, adonitol, dulcitol, inositol, mannitol, melizitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-xyloside, sorbitol, D-raffinose, L-sorbose, inulin, L-arabitol, or L-xylose

Isolation, Enrichment and Maintenance Procedures

Isolation and Enrichment

A wide range of isolation techniques and isolation media were reported in the original descriptions of *Promicromonosporaceae* type strains. Rather than listing the techniques individually, the most salient isolation features are compiled in *Table 35.9*. In some cases, antibiotics were used in the isolation medium to suppress the growth of Gram-negative bacteria and eukaryotes (e.g., 20–25 mg nalidixic acid, 50 mg L^{-1} cycloheximide, and 50–100 mg L^{-1} nystatin). Variations and different combinations of the methods and media listed were also used by other authors, such as Al-Awadhi et al. (2007), who studied alkaliphilic and halophilic hydrocarbon-utilizing bacteria from the Kuwaiti coasts of the Arabian Gulf; Radwan et al. (2010), who isolated epilithic hydrocarbon-utilizing diazotrophic bacteria from the Arabian Gulf coasts; and Ten et al. (2005) who used dye-labelled substrates for simultaneous screening of polysaccharide-degrading and protein-degrading micro-organisms. Snow samples spiked with different concentrations of glucose, starch, and a combination of both carbohydrates were used in the isolation of promicromonosporas from Antarctic snow (Antony et al. 2009, 2012).

Maintenance Procedures

Most members of the family can be maintained in trypticase-soy-yeast extract (TSY) medium (per liter: trypticase soy broth 30.0 g, yeast extract 3.0 g, agar 15.0 g, pH 7.0–7.2) or in glucose-yeast extract-malt extract (GYM) Streptomyces medium (per liter: glucose 4.0 g, yeast extract 4.0 g, malt extract 10.0 g, CaCO3 2.0 g, agar 12.0 g, with pH adjusted to 7.2 before adding agar and $CaCO_3$ deleted if liquid medium is used). See the DSMZ catalogue of strains (DSMZ; http://www.dsmz.de/catalogues/catalogue-microorganisms.html) for the maintenance media of family members.

The recommended provisions for preservation are serial transfers at 4-week intervals, followed by maintenance at 4 °C and storage of cells as 20 % (w/v) glycerol suspensions at −20 °C and −80 °C. Long-term preservation methods include freeze-drying in skim milk or in liquid TSY medium supplemented with 5 % dimethyl sulfoxide and maintenance in the vapor phase of liquid nitrogen at −196 °C.

Ecology

As of July 2012, BLAST 16S rRNA gene sequence hits greater than 98 % confirmed and extended the range of habitats of

Table 35.9

Isolation source, sample treatment, isolation medium and isolation conditions described for members of *Promicromonosporaceae*

Type strain of	Origin	Sample treatment	Isolation medium	Isolation conditions
Promicromonospora				
P. xylanilytica	Leaves of *Maytenus austroyunnanensis*	Qin et al. (2008, 2009)	Xylan-arginine agar [per liter: 2.5 g xylan, 1.0 g arginine, 1.0 g $(NH_4)_2SO_4$, 2.0 g $CaCl_2$, 1.0 g K_2HPO_4, 0.2 g $MgSO_4$, $7H_2O$, 10 mg $FeSO_4 \times 7H_2O$, 15.0 g agar; pH 7.2]	28 °C, 2 weeks
P. endophytica	Root of Grey Box tree	Sterilization with 70 % ethanol and 6 % hypochlorite for 5 min each, rinsed several times with sterile water and then with 10 % $NaHCO_3$ for 10 min followed by rinsing with sterile water	VL70 medium (Schoenborn et al. 2004)	27 °C, 3 weeks
P. thailandica	Marine sediment	Thawai et al. (2005)	HV agar (Hayakawa and Nonomura 1987)	n.i.[a]
P. enterophila	Fresh excrement from *Chromatoiulus projectus* Verh. (Diplopoda)	Direct plating	Diverse, such as nutrient agar (Difco); synthetic glucose agar (Márialigeti 1979); glycerol-arginine agar (El-Nakeeb and Lechevalier 1963)	28 °C, 2 weeks
P. kroppenstedtii	Sandy soil	Direct plating	Yeast extract-malt extract agar (Shirling and Gottlieb 1966)	28 °C, 5 days
P. flava	Sediment sample Baltic Sea, Germany	Direct plating	Fucose-proline medium [per liter Baltic Sea water: 5 g fucose, 1 g proline, 1 g $(NH_4)_2SO_4$, 1 g NaCl, 2 g $CaCl_2$, 1 g K_2HPO_4, 1 g $MgSO_4 \times 7H_2O$, 20 g agar; pH 7.2]	28 °C, 3 weeks
P. umidemergens	Wall colonized with molds	1 g sample material was shaken for 15 min in 10 ml 0.9 % NaCl solution containing 0.01 % (v/v) Tween 80	M79 agar (Prauser and Falta 1968)	28 °C, 2 weeks
P. vindobonensis *P. aerolata*	Air of the 'Virgilkapelle'; Vienna, Austria	BIOTEST Hycon RCS Plus air-sampler	PYES agar (Zlamala et al. 2002)	n.i.
P. citrea	Garden soil (Krasil'nikov et al. 1961)	n.i.	n.i.	n.i.
P. sukumoe	Soil sample collected at Sukumo city (Japan) Takahashi et al. (1987)	n.i.	n.i.	n.i.
Cellulosimicrobium				
C. cellulans	Soil beneath a chalk grassland plant community, UK	Washed filter paper placed on the surface of basal medium impregnated silica gel (Metcalfe and Brown 1957) was seeded with minute soil crumbs	Alternate growth in yeast-extract peptone agar and tubes containing liquid basal medium with strips of washed filter paper	25 °C, 2–3 days for first growth, duration for obtaining pure cultures not indicated
C. funkei	Blood of an endocarditis patient	Isolation protocol for the type strain or related strains is not recorded	Isolates of related strains were inoculated onto heart infusion agar with 5 % rabbit blood (BBL Microbiology Systems)	n.i.
C. terreum	Soil sample from Dokdo, Korea	Dilution plating	10× diluted nutrient agar (Difco)	25 °C
Isoptericola				
I. variabilis	Gut contents of *Mastotermes darwiniensis* (Froggatt)	Intestinal content of 10 termites was suspended in 10 ml sterile 0.85 % (w/v) NaCl solution. Aliquots of 0.5 ml were suspended in 4.5 ml of Medium A containing 0.2 g/l yeast extract and 5 g/l xylan. After 3 days 100 µl of enrichment culture was plated on Medium A solidified by 15 g/l agar (Schäfer et al. 1996)	Medium A (Kuhnigk et al. 1994): 10.8 mM K_2HPO_4, 6.9 mM KH_2PO_4, 21.5 mM KCl, 24.1 mM NaCl, 5.3 mM $MgSO_4$, 0.53 mM $CaCl_2$, 10 ml/l vitamin and trace element solution (Balch et al. 1979)	aerobic cultivation at 28 °C

Table 35.9 (continued)

Type strain of	Origin	Sample treatment	Isolation medium	Isolation conditions
I. hypogeus	Tufa sample from the catacomb of Domitilla Rome, Italy	Dilution plating	PY-BHI agar (Yokota et al. 1993)	n.i.
I. halotolerans	Saline soil, China	Dilution plating	Modified Horikoshi medium (Horikoshi and Grant 1998).	n.i.
I. dokdonensis	Soil sample from Dokdo, Korea	Dilution plating	Nutrient agar (Difco).	25 °C
I. chiayiensis	Mangrove soil sample, Chiayi County, Taiwan	Direct plating	HV agar (Hayakawa and Nonomura 1987)	30 °C, 4 weeks
I. nanjingensis	Soil sample, Nanjing, Jiangsu Province, China	Dilution plating	Sucrose–mineral salts medium: [per liter: 10.0 g sucrose, 0.5 g yeast extract, 1 g $(NH_4)_2SO_4$, 2 g K_2HPO_4, 0.5 g $MgSO_4$, 0.1 g NaCl, 0.5 g, $CaCO_3$ and 15 g agar	n.i.
I. jiangsuensis	Beach sand, Lianyungang, Jiangsu Province, China	Direct plating	Colloidal chitin as a sole carbon source. No medium indicated	n.i.
Myceligenerans				
M. halotolerans	Soil sample Qijiaojing salt lake, Xinjiang province, China	Direct plating	Glucose-tryptone-yeast medium with 5 % (w/v) NaCl (Tang et al. 2010).	37 °C, 2 weeks
M. crystallogenes	Tufa sample from the catacomb of Domitilla Rome, Italy	Dilution plating	PY-BHI agar (Yokota et al. 1993)	28 °C, 10 days
M. xiligouense	Pasture near an alkaline salt marsh in the Qinghai province, China	Dilution plating	Marine agar, pH 7.2 (Bacto)	28 °C
Xylanibacterium ulmi	*Ulmus nigra* wood decay, Spain	1 g, suspended in sterile water, was stirred for 60 min	100 µl was spread on XED medium (0.7 % xylan, 0.3 % yeast extract, 2.5 % agar)	28 °C, 10 days
Xylanimonas cellulosilytica	*Ulmus nigra* sawdust from a decayed tree, Spain	1 g, suspended in sterile water, was stirred for 30 min	100 µl was spread on XED medium (0.7 % xylan, 0.3 % yeast extract, 2.5 % agar)	28 °C
Xylanimicrobium pachnodae	Hindgut of larvae of the rose chafer *Pachnoda marginata* (Scarabaeidae, Coleoptera)	Isolated after enrichment of bacteria by using Media I and II under aerobic and anaerobic conditions. The hindgut suspension in medium I was plated on solidified Medium II containing xylan (1 g/l) or carboxymethylcellulose (10 g/l). Colonies with cellulolytic or hemicellulolytic activity were selected for restreaking on Medium II plates with xylan or carboxymethylcellulose (Cazemier et al. 2003)	Medium I: peptone, 5 g/l; yeast extract, 2 g/l; K_2HPO_4, 1 g/l; $MgSO_4.7H_2O$, 0.2 g/l; $Na_2S.9H_2O$, 0.16 g/l; cysteine, 0.32 g/l.; Na_2CO_3, 10 g/l (sterilized separately); pH 10.3. Medium II: K_2HPO_4, 1.9 g/l; KH_2PO_4, 0.94 g/l; $NaHCO_3$, 1.68 g/l; KC1, 1.6 g/l; NaCl, 1.43 g/l; NH_4Cl, 0.15 g/l; $MgSO_4.7H_2O$, 0.037 g/l; $CaCl_2.2H_2O$, 0.017 g/l; yeast extract, 0.1 g/l; $Na_2S.9H_2O$, 0.16 g/l and cysteine, 0.32 g/l; 0.2 ml/l trace element solution (Vishniac and Santer 1957); pH 7–7.2. Resazurine (0.0001 %, w/v) was added as a redox indicator to the anaerobic media The gas phase was N_2/CO_2 (80/20, at 0.5 atm overpressure). For cultivation under aerobic conditions $Na_2S.9H_2O$, cysteine and resazurine were omitted from media I and II and $NaHCO_3$ from medium II	Cultures were incubated on a rotary shaker (100 rpm) at 30 °C

[a] *n.i.* not indicated

cultured and as as-yet uncultured strains. Many records, however, do not indicate the isolation source of organisms and DNA. *Promicromonospora* strains were recorded as endophytes of *Jatropha* spp. (Madhaiyan and Ji unpublished; JQ659853); medicinal plants in Panxi plateau, China (Zhao et al. 2011); scabby potatoes (Wanner unpublished; EU080946), tropical plants (Qin et al. 2009), and native plants of unrecorded origin (Kaewkla and Franco unpublished; GU434253);

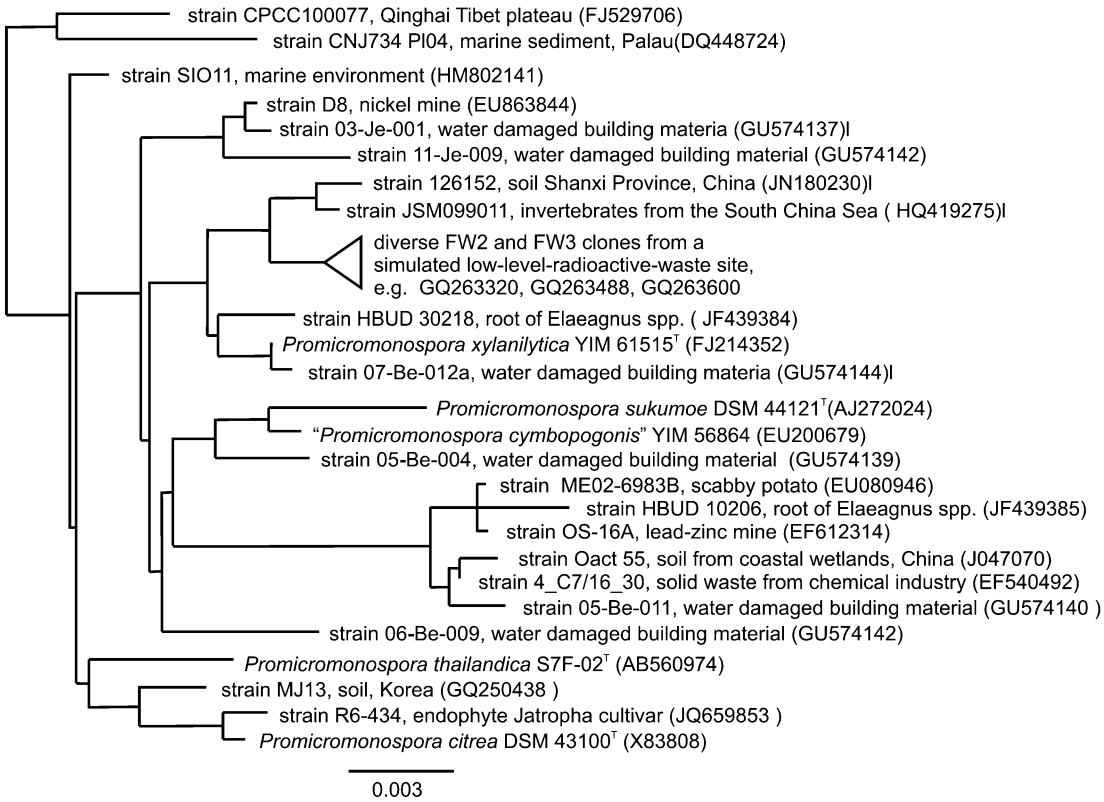

Fig. 35.4
BLAST Fast Minimum Evolution tree of 16S rRNA sequences of *Promicromonospora* clones and isolates

laboratory-scale composters (Xu et al. 2011); in root nodules of *Elaeagnus angustifolia* (Chen and Zhang unpublished; JF439384); marine invertebrates (Chen unpublished; HQ419275); soil (Lee unpublished; GQ250438; Kim unpublished; DQ906913), straw-decomposing microorganisms (Wang and Sun unpublished; JQ798496), members of communities in coal beds (Tan et al. unpublished; JF417733), nickel mines (Lan and Rahman unpublished; EU863844), and lead-zinc mines (Mendez et al. 2008); chemically contaminated waste (Vedler et al. unpublished; EF540492) and hydrocarbon-contaminated soil (Paidisetti unpublished; DQ297997); marine microbial communities (Xu et al. unpublished; HM802141; Donachie et al. unpublished; AY345426; Yu et al. unpublished; HM802141); and brackish water (Jia et al. unpublished; JQ801173). Several entries for the strain originate from mold-colonized water in damaged building materials (Schäfer et al. 2010) and for clones from a low-level radioactive waste site (Field et al. 2010) (see *Fig. 35.4*).

A similar broad range of habitats has also been reported for strains and clones from *Isoptericola* (*Fig. 35.5*), ranging from soil (Valenzuela-Encinas et al. 2009; Cao et al. unpublished, EU910875; Zhang et al. unpublished, FJ906972; Yamaguchi et al. unpublished, AB637292; Osman unpublished, JF508368; Tudahong et al. unpublished, HM448811), rhizosphere (Lee et al. unpublished, e.g., FN178383), a lake (Xia et al. unpublished, HQ738825), river delta (Jia et al. unpublished, e.g., JQ7162209), mangrove sediment (Liao et al. unpublished, FJ176393), deep-sea sediment (Xu et al. unpublished, EU181261), the gut of the moth *Helicoverpa armigera* (Rajagopal and Gayatri Priya unpublished; JF714416), compost (Narihiro et al. 2004; Hayakawa et al. unpublished, AB562485), and phenol-chemostat enrichment (Futamata et al. 2005; AB167235).

Cellulosimicrobium spp. have been found in compost (Narihiro et al. 2004; Hiraishi et al. 2003; Ohnishi et al. unpublished; AB210965), degradation of phenol (Chen and Wei unpublished; HQ619223), bagasse (Tong et al. unpublished; DQ530364), toluene (Chen et al. unpublished; GU966673), cellulose hydrolysis (Lo et al. 2009), Indian lake water (Joshi et al. 2008), deep sea sediment (Luo et al. unpublished; HM222665), hexane-degrading biofilters (Friedrich unpublished; AJ313025), poly(L-lactic acid) depolymerization (Wang et al. unpublished; GQ274926), the gut of *Holotrichia parallela* larvae (Huang et al. 2012a; JQ291586), the gut of the red turpentine beetle *Dendroctonus valens* (Morales-Jiménez et al. 2009), the intestinal tracts of earthworms *Eisenia fetida* (Hong et al. 2011), and formation of 7-prenylindole (Zhu et al. unpublished; HM623867). Strains of *C. funkei* were identified as culturable airborne bacteria from duck houses (Martin et al. 2010a), whereas Murphy et al. (2005) isolated *Cellulosimicrobium* strains from duck feces. No high scores are reported for *C. terreum*.

The presence of a strain of *Xylanimicrobium pachnodae* in the midgut and hindgut of *Pachnoda ephippiata* was reported by Egert et al. (2003). No close relatives were found for *Xylanimonas*

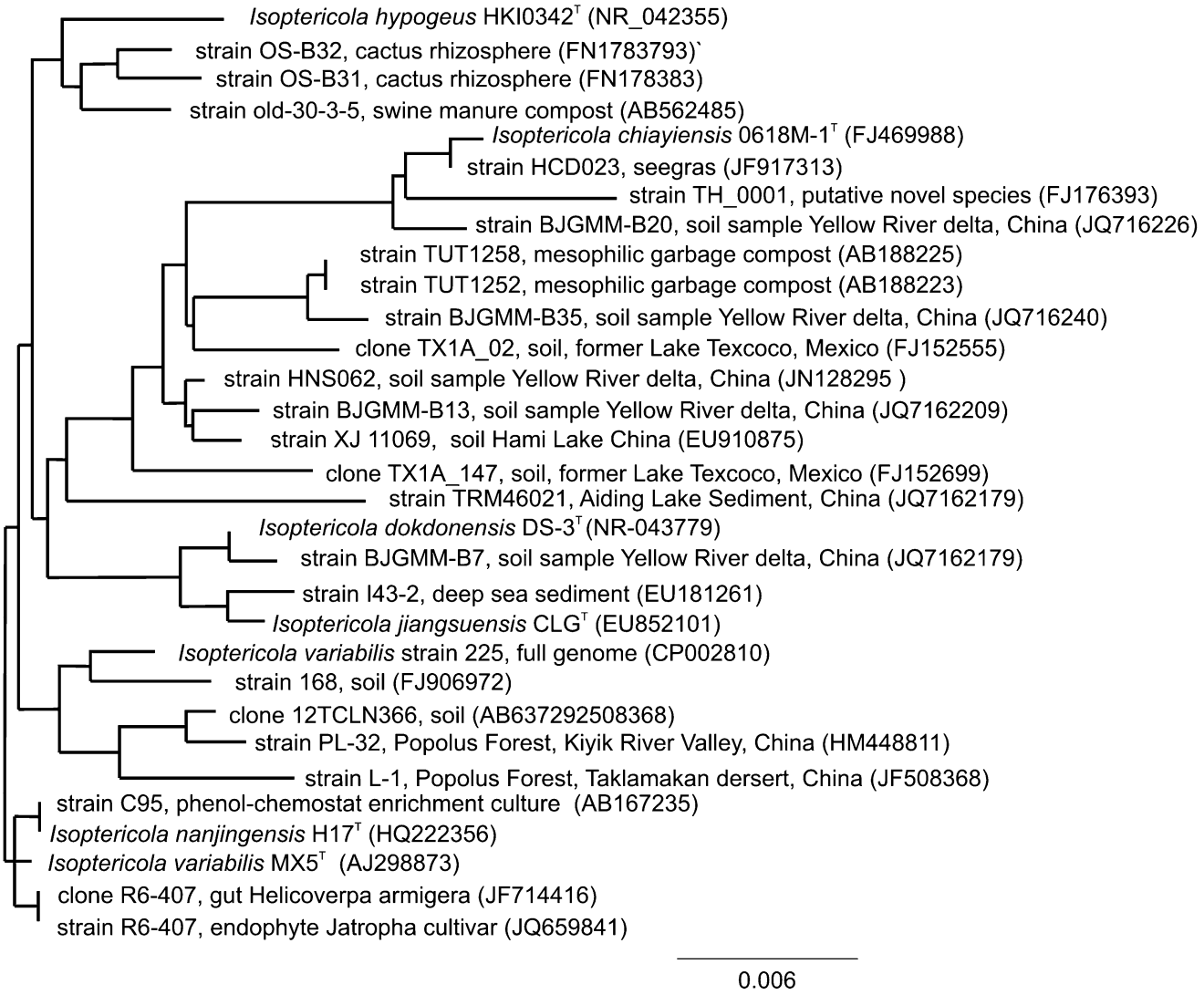

Fig. 35.5
BLAST Fast Minimum Evolution tree of 16S rRNA sequences of *Isoptericola* clones and isolates

cellulosilytica or Xylanibacterium ulmi (see Fig. 35.6). Likewise, besides entries for type strains of *Myceligenerans*, public databases only list two additional strain entries: one for an isolate from soil of Iriomote Island, Japan (Hayakawa et al. unpublished; AB546279) and the other for isolate LC 347 from a Lechuguilla cave, New Mexico (Barton et al. unpublished; JQ024522).

Pathogenicity, Clinical Relevance

Strains of *Cellulosimicrobium cellulans* were isolated from fetal tissues or placentas from cases of equine abortion, premature birth, and term pregnancies of horses, suggesting the causative role of these bacteria (Bolin et al. 2004). *Cellulosimicrobium*

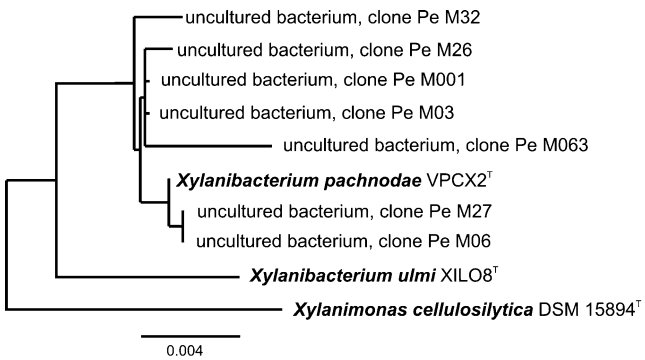

Fig. 35.6
BLAST Fast Minimum Evolution tree of 16S rRNA sequences of *Xylanimicrobium* clones and isolates

Table 35.10
Examples of recent case studies involving members of *Cellulosimicrobium*

Taxon	Clinical aspect	Identification	Treatment	References
C. cellulans	Human: Chronic tongue ulcer	16S rRNA gene sequence	Penicillin V, Azithromycin	Heym et al. (2005)
C. cellulans	Human: catheter-related bacteremia	API Coryne strip,16S rRNA gene sequence	Vancomycin, Rifampin	Rowlinson et al. (2006)
C. cellulans	Human: Pyogenic Flexor Tenosynovitis	API Coryne strip	Trimethoprim-sulfamethoxazole, Rifampin	Tucker et al. (2008)
C. cellulans	Human: Early-onset neonatal sepsis	API Coryne strip	Vancomycin	Casanova-Román et al. (2010)
C. cellulans	Human: Septic arthritis	MALDI-TOF MS, Vitek-II system, 16S rRNA gene sequence (JN695266)	Levofloxacin, Rifampin, Linezolid	Magro-Checa et al. (2011)
C. cellulans	Human: Endophthalmitis	Not indicated	Vancomycin, Moxifloxacin	Jaru-ampornpan et al. (2011)
C. cellulans	Equine: abortion and premature birth	API Coryne strip, 16S rRNA gene sequence	none	Bolin et al. (2004)
C. funkei	Human: peritoneal infection	16S rRNA gene sequence	Cotrimoxazole, Diprofloxacin, Vancomycin	Betancourt Castellanos et al. (2011)
C. funkei	Human: bacteremia and possibly prosthetic valve endocarditis	API coryne strip, 16S rRNA gene sequence (HQ402902), physiological tests	Vancomycin Gentamicin	Petkar et al. (2011)

cellulans was discussed as etiological agent of chronic tongue ulcerations in an immunocompromised patient (Heym et al. 2005). The strains of *Cellulosimicrobium funkei* are isolates from human sources that were received by the Special Bacteriology Section and the Actinomycete Reference Laboratory at the Center for Disease Control (Atlanta, Georgia, USA) between 1957 and 1977 and were classified earlier as strains of *Oerskovia turbata* (McNeil et al. 2004). The type strain W6122^T and strain W4083 were isolated from blood from patients from Colorado and California, respectively, and the lung isolate W2796 and strain W6123 originated from patients from New York. Although the isolation sites were suggestive of invasive infections, the clinical significance of these strains cannot be elucidated because no satisfactory case histories were available. The only strain of *Cellulosimicrobium terreum* was isolated from Korean soil (Yoon et al. 2007).

Of all the species included in the family, only two species of *Cellulosimicrobium* have been associated with human infection, although rarely (*Table 35.10*). Reports on clinical significance are available for *Cellulosimicrobium cellulans* (formerly *Oerskovia xanthineolytica*, to which the older literature refers) and *Cellulosimicrobium funkei;* these are considered to be opportunistic pathogens that mostly enter a wound by foreign bodies or cause infections due to medical devices, which have to be removed for resolution of the infection. *C. funkei* was misleadingly named *Oerskovia turbata* by Betancourt Castellanos et al. (2011) [see Chap. 9, "The Family Cellulomonadaceae" by Stackebrandt and Schumann, this volume]. Several case reports have been published describing infection due to *Oerskovia* spp. and *Cellulosimicrobium spp*., including bacteremia, endophthalmitis, endocarditis, peritonitis, meningitis, gangrenous cholecystitis, keratitis, pyonephrosis, and soft-tissue infection, as well as an association with bone marrow transplantation (compiled by Rowlinson et al. 2006; Magro-Checa et al. 2011 and Betancourt Castellanos et al. 2011); however, identification might not have been reliable in some of the early literature. Infections often occur in patients with chronic underlying illnesses such as neoplasms, chronic renal failure, Crohn disease, and acquired immunodeficiency syndrome (Lujan-Zilbermann et al. 1999; Kailath et al. 1988; Niamut et al. 2003; Tucker et al. 2008).

C. cellulans has been reported to be resistant to Amikacin (minimum inhibitory concentration [MIC] $\geq$ 64 µg/ml), ciprofloxacin (MIC $\geq$ 4 µg/ml), imipenem, erythromycin and other macrolides, lincosamides, aminoglycosides, and penicillin. It is considered to be variably susceptible to cephalosporins (but resistant to ceftazidime) and ciprofloxacin, but susceptible to vancomycin, cotrimoxazol, rifampin, moxifloxacin, and amikacin (see *Table 35.9*).

Strains of *Cellulosimicrobium funkei* are also resistant to Amikacin (MIC $\geq$ 64 µg/ml), trimethoprim-sulfamethoxazole (MIC $\geq$ 4–76 µg/ml), and CIprofloxacin (MIC $\geq$ 4 µg/ml) and sensitive to imipenem (MIC $\geq$ 16 µg/ml) (Brown et al. 2006).

All *Isoptericola* type strains are susceptible to carbenicillin (100 mg), chloramphenicol (30 mg), gentamicin (10 mg), neomycin (30 mg), novobiocin (30 mg), polymyxin B (30 mg), roxithromycin (15 mg), tetracycline (30 mg), and vancomycin (30 U). They are resistant to kanamycin (30 mg).

Table 35.11

Examples of the presence and characterization of genes of putative industrial importance

Strains of	Product	Genes and proteins, e.g.	Mode of action	References
Cellulosimicrobium cellulans (investigated as *Oerskovia xanthineolytica* LL G109), *C. cellulans* DSM 10297, and *C. cellulans* (investigated as *Arthrobacter luteus* ATCC 21606 (strain 73–14)	Wide heterogeneity of glucanases, strain dependent	β*gllIA* 28.6, kDa and β*gll* 40.8 kDa (family 16 of glycosyl hydrolases, release of biose and glucose). 54.5 kDa (family 64 of glycosyl hydrolases, release of pentoses)	Lyse viable yeast cells, spheroplasting for structural analyses of yeast and fungal cell wall, in cell wall permeabilization for the selective recombinant protein recovery from yeast cells or in biocatalysis	Review Ferrer (2006)
Cellulosimicrobium cellulans DK-1	Carbohydrate-binding module (CMB) appended recombinant endo-1,3-β-glucanase	Carbohydrate-binding module family 13	Hypothesis: insoluble carbohydrates bind to the catalytic domain and to the CBM of an endo-1,3-β-glucanase molecule; hence, the recombinant protein could accelerate hydrolysis, releasing shorter glucans as products.	Tamashiro et al. (2012)
Cellulosimicrobium cellulans DK-1	Endo-1,3-β-glucanase isolated from Tunicase, a crude enzyme preparation	Recombinants of a 41-kDa protein (glycoside hydrolase family 16); Accession no. EU589324	Hydrolysis of β-1,3- or both β-1,3- and β-1,4-glucosidic linkages; Hydrolysis of laminaripentaose and laminariheptaose	Tanabe and Oda (2011)
Cellulosimicrobium sp. strain HY-13	Endo-β-1,4-mannanases	Man H. 44.0 kDa mannanase; Accession no. JF519824	High specific activity towards ivory nut mannan and locust bean gum	Kim et al. (2011a)
Cellulosimicrobium sp. strain HY-13	Endo- β -1, 4-xylanase, lacking cellulase activity	XylK1. 58.3 kDa, unique modular xylanase; Accession no. FJ859907	Birch wood xylan, oat spelt xylane, p-nitrophenyl-β-D-cellobioside	Kim et al. (2009)
Cellulosimicrobium sp. strain HY-13	Endo- β -1,4-xylanase, displaying transglycosylation activity	XylK2. 79.6 Da, structurally analogous to XylK1t (90 %) and to *Xylanimonas cellulosilytica* DSM 15894 β-1,4-cellobiohydrolase (ACZ30181) (68 %)	Degradation of birchwood xylan, xylotriose, and xylotetraose	Kim et al. (2012)
Cellulosimicrobium sp. strain HY-13	Endo-β-1,4-mannanases	ManK. 34.93 Da	Highly specific for locust bean gum, guar gum, mannotetraose, and mannopentaose	Kim et al. (2011b)
Cellulosimicrobium sp. HY-12	Endo-β-1,4-xylanase, lacking cellulose activity	XylACspHY-12. 39.0 kDa (glycoside hydrolase family 10 endoxylanases)	Beechwood xylan Oat spelt xylan, Birchwood xylan, low p-nitrophenyl-β-D-cellobioside activity	Oh et al. (2008)
Cellulosimicrobium cellulans ST26 (from a petal of Casa Blanca Lily)	Hypothetical pathway: Glucose-glycogen-trehalose via malto-oligosyltrehalose synthase and malto-oligosyltrehalose trehalohydrolase		Trehalose (α-D-glucopyranosyl-[1,1]-α-D-glucopyranose) production	Seto et al. (2004)
Cellulosimicrobium cellulans GS6 from farmland in central Taiwan	Entrapped in modified calcium alginate capsules		Solubilizes insoluble phosphate complexes [$CaHPO_4$, $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$]	Liu et al. (2008)
Promicromonospora sp. RL26 from mangrove soil	Macrocyclic dilactone—JBIR-101		Cytotoxic activities against human malignant pleural mesothelioma ACC-MESO-1 cells and human cervical carcinoma HeLa cells	Izumikawa et al. (2011)
Isoptericola jiangsuensis CLG	Chitobiosidase Is-chiA endochitinase Is-chiB	Recombinant proteins Is-chiA 92 kDa and Is-chiB 60 kDa, glycosyl hydrolase family 18		Wu et al. (2011)

Application

The biological interest in *Promicromonospora* strains originates from a study by Lyons et al. (1969) who detected alpha-galactosidase activity in several members of Actinomycetales. Alpha-galactosidase activity can be exploited in several fields, such as the possible elimination of the flatus-inducing factor(s) in beans and soy, elimination of raffinose during beet-sugar processing, and the analytical determination of raffinose.

Most publications, however, are related to members of *Cellulosimicrobium* (see Table 35.11), which are rich sources of endo-1,3-β-glucanase, endo-β-1,4-mannanases, and endo-β-1,4-xylanases. The latter two enzymes were isolated from an exo-symbiotic bacterium capable of hydrolyzing xylan in the gut of the mole cricket, *Gryllotalpa orientalis*. In particular, the endo-β-1,4-xylanase lacking cellulose activity XylK1 was praised by the authors (Kim et al. 2009) for its potential role in removing hemicellulose without attacking cellulase and for its use as a biocatalyst in the bleaching process in the paper and pulp industry. Its pH activity, which is around 6.0, would make it superior to commercial fungal xylanases, which generally have an optimum pH of 5.0; when used to improve the digestion of foods by herbivorous animals, they are most active at a pH of 6.0–6.5 (Morgavi et al. 2000).

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36 The Family *Propionibacteriaceae*: Genera other than *Propionibacterium*

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Abstract

The family *Propionibacteriaceae* constitutes a phylogenetically coherent family. Together with the family *Nocardioidaceae*, it is a member of the order *Propionibacteriales* which, according to the Road Map of the Phylum Actinobacteria (Ludwig et al. 2012), form a large clade containing also the orders *Actinopolysporales*, *Corynebacteriales*, *Glycomycetales*, *Jiangellales*, *Micromonosporales*, and *Pseudonocardiales* (note, that the names of these orders have not yet been validated). Besides the genus *Propionibacterium*, that will be dealt with in a separate chapter, the family embraces 14 genera, most of which are monospecific. Members of the family have been isolated from a broad range of different habitats, including activated sludge, the marine environment, contaminated soil, human samples, and spiders and their webs. Chemotaxonomically the family is defined by similar menaquinone types, fatty acid composition, polar lipids, and, if investigated, polyamine pattern, but shows a wider spectrum of different peptidoglycan types and variations as well as more than 15 mol% differences in the G+C content of DNA.

Taxonomy: Historical and Current

A systematic-phylogenetic reevaluation of Gram-positive bacteria with a base composition G+C of DNA higher than 50 mol% has led to the description of the class *Actinobacteria* Stackebrandt et al. 1997 that embraces six orders. The order *Actinomycetales* Buchanan 1917, emend Stackebrandt, Rainey and Ward-Rainey 1997, has been defined to include 10 suborders, one of which was *Propionibacterineae*, containing the family *Propionibacteriaceae* Delwiche 1957, emend Rainey, Ward-Rainey and Stackebrandt 1997. Recently, the order *Actinomycetales* and the suborder *Propionibacterineae* were elevated to ranks of class and order, respectively (Ludwig et al. 2012). At that time of the description of the suborder, it contained the genera *Propionibacterium* (Orla-Jensen 1909), *Luteococcus* (Tamura et al. 1994), *Microlunatus* (Nakamura et al. 1995a), and *Propioniferax* (Yokota et al. 1994). The suborder was emended by Zhi et al. (2009) and the pattern of 16S rRNA signatures revised to consist of nucleotides at positions 127 : 234 A–U, 598 : 640 U–A, 657 : 749 G–C, 828 U, 829 : 851 A–C, 832 : 854 U–C, 833 : 853 G–U, 952 : 1229 C–G and 986 : 1219 U–A. In the same communication

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also, the family *Propionibacteriaceae* was emended and a new set of 16S rRNA signatures provided [328 U, 407 : 435 C–G, 451 A, 453 G, 819 G, 825 : 875 A–U, 827 C, 828 U, 832 : 854 U–C, 833 : 853 G–U and 844 U] for all members that were described until 2007: In addition to those existing in 1997, these were *Aestuariimicrobium* (Jung et al. 2007), *Brooklawnia* (Bae et al. 2006a), *Friedmanniella* (Schumann et al. 1997), *Granulicoccus* (Maszenan et al. 2007), *Micropruina* (Shintani et al. 2000), *Propionicicella* (Bae et al. 2006b), *Propionicimonas* (Akasaka et al. 2003), *Propionimicrobium* (Stackebrandt et al. 2002), and *Tessaracoccus* (Maszenan et al. 1999a). Recently, the genus *Auraticoccus* (Alonso-Vega et al. 2011) and *Propioniclava* (Sugawara et al. 2011) have been added to the family (*Table 36.1*).

The phylogenetic tree (*Fig. 36.1*), based upon the RAxML algorithm of Stamatakis (2006) sees members of the family as a phylogenetically coherent group, consisting of several subgroups. One subgroup contains the genera *Friedmaniella*, *Microlunatus*, and *Auraticoccus*, while a second one embraces *Micropruina*, *Propionicimonas*, *Propionicicella*, and *Propioniciclava*. A third, small subgroup includes *Granulicoccus* and *Propioniferax*, while all other genera, including *Propionibacterium*, are members of a fourth subgroup. The membership to genera is consistent with most of the dendrograms included in the species descriptions, though the topology may differ. For example, while three species of *Tessaracoccus* and *Propionibacterium* are sister lineages in the RaxML tree, *Propionciclava* tarda branches with the genus *Propionibacterium* in the Neighbor-Joining tree (not shown). In both trees, *Tessaracoccus oleiagri* clusters more closely to *Brooklawnia cerclea* and *Propionicimicrobium lymphophilum* than with the other three species of *Tessaracoccus*. The branching pattern of genera does not correlate with the distribution of peptidoglycan types and variations.

Another member of the family has been proposed by Lee and Lee (2008b), "*Ponticoccus gilvus*." The generic name has already been used for a taxon in the alpha-proteobacterial family *Rhodobacteraceae*, the proposal to name a second genus with the same name has been denied.

As the majority of the recently described genera are monospecific, the description of metabolic properties may change with new species included. Also, the descriptions of the taxa were not always done in a comparable manner and many properties should be re-evaluated. This should not only be done for general metabolic properties but also for chemotaxonomic markers such as amino acid composition and linkage of the peptidoglycan, polar lipids, whole cell sugars, and polyamine composition. Membership to the family is based on distinct phylogenetic position and an exclusive set of some 16S rDNA signature nucleotides not found in other families. These signatures should be re-evaluated when new members are added to the family.

Members of the *Propionibacteriaceae* thrive in diverse habitats, covering human epidermal surfaces, dairy products, silage, soil, water, Antarctic sandstone, and sewage treatment plants. They are either aerobic or facultatively anaerobic, cover different morphologies, exhibit different peptidoglycan types and ariations, and the base composition of DNA ranges between 53 and 73 mol%. The peptidoglycan composition sees four variations, either the directly linked meso-A_2pm diaminopimelic acid at position 3 of the peptide subunit [variation A1γ according to Schleifer and Kandler (1972); or A31 according to Schumann (2011)], LL-A_2m at position 3 and glycine as the interpeptide bridge. This type has two variations:

Table 36.1
Validly published genera and species in the family *Propionibacteriaceae* except *Propionibacterium*. Type strains numbers relate to those species indicated in *Figure 36.1* and in *Tables 36.3–36.6*

Genus	Species	Type strain
Aestuariimicrobium	*kwangyangense*	R27^T
Auraticoccus	*monumenti*	MON 2.2^T
Brooklawnia	*cerclae*	BL-34^T
Friedmanniella	*antarctica*	DSM 11053^T
	capsulata	Ben 108^T
	lacustris	EL-17A^T
	spumicola	Ben 107^T
	sagamiharensis	FB2^T
	okinawensis	FB1^T
	lucida	FA2^T
	luteola	FA1^T
Granulicoccus	*phenolivorans*	PG-02^T
Luteococcus	*japonicus*	IFO 12422^T
	peritonei	CCUG38120^T
	sanguinis	CCUG 33897^T
Microlunatus	*phosphovorus*	DSM 10555^T
	aurantiacus	YIM 4572^T
	soli	CC-012602^T
	parietis	12-Be-011^T
	gingengisoli	Gsoil 633^T
	panaciterrae	Gsoil 954^T
Micropruina	*glycogenica*	Lg2^T
Propionicella	*superfundia*	BL-10^T
Propioniciclava	*tarda*	WR061^T
Propionicimonas	*paludicola*	JCM 11933^T
Propioniferax	*innocua*	ATCC49929^T
Propionimicrobium	*lymphophilum*	DSM 4903^T
Tessaracoccus	*bendigoensis*	Ben 106^T
	flavescens	SST-39^T
	oleiagri	SL014B-20A1^T
	lubricantis	KSS-17Se^T

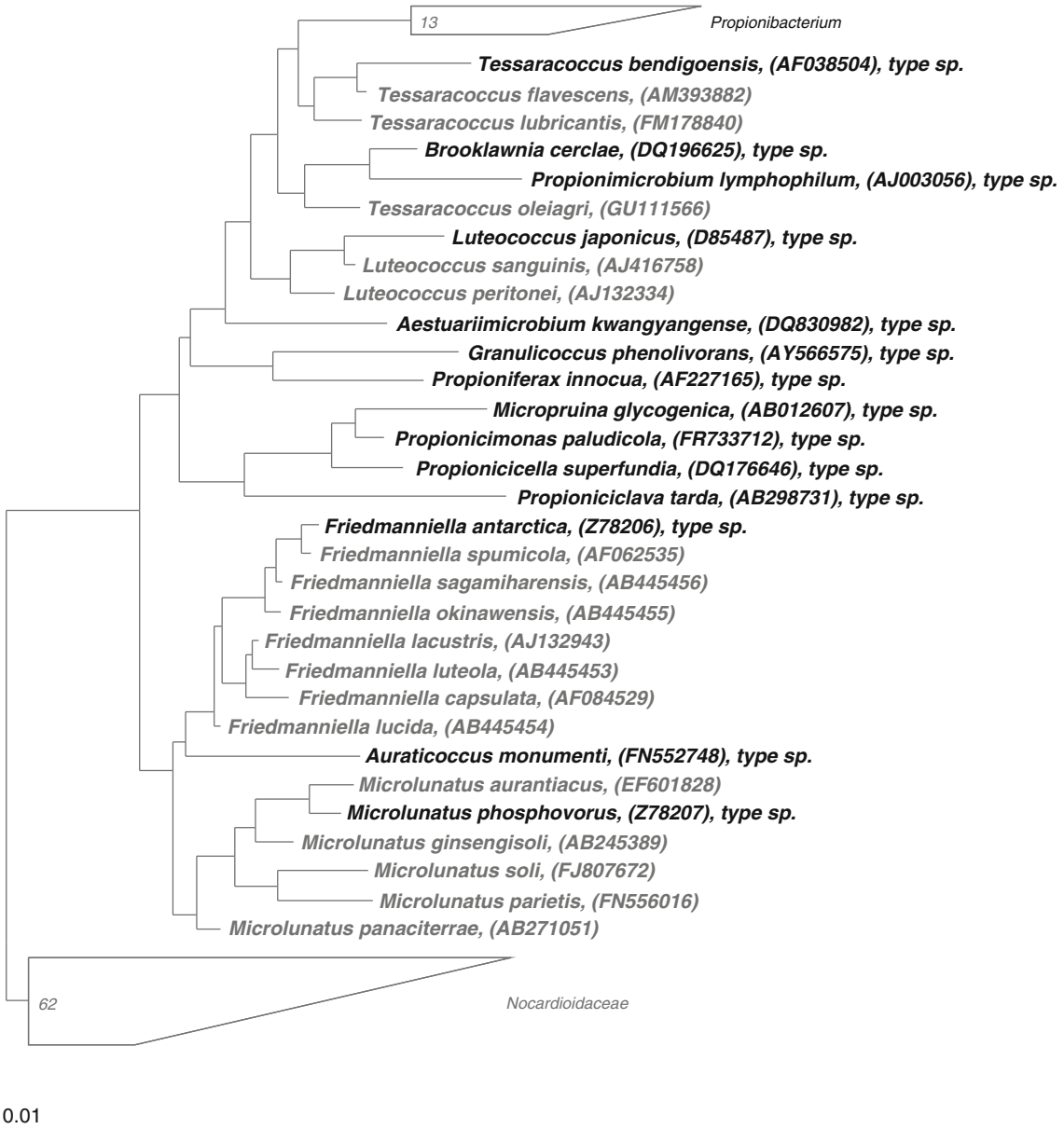

Fig. 36.1

Maximum likelihood genealogy reconstruction based on the RAxML algorithm (Stamatakis 2006) of the sequences of all members of the family *Propionibacteriaceae* 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 60 % conservational filter for the whole bacterial domain was used to remove hypervariable positions. The bar indicates 1 % sequence divergence. Type strain numbers are included in *Table 36.1*

one with alanine at position 1 of the subunit (A3γ, A41.1), the other with glycine at position A3γ (A42.1), the fourth one with L-lysine at position 3 and D-aspartic acid as the interpeptide bridge (A4α, A11.31). The three diaminopimelic variations are also found among members of *Propionibacterium*. However, with respect to certain chemotaxonomic properties, such the combination of the few patterns of polyamines available (Busse and Schumann 1999), major menaquinones, and fatty acids, they appear rather homogeneous. Salient features differentiating members of the family are indicated in *Table 36.2*.

Molecular Analyses

DNA-DNA Similarities

DNA-DNA hybridization experiments, using different formats such as the methods of Ezaki et al. (1989), DeLey et al. (1970), and Ziemke et al. (1998), were performed to confirm the presence of genomospecies (Maszenan et al. 1999b; Collins et al. 2003; Wang et al. 2008; Kämpfer et al. 2009) and to affiliate isolates to the same species (Tamura et al. 1994; Jung et al. 2007).

Table 36.2

Properties differentiating genera of the family *Propionibacteriaceae*. The order is based on the phylogenetic position of the genera, as shown in the RaXML dendrogram *Fig. 36.1*

Genus	Cell morphology	Diagnostic amino acids in peptidoglycan	Position 1 in peptide subunit of peptidoglycan	Major menaquinone	Polar lipids	Major fatty acids	Mol% DNA G+C
Tessaracoccus	Spherical or rods	LL-A_2pm-Gly[a]	Glycine[a]	MK-9H_4, MK7H_4 or MK8 in some species	DGP, PG. PI, PE, PL, GL may occur	ai-$C_{15:0}$. i-$C_{16:0}$ may occur[b]	68.4–74
Brooklawnia	Pleomorphic rods	meso-A_2pm-direct	nd	MK-9H_4	nd	ai-$C_{15:0}$, $C_{15:0}$[c]	67.6
Propionimicrobium	Pleomorphic rods, spherical cells may occur	Lys-D-Asp	Alanine	MK-9H_4	nd	$C_{18:1}\omega9c$, ai-$C_{15:0}$, $C_{16:0}$	53–56
Luteococcus	Spherical or pleomorphic rods	LL-A_2pm-Gly[a]	Alanine[a]	MK-9H_4	DPG, PG, PI[a]	$C_{16:1}$, $C_{17:1}$, $C_{15:1}$. $C_{18:1}$ may occur	64–67
Aestuariimicrobium	Spherical to short rods	LL-A_2pm-nd	nd	MK-9H_4	nd	ai-$C_{15:0}$	69
Granulicoccus	Spherical, single, pairs	LL-A_2pm-Gly	Alanine	MK-9H_4, MK-8H_4	DPG, PG, 3GL, 3PL	i-$C_{15:0}$, i-$C_{15:0}$DMA	69
Propioniferax	Pleomorphic rods	LL-A_2pm-Gly	Alanine	MK-9H_4	PG, PE, PL, GL	nd	59–63
Micropruina	Spherical	meso-A_2pm-direct	nd	MK-9H_4	nd	i-$C_{14:0}$, ai-$C_{15:0}$	70.5
Propionicella	Rods	meso-A_2pm-direct	nd	MK-9	nd	ai-$C_{15:0}$, $C_{15:0}$, i-$C_{16:0}$	69.9
Propioniciclava	Pleomorphic rods	meso-A_2pm-direct	nd	MK-9H_4	nd	ai-$C_{15:0}$, i-$C_{15:0}$	69.5
Auraticoccus	Spherical, single, pairs, clusters	LL-A_2pm-Gly	Alanine	MK-8H_2, MK-9H_2, MK-9H_4	DPG, PG, PG2, 2PL, 4GL, 2 L	ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{15:1}$, ai-$C_{17:0}$, i-$C_{14:0}$	73.5
Friedmanniella	Spherical, single, pairs, clusters	LL-A_2pm-Gly	Glycine[a]	MK-9H_4. MK-9H_2 and MK-7H_2 may occur	PG, PI. DPG and PC in most species. PL, GL may occur	ai-$C_{15:0}$, i-$C_{15:0}$. $C_{18:1}$ may occur	69–75
Microlunatus	Spherical	LL-A_2pm-Gly[a]	Glycine[a]	MK-9H_4. MK-8H_2 may occur	DGP, PG. PI, PE, PL, GL may occur[a]	ai-$C_{15:0}$. i-$C_{15:0}$, i-$C_{16:0}$ in most species. One species with $C_{18:1}\omega9c$	65–71
Propionicimonas	Irregular rods	meso-A_2pm-direct	nd	MK-9H_4, MK-10H_4	nd	$C_{13:0}$, $C_{15:0}$, ai-$C_{15:0}$	68.7
Propionibacterium	Spherical or pleomorphic rods,	LL-A_2pm-Gly, or meso-A_2pm-direct	Alanine (most species) or glycine	MK-9H_4[d]	nd	branched, or straight, or ω–cyclohexane	57–68

Abbreviation: *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *GL* unknown glycolipid, *PL* unknown phospholipid, *L* unknown lipid

[a]Properties only detected in a single or a few species

[b]For some species Lee et al. (2007) and Kämpfer et al. (2009) report a different composition

[c]No $C_{15:0}$ but $C_{16:0}$ has been reported by Cai et al. (2011)

[d]Kusano et al. (1997)

Within Friedmanniella, *F. antarctica* shares 98.8 % 16S rRNA gene sequence similarity and 50 % DNA-DNA reassociation DDH with *F. spumicola* (Maszenan et al. 1999b). With sequence similarity levels of >97 %, *F. luteola* and *F. lucida* are moderately related to the closely related pair *F. capsulata* and *F. lacustris* (99 %). The two spider isolates, showing 46 % DDH among themselves, showed 29 % and 43 % DDH relatedness to *F. lacustris* and *F. capsulata*, respectively (Iwai et al. 2010). No DDH values are available for the latter strain pair. With sequence similarity levels of 98 % (19 % DDH), *F. sagamiharensis* and *F. okinawensis* are most closely related to *F. antarctica* and *F. spumicola*. The DDH values obtained for these two pairs of strains were between 11 % and 30 % (Iwai et al. 2010).

Probes

The presence of *Micropruina* ssp. cells in an aerobic: anaerobic sequencing batch reactor with no phosphorus removal was verified by using the fluorescent in situ hybridization FISH in combination with microautoradiography FISH/MAR. The fluorescent 16S rRNA gene probe MIC 184 with the sequence CATTCCTCAAGTCTGCC was derived from the sequences of *Micropruina glycogenica* (Kong et al. 2001).

Another FISH probe was designed for the identification of *Microlunatus phosphoreus* in sludge of an enhanced biological phosphorous removal process. The sequence of the fluorescently labeled 16S rRNA targeting MP2 probe is GAGCAAGCTCTTCTCAACCG (Kawaharasaki et al. 1998).

Phages

Phages have been reported for *Microlunatus phosphovorus* (Lee et al. 2006). All of the 13 phages, obtained from an activated sludge reactor by the agar overlay plate technique, were double-stranded DNA phages. Based upon restriction patters, two types were recognized. The genome size was determined to be 42–48 kb for MP1 and 21–28 kb MP2. Both bacteriophages, belonging to the family *Siphoviridae*, had an icosahedral head and a long flexible tail, which appeared to be non-contractile. Host range studied showed the phages to be specific for *M. phosphovorus* JCM9379. Other members of the family, such as *Micropruina glycogenica* JCM10248, *Friedmanniella antarctica* NBRC16127, *F. capsulata* strain Ben108^T, *F. spumicola* strain Ben107^T, and *Tessaracoccus bendigoensis* NBRC16165, were not lysed. Using the fluorescently labeled phage technique, both phage types were able to tag *M. phosphoreus* added to activated sludge (Lee et al. 2006).

Genome Sequence

The genome sequence of *Microlunatus phosphovorus* NM-1^T has been deposited under the accession number AP012204 (Hosoyama et al., Unpublished).

Phenotypic Analyses

As seen in ● *Table 36.1* the family, excluding *Propionibacterium*, consists of 14 genera and 4 of these contain more than the type species only *Friedmanniella*: 8 species, *Microlunatus*: 6 species, *Tessaracoccus*: 4 species, and *Luteococcus*: 3 species. Species of each genus share the same relationship to oxygen and the same peptidoglycan type and variation, but differ in many other chemotaxonomic and metabolic properties which, together with moderate 16S rRNA gene sequence similarities below 97 %, were used for their definition. The following description of genera will point out only some of the species properties and the reader is referred to the original descriptions for a more comprehensive information which are helpful in the identification of novel isolates.

Friedmanniella Schumann, Prauser, Rainey, Stackebrandt, Hirsch 1997, 282^{VP}

Fried. man.ni.el'la. M. L. dim. ending *-ella;* M. L. fem. n. *Friedmanniella*, named after E. Imre Friedmann, an American microbiologist, in recognition of his contributions to Antarctic microbiology.

The genus *Friedmanniella* contains eight Gram-positive, nonmotile, aerobic coccoid, packets-forming species, two of which, *F. antarctica* (Schumann et al. 1997) and *F. lacustris* (Lawson et al. 2000), originate from Antarctic environment; two other species, *F. spumicola* and *F. capsulata* (Maszenan et al. 1999b), were isolated from sewage treatment plants in Australia, while the other four species were isolated from spiders or spider webs (Iwai et al. 2010). Generally, the cells occurred in packets which resulted from cell division in three perpendicular planes, no thin sections are available for the spider and spider web isolates. The packets adhered to one another, forming aggregates. Packets are often surrounded by extracellular polymer.

Most strains are catalase positive, oxidase negative, do not reduce NO_3 to NO_2 and produce extracellular polymers. Some strains accumulate polyphosphate (● *Table 36.3*) and, if investigated, polyamines are spermidine and spermine. Strains exhibit the same rare peptidoglycan type in which one glycine residue is defining the interpeptide bridge, while a second one is found at position 1 of the peptide subunit. Other properties of the genus are indicated in ● *Table 36.2*. The metabolic properties clearly differentiate between the eight species, though the intra-species diversity of strains is yet to be elucidated for more strains.

Microlunatus Nakamura, Hiraishi, Yoshimi, Kawaharasaki, Masuda, Kamagata 1995, 21^{VP}

Mi.cro.lu.na'tus. Gr. adj. *micros*, small; M. *L.* n. *lunatus*, half moon; M. L. n. *Microlunatus*, small moon-like microorganism.

Table 36.3

Comparative phenotypic properties of type strains of species of *Friedmanniella* (Data were taken from the compilations of Iwai et al. (2010), Stackebrandt and Schaal (2002), and the original species descriptions)

Phenotypic properties	*F. antarctica*	*F. spumicola*	*F. capsulata*	*F. lacustris*	*F. sagamiharensis*	*F. okinawensis*	*F. lucida*	*F. luteola*
Isolated from	Australian sandstone	Sewage treatment plant	Sewage treatment plant	Antarctic hypersaline lake	Spider web	Spider web	Spider web	Spider body
Size μm	0.5–2.2	0.5–1.4	0.6–1.2	1.0–1.3	0.7–1.0	0.6–0.7	0.5–0.8	1.1–1.6
Color of colonies	Beige-orange	Yellow-orange	Bright orange	Brownish to orange	Orange	Orange	Creamy yellow	Creamy yellow
Growth temperature °C								
Optimum	22	25	20–25	26	25–28	22–28	22–28	25–27
Range	9–25	15–37	15–30	3–43.5	18–32	18–32	18–32	18–28
pH for growth								
Optimum	6–7.2	7–7.5	6.5–7	7–8	7.0–7.7	6.0–7.0	6.0–7.0	6.0–7.7
Range	5.1–8.7	5.5–8.0	5.5–7.5	5.5–9.5	5.0–9.0	5.0–9.0	5.0–9.0	5.3–8.5
NaCl range %, w/v	0–2	Inhibition	Inhibition	0–8	0–6	0–4	0–2	0–2
Storage products	Polyphosphate	Polyphosphate	Polyphosphate	nd	nd	nd	nd	nd
Extracellular polymer	–	+	+	+	+	+	+	–
Oxidase	–	–	–	w	–	–	–	–
Catalase	+	+	+	+	–	+	+	+
Nitrate reduction	–	–	–	w	–	–	–	–
Urease	+	+	+	–	+	+	+	+
Indole production	–	–	–	–	–	–	–	–
H_2S production	+	+	+	–	–	–	–	–
Acid produced from D-Glucose	–	–	–	+	–	–	–	–

Carbohydrates utilized								
Lactose	–	–	–	+	nd	nd	nd	nd
Raffinose	–	–	–	+	+	+	+	w
Mannitol	–	+	–	+	+	+	+	–
Glycerol	–	–	–	V	nd	nd	nd	nd
L-Arabinose	w	+	+	+	+	+	–	w
myo-Inositol	–	–	–	–	w	w	–	w
Sucrose	–	–	+	+	+	+	+	–
D-Fructose	–	+	+	+	+	+	–	w
Glucose	–	+	–	+	+	+	+	–
Mannose	–	–	–	+	nd	nd	nd	nd
Galactose	–	+	–	+	+	+	w	w
Trehalose	–	–	–	+	nd	nd	nd	nd
Maltose	–	–	+	+	nd	nd	nd	nd
D-Ribose	+	+	+	–	+	w	–	–
D-Xylose	w	+	+	+	+	+	–	–
L-Rhamnose	–	–	+	–	w	+	–	–
Adonitol	–	–	–	+	nd	nd	nd	nd
Cellobiose	–	nd	nd	+	nd	nd	nd	nd
Hydrolysis of								
Gelatin	–	+	–	+	+	+	+	+
Starch	+	–	–	+	–	–	–	–

+ positive,– negative, *nd* not determined, *w* weak
v, different reactions between Biolog and API 50 CH tests

Table 36.4

Comparative phenotypic properties of type strains of the genus *Microlunatus* (Data were taken from the compilations of Kämpfer et al. (2010a), Stackebrandt and Schaal (2002), and the original species descriptions)

Phenotypic properties	*M. phosphovorus*	*M. soli*	*M. aurantiacus*	*M. parietis*	*M. ginsengisoli*	*M. paniciterrae*
Isolated from	Activated sludge	Mushroom spawn	Rhizosphere soil	Indoor wall	Ginseng soil	Ginseng soil
Size μm	0.8–2.0	1.0–1.5	0.9–1.3	nd	0.5–0.8	0.3–0.7
Color of colonies	Cream	nd	Orange-yellow	Beige	Yellowish	Yellowish
Growth temperature °C						
Range/optimum	5–35/25–30	nd/25–30	20–37/15–37	nd/25–30	16–37/20–30	16–37/20–30
Range pH for growth	5.0–9.0	nd	7.0–7.5	nd	5.5–8.5	5.0–9.0
NaCl range %, w/v	0–6	nd	0–?	0–5	0–5	0.5
Oxidase	+	+	–	+	–	nd
Catalase	+	+	+	nd	+	+
Nitrate reduction	+	nd	+	nd	+[a]	+
Assimilation of						
Acetate	–	+	w	–	w	–
N-acetyl-glucosamine	+	+	+	w	+	–
Adonitol	+	+	–	+	+	–
p-Arbutin	+	+	–	–	+	–
Cellobiose	+	+	+	+	+	–
D-Fructose	+	+	+	+	+	–
D-Galactose	+	+	w	+	+	–
L-Histidine	–	w	–	–	w	–
i-Inositol	+	+	+	+	+	–
L-Malate	+	w	w	–	+	–
Maltitol	+	+	+	+	+	–
D-Mannitol	+	+	+	+	+	–
L-Proline	–	w	–	–	w	–
Propionate	–	+	–	–	–	–
Salicin	+	+	–	–	+	+
L-Serine	–	w	–	–	–	–
Sucrose	–	+	+	+	+	+
Trehalose	+	+	+	+	+	–
D-Xylose	+	+	+	+	+	–

nd not determined, *w* weak
[a]Negative in Wang et al. (2008)

This genus has been described to accommodate the Gram-positive, nonmotile, coccoid, and aerobic organism *Microlunatus phosphovorus* (Nakamura et al. 1995a). The description is based on the properties of two strains NM-1 and NM-2; the latter strain differing from the type strain NM-1^T JCM 9379^T only by flocculent growth. These strains, isolated from activated sludge in Japan, are chemoorganotrophic organisms which store polyphosphate under aerobic condition and take up organic substances such as glucose by utilizing polyphosphate as the energy source under anaerobic condition (Nakamura et al. 1995a, b). It also stores polyhydroxyalkanoates, mainly polyhydroxybutyrate (Akar et al. 2006).

The original description did not link *M. phosphovorus* to the genera *Luteococcus*, *Propioniferax*, and *Propionibacterium* but was closely affiliated to *Aeromicrobium* and *Nocardioides*, later included in the family *Nocardioidaceae* (Stackebrandt et al. 1997). Only with the inclusion of novel members of *Propionibacteriaceae*, the membership of *Microlunatus* to this family became obvious with which it shares the same peptidoglycan composition (*Table 36.2*). The other members of the genus match the circumscription as given above except for *M. parietis*, for which irregular rods in young cultures and short rods and spherical forms were described in older cultures >5 days of growth (Kämpfer et al. 2010b). Spermidine and spermine has been identified as polyamines in one species (Kämpfer et al. 2010a). Chemotaxonomic properties and some differentiating metabolic and cultural properties are indicated in *Tables 36.2* and *36.4*, respectively. All strains were positive

for utilization of L-arabinose, D-glucose, D-mannose, maltose, melibiose, L-rhamnose, D-ribose, and D-sorbitol. All strains were negative for the utilization of: D-gluconate, adipate, itaconate, L-alanine, 3-hydroxybenzoate, and phenylacetate.

Tessaracoccus Maszenan, Seviour, Patel, Schumann, Rees 1999, 466[VP]

Tes.sa.ra'coc.cus. Gr. adj. *tessara* four;Gr. n. *coccus* grain; L. n. *Tessaracoccus* four round cells.

The genus *Tessaracoccus* has been described for the nonmotile, coccoid, facultatively anaerobic Gram-positive strain *T. bendigoensis* Ben 106 [T], isolated from activated sludge biomass (Maszenan et al. 1999a). The sludge, originating from the Biological Nutrient Removal plant in Bendigo, Australia, had been processed in a laboratory scale sequencing batch reactor. The morphology of this strain, i.e., spherical or clusters of cocci arranged in tetrads, resembles that of the Gram-negative so-called G-bacteria (Carucci et al. 1994; Cech and Hartman 1993), commonly detected in activated sludge samples.

The G-bacteria constitute a phylogenetically diverse group of organisms belonging to the Actinobacteria and Proteobacteria (Seviour et al. 2000). The description of *Tessarococcus bendigoensis* extends the range of spherical species described to thrive in similar habitats, e.g., four Gram-negative species of *Amaricoccus* (Maszenan et al. 1997) from wastewater treatment plants in Italy, Czech Republic, Macau, and Australia, and the Gram-positive species *Microlunatus phosphovorus* (Nakamura et al. 1995a) from activated sludge, and *Microsphaera multipartita* [*Nakamurella multipartita*] (Yoshimi et al. 1996) from sugar containing synthetic wastewater, both from Japanese plants. While *Amaricoccus* is a member of the *Alphaproteobacteria*, *Microsphaera* is a member of the actinobacterial suborder *Frankineae*. The role of these organisms in this habitat is no yet settled, but they are all defined by depositing storage polymers intracellularly under aerobic conditions. *Tessarococcus bendigoensis* stores polyphosphate and may thus participate in phosphate removal (Seviour et al. 2000). The presence of storage compounds was not reported in the other species of the genus.

The type strains of two species, *T. lubricantis* and *T. flavescens*, were analyzed with respect to their polyamine pattern. Like in related genera, both strains contain spermidine and spermine and small amounts of putrescine, 1,3-diaminopropan and cadaverine were present in the latter strain (Kämpfer et al. 2009). *T. flavescens* differs from other members of the genus in the composition of menaquinones, as it has, in addition to the common MK-9H_4 type, MK-8H_0 that is present in considerable amounts (12 %) (Cai et al. 2011). The phospholipid composition of *T. flavescens* consists of diphosphoglycerol and phosphoglycerol only, while those of the other species is more complex.

All strains are Gram-positive, positive for catalase, negative for oxidase, urease, and indole production. Acid is produced from D-glucose, L-rhamnose, and maltose (Cai et al. 2011). Other properties are listed in ❯ *Tables 36.2* and *36.5* and in the original species descriptions.

The species *Tessaracoccus profundi*, described by Finster et al. (2009), has been effectively published but not yet validated. The type strain, isolated from the Chesapeake meteor impact crater drill core sample at 940-m depth, shows 95–96 % identity to other members of the genus. Morphology, oxidase and catalase reaction, relationship to oxygen, and peptidoglycan type are as reported for the other genus members. The G+C content of DNA is 68 mol%. Differences are seen in menaquinone composition and metabolic properties.

Luteococcus Tamura, Takeuchi, Yokota 1994, 355[VP]

Lu.te.o.- coc'cus. Lat. adj. *luteus* yellow; Gr. masc. n. *coccus* a grain; M.L. masc. n. *Luteococcus* yellow coccus.

This genus comprises three species, which are morphologically distinct but share chemotaxonomic and phylogenetic similarities. The type species *Luteococcus japonicus* (Tamura et al. 1994), isolated from soil and water, and *Luteococcus sanguinis* (Collins et al. 2003), isolated from human blood, are spherical. *L. peritonei* (Collins et al. 2000), isolated from human specimen exhibits, has a pleomorphic rod morphology. The most salient feature of the genus is the presence of monounsaturated long-chain fatty acids (>80 % of total), i.e., $C_{15:1}$, $C_{16:1}$, $C_{17:1}$, and $C_{18:1}$. If investigated, polyamines are spermidine and spermine. Strains are catalase positive. Acid is produced from glucose, maltose, D-mannitol, and sucrose, but not from D-xylose. The main end-product of glucose fermentation of *T. japonicus* is propionic acid. Other properties are indicated in ❯ *Tables 36.2* and *36.6* and in the original species descriptions.

Other Genera

The remaining genera of the family are monospecific, and they will be listed in alphabetical order together with some specific properties which are not already indicated in ❯ *Table 36.2*.

Aestuariimicrobium Jung, Kim, Song, Lee, Oh, Yoon 2007, 2117[VP]

Aes.tu.a.ri.i.mi.cro'bi.u.m. L. n. *aestuarium* part of the sea coast which, during the flood-tide, is overflowed, but at ebb-tide is left covered with mud or slime, a tidal flat; N.L. n. *microbium* microbe; N.L. neut. n. *Aestuariimicrobium* a microbe isolated from tidal flat.

Table 36.5

Comparative phenotypic properties of type strains of species of *Tessaracoccus* (Data were taken from the compilations of Cai et al. (2011) and the original species descriptions)

Phenotypic properties	*T. bendigoensis*	*T. flavescens*[a]	*T. oleiagri*	*T. lubricantis*
Morphology	Spherical, aggregates	Short rods	Oval to rod-shaped	Rods
Isolated from	Activated sludge	Marine sediment	Pollutes saline soil	Metalworking fluid
Size μm	0.5–1.1	0.6 × 1.2	0.48 × 0.5–1.0	0.5–1.0 × 0.8–2.0
Color of colonies	Beige	Brilliant yellow	Bright yellow	Yellow
O_2 requirement	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic	Aerobic
Optimum growth temperature °C/range	25/20–37	nd/20–30	28/4–50	25/15–36
Optimum growth pH/range	7.5/5.5–9.3	nd/6.0–12.0	7.0/6.0–9.0	nd/6.5–9.5
Oxidase	–	–	nd	–
Catalase	+	+	nd	+
Nitrate reduction	+	+	+	nd
Urease	–	–	–	nd
Aesculin degradation	+	w	+	nd
Indole production	–	–	nd	–
H_2S production	–	–	–	nd
Cell wall sugars[b]	Rib, xyl, man, gal, glu	Rib, xyl, man, gal, glu	Rib, rha, man, gal, glu	nd
Acid production from				
L-Arabinose	+	+	+	–
D-Xylose	+	+	–	–
D-Adonitol	–	+	–	–
D-Galactose	+	+	–	+
D-Mannose	+		+	–
D-Mannitol	+	–	–	–
D-Sorbitol	+	–	–	–
Methyl-α-D-Glucoside	+	–	–	–
Lactose	+	+	–	+
Melibiose	+	–	–	–
Raffinose	+	–	–	–
D-Arabitol	+	–	–	–
Enzyme activities				
Valine arylamidase	+	–	+	nd
Cystine arylamidase	+	–	+	nd
Naphthol-AS-BI-phosphohydrolase	+	–	+	nd
α-Galactosidase	+	–	+	nd
β-Galactosidase	+	+	–	nd
β-Glucuronidase	–	–	+	nd

nd not determined, *w* weak

[a] *rib* ribose, *xyl* xylose, *man* mannose, *gal* galactose, *glu* glucose

[b] Lee et al. 2008a

The type species *A. kwangyangense* consists of aerobic, catalase positive, and oxidase negative short rods or spherical cells (0.6 – 1.0 × 1.2 – 2.0 μm). It grows between 4 °C and 40 °C, with an optimum of 30 °C and at a pH range of 7.5–8.5. Phylogenetically it is moderately related to the *Tessaracoccus/Luteococcus* cluster (Fig. 36.1).

Auraticoccus Alonso-Vega, Carro, Martínez-Molina, Trujillo 2011, 1101[VP]

Au.ra.ti.coc'cus. L. adj. *auratus* golden; N.L. masc. n. *coccus* from Gr. masc. n. kokkos a grain, seed a coccus; N.L. masc. n. *Auraticoccus* golden coccus.

Table 36.6

Comparative phenotypic properties of type strains of species of *Luteococcus* (Data from the original species descriptions)

Phenotypic properties	*L. japonicus*	*L. peritonei*	*L. sanguinis*
Morphology	Spherical	Pleomorphic rods	Spherical
Isolated from	Soil	Human peritoneum	Human blood
Size μm	0.7–1.0	nd	nd
Color of colonies	Cream to yellow	Non-pigmented	nd
O_2 requirement	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic
Optimum growth temperature °C	26–28	nd	nd
Oxidase	+	nd	nd
Nitrate reduction	–	+	+
Urease	–	nd	–
Aesculin degradation			
Indole production	–	nd	nd
H_2S production	–	nd	nd

nd not determined

The type species *A. monumenti* is an aerobic, catalase and oxidase positive coccus (0.8–1.2 μm). It grows between 15 and 30 °C, with an optimum of 28 °C and at a pH range of 6.5–9.0. It tolerates up to 5 % NaCl. Phylogenetically, it is moderately related to the *Friedmanniella/Microlunatus* cluster ❯ *Fig. 36.1* but contains alanine instead of glycine at position 1 of the peptide subunit.

Brooklawnia Rainey, da Costa and Moe 2006, 1981[VP]

Brook.law'ni.a. N.L. fem. n. *Brooklawnia* named after Brooklawn, the contaminated site from which members of the genus were first isolated.

The type species *B. cerclae* is a facultative anaerobic, catalase positive, and oxidase negative pleomorphic rod that grows between 10 and 40 °C, with an optimum of 37 °C and at a pH range of 4.5–8.0 optimum 6.5. It tolerates up to 3 % NaCl. Phylogenetically it is related to *Propionimicrobium lymphophilum* (❯ *Fig. 36.1*) but differ from this species in several chemotaxonomic properties (❯ *Table 36.2*).

Granulicoccus Maszenan, Jiang, Tay, Schumann, Kroppenstedt, Tay 2007, 733[VP]

Gra.nu.li.coc'cus. L. neut. n. *granulum* a small grain; L. masc. n. *coccus* a berry; N.L. masc. n. *Granulicoccus* a coccus from sludge granules.

The type species *G. phenolivorans* is a facultative anaerobic, catalase positive, and oxidase negative coccus (0.3–1.4 μm) that grows between 17 and 37 °C, with an optimum of 30 °C and at a pH range of 5.0–8.5 optimum 7.0. It contains intracellular polyphosphate granules and produces extracellular material. Phylogenetically it is related to *Propioniferax innocua* (❯ *Fig. 36.1*) with which it shares several common chemotaxonomic properties but differs in cell morphology and mol% G+C content of DNA (❯ *Table 36.2*).

Micropruina Shintani, Liu, Hanada, Kamagata, Miyaoka, Suzuki, Nakamura 2000, 205[VP]

Mi.cro.prui'na. Gr. adj. *micros* small, fine; M. L. fem. n. *pruina* hoarfrost; M. L. fem. n. *Micropruina* fine hoarfrost.

The type species *M. glycogenica* is an aerobic, catalase and oxidase positive coccus (0.5–2.2 μm) that grows between 20 °C and 30 °C, with an optimum of 30 °C and at a pH range of 6.0–8.0, optimum 7.0. It accumulates glycogen intracellularly. Phylogenetically it is related to *Propionicimonas paludicola* and *Propionicella superfundia* (>98 % similarity, ❯ *Fig. 36.1*) with which it shares the same meso-A_2pm peptidoglycan composition properties but differs in cell morphology and in the composition of other chemotaxonomic properties (❯ *Table 36.2*).

Propionicicella Rainey, da Costa and Moe 2006, 411, effective publication Validation List N° 111

Pro.pi.o.ni.ci.cel'la, N.L. n. *acidum propionicum*, propionic acid; L. fem. n. *cella*, a storeroom, chamber and in biology a cell; N.L. fem. n. Propionicicella, propionic acid-producing cells.

The type species *P. superfundia* is a facultative anaerobic, catalase and oxidase negative rod (0.5 × 1.7–2.5 μm) that grows between 15 °C and 37 °C, with an optimum of 30 °C and at a pH range of 4.5–8.5, optimum 6.5. It tolerates up to 4 % NaCl. Propionic acid, acetic acid, and formic acid are end-products of

glucose fermentation. Phylogenetically it is related to *Propionicimonas paludicola* and *Micropruina glycogenica* (Fig. 36.1), with which it shares the same meso-A_2pm peptidoglycan composition. The menaquinone is fully unsaturated MK-9 which is unique among members of the family.

Propionicimonas Akasaka, Ueki, Hanada, Kamagata, Ueki 2003, 1996[VP]

Pro.pi.on.i.ci.mo'nas. N.L. n. *acidum propionicum* propionic acid; Gr. fem. n. *monas* a unit, monad; N.L. fem. n. *Propionicimonas* propionic acid-producing monad.

The type species *P. paludicola* is a facultative anaerobic, catalase and oxidase negative irregular rod (0.4–0.5 × 1.8–2.0 µm) that grows between 10 °C and 40 °C, with an optimum of 35 °C and at a pH range of 4.5–7.5, optimum 6.5. It tolerates up to 2 % NaCl and has a requirement for cobalamin. Propionic acid, acetic acid, lactic acid, and minor succinic acid are end-products of glucose fermentation. Phylogenetically it is related to *Propionicicella* and *Micropruina* (Fig. 36.1), with which it shares the same meso-A_2pm peptidoglycan composition.

Propioniciclava Sugawara, Ueki, Abe, Kaku, Watanabe, Ueki 2011, 2302[VP]

Pro.pi.on.i.ci.cla'va. N.L. n. acidum propionicum propionic acid; N.L. fem. n. *clava* club; N.L. fem. *Propioniciclava* propionic acid-producing club.

The type species *P. tarda* is a facultative anaerobic, catalase positive and oxidase negative irregular rod (0.5–0.8 × 0.6–1.8 µm) that grows between 20 °C and 37 °C, with an optimum of 35 °C and at a pH range of 5.3–8.0, optimum 7.5. Growth is inhibited in the presence of NaCl, but cobalamin or a B-vitamin mixture stimulates growth. Propionic acid and acetic acid are the main end-products of carbohydrate fermentation. *P. tarda*, too, is defined by a meso-A_2pm peptidoglycan type. The 16S rRNA gene sequence similarities with other members of the family are low <92 % and its phylogenetic position is not clear-cut. Branching remotely to the *Propionicicella/Propionicimonas/Micropruina* lineage in the RaxML tree, it appears as a sister lineage to the genus *Propionibacterium* in the neighbor-joining tree of the LTP. According to the NJ tree of Sugawara et al. (2011), the *Propioniclava* lineage branches even deepest within *Propionibacteriaceae*.

Propioniferax Yokota, Tamura, Takeuchi, Weiss, Stackebrandt 1994, 581[VP]

Pro.pi.on.i.fe'rax. M.L. *n. acidum propionicum*, propionic acid; L. adj. *ferax*, fertile; M.L. n. *Propioniferax*, propionic acid-producing.

Propioniferax innocua is a facultative anaerobic, catalase and oxidase positive pleomorphic rod (0.5 × 1.2 µm) that grows between 10 °C and 40 °C, with an optimum of 37 °C and at a pH optimum of 7.0. Isolated from human epidermal surface. Originally, the species was affiliated to *Propionibacterium* because of the presence of genus-specific characteristics such as the presence of LL-diaminopimelic acid in the peptidoglycan, coryneform morphology, base composition of DNA, and the formation of propionic acid as the main end-product of glucose fermentation. 16S rRNA analysis placed *Propionibacterium innocuum* as a remotely related member of the genus *Propionibacterium*. However, outside reference organisms that would have shown the position of *P. innocuum* in relation to other coryneform and actinomycete species were not included in the phylogenetic analysis. With the description of *Luteococcus japonicus*, *P. innocuum* emerged as the nearest phylogenetic neighbor (94.5 % 16S rRNA gene sequence similarity), and both species branched as a sister clade to *Propionibacterium* species. In the light of this information, certain properties of *P. innocuum* were re-evaluated: In contrast to the descriptions of most *Propionibacterium* species (Charfreitag et al. 1988; Kusano et al. 1997), strains of *P. innocuum* show aerobic and facultatively anaerobic growth, contain arabinose in cell wall hydrolysates, and do not require blood, serum, or Tween 80 for growth. The principal carboxylic acid produced from glucose, sucrose, maltose, trehalose, fructose, mannose, and glycerol is propionic acid. Polyamines are spermidine and spermine. Phylogenetically it is remotely related to *Granulicoccus phenolivorans*, with which it shares the same peptidoglycan composition.

Propionimicrobium Stackebrandt, Schumann, Schaal, Weiss 2002, 1926[VP]

Pro.pi.on.i.mi.cro«bi.um. N.L. n. *acidum propionicum* propionic acid; Gr. adj. *micros* small; Gr. masc. n. *bios* life. N.L. neut. n. *Propionimicrobium* propionic acid-producing microbe.

Originally described as "*Bacillus lymphophilus*" Torrey 1916, "*Corynebacterium lymphophilum*" Torrey 1916 Eberson 1918 and "*Mycobacterium lymphophilum*" Torrey 1916 Krasil'nikov 1949, strain VIP 0202 has been included in a taxonomic study by Johnson and Cummins (1972) on coryneforms and propionibacteria. The type strain ATCC 27520^T was tentatively classified as *Propionibacterium lymphophilum*, because of low DNA reassociation with members of *Corynebacterium*, anaerobic growth, and the formation of propionic acid. *Propionibacterium lymphophilum* was transferred to a new genus *Propionimicrobium* as it shared less than 91.8 % sequence similarity with the other *Propionibacterium* species (Dasen et al. 1998) which themselves show higher than 93 % similarity among each other.

Main end-products of glucose fermentation are propionic acid, acetic acid, succinic acid, and iso-valeric acid. Formic acid is produced in smaller amounts. Lysine instead of A_2pm is the dicarboxylic amino acid of the peptidoglycan (Johnson and Cummins 1972; Stackebrandt et al. 2002) and aspartic acid

forms the interpeptide bridge. The DNA base composition of 56 mol% G+C is significantly lower than those described for other family members.

Isolation, Enrichment, and Maintenance Procedures

Due to their isolation from different habitats and by different research groups, the isolation procedures and the incubation time from 2 to 10 days to up to 4 weeks of members of the family are quite different. Rather than listing detailed protocols for all species, organisms will be grouped according to similar procedures and only examples will be given. Several strains were reported to be anaerobic or facultative anaerobic. The recently enriched and isolated strains were isolated in an anaerobic chamber on plate count agar 9 (Bae et al. 2006a) or sheep-blood agar (Bae et al. 2006b). Other authors used the anaerobic role tube method of Holdemann et al. (1977) using either PYAS medium and a vitamin B mixture (Sugawara et al. 2011) or a peptone-yeast medium supplemented with carbohydrates, a plant residue extract, and cobalamin (Akasaka et al. 2003).

Most of these strains can be maintained in modified PYG–medium 104 according to the DSMZ catalogue (2001); http://www.dsmz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html (*Table 36.7*).

A nutrient complex medium were used for the isolation/maintenance of most of the aerobic members of the family, such as standard agar medium plus 1 % horse serum (Maszenan et al. 1999b), nutrient broth Difco (Tamura et al. 1994), ISP medium (Wang et al. 2008), Luedeman agar (Luedemann 1968) (Alonso-Vega et al. 2011), nutrient agar (Kämpfer et al. 2010a), and modifications of DSMZ media 776, 65, and 92 for strains of *Micropruina*, *Friedmanniella*, and *Microlunatus*. R2A (An et al. 2008) and 1/5 strength R2A media (Cui et al. 2007) media were used in the isolation of other *Microlunatus* strains. Inorganic salt agar media, such as the one recommended by the SIM Committee on microbiological deteriorations of fuels (Bushnell and Haas 1941), were used for the isolation of *Aestuariimicrobium kwangyangense* (Jung et al. 2007) and for *Tessaracoccus oleiagri* (Cai et al. 2011). Some Friedmanniella type strains were isolated on PYGV medium (*Table 36.8*).

Friedmaniella spumicola strain Ben 107^T *Friedmaniella capsulata* strain Ben 108^T ACM 5121^T was isolated by micromanipulation (Skerman 1968).

If indicated, strains can be maintained as glycerol 20 % cultures. Long-term preservation is done by lyophilization and under a N_2 atmosphere.

Table 36.7
Composition of DSM medium 104

Trypticase peptone	5.00 g
Peptone	5.00 g
Yeast extract	10.00 g
Beef extract	5.00 g
Glucose	5.00 g
K_2HPO_4	2.00 g
Tween 80	1.00 mL
Cysteine-HCl × H_2O	0.50 g
Resazurin 1.00 mg	
Salt solution, see below	40.00 mL
Distilled water	950.00 mL
Haemin solution, see below	10.00 mL
Vitamin K1 solution, see below	0.20 mL
The vitamin K1, haemin solution, and the cysteine are added after the medium has been boiled and cooled under CO_2. Adjust pH to 7.2 using 8 N NaOH. Distribute under N_2 and autoclave	
Salt solution:	
$CaCl_2$ × $2H_2O$	0.25 g
$MgSO_4$ × $7H_2O$	0.50 g
K_2HPO_4	1.00 g
KH_2PO_4	1.00 g
$NaHCO_3$	10.00 g
NaCl	2.00 g
Distilled water	1000.00 mL
Haemin solution:	
Dissolve 50-mg haemin in 1-mL 1 N NaOH; make up to 100 mL with distilled water. Store it refrigerated	
Vitamin K1 solution:	
Dissolve 0.1-mL of vitamin K1 in 20-mL 95 % ethanol and filter-sterilize. Store it refrigerated in a brown bottle	

Ecology

As indicated above, members of *Propionibacteriaceae* genera have been isolated from a wide range of habitats. As in most cases, a single strain only was taxonomically characterized, questions about the actual ecological niche and the functional role of most of the species must remain unanswered. The presence and action of *Microlunatus phosphovorus*, *Micropruina glycogenica*, and *Tessaracoccus bendigoensis* in activated sludge has been reported (Nakamura et al. 1989, 1991, 1995a, b; Seviour et al. 2000; Mino 2000; Kong et al. 2001) but the presence of *Granulicoccus phenolivorans*, *Propioniciclava tarda*, *Friedmanniella spumicola*, and *F. capsulata*, also isolated from similar habitats, has not been reported in other studies. Several strains of different genera were isolated from the Antarctic environment (Lawson et al. 2000; Schumann et al. 1997; Peeters et al. 2011; Shiwaji et al., Unpublished [accession numberJQ396532]), others from soil, the marine environment, associated invertebrates, or freshwater sediments (Krett and Palatinszky 2009). Most additional reports about the presence of family members in different habitats than that indicated for the type strain of species come from non-culture studies.

Table 36.8
Medium PYGV Staley 1968, medium DSM 621

Mineral salt sol. "Hutner/Cohen-Bazire"	20.00 mL
Peptone Bacto	0.25 g
Yeast extract Bacto	0.25 g
Agar Bacto	15.00 g
Distilled water	965.00 mL
Sterilize 20 min/121 °C. After cooling to 60 °C add to the medium:	
Glucose sol. 2.5 %, sterile-filtered	10.00 mL
Vitamin sol. double conc.	5.00 mL
Adjust pH to 7.5, the medium is only weakly buffered; one needs approx. 10 drops/L medium of 6 N KOH	
Mineral salt solution:	
Nitrilotriacetic acid NTA	10.00 g
$MgSO_4 \times 7H_2O$	29.70 g
$CaCl_2 \times 2H_2O$	3.34 g
$Na_2MoO_4 \times 2H_2O$	12.67 mg
$FeSO_4 \times 7H_2O$	99.00 mg
Metall salt sol. "44" see medium 590	50.00 mL
Distilled water	900.00 mL
Dissolve NTA first by neutralizing with KOH, then add other salts. Adjust pH to 7.2 with KOH or H_2SO_4. Adjust volume to 1,000.0 mL with distilled water	
Vitamin solution double conc.:	
Biotin	4.00 mg
Folic acid	4.00 mg
Pyridoxine-HCl	20.00 mg
Riboflavin	10.00 mg
Thiamine-HCl $\times 2H_2O$	10.00 mg
Nicotinamide	10.00 mg
D-Ca-pantothenate	10.00 mg
Vitamin B12	0.20 mg
p-Aminobenzoic acid	10.00 mg
Distilled water	1.0 L
Store in the dark and cold at 5 °C	

Each of the type strain of the type species of *Microlunatus*, *Friedmanniella*, and *Propionimicrobium* is closely to highly related (>96 % BLAST similarity) to clone sequences obtained from DNA which was retrieved from even other sides than those reported for the type strains. The data indicate the presence of many more species to be described once the respective organisms will have been cultured. Only a few reports have been published, e.g., a single clone, originating from DNA of a *Microlunatus* strain, which could not be isolated on any of the seven media provided, was found to be associated with the marine sponge *Hymeniacidon perleve* from the South China Sea (Sun et al. 2010). *Microlunatus* spp. were also found in natural nests of the temperate fungus-growing ant *Trachymyrmex septentrionalis* by culture-independent 16S rRNA gene amplicon 454-sequencing but not in an accompanying culture-dependent study, using a chitin, minimum carbon medium. The results of the culture-independent studies showed a diverse actinobacteria populations, including most prominently *Solirubrobacter* (12.2–30.9 % of sequence reads), *Pseudonocardia* (3.5–42.0 %), but also an abundant presence of *Microlunatus* spp. (0.4–10.8 %). *Microlunatus* were associated with ant garden workers, outside workers and, between July and August, with reproductive females (Ishak et al. 2011). A single clone sequence similar to that of *Propionicimonas paludicola* was found as an ectosymbiont of the ant *Acromyrmex subterraneus brunneus* (Zucchi et al. 2011). The phylogenetic positions of some of the clone sequences have been included in the descriptions of *Auraticoccus monumenti* (Alonso-Vega et al. 2011), *Brooklawnia cerclae* (Bae et al. 2006a), and *Propionicicella superfundia* (Bae et al. 2006b).

Pathogenicity: Clinical Relevance

No clinical significance has been reported for *Luteococcus sanguinis*, *L. peritonei*, and *Propionimicrobium lymphophilum* though they were originally isolated from human material (the reisolate of *P. lymphophilum* originated from urinary tract infections and mesenteric ganglion of a monkey). Schofield and Schaal (1981) indicate a submaxillary tissue as the origin of the type strain ATCC 27529^T. There are neither additional clinical reports nor additional clone sequences related to the 16S rRNA gene sequences of the two *Luteococcus* species. *Propionimicrobium lymphophilum* has been reported in the vaginal epithelium (Hyman et al. 2005; Isaacs, Unpublished [HM021413]; Sizova et al., Unpublished [JN809767]), joint arthrodesis (Goldenberger, Unpublished [HM135521]) and several clone sequences, e.g., HM257519, from the NIH Intramural Skin Microbiome Consortium (Kong et al., Unpublished). *Propioniferax innocua* was isolated from human epidermal surface. The latter study also report several clone sequences to be closely related to *P. innocua*. Even higher related are clone sequences from samples collected from human skin and the surface of human habitations (Taubel et al. 2009).

Application

Microlunatus phosphovorus is physiologically close to other phosphate-accumulating bacteria (PAO) found in enriched sludges and it is one of the few successfully isolated PAOs so far. In a reactor containing about 9 % PAOs of the total bacteria as detected by DAPI stain for polyphosphate, about 2.7 % of the total bacteria belonged to *M. phosphoreus* (Kawaharasaki et al. 1998).

It accumulates large amounts of polyphosphate under aerobic conditions (up to than 48 % of its dry weight in glucose medium), which is then consumed along with the anaerobic uptake of carbon sources like glucose and casamino acids, accompanied by the accumulation of glycogen under anaerobic

conditions (Nakamura et al. 1995a, b). However, it lacks key metabolic characteristics of other organisms involved in enhanced biological phosphate removal as it neither takes up acetate nor accumulates PHA under anaerobic conditions (Minoa et al. 1998; Seviour et al. 2003). A conceptual model for anaerobic carbon metabolism in an EBPR process has been reviewed by Mino (2000). According to Akar et al. (2006), it does produce significant amounts of PHAs under various growth conditions and with different carbon sources. Polyhydroxybutyrate was produced at about 20–30% of the cellular dry weight.

M. phosphoreus contains an ATP-dependent polyphosphate-dependent glucokinase that produces glucose-6-P from glucose and polyphosphate (Tanaka et al. 2003). As determined by tracer studies, *M. phosphovorus* fermented glucose to acetate via an Embden-Meyerhof pathway but was unable to grow under anaerobic *conditions* (Santos et al. 1999). In the aerobic period of the process, acetate was oxidized and growth was strictly dependent on the availability of external phosphate.

The rate of glucose consumption by *M. phosphovorus* was stimulated by phosphate release and, reciprocally, the availability of glucose caused an increase in the rate of phosphate release.

Micropruina glycogenica was originally isolated from an enhanced biological phosphorus removal sequencing batch reactor (SBR) fed with acetate and peptone (Liu et al. 1997). It is one of the few so-called G-bacteria that has been isolated and formally described (another actinobacterium being *Amaricoccus* Maszenan et al. 1997). *M. glycogenica* assimilates organic substrates anaerobically and accumulates glycogen in pure culture; this process has been studied in an aerobic-anaerobic non-P removing SBR biomass fed a mixture of acetate and glucose with fluorescent in situ hybridization FISH in combination with microautoradiography FISH/MAR (Kong et al. 2001). *M. glycogenica*, seen as small aggregate cocci assimilating glucose and acetate while producing polyhydroxybutyrate, was detected by the FISH probe MIC 184 to constitute about 76 % of the high G+C bacteria (22 % of the total bacteria number) which themselves were present in 29 % of total bacteria as detected by molecular analysis.

For other species, a direct application has not been demonstrated but their ability to thrive in the presence of oil and hazardous chemical compounds provides a bioremediation potential. *Brooklawnia cerclae* (Bae et al. 2006a) and *Propionicicella superfundia* (2006b) were isolated from chlorosolvent-contaminated groundwater. Though both type strains did not utilize 1,2-dichloroethane (1,2-DCA) and 1,1,2-trichloroethane (1,1,2-TCA), both strains fermented glucose in the presence of high concentrations of these compounds (*B. cerclae* tolerated up to 9.8 mm of both compounds, while *P. superfundia* grew in the presence of 9.8 mM 1,2-DCA and 5.9 mM 1,1,2-TCA). The type strains of *Tessaracoccus oleiagri* (Cai et al. 2011) and *Aestuariimicrobium kwangyangense* (Jung et al. 2007) were isolated from crude-oil-contaminated saline soil and oil-contaminated tidal flat sediment, respectively. However, in none of the two descriptions, experiments on their oil-degrading properties have been evaluated.

A strain distantly related to *Luteococcus peritonei* was recently isolated from a highly diluted sample of activated sludge of paper mill effluent (Thorenoor et al. 2009). This strain was able to grow on anisole, phenetole, benzene, toluene, phenol, styrene, and biphenyl, producing 2-methoxyphenol and 2-ethoxyphenol from the degradation of anisole and phenetole, respectively. PCR analysis revealed that the genomic DNA encoded at least three ring-hydroxylating dioxygenase large subunits. The authors suggest that the isolate may play a major role in the degradation of lignin-like *O*-aryl alkyl ethers and various aromatic hydrocarbon pollutants in activated sludge of paper mill effluent.

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37 The Order *Pseudonocardiales*

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Abstract

The order *Pseudonocardiales* is made of up a single family *Pseudonocardiaceae* as it now contains all the genera that belonged to the other family in the order, the *Actinosynemmataceae*. It also covers the suborder *Pseudonocardineae* which forms a distinct cluster between the *Frankineae* and *Streptomycineae* when members of the taxa are subjected to 16S rRNA gene phylogenetic analysis. The family has 26 genera *Pseudonocardia*, *Actinoalloteichus*, *Actinokineospora*, *Actinomycetospora*, *Actinophytocola*, *Actinosynnema*, *Alloactinosynnema*, *Allokutzneria*, *Amycolatopsis*, *Crossiella*, *Goodfellowiella*, *Haloechinothrix*, *Kibdelosporangium*, *Kutzneria*, *Labedaea*, *Lechevalieria*, *Lentzea*, *Prauserella*, *Saccharomonospora*, *Saccharopolyspora*, *Saccharothrix*, *Sciscionella*, *Streptoalloteichus*, *Thermocrispum*, *Umezawaea*, and *Yuhushiella*. These genera contain strains which are heterogenous in their morphological and chemotaxonomic features. In general, they are predominantly mycelial actinobacteria with 8 genera having sporangia, pseudosporangia and synnemata, and motile spores as a feature of at least one genus. There are no common chemotaxonomic features though the majority contain galactose in their cell walls and phophatidylethanolamine and the menaquinone MK9 (H_4), though the latter is absent in the type species of the family.

There are 8 monophyletic genera and another 8 that contain between 2 and 5 species, with three genera *Amycolatopsis* (60), *Pseudonocardia* (47), and *Saccharopolyspora* (22) containing at least half the 237 species of the family. For ease of differentiating the taxa, the majority of the morphological, chemotaxonomic, and physiological traits have been placed in comprehensive tables.

Like most actinobacteria, members of the family are predominantly soil dwelling chemoorganotrophs, but some metabolic diversity is reported with growth on carbon dioxide, hydrocarbons, and recalcitrant compounds. Some are halophiles but very few have been isolated from marine sources. Recently new species have been sourced as endophytes indicating that members of certain genera have adapted to living within plant hosts. There have been a few reports of strains isolated from human clinical samples indicating they are opportunistic pathogens. However, some *Crossiella* species have been implicated in equine placentitis. A number of genera including those with large numbers are known to produce antibiotics and other bioactive secondary metabolites, some of which have found clinical use.

Order *Pseudonocardiales* Labeda and Goodfellow 2012, 1301^{VP}

Pseu.do.no.cardi.a.les. N.L. fem. n. *Pseudonocardia* the type genus of the order; suff. -*ales* ending to denote an order; *Pseudonocardiales* the *Pseudonocardia* order.

The removal of the family *Actinosynnemmataceae* Labeda and Kroppenstenstedt 2000 by Labeda et al. (2010a) emended the family *Pseudonocardiaceae* to include genera that belonged to this extant family and consequently elevated the suborder *Pseudonocardineae* Stackebrandt, Rainey and Ward-Rainey 1997, 486^{VP} emend. Zhi, Li and Stackebrandt 2009, 599, to the order *Pseudonocardiales*. Therefore, this order contains a single family *Pseudonocardiaceae* with the type genus *Pseudonocardia* Henssen 1957, 408^{AL}.

Family 1. *Pseudonocardiaceae* Embley, Smida, and Stackebrandt 1989, 205^{VP} emend. Labeda, Goodfellow, Chun, Zhi, and Li 2010a

Pseu.do.no.cardi.a.ce'a.e. N.L. fem. n. *Pseudonocardia* the type genus of the family; suff. -*aceae* ending to denote a family; N.L. fem. pl. n *Pseudonocardiaceae* the *Pseudonocardia* family.

The 16S rRNA signatures consist of nucleotides at positions 127:234 (G–C), 564 (U), 72:734 (U–G), 831:855 (U–G), 832:854 (G–Y), 833:853 (U–G), 952:1229 (U–A), and 986 (U–A).

This family comprises actinobacteria that are aerobic, Gram-stain positive, nonacid fast, and catalase positive and grow in the mesophilic or thermophilic temperature range. The type species is *Pseudonocardia* (Henssen 1957) emend. Park et al. (2008) and 25 other genera: *Actinoalloteichus* Tamura et al. (2000), *Actinokineospora* (Hasegawa 1988) Labeda et al. (2010b), *Actinomycetospora* Jiang et al. (2008), *Actinophytocola* Indananda et al. (2010), *Actinosynnema* Hasegawa et al. (1978), *Alloactinosynnema* Yuan et al. (2010), *Allokutzneria* Labeda and Kroppenstedt (2008), *Amycolatopsis* (Lechevalier et al. 1986) Lee (2009), *Crossiella* Labeda (2001), *Goodfellowiella* Labeda et al. (2008), *Haloechinothrix* Tang et al. (2010b), *Kibdelosporangium* Shearer et al. (1986a), *Kutzneria* Stackebrandt et al. (1994), *Labedaea* Lee (2012), *Lechevalieria* Labeda et al. (2001), *Lentzea* (Yassin et al. 1995) Labeda et al. (2001), *Prauserella* (Kim and Goodfellow 1999) Li et al. (2003b), *Saccharomonospora* Nonomura and Ohara 1971, *Saccharopolyspora* (Lacey and Goodfellow 1975) Li et al. (2009a), *Saccharothrix* (Labeda et al. 1984) Labeda and Lechevalier (1989a), *Sciscionella* Tian et al. (2009), *Streptoalloteichus* (Tomita et al. 1987) Tamura et al. (2008b), *Thermocrispum* Korn-Wendisch et al. (1995), *Umezawaea* Labeda and Kroppenstedt (2007), and *Yuhushiella* Mao et al. (2011).

Figure 37.1 shows the phylogenetic tree of the genera and species of the family *Pseudonocardiaceae* in the order *Pseudonocardiales* based on 16S rRNA gene sequence data analysis.

The morphological characteristics of the members of the family vary with vegetative mycelium that branches and aerial mycelium when present fragments into chains of rod-shaped or square-shaped spores, generally with smooth surfaces. In some species aerial mycelium is not produced. Spores are found on aerial mycelia and can be single or in short chains that have rod-shaped elements with smooth surfaces for some genera or in some taxa are in sporangia, pseudosporangia, or synnemata. Other taxa may fail to produce aerial mycelia, while some genera produce motile spores. While most species of the family are chemoorganotrophs, some autotrophs are notably also present. A few species are implicated as human or animal pathogens, but the majority has been isolated predominantly from soils and other environmental sources such as manure and plant material. In recent years, many members of the family have been isolated as endophytes from a range of plant species including trees and medicinal plants.

Chemotaxonomic properties of the genera of the order are shown in *Table 37.1*. *Meso*-diaminopimelic acid is present in the cell walls of all members of the family and the majority of genera contain galactose as a whole-cell sugar except for some members of the genus *Kutzneria* and the single *Thermocrispum* species. Most genera contain tetrahydrogenated menaquinones with nine isoprene units, but three genera, including the type genus, contain eight isoprene units, and two genera have 9 isoprene units. No mycolic acids are present in any members of the family. The phospholipid composition varies between genera with phosphotidylethanolamine or its hydroxide being the most prevalent, followed by diphosphatidylglycerol, with phosphatidylinositol also present in more than half the species.

The G = C content is 64.1–79.6 (mol%)

Type genus: *Pseudonocardia* Henssen 1957, 408^{AL}, emend. Park, Park, Lee and Kim 2008, 2477^{T}

Further Taxonomic Information

The family *Pseudonocardiaceae* Embley, Smida, and Stackebrandt 1989, 205^{VP} emend. Zhi, Li, and Stackebrandt, 2009, 599 was

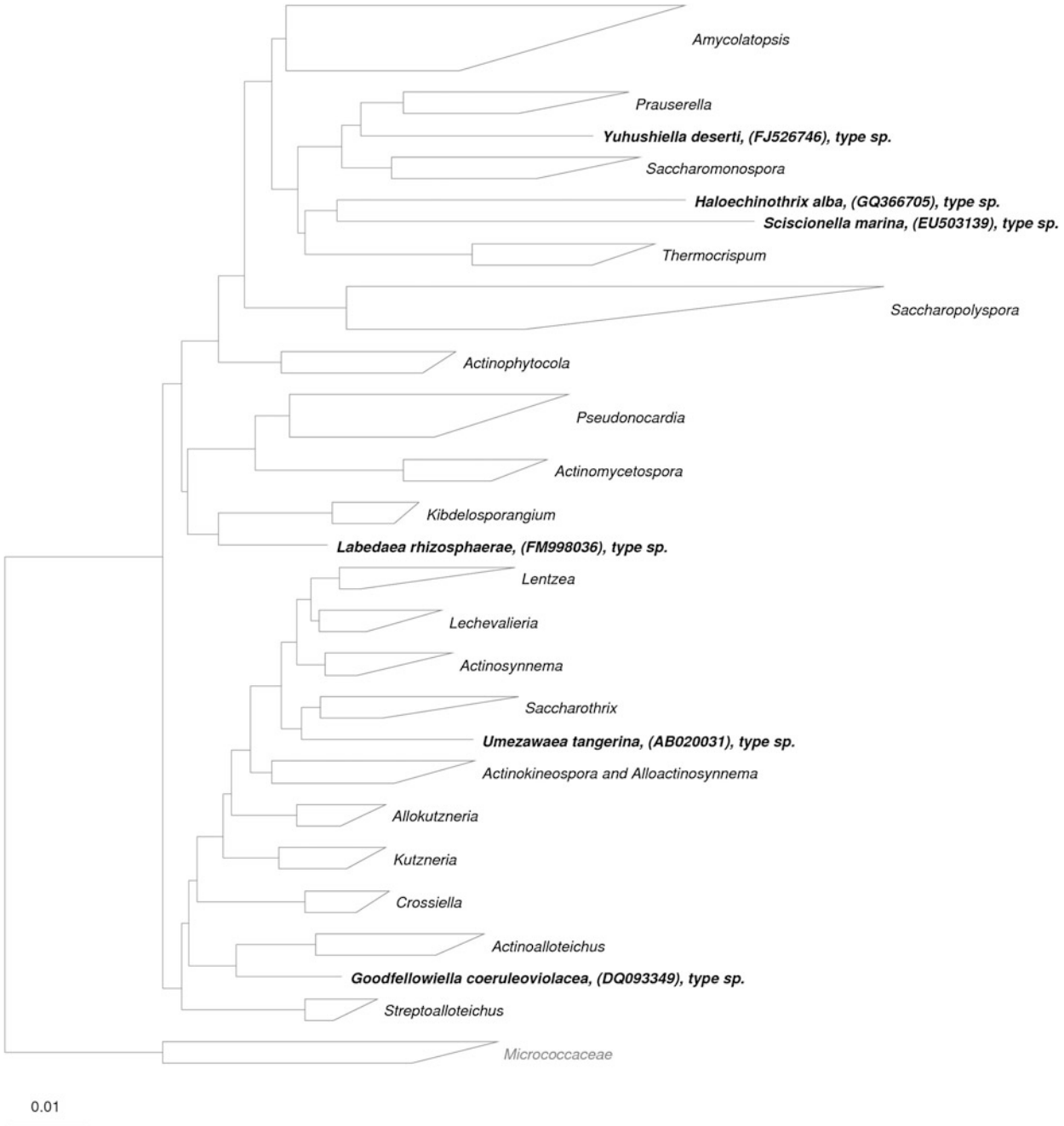

Fig. 37.1

Phylogenetic reconstruction of the family *Pseudonocardiales* based on 16S rRNA gene sequences and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). These sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. In addition, a 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

first proposed by Embley et al. (1988, 1989) and later by Warwick et al. (1994) who used 16S rRNA phylogeny to include the genera *Actinokineospora* and *Saccharothrix* and combine the *Amycolata* into an emended genus *Pseudonocardia*. After the family was emended by Stackebrandt et al. (1997), Labeda and Kroppenstedt (2000) proposed the removal of the genera *Actinosynnema, Actinokineospora, Lentzea*, and *Saccharothrix* into a new family *Actinosynnemataceae* based on 16S rRNA phylogenetic analyses. Recently, Labeda et al. (2010a) studied all the 16S rRNA gene sequences available at the time for members of the families *Actinosynnemataceae* and *Pseudonocardiaceae* and reached the conclusion that the genera within the family *Actinosynnemataceae* be placed into the family *Pseudonocardiaceae* (Fig. 37.2).

Table 37.1

Comparison of selected features of genera within the order *Pseudonocardiales*

Genus	Sporangia produced	Motile spores	Whole-cell sugar pattern	Phospholipids[a]	Predominant menaquinones	DNA Mol% G+C
Pseudonocardia	None	No	Arabinose, galactose, (mannose, ribose, glucose)	PC, PE, PME, PI, PIM, OH-PE, PG	MK-8(H_4)	68.2–78.8
Actinoalloteichus	None	No	Glucose, galactose, mannose, ribose, rhamnose	PI, PG, (PE, PIM, DPG, PME, GluNu)	MK-9(H_4) MK-9(H_6)	72–76.6
Actinokineospora	None	Variable	Galactose, arabinose, (rhamnose, mannose, glucose, glucosamine)	PE	MK-9(H_4) MK-10	68.2–73.8
Actinomycetospora	None	No	Arabinose, galactose, (glucose)	PC, PC, DPG	MK-8(H_4)	69.0–74.0
Actinophytocola	None	No	Arabinose, galactose, rhamnose	PE, OH-PE,(DPG)	MK-9(H_4) MK-10(H_4)	69.7–72.5
Actinosynnema	Synnemata	Yes	Galactose, mannose	PE, OH-PE, DPG	MK-9(H_6)	71.0–73.0
Alloactinosynnema	Pseudosporangia	No	Galactose, ribose	DPG, PG, PC	MK-9(H_4)	68.2
Allokutzneria	Yes, no spores	No	Arabinose, galactose, mannose	PE, PME, OH-PE, PI, lyso-PME, DPG, PG, lyso-PE	MK-9(H_4)	71.7
Amycolatopsis	None	No	Arabinose, galactose,	PE, (DPG, PG)	MK-9(H_4) MK-8(H_4) MK-9(H_2) MK-11(H_4)	65.8–75.0
Crossiella	(Pseudosporangia)	No	Galactose, mannose, rhamnose, ribose	PE, DPG, PME, PI, PIM	MK-9(H_4)	71.4
Goodfellowiella	None	No	Galactose, ribose	PE, DPG, OH-PE, PI, PIM	MK-9(H_4), MK-10(H_4)	68.2
Haloechinothrix	None	No	Glucose, mannose, glucosamine, unknown sugar	DPG, PG, PE, PI, PIM, PL	MK-8(H_4)	68.1
Kibdelosporangium	Yes	No	Arabinose, galactose	PE, PI, PME, (PG, DPG, PIM)	MK-9(H_4)	66.0–67.2
Kutzneria	Yes	No	Rhamnose (galactose),	PE, DPG, PI, OH-PE	MK-9(H_4)	70.3
Labedaea	None	No	Glucose, rhamnose, galactose, ribose, mannose, arabinose, xylose	DPG, PME, PG, PI, PL	MK-9(H_4)	64.2
Lechevalieria	None	No	Galactose, mannose, (rhamnose)	PE, (DPG)	MK-9(H_4)	68.0–70.5
Lentzea	None	No	Galactose, mannose	PE, DPG, PI, PG	MK-9(H_4)	64.1–79.6
Prauserella	None	No	Arabinose, galactose, ribose	DPG, PG, PI	MK-9(H_4) MK-9(H_2)	65.8–70.1
Saccharomonospora	None	No	Arabinose, galactose	(PI, DPG, PG, PE-OH)	MK-9(H_4)	68.1–71.8
Saccharopolyspora	None	No	Arabinose, galactose	PC, DPG, PG	MK-9(H_4), MK-9(H_6)	66.3–76.9
Saccharothrix	None	No	Galactose, rhamnose,	PE	MK-9(H_4), MK-9(H_6), MK-9(H_8), MK-10(H_4)	67.0–74.0
Sciscionella	None	No	Arabinose, galactose, glucose	DPG, PC, PE, PI, PL, PME	MK-9(H_4)	69.0
Streptoalloteichus	Pseudosporangia	Variable	Galactose, mannose, (rhamnose, glucose)	PE	MK-9(H_6), MK-10(H_6)	71.6
Thermocrispum	Pseudosporangia	No	Arabinose, mannose, glucose,	PE, PI, OH-PE	MK-9(H_4)	69.0–73.0
Umezawaea	None	No	Galactose, mannose, ribose, rhamnose	PE, PI, OH-PE, *lyso*-PE, DPG, PIM	MK-9(H_4),	74.0
Yuhushiella	Pseudosporangia	No	Arabinose, galactose, glucose, ribose	PE, PIM, PME, DPG, PL, GluNu	MK-9(H_4)	69.9

Values in brackets are found in the majority, not all species

[a]Abbreviations: *DPG* diphosphatidylglycerol, *GluNu* phospholipids of unknown structure containing glucosamine, *OH-PE* phosphatidylethanolamine with hydroxy fatty acids, *lyso*-PE, phosphatidylethanolamine where one fatty acid chain is missing from the glycerol backbone, *lyso*-PME phosphatidylmethylethanolamine where one fatty acid chain is missing from the glycerol backbone, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PL* unknown phospholipids, *PME* phosphatidylmethylethanolamine

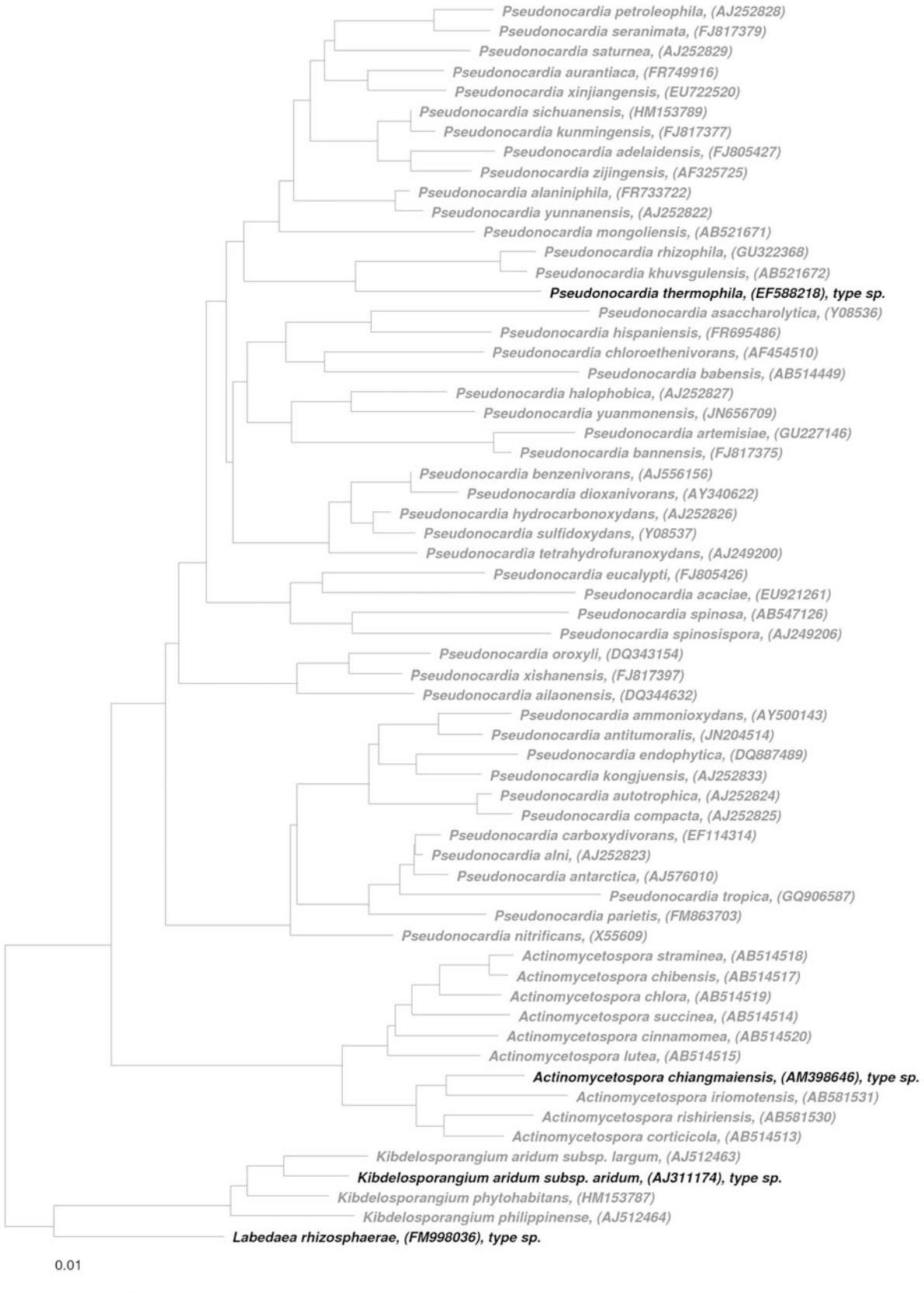

Fig. 37.2
Phylogenetic tree of the genera *Pseudonocardia, Actinomycetospora, Kibdelosporangium* and *Labedaea*

Pseudonocardia Henssen 1957, 408[AL], emended Park, Park, Lee and Kim 2008, 2477[VP]

Pseu.do.no.cardi.a. Gr. adj. *pseudês* false; N.L. fem. n. *Nocardia* a bacterial genus name; N.L. fem. n. *Pseudonocardia* false *Nocardia*.

Phenotypic Analyses

Pseudonocardia species are aerobic, Gram-stain positive, nonacid fast, nonmotile, catalase positive, and mesophilic or thermophilic. Substrate and aerial mycelium can form apical or intercalary swellings and produce spores that are smooth with two species known to have spiny or "knobby" spore surfaces. Thickness of hyphae is variable and can fragment into rod-shaped elements or chains that are oval or square-shaped or chains with two or more spores. Some species are facultative autotrophs. Chemotaxonomic analysis of the cell wall shows that most *Pseudonocardia* species possess *meso*-diaminopimelic acid and whole-cell hydrolysates contain arabinose and galactose. Menaquinone MK-8 (H_4), or MK-8 (H_2) or MK-9 within a limited number of taxa, is the predominant isoprenoid quinone, and iso-branched hexadecanoic acid is the predominant fatty acid. No mycolic acids are detected and the phospholipids are type II, III or IV depending on the species, but the following are commonly found: phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl methylethanolamine, and glucosamine-containing phospholipids.

The G+C content of the DNA of members of the genus *Pseudonocardia* ranges from 68.2 to 78.8 (mol%).

The type species in *Pseudonocardia thermophila* ATCC 19285^T (Huang and Goodfellow 2012).

Isolation Procedures

A range of isolation techniques that were utilized to obtain the type species of *Pseudonocardia* are presented in *Table 37.2*.

Taxonomy, Historical, and Current

The phylogenetic position of *Pseudonocardia*, as determined by 16S rRNA gene sequence analysis, forms a distinct clade in the order *Pseudonocardiales* (*Figs. 37.1*, *37.2*, *37.3*, *37.4*, and *37.5*: 16S rRNA gene tree of the order *Pseudonocardiales*). The closest genera are *Actinomycetospora*, which have no aerial mycelium, and *Kibdelosporangium*, which form pseudosporangia and contain MK-9 (H_4) as the predominant menaquinone, whereas most species of the genus *Pseudonocardia* contain MK-8(H_4). *Pseudonocardia* spp. have distinct common 16S rRNA gene sequence regions which correspond to nucleotide positions 179–219 and 813–833 of the *Streptomyces ambofaciens* 16S rRNA gene (Pernodet et al. 1989).

The genus *Pseudonocardia* was proposed for a thermophilic strain and two other actinomycetes which formed aerial spores in chains by exhibiting acropetal budding of substrate and aerial hyphae (Henssen 1970; Henssen and Schäfer 1971; Henssen and Schnepf 1967). These strains were *Pseudonocardia thermophila*, (Henssen 1957). *Pseudonocardia compacta* (Henssen 1957) and *Pseudonocardia spinosa* (Henssen and Schäfer 1971; Schäfer 1969). Huang et al. (2002) found that the *Actinobispora* and *Pseudonocardia* species shared key chemical markers with MK-8(H_4) as the major menaquinone and proposed that the genus *Actinobispora* (Xu et al. 1999) which contained *Actinobispora alaniniphila*, *Actinobispora aurantiaca*, *Actinobispora xinjiangensis*, and *Actinobispora yunnanensis* should become part of the genus *Pseudonocardia*. In 1994, Warwick et al. (1994) analysed the 16S rRNA gene sequences of the type strains of *Amycolata* and showed that they were more closely related to *Pseudonocardia* species. *Amycolata autotrophica* was classified to a new genus *Pseudoamycolata* (Akimov et al. 1989) as it lacked phosphatidylcholine. However, apart from this difference other characteristics were very similar to *Pseudonocardia* species and McVeigh et al. (1994) proposed its reclassification as *Pseudonocardia halophobica*.

The chemotaxonomic and physiological properties of the species of the genus *Pseudonocardia* are shown in *Tables 37.3* and *37.4*.

Ecology

Pseudonocardia species have a versatile metabolism with some species being facultative autotrophs. *Pseudonocardia autotrophica*, *Pseudonocardia petroleophila*, and *Pseudonocardia saturnae* can utilize CO_2 and oxidize hydrogen (Lechevalier et al. 1986), whereas *Pseudonocardia carboxydivorans* can utilize CO_2 as sole carbon source (Park et al. 2008) and *Pseudonocardia ammonioxydans* can grow both autotrophically on nitrifying medium as well as heterotrophically on a complex nitrogen source (Liu et al. 2006). *Pseudonocardia hydrocarboxydans* and *Pseudonocardia petroleophila* can utilize simple hydrocarbons as well as hexanes (Hirsch and Engel 1956; Nolof and Hirsch 1962); *Pseudonocardia ascaccharolytica* and *Pseudonocardia sulfidoxydans*, *Pseudonocardia benzenivorans*, *Pseudonocardia chloroethenivorans*, *Pseudonocardia dioxanivorans*, and *Pseudonocardia dinitrificans* can utilize DMSO, chlorobenzenes, chloroethane, dioxane, and urethane, respectively (Schatz et al. 1954; Reichert et al. 1998; Kämpfer and Kroppenstedt 2004; Lee et al. 2004; Mahendra and Alvarez-Cohen 2005).

The nutritional requirements, degradation, and enzyme activity of selected members of this genus are described in *Table 37.5*.

More than half of the 45 species of the genus *Pseudonocardia* have been reported over the last 10 years with a high proportion of these new species isolated from plant or plant-associated samples (Gu et al. 2006; Chen et al. 2009; Duangmal et al. 2009; Kaewkla and Franco 2010, 2011; Li et al. 2012; Qin et al. 2010b, 2011; Reichert et al. 1998; Zhao et al. 2011a, b, c, d, 2012a, b).

Table 37.2
Origin of isolate and method of isolation for *Pseudonocardia* species

Species	Source	Province/country	Method of isolation	References
Pseudonocardia thermophila	Fresh and rotten manure	nr	nr	Huang and Goodfellow (2012)
Pseudonocardia acaciae	Roots of *Acacia auriculiformis* A. Cunn. ex Benth. (earpod wattle)	Bangkok, Thailand	Surface-sterilized roots using starch–casein agar (Küster and Williams 1964) supplemented with antibacterial and antifungal agents (nalidixic acid and ketokonazole at 25 and 50 μg ml^{-1}, respectively); incubated at 28 °C for 21 days	Duangmal et al. (2009)
Pseudonocardia adelaidensis	Stem of a gray box tree (*Eucalyptus microcarpa*)	Adelaide, South Australia	Internal tissue of a surface-sterilized stem sample was inoculated onto 10 isolation media supplemented with nalidixic acid (20 μg ml^{-1}) and nystatin (100 units ml^{-1}); incubated at 27 °C for 8 weeks	Kaewkla and Franco (2010)
Pseudonocardia ailaonensis	Soil sample	Ailao Mountain, Yunnan Province, China	Isolated from Yunnan soil sample on modified starch casein agar [0.3 g mannose, 2 g KNO_3, 0.3 g casein, 0.05 g $MgSO_4 \cdot 7H_2O$, 2 g NaCl, 2 g K_2HPO_4, 0.02 g $CaCO_3$, 10 mg $FeSO_4 \cdot 7H_2O$, and 15 g agar in 1,000 ml tap water (pH 7.2–7.4)]; incubated at 28 °C for 2 weeks	Qin et al. (2008b)
Pseudonocardia alaniniphila	Soil sample	Xichou, Yunnan Province, China	Strains were isolated using AV agar (Ochi 1995; Nonomura and Ohara 1971) and HV agar (Hayakawa 1990) from soil samples. Plates were incubated at 28 °C for 21–28 days	Xu et al. (1999), Huang et al. (2002)
Pseudonocardia alni	Root nodules and rhizospheres of alders *Alnus glutionosa* (L.) Gaerth. and *Alnus incana* (L.) Moench. and from marine sediments	Pushchino, USSR	nr	Evtushenko et al. (1989), Warwick et al. (1994)
Pseudonocardia ammonioxydans	Coastal sediment	Jiao-Dong peninsula, near Qingdao, Shandong Province, China	Isolated from coastal sediment using modified nitrifying medium; incubated at 30 °C for 1 month	Liu et al. (2006)
Pseudonocardia antarctica	Rock and soil samples	McMurdo Dry Valley, Antarctica	Isolated from moraine sample using YPD agar; incubated at 25 °C for 4 days	Prabahar et al. (2004)
Pseudonocardia antitumoralis	Deep -sea sediment	Northern South China	Strain was isolated through a serial dilution plate method followed by incubation at 28 °C for 3 weeks on Gause No. 1 medium prepared with seawater	Tian et al. (2013)
Pseudonocardia artemisiae	Roots of *Artemisia annua* L	Yunnan Province, China	Isolated from surface-sterilized tissue of *Artemisia annua* L. on TWYE agar plates; incubated at 28 °C until the outgrowth of endophytic actinomycetes were discerned	Zhao et al. (2011b)
Pseudonocardia asaccharolytica	Tree-bark compost biofilters from an animal-rendering plant	nr	Isolated using tree-bark compost enrichment using biofilters supplied with methyl sulfide containing off-gas from an animal-rendering plant; Tubes (50 ml) sealed with Teflon lined screw caps and were filled with mineral salts medium and incubated at 25 °C for several weeks	Reichert et al. (1998)

Table 37.2 (continued)

Species	Source	Province/country	Method of isolation	References
Pseudonocardia aurantiaca	Soil sample	Jianchuan, Yunnan Province, China	Strains were isolated using AV agar (Ochi 1995; Nonomura and Ohara 1971) and HV agar (Hayakawa 1990) from soil samples. Plates were incubated at 28 °C for 21–28 days	Xu et al. (1999), Huang et al. (2002)
Pseudonocardia autotrophica	Phosphate buffer solution, aluminum hydroxide gel, vegetable matter, soil, and clinical specimens	nr	nr	Huang and Goodfellow (2012), Warwick et al. (1994)
Pseudonocardia babensis	Plant litter	Ba Be National Park, Vietnam	Isolated from plant litter samples using yeast extract soluble starch medium; incubated at 28 °C for 10–14 days	Sakiyama et al. (2010)
Pseudonocardia bannensis	Roots of *Artemisia annua* L	Yunnan Province, China	Surface sterilized from the roots of *Artemisia annua* L. on HV agar plates; incubated at 28 °C for 4–6 weeks	Zhao et al. (2011c)
Pseudonocardia benzenivorans	Soil sample	Bitterfeld, Germany	Isolated from contaminated soil using selective medium containing 1,2,3,5-tetrachlorobenzene as the sole carbon source; incubated at 25 °C	Kämpfer and Kroppenstedt (2004)
Pseudonocardia carboxydivorans	Soil sample	Seoul, Korea	Isolated from soil using enrichment medium [400 p.p.m. CO in air, 0.005 % (w/v) yeast extract] and solid mineral medium; incubated at 30 °C for 2–6 weeks	Park et al. (2008)
Pseudonocardia chloroethenivorans	Aerobic laboratory enrichment	Department of Civil and Environmental Engineering, University of Washington, Seattle, USA	Isolated from laboratory enrichment using mineral-medium agar plates; incubated at 25 °C for 5 days	Lee et al. (2004)
Pseudonocardia compacta	Garden soil	Wohra, near Marburg, Germany	nr	Henssen et al. (1983)
Pseudonocardia dioxanivorans	1,4-dioxane-contaminated sludge sample	Darlington, South Carolina, USA	Isolated from enriched 1,4-dioxane contaminated industrial sludge, grown aerobically in ammonium mineral salts (AMS) liquid medium (Parales et al. 1994) at 30 °C with 5 mM 1,4-dioxane	Mahendra and Alvarez-Cohen (2005)
Pseudonocardia endophytica	Tissue sample of plant *Lobelia clavata*	Xishuangbanna, Yunnan Province, China	Surface sterilized tissue of *L. Clavata* plated onto TWYE containing nalidixic acid (10 mg ml^{-1}), nystatin and cycloheximide (each at 50 mg ml^{-1}); incubated at 28 °C for 4 weeks	Chen et al. (2009)
Pseudonocardia eucalypti	Roots of a native Australian eucalyptus tree (*Eucalyptus microcarpa*)	Adelaide, South Australia	Surface sterilized root tissue were plated onto Humic acid–vitamin B (HV) medium (Hayakawa and Nonomura 1987) but solidified with 0.8 % of gellan gum instead of agar; plates incubated at 27 °C for up to 12 weeks	Kaewkla and Franco (2011)
Pseudonocardia halophobica	Soil sample	nr	nr	Huang and Goodfellow (2012), McVeigh et al. (1994)

Table 37.2 (continued)

Species	Source	Province/country	Method of isolation	References
Pseudonocardia hispanensis	Sample from oil refinery wastewater treatment plant	Palos de la Frontera, Huelva, Spain	Isolated by using a modified Czapek agar (sucrose, 2 %, w/v; yeast extract, 0.2 %, w/v, $FeSO_4$, 0.001 %; KCl, 0.001 %; K_2HPO_4, 0.1 %; $MgSO_4$. $7H_2O$, 0.05 %; $NaNO_3$, 0.2 %; agar 1.5 %, w/v; distilled water, 1 l) plate supplemented with nalidixic acid (20 mg l^{-1}); incubated for 14 days at 28 °C	Cuesta et al. (2013)
Pseudonocardia hydrocarbonoxydans	Air contaminant	nr	Isolated from a silica gel plate	Huang and Goodfellow (2012), Warwick et al. (1994)
Pseudonocardia khuvsgulensis	Soil sample from Khuvsgul Lake	Khuvsgul province, Mongolia	Dried soil samples inoculated using the rehydration–centrifugation method (Hayakawa et al. 2000) on humic acid–vitamin agar (Hayakawa and Nonomura 1987) containing trimethoprim (20 mg l^{-1}) and nalidixic acid (10 mg l^{-1}), incubated at 28 °C for 3–4 weeks	Ara et al. (2011b)
Pseudonocardia kongjuensis	Gold mine cave	Kongju, Republic of Korea	Isolated from soil by dilution plating method on tap water agar (Lee 1996). Cells were obtained after the organisms were grown on trypticase soy broth (BBL) with shaking at 30 °C for 3 days	Lee et al. (2001)
Pseudonocardia kunmingensis	Surface-sterilized roots of *Artemisia annua* L	Yunnan Province, China	Isolated from surface-sterilized roots followed by plating on HV agar plates (Hayakawa and Nonomura 1987); incubated at 28 °C until the outgrowth of endophytic actinomycetes were discerned	Zhao et al. (2011a)
Pseudonocardia mongoliensis	Soil sample	Khuvsgul province, Mongolia	Dried soil samples inoculated using the rehydration–centrifugation method (Hayakawa et al. 2000) on humic acid–vitamin agar (Hayakawa and Nonomura 1987) containing trimethoprim (20 mg l^{-1}) and nalidixic acid (10 mg l^{-1}), incubated at 28 °C for 3–4 weeks	Ara et al. (2011b)
Pseudonocardia nitrificans	Soil sample	nr	Isolated on distilled water basal medium supplemented with 0.2 % urethane and 0.02 % trace metals. All the samples were incubated aerobically at 28 °C to 30 °C in the dark	Warwick et al. (1994)
Pseudonocardia oroxyli	Root of *Oroxylum indicum*	Yunnan Province, China	Surface-sterilized root elongation zone of *Oroxylum indicum*, plated onto BL-2 agar plates supplemented with penicillin (100 μg ml^{-1}); incubated at 27 °C for 2–4 weeks	Gu et al. (2006)
Pseudonocardia parietis	Wall of an indoor environment colonized with moulds	Stuttgart, Germany	Isolated from a wall colonized with moulds. After extraction of 1 g material sample for 15 min in 10 ml 0.9 % NaCl solution containing 0.01 % (v/v) Tween 80 and dilution on M79 agar (containing 10 g glucose, 10 g Bacto peptone, 2 g casein hydrolysate, 2 g yeast extract, 6 g NaCl, 15 g agar) for 2 weeks at 28 °C	Schäfer et al. (2009)

Table 37.2 (continued)

Species	Source	Province/country	Method of isolation	References
Pseudonocardia petroleophila	Soil sample	Germany	nr	Warwick et al. (1994)
Pseudonocardia rhizophila	Rhizosphere soil of *Tripterygium wilfordii Hook* F	Yunnan Province, China	Isolated from the rhizosphere soil of *Tripterygium wilfordii* Hook. f., by the standard serial dilution technique using raffinose–histidine agar (Vickers et al. 1984) and the plates were incubated at 28 °C for 2–3 weeks	Li et al. (2010)
Pseudonocardia saturnea	Air and compost	Germany	nr	Huang and Goodfellow (2012), Warwick et al. (1994)
Pseudonocardia seranimata	Leaves of *Artemisia annua* L	Yunnan Province, China	Isolated from surface-sterilized leaves followed by plating on sodium propionate asparagine–plant extract agar plates (Li et al. 2010) containing nalidixic acid (25 mg l^{-1}), nystatin (50 mg l^{-1}) and cycloheximide (50 mg l^{-1}); incubated at 28 °C for 4–8 weeks	Zhao et al. (2011d)
Pseudonocardia sichuanensis	Root of *Jatropha curcas* L	Sichuan Province, China	Isolated from surface sterilized root and plated onto tap water–yeast extract agar (TWYE) (Crawford et al. 1993); incubated at 28 °C for 2–8 weeks	Qin et al. (2011)
Pseudonocardia spinosa	Soil sample	Turkey	nr	Huang and Goodfellow, (2012)
Pseudonocardia spinosispora	Soil from a gold mine	Kongju City, Korea	Isolated by dilution plating of soil sample on tap-water agar (Lee 1996)	Lee et al. (2002)
Pseudonocardia sulfidoxydans	Tree-bark compost biofilters from an animal-rendering plant	nr	Isolated using tree-bark compost enrichment using biofilters supplied with methyl sulfide containing off-gas from an animal-rendering plant; tubes (50 ml) sealed with Teflon lined screw caps and were filled with mineral salts medium and incubated at 25 °C for several weeks	Reichert et al. (1998)
Pseudonocardia tetrahydrofuranoxydans	Sludge of a wastewater treatment plant	Göttingen, Germany	Enriched and recovered on a selective medium containing tetrahydrofuran (THF) as the single carbon source from sludge from a wastewater plant	Kämpfer et al. (2006)
Pseudonocardia tropica	Stem of *Maytenus austroyunnanensis*	Yunnan Province, China	Isolated from surface-sterilized stems and were spread onto sodium propionate agar (Qin et al. 2009), incubated at 28 °C for 3 weeks	Qin et al. (2010b)
Pseudonocardia xinjiangensis	Soil sample	Xinjiang Province, China	Strains were isolated using AV agar (Nonomura and Ohara 1971) and HV agar (Hayakawa 1990) from soil samples. Plates were incubated at 28 °C for 21–28 days	Huang et al. (2002), Xu et al. (1999)
Pseudonocardia xishanensis	Roots of *Artemisia annua* L	Yunnan Province, China	Isolated by surface-sterilizing root samples described by Li et al. (2008), incubated on humic acid–vitamin agar (Hayakawa and Nonomura 1987) supplemented with 25 mg l^{-1} nalidixic acid and 50 mg l^{-1} nystatin at 28 °C for 35 days	Zhao et al. (2012b)

Table 37.2 (continued)

Species	Source	Province/country	Method of isolation	References
Pseudonocardia yuanmonensis	Soil sample	Yunnan Province, China	Soil sample was air dried at room temperature followed by serial dilution and was incubated on ISP 5 medium agar plate, supplemented with nalidixic acid (25 mg l^{-1}) and nystatin (50 mg l^{-1}). The plate was incubated at 28 °C for 1 week	Nie et al. (2012b)
Pseudonocardia yunnanensis	Soil sample	Yunnan Province, China	The strain was isolated by plating the soil sample on glycerine–asparagine agar and incubated for 2 weeks at 28 °C	Jiang et al. (1991), Huang et al. (2002)
Pseudonocardia zijingensis	Soil sample	Yunnan Province, China	Isolated on a yeast extract/starch agar (Emerson, 1958) plate, which had been seeded with a soil suspension and incubated at 28 °C for 3 weeks	Huang et al. (2002)

Symbols: *nr* not reported

Another well-established relationship, in which Pseudonocardiae plays a major role, is the mutualistic association in Attine leaf-cutting ants. The role of the actinobacterial partner is believed to be in inhibiting the growth of the *Escovopsis* parasites that would attack the fungal gardens (Cafaro and Currie 2005; Currie et al. 1999a, b, 2003; Kost et al. 2007), which are a nutrient source for the ants (Chapela et al. 1994; Martin 1970; Weber 1966, 1972).

Application

Pseudonocardia are well known as a source of biologically active natural products, such as vancomycin, erythromycin, and rifamycin (Platas et al. 1998; Bredholdt et al. 2007). *Pseudonocardia* strains produce antimicrobial compounds such as the novel glycopeptides, helvecardins A and B (Takeuchi et al. 1991), and phenazostatin D (Maskey et al. 2003) and eight new quinolone compounds with selective and potent anti-*Helicobacter pylori* activity (Dekker et al. 1998). *Pseudonocardia autotrophica* produces a broad spectrum antifungal compound (Kim et al. 2009). *Pseudonocardia* strain No. G495-11 (ATCC 53205) produces Boxazomycin A and B which inhibited growth of aerobic Gram-positive bacteria and anaerobes (Kusumi et al. 1988).

Pseudonocardia autotrophica has been reported recently to be able to bioconvert vitamin D_3 to its physiologically active forms, namely, 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3 clinically used for chronic renal failure, hypoparathyroidism, osteoporosis, and psoriasis (Takeda et al. 2006; Fujii et al. 2009). Vitamin D_3 exerts its physiological activity by being converted to 25-hydroxyvitamin D_3 in the mammalian liver and subsequently to 1α,25-dihydroxyvitamin D_3 in the kidney via approximately 20 steps, but the yield is very low (approximately 1 %). The microbial reaction has an advantage to reduce the number of steps of synthesis.

Genetics and Molecular Analyses

There have been a number of genetic studies on *Pseudonocardia* strains because of their metabolic versatility and their ability to utilize simple and recalcitrant carbon sources as well as their ability to produce antibiotics (Huang and Goodfellow 2012).

Pseudonocardia dioxanivorans CB1190 was selected as the first member of the genus to have its annotated genome sequence published as it was the first actinobacterium capable of growing on 1,4-dioxane (Sales et al. 2011). The genome consists of four replicons, namely, the 7.1 Mb chromosome, two circular plasmids (137 with gaps, and 192 kb), and a 15 kb linear plasmid. Eight putative gene clusters encoding bacterial multicomponent monooxygenases were found, that could be involved in the aerobic degradation of 1,4 dioxane, as well as degradation of tetrahydrofuran, similar to that found in *Pseudonocardia tetrahydrofuranoxydans* strain K1 (Vainberg et al. 2006).

Pathogenicity

Pseudonocardia strains are not known or their pathogenicity though some *Pseudonocardia autotrophica* have been isolated from clinical specimens (Mishra et al. 1980; Schaal and Beaman 1984) indicating their presence in immunocompromised patients (*Fig. 37.3*).

Actinoalloteichus Tamura, Zhiheng, Yamei, and Hatano 2000, 1439^{VP}

Ac.ti.no.al.lo.tei.chus. Gr. n. *aktis*, *aktinos* ray -used to refer to actinomycetes; Gr. adj. *allos* another, the other; Gr. masc. n. *teichos* wall; N.L. masc. n. *Actinoalloteichus* actinomycete with a different wall.

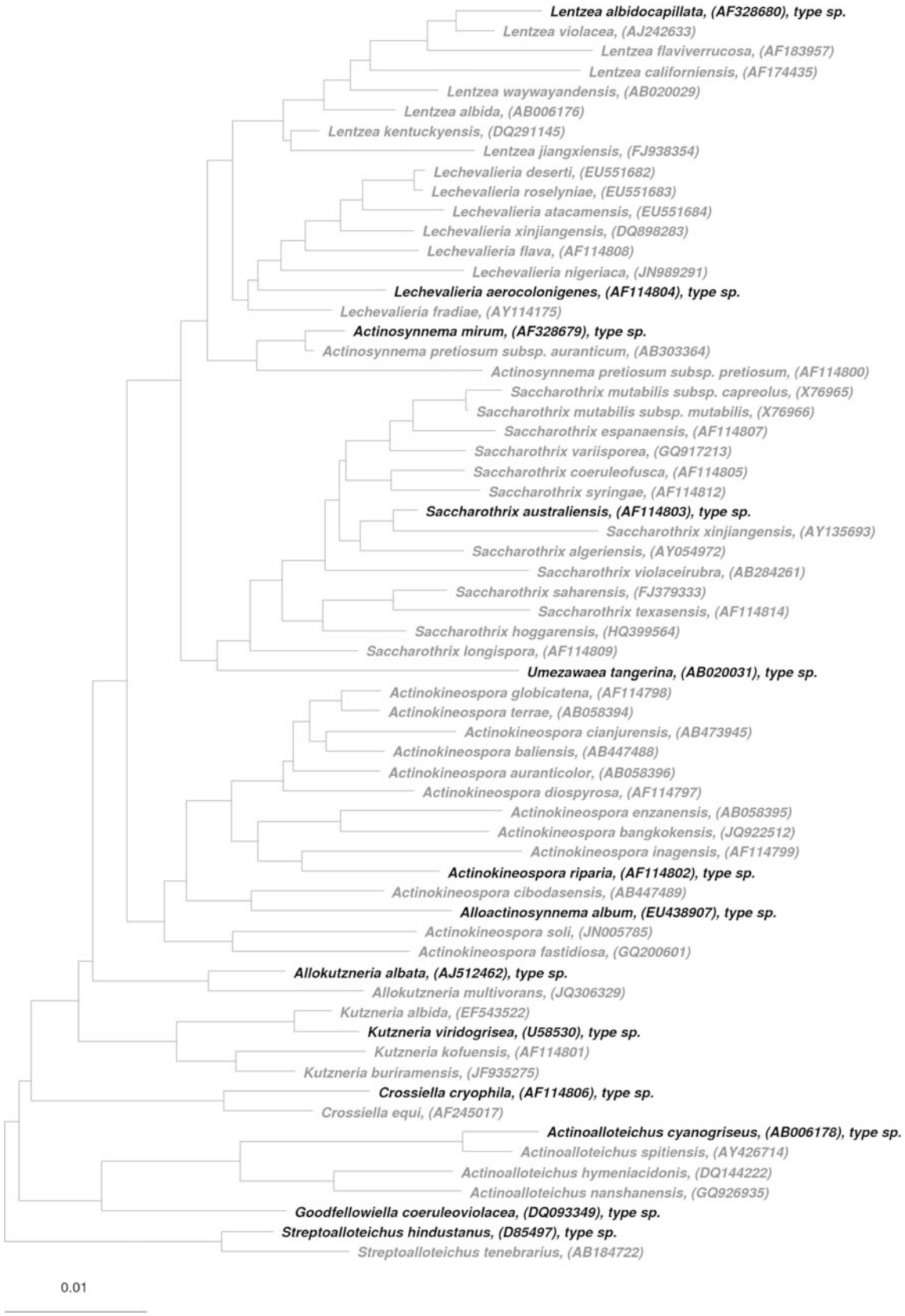

Fig. 37.3

Phylogenetic tree of the genera *Lentzea, Lechevaliera, Actinosynnema, Saccharothrix, Umezawaea, Actinokineosporia, Alloactinosynnema, Allokutzneria, Kutzneria, Crossiella, Actinoalloteichus, Goodfellowiella* and *Streptoalloteichus*

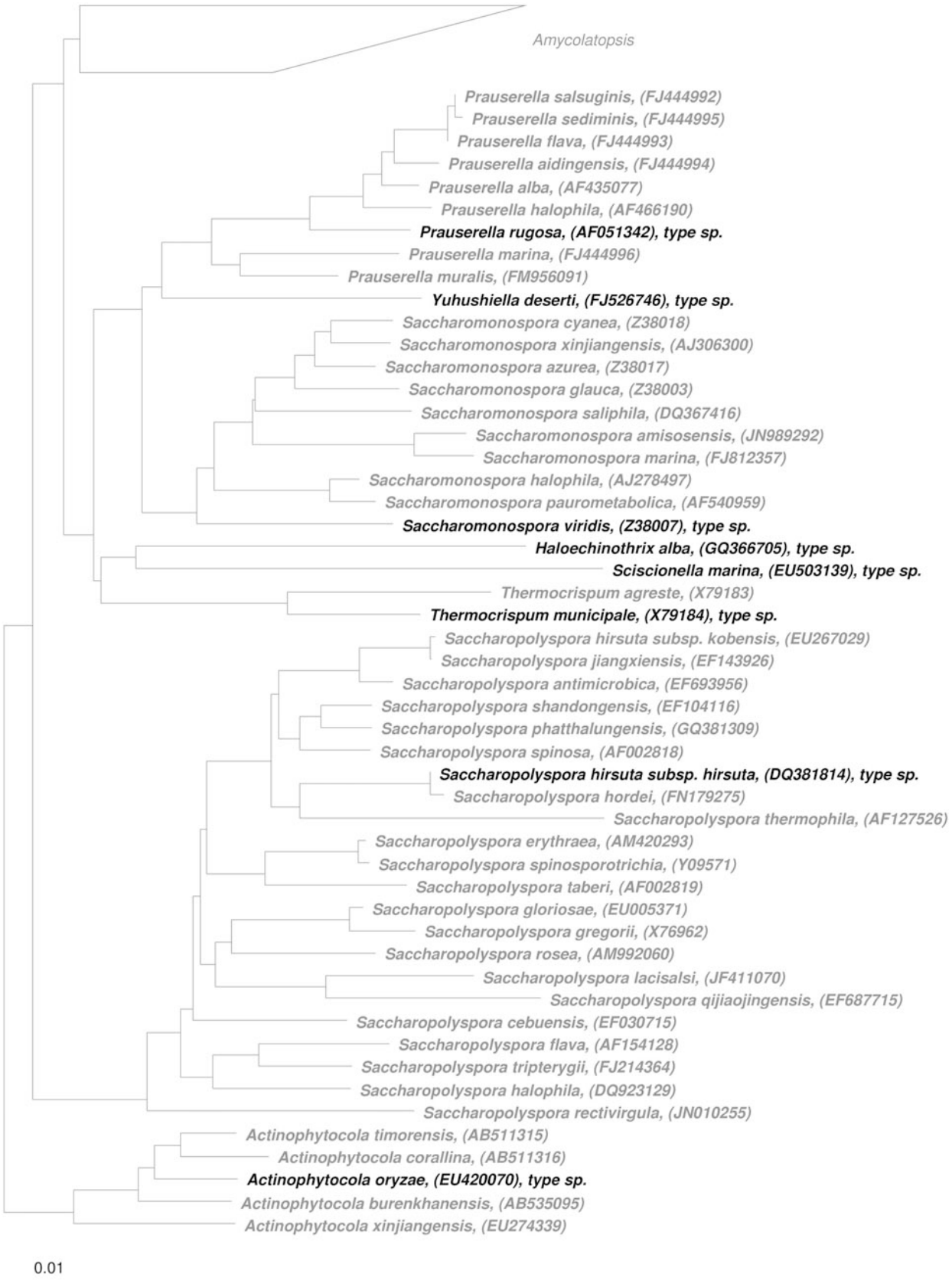

Fig. 37.4

Phylogenetic tree of the genera *Prauserella, Yuhushiella, Saccharomonospora, Haloechinothrix, Sciscionella, Thermocrispum, Saccharopolyspora* and *Actinphytocola*

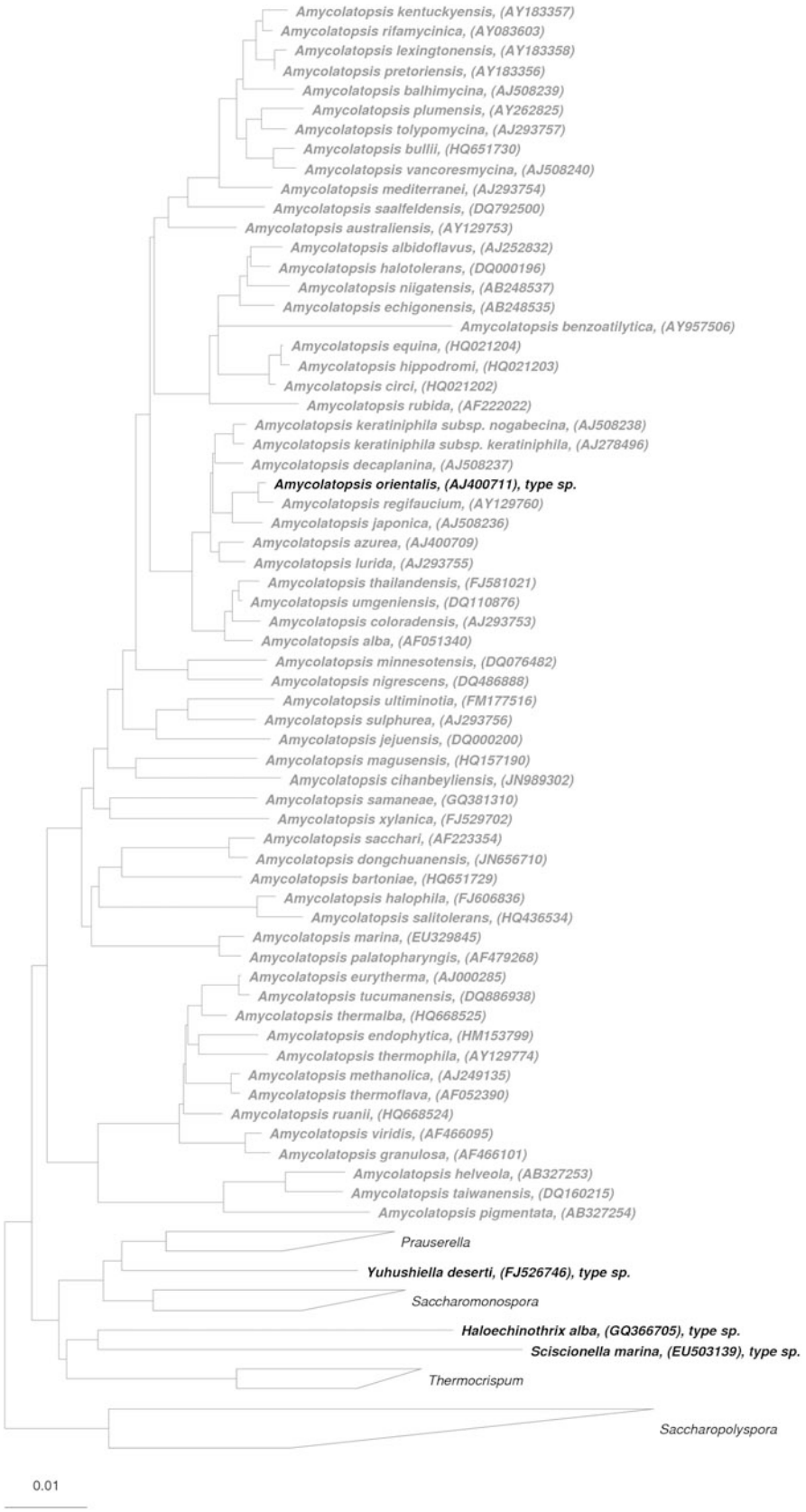

Fig. 37.5
Phylogenetic tree of the genus *Amycolatopsis*

Table 37.3
Chemotaxonomic characteristics of *Pseudonocardia* species

Species	Major menaquinone(s)			Whole-cell sugars					Phospholipids	Major Fatty acids	DNA G+C content (mol %)
	MK-8(H2)	MK-8(H4)	MK-9	Arabinose	Mannose	Ribose	Glucose	Galactose			
P. thermophila		+		+	nr	nr	nr	+	nr	nr	nr
P. acaciae		+		+	nr	nr	nr	+	nr	*iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$, $C_{17:1}$ ω6*c*	71.6
P. adelaidensis		+		nr	nr	nr	nr	nr	nr	*iso*-$C_{15:0}$, *iso*-$C_{16:1}$H, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl	78.8
P. ailaonensis		+		+	nr	+	+	+	DPG, PG, PI	*iso*-$C_{16:0}$ 2-OH, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl	74.1
P. alaniniphila		+		nr	nr	nr	nr	nr	PC, PE, PG, PME, GluNu	nr	69.3
P. alni		+		nr	nr	nr	nr	nr	PC, PE	*iso*-$C_{16:1}$, $C_{16:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$, $C_{17:0,}$ $C_{18:0}$ methyl	72–74
P. ammonioxydans		+		+	nr	nr	nr	+	DPG, PC, PG, PIM, PME	*iso*-$C_{16:1}$, *iso*-$C_{16:0}$, $C_{17:1}$ ω8*c*	69.6
P. antarctica		+		+	nr	nr	nr	+	DPG, PC, PE, PG, PI, PME	$C_{16:0,}$ *iso*-$C_{16:0}$	71
P. antitumoralis		+		+	nr	nr	nr	+	DPG, PC, PE, PG, PI, PL	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{16:1}$H, *iso*-$C_{17:1}$ ω9*c*, $C_{16:0}$ 10-methyl.	73.2
P. artemisiae		+		+	+	+	nr	+	DPG, PC, PE, PME, PI, PIM	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:1}$ H, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl	68.2
P. asaccharolytica		+		+	nr	nr	nr	+	DPG, OH-PE, PG, PIM, PME,	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$	nr
P. aurantiaca		+		nr	nr	nr	nr	nr	PC, PE, PG, GluNu	nr	71.5
P. autotropica		+		nr	nr	nr	nr	nr	DPG, PC, PG, PI, PIM, PME.	*iso*-$C_{16:0}$, *iso*-$C_{17:0}$, $C_{17:1}$ ω8*c*	70
P. babensis		+		+	+	nr	+	+	PME, PC, DPG, PI, PL	*iso*-$C_{15:0}$, *iso*-$C_{16:1}$ H, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$	73
P. bannensis		+		+	+	nr	+	+	DPG, PE, PME, PC, PI, PIM	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:1}$ H, *iso*-$C_{16:0}$	69.4
P. benzenivorans		+		nr	nr	nr	nr	nr	DPG, PE, PI,	*iso*-$C_{16:1}$*cis*9, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$, $C_{17:1}$ *cis*9, $C_{17:0}$ 10-methyl	nr
P. carboxydivorans			+	nr	nr	nr	nr	nr	nr	*iso*-$C_{15:0}$, *iso*-$C_{16:1}$, *iso*-$C_{16:1}$*cis*9, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, *iso*-$C_{17:0}$	77

P. chloroethenivorans		+		+	nr	nr	nr	+	nr	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$	nr
P. compacta	nr	nr	nr	nr	nr	nr	nr	nr	DPG, PC, PE, PI, PIM, PME	$C_{15:0}$, *iso*-$C_{16:0}$, $C_{17:0}$	nr
P. dioxanivorans		+		nr	nr	nr	nr	nr	nr	*iso*-$C_{16:1}$ *cis*9, *iso*-$C_{16:0}$, *iso*-$C_{17:1}$*cis*9	74
P. endophytica		+	+	+	nr	nr	nr	+	DPG, PC, PE, PME	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{17:1}$ *cis*9	70.3
P. eucalypti		+		+	nr	nr	nr	+	nr	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$, $C_{17:0}$	72.9
P. halophobica	nr	nr	nr	nr	nr	nr	nr	nr	DPG, OH- PE, PE, PG, PI, PIM, PME	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$,	72
P. hispanensis		+		+	nr	nr	nr	+	DPG, PE, PG, PI, PME	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{17:0}$, *iso*-$C_{16:1}$ H, $C_{17:1}$ ω6*c*, *iso*-$C_{17:0}$ω9*c*/$C_{16:0}$ 10-methyl	69.7
P. hydrocarbonoxydans	+	+		+	nr	nr	nr	+	DPG, PC, PG, PI, PIM, PME,	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$	69
P. khuvsgulensis		+		+	+	+	+	+	nr	*iso*-$C_{15:0}$, *iso*-$C_{16:1}$ H, *iso*-$C_{16:0}$ 2-OH, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, $C_{17:0}$ 10-methyl	73
P. kongjuensis		+		nr	nr	nr	nr	nr	DPG, PC, PE, PG, PI	$C_{16:1}$, *iso*-$C_{16:1}$, $C_{16:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:1}$, $C_{17:1}$, $C_{18:0}$	71
P. kunmingensis		+		+	+	+	+	+	DPG, PME, PC, PI, PG, PL	$C_{16:0}$, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, ante*iso*-$C_{17:0}$	73.3
P. mongoliensis	+	+		+	+	+	+	+	nr	*iso*-$C_{14:0}$, *iso*-$C_{16:1}$ H, *iso*-$C_{16:0}$ 2-OH, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl	73
P. nitrificans		+		+	nr	nr	nr	+	nr	nr	nr
P. oroxyli		+		+	nr	nr	nr	+	PE, PI, PME, GluNu,	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, $C_{17:1}$ ω6*c*	70.6
P. parietis	+	+		nr	nr	nr	nr	nr	DPG, PME, PC, PG, PL	*iso*-$C_{16:1}$, *iso*-$C_{16:0}$	nr
P. petroleophila		+		+	nr	nr	nr	+	nr	nr	nr
P. rhizophila		+		+	+	nr	nr	+	DPG, PC, PE, PI, PIM	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, ante*iso*-$C_{17:0}$	69.7
P. saturnea	+	+		nr	nr	nr	nr	nr	PC, PME	nr	72
P. serianimata		+		+	+	nr	+	+	DPG, PE, PME, PC, PI, PIM, PL	*iso*-$C_{15:0}$, $C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, $C_{17:1}$ ω8*c*	72
P. sichuanensis		+		+	nr	nr	nr	+	DPG, PC, PE, PI	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl, ante*iso*-$C_{17:0}$, *iso*-$C_{17:1}$	69.8
P. spinosa		+		+	nr	nr	nr	+	nr	nr	nr
P. spinosispora		+		+	nr	nr	nr	+	DPG, PC, PE, PG, PI, PIM, PME, OH- PE, PL	*iso*-$C_{15:0}$, *iso*-$C_{16:1}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$, *iso*-$C_{17:1}$, $C_{17:1}$	70.4

Table 37.3 (continued)

Species	Major menaquinone(s)			Whole-cell sugars					Phospholipids	Major Fatty acids	DNA G+C content (mol %)
	MK-8(H2)	MK-8(H4)	MK-9	Arabinose	Mannose	Ribose	Glucose	Galactose			
P. sulfidoxydans		+		+	nr	nr	nr	+	DPG, OH-PE, PE, PG, PI, PIM, PME	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$	nr
P. tetrahydrofuranoxydans		+		nr	nr	nr	nr	nr	nr	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$	71.3
P. tropica		+		+	nr	nr	+	+	DPG, PC, PE, PG, PI, PIM	*iso*-$C_{15:0}$, *iso*-$C_{16:1}$ H, *iso*-$C_{16:1}$ *cis*9, $C_{16:0}$ 9-methyl, *iso*-$C_{16:0}$	72.4
P. xinjiangensis		+		nr	nr	nr	nr	nr	PC, PE, PG, PI, GluNu	nr	72.1
P. xishanensis		+		+	+	nr	+	+	DPG, PG, PME, PE, PC, PI, PL	*iso*-$C_{16:1}$, $C_{16:0}$, *iso*-$C_{16:0}$, $C_{16:0}$ 10-methyl	72.1
P. yuanmonensis		+		+	+	nr	+	+	DPG, PG, PME, PE, PC, PI, PIM, PL	$C_{16:0}$, *iso*-$C_{16:0}$	70.6
P. yunnanensis		+		+	nr	nr	nr	+	PE, PME, GluNu	nr	73.4
P. zijingensis		+		nr	nr	nr	nr	nr	DPG, PC, PG, PIM, PME	nr	70.9

Symbols: + positive, *nr* not reported, *DPG* diphosphatidylglycerol, *GluNu* phospholipids of unknown structure containing glucosamine, *OH-PE* phosphatidylethanolamine with hydroxyl fatty acids, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PL* unknown phospholipids, *PME* phosphatidylmethylethanolamine

Table 37.4

Physiological and cultural characteristics for *Pseudonocardia* species

Species	Characteristic																										
	Fragmentation of:				Carbon utilization/acid production from:														Decomposition of:				Growth in NaCl (w/v):				Urease production
	Substrate mycelium	Aerial mycelium	Single spores	Pairs of spores	Adonitol	l – Arabinose	Cellobiose	Erythritol	Fructose	Glucose	Lactose	Maltose	Mannitol	Rhamnose	Salicin	Sorbitol	Trehalose	d – Xylose	Adenine	Hypoxanthine	l – Tyrosine	Xanthine	3 %	4 %	5 %	7 %	
P. thermophila	+	+	–	–	nr	+	+	+	+	+	+	+	+	+	+	+	+	+	nr	nr	–	nr	+	nr	nr	nr	+
P. acaciae	+	+	+	+	+	+	nr	nr	+	+	+	–	(+)	+	nr	–	nr	+	nr	nr	+	nr	+	+	+	–	+
P. adelaidensis	+	(+)	+	–	nr	–	–	nr	+	+	–	–	+	+	nr	+	w	+	–	+	+	–	+	+	+	+	nr
P. ailaonensis	+	+	–	–	+	+	+	+	+	+	–	+	+	+	+	–	+	+	–	+	+	+	+	+	+	–	+
P. alaniniphila	–	–	+	+	–	–	+	–	–	(+)	–	–	+	+	–	–	–	+	–	+	–	–	–	–	–	–	–
P. alni	+	+	–	–	+	+	–	+	+	+	–	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
P. ammonioxydans	+	+	–	–	+	+	–	nr	+	+	–	+	nr	–	nr	–	+	(+)	nr	–	+	–	+	+	+	+	+
P. antarctica	nr	+	–	–	–	–	–	–	–	nr	nr	+	(+)	+	nr	–	–	+	nr	+	+	–	+	+	+	+	+
P. antitumoralis	+	+	+	nr	nr	nr	nr	nr	–	nr	+	nr	+	+	nr	+	nr	–	nr	nr	nr	nr	+	+	+	+	–
P. artemisiae	+	+	+	+	nr	+	nr	nr	+	nr	+	+	+	nr	nr	nr	nr	+	nr	nr	nr	nr	+	–	–	–	–
P. asaccharolytica	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P. aurantiaca	–	–	+	+	–	(+)	+	–	+	–	–	+	+	+	(+)	–	–	–	–	+	–	–	–	–	–	–	+
P. autotropica	+	+	–	–	+	+	+	+	+	+	–	+	+	–	–	+	+	+	+	–	+	–	+	+	+	+	+
P. babensis	+	+	+	+	–	+	+	nr	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	–	–
P. bannensis	+	+	+	+	nr	+	nr		+	+	+	+	+	nr	nr	nr	nr	nr	nr	+	+	–	–	–	–	–	–
P. benzenivorans	+	+	–	–	nr	–	–	–	+	+	–	–	–	nr	–	–	–	+	–	+	–	–	+	+	–	–	nr
P. carboxydivorans	nr	+	–	–	nr	nr	–	nr	–	nr	–	–	–	nr	nr	–	–	–	–	nr	nr	nr	+	+	–	–	+
P. chloroethenivorans	–	–	+	–	–	+	+	–	+	+	+	+	+	+	+	–	+	+	–	+	+	+	(+)	–	–	–	–
P. compacta	+	+	–	–	–	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P. dioxanivorans	+	+	–	–	+	–	–	–	+	+	–	–	–		–	–	+	(+)	–	+	–	+	+	+	(+)	–	nr
P. endophytica	+	–	–	–	–	–	–	+	(+)	+	–	+	–	–	+	+	+	–	nr	+	+	nr	+	+	+	–	–
P. eucalypti	–	+	+	+	+	+	–	nr	+	+	nr	–	+	+	nr	(+)	+	+	nr	nr	+	nr	+	nr	nr	nr	+
P. halophobica	+	+	–	–	–	+	–	+	–	+	–	+	+	+	–	–	+	(+)	(+)	+	–	+	+	+	(+)	(+)	–
P. hispanensis	+	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	+	–	–	–	–
P. hydrocarbonoxydans	+	+	–	–	–	+	+	–	–	+	+	–	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–
P. khuvsgulensis	+	+	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	+	+	nr	+	–	–	–	+
P. kongjuensis	nr	+	–	–	+	–	+	–	(+)	(+)	–	+	+	nr	–	–	+	+	nr	+	+	–	+	+	+	+	+
P. kunmingensis	+	+	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	+	+	+	+
P. mongoliensis	+	+	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	–	–	nr	–	nr	–	–	+

Table 37.4 (continued)

Species	Characteristic																									
	Fragmentation of:				Carbon utilization/acid production from:														Decomposition of:				Growth in NaCl (w/v):				Urease production
	Substrate mycelium	Aerial mycelium	Single spores	Pairs of spores	Adonitol	l − Arabinose	Cellobiose	Erythritol	Fructose	Glucose	Lactose	Maltose	Mannitol	Rhamnose	Salicin	Sorbitol	Trehalose	d − Xylose	Adenine	Hypoxanthine	l − Tyrosine	Xanthine	3 %	4 %	5 %	7 %	
P. nitrificans	nr	+	−	−	+	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
P. oroxyli	+	+	−	−	nr	−	−	−	+	+	−	+	+	nr	−	−	+	−	+	+	+	+	+	+	+	(−)	−
P. parietis	+	+	+	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
P. petroleophila	+	+	−	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	+	+	+	nr
P. rhizophila	+	+	+	+	nr	nr	nr	nr	nr	nr	+	nr	nr	+	nr	nr	nr	+	nr	nr	nr	nr	+	+	+	+	nr
P. saturnea	+	+	−	−	−	+	+	−	+	+	−	+	+	−	−	−	+	+	+	+	+	+	+	nr	−	−	+
P. serianimata	+	+	−	−	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	+	+	+	nr	+	nr	nr
P. sichuanensis	+	+	+	+	nr	nr	+	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	+	nr	+	nr	+	nr	−	nr	nr
P. spinosa	+	+	−	−	nr	−	−	−	−	−	−	−	−	−	−	−	−	−	nr	−	−	−	−	−	−	−	−
P. spinosispora	nr	+	−	−	+	+	−	+	+	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+
P. sulfidoxydans	+	+	−	−	−	+	−	+	+	+	−	−	−	−	−	−	+	+	−	+	+	+	+	−	−	−	−
P. tetrahydrofuranoxydans	nr	+	−	−	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr		nr	nr	nr	+	+	−	−	nr
P. tropica	+	+	−	−	nr	nr	+	nr	+	+	nr	+	+	nr	nr	nr	nr	+	nr	nr	nr	nr	+	+	+	+	nr
P. xinjiangensis	−	−	+	+	+	+	+	−	+	+	+	(+)	+	+	(+)	−	−	+	−	+	−	−	−	−	−	−	+
P. xishanensis	+	+	−	−	nr	nr	nr	nr	+	+	+	+	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	nr	(+)	+
P. yuanmonensis	+	+	−	−	nr	+	nr	nr	+	+	nr	nr	nr	nr	nr	nr	+	+	nr	nr	nr	nr	+	nr	nr	−	nr
P. yunnanensis	−	−	+	+	+	−	+	−	+	−	−	−	+	+	(+)	−	−	+	−	+	−	(+)	−	−	−	−	−
P. zijingensis	+	+	−	−	+	(+)	+	−	−	+	+	(+)	(+)	+	−	+	+	+	−	+	+	+	+	+	(+)	−	−

Symbols: + positive, − negative, (+) weakly positive, *nr* not reported

Table 37.5

Nutritional requirements, degradation, and enzyme activity of *Pseudonocardia* species

Species	Nutrition and growth media	Degradation and enzyme activity	References
P. thermophila	nr	Possesses an inducible exo- and intracellular carboxymethylcelluase and β-D-glucosidase activity; calcium alginate-immobilized cells of this strain have been used to produce optically active a-hydroxy-esters; produces cobalt-containing nitrile hydratases; Constitutively expressed thermostable amidase; exhibits cellulase and xylanase activity	Malfait et al. (1984), Ishihara et al. (1997), Miyanaga et al. (2001), Peplowski et al. (2007), Egorova et al. (2004), Li et al. (1984), Zimmermann et al. (1988)
P. ammonioxydans	Oxidizes ammonia to nitrate as an energy source; grows autotrophically on modified nitrifying medium and heterotrophically on Luria-Bertani medium	nr	Liu et al. (2006)
P. asaccharolytica	Uses dimethyl disulfide as an energy source	nr	Reichert et al. (1998)
P. autotrophica	Autotroph; growth in the presence of CO_2, H_2, and O_2	Converts vitamin D3 into hydroxylated active forms, such as 1a and 2a, 25-dihydroxyvitamin D3; metabolizes 4-ethylpyridine and 4-methylpyridine and polyethylene glycol; degrades lignin, vanillinic acids, and its derivatives	Hirsch and Engel (1956), Kim et al. (2002b), Takeda et al. (2006), Lee et al. (2006), Yamashita et al. (2004), Malarczyk et al. (1987)
P. benzenivorans	Uses 1, 2, 3, 5-tetrachlorobenzene as a sole carbon source; grows on 4-aminobutyrate, 4-hydroxybutyrate, serine, and succinate	Degrades tetrahydrofuran (THF)	Kämpfer and Kroppenstedt (2004), Kämpfer et al. (2006)
P. carboxydivorans	Uses CO as a sole carbon source	nr	Park et al. (2008)
P. chloroethenivorans	Degrades chloroethene, *cis*-1,2-dichloroethene, and trichloroethene, and metabolizes phenol as a source of carbon and energy	nr	Lee et al. (2004)
P. dioxanivorans	Autotroph; degrades 1,4-dioxane	Degrades tetrahydrofuran (THF)	Mahendra and Alvarez-Cohen (2005), Kämpfer et al. (2006)
P. hydrocarbonoxydans	Good growth in the presence of simple hydrocarbons; oxidizes hydrocarbons	Degrades tetrahydrofuran (THF)	Hirsch and Engel (1956), Kämpfer et al. (2006), Lechevalier et al. (1986)
P. hydrocarbonoxydans	Able to partially oxidize sebacic and succinic acid	nr	Lacey (1988), Nolof (1962), Nolof and Hirsch (1962)
P. nitrificans	Converts ethyl-, ethyl-*N*-ethyl, *n*-butyl-, and *n*-propyl-carbamates to nitrite and uses these compounds as sole carbon, energy, and nitrogen sources; oxidizes urethane to nitrite as a sole source of carbon, nitrogen, and energy	nr	Isenberg et al. (1954), Schatz et al. (1954)

Table 37.5 (continued)

Species	Nutrition and growth media	Degradation and enzyme activity	References
P. petroleophila	Poor growth on most media; autotroph; grow in the presence of CO_2, H_2, and O_2	nr	Hirsch and Engel (1956), Lechevalier et al. (1986)
P. petroleophila	Good growth in the presence of simple hydrocarbons; oxidizes the cyclohexane ring of methylcyclohexane	nr	Nolof and Hirsch (1962), Tonge and Higgins (1974)
P. saturnea	Autotroph; grows in the presence of CO_2, H_2, and O_2	nr	Takamiya and Tubaki (1956)
P. sulfidoxydans	Uses dimethyl disulfide as an energy source; grows on 4-aminobutyrate, 4-hydroxybutyrate, serine, and succinate	Degrades tetrahydrofuran (THF)	Reichert et al. (1998), Kämpfer et al. (2006)
P. tetrahydrofuranoxydans	nr	Degrades tetrahydrofuran (THF)	Kämpfer et al. (2006)
P sp. *strain TY-7*	Oxidizes propane to 1-propanol and 2-propanol	Degrades 1.4-dioxane and other ether pollutants; used for in situ and ex situ systems to treat recalcitrant pollutants	Kotani et al. (2006), Vainberg et al. (2006)

Symbols: *nr* not reported

Phenotypic Analyses

Actinoalloteichus species are aerobic, Gram-stain-positive, nonacid-fast, nonmotile actinobacteria. Substrate mycelia are fine, branched, and non-fragmenting. Aerial mycelia are formed and fragment, with straight spore chains forming from the aerial mycelium. Good growth occurs at 20–37 °C. As found in all genera of this order, the cell wall contains *meso*-diaminopimelic acid, and whole-cell hydrolysates contain glucose, galactose, mannose, and ribose. Present in all species are the cellular fatty acids *iso*-$C_{16:0}$, *anteiso*-$C_{15:0}$, anteiso-, $C_{17:0}$ anteiso-, and $C_{15:0}$ anteiso- are the major cellular fatty acids present in all species. The major menaquinone is MK-9(H_4); small amounts of MK-9(H_6) are also present. Phosphatidylglycerol and phosphatidylinositol are present as the major diagnostic phospholipids. Mycolic acids are not detected. The acyl type of the cell wall is acetyl.

The G+C content of the DNA of members of this genus ranges between 72 and 76.6 (mol%).

The type species is *Actinoalloteichus cyanogriseus* NBRC 14455^T (Tamura et al. 2000).

Isolation Procedures

Isolation procedures for species of this genus are described in *Table 37.6*.

Taxonomy, Historical, and Current

The genus *Actinoalloteichus* forms a distinct clade and all four species have less than 95 % sequence similarity with the nearest genera *Goodfellowiella* (Labeda and Kroppenstedt 2006), *Streptoalloteichus* (Tomita et al. 1987), and *Crossiella* (Labeda 2001). The genus *Goodfellowiella* has $C_{17:0}$10-methyl and anteiso-branched 2-hydroxy fatty acids. *Streptoalloteichus* develops motile spores in sporangia, and *Crossiella* has substrate mycelia that fragment into rods and can develop sclerotia-like pseudosporangia. Species of the genus *Actinoalloteichus* do not develop sporangia or pseudo-sporangia and do not have motile spores (Tamura et al. 2008a).

Liu et al. (1984) described *Actinoalloteichus cyanogriseus* as a new taxon with blue and gray aerial mycelium with straight spore chains and bluish-violet soluble pigment. Itoh et al. (1987) found it contained glutamic acid, alanine and *meso*-diaminopimelic acid, and menaquinone 9(H_4). The phylogenetic position of *Actinoalloteichus cyanogriseus* was placed in the order *Pseudonocardiales* based on its 16S rRNA gene sequence (Stackebrandt et al. 1997; Tamura and Hatano 1998). Later, based on significant taxonomic differences as described above, it was proposed as a new genus with *A. cyanogriseus* gen. nov., sp. nov. as the type species of the new genus (Tamura et al. 2000).

Streptomyces caeruleus was reclassified as *Actinoalloteichus cyanogriseus* on the basis of 16S rRNA gene sequence analysis and DNA–DNA hybridization (Tamura et al. 2008a).

Table 37.6

Physiological characteristics and isolation procedures of *Actinoalloteichus* species[a]

Species	Aerial mycelium	Utilization of:													Decomposition of casein	Hydrolysis of starch	Resistance to methyl violet	Pigmentation on:		Origin of isolation sample	Isolation method	References
		Arabinose	Fructose	Glucose	Maltose	Mannitol	Mannose	Raffinose	Rhamnose	Sucrose	Sorbitol	Sodium citrate	Sodium succinate	Xylose				ISP 1	ISP 6			
A. cyanogriseus	+	w	–	+	+	+	+	–	+	w	+	–	–	+	–	w	–	Absent	Black	Cultivated soil, Yunnan Province, China	Yeast extract and glucose broth; pH 7.0; at 28 °C for 5 days	Tamura et al. (2000)
A. hymeniacidonis	+	–	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	Black	Black	Marine sponge *Hymeniacidon perleve*, Dalian, China	Surface sterilization, homogenization, serial dilution followed by plating on ISP medium 5; incubated at 28 °C for 4 weeks	Zhang et al. (2006)
A. nanshanensis	+	–	+	+	+	+	+	–	+	+	–	–	–	+		–	nr	nr	Black	Rhizosphere soil of *Ficus religiosa*, Guanxi province, China	Humic acid–vitamin agar (HV) (Hayakawa and Nonomura 1987), supplemented with nystatin (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}); incubated at 28 °C for 21 days	Xiang et al. (2011)
A. spitiensis	–	–	–	–	–	–	+	–	+	–	+	–	+	+	–	+	+	–	Absent	Absent	Cold desert soil, Himachal Pradesh, India	Dilution plating technique on *Actinomycetes* isolation agar (sodium caseinate, 0.2 %; asparagine, 0.01 %; sodium propionate, 0.4 %; dipotassium phosphate; incubated at −70 °C)	Singla et al. (2005)

Symbols: + positive, *w* weakly positive, – negative, *nr* not reported

Characteristics that differentiate the four *Actinoalloteichus* species are shown in Tables 37.6 and 37.7.

The four different species of the genus *Actinoalloteichus* are *Actinoalloteichus cyanogriseus* CIP 106755^T = DSM 43889^T = NBRC 14455^T = JCM 6095^T = NRRL B-16252^T (Tamura et al. 2000); *Actinoalloteichus hymeniacidonis* CGMCC 4.2500^T = JCM 13436^T (Zhang et al. 2006); *Actinoalloteichus nanshanensis* CGMCC 4.5714^T = NBRC 106685^T. (Xiang et al. 2011); and *Actinoalloteichus spitiensis* DSM 44848^T = JCM 12472^T = MTCC 6194^T = NBRC 102582^T (Singla et al. 2005).

Actinokineospora Hasegawa 1988, 449^{VP}

Ac.ti.no'ki.ne.o.spo.ra actis, *actinos* Gr. n., ray; *kinesis* Gr. n., motion; *spora* L. n., spore; *Actinokineospora*, actinomycete bearing zoospores.

Phenotypic Analyses

Actinokineospora strains form hyphae (approximately 0.5 µm in diameter) which differentiate into a vegetative mycelium that penetrates the agar medium forming colonies on the surface from which aerial mycelium grow. The aerial hyphae form chains of conidia capable of producing flagella in a suitable aqueous environment in all but two species, *Actinokineospora fastidiosa* and *Actinokineospora soli*. Cells are Gram-stain positive, catalase positive, and aerobic. The cell wall contains *meso*-diaminopimelic acid as the diamino acid along with glycine, D-glutamic acid, and L-alanine, properties characteristic of a type A1γ peptidoglycan. All species contain the cellular fatty acid iso-$C_{16:0}$, with iso-$C_{15:0}$ present in 9 of the 13 species. The major menaquinone is MK-9(H_4), except for 1 member which contains MK-10 as the sole menaquinone. Phosphatidylethanolamine is present as the major diagnostic phospholipid. Mycolic acids are not detected. Chemotaxonomic properties of the genus are shown in Table 37.8.

The G+C content of the DNA of members of this genus ranges between 68.2 and 73.8 (mol%).

The type species is *Actinokineospora riparia* ATCC 49499^T = DSM 44259^T = NBRC 14541 = JCM 7471^T = NRRL B-16432^T = VKM Ac-1980^T(Hasegawa 1988).

Isolation Procedures

Isolation procedures for species of this genus are described in Table 37.8.

Taxonomy, Historical, and Current

Differentiation of *Actinokineospora* species has generally relied on both 16S rRNA gene sequence phylogeny and physiological characterization utilizing variations on the methods of Shirling and Gottlieb (1966), Tamura et al. (1995), and Otoguro et al. (2001) although the characterization of the nonmotile species *Actinokineospora fastidiosa* and *Actinokineospora soli* utilized methods more typically applied to strains of *Amycolatopsis*. The comparison of the morphological and physiological characteristics of the species within *Actinokineospora* can be seen in Table 37.9. The two species that have not been observed to produce motile spores appear to be phylogenetically separated from the species of *Actinokineospora* sensu *strictu* and could represent a separate genus (Fig. 37.3). The sole *Alloactinosynnema* sp. fall within this 16S rRNA gene cluster but can be differentiated on the basis of its ability to produce pseudosporangia and because it has ribose, phosphatidylcholine, and phospatidylglycerol in its cell wall.

The twelve species of the genus *Actinokineospora* are *Actinokineospora riparia* ATCC 49499^T = DSM 44259 = NBRC 14541 = JCM 7471^T = NRRL B-16432^T = VKM Ac-1980^T (Hasegawa 1988); *Actinokineospora auranticolor* YU 961-1^T = DSM 44650^T = NRBC 16518^T = JCM 11646^T (Otoguro et al. 2001); *Actinokineospora baliensis* ID03-0561^T = BTCC B-554^T = NBRC 104211^T; *Actinokineospora cianjurensis* ID03-0810^T = BTCC B-558^T = NBRC 105526^T; *Actinokineospora cibodasensis* ID03-0748^T = BTCC B-555^T = NBRC 104212^T (Lisdiyanti et al. 2010); *Actinokineospora diospyrosa* YU8-1^T = DSM 44255^T = NRBC 15665^T = JCM 9921^T = NRRL B-24047^T = VKM Ac-1984^T (Tamura et al. 1995); *Actinokineospora enzanensis* YU 924-101^T = DSM 44649^T = NRBC 16517^T = JCM 11647^T (Otoguro et al. 2001); *Actinokineospora fastidiosa* ATCC 31181^T = DSM 43855^T = JCM 3276^T = NBRC 14105^T = NRRL B-16697^T = VKM Ac-1419^T (Labeda et al. 2010b); *Actinokineospora globicatena* YU6-1^T = DSM 44256^T = NRBC 15664^T = JCM 9922^T = NRRL B-24048^T = VKM Ac-1981^T; *Actinokineospora inagensis* YU4-1^T = DSM 44258^T = NRBC 15663^T = JCM 9923^T = NRRL B-24050^T = VKM Ac-1982^T (Tamura et al. 1995); *Actinokineospora soli* YIM 75948^T = DSM 45613^T = JCM 17695^T (Tang et al. 2012); and *Actinokineospora terrae* YU6-3^T = DSM 44260^T = NRBC 15668^T = JCM 9924^T = NRRL B-24049^T = VKM Ac-1983^T (Tamura et al. 1995).

Actinomycetospora Jiang, Wiese, Tang, Xu, Imhoff, and Jiang 2008, 408^{VP}, emended Tamura, Ishida, Hamada, Otoguro, Yamamura, Hayakawa, and Suzuki 2011, 1275

Ac.ti′no.my.ce.to.spo′ra. N. Gr. n.*actinomyces -etos* an actinomycete; Gr. fem. n. *spora* a seed and, in bacteriology, a spore; N.L. fem. n. *Actinomycetospora* referring to an actinomycete with spore chains.

Phenotypic Analyses

Actinomycetospora species are aerobic, Gram-stain-positive, nonacid-fast, nonmotile actinomycetes. Substrate mycelium

Table 37.7

Chemotaxonomic characteristics of *Actinoalloteichus* species

Species	Menaquinone (s) (%):						Phospholipids[a]	Major fatty acids	Whole-cell sugars					DNA G+C content (mol %)	References
	MK – 8(H4)	MK – 9(H2)	MK – 9(H4)	MK – 9(H6)	MK – 9(H8)	MK – 10(H4)			Galactose	Mannose	Glucose	Rhamnose	Ribose		
A. cyanogriseus	10	9	75	2		3	PE, PI, PG	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, *iso*-$C_{16:1}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$, others	+	+	+	+	nr	73	Tamura et al. (2000)
A. hymeniacidonis			64	23	12		PE, PG, PI, PIM, GluNu	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, $C_{15:0}$, *iso*-$C_{16:0}$ G, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:1}$ ω8*c*, $C_{17:0}$, others	+	+	+	nr	+	nr	Zhang et al. (2006)
A. nanshanensis	1.40	0.96	78.91	1.96		16.76	PE, PG, PI, PIM	*iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, $C_{15:0,}$ $C_{16:1}$, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:1}$ ω8*c*, $C_{17:0}$, $C_{18:0}$	+	+	+	+	+	76.6	Xiang et al. (2011)
A. spitiensis	5		82	2		9	PME, PI, PG, PIM, DPG, PE	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, *iso*-$C_{16:1}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$, others	+	+	+	+	+	72	Singla et al. (2005)

Symbols: *nr* not reported

[a]*DPG* diphosphatidylglycerol, *GluNu* phospholipids of unknown structure containing glucosamine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositolmannosides, *PME* phosphatidymethylethanolamine

Table 37.8

Chemotaxonomic properties and method of isolation of *Actinokineospora* and *Alloactinosynnema* species

Species	Phospholipids[a]	Major Fatty acids	Whole-cell sugars[b]	Major menaquinone	DNA G+C content (mol %)	Origin of isolation sample	Method of isolation	References
Actinokineospora auranticolor NBRC 16518^T	PE, OH-PE	*iso*-$C_{16:0}$	Ara, Gal, Rha	MK-9(H_4)	71.3	Fallen leaves sample; Japan	Surface sterilized samples processed with calcium carbonate, centrifugation (1,500 *g* for 20 min), enrichment of supernatant with zoospores followed by serial dilution with sterile tap water were plated on HV agar with or without antibacterial agents. Incubated at 30 °C for 2–3 weeks	Otoguro et al. (2001)
Actinokineospora baliensis NBRC 104211^T	PE	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{17:0}$	Gal, Ara	MK-9(H_4)	71.4	Soil sample; Bali, Indonesia	Humic acid-vitamin (HV) agar supplemented with 50 μg ml^{-1} cycloheximide and 20 μg ml^{-1} nalidixic acid was used as the isolation medium (Hayakawa and Nonomura 1987, 1989)	Lisdiyanti et al. (2010)
Actinokineospora bangkokensis NBRC 108932^T	PE, OH-PE, DPG, PL,	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$ 2-OH	Ara, Gal, Glc, Man, Rha	MK-9(H_4)	74	Rhizosphere soil under an Elephant ear plant (*Colocasia esculenta*); Bangkok, Thailand	Soil suspensions were serially diluted after pretreatment using dry heat at 120 °C for 1 h and spread onto water-proline agar (proline 1.0 %, agar 1.2 %, tap water, pH 7.0) supplemented with 25 μg ml^{-1} nalidixic acid and 50 μg ml^{-1} cycloheximide; incubated for 4 weeks at 28 °C	(Intra et al. 2013)
Actinokineospora cianjurensis NBRC 105526^T	PE	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$ 2-OH	Gal, Ara	MK-9(H_4)	71.4	Leaf-litter sample; West Java, Indonesia	Rehydration and centrifugation method, as described by Hayakawa et al. (2000) Otoguro et al. (2001). Humic acid–vitamin (HV) agar supplemented with 50 μg cycloheximide ml^{-1} and 20 μg nalidixic acid ml^{-1} was used as the isolation medium (Hayakawa and Nonomura 1987, 1989)	Lisdiyanti et al. (2010)
Actinokineospora cibodasensis NBRC 104212^T	PE	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$ 2-OH	Gal, Ara	MK-9(H_4)	70.2	Leaf-litter sample; West Java, Indonesia	Rehydration and centrifugation method, as described by Hayakawa et al. (2000) and Otoguro et al. (2001a). Humic acid–vitamin (HV) agar supplemented with 50 μg cycloheximide ml^{-1} and 20 μg nalidixic acid ml^{-1} was used as the isolation medium (Hayakawa and Nonomura 1987, 1989)	Lisdiyanti et al. (2010)

Actinokineospora diospyrosa NBRC 15665^T	PE	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{17:0}$, $C_{16:0}$	Ara, Gal, Glc, Rha, Man	MK-9(H_4)	69.3	Fallen Persimmon leaves; Yamanashi, Japan	Isolated on humic acid–vitamin (HV) agar by using the modified method of Makkar and Cross. Incubated at 28 °C for 14 days	Tamura et al. (1995)
Actinokineospora enzanensis NBRC 16517^T	PE, OH-PE	*iso*-$C_{16:0}$	Ara, Gal, Rha	MK-9(H_4)	70.0	Level- land forest soil sample; Yamanashi, Japan	Surface-sterilized samples processed with calcium carbonate, centrifugation (1,500 *g* for 20 min), enrichment of supernatant with zoospores followed by serial dilution with sterile tap water were plated on HV agar with or without antibacterial agents. Incubated at 30 °C for 2–3 weeks	Otoguro et al. (2001)
Actinokineospora fastidiosa NRRL B-16697^T	PE, OH-PE, DPG, PI	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, $C_{16:0}$, $C_{17:1}\omega6c$	Ara, Gal, Glc, Rha, Man	MK-9(H_4), MK-9(H_2)	73.0	Soil sample; Egypt	nr	Labeda et al. (2010b)
Actinokineospora globicatena NBRC 15664^T	PE	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{17:0}$, $C_{16:0}$	GlcN, Ara, Gal, Glc, Rha, Man	MK-9(H_4)	69.5 to 69.8	Soil sample; Yamanashi, Japan	Isolated on humic acid–vitamin (HV) agar by using the modified method of Makkar and Cross. Incubated at 28 °C for 14 days	Tamura et al. (1995)
Actinokineospora inagensis NBRC 15663^T	PE	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, $C_{16:0}$	GlcN, Ara, Gal, Glc, Rha, Man	MK-9(H_4)	69.1	Fallen leaves; Yamanashi, Japan	Isolated on humic acid–vitamin (HV) agar by using the modified method of Makkar and Cross. Incubated at 28 °C for 14 days	Tamura et al. (1995)
Acinokineospora riparia NBRC 14541^T	PE, DPG, PI	nr	Gal, Glc, Man, Ara, Rha	MK-10	72	Soil sample; Ado river, Japan	nr	Hasegawa (1988)
Actinokineospora soli YIM 75948^T	PE, OH-PE, DPG, PI	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, $C_{16:0}$	Ara, Gal, Glc, Rha, Man	MK-9(H_4)	73.8	Soil sample; Yunnan Province, China	Isolated by plating dilutions of soil sample on ISP 2 agar. The pure culture was preserved in 20 % (v/v) glycerol at -80 °C	Tang et al. (2012)
Actinokineospora terrae NBRC 15668^T	PE	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{17:0}$, $C_{16:0}$	GlcN, Ara, Gal, Glc, Rha, Man	MK-9(H_4)	70.0	Soil sample; Yamanashi, Japan	Isolated on humic acid–vitamin (HV) agar by using the modified method of Makkar and Cross. Incubated at 28 °C for 14 days	Tamura et al. (1995)
Alloactinosynnema album DSM 45114^T	DPG, PG, PC	*iso*-$C_{16:0}$, *iso*-$C_{16:1}$ H	Gal, Rib	MK-9(H_4)	68.2	Soil sample, Xinjiang Province, China	Isolated on Czapek's agar (Waksman 1961). Incubated at 28 °C for 3 weeks	Yuan et al. (2010)

Symbols: *nr* not reported

[a]*PE* phosphatidylethanolamine, *OH-PE* hydroxy phosphatidylethanolamine, *DPG* diphosphatidylglycerol, *PI* phosphatidylinositol, *PC* phosphatidylcholine

[b]*Gal* galacatose, *Man* mannose, *Glc* glucose, *Ara* arabinose, *Rha* rhamnose, *GlcN* glucosamine, *Rib* ribose

Table 37.9
Morphological and physiological properties of *Actinokineospora* species and *Alloactinosynnema album*

	Actinokineospora auranticolor NBRC 16518^T	*Actinokineospora baliensis* NBRC 104211^T	*Actinokineospora bangkokensis* NBRC 108932^T	*Actinokineospora cianjurensis* NBRC 105526^T	*Actinokineospora cibodasensis* NBRC 104212^T	*Actiokineospora diospyrosa* NBRC 15665^T	*Actinokineospora enzanensis* NBRC 16517^T	*Actinokineospora fastidiosa* NRRL B – 16697^T	*Actinokineospora globicatena* NBRC 15664^T	*Actinokineospora inagensis* NBRC 15663^T	*Acinokineospora riparia* NBRC 14541^T	*Actinokineospora soli* YIM 75948^T	*Actinokineospora terrae* NBRC 15668^T	*Alloactinosynnema album* DSM 45114^T
Colony reverse color	Orange	Yellow	Bright melon yellow, flesh pink, apricot, bisque, light tan	Yellow/ tan	Yellow/ brown	Yellow/ brown	Gray	Yellow/ salmon	Yellow/ brown	Yellow/ brown	Yellow/ brown	Yellow/ white	Yellow/ brown	White/ buff to pink
Motile spores produced	+	+	–	+	+	+	+	–	+	+	+	–	+	–
Aerial mycelium color	White to gray	White	White	White	White	White to gray	White	White to pale pink	White to gray	White to gray	White	White to pale yellow	White to gray	White
Vegetative mycelium color	Yellow-orange	Pale yellow	Yellow	Yellow to brown	Yellow to tan	Yellow to tan	Greenish gray	Pale yellow to pale salmon	Yellow to tan	Yellow to tan	Colorless to brownish	White to pale yellow	Yellow to tan	White or buff to pink
Hydrolysis of:														
Calcium malate	+	–	nr	–	–	–	+	nr	+	–	–	nr	v	nr
Elastin	+	+	nr	+	+	+	+	nr	+	+	–	nr	+	+
Gelatin	+	–	+	–	–	+	+	–	+	–	–	–	+	+
Milk (peptonization)	nr	+	nr	–	–	+	nr	+	–	–	–	nr	+	+
Starch	nr	nr	+	nr	nr	+	nr	–	+	–	–	–	+	+
Testosterone	+	–	nr	+	+	+	+	nr	v	–	+	nr	+	nr
Production of:														
Nitrate reductase	–	+	–	+	+	–	+	+	–	+	+	–	–	+
Hydrogen sulfide	+	nr	–	nr	nr	+	+	–	+	+	–	–	+	–

Growth on sole carbon source (1.0 % w/v):														
Arabinose	–	–	–	–	–	–	–	+	–	–	–	–	w	nr
D-Fructose	–	nr	+	+	+	+	–	+	+	–	w	nr	+	nr
Galactose	nr	–	nr	+	+	–	nr	–	–	–	–	+	–	
Glycerol	+	nr	nr	nr	nr	+	+	nr	+	–	+	–	v	nr
Maltose	+	+	nr	+	+	+	–	nr	+	w	–	+	+	nr
D-Mannose	–	+	nr	+	+	w	–	nr	+	–	w	+	+	+
Rhamnose	–	–	–	–	–	w	–	–	+	w	–	–	w	+
Sodium acetate	v	nr	nr	nr	nr	–	v	nr	–	+	–	nr	v	–
Sorbitol	nr	–	nr	–	–	–	nr	nr	–	–	–	nr	–	+
Sucrose	+	+	+	+	+	+	–	nr	+	–	–	+	+	+
Trehalose	+	+	nr	+	+	nr	+	nr	nr	nr	nr	–	nr	+
Growth on sole nitrogen source (0.1 % w/v):														
D-Alanine	v	nr	nr	nr	nr	v	+	nr	v	–	–	+	+	nr
L-Proline	+	nr	nr	nr	nr	+	+	nr	–	+	–	+	+	nr
Growth in the presence of (w/v):														
Bismuth citrate, 0.001 %	+	nr	nr	nr	nr	v	+	nr	–	–	+	nr	v	nr
Brilliant green, 0.001 %	–	nr	nr	nr	nr	v	–	nr	–	–	–	nr	+	nr
Furazolidone, 0.004 %	–	nr	nr	nr	nr	+	–	nr	+	+	–	nr	+	nr
Potassium tellurite 0.01 %	+	nr	nr	nr	nr	+	+	nr	+	–	–	nr	+	nr
Sodium chloride, 2.0 %	+	–	nr	–	–	+	+	+	+	–	+	+	+	nr
Sodium chloride, 3.0 %	v	–	nr	–	–	+	–	+	+	–	–	+	+	nr
Vanillin, 0.05 %	v	nr	nr	nr	nr	–	v	nr	v	+	–	nr	+	nr

Table 37.9 (continued)

	Actinokineospora auranticolor NBRC 16518^T	*Actinokineospora baliensis* NBRC 104211^T	*Actinokineospora bangkokensis* NBRC 108932^T	*Actinokineospora cianjurensis* NBRC 105526^T	*Actinokineospora cibodasensis* NBRC 104212^T	*Actiokineospora diospyrosa* NBRC 15665^T	*Actinokineospora enzanensis* NBRC 16517^T	*Actinokineospora fastidiosa* NRRL B – 16697^T	*Actinokineospora globicatena* NBRC 15664^T	*Actinokineospora inagensis* NBRC 15663^T	*Acinokineospora riparia* NBRC 14541^T	*Actinokineospora soli* YIM 75948^T	*Actinokineospora terrae* NBRC 15668^T	*Alloactinosynnema album* DSM 45114^T
Resistance to antibiotics:														
Ampicillin, 10 μg/ml	+	nr	nr	nr	nr	–	+	nr	–	–	–	+	–	nr
Benzyl penicillin, 10 μg/ml	–	nr	nr	nr	nr	–	+	nr	–	+	–	–	–	nr
Cephaloridine, 10 μg/ml	–	nr	nr	nr	nr	–	+	nr	+	+	–	nr	v	nr
Chloramphenicol, 10 μg/ml	+	nr	nr	nr	nr	v	–	nr	–	–	–	–	v	nr
Lincomycin, 20 μg/ml	+	nr	nr	nr	nr	v	–	nr	v	–	–	nr	v	nr
Norfloxacin, 40 μg/ml	+	nr	nr	nr	nr	v	v	nr	–	–	–	+	–	nr
Oleandomycin, 5 μg/ml	+	nr	nr	nr	nr	+	–	nr	v	–	–	nr	v	nr
Rifampicin, 20 μg/ml	–	nr	nr	nr	nr	–	+	nr	–	–	+	–	–	nr
Growth at:														
10 °C	+	–	–	–	–	+	–	+	+	–	–	–	+	–
37 °C	+	–	+	–	–	v	–	+	–	–	+	+	v	+

Data from: Otoguro et al. (2001), Lisdiyanti et al. (2010), (Intra et al. 2013), Tamura et al. (1995), Labeda et al. (2010b), Hasegawa (1988), Tang et al. (2012), Yuan et al. (2010)
Symbols:+ positive, – negative, *v* variable reaction, *w* weak positive reaction, *nr* not reported

fragments into rod-shaped elements and is yellow, greenish yellow, or orange. No aerial mycelium is produced. Bud-like short spore chains are formed directly from substrate mycelium. Spores are white short rods or have a slight oval shape with smooth surfaces ranging from approximately 0.4–0.8 × 0.7–2.0 μm. The cell wall contains *meso*-diaminopimelic acid; whole-cell hydrolysates contain arabinose and galactose. Phosphatidylcholine and phosphatidylethanolamine are the diagnostic phospholipids. The predominant menaquinone is MK-8 (H_4). The major cellular fatty acid is iso-$C_{16:0}$, with $C_{16:1}$*cis-9*, $C_{17:1}$*cis-9*, iso-$C_{16:0}$2-OH, and $C_{17:0}$10-methyl also present.

The G+C content of the DNA of members of the genus ranges between 69 and 74.2 (mol%).

The type species is *Actinomycetospora chiangmaiensis* CCTCC AA 205017^T (=DSM 45062^T). The strain was isolated from soil collected from a tropical rainforest in Thailand (Jiang et al. 2008).

Isolation Procedures

Isolation procedures for the species of this genus are described in *Table 37.10*.

Taxonomy, Historical, and Current

The phylogenetically nearest neighboring genus within the order *Pseudonocardiales* is *Pseudonocardia* (*Figs. 37.6* and *37.7*), but the ten *Actinomycetospora* species form a distinct clade and showed less than 95.1 % sequence similarity to *Pseudonocardia* strains. In addition, the *Actinomycetospora* species are distinct as they do not produce aerial mycelia and bud-like spore chains are formed on the substrate mycelia (*Actinomycetospora* *Figs. 37.8*, *37.9*, and *37.10*).

The type species *Actinomycetospora chiangmaiensis* was originally described by Jiang et al. (2008) as containing MK-9(H_4) as the major menaquinone and phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol. This was emended by Tamura et al. (2011) who found that the type species contained MK-8(H_4) as the major menaquinone with no MK-9(H_4) and that the major phospholipids included phosphatidylethanolamine and phosphatidylcholine, as was found in all seven of the new *Actinomycetospora* species proposed.

The ten species of the genus *Actinomycetospora* can be readily differentiated by physiological characteristics and chemotaxonomic properties as can be seen in *Tables 37.10* and *37.11*, respectively.

These species are *Actinomycetospora chiangmaiensis* NBRC 104400^T (Jiang et al. 2008); *Actinomycetospora chibensis* NBRC103694^T = KACC 14256^T; *Actinomycetospora chlora* NBRC105900^T = KACC 14252^T; *Actinomycetospora cinnamomea* NBRC105527^T = KACC 14250^T; *Actinomycetospora corticicola* NBRC103689^T = KACC 14253^T (Tamura et al. 2011); *Actinomycetospora irimotoensis* NBRC106365^T = KCTC 19783^T (Yamamura et al. 2011a); *Actinomycetospora lutea* NBRC103690^T = KACC 14254^T (Tamura et al. 2011); *Actinomycetospora rishiriensis* NBRC106356^T = KACC19782^T (Yamamura et al. 2011b); *Actinomycetospora straminea* NBRC105528^T = KACC 14251^T; and *Actinomycetospora succinea* NBRC103691^T = KACC 14255^T (Tamura et al. 2011) (*Fig. 37.4*).

Actinophytocola Indananda, Matsumoto, Inahashi, Takahashi, Duangmal, and Thamchaipenet, 1141^{VP}

Ac.ti.no.phy.toco.la. Gr. n. *aktis, aktinos* ray; Gr. n. phyton, a plant; L. masc. suff. -cola (from L. n. incola) a dweller, inhabitant; N.L. masc. n. *Actinophytocola* actinobacterial dweller inside a plant.

Phenotypic Analyses

Actinophytocola species are aerobic, Gram-stain positive, nonacid fast, and nonmotile. Vegetative hyphae are non-fragmenting and aerial mycelium, which are produced on some media, fragments into cylindrical spores (0.5 × 2 μm) with smooth surfaces but no sporangium-like structures (*Actinophytocola* *Figs. 37.11* and *37.12*). Good growth occurs at 18–28 °C on oatmeal agar and oatmeal nitrate agar. The cell wall contains *meso*-diaminopimelic acid, alanine, glutamic acid, and acetylated muramic acid. Mycolic acids are absent. Whole-cell sugars are arabinose, galactose, and rhamnose. Mycolic acids are absent. The major fatty acid is iso-$C_{16:0}$, and the major menaquinone is MK-9(H_4) with MK-10(H_4) as the sole menaquinone in one species. The polar phospholipids are phosphatidylethanolamine in all species, with diphosphatidylglycerol and ninhydrin-positive phosphoglycolipids in 4 of 5 species. Chemotaxonomic and physiological properties distinguishing the *Actinophytocola* species are detailed in *Tables 37.12* and *37.13*, respectively.

The G+C content of the DNA of members of this genus ranges between 69.7 and 72.5 (mol%).

The type species is *Actinophytocola oryzae* NBRC 105245^T (Indananda et al. 2010).

Taxonomy, Historical, and Current

The genera most closely related to *Actinophytocola* include *Kibdelosporangium*, *Amycolatopsis*, *Pseudonocardia*, *Saccharopolyspora*, and *Thermocrispum* (Korn-Wendisch et al. 1995)

Table 37.10

Physiological characteristics and method of isolation of species of the genus *Actinomycetospora*

Characteristic	*A. chiangmaiensis* NBRC 104400^T	*A. chibensis* NBRC103694^T	*A. chlora* NBRC105900^T	*A. cinnamomea* NBRC105527^T	*A. corticicola* NBRC103689^T	*A. lutea* NBRC103690^T	*A. straminea* NBRC105528^T	*A. succinea* NBRC103691^T	*A. irimotoensis* NBRC106365^T	*A. rishiriensis* NBRC106356^T
Colour of substrate mycelium	Light to strong yellow	Light to strong yellow	Light greenish yellow to strong yellow	Orange	Orange yellow	Light to strong yellow	Light greenish yellow to yellow	Orange yellow	Cream yellow	Light orange-yellow
Aerial mycelium.	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Claimed present	Not observed
Spore chains	White spore chains	White spore chains	White spore chains	White spore chains	White spore chains	White spore chains	White spore chains, form loops and spirals	Bud-like spore chains	Bud-like spore chains off substrate mycelium	Bud-like spore chains
Spores	Smooth; rod-shaped	Smooth; rod-shaped	Smooth; rod-shaped	Smooth; rod-shaped	Smooth; rod-shaped	Smooth; rod-shaped	Smooth; rod-shaped	Smooth; rod-shaped, 0.3–0.6 × 0.8–1.2 μm	Smooth; rod- and oval shaped, 0.4–0.5 × 0.9–1.1 μm	Smooth; rod- and oval shaped, 0.4–0.6 × 0.9–1.3 μm
Nitrate reduction	−	+	−	+	−	−	+	−	−	−
Pyrrolidonyl arylamidase	W	+	+	−	+	−	+	−	−	−
Aesculin hydrolysis	−	−	−	−	+	−	−	+	+	+
Urea hydrolysis	+	−	−	−	+	−	−	+	+	−
Esterase (C-4)	W	+	−	+	−	+	+	+	+	−
Valine aminopeptidase	+	W	+	−	+	−	+	W	−	+
Utilization of:										
Erythritol	−	−	−	−	−	V	−	+	−	−
Trehalose	+	−	−	−	+	V	+	+	+	+
Turanose	+	+	+	−	+	+	+	V	−	+
L-Arabitol	−	−	−	−	V	−	+	−	−	−
Growth pH	5–8	6–7	5–8	5–8	5–9	5–8	6–8	5–9	5–8	5–9
Tolerance of NaCl (% w/v)	0–3	0–1	0–1	0–7	0–7	0–1	0–5	0–7	0–3	0–3
Growth temperature (°C)	20–37	15–28	15–28	15–37	10–37	20–28	10–28	15–37	20–30	10–30
Origin of isolation sample	Rainforest soil, Chiang Mai, Thailand	Paddy soil, Chiba, Japan	Paddy soil, Iriomote Island, Japan	Paddy soil, Iriomote Island, Japan	Mangrove tree, Iriomote Island, Japan	Vegetable field soil, Amami Island, Japan	Paddy soil, Iriomote Island, Japan	Paddy soil, Iriomote Island, Japan	Lichen sample, Iriomote Island, Japan	Lichen sample, Rishiri Island, Japan
Isolation method	Starch–glycerol medium (Jiang et al. 2006).	Humic acid–vitamin (HV) agar (Hayakawa and Nonomura 1987) with SDS/yeast extract method (Hayakawa and Nonomura 1989).							Humic acid–vitamin (HV) agar (Hayakawa and Nonomura 1987) with cycloheximide, (50 μg ml^{-1}), nalidixic acid (20 μg ml^{-1}).	

Data from: Jiang et al. (2008), Tamura et al. (2011), Yamamura et al. (2011a, b)

Symbols: + positive, − negative, *W* weakly positive, *V* variable

Fig. 37.6
Scanning electron micrograph of *Pseudonocardia eucalypti* EUM374 showing spore chains with knobby protrusions on their spore surface (Micrograph courtesy of Onuma Kaewkla)

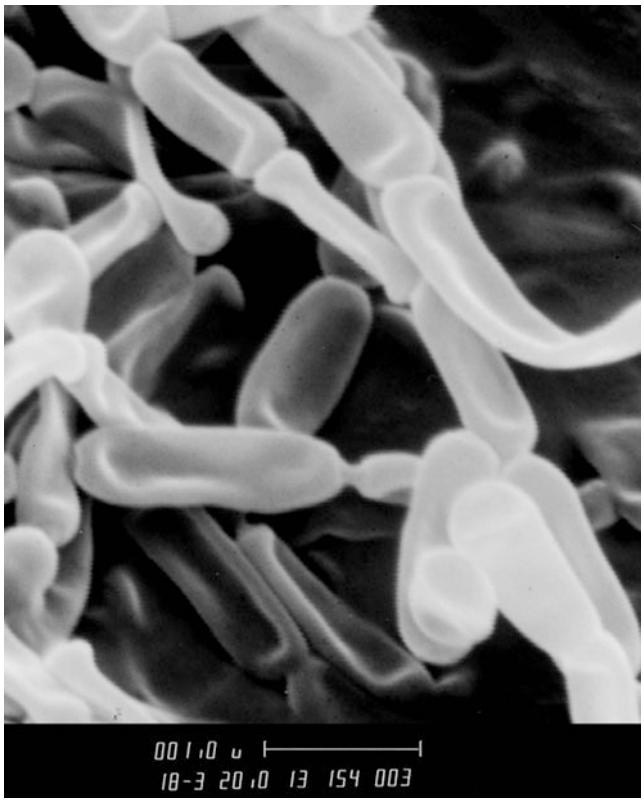

Fig. 37.7
Scanning electron micrograph of *Pseudonocardia adelaidensis* EUM221 showing spore chains (Micrograph courtesy of Onuma Kaewkla)

with 94–95 % nucleotide similarity. Rhamnose as a whole-cell sugar is found only in *Actinophytocola* and not in the other related genera. It can be differentiated from *Kibdelosporangium* and *Thermocrispum* which have sporangium-like structures and a fragmentation of substrate mycelium, while *Actinoalloteichus* species do not have these structures. The phospholipid profile of *Actinophytocola* is clearly distinct from members of closely related genera as all strains contain phosphatidylethanolamine and (except for the type species) diphosphatidylglycerol but lack phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol.

Isolation Procedures

Methods for the isolation of species of the genus *Actinophytocola* are shown in *Table 37.12*.

The five species of the genus *Actinophytocola* are *Actinophytocola oryzae* BCC 31372^T = JCM 17236^T = NBRC 105245^T (Indananda et al. 2010); *Actinophytocola burenkhanensis* NBRC 105883^T = VTCC D9-23^T (Ara et al. 2011a); *Actinophytocola coralline* BTCC B-674^T = NBRC 105525^T; *Actinophytocola timorensis* BTCC B-673^T = NBRC 105524^T (Otoguro et al. 2011); and *Actinophytocola xinjiangensis* CGMCC 4.4663^T = NBRC 106673^T (Guo et al. 2011).

Actinosynnema Hasegawa, Lechevalier, and Lechevalier 1978, 305^{AL}

Ac.ti.no.syn'ne.ma Gr. n. *aktis*, *aktinos* ray; Gr. adv. *syn* together; Gr. n.*nema*, *nematos* thread; *Actinosynnema* indicates a synnema-forming actinomycete.

Phenotypic Analyses

Actinosynnema species are mesophilic, aerobic, Gram-stain-positive, nonacid-fast, and catalase-positive filamentous actinomycetes, typically isolated directly from plant tissue. The fine mycelia (approximately 0.5 μm in diameter) differentiate into long-branching substrate mycelia that penetrate the growth medium and also form dome-like bodies, also called synnemata or flat colonies on the agar surface. Aerial hyphae (0.5–1.0 μm in diameter) arise from synnemata or flat colonies and bear chains of spores that can become motile in a suitable aqueous environment. The cell walls contain *meso*-diaminopimelic acid. The whole-cell sugar pattern consists of galactose and mannose. Principal phospholipids include phosphatidylethanolamine, phosphatidylethanolamine containing 2-hydroxy fatty acids, and diphosphatidylglycerol. Menaquinones are predominantly MK-9(H_6).

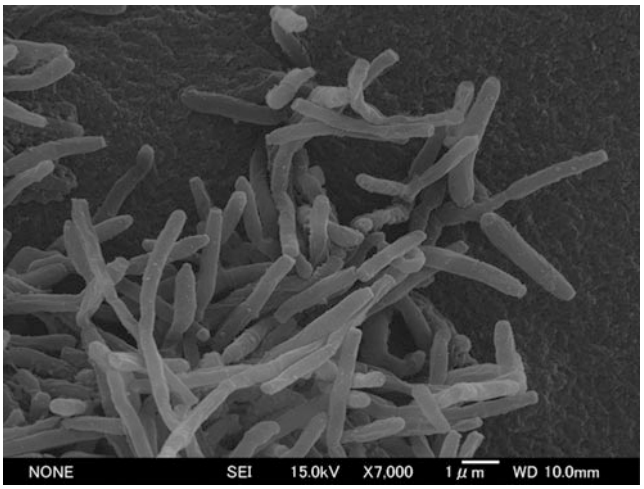

Fig. 37.8
Scanning electron micrograph of *Actinophytocola rishiriensis* RI109-Li102 showing spore chains (Micrograph courtesy of Hideki Yamamura)

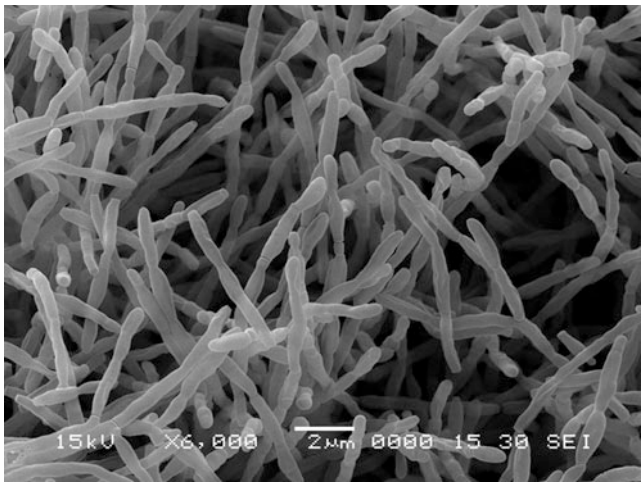

Fig. 37.10
Scanning electron micrograph of *Actinomycetospora lutea* TT00-04 showing spore chains

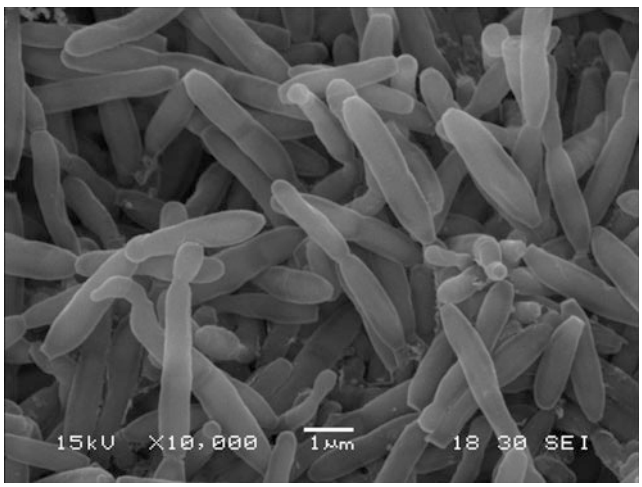

Fig. 37.9
Scanning electron micrograph of *Actinomycetospora corticicola* 014–5 showing bud-like spores (Micrograph courtesy of H. Yamamura)

The G+C of the DNA of members of this genus ranges from 71 to 73 (mol%).

The type species is *Actinosynnema mirum* ATCC 29888^T = DSM 43287^T = NBRC 14064^T = IMRU 3971^T = JCM 3225^T = NRRL B-12336^T = VKM Ac-843^T (Hasegawa et al. 1978).

Taxonomy, Historical, and Current

Phylogenetic analyses based on the sequence of the 16S rRNA gene demonstrates that the genus *Actinosynnema* is related to the genera *Lechevalieria*, *Lentzea*, and *Saccharothrix*, being intermediate between the first two genera and *Saccharothrix* and was the type genus for the family *Actinosynnemataceae* (Labeda and Kroppenstedt 2000).

The genus *Actinosynnema* was described by Hasegawa et al. (1978) to contain actinomycetes that produce unique morphological structures called synnemata (*Actinosynnema* *Fig. 37.13*) or dome-like bodies on most media. Aerial mycelia are produced on these synnemata or dome-like bodies and are initially whitish in color and become yellow to yellowish orange in color. Regular septation occurs in mature aerial hyphae making it look bamboo-like when observed microscopically and then the hyphae become chains of spores. Suspension of the aerial mycelia in liquid media under a coverslip permits the observation of peritrichously motile zoospores (*Actinosynnema* *Fig. 37.14*) within 30 min to 1 h.

Strains of *Actinosynnema* can be easily differentiated from other actinomycetes by observation of the characteristic synnemata produced on most growth media. Numerous other actinobacterial genera produce motile zoospores, notably *Actinokineospora*, *Actinoplanes*, *Planobispora*, *Planomonospora*, *Spirillospora*, and several others, but none produce synnemata. The chemotaxonomic profile of *Actinosynnema* species (*Table 37.14*) is different from the other genera in the order, except *Lentzea*, particularly the whole-cell sugar pattern consisting of only galactose and mannose as the diagnostic sugars. The phospholipid pattern of *Actinosynnema* strains is quite similar to that of *Saccharothrix* species, but the lack of rhamnose in the whole-cell sugar pattern and the presence of MK-9(H_6) and lack of MK-10(H_4) menaquinones differentiate them from this genus.

The physiological characteristics of *Actinosynnema* species are summarized in *Tables 37.15* and *37.16* can be used to differentiate between species.

Table 37.11

Chemotaxonomic characteristics of species of the genus *Actinomycetospora*

Characteristic	*A. chiangmaiensis* NBRC 104400^T	*A. chibensis* NBRC103694^T	*A. chlora* NBRC105900^T	*A. cinnamomea* NBRC105527^T	*A. corticicola* NBRC103689^T	*A. lutea* NBRC103690^T	*A. straminea* NBRC105528^T	*A. succinea* NBRC103691^T	*A. irimotoensis* NBRC106365^T	*A. rishiriensis* NBRC106356^T
Whole-cell sugar[a]	Ara, Gal	Ara, Gal, Glc	Ara, Gal, Glc	Ara, Gal, Glc	Ara, Gal, Glc	Ara, Gal, Glc	Ara, Gal, Glc	Ara, Gal, Glc	Ara, Gal	Ara, Gal
Phospholipids[b]	PC, PE, PI, PG	PE, PC, DPG	PE, PC, DPG	PE, PC, DPG	PE, PC, DPG	PE, PC, DPG	PE, PC, DPG	PE, PC, DPG	PE, PC, PI, DPG	PE, PC, DPG
Major menaquinone(s)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
DNA G + C content (mol %)	69	73	73	74	74	73	74	73	74	73.1
Fatty acid composition(%):										
$C_{9:0}$	–	–	–	–	–	0.8	–	–	–	–
$C_{11:0}$	–	–	–	–	–	0.5	–	–	–	–
iso-$C1_{2:0}$	–	–	–	–	–	0.7	0.5	–	–	–
$C_{12:0}$	–	–	–	–	–	0.6	–	–	–	–
iso-$C_{13:0}$	–	–	–	–	–	–	–	–	–	–
$C_{13:0}$	–	–	–	–	–	1.7	–	–		
iso-$C_{14:0}$	1.0	2.5	2.5	1.0	–	4.7	4.6	1.5	4.1	1.4
$C_{14:0}$	–	1.2	1.0	0.8	–	2.4	–	–	–	–
iso-$C_{15:0}$	1.5	11.6	1.3	4.7	–	1.2	2.7	1.9	1.6	–
ante*iso*-$C_{15:0}$	–	7.2	–	1.1	–	–	–	–	–	–
$C_{15:1}$ A	–	–	–	–	–	0.9	–	–	–	–
$C_{15:1}$ B	–	–	1.1	1.5	–	4.3	–	1.1	–	–
$C_{15:0}$	–	0.6	1.6	4.5	–	14.4	0.7	7.7	–	–
iso-$C_{16:1}$ H	2.2	4.2	5.9	3.5	7.7	0.9	11.1	0.8	8.9	12.5
iso-$C_{16:0}$	43.5	42	51.4	20.4	49.5	23.5	62.3	37.6	58.1	49.2
$C_{16:1}$ A	–	1.7	–	–	–	–	–	–	–	–
$C_{16:1}$ *cis*-9	15.5	4.4	12.4	8.9	7.5	10.1	2.1	5.0	7.5	7.3

Table 37.11 (continued)

Characteristic	*A. chiangmaiensis* NBRC 104400^T	*A. chibensis* NBRC103694^T	*A. chlora* NBRC105900^T	*A. cinnamomea* NBRC105527^T	*A. corticicola* NBRC103689^T	*A. lutea* NBRC103690^T	*A. straminea* NBRC105528^T	*A. succinea* NBRC103691^T	*A. irimotoensis* NBRC106365^T	*A. rishiriensis* NBRC106356^T
$C_{16:1}$ C	–	0.5	–	–	–	–	–	–		
C16:0	10.0	4.4	5.5	6.7	2.4	4.8	–	4.7	4.5	3.6
$C_{16:0}$ 9-methyl	–	–	–	1.9	–	–	–	–	–	–
$C_{16:0}$ 10-methyl	8.9	2.1	6.0	–	17.0	1.7	–	1.2	4.6	9.4
ante*iso*-$C_{17:1}$	–	2.1	–	–	–	–	–	–	–	–
iso-$C_{17:0}$	1.3	–	–	3.8	–	–	–	–	–	–
ante*iso*-$C_{17:0}$	4.6	2.3	–	8.4	–	–	2.1	–	–	–
$C_{17:1}$ *cis*-9	2.4	3.4	4.6	18.9	4.4	15.2	9.0	17.3	2.3	5.2
iso-$C_{16:0}$ 2-OH	2.2	2.5	2.6	1.3	4.0	3.6	2.1	6.9	1.6	3.3
$C_{17:0}$	–	0.9	1.1	7.0	–	4.4	1.0	9.4	–	–
$C_{17:0}$ 10-methyl	1.3	2.0	1.8	1.6	7.6	2.2	1.9	4.0	1.6	7.9
iso-$C_{18:0}$	–	1.5	–	–	–	–	–	–	–	–
$C_{18:1}$ *cis*-9	2.4	1.0	1.3	1.7	–	1.0	–	–	–	–
$C_{18:0}$	3.3	0.8	–	2.4	–	–	–	1.0	–	–
Others	–	1.1	–	–	–	0.4	–	–	–	–

Data from: Jiang et al. (2008), Tamura et al. (2011), Yamamura et al. (2011a, b)

Symbols: + Positive, – not reported or trace amount (<0.5 %)

[a]*Ara* arabinose, *Gal* galactose, *Glc* glucose

[b]*PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol

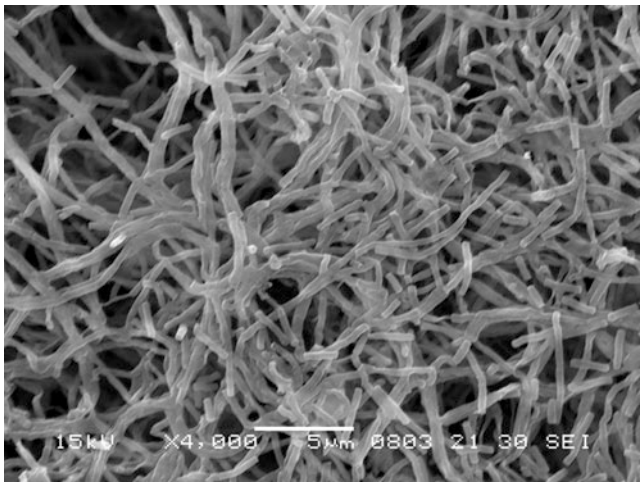

Fig. 37.11
Scanning electron micrograph of *Actinophytocola coralline* ID06-A0464 grown on HV agar at 28 °C for 2 weeks showing spore formation (Micrograph courtesy Misa Otoguro)

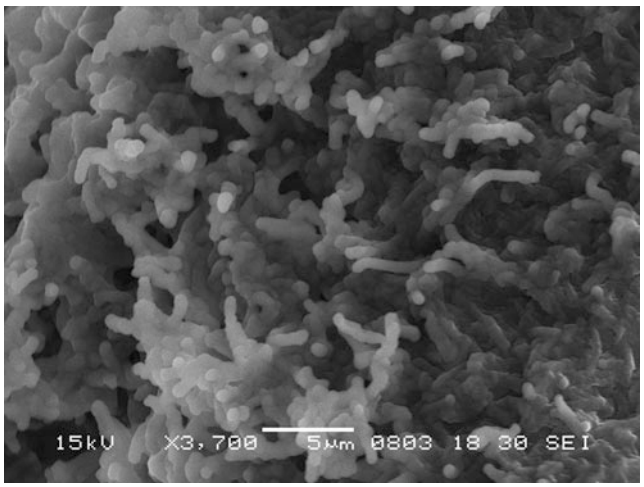

Fig. 37.12
Scanning electron micrograph of *Actinophytocola timorensis* sp. nov. ID05-A0653 grown on HV agar at 28 °C for 2 weeks showing spore formation (Micrograph courtesy Misa Otoguro)

Isolation, Enrichment, and Maintenance Procedures

The isolation procedures of *Actinosynnema* species from blades of grass are outlined in *Table 37.14*. Working cultures of *Actinosynnema* can be maintained as plate or agar slant cultures on an appropriate medium such as NZ-amine agar (DSMZ medium 554) with biweekly subculturing. Survival of the cultures is often better at room temperature than at 4 °C. Longer-term preservation of strains is best accomplished as frozen stocks in 40 % (v/v) aqueous glycerol at −80 °C (mechanical freezer) to −172 °C (liquid nitrogen vapour phase) or by using traditional lyophilization techniques.

Alloactinosynnema Yuan, Zhang, Yu, Liu, Guan, Lee, Kim, and Zhang 2010, 21[VP]

Al.lo.ac.ti.no.syn'ne.ma.Gr. adj *allos* other; N. L. neut. N. *Actinosynnema* a bacterial genus name; N.L. neut . n. *Alloactinosynnema*, the other *Actinosynnema*, referring to the fact that it is morphologically similar to *Actinosynnema*, but chemotaxonomically and phylogenetically distinct.

Phenotypic Analyses

The sole *Alloactinosynnema* species is an aerobic, Gram-stain-positive, lysozyme-resistant actinomycete that forms extensively branched, white- or buff- to pink-colored substrate mycelium that may fragment into rod-shaped elements. White-colored aerial hyphae are produced and these differentiate into long chains of smooth-surfaced spores which may aggregate with the aerial mycelia into clusters. Sporangium-like structures are also produced on some media. Cell walls contain the *meso*-isomer of diaminopimelic acid, and whole-cell hydrolysates contain galactose and ribose. Phosphatidylcholine is the predominant phospholipid, along with phosphatidylglycerol and diphosphatidylglycerol. The principal menaquinone is MK-9(H_4).

The G+C content of the DNA of the type species is 68.2 (mol%).

The type species is *Alloactinosynnema album* DSM 45114^T = JCM 17965^T = KCTC 19294^T (Yuan et al. 2010), the only species in the genus.

Taxonomy, Historical, and Current

The genus *Alloactinosynnema* is phylogenetically most near the genus *Actinokineospora*, as can be seen in the *Pseudonocardiales* phylogenetic tree (*Fig. 37.3*). The polar lipid content of *Alloactinosynnema* is distinct from phylogenetically near genera in that it contains ribose in its cell wall, and phosphatidylcholine as the predominant phospholipid, along with phosphatidylglycerol and diphosphatidylglycerol. It also forms pseudosporangia which are not formed by members of the genus *Actinokineosporia*.

The 16S rRNA gene contains a genus-specific pattern of diagnostic nucleotide signatures, namely, 603:635 (C–G), 617:623 (U–C), and 619 (U).

The chemotaxonomic, morphological, and physiological characteristics of the type species *Alloactinosynnema album* are shown in *Tables 37.8* and *37.9*.

Allokutzneria Labeda and Kroppenstedt 2008, 1474[VP]

Al.lo.kutz.ne′ri.a Gr. adj. *allos* other; N. L fem. n. *Kutzneria* a bacterial generic name; N. L. fem. n. *Allokutzneria* the "other *Kutzneria*" referring to the fact that it is phylogenetically close to *Kutzneria* but chemotaxonomically distinct.

Table 37.12

Chemotaxonomic properties and method of isolation of the species of the genus *Actinophytocola*

Characteristic	*A. oryzae* NBRC 105245^T	*A. burenkhanensis* NBRC 105883^T	*A. corallina* NBRC 105525^T	*A. timorensis* NBRC 105524^T	*A. xinjiangensis* QAIII60^T
Whole-cell sugar[a]	Ara, Gal, Man, Rha, Rib	Ara, Gal, Glc, Man, Rha	Ara, Gal, Rha	Ara, Gal, Rha	Ara, Gal, Glc, Rha, Rib
Phospholipids[b]	PE, OH-PE	DPG, PE, OH-PE, NPG	DPG, PE, NPG	DPG, PE, NPG	DPG, PE, OH-PE, NPG
Major menaquinone(s)	MK-9(H_4)	MK-10(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-10(H_2)
Major fatty acids (>10 %)	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$ 2-OH	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$	$C_{16:0}$, *iso*-$C_{16:0}$	*iso*-$C_{14:0}$, *iso*-$C_{16:0}$, *iso*-$C_{16:1}$H, $C_{17:1}$ ω6c
DNA G+C content (mol %)	71.1	70.6	71.2	69.7	72.5
Origin of Isolation Sample	Rice roots, Thani Province, Thailand	Soil, Khuvsgul province, Mongolia	Soil, Lombok Island, Indonesia	Soil, West Timor, Indonesia	Virgin forest soil, Xinjiang province, China
Isolation method	Surface sterilization followed by plating on starch casein (Küster and Williams 1964) + penicillin G (2.5 U ml^{-1}) and cycloheximide (50 mg ml^{-1})	Humic acid–vitamin (HV) agar (Hayakawa and Nonomura 1987) + rehydration and centrifugation method (Hayakawa et al. 2000) + trimethoprim (20 mg L^{-1}) and nalidixic acid (10 mg L^{-1})	Humic acid–vitamin (HV) agar (Hayakawa and Nonomura 1989) + rehydration and centrifugation method (Hayakawa et al. 2000)	Humic acid–vitamin (HV) agar (Hayakawa and Nonomura 1987) + SDS/yeast extract method (Hayakawa and Nonomura 1989)	Pretreated with 1.5 % phenol on 100-fold-diluted nutrient agar + cycloheximide, nystatin, nalidixic acid (each at 50 μg ml^{-1}) + novobiocin (25 μg ml^{-1}) (Qiu et al. 2008)

Data from: Ara et al. (2011a), Otoguro et al. (2011), Indananda et al. (2010), Guo et al. (2011)

[a]*Ara* arabinose, *Gal* galactose, *Glc* glucose, *Man* mannose, *Rha* rhamnose, *Rib* ribose

[b]*DPG* diphosphatidylglycerol, *NPG* ninhydrin-positive phosphoglycolipids, *HO-PE* hydroxyphosphatidylethanolamine, *PE* phosphatidylethanolamine

Phenotypic Analyses

Allokutzneria species are aerobic, Gram-staining-positive, nonacid-fast, nonmotile actinomycetes that produce sporangium-like bodies containing hyphae but no spores (*Allokutzneria* ◉ *Fig. 37.15*). Chains of aerial spores are also produced and the vegetative mycelium also fragments to varying degrees. Cell walls contain *meso*-diaminopimelic acid as the diamino acid, the muramic acid in the peptidoglycan is acetylated and the whole-cell sugar pattern consists of arabinose, galactose, and mannose. The principal phospholipids include phosphatidylethanolamine, phosphatidylmethylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylinositol, *lyso*-phosphatidylmethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, and *lyso*-phosphatidylethanolamine. The menaquinones present are predominantly MK-9(H_4) with traces of MK-9(H_2), MK-9(H_6), and MK-10(H_4). Mycolic acids are not present and the major fatty acid components are iso- and anteiso-fatty acids.

The G+C content of the DNA of the type species is 71.7 (mol%).

The type species is *Allokutzneria albata* DSM 44149^T (ATCC 55061^T = JCM 9917^T = NBRC 101910^T = NRRL B-24461^T) (Tomita et al. 1993; Labeda and Kroppenstedt, 2008).

Isolation Procedures

A soil sample collected in Mindanao Island, Philippines, was dried, ground, and transferred with a nylon sponge to agar medium containing 5 g of soluble starch, 5 g of glucose, 1 g of yeast extract, 1 g of NZ-case, 1 g of $CaCO_3$, 20 ml of V-8 juice (Campbell Japan Corp.), and 16 g agar made up to 1 L with distilled water, pH 7.2. This was supplemented with 4 μg/ml ampicillin, 10 μg/ml nalidixic acid, and 80 μg/ml nystatin. The plates were incubated at 43 °C (Tomita et al. 1993).

Taxonomy, Historical, and Current

Kibdelosporangium albatum was described by Tomita et al. (1993) for the actinomycete strain producing the antiviral antibiotics, the cycloviracins, based on morphological and chemotaxonomic characteristics. A phylogenetic analysis using 16S rRNA gene sequences for species within the order *Pseudonocardiales* by Labeda and Kroppenstedt (2000) demonstrated that this species clearly did not belong to the genus *Kibdelosporangium* (◉ *Fig. 37.18*) and chemotaxonomic analyses, specifically the presence of arabinose in the whole-cell sugar pattern and the presence of *lyso*-phospholipids in the polar lipid profile,

Table 37.13

Cultural and physiological properties of the species of the genus *Actinophytocola*

Characteristic	*A. oryzae* NBRC 105245^T	*A. burenkhanensis* NBRC 105883^T	*A. coralline* NBRC 105525^T	*A. timorensis* NBRC 105524^T	*A. xinjiangensis* QAIII60^T
Colour of substrate mycelium	Light melon yellow	Light yellow to moderate yellowish brown on different agar media	Coral on ISP7	Orange-yellow on ISP7	Beige to yellow brown on IPS3, ISP4, modified Bennett's agar and NZ-amine medium
Aerial mycelium	Pale peach – ISP3	Pale yellow	White to yellowish-white on ISP 5 and 7 and NBRC medium 266	White to yellowish-white on ISP 5 and 7 and NBRC medium 266	White
Others	Good growth on ISP3 and oatmeal-nitrate agar	Good growth on Bennett's agar	Good growth on ISP2, 3, 4, 5, and 7 and NBRC medium 266	Good growth on ISP2, 3, 4, 5, and 7 and NBRC medium 266	Grayish-black on ISP7
	Moderate growth on ISP2 and 1/10 yeast extract-starch agar	Moderate growth on ISP2, ISP3, and ISP6,			
	Poor growth on ISP4 and 5	Weak growth on YS, ISP7, and water agar			
		No growth on ISP5			
Reduction of nitrate	w	+	–	+	+
Liquefaction of gelatin	w	–	+	+	+
Peptonization of milk	–	–	+	+	+
Ranges for growth					
pH	4.0–9.0	5.0–8.0	4.0–11.0	4.0–11.0	6.0–9.0
Temperature (°C)	15–30	15–37	15–30	15–37	10–30
NaCl (%)	≤ 2	≤ 2	≤ 7	≤ 5	≤ 4
Decomposition of:					
Casein	–	+	+	+	+
Hypoxanthine	–	–	–	–	w
Guanine	–	–	+	+	–
L-Tyrosine	–	–	w	+	–
Starch	+	+	–	w	+
Urea	+	–	–	+	+
Utilization of:					
Adonitol	–	–	w	+	+
D-Fructose	w	–	+	+	+
Lactose	–	+	w	w	–
Maltose	w	–	+	+	+
D-Mannose	–	–	+	+	+
(+)-D-Xylose	–	–	–	+	+
D-Sorbitol	–	–	w	–	w
Raffinose	–	–	–	w	–
L-Rhamnose	–	–	+	+	+
Sucrose	–	–	w	+	w

Table 37.13 (continued)

Characteristic	*A. oryzae* NBRC 105245^T	*A. burenkhanensis* NBRC 105883^T	*A. coralline* NBRC 105525^T	*A. timorensis* NBRC 105524^T	*A. xinjiangensis* QAIII60^T
Acid production from					
L-Arabinose	–	+	–	–	+
Cellobiose	w	–	+	+	+
Fructose	+	w	–	–	–
Glucose	w	–	–	+	w
Maltose	–	–	–	+	w
L-Rhamnose	w	–	–	–	w
Ribose	+	+	+	–	+
Sorbitol	+	+	–	w	–

Data from: Ara et al. (2011a), Otoguro et al. (2011), Indananda et al. (2010), Guo et al. (2011)
Symbol: + positive, *w* weak positive, – negative

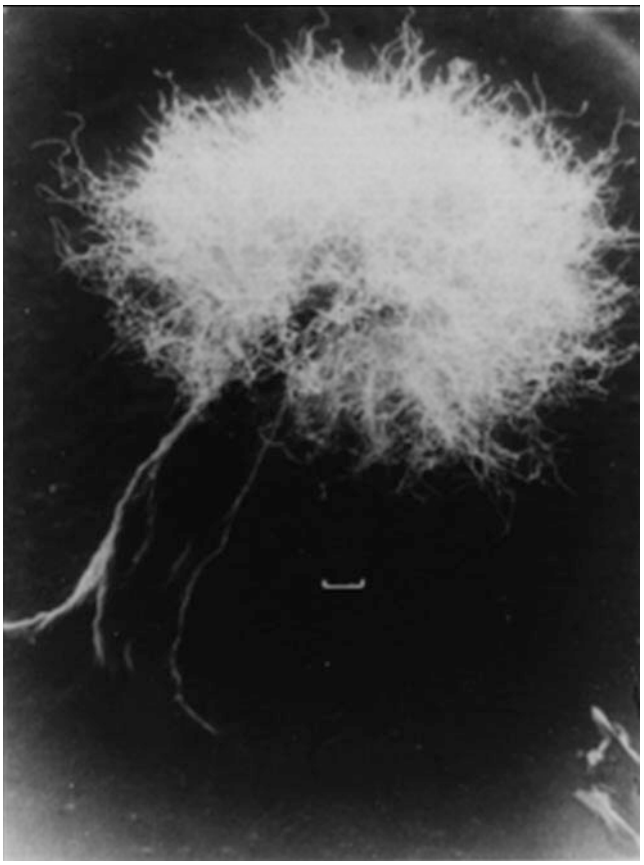

Fig. 37.13
Scanning electron micrograph of a synnemata. Bar represents 10 μm

indicated that it is distinct from the phylogenetically nearest genus *Kutzneria* as is shown in the Table 37.17. The comparison of the morphological and physiological characteristics with those of the species of *Kutzneria* can be seen in Table 37.18 (Fig. 37.5).

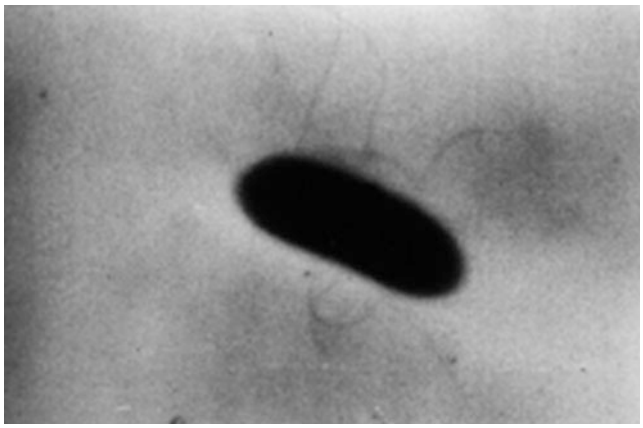

Fig. 37.14
Electron micrograph of a zoospore of *Actinosynnema mirum*

Amycolatopsis Lechevalier, Prauser, Labeda and Ruan 1986, 34^{VP}emend. Lee 2009, 1403

A.my.co.la.top'sis.N.L. fem. n. *Amycolata* genus belonging to the order *Actinomycetales*; Gr. fem. n. *opsis* aspect, appearance; N.L. fem. n. *Amycolatopsis* that which appears similar to *Amycolata*.

Phenotypic Analyses

Amycolatopsis are Gram-stain-positive, aerobic to facultatively anaerobic, nonacid-fast, nonmotile, catalase-positive actinobacteria that grow in the mesophilic to thermophilic temperature range. The substrate hyphae branches and fragments into squarish and rod-shaped elements. Aerial hyphae may not bear spores or can differentiate into chains of spore-like structures similar in shape to the hyphal fragments.

Table 37.14

Chemotaxonomic characteristics and method of isolation for *Actinosynnema* sp.

Species	Whole-cell sugars[a]	Phospholipids[b]	Major menaquinone(s)	Major fatty acids	DNA G+C content (mol %)	Origin of isolate	Method of isolation
Actinosynnemamirum NBRC 14064^T	Gal, Man	PE, OH-PE, DPG	MK-9(H_6)	nr	73 ± 1	Surfaces of blades of *Carex* sp. grass; Raritan River, New Jersey	Grass blade was placed on yeast extract agar (0.02 % yeast extract [Difco], 1.5 % agar), and incubated for 3 weeks at 28 °C
Actinosynnemapretiosum subsp. *pretiosum* ATCC 31281^T	Gal, Man	PE, OH-PE, DPG	MK-9(H_6)	$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:0}$, $C_{18:0}$.	71 ± 1	Surfaces of blades of *Carex* sp. grass; Shiga Prefecture, Japan	As above
Actinosynnemapretiosum subsp. *auranticum* ATCC 31309^T	Gal, Man	PE, OH-PE, DPG	MK-9(H_6)	*iso*-$C_{16:0}$, $C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:0}$, $C_{18:0}$.	71 ± 1	Surfaces of blades of *Carex* sp. grass; Shiga Prefecture, Japan	As above

Data from: Hasegawa et al. (1978) and Hasegawa et al. (1983)

Symbols: *nr* not reported

[a]*Gal* galactose, *Man* mannose

[b]*PE* phosphatidylethanolamine, *OH-PE* phosphatidylethanolamine containing 2-hydroxy fatty acids, *DPG* diphosphatidyl glycerol

Chemotaxonomic analysis of the cell wall shows that most *Pseudonocardia* species possess *meso*-diaminopimelic acid and the muramic acid is *N*-acetylated, and whole-cell hydrolysates contain arabinose and galactose. Menaquinone MK-9(H_4) is the predominant isoprenoid quinone, with two taxa possessing MK-8(H_4) and one taxon each containing only MK-9(H_2), MK-11(H_4), or MK-12(H_4). The diagnostic phospholipid is phosphatidylethanolamine, with a majority of species also containing diphosphatidylglycerol and phosphatidylglycerol. No mycolic acids were detected.

The physiological characteristics and chemotaxonomic traits reported for the type species of the genus *Amycolatopsis* are described in *Tables 37.19* and *37.20*, respectively.

G+C content of the DNA of the members of the genus ranges from 65.8 to 75 (mol%).

The type species is *Amycolatopsis orientalis* (Pittenger and Brigham 1956).

Isolation Procedures

Members of this genus are nutritionally versatile and include facultative autotrophs and can be isolated from soil and other environmental samples using a range of media. Isolation procedures of all the species are shown in *Table 37.21*.

Taxonomy, Historical, and Current

The genus *Amycolatopsis* was proposed for actinobacteria that possessed substrate hyphae that fragmented into square elements and aerial mycelia which were either sterile or also fragmented into square to oval spore-like structures. Initially the members of this genus when first isolated were described as either *Actinomyces* or *Nocardia* or *Proactinomyces* or *Streptomyces*. The type species *Amycolatopsis orientalis* (Krasil'nikov 1981) was classified according to these genera in the same order (Pridham 1970; Rautenstein et al. 1975; Pittenger and Brigham 1956). In a similar fashion *Amycolatopsis mediterranei* was initially termed *Nocardia mediterranei* (Thiemann et al. 1969) and then "*Streptomyces mediterranei*" (Margalith and Beretta 1960). When *Amycolatopsis orientalis* subsp. *lurida* (Grundy et al. 1957; Lechevalier et al. 1986) was accepted as a valid type species of the new genus, it became *Amycolatopsis orientalis* subsp. *orientalis* (Pittenger and Brigham 1956) which reverted to its earlier name *Amycolatopsis orientalis* (Pittenger and Brigham 1956; Lechevalier et al. 1986). *Amycolatopsis orientalis* subsp. *lurida* was later afforded its own species name to become *Amycolatopsis lurida* (Stackebrandt et al. 2004).

Amycolatopsis species can be differentiated from other members of the family *Pseudonocardiaceae* using 16S rRNA and *gyrB* gene sequencing (Everest and Meyer 2009) as well as MALDI-TOF MS profiles (Groth et al. 2007) and ribotyping patterns (Wink et al. 2003, 2004).

Table 37.15
Physiological properties of *Actinosynnema* species

	Actinosynnema mirum NBRC 14064^T	*Actinosynnema pretiosum* subsp. *pretiosum* ATCC 31281^T	*Actinosynnema pretiosum* subsp. *auranticum* ATCC 31309^T
Gross morphology			
Substrate mycelium	Yellow to orange-yellow	Pale orange-yellow	Yellowish-orange or orange
Aerial mycelium	White to pale yellow	White to pale yellow	White to pale yellow
Synnemata	+	+	+
Soluble pigments	Yellowish brown/pale greenish	Yellowish brown-purplish brown	Yellowish brown-purplish brown
Natural products			
Decomposition of			
Adenine	−	nr	nr
Casein	+	+	+
Esculin	nr	+	+
Gelatin	+	±	±
Hypoxanthine	−	−	−
Starch	+	+	+
Tyrosine	+	+	+
Urea	−	−	−
Xanthine	−	−	−
Growth on			
Arabinose	±	+	−
Galactose	+	+	+
Glucose	+	+	+
Glycerol	+	−	+
Inositol	−	−	−
Lactose	−	−	±
Mannitol	+	+	+
Maltose	+	±	+
Mannose	+	+	+
Melibiose	−	+	±
Raffinose	−	±	±
Rhamnose	+	+	+
Soluble starch	±	+	+
Sorbitol	−	−	−
Sucrose	+	+	+
Trehalose	+	+	+
Xylose	+	+	+
Production of			
Catalase	+	+	+
Hydrogen sulfide	nr	−	−
Nitrate reductase	+	+	+
Phosphatase	+	+	+
Growth in the presence of			
2 % (w/v) NaCl	nr	+	+
Lysozyme	+	+	+
Amphotericin B (100 μg/ml)	nr	+	+

Table 37.15 (continued)

	Actinosynnema mirum NBRC 14064^T	*Actinosynnema pretiosum* subsp. *pretiosum* ATCC 31281^T	*Actinosynnema pretiosum* subsp. *auranticum* ATCC 31309^T
Candicidin (50 μg/ml)	+	nr	nr
Chloramphenicol (20 μg/ml)	−	−	−
Dihydrostreptomycin (10 μg/ml)	−	nr	nr
Nystatin (100 μg/ml)	+	nr	nr
Streptomycin (20 μg/ml)	nr	−	−
Sulbenicillin (100 μg/ml)	nr	+	+
Tetracyline (1 μg/ml)	−	nr	nr
Tetracyline (20 μg/ml)	nr	−	−
Growth at			
10 °C	+	−	−
37 °C	−	+	+

Data from: Hasegawa et al. (1978) and Hasegawa et al. (1983)
Symbols: + positive, − negative, ± doubtful response, *nr* not reported

Application

The importance of this genus is due to their ability to produce bioactive secondary metabolites belonging to a number of chemical structural classes, as described in *Table 37.22*. Of these classes the two that are effective in human antibacterial therapy are the ansamycins, e.g., rifamycin, and glycopeptides, e.g., vancomycin and balhimycin.

Pathogenicity

Some evidence of human and equine pathogenicity has been published which implicates *Amycolatopsis benzoatilytica* as an agent of submandibular mycetoma (Majumdar et al. 2006; Scharfen 1971) in humans. Members of the genus have also been found in clinical samples such as *Amycolatopsis orientalis* in cerebrospinal fluid and *Amycolatopsis palatopharyngis* from the palatopharygeal mucosa of a 70-year-old male (Huang et al. 2004), *Amycolatopsis kentuckyensis*, *Amycolatopsis lexingtonensis*, and *Amycolatopsis pretoriensis*, as the causal agent of placentitis and abortion in horses (Donahue and Williams 2000; Giles et al. 1993; Hong et al. 1993; Labeda et al. 2003).

Ecology and Metabolism

Apart from the strains isolated from clinical specimens, the majority of taxa have been isolated from soils of different types, including rhizosphere soils, dusts from catacombs, and ocean sediments. To date only one species has been recovered as an endophyte. Some of the facultative autotrophs can degrade a range of organic substrates and use different carbon sources as their sole source of energy and growth (Chun et al. 1999; De Boer et al. 1990; Groth et al. 2007). *Amycolatopsis benzoatilytica* is the only member of this genus which can grow on *m*-hydroxybenzoate.

Molecular Analyses

The genetic analyses of some members of the genus *Amycolatopsis* is based on species which produce economically important antibiotics (as described in detail by Tan and Goodfellow 2012).

To date three genome sequences of *Amycolatopsis* species have been fully annotated.

Amycolatopsis mediterranei U3, an industrial strain producing the antimycobacterial antibiotic rifamycin, has a circular chromosome containing 10,236,715 base pairs (bp) with a G+C content of 71.3 (mol%) and a predicted 9,228 protein-coding genes (Zhao et al. 2010). It has a core region which carries the rifamycin biosynthesis gene cluster with the majority of *rif* genes encoded on the leading strand of replication and a novel "quasi-core" region which has 21 out of the total 26 gene clusters for secondary metabolite production – 4 polyketides (PK), 11 non-ribosomal peptides (NRP), PK-NRP hybrids, and 4 terpenoids, with the rest being other chemical structures. There are 55 genes encoding cytochrome P450 enzymes (notably including the enzyme involved in the conversion of rifamycin SV to B) and 86 antibiotic-resistant genes, which are evenly distributed along the chromosome.

The genome sequence of another more thoroughly investigated rifamycin producer, *Amycolatopsis mediterranei* S699 (GenBank accession number CP002896), was reported a year later (Verma et al. 2011). Strain S699 has a gene order and

Table 37.16
Applications. Antibiotics produced by the genera *Actinosynnema, Allokutzneria, Lechevaliera, Saccharothrix*, and *Umezawaea*

Species	Strain no.	Antibiotic	Reference
Actinosynnema pretiosum subsp. *auranticum*	ATCC 31309^T	Ansamitocins	Higashide et al. (1977)
Actinosynnema pretiosum subsp. *pretiosum*	ATCC 31281^T	Ansamitocins	Higashide et al. (1977)
Allokutzneria albata	NRRL B-24461^T	Cycloviracins	Tsunakawa et al. (1992a)
Lechevalieria aerocolonigenes	ATCC 39243	Rebeccamycin	Bush et al. (1987)
Lechevalieria flava	INA 2171^T	Madumycin	Gauze et al. (1974)
Lechevalieria species	VK-A9	Thiobutacin	Lee et al. (2004a)
Saccharothrix australiensis	NRRL 11239^T	LL-BM782 complex	Tresner et al. (1980)
Saccharothrix espanaensis	NRRL 15764^T	LL-C19004	Kirby et al. (1987)
Saccharothrix mutabilis subsp. *capreolus*	NRRL 2773^T	Capreomycin	Stark et al. (1962)
Saccharothrix mutabilis subsp. *mutabilis*	ATCC 31520^T	Polynitroxin	Jain (1982)
Saccharothrix syringae	INA 2240^T	Nocamycin	Gauze et al. (1977)
Saccharothrix sp.	DSM 12931	Pluraflavins	Vertesy et al. (2001)
Saccharothrix sp.	SA 103	Mutactimycin PR	Zitouni et al. (2004a)
Saccharothrix sp.	SA 233	Dithiopyrrolones	Lamari et al. (2002)
Umezawaea tangerinus	JCM 10302^T	Formamicin	Kinoshita et al. (1999)

nucleotide identity very similar to that of *Amycolatopsis mediterranei* U32. However, it is reported to contain a 10,236,779 bp circular chromosome and 9,575 coding sequences, and in addition to the well-known 90-kb rifamycin gene cluster, five other PK, twelve NRPS, and three PK-NRPS hybrid clusters were observed.

Vongsangnak et al. (2012) not only performed genome sequencing and analysis of *Amycolatopsis balhimycina* but also used the information to construct a genome-scale metabolic model. The purpose of the model was to facilitate the application of rational engineering strategies to increase the production of balhimycin. The *Amycolatopsis balhimycina* genome sequence is reported to have 10,562,587 base pairs with a G+C content of 69 (mol%), which includes 8,585 open reading frames (ORFs).

Amycolatopsis sp. strain ATCC 39116 was selected for genome sequencing as it can depolymerize lignin (Antai and Crawford 1981) and catabolize the resulting aromatic components (Sutherland 1986). It has a genome size of 8,442,518 bp (AFWY00000000) with a G+C content of 71.9 (mol%) and predicted 8,264 candidate protein-encoding genes (Davis et al. 2012).

Crossiella Labeda 2001, 1578^{VP}

Cross.i.el'la.M.L. dim. ending *-ella*; M.L, fem. n. *Crossiella* named for Thomas Cross, a microbiologist at the University of Bradford who made many contributions to actinomycete biology and systematics.

Phenotypic Analyses

Crossiella species are aerobic, Gram-stain-positive, nonacid-fast, catalase-positive, nonmotile actinomycetes. Generally, branched substrate mycelium (approximately 0.5 µm diameter) is produced on all growth-supported media, and aerial mycelia may also be produced on some media. The vegetative mycelium may fragment into rod-shaped elements with age and sclerotia-like pseudosporangia may also be produced on the substrate mycelium (*Crossiella* ❯ *Fig. 37.16a*). Swelling of the aerial hyphae may be observed, particularly at or near the apical tip (see *Crossiella* ❯ *Fig. 37.16b*). The peptidoglycan is acylated, containing the *meso*-isomer of diaminopimelic acid as the diamino acid and whole-cell hydrolysates contain galactose, mannose, rhamnose, and ribose as the diagnostic sugars. Phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol, diphosphatidylglycerol, and phosphatidylinositol mannosides are observed as the characteristic phospholipids. The predominant menaquinone present is MK-9(H_4) and mycolic acids are not observed. Both species have a fatty-acid profile rich in branched chain and saturated components.

The G+C content of the DNA of both *Crossiella* species is 71.4 (mol%).

Type species is *Crossiella cryophila* NRRL B-16238^T = ATCC 51143^T = DSM 44230^T = IFO 14475^T = NBRC 14475^T(Labeda 2001).

Isolation Procedures

Isolation procedures of all the species are shown in ❯ *Table 37.23*.

Taxonomy, Historical, and Current

The phylogenetically nearest neighboring genera within the order *Pseudonocardiales* are *Streptoalloteichus, Goodfellowiella*, and *Actinoalloteichus* (❯ *Fig. 37.3*), but both *Crossiella* species

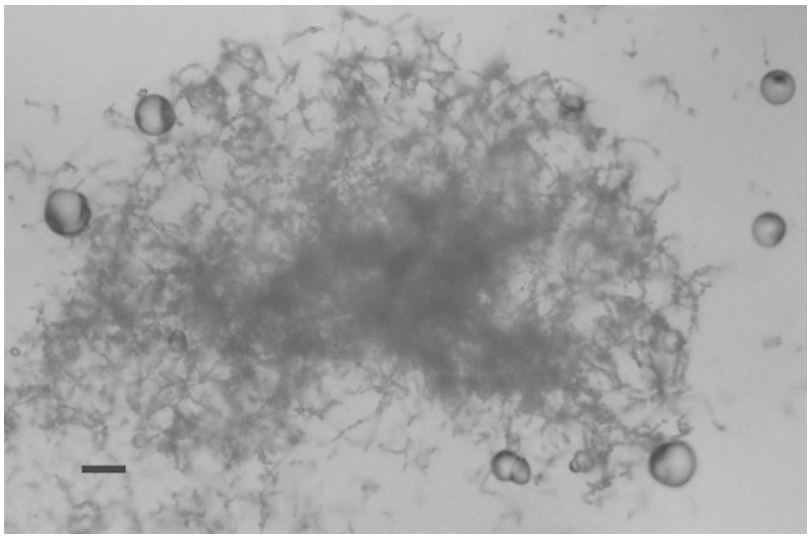

Fig. 37.15
Light micrograph of 21-day growth of *Allokutzneria albata* NRRL B-24461^T on Czapek's sucrose agar. Note the sporangium-like bodies produced on the substrate mycelium. The bar represents 20 μm

are quite distinct from them based on chemotaxonomy and morphology, since neither produces sporangia.

The type species, a novel soil isolate that produced the antibiotic dopsisamine, was originally described by Takahashi et al. (1986) as *Nocardiopsis mutabilis* subspecies *cryophilis*. On the basis of more complete chemotaxonomic investigations, the transfer of *Nocardiopsis mutabilis* and its subspecies to the genus *Saccharothrix* was proposed by two independent studies (Grund and Kroppenstedt 1989; Labeda and Lechevalier 1989). The DNA relatedness of *Nocardiopsis mutabilis* subspecies *cryophilus* to the other valid species of the genus *Saccharothrix* was very low (2–11 %) and a new species named *Saccharothrix cryophila* was proposed. Subsequent phylogenetic analysis of the species of the genera within the suborder *Pseudonocardineae* based on 16S rRNA gene sequences (Labeda and Kroppenstedt 2000) demonstrated that a number of *Saccharothrix* species were misclassified and consideration of the morphological, chemotaxonomic, and phenotypic properties of the type strain of *Saccharothrix cryophilus* resulted in the proposal for the new genus *Crossiella* to accommodate it (Labeda 2001).

The species of the genus *Crossiella* can be readily differentiated by chemotaxonomic and physiological characteristics, as can be seen in *Tables 37.23* and *37.24*, respectively. An interesting observation during the assessment of numerous strains of *Crossiella equi* was that during testing for acid production from carbohydrates, all strains absorbed the pH indicator dye bromothymol blue from the growth medium.

Pathogenicity and Clinical Relevance

Nocardioform placentitis is an emergent disease causing placentitis and abortions in thoroughbred mares, first recognized in Kentucky in 1986 (Donahue and Williams 2000; Giles et al. 1993; Hong et al. 1993). The infection is typically diagnosed because of lesions in the chorionic surface of the placenta and the isolation of Gram-stain-positive branching microorganisms. *Crossiella* biomass may infiltrate up to 30 % of the surface area of a placenta but does not invade the fetus and likely contributes to death or weakening of the unborn foal by competing for nutrients. The placental infection may result in spontaneous abortions, stillbirth, and full-term deliveries of weakened foals, or there may be no apparent effect with the birth of healthy foals. Strains isolated from placental tissues are phylogenetically very near to *Crossiella cryophila*, having 16S rRNA gene sequences that are 98.1 % similar, exhibiting morphological and chemotaxonomic characteristics that are typical for this genus, and therefore the species *Crossiella equi* was proposed for these isolates (Donahue et al. 2002). The manner in which *Crossiella equi* strains infect equine placentas is still unknown and studies have been hampered by the fact that the incidence of *Crossiella equi* equine placentitis is quite variable and numbers of infected placentas observed is relatively low in most years.

Goodfellowiella Labeda, Kroppenstedt, Euzeby, and Tindall 2008, 1048^{VP}

Good.fel.low'i.el.la M.L., fem. n. *Goodfellowiella* named for Michael Goodfellow, a microbiologist at University of Newcastle, in recognition of his contributions to microbial systematics.

Phenotypic Analyses

Goodfellowiella species are aerobic, Gram-stain-positive, nonacid-fast, nonmotile actinomycetes that produce branching

Table 37.17

Chemotaxonomic characteristics and methods of isolation of *Allokutzneria* and *Kutzneria* species

Species	Whole-cell sugars[a]	Phospholipids[b]	Major menaquinone(s)	Major fatty acids (>5 %)	DNA G+C (mol %)	Origin of isolate	Method of isolation
Allokutzneria albata NRRL B-24661^T	Ara, Gal, Man	PE, PME, OH-PE, PI, *lyso*-PME, DPG, PG, *lyso*-PE	MK-9(H_4)	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:1}$, *iso*-$C_{16:0}$, C-$_{17:1}$ (c9)	71.7	Soil sample; Mindanao Island, Philippines	The pulverized dry soil sample was transferred with a nylon sponge to an agar medium, which contained (per 1 L of distilled water) 5.0 g of soluble starch, 5.0 g of glucose, 1.0 g of yeast extract, 2.0 g of NZ-case, 1.0 g of $CaCO_3$, 20 ml of V-8 juice (Campbell Japan Corp.), and 16 g of agar (pH 7.2). The agar medium was supplemented with 4 μg ml^{-1} of ampicillin, 10 μg ml^{-1} of nalidixic acid, and 80 μg ml^{-1} of nystatin. The agar plate was incubated at 43 °C
Allokutzneria multivorans DSM 45532^T	Ara, Gal, Glc, Man, Rha	DPG, PG, PE, OH-PE, PME, PI, PL	MK-9(H_4)	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$ 3-OH, C-$_{17:1}$ ω8*c*	69.1	Soil sample; Nujiang River, Yunnan Province, South-west China	Isolated on glycerol-proline agar (5 g glycerol, 1 g proline, 0.5 g $MgSO_4$. $7H_2O$, 1 g K_2HPO_4, 0.3 g $CaCO_3$, 15 g agar, pH 7.7); incubated at 28 °C for 7 days
Kutzneria albida DSM 43870^T	Rha	PE, OH-PE, DPG, PI	MK-9(H_4)	*iso*-$C_{16:0}$, $C_{16:0}$, $C_{16:0}$ 10-methyl, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{16:0}$ 2-OH, C-$_{17:0}$ 10-methyl	70.3	nr	nr
Kutzneria buriramensis NBRC 107931^T	Gal, Glc, Man, Rha, Rib	PE, DPG, PME, OH-PE, PI	MK-9(H_4)	*iso*-$C_{16:0}$, $C_{16:0}$ 10 methyl, *iso*-$C_{16:0}$ 2-OH	72	Forest soil, Thailand	Isolated on humic acids- salts vitamin agar (Nonomura and Hayakawa, 1988), supplemented with nalidixic acid (25 mg l^{-1}), cycloheximide (50 mg l^{-1}) and terbinafin (1 mg l^{-1})

Table 37.17 (continued)

Species	Whole-cell sugars[a]	Phospholipids[b]	Major menaquinone(s)	Major fatty acids (>5 %)	DNA G+C (mol %)	Origin of isolate	Method of isolation
Kutzneria kofuensis DSM 43851^T	Gal, Rha	PE, OH-PE, DPG, PI	MK-9(H_4)	*iso*-$C_{16:0}$, $C_{16:0}$, $C_{16:0}$ 10 methyl, *iso*-$C_{17:0}$, anteiso-$C_{17:0}$, *iso*-$C_{16:0}$ 2-OH, $C_{17:0}$ 10-methyl	70.3	Kofu district, Japan	nr
Kutzneria viridogrisea DSM43850^T	Gal, Rha	PE, OH-PE, DPG, PI	MK-9(H_4)	*iso*-$C_{16:0}$, $C_{16:0}$, *iso*-$C_{17:0}$ 10-methyl, anteiso-$C_{17:0}$, *iso*-$C_{16:0}$ 2-OH, $C_{17:0}$ 10 methyl	70.3	nr	nr

Data from: Cao et al. (2013), Labeda and Kroppenstedt (2008), Tomita et al. (1993), Stackebrandt et al. (1994) and Suriyachadkun et al. (2013)
Symbols: *nr* not reported
[a]*DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *OH-PE* hydroxyphosphatidylethanolamine, *PI* phosphatidylinositol, *PME* phosphatidylmethylethanolamine
[b]*Ara* arabinose, *Gal* galactose, *Rha* rhamnose, *Rib* ribose

substrate mycelium (approximately 0.5 μm in diameter). Aerial mycelia are also produced on some growth media and the substrate mycelia fragment into coccoid conidia. Typical of all genera within the *Pseudonocardiales*, the cell wall contains *meso*-diaminopimelic acid as the diamino acid, and whole-cell hydrolysates contain galactose and ribose. As can be seen in *Pseudonocardiales* Chemotaxonomy Table (*Table 37.1*), the polar lipid content includes diphosphatidylglycerol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, and traces of phosphatidylinositol and phosphatidylinositol mannosides. The predominant menaquinones are MK-9(H_4) and MK-10(H_4). The fatty-acid profile of *Goodfellowiella* is rich in branched chain and saturated components including 10-methyl branched heptadecanoic acid and anteiso-branched 2-hydroxy fatty acids. This single species genus is to be phylogenetically nearest to the genus *Actinoalloteichus* as can be seen in *Figs. 37.1*, *37.2*, *37.3*, *37.4*, and *37.5*.

The G+C content of the DNA of the type species is 68.2 (mol%).

The genus only contains a single species, *Goodfellowiella coeruleoviolacea* NRRL B-24058^T = DSM 43935^T = INA 3564^T = JCM9110^T = NBRC14988^T = VKM Ac-1083^T (Preobrazhenskaya and Terekhova 1987; Labeda and Kroppenstedt 2006; Labeda et al. 2008), whose characteristics are shown in *Tables 37.23* and *37.24*.

Taxonomy, Historical, and Current

Preobrazhenskaya and Terekhova (1987) and Preobrazhenskaya et al. (1976) first described *Goodfellowiella coeruleoviolacea* as *Actinomadura coeruleoviolacea* that was subsequently transferred by Kroppenstedt et al. (1990, 1991)into the genus *Saccharothrix* as *Saccharothrix coeruleoviolacea*. During a phylogenetic evaluation of the species within the genus *Saccharothrix* based on almost complete 16S rRNA sequences (Labeda and Kroppenstedt 2000), it was noted that the type strain of *Saccharothrix coeruleoviolacea* was not near to the other species of *Saccharothrix* and likely represented a new genus within the suborder *Pseudonocardineae*. The strain was subsequently shown to be chemotaxonomically distinct from *Saccharothrix* as well as other genera within the suborder, and the new genus *Goodfellowia* was proposed by Labeda and Kroppenstedt (2006). This genus name was afterward discovered to be taxonomically illegitimate because it had been used previously in 1903 as the name of an avian genus. This situation was corrected by the publication of a proposal to emend the genus name to *Goodfellowiella* (Preobrazhenskaya and Terekhova 1987; Labeda et al. 2008).

Goodfellowiella coeruleoviolacea is most closely related to the genus *Actinoalloteichus* (*Fig. 37.3*) but can be distinguished from this genus based on chemotaxonomic properties (*Table 37.1*). The chemotaxonomic properties of

Table 37.18

Morphological and physiological characteristics of *Allokutzneria* and *Kutzneria* species

	Allokutzneria albata NRRL B-24661^T	*Allokutzneria multivorans* DSM 45532^T	*Kutzneria albida* DSM 43870^T	*Kutzneria buriramensis* NBRC 107931^T	*Kutzneria kofuensis* DSM 43851^T	*Kutzneria viridogrisea* DSM43850^T
Presence of *lyo*-phospholipids	+	−	−	−	−	−
Sporangium						
11–15 μm	+	nr	(+)	+	+	−
16–20 μm	+	nr	(+)	+	+	−
21–30 μm	−	nr	+	−	−	+
31–50 μm	−	nr	−	−	−	+
Sporangiospores						
Short (10 μm)		nr	−	−	+	−
Long (50 μm)		nr	+	+	+	+
Spores						
Spherical to ovoid	−	−	+	+	−	+
Rods	+	+	−	+	+	−
Color of spore mass	Yellow to yellowish white	nr	White	Pale yellow; white	Greenish-gray	Greenish-gray
Color of substrate mycelium	Yellow to light brown	White	Yellowish-brown to brown	Moderate yellow; greenish white	Yellowish-brown to brown	Yellowish-brown to brown
Production of:						
Soluble pigments	+	−	−	−	−	−
Nitrate reductase	−	−	+	+	−	+
Hydrolysis of:						
Cellulose	nr	−	−	nr	nr	−
Gelatin	+	−	−	−	+	+
Milk (peptonization)	nr	−	+	+	w	+
Starch	+	−	−	−	+	+
Growth as sole carbon source:						
Arabinose	+	+	+	−	+	w
Dextrin	+	+	−	nr	nr	nr
Dulcitol	−	+	nr	nr	nr	−
Fructose	+	+	+	−	nr	+
Galactose	+	+	nr	+	nr	+
Glucose	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+
Inositol	+	+	+	nr	+	w
Inulin	nr	nr	nr	nr	nr	−
Lactose	±	+	+	−	nr	w
Maltose	+	+	nr	nr	nr	+
Mannitol	+	+	+	+	nr	+
Mannose	+	+	+	nr	nr	+
Raffinose	−	+	+	+	nr	+
Rhamnose	+	+	+	−	w	w
Salicin	+	nr	−	−	nr	−
Sorbitol	−	+	nr	nr	nr	+

Table 37.18 (continued)

	Allokutzneria albata NRRL B-24661^T	*Allokutzneria multivorans* DSM 45532^T	*Kutzneria albida* DSM 43870^T	*Kutzneria buriramensis* NBRC 107931^T	*Kutzneria kofuensis* DSM 43851^T	*Kutzneria viridogrisea* DSM43850^T
Starch	+	nr	nr	nr	nr	+
Sucrose	+	−	+	nr	nr	+
Xylose	+	+	+	+	nr	w
Assimilation of:						
Malate	nr	nr	+	nr	nr	+
Growth at pH:	nr	6 to 9	nr	nr	nr	4 to 9
Growth at:						
10 °C	−	+	nr	nr	nr	−
27 °C	+	+	+	+	+	+
37 °C	+	+	nr	+	+	+
42 °C	+	−	nr	nr	+	nr
50 °C	−	−	nr	nr	±	±

Data from: Cao et al. (2013), Labeda and Kroppenstedt (2008), Tomita et al. (1993), Stackebrandt et al. (1994) and Suriyachadkun et al. (2013)
[a]Symbols: + positive, (+) sometimes observed, ± doubtful response, *w* weak positive reaction, − negative, *nr* not reported

Goodfellowiella distinguish it from *Actinoalloteichus* by the lack of mannose in whole-cell hydrolysate sugar pattern as well as the lack of inositol-containing phospholipids and the presence of hydroxylated phosphatidylethanolamine in its polar lipid profiles. Substantial quantities of menaquinone MK-10(H_4) are present and this is distinct from other taxa within the suborder. Scanning electron microscopic observations of colony growth on several different media have not revealed the presence of sporangia, and the substrate mycelium appears to fragment into coccoidal rod elements (*Goodfellowia* ❯ *Fig. 37.17*). Spore chains typical of those observed in *Actinoalloteichus* species were not found.

Haloechinothrix Tang, Wang, Zhang, Lee, Lou, Kim, and Li 2254^{VP}

Ha.lo.e.chi.no'thrix. Gr. n. hals, halos salt; Gr. n. echinos hedgehog; Gr. fem. n. thrix hair; N.L. fem. n. Haloechinothrix halophilic, hedgehog-like filament, referring to halophilic filamentous actinobacterium with spiny aerial mycelium.

Phenotypic Analyses

Haloechinothrix species are strictly aerobic, Gram-stain-positive, nonacid-fast, moderately halophilic, filamentous actinobacteria. The substrate mycelium is well developed and fragments into rodlike elements, while the aerial mycelium is "hedgehog-like" or spiny and does not form spores at maturity. As shown in ❯ *Table 37.25*, the aerial mycelium is white and the substrate mycelium yellow–white. The cell wall contains *meso*-diaminopimelic acid as the diamino acid and whole-cell sugars consist of glucose, glucosamine, mannose, and an unknown sugar. The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, and an unknown phospholipid. The predominant menaquinone is MK-8(H_4) and the major fatty acid is iso-$C_{16:0}$ (❯ *Table 37.26*).

The mol% G+C content of the DNA of the sole species is 68.1 (mol%).

The type species is *Haloechinothrix alba* YIM 93221^T = DSM 45207^T; CCTCC AB 208140^T (Tang et al. 2010b).

Isolation Procedures

As shown in ❯ *Table 37.26*, the strain was isolated from a soil sample collected from Qijiaojing Lake, a salt lake in Xinjiang Province, China, after 3 weeks of incubation at 37 °C on cellulose–casein multi-salts medium (Tang et al. 2010b).

Taxonomy, Historical, and Current

Phylogenetic analysis based on 16S rRNA gene sequences indicated that *Haloechinothrix alba* YIM 93221^T formed a distinct lineage within the order *Pseudonocardiales* and has the highest sequence similarity to a *Saccharopolyspora* species. The neighbor-joining algorithm however places the genera *Haloechinothrix* and *Sciscionella* in a distinct clade supported by a high bootstrap value (66 %).

The type strain is a halophilic actinobacterium that cannot grow without NaCl, whereas members of the genera *Sciscionella, Thermocrispum*, and *Saccharopolyspora* (except for *Saccharopolyspora halophila* and *Saccharopolyspora qijiaojingensis*) are

Table 37.19

Physiological characteristics differentiating the species of the genus *Amycolatopsis*

Species	Characteristic															
	Acid production from:															
	Adonitol	Arabinose	Cellobiose	Dextrin	meso-Erythritol	Fructose	Galactose	Glucose	Glycerol	myo- Inositol	Lactose	Maltose	Mannitol	Mannose	Melezitose	Melibiose
A. orientalis	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
A. alba	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+
A. albidoflavus	+	+	+	nr	+	+	+	–	nr	+	+	–	–	+	–	–
A. australiensis	–	+	+	v	nr	+	+	+	+	+	+	v	+	+	–	–
A. azurea	+	+	+	nr	+	+	+	nr	nr	+	+	nr	+	nr	+	nr
A. balhimycina	nr	+	nr	nr	nr	+	nr	nr	nr	+	nr	nr	+	nr	nr	+
A. bartoniae	nr	nr	nr	nr	nr	–	–	nr	nr	nr	–	+	nr	nr	nr	–
A. benzoatilytica	+	+	+	+	+	+	+	+	nr	+	+	–	+	+	nr	nr
A. bullii	nr	nr	nr	nr	nr	+	+	nr	nr	nr	+	+	nr	nr	nr	+
A. cihanbeyliensis	+	+	+	+	nr	+	+	nr	nr	+	+	+	+	+	nr	nr
A. circi	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
A. coloradensis	–	–	+	+	–	+	+	nr	nr	+	–	+	+	nr	–	–
A. dongchuanensis	nr	nr	nr	nr	nr	+	nr	+	nr	nr	nr	nr	nr	+	nr	nr
A. decaplanina	nr	+	nr	nr	nr	+	nr	+	nr	–	nr	nr	+	nr	nr	nr
A. echigonensis	+[w]	nr	nr	nr	+[w]	nr	+	+[w]	nr	+	+	nr	–	–	nr	nr
A. endophytica	nr	+	–	nr	nr	+	+	+	nr	nr	nr	nr	nr	+	nr	nr
A. equina	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
A. eurytherma	+	+	+	+[w]	+	+	+	nr	nr	+	+[w]	–	+	nr	–	–
A. granulosa	nr	+	nr	+	+	nr	+	nr	nr	–	+	–	–	nr	nr	–
A. halophila	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
A. halotolerans	+	–	+	nr	–	+	+	–	+	+	–	–	+	+	–	+
A. helveola	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
A. hippodromi	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
A. japonica	+	+	+	+	+	+	+	nr	nr	+	+	+	+	nr	–	+
A. jejuensis	+	+	–	nr	–	+	+	–	–	+	–	–	+	+	–	–
A. kentuckyensis	+	+	+	+	–	+	+	+	+	+	+	+	–	+	–	+
A. keratiniphila subsp. *keratiniphila*	+	+	+	nr	nr	+	+	nr	+	+	+	nr	+	nr	nr	+
A. keratiniphila subsp. *nogabecina*	nr	+	nr	nr	nr	+	nr	nr	nr	+	nr	nr	+	nr	nr	nr
A. lexingtonensis	+	+	+	+	+[w]	+	+	+	+	+	+	+	+[w]	+	–	+
A. lurida	+	+	+	+	+	+	+	nr	+	+	+	+	+	nr	nr	–
A. magusensis	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
A. marina	nr	–	+	nr	nr	+	+	+	nr	+	–	+	+	nr	nr	nr
A. mediterranei	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+
A. methanolica	+	–	+	–	+	+	+	nr	nr	–	–	nr	+	nr	+	–
A. minnesotensis	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–

								Decomposition of:							Production of:			Growth in/at:		
Methyl-D-glucoside	Raffinose	Rhamnose	Salicin	D(-)- sorbitol	Sucrose	Trehalose	Xylose	Allantoin	Casein	Aesculin	Gelatin	Hypoxanthine	Tyrosine	Xanthine	Amylase	Nitrate reductase	Urease	5 % NaCl	10 °C	45 °C
+	−	+	+	−	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	−
+	+	−	+	−	−	+	+	+	+	+	+	+	nr	+	+	−	+	+	−	−
−	−	nr	−	−	+	+	+	nr	+	+	+	+	+	+	+	+	+[w]	+	+	−
nr	v	nr	nr	−	v	v	+	v	+	+	+	v	nr	−	+	+	−	−	v	+
nr	+	nr	nr	nr	nr	nr	+	−	+	+	+	−	nr	−	−	+	+	+	+	−
nr	+	+	nr	−	+	nr	−	nr	nr	nr	nr	nr	nr	−	−	−	+	nr	nr	−
nr	+	nr	nr	−	−	nr	+	nr	+	nr	nr	+	nr	nr	nr	−	−	−	−	−
nr	nr	+	nr	−	−	+	+	+	−	+	−	−	nr	−	−	nr	+	nr	nr	nr
nr	+	nr	nr	−	+	nr	+	nr	+	nr	nr	−	nr	nr	nr	−	−	−	−	−
nr	nr	nr	nr	+	−	nr	+	nr	−	nr	nr	+	+	+	nr	−	+	+	−	−
nr	nr	nr	nr	nr	nr	nr	nr	+[w]	+	nr	+	+	+	+[w]	nr	nr	nr	+	nr	−
+	−	−	+	−	+	+	+	−	+	+	+	+	+	−	+	+	−	+	+	−
nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	+	+	nr	nr	+	+	+	+
nr	−	−	nr	nr	−	nr	−	nr	nr	nr	+	nr	nr	nr	nr	nr	+	nr	nr	nr
nr	nr	+	nr	+	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	+	+	nr	+	+
nr	−	+	nr	nr	+	−	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	+
nr	nr	nr	nr	nr	nr	nr	nr	+	+	+	+	+	+	−	nr	nr	nr	+	nr	−
−	−	+	−	+	−	+	+	+	+	−	+	+[w]	nr	+	−	+	+	+	−	+
+	+	nr	nr	−	+	+	+	−	nr	nr	+	+	−	−	nr	nr	nr	−	−	+
nr	nr	nr	nr	nr	nr	nr	nr	nr	−	nr	nr	nr	nr	nr	nr	nr	nr	+	−	+
−	−	−	−	−	+	−	−	nr	+	nr	+	+	+	+	−	+	+	+	+	−
nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	−	−	nr	nr	nr	nr	nr	nr	+	−	−
nr	nr	nr	nr	nr	nr	nr	nr	+[w]	+	nr	+	+	+	+[w]	nr	nr	nr	+	nr	−
+	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	−	−	+	+	+
−	−	−	−	−	+	−	−	nr	+	nr	+	+	+	−	−	+	+[w]	−	+	−
+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	−	+	+	−	+
nr	+	+	nr	nr	+	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	+	+	+	+	−
nr	−	+	nr	nr	+	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	+	−	nr	nr	nr
+	+	+	+	−	+	−	+	−	+	+	+	+	+	−	−	+	+	+	nr	+
+	−	−	+	−	+	+	+	nr	+	+	+	+	+	+	−	+	+	−	+	−
nr	nr	nr	nr	nr	nr	nr	nr	+	−	+	nr	nr	+	−	nr	−	+	+	−	−
nr	−	+	nr	−	−	+	+	nr	−	nr	+	+	−	nr	+[w]	−	−	+	+	+
+	+	+	+	+[w]	+	+	+	nr	+	+	+	+	+	−	−	+	+	−	+	−
−	−	+	+[w]	+	+	+	+	−	+	+[w]	+	+	+	−	−	+	−	nr	−	+
−	−	+	+	+	+	+	+	nr	+	nr	+	+	+	+	−	+	+	+	+	−

Table 37.19 (continued)

Species	Characteristic: Acid production from:															
	Adonitol	Arabinose	Cellobiose	Dextrin	meso-Erythritol	Fructose	Galactose	Glucose	Glycerol	myo- Inositol	Lactose	Maltose	Mannitol	Mannose	Melezitose	Melibiose
A. nigrescens	nr	+	nr	nr	nr	+	nr	+	nr	+	nr	nr	+	nr	nr	nr
A. niigatensis	+^{w}	nr	nr	nr	+^{w}	nr	+	+^{w}	nr	–	–	nr	+	–	nr	nr
A. palatopharyngis	+	+	–	–	+	+	+	nr	nr	+	–	–	–	nr	–	–
A. pigmentata	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
A. plumensis	+	+	+	nr	nr	+	–	nr	nr	nr	–	+	+	nr	nr	–
A. pretoriensis	–	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+
A. regifaucium	–	+	+	–	–	nr	+	nr	nr	+	–	nr	nr	+	–	–
A. rifamycinica	+	+	+	nr	nr	+	+	nr	nr	+	–	–	–	nr	–	nr
A. ruanii	nr	+	nr	+	+	nr	+	nr	nr	+	+	+	+	nr	nr	–
A. rubida	+	+	+	–	+	+	+	nr	nr	+	–	–	+	nr	–	–
A. saalfeldensis	nr	+	nr	nr	nr	+	nr	+	nr	+	nr	nr	+	nr	–	nr
A. sacchari	+	+	+	+	+	+	+	nr	nr	–	+	+	+	nr	–	–
A. salitolerans	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
A. samaneae	+	–	+	nr	nr	+	+	+	+	+	+	–	+	+	nr	–
A. sulphurea	–	–	–	+	–	nr	+	nr	nr	+	–	+	+	nr	nr	–
A. taiwanensis	nr	+	nr	nr	nr	–	nr	+	nr	nr	nr	nr	nr	nr	–	nr
A. thailandensis	nr	–	+	nr	nr	+	+	+	nr	nr	+	+	+	+	nr	+
A. thermalba	nr	+	nr	+	+	nr	+	nr	nr	+	+	+	+	nr	nr	–
A. thermoflava	+	+	+	–	+	+	+	nr	nr	–	+	–	+	nr	–	+
A. thermophila	nr	–	nr	–	–	nr	–	nr	nr	–	–	nr	–	nr	nr	–
A. tolypomycina	–	+	+	nr	nr	+	+	nr	nr	+	–	nr	+	nr	nr	–
A. tucumanensis	nr	nr	nr	nr	nr	+^{w}	nr	nr	nr	–	nr	nr	nr	nr	nr	nr
A. ultiminotia	–	–	+	nr	–	–	+	+	+	+	+	+	+	+	–	–
A. umgeniensis	+^{w}	+	–	nr	+^{w}	+	+	nr	+	+^{w}	+	+	+^{w}	nr	–	+
A. vancoresmycina	–	+	+	nr	nr	+	+	nr	nr	+	–	nr	+	nr	nr	–
A. viridis	nr	+	nr	–	–	nr	+	nr	nr	–	+	nr	–	nr	nr	–
A. xylanica	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr

Symbols: + positive, ^{w} weakly positive,– negative, *v* variable, *nr* not reported

								Decomposition of:							Production of:			Growth in/at:		
Methyl-D-glucoside	Raffinose	Rhamnose	Salicin	D(-)- sorbitol	Sucrose	Trehalose	Xylose	Allantoin	Casein	Aesculin	Gelatin	Hypoxanthine	Tyrosine	Xanthine	Amylase	Nitrate reductase	Urease	5 % NaCl	10 °C	45 °C
nr	+	+^{w}	nr	nr	–	nr	+	nr	+	+	nr	+	+	+	–	+	+	+	–	–
nr	nr	+	nr	+^{w}	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	–	+	nr	+	+
–	+	–	–	–	–	+	+	–	+	–	+	+	+	+	–	+	+^{w}	+	+	–
nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	–	nr	nr	nr	nr	nr	nr	–	–	–
nr	–	–	nr	–	–	+	–	nr	+	nr	+	–	+	–	+	nr	+	–	–	–
+	+	+	+	+^{w}	+	+	+	–	+	+	+	+	+^{w}	–	–	–	+^{w}	+	nr	–
nr	–	nr	nr	–	–	nr	+	–	nr	+	nr	+	+	+	+	+	–	+	+	–
nr	–	–	nr	–	nr	nr	+	–	+	+	+	+	nr	–	–	nr	+	+^{w}	+	–
+	+	nr	nr	+	+	–	+	–	nr	nr	+	+	–	–	nr	nr	nr	nr	–	+
–	–	+	+	–	+	+	+	–	+	+	+^{w}	+	nr	+	–	+	+	+	+	–
nr	–	+^{w}	nr	nr	+	nr	+	nr	+	+	+	–	+	+	–	–	+	–	+^{w}	–
+	nr	+	+	–	+	+	+	+	+	+	+	–	+	+	–	+	+	+	–	+
nr	nr	nr	nr	nr	nr	nr	nr	nr	–	+	nr	nr	nr	nr	nr	nr	nr	+	–	+
nr	–	–	nr	–	–	+	–	nr	nr	+	+	+	+	+	nr	nr	+	+	+	–
–	–	–	–	–	+	+	–	nr	+	+	+	+	+	–	–	+	–	+	–	–
nr	nr	nr	–	nr	+	nr	nr	nr	+	+	+	+^{w}	nr	–	–	+	–	–	–	–
nr	+	–	nr	nr	nr	+	+	nr	+	+	nr	nr	+	nr	nr	nr	+	+	+	–
+	+	nr	nr	+	–	+	+	+	nr	nr	+	–	–	–	nr	nr	nr	nr	–	+
+	+	–	+	+	–	+	+	+	+	+	–	+	nr	+	–	+	+	+	–	+
–	–	nr	nr	–	–	–	–	nr	+	nr	+	–	–	+	nr	nr	–	nr	+	+
nr	–	+	nr	–	+	nr	–	nr	+	nr	+	nr	nr	+	–	+	+	+	nr	+
nr	–	+^{w}	nr	nr	–	+^{w}	+^{w}	nr	+	+	nr	nr	nr	nr	nr	–	+	nr	nr	nr
–	–	+	–	–	–	–	+	nr	+	+	+	+	+	+	–	–	–	+	+	–
+	+	–	+^{w}	nr	–	+^{w}	+	+^{w}	nr	+	+	+	+	+	nr	+	nr	nr	nr	–
nr	+	+	nr	–	+	nr	–	nr	–	nr	–	nr	nr	–	–	–	+	+	nr	–
–	–	nr	nr	–	–	+	–	nr	–	nr	–	+	+	+	nr	nr	–		+	+
nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	+	nr	+	+	+	+

Table 37.20

Chemotaxonomic characteristics of *Amycolatopsis* sp.

Species	Major fatty acids (>10 %)	Phospholipids[a]	Major menaquinones	Whole-cell sugars[b]	DNA G+C content (mol %)
A. orientalis	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:0}$	PE	MK-9(H_2), MK-9(H_4)	Gal, Ara	66
A. alba	*iso*-$C_{15:0}$, $C_{17:0}$	PE	MK-9(H_4)	Gal, Ara	nr
A. albidoflavus	*iso*-$C_{16:0}$, $C_{16:0}$, *iso*-$C_{14:0}$	PE, PI, PME, DPG	MK-9(H_4)	Gal, Ara	68.5
A. australiensis	nr	DPG, PE, PG, PI, PME	MK-9(H_4), MK-8(H_2)	Gal, Ara	nr
A. azurea	*iso*-$C_{15:0}$, $C_{17:0}$, *iso*-$C_{16:0}$, $C_{16:0}$	DPG, PE, PG, PI, PIM	MK-9(H_4), MK-9(H_2)	Gal, Ara	66
A. balhimycina	*iso*-$C_{16:0}$	DPG, PE, PG, PI	MK-9(H_4)	Gal, Ara	nr
A. bartoniae	*iso*-$C_{16:0}$, $C_{16:0}$, ante*iso*-$C_{17:0}$, ante*iso*-$C_{18:0}$, $C_{18:2}$ ω6, 9c	DPG, PE, PG, PI, PME	MK-9(H_4), MK-8(H_4)	Gal, Ara	nr
A. benzoatilytica	*iso*-$C_{14:0}$, $C_{15:0}$, $C_{15:1}$ ω6*c*, *iso*-$C_{16:0}$, $C_{17:0}$, $C_{17:1}$ ω8*c*	DPG, PE, PG, PI, PME	MK-9(H_4)	Gal, Ara	nr
A. bullii	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$, ante*iso*-$C_{17:0}$	DPG, PE, PG, PI, PME	MK-9(H_2), MK-9(H_4)	Gal, Ara	nr
A. cihanbeyliensis	*iso*-$C_{16:0}$ 2-OH, *iso*-$C_{16:0}$	DPG, PE, PI, PME, PL, AL	MK-9(H_4)	Gal, Ara, Glc	68.8
A. circi	$C_{16:0}$, $C_{17:0}$, $C_{17:1}$ ω6c	PE, PI, PG, PIM	MK-9(H_4)	Gal, Ara, Glc	nr
A. coloradensis	$C_{15:0}$, $C_{15:1}$ ω6*c*, *iso*-$C_{16:0}$, $C_{17:0}$	PE	MK-9(H_2), MK-9(H_4)	Gal, Ara	66
A. decaplanina	*iso*-$C_{15:0}$, $C_{17:0}$, *iso*-$C_{16:0}$	DPG, PE, PG, PI, PIM, OH-PE	MK-9(H_4), MK-8(H_4)	Gal, Ara	68.5
A. dongchuanensis	ante*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{15:0}$	DPG, PG, PE, OH-PE, PI, PIM, PL	MK-9(H_4)	Gal, Ara, Glc	nr
A. echigonensis	ante*iso*-$C_{15:0}$, *iso*-$C_{16:0}$	nr	MK-9(H_4)	Gal, Ara	72.4
A. endophytica	*iso*-$C_{16:0}$	DPG, PG, PE, OH-PE, PI, PL	MK-9(H_4)	Gal, Ara	73.3
A. equina	$C_{16:0}$, $C_{17:0}$, $C_{17:1}$ω6c	PE, PI, PG, PIM	MK-9(H_4)	Gal, Ara, Glc	nr
A. eurytherma	*iso*-$C_{16:0}$, $C_{16:0}$	DPG, PE, PG, PI, PIM, PME	MK-9(H_4)	Gal, Ara	72–74
A. granulosa	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{16:0}$	DPG, PE, PME, PG, PI	MK-9(H_4)	Gal, Ara	nr
A. halophila	*iso*-$C_{16:0}$, $C_{16:0}$, $C_{16:1}$ω7c, *iso*-$C_{15:0}$ 2-OH	DPG, PE, OH-PE, PI, PIM, PL	MK-8(H_4)	Glu, Gal	66.1
A. halotolerans	*iso*-$C_{15:0}$, $C_{17:0}$, *iso*-$C_{16:0}$, $C_{16:0}$, $C_{18:0}$	DPG, PE, PG, PI	MK-9(H_4)	Gal, Ara	72.5
A. helveola	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$ 2-OH	PE	MK-9(H_4)	Gal, Ara, Man, Glc	68.9
A. hippodromi	$C_{16:0}$, $C_{17:0}$, $C_{17:1}$ ω6c	PE, PI, PG, PIM	MK-9(H_4)	Gal, Ara, Glc	nr
A. japonica	*iso*-$C_{16:0}$, $C_{16:0}$, *iso*-$C_{15:0}$	DPG, PE, PG, PI, PIM	MK-9(H_4)	Gal, Ara	69.5
A. jejuensis	*iso*-$C_{16:0}$, $C_{16:0}$, *iso*-$C_{15:0}$, $C_{18:0}$	DPG, PE, PG, PI, PIM	MK-9(H_4)	Gal, Ara	71.7
A. kentuckyensis	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, $C_{17:1}$ *cis* 9	PE, PME	MK-9(H_4), MK-9(H_2)	Gal, Ara	nr
A. keratiniphila	*iso*-$C_{14:0}$, *iso*-$C_{16:0}$, $C_{17:1}$	PI, PE, OH-PE, DPG	MK-9(H_4)	Gal, Ara	nr
A. keratiniphila subsp. *keratiniphila*	*iso*-$C_{14:0}$, *iso*-$C_{16:0}$, $C_{17:1}$	PI, PE, OH-PE, DPG	MK-9(H_4)	Gal, Ara	nr
A. keratiniphila subsp. *nogabecina*	*iso*-$C_{14:0}$, *iso*-$C_{16:0}$, $C_{17:0}$	PI, PE, OH-PE, DPG	MK-9(H_4)	Gal, Ara	nr
A. lexingtonensis	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$	PE, PME	MK-9(H_4), MK-9(H_2)	Gal, Ara	nr
A. lurida	*iso*-$C_{16:0}$, $C_{17:0}$, *iso*-$C_{15:0}$	DPG, PE, PG, PI, PIM	MK-9(H_4), MK-9(H_2)	Gal, Ara	67
A. magusensis	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{14:0}$	PE, PG, PI, OH-PE	MK-9(H_4)	Gal, Ara, Gls, Rib	70.8
A. marina	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$2-OH	DPG, PE, PG, PI, PIM, PME	MK-9(H_4)	Gal, Ara	70.1
A. mediterranei	*iso*-$C_{16:0}$	PE, PME	MK-9(H_4), MK-9(H_6)	Gal, Ara	67–69

Table 37.20 (continued)

Species	Major fatty acids (>10 %)	Phospholipids[a]	Major menaquinones	Whole-cell sugars[b]	DNA G+C content (mol %)
A. methanolica	*iso*-$C_{16:0}$, $C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{16:1}$	DPG, PE, PIM, PME	MK-9(H_4), MK-9(H_2)	Gal, Ara	nr
A. minnesotensis	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, $C_{17:0}$	PME, DPG, PI	MK-9(H_4)	Gal, Ara	69.5
A. nigrescens	*iso*-$C_{16:0}$	PG, OH-PE, DPG, PI, PS	MK-11(H_4), MK-12(H_4)	Gal, Ara	nr
A. niigatensis	*iso*-$C_{16:0}$	nr	MK-9(H_4)	Gal, Ara	72.4
A. palatopharyngis	*iso*-$C_{16:0}$, $C_{16:0}$, ante*iso*-$C_{17:0}$	PE, DPG, PI	MK-9(H_4)	Gal, Ara	65.8
A. pigmentata	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$2-OH, $C_{17:0}$, $C_{17:1}\omega9c$	PE	MK-9(H_4)	Gal, Ara, Man, Glc	67.2
A. plumensis	nr	nr	MK-9(H_4)	Gal, Ara	nr
A. pretoriensis	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$ 2-OH	PE, PME	MK-9(H_4), MK-9(H_2)	Gal, Ara	nr
A. regifaucium	nr	DPG, PE, PG, PI, PIM, PME	MK-9(H_4), MK-9(H_6)	Gal, Ara	nr
A. rifamycinica	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{18:1}$	PE, PG, PI	nr	nr	nr
A. ruanii	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{16:0}$	DPG, PE, PME, PG, PI	MK-9(H_4)	Gal, Ara	nr
A. rubida	*iso*-$C_{14:0}$, $C_{15:0}$, $C_{15:1}\omega6c$, *iso*-$C_{16:0}$, $C_{17:0}$	PIM, PME, DPG, PE	MK-9(H_4)	Gal, Ara	67.4
A. saalfeldensis	*iso*-$C_{16:0}$	DPG, PE, PG, PS, OH-PE	MK-9(H_4)	Gal, Ara	nr
A. sacchari	ante*iso*-$C_{17:0}$, $C_{16:0}$	DPG, PE, PG, PI	MK-9(H_4)	Gal, Ara	nr
A. salitolerans	*iso*-$C_{16:0}$, $C_{16:0}$	DPG, PE, PME, GluNu	MK-8(H_4)	Rib, Glc, Gal	66.4
A. samaneae	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, iso $C_{16:0}$2-OH, *iso*-$C_{17:0}$	DPG, OH-PE, PE, PG, PI, PIM	MK-9(H_4)	Gal, Ara	71.7
A. sulphurea	$C_{16:0}$, *iso*-$C_{16:0}$	PE, PME	MK-9(H_4), MK-9(H_2)	Gal, Ara	67
A. taiwanensis	*iso*-$C_{16:0}$, $C_{17:1}$	PE	MK-9(H_4)	Gal, Ara	68.9
A. thailandensis	*iso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$	DPG, PG, PE, PI, PME	MK-9(H_4)	Gal, Ara	67
A. thermalba	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{16:0}$	DPG, PE, PME, PG, PI	MK-9(H_2)	Gal, Ara	nr
A. thermoflava	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-a-$C_{16:0}$OH	nr	MK-9(H_4)	Gal, Ara	75
A. thermophila	nr	DPG, PE, PME, PG, PI	MK-9(H_4)	Gal, Ara	nr
A. tolypomycina	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{15:0}$	DPG, PE, PG, PI, OH-PE	MK-9(H_4)	Gal, Ara	nr
A. tucumanensis	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{16:0}$	DPG, PI, OH-PE	MK-9(H_4)	Gal, Ara	nr
A. ultiminotia	$C_{17:0}$, $C_{15:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$	PME, PL	MK-9(H_4)	Gal, Ara	67.5
A. umgeniensis	$C_{17:0}$, $C_{15:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$, ante*iso*-$C_{17:0}$	DPG, PE, PME, PI, PIM, PL,	MK-9(H_4)	Gal, Ara, Glc	nr
A. vancoresmycina	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$ 2-OH	PE, OH-PE, DPG, PI	MK-9(H_4)	Gal, Ara	nr
A. viridis	nr	DPG, PE, PME, PG, PI	MK-9(H_4)	Gal, Ara	nr
A. xylanica	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{14:0}$, $C_{16:1}$*cis*9, $C_{17:1}$*cis*9	PE, PME, PC	MK-9(H_4)	Gal, Ara	65.9

Symbols: *nr* not reported

PL unknown phospholipids, *PME* phosphatidylmethylethanolamine, *OH- PE* hydroxyl phosphatidylethanolamine, *PIM* phosphatidylinositol mannosides

PL unknown phospholipids, *GluNu* phospholipids containing glucosamine, *AL* unknown aminolipid

[a]PC phosphatidylcholine, *PE* phosphatidylethanolamine, *PS* phosphatidylserine, *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol

[b]*Gal* galactose, *Ara* arabinose, *Glc* glucose, *Rib* ribose, *Man* mannose

not halophilic. *Haloechinothrix* has MK-8(H_4) as the predominant menaquinone and phospholipids containing phosphatidylglycerol (no phosphatidylcholine), whereas the genera *Sciscionella*, *Thermocrispum*, *Yuhushiella*, and *Saccharopolyspora* have arabinose and/or galactose as the diagnostic sugars, MK-9(H_4) as the predominant menaquinone and phospholipids containing phosphatidylcholine (no phosphatidylglycerol).

Table 37.21
Method of isolation for the species of the genus *Amycolatopsis*

Species	Source	Province/country	Method of isolation	References
Amycolatopsis orientalis	Soil, vegetable matter, and clinical specimens	nr	nr	Krasil'nikov (1981)
Amycolatopsis alba	Soil	nr	Isolated from soil by using the dilution plating technique. Cells were grown on a rotary shaker (250 rpm) for 72 h at 30 °C in a medium containing 30 g of trypticase soy broth (BBL, Becton Dickinson, Cockeysville, Md.), 3 g of yeast extract, 2 g of $MgSO_4 \cdot 7H_2O$, 5 g of glucose, and 4 g of maltose in 1 L of deionized water	Mertz and Yao (1993)
Amycolatopsis albidoflavus	Soil	nr	nr	Lee and Hah (2001)
Amycolatopsis australiensis	Arid soil	Western Australia	Isolated on SM2 agar plates that had been inoculated with suspensions of a composite arid Australian soil sample and incubated at 28 °C for 3 weeks, as described by Tan et al. (2006)	Tan et al. (2006)
Amycolatopsis azurea	Soil	Japan	nr	Henssen et al. (1987)
Amycolatopsis balhimycina	Soil	India	nr	Wink et al. (2003)
Amycolatopsis bartoniae	Dry sandy soil	Kings Canyon, Australia	Isolated on SM2 agar plates that had been inoculated with suspensions of arid sandy soil sample and incubated at 28 °C for 3 weeks, as described by Tan et al. (2006)	Zucchi et al. (2012a)
Amycolatopsis benzoatilytica	Submandibular mycetoma tissue	Czechoslovakia	nr	Majumdar et al. (2006), Yuan et al. (2012)
Amycolatopsis bullii	Dry sandy soil	Kings Canyon, Australia	Isolated on SM2 agar plates that had been inoculated with suspensions of arid sandy soil sample and incubated at 28 °C for 3 weeks, as described by Tan et al. (2006)	Zucchi et al. (2012a)
Amycolatopsis cihanbeyliensis	Soil	Cihanbeyli Salt Mine; Central Anatolia region of Turkey	Soil suspension was inoculated on modified Bennett's agar supplemented with 5 % NaCl (w/v); incubated at 28 °C for 3 weeks	(Tatar et al. 2013)
Amycolatopsis circi	Soil sample	Kenilworth Racecourse, Cape Town, South Africa	Soil sample was ground with a sterile pestle and mortar before being heated at 60 °C for 1 h. 0.1 g of soil was then added to 1 ml of sterile distilled water and agitated by vortexing for 1 min. The sample was serially diluted in sterile distilled water and spread plated on SM1 agar, SM3 agar (Tan et al. 2006) and soil extract agar (Hamaki et al. 2005) adjusted to pH8, all containing cycloheximide (50 μg ml^{-1}) and nalidixic acid (μg ml^{-1}). Strain was then isolated from SM1 agar after incubation for 21 days at 30 °C	Everest and Meyers (2011)
Amycolatopsis coloradensis	Soil	Colorado, USA	nr	Labeda (1995)
Amycolatopsis decaplanina	Soil	India	nr	Wink et al. (2004)
Amycolatopsis dongchuanensis	Soil sample from dry and hot river valley	Yunnan Province, China	2 g of air-dried soil sample was shaken in a flask with 18 ml of sterile water and several glass beads at 200 rpm for 1 h at 30 °C. The resultant suspension	Nie et al. (2012a)

Table 37.21 (continued)

Species	Source	Province/country	Method of isolation	References
			was serially diluted (1000-fold) with sterile water and spread onto modified ISP (International Streptomyces Project; Shirling and Gottlieb 1966) 5 medium supplemented with nalidixic acid (25 mg l^{-1}) and nystatin (50 mg l^{-1}) and incubated at 28 °C for one week	
Amycolatopsis echigonensis	Filtration material made from volcanic soil	Niigata, Japan	Isolated using a filtration substrate as the substrate on NY medium (1.6 g nutrient broth, 0.5 g yeast extract, 1 L deionized water and 1.5 % agar, pH 7.0), with cycloheximide (0.05 l^{-1}) and kabicidin (0.1 g l^{-1}); maintained on NY medium or oatmeal agar at 27 °C	Ding et al. (2007)
Amycolatopsis endophytica	Seeds of *Jatropha curcas* L	Sichuan Province, China	Seeds were surface-sterilized according to the five-step sterilization procedure (Qin et al. 2008). Surface sterilized seeds were aseptically crumbled into smaller fragments using a commercial Joyoung blender, spread onto sodium propionate agar (Qin et al. 2009), and incubated at 28 °C for 2–6 weeks	Miao et al. (2011)
Amycolatopsis equina	Soil sample	Kenilworth Racecourse, Cape Town, South Africa	Soil sample was ground with a sterile pestle and mortar before being heated at 60 °C for 1 h. 0.1 g of soil was then added to 1 ml of sterile distilled water and agitated by vortexing for 1 min. The sample was serially diluted in sterile distilled water and spread plated on SM1 agar, SM3 agar (Tan et al. 2006) and soil extract agar (Hamaki et al. 2005) adjusted to pH 8, all containing cycloheximide (50 μg ml^{-1}) and nalidixic acid (10 μg ml^{-1}). Strain was then isolated from soil extract agar after incubation for 10 days at 30 °C	Everest and Meyers (2011)
Amycolatopsis eurytherma	Scrubland soil	India and Turkey	Isolated on starch casein agar (Küster and Williams 1964) that had been supplemented with cycloheximide (50 μg ml^{-1}) and rifampicin (0.5 μg ml^{-1}) and incubated at 55 °C for 7 days	Kim et al. (2002a)
Amycolatopsis granulosa	Arid soil sample	Marla, Australia	Isolated from SM2 agar plates (Tan et al. 2006)	Zucchi et al. (2012b)
Amycolatopsis halophila	Soil sample	Xinjiang, China	Isolated on cellulose–casein multi-salt medium (Tang et al. 2008), incubated at 37 °C for 3 weeks	Tang et al. (2010a)
Amycolatopsis halotolerans	Soil	Natural cave on Jeju Island, Republic of Korea	Serial dilution of sample suspensions were transferred onto starch casein agar; incubated at 30 °C for 14 days	Lee (2006)
Amycolatopsis helveola	Soil	Amami Oshima Island, Japan	Isolated using the yeast extract–SDS method (Hayakawa and Nonomura 1989) with humic acid–vitamin (HV) agar (Hayakawa and Nonomura 1987)	Tamura et al. (2010)
Amycolatopsis hippodromi	Soil sample	Kenilworth Racecourse, Cape Town, South Africa	Soil sample was ground with a sterile pestle and mortar before being heated at 60 °C for 1 h. 0.1 g of soil was then added to 1 ml of sterile distilled water and agitated by vortexing for 1 min. The sample was serially diluted in sterile distilled water and spread plated on SM1 agar, SM3 agar (Tan et al. 2006), and soil extract agar (Hamaki et al. 2005) adjusted to pH8, all containing cycloheximide (50 μg ml^{-1}) and nalidixic acid (10 μg ml^{-1}). Strain was then isolated from soil extract agar after incubation for 10 days at 30 °C	Everest and Meyers (2011)
Amycolatopsis japonica	Soil	Japan	nr	Goodfellow et al. (1997)

Table 37.21 (continued)

Species	Source	Province/country	Method of isolation	References
Amycolatopsis jejuensis	Dried bat dung	Jeju Island, Republic of Korea	Serial dilution of sample suspensions were transferred onto starch casein agar and incubated at 30 °C for 14 days	Lee (2006)
Amycolatopsis kentuckyensis	Equine placentas	Kentucky, USA	nr	Labeda et al. (2003)
Amycolatopsis keratiniphila	Marsh soil	Kuwait	Isolated using animal wool as bait	Al-Musallam et al. (2003)
Amycolatopsis keratiniphila subsp. *keratiniphila*	Marsh soil	Kuwait	Isolated using animal wool as bait	Wink et al. (2003)
Amycolatopsis keratiniphila subsp. Nogabecina	Soil	India	nr	Wink et al. (2003)
Amycolatopsis lexingtonensis	Equine placentas	Kentucky, USA	nr	Labeda et al. (2003)
Amycolatopsis lurida	Soil	na	nr	Stackebrandt et al. (2004)
Amycolatopsis magusensis	Arid soil	Magusa, northern Cyprus	Isolated on Stevenson's medium No. 2 (Tan et al. 2006) supplemented with cycloheximide (50 μg ml^{-1}), nystatin (50 μg ml^{-1}), neomycin sulfate (4 μg ml^{-1}) and melezitose (1 %, w/v); incubated at 28 °C for 21 days	(Camas et al. 2013a)
Amycolatopsis marina	Ocean sediment	South China Sea	Serial dilutions of sample suspensions were transferred onto the selective isolation medium (SM1; Tan et al. 2006) for the genus *Amycolatopsis* and incubated at 28 °C for 4 weeks	Bian et al. (2009)
Amycolatopsis mediterranei	Soil sample collected in a Pinus arboretum	St. Raphael, France	nr	Lechevalier et al. (1986)
Amycolatopsis methanolica	Soil	New Guinea	nr	De Boer et al. (1990)
Amycolatopsis minnesotensis	Prairie soil	Minnesota, USA	Isolated from a prairie soil by using the dilution plating method with oatmeal agar and were maintained as 20 % (v/v) glycerol suspensions at −70 and −20 °C	Lee et al. (2006b)
Amycolatopsis nigrescens	Wall of St. Callistus hypogean Roman catacomb	St. Callistus	Isolated from the wall of the last arcosolium near the exit of the Roman catacomb of St. Callistus by touching the stone with a sterile cotton swab and suspending the adherent bacteria in 1:10-diluted organic medium 79 (Prauser and Falta 1968). Aliquots of this suspension were spread over nutrient agar (Difco) plates, which were incubated at 28 °C for 14 days	Groth et al. (2007)
Amycolatopsis niigatensis	Filtration material made from volcanic soil	Niigata, Japan	Isolated using a filtration substrate as the substrate on NY medium (1.6 g nutrient broth, 0.5 g yeast extract, 1 L deionized water and 1.5 % agar, pH 7.0), with cycloheximide (0.05 g L^{-1}) and kabicidin (0.1 g L^{-1}); maintained on NY medium or oatmeal agar at 27 °C	Ding et al. (2007)
Amycolatopsis orientalis subsp. *orientalis*	Soil, vegetable matter, and clinical specimens	nr	nr	Yuan et al. (2012)

Table 37.21 (continued)

Species	Source	Province/country	Method of isolation	References
Amycolatopsis palatopharyngis	Infected palatopharyngeal mucosa of a 70-year-old male patient	nr	Isolated on a brain–heart infusion agar plate that had been seeded using swabs and incubated at 37 °C for 5 days under microaerophilic conditions	Huang et al. (2004)
Amycolatopsis pigmentata	Soil sample from pineapple field	Iriomote Island, Japan	Isolated using the yeast extract–SDS method (Hayakawa and Nonomura 1989) with humic acid–vitamin (HV) agar (Hayakawa and Nonomura 1987)	Tamura et al. (2010)
Amycolatopsis plumensis	Brown hypermagnesian ultramafic soil	New Caledonia	Isolated from a suspension of a brown hypermagnesian ultramafic soil, which was used to inoculate a yeast extract/malt extract agar plate (ISP 2 medium; Shirling and Gottlieb 1966) supplemented with 10 μg ml^{-1} streptomycin sulfate and 100 μg ml^{-1} cycloheximide (Vobis 1992) and incubated at 30 °C for 2 weeks	Saintpierre-Bonaccio et al. (2005)
Amycolatopsis pretoriensis	Equine placentas	Pretoria, South Africa	nr	Labeda et al. (2003)
Amycolatopsis regifaucium	Arid soil	Australia	Strains were isolated on SM2 agar plates that had been inoculated with tenfold dilutions of a composite Australian soil sample and incubated at 28 °C for 21 days, as described by Tan et al. (2006b)	Tan et al. (2007)
Amycolatopsis rifamycinica	Arid soil	Alice Springs, Australia	nr	Bala et al. (2004)
Amycolatopsis ruanii	Arid soil sample	nr	Isolated from starch–casein agar plate (Küster and Williams 1964) which had been inoculated with soil suspension, incubated at 28 °C for 21 days	Zucchi et al. (2012b)
Amycolatopsis rubida	Soil sample from conifer forest	Guangxi Province, China	Isolated on a glucose–asparagine agar (GAA); glucose, 10 g; L-asparagine, 0.5 g; K_2HPO_4, 0.5 g; distilled water, 1 L; (pH 7.2) plate, which had been seeded with a soil suspension and incubated at 28 °C for 14 days	Huang et al. (2001)
Amycolatopsis saalfeldensis	Surfaces of acidic and heavy-metal-containing rocks; medieval alum slate mine	Thuringia, Germany	Isolated from rock in the central grotto (second level of the mine) by touching it with a sterile cotton swab and dispersing adhering bacteria in 1 ml sterile distilled water. Aliquots of the resultant suspension were spread over casein mineral agar plates (Altenburger et al. 1996), supplemented with cycloheximide (50 μg ml^{-1}), and incubated at 28 °C for 4 weeks	Carlsohn et al. (2007)
Amycolatopsis sacchari	Floor dust	Lucknow, India	Isolated using a wind-tunnel technique (Lacey 1971; Lacey and Dutkiewicz 1976); the Andersen sampler was loaded with petri dishes containing half-strength nutrient agar supplemented with 50 μg ml^{-1} actidione (Gregory and Lacey 1963)	Goodfellow et al. (2001)
Amycolatopsis salitolerans	Hypersaline habitat	Xinjiang Province, China	Isolated under aerobic conditions on GTY agar (5 g glucose, 0.5 g tryptone, 2 g yeast extract, 1 g $CaCO_3$, 0.5 K_2PO_4, 16.0 g agar, 1 L distilled water; pH 7.0–7.5) supplemented with 10 % (w/v) NaCl	Guan et al. (2012)

Table 37.21 (continued)

Species	Source	Province/country	Method of isolation	References
Amycolatopsis samaneae	Roots of *Samanea saman* (Jacq.) Merr	Bangkok, Thailand	Isolated from a leguminous plant using starch casein agar (Küster and Williams 1964) supplemented with ketokonazole (100 μg ml^{-1}) and nalidixic acid (25 μg ml^{-1}). Plates that had been inoculated with surface-sterilized crushed root suspensions of *Samanea saman* were incubated at 28 °C for 14 days	Duangmal et al. (2011)
Amycolatopsis sulphurea	Soil	nr	nr	Lechevalier et al. (1986)
Amycolatopsis taiwanensis	Soil sample	Yilan County, Taiwan	Isolated using HV agar (Hayakawa and Nonomura 1987), and incubated at 28 °C for 4 weeks	Tseng et al. (2006)
Amycolatopsis thailandensis	Soil sample	Northern Thailand	1 g soil sample was suspended in 10 ml basal medium [containing 0.4 % $(NH4)_2SO_4$, 0.2 % K_2HPO_4, 0.05 % $MgSO_4 \cdot 7H_2O$, and 0.1 % yeast extract] with a 25 mg sample of 0.1 % (w/v) PLA film, as described by Tomita et al. (1999) incubated at 30 °C with rotary shaking at 150 r.p.m for 14 days. The enriched culture broth was spread onto 0.1 % (w/v) emulsified PLA agar and incubated at 30 °C for 14 days	Chomchoei et al. (2011)
Amycolatopsis thermalba	Arid soil sample	Marla, Australia	Isolated from SM3 agar plates (Tan et al. 2006b)	Zucchi et al. (2012b)
Amycolatopsis thermoflava	Heat treated soil sample	Hainan Island, China	nr	Chun et al. (1999)
Amycolatopsis thermophila	Composite arid soil sample	Australia	Isolated from SM1 agar plates (Tan et al. 2006b), inoculated with suspension of composite soil sample, incubated at 28 ° C for 21 days	Zucchi et al. (2012c)
Amycolatopsis tolypomycina	Soil	Tokyo, Japan	nr	Wink et al. (2003)
Amycolatopsis tucumanensis	Groundwater sediments polluted with copper	Tucuman, Argentina	Isolated from groundwater sediments polluted with copper (Albarracín et al. 2005). It was stored at 4 °C on starch–casein agar slants, containing (L^{-1}): 10.0 g starch, 1.0 g casein, 0.5 g K_2HPO_4, 15 g agar; pH 7.0	Albarracín et al. (2010)
Amycolatopsis ultiminotia	Rhizosphere soil of *Peucedanum japonicum* Thunb plant	Mara Island, Jeju, Republic of Korea	Serially diluted rhizosphere soil samples (10-fold dilutions) were inoculated on starch–casein agar and incubated at 30 °C for 14 days, as described by Lee (2006)	Lee (2009)
Amycolatopsis umgeniensis	Soil	KwaZulu Natal province, South Africa	Soil samples were air dried and 0.1 g of soil was added to 1 ml of sterile distilled water and vortexed for 1 min. Serially diluted (in sterile distilled water) samples were spread onto Czapek Solution Agar (Atlas, 2004) and incubated for 14 days at 30 °C	Everest et al. (2013)
Amycolatopsis vancoresmycina	Soil	India	nr	Wink et al. (2003)
Amycolatopsis viridis	Composite arid soil sample	Australia	Isolated from SM2 agar plates (Tan et al. 2006), inoculated with suspension of composite soil sample, incubated at 28 °C for 21 days	Zucchi et al. (2012c)
Amycolatopsis xylanica	Soil sample	Qinghai Province, China	Selective isolation on medium MY10S, which contained xylan as the only carbon and energy source, after incubation at 28 °C for 2 weeks	Chen et al. (2010)

nr not reported

Table 37.22

Diversity of antibiotic compounds discovered as products of *Amycolatopsis* species

Species	Antibiotic	Class of compound	References
Amycolatopsis alba	Compound VIcd	1-(10 aminodecyl) pyridinium	Dasari et al. (2012)
Amycolatopsis azurea	Octacosamicin A and B	Guanidines	Dobashi et al. (1988)
	Azureomycins A and B	Glycopeptide	Omura et al. (1979)
Amycolatopsis balhimycina	Balhimycin	Glycopeptide	Nadkarni et al. (1994)
Amycolatopsis coloradensis	Avoparcin	Glycopeptide	Kunstmann et al. (1968)
Amycolatopsis decaplanina	Decaplanin	Glycopeptide	Wink et al. (2004)
Amycolatopsis keratiniphila subsp. *nogabecina*	Nogabecin	Glycopeptide	Shorin et al. (1957)
Amycolatopsis lurida	Benzathrins	Quinones	Philip et al. (1957), Theriault et al. (1986), Rasmussen et al. (1986)
	Ristocetin	Glycopeptide	Grundy et al. (1957)
Amycolatopsis mediterranei	Rifamycin W, 34a-deoxy Proansamycin B	Polyketide	Stratmann et al. (2002)
	31-Homorifamycin W	Quinones	Wang et al. (1994)
	Dethymicin	Unknown	Ueno et al. (1992)
	3-Hydroxyrifamycin S	Ansamycin	Traxler et al. (1981)
	Protorifamycins	Ansamycin	Ghisalba et al. (1978), Ghisalba et al. (1979), Ghisalba et al. (1980)
	Kanglemycin A	Ansamycin	Wang et al. (1988)
	Rifamycin SV	Ansamycin	Krishna et al. (1999)
Amycolatopsis orientalis	Orienticin A, chloro Orienticin B, chloro Orienticin C, chloro Orienticin D, chloro, Orienticin E	Glycopeptide	Tsuji et al. (1988)
	UK-69,753	Pyrans	Pacey et al. (1989)
	MM 47761 and MM 49721	Glycopeptide	Box et al. (1990)
	MM 55266 and MM 55268	Glycopeptide	Box et al. (1991)
	Quartromicin (A1, A2, and A3) and (D1, D2, and D3)	Lactones	Tsunakawa et al. (1992b)
	Vancomycin	Glycopeptide	Pittenger and Brigham (1956)
	Muraceins	Muramyl peptide	Bush et al. (1984)
	N-Demethylvancomycin	Vancomycin analog	Boeck et al. (1984)
Amycolatopsis regifaucium	Kigamicin A, B, C, D, and E	Oxazoles	Kunimoto et al. (2003)
Amycolatopsis sulphurea	Azicemicin A(1) and B(2)	Benz (a) Anthracenes	Tsuchida et al. (1995)
Amycolatopsis sp.	Amythiamicin A, B, C, and D	Thiazoles	Shimanaka et al. (1994)
Amycolatopsis sp.	Ochracenomicins A, B and C	Benz[a]anthraquinone	Igarashi et al. (1995)
Amycolatopsis sp.	XR651	Naphthacenes	Bahl et al. (1997)
Amycolatopsis sp.	Epoxyquinomicin A, B, C and D	Quinones	Matsumoto et al. (1997)
Amycolatopsis sp.	Tigloside	Tigloylated tetrasaccharide	Breinholt et al. (1998)
Amycolatopsis sp.	Actinotetraose hexatiglate	Hexa-ester	Rickards et al. (1998)
Amycolatopsis sp.	MJ347-81 F4 (A and B)	Peptides	Sasaki et al. (1998)
Amycolatopsis sp.	A-102395	Nucleoside	Murakami et al. (2007)
Amycolatopsis sp.	Pargamicin A	Cyclic peptide	Igarashi et al. (2008)
Amycolatopsis tolypomycina	Tolypomycin	Ansamycin type	Hasegawa et al. (1971), Kishi et al. (1972)
Amycolatopsis vancoresmycina	Homorifamycin; Vancoresmycin	Ansamycin type; polyketide	Hopmann et al. (2002)

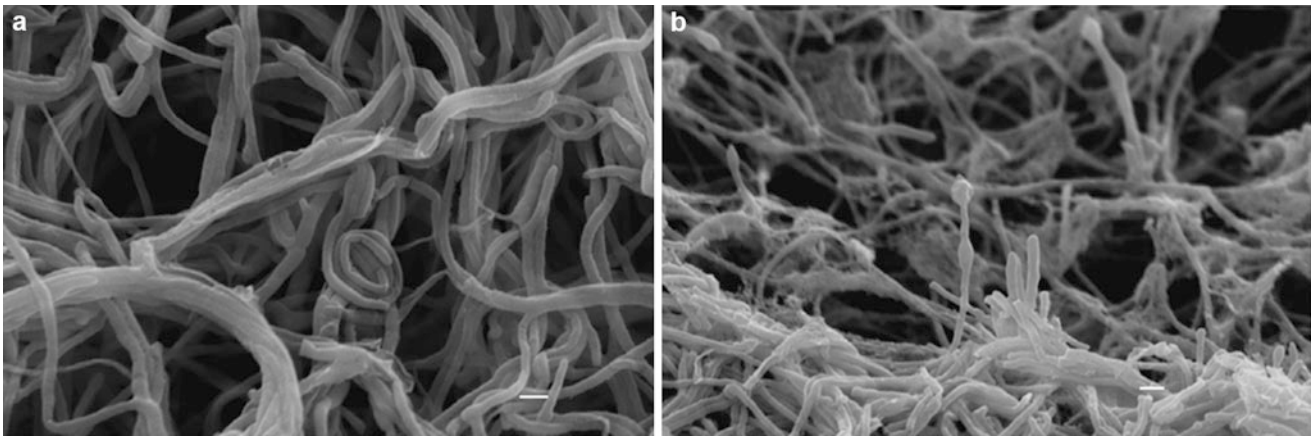

Fig 37.16
Micromorphological properties of *Crossiella cryophilus* NRRL B-16238^T. Note the pseudosporangia on the substrate mycelium in (a) and the swollen mycelial tips in (b). Bars, 1 μm

Kibdelosporangium Shearer, Colman, Ferrin, Nisbet, and Nash 1986, 48^{VP}

Kib.del′o.spo.ran.gi.um.Gr. adj *kibdelos* false, ambiguous; Gr. n. *spora* seed; Gr. n. *angeion* a vessel; M. L. neut. n. *Kibdelosporangium* false or ambiguous sporangium.

Phenotypic Analyses

Kibdelosporangium species are aerobic, catalase-positive, Gram-stain-positive, non acid-fast, filamentous actinobacteria that forms both substrate mycelium that penetrates the agar and forms a compact layer on top of the agar, as well as aerial mycelium that develops from the substrate mycelium. Fragmentation of the substrate mycelium varies and generally features specialized structures which appear to be dichotomously branched, with septate hyphae radiating from a common stalk. Long chains of nonmotile spores and sporangium-like structures may also be observed on the aerial mycelium. These sporangium-like elements are surrounded by well-defined walls which do not contain spores but produce one or more germ tubes when placed on solid growth media. As can be seen in ❯ *Table 37.27*, the cell walls contain *meso*-diaminopimelic acid as the diamino acid and whole-cell sugars consist of arabinose and galactose. The major phospholipids are phosphatidylethanolamine, phosphatidylinositol, and phosphatidylmethylethanolamine. Mycolic acids are not present.

The species within this genus produce sporangium-like structures of the substrate mycelium, whereas the spore chains are on the aerial mycelium and consist of rod-shaped spores (*Kibelosporangium* ❯ *Fig. 37.18*).

The sporangium-like structures originate as small round swellings at the tips of the hyphae that enlarge to 9–35 μm in diameter when mature (*Kibdelosporangium* Fig. ❯ *37.19*). The sporangia are surrounded by a well-defined wall and contain septate, branched hyphae in an amorphous matrix (*Kibdelosporangium* ❯ *Fig. 37.20*), and will germinate within 24–48 h when placed on a suitable medium, producing one or more germ tubes.

The G+C content of the DNA of members of this genus range from 66 to 67.2 (mol%).

The type species is *Kibdelosporangium aridum* ATCC 39323^T = DSM 43828^T = NBRC 14493^T = JCM 7912^T = NRRL B-16436^T = VKM Ac-1316^T.

Isolation and Maintenance Procedures

The isolation methods used to obtain some of the species is shown in ❯ *Table 37.27*.

Serial transfer of strains is not an acceptable method of maintenance for *Kibdelosporangium* species as they cannot be subcultured by serial plating because the sporangium-like structures are often lost on transfer. Therefore frozen stocks in glycerol (10–20 %, v/v) are maintained at −80 °C for medium-term storage or lyophilized in skim milk for long-term storage.

Taxonomy, Historical, and Current

The genus *Kibdelosporangium* is phylogenetically within the order *Pseudonocardiales* based on 16S rRNA gene sequences as can be seen in *Pseudonocardiales* phylogenetic tree (❯ *Figs. 37.1*, ❯ *37.2*, ❯ *37.3*, ❯ *37.4*, and ❯ *37.5*) and also shares similar chemotaxonomic characteristics with many of the genera within the order as can be seen in ❯ *Table 37.1*.

The morphological and physiological characteristics of the species of *Kibdelosporangium* can be seen in *Kibdelosporangium* ❯ *Table 37.28*. Gross morphology and 16S rRNA gene sequences

Table 37.23

Chemotaxonomic characteristics and methods of isolation of *Crossiella* and *Goodfellowiella* species

Species	Whole-cell sugars[a]	Phospholipids[b]	Major menaquinone(s)	Major fatty acids (>3 %)	DNA G+C (mol %)	Origin of Isolate	Method of Isolation
Crossiella cryophila NRRL B-16238^T	Gal, Man, Rib, Rha	PE, DPG, PI, PIM, PME	MK-9(H_4)	*iso*-$C_{15:0}$, $C_{15:1}$ B, *iso*-$C_{16:1}$ H, *iso*-$C_{16:0}$, $C_{16:1}$*cis*9, $C_{16:0}$ 9-methyl, *iso*-$C_{17:0}$	71.4	Soil; Yamanashi, Japan.	The isolation medium 4PC consisted of the mixture of ISP No. 4 (1 vol) and potato–carrot extract (2 vol), 1.5 % agar, and 20 g ml^{-1} of two different AG antibiotics (istamycin B and sisomicin). The potato–carrot extract consisted of the filtrate of potato (30 g) and carrot (2.5 g) boiled in 1 L of water for 30 min. Incubated at 27 °C for 7–14 days.
Crossiella equi NRRL B-24104^T	Gal, Man, Rib, Rha	PE, DPG, PI, PIM, PME	MK-9(H_4)	*iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, $C_{15:1}$ B, *iso*-$C_{16:0}$, $C_{16:0}$ 9-methyl, *iso*-$C_{17:0}$, *iso*-$C_{17:0}$ 3-OH	71.4	Equine placenta; Lexington, Kentucky, USA	Isolated from placental tissues on tryptic soy agar (Difco) plus 5 % blood.
Goodfellowiella coeruleoviolacea DSM43935^T	Gal, Rib	PE, DPG, OH-PE, PI, PIM	MK-9(H_4), MK-10(H_4)	*iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, $C_{15:0}$, *iso*-$C_{16:1}$ H, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:0}$, $C_{17:0}$ 10-methyl, ante*iso*-$C_{17:0}$ 2-OH	68.2	Soil; Russia	nr

Data from: Labeda (2001), Donahue et al. (2002), Takahashi et al. (1986), Labeda and Kroppenstedt (2006), Labeda et al. (2008)

Symbols: *nr* not reported

[a]*DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *OH-PE* hydroxyphosphatidylethanolamine, *PI* phosphatidylinositol, *PIM* phosphatidylinositolmannosides, *PME* phosphatidylmethylethanolamine

[b]*Gal* galactose, *Rha* rhamnose, *Man* mannose, *Ri* ribose

Table 37.24
Physiological characteristics of species of the genus *Crossiella* and the genus *Goodfellowiella*

	Crossiella cryophila NRRL B-16238^T	*Crossiella equi* NRRL B-24104^T	*Goodfellowiella coeruleoviolacea* DSM43935^T
Morphology			
Substrate mycelium	Pale yellow/ light-brown	Pale orange/ light brown	Pale yellow/dark-brown yellow
Aerial mycelium	White/ yellowish-white	Copious white	Blue
Decomposition of			
Adenine	–	–	–
Allantoin	–	–	–
Casein	–	+	+
Esculin	+	+	+
Gelatin	+	+	+
Hippurate	–	+	–
Hypoxanthine	–	–	+
Starch	+	+	+
Tyrosine	+	+	+
Urea	+	–	+
Xanthine	–	–	–
Acid from			
Adonitol	–	–	+
Arabinose	–	±	+
Cellobiose	w	+	+
Dextrin	w	+	+
Dulcitol	–	–	–
Erythritol	–	–	+
Fructose	+	+	+
Galactose	+	+	+
Glucose	+	+	+
Glycerol	+	+	+
Inositol	+	+	+
Lactose	w	–	+
Maltose	+	+	+
Mannose	+	+	+
Mannitol	–	–	+
Melibiose	w	+	+
α-Methyl-D-glucoside	–	–	+
β-Methyl-D-xyloside	–	–	+
Raffinose	w	+	+
Rhamnose	–	+	+

Table 37.24 (continued)

	Crossiella cryophila NRRL B-16238^T	*Crossiella equi* NRRL B-24104^T	*Goodfellowiella coeruleoviolacea* DSM43935^T
Salicin	–	+	+
Sorbitol	–	–	+
Sucrose	–	–	+
Trehalose	+	+	+
Xylose	–	+	+
Utilization of			
Acetate	+	w	+
Benzoate	–	w	–
Citrate	–	±	+
Lactate	+	–	–
Malate	+	–	+
Mucate	–	–	–
Oxalate	+	–	+
Propionate	+	±	+
Succinate	+	w	+
Tartrate	–	–	–
Production of			
Nitrate reductase	+	+	w
Phosphatase	+	+	nr
Growth in the presence of			
4 % NaCl	+	+	w
5 % NaCl	–	+	–
Growth at			
10 °C	+	+	–
37 °C	–	+	+
42 °C	–	+	+
45 °C	–	–	+

Data from: Labeda (2001), Donahue et al. (2002), Takahashi et al. (1986), Labeda and Kroppenstedt (2006), Labeda et al. (2008)
Symbols: + positive, ± doubtful response, *w* weak positive, *nr* not reported

can be used to differentiate between the *Kibdelosporangium aridum* subspecies and *Kibdelosporangium philippinense*.

The four different species of the genus *Kibdelosporangium* are *Kibdelosporangium aridum* subsp. *aridum* ATCC 39323^T = DSM 43828^T = NBRC 14493^T = JCM 7912^T = NRRL B-16436^T = VKM Ac-1316^T (Shearer et al. 1986a); *Kibdelosporangium aridum* subsp. *largum* ATCC 39922^T = DSM 44150^T = NBRC 15152^T = JCM 9107^T (Shearer et al. 1986b); *Kibdelosporangium philippinense* ATCC 49844^T = DSM 44226^T = JCM 9918^T = NBRC 15154^T = NRRL 18198^T (Mertz and Yao 1988); and *Kibdelosporangium phytohabitans* KLBMP 1111^T = CCTCC AA 2010001^T = KCTC 19775^T (Xing et al. 2012).

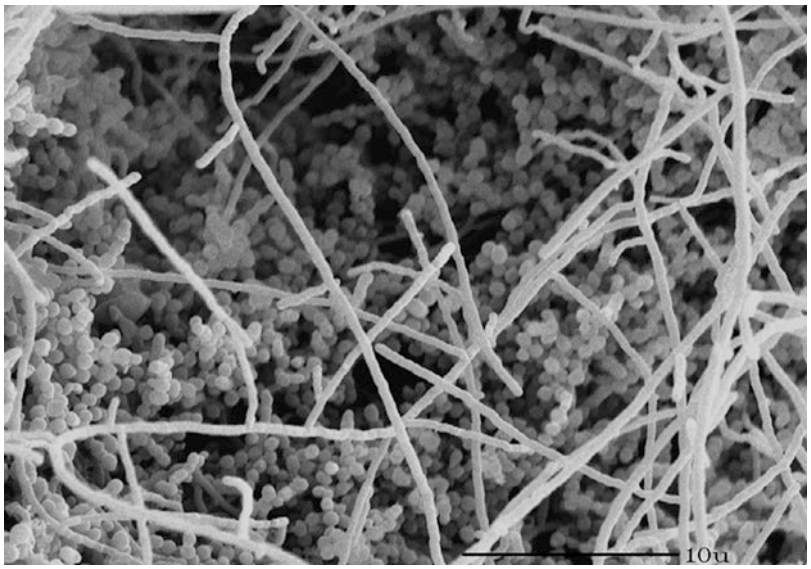

Fig. 37.17
Scanning electron micrograph of 21-day growth of *Goodfellowiella coeruleoviolacea* NRRL B-24058^T on yeast extract-malt extract agar. Note that spores are produced by fragmentation of the vegetative mycelium. The bar represents 10 μm

Kutzneria Stackebrandt, Kroppenstedt, Jahnke, Kemmerling, and Gürtler 1994, 267^{VP}

Kutz.ne´ri.a. M. L. fem. n. *Kutzneria*, named for Hans-Jürgen Kutzner, a German microbiologist.

Phenotypic Analyses

Kutzneria species are aerobic, Gram-stain-positive, mesophilic actinomycetes that form branched, cottony aerial mycelium and large (10–48 μm in diameter) globose sporangia with thick and strong sporangial walls. The sporangiophores within the sporangia may be more than 50 μm long and are the coiled, unbranched hyphae septate into spherical, rod-shaped, or ovoid nonmotile conidia. Some species are thermotolerant and all are chemoorganotrophic. As can be seen in *Table 37.17*, the cell walls of *Kutzneria* strains contain *N*-acetylated muramic acid and *meso*-diaminopimelic acid, and usually galactose is the characteristic sugar although rhamnose may also be present in some species. The predominant menaquinones are MK-9(H_4) and the predominant phospholipids present include diphosphatidylglycerol, hydroxyphosphatidylethanolamine, phosphatidylethanolamine, and phosphatidylinositol. The fatty-acid profile of *Kutzneria* species contains iso-$C_{16:0}$, 2-OH-iso-$C_{16:0}$, 10-methyl-$C_{16:0}$, anteiso-$C_{17:0}$, and 2-OH-anteiso-$C_{17:0}$ as the major components, while $C_{10:0}$, iso-$C_{14:0}$, and $C_{14:0}$ fatty acids are absent.

The G+C content of the DNA of all 3 members of this genus is 70.3 (mol%).

The type species is *Kutzneria viridogrisea* DSM43850^T (Stackebrandt et al. 1994).

Taxonomy, Historical, and Current

The species currently classified within the genus *Kutzneria* were originally described as *Streptosporangium* species, based on their production of sporangia (*Kutzneria* *Fig. 37.21*). The sporangia are produced on long sporophores (*Kutzneria* *Fig. 37.22*) containing chains of nonmotile spores. The new genus *Kutzneria* was proposed for these species by Stackebrandt et al. (1994) because phylogenetic analysis based on 16S rRNA genes (Kemmerling et al. 1993), as well as earlier studies based on 5S rRNA genes (Kudo et al. 1993) and electrophoretic mobility of ribosomal protein AT-L30 (Ochi and Miyadoh 1992), clearly demonstrated that they were phylogenetically distinct from species of the genus *Streptosporangium*. The chemotaxonomic characteristics of the species transferred to *Kutzneria* were also observed to be distinct from those of *Streptosporangium* sensu *strictu*, as can be seen in *Pseudonocardiaceae* *Table 37.1*, particularly with regard to the lack of madurose in the whole-cell sugar pattern, the lack of ninhydrin-positive and sugar-containing phospholipids in their polar lipid patterns (Labeda 2012).

The physiological and morphological characteristics of the three species in the genus *Kutzneria*: *Kutzneria albida* DSM 43870^T = ATCC 25243^T = NBRC 13901^T = JCM 3240^T = NRRL B-24060^T; *Kutzneria kofuensis* DSM 43851^T = ATCC 27102^T = NBRC 13989^T = JCM 3157^T = NRRL B-24061^T; and *Kutzneria viridogrisea* DSM43850^T = ATCC 25242^T = NBRC 15561^T = JCM 3282^T = NRRL B-24059^T = VKM Ac-1297^T (Stackebrandt et al. 1994) are shown in *Table 37.18*.

Table 37.25
Physiological characteristics of *Haloechinothrix, Sciscionella* and *Yuhushiella* species

	Haloechinothrix alba DSM 45207^T	*Sciscionella marina* KCTC 19433^T	*Yuhushiella deserti* CGMCC 4.5579^T
Growth on:			
Inorganic salts–starch agar (ISP 4)	+	+	+
Benett's agar	nr	nr	+
Oatmeal agar (ISP 3)	+	−	+
Czapek's agar	±	+	nr
Gause's asparagine agar	nr	nr	+
Sauton's agar	nr	nr	+
Nutrient agar	±	+	nr
Glycerol/asparagine agar (ISP 5)	w	+	+
Yeast extract and malt extract agar (ISP 2)	−	+	+
Potato agar	−	+	nr
Aerial mycelium	Abundant	Sparse	Absent
Colour of aerial mycelium	White	White	None
Colour of substrate mycelium	Yellow – white	Yellow – white	Pale yellow to light yellow
Spiny aerial mycelium	+	−	−
Spores	−	−	−
Soluble pigments colour	None	None	Brown
Hydrolysis of:			
Starch	+	−	+
Tween	+	+	nr
Aesculin	−	nr	nr
Casein	−	−	+
Cellulose	−	nr	nr
Chitin	−	nr	nr
Urea	−	−	+
Gelatin liquefaction	−	+	nr
Nitrate reduction	−	−	nr
Milk peptonization	−	−	nr
H_2S production	−	−	nr
Melanin production	−	−	nr
Utilization of:			
D- mannose	+	+	nr
Rhamnose	+	−	nr
Trehalose	+	+	nr
Erythritol	+	nr	nr
Dulcitol	+	−	nr
Xylitol	+	−	nr
Starch	+	−	nr
Trisodium citrate	+	nr	nr
L- lysine	+	nr	nr
Alanine	+	nr	nr
L- arginine	+	nr	nr
L- asparagine	+	nr	nr
Glycine	+	nr	nr

Table 37.25 (continued)

	Haloechinothrix alba DSM 45207^T	*Sciscionella marina* KCTC 19433^T	*Yuhushiella deserti* CGMCC 4.5579^T
L- histidine	+	nr	nr
L- proline	+	nr	nr
L- serine	+	nr	nr
L- threonine	+	nr	nr
L- tyrosine	+	nr	nr
Hypoxanthine	+	nr	–
D- arabinose	–	–	nr
Cellobiose	–	+	nr
D- fructose	–	+	nr
Galactose	–	+	nr
D- glucose	–	+	nr
Lactose	–	+	nr
Maltose	–	–	nr
Raffinose	–	–	nr
D- ribose	–	+	nr
Sucrose	–	–	nr
D- xylose	–	–	nr
Glycerol	–	nr	nr
Inositol	–	–	nr
Mannitol	–	+	nr
Sorbitol	–	–	nr
Adenine	–	nr	–
Methionine	–	nr	nr
Xanthine	–	nr	–
Acetate	nr	–	nr
NaCl (w/v) range (%):	9–23	0–13	0–3.5
Temperature range (°C):	20–45	10–37	37–45

Data from: Tang et al. (2010b), Tian et al. (2009), Mao et al. (2011)
Symbols: + positive, – negative, ± moderate, *nr* not reported

Labedaea Lee 2012, 1775^{VP}

La.be.da'e.a. N. L. fem. N. *Labedaea* named after David P. Labeda, a microbiologist who has contributed significantly to the systematics of actinomycetes.

Phenotypic Analyses

Labedaea species are mesophilic, aerobic, Gram-stain-positive, nonacid-fast, nonmotile filamentous actinomycetes. Branched aerial hyphae are produced and fragment into rod-shaped elements. Hyphal swelling occurs at the tips of some of the branched aerial mycelia, and single spherical spores (1 μm) can be observed arising from the substrate mycelium on minimal media such as soil extract agar. The substrate mycelium is not observed to fragment and sporangium-like structures are also not observed. The chemotaxonomic profile is quite similar to the other genera in the *Pseudonocardiales* (Table 37.1), having *meso*-diaminopimelic acid as the diamino acid in the cell wall, glucose, rhamnose, galactose, ribose, mannose, arabinose, and xylose in whole-cell hydrolysates and MK-9(H_4) as the predominant menaquinone. The polar lipid profile is unique compared to other genera in the *Pseudonocardiales*, containing phosphatidyl dimethylethanolamine along with phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unknown phospholipid, as shown in Table 37.29.

The G+C content of the DNA of the type species is 64.2. (mol%).

The type species is *Labedaea rhizosphaerae* DSM 45361^T = KCTC 19662^T (Lee 2012).

Table 37.26
Chemotaxonomic properties and method of isolation for *Haloechinothrix, Sciscionella* and *Yuhushiella* species

Characteristics	*Haloechinothrix alba* DSM 45207^T	*Sciscionella marina* KCTC 19433^T	*Yuhushiella deserti* CGMCC 4.5579^T
Mol% G + C of DNA	68.1	69	69.9
Phospholipids[a]	DPG, PG, PE, PI, PIM, PL	DPG, PC, PE, PME, PI, PL	DPG, PE, PME, PIM, PL, GluNu
Major menaquinone	MK-8(H_4)	MK-9(H_4)	MK-9(H_4)
Whole-cell sugars[b]	Glc, Man, GlcN, UK	Ara, Gal, Glc	Ara, Rib, Glc, Gal
Major fatty acids	$C_{16:0}$10-methyl, *iso*-$C_{16:0}$, $C_{16:0}$	*iso*-$C_{16:0}$ 2-OH, *iso*-$C_{16:0}$	ante*iso*-$C_{17:0}$, *iso*-$C_{16:0}$, $C_{16:1}\omega 7c$, *iso*-$C_{15:0}$ 2-OH, $C_{17:1}\omega 6c$, $C_{16:0}$, $C_{18:0}$
Origin of isolation sample	Soil sample; Qijiaojing Lake, China	Marine sand sediment; northern South China Sea	Soil sample; Xinjiang Uigur Autonomous Region, China
Method of isolation	Strain was isolated on cellulose–casein multi-salts (CCMS) medium (Tang et al. 2008) and incubated at 37 °C for 3 weeks.	Strain was isolated through a serial dilution plate method followed by incubation at 28 °C for 3 weeks on Gause No. 1 medium prepared with seawater	Isolated through a serial dilution plate technique after 2 weeks of incubation at 28 °C on Sauton's agar (10 g tryptone, 2 g yeast extract, 10 g glucose, 6 g NaCl, 2 g acid-hydrolyzed casein, 1 L distilled water, pH 7.0). To inhibit fungal growth, cycloheximide and nystatin (50 μg ml^{-1} each) were added to all media

Data from: Tang et al. (2010b), Tian et al. (2009), Mao et al. (2011)
PL unknown phospholipids, *PC* phosphatidylcholine, *PME* phosphatidylmethylethanolamine, *GluNu* phospholipids of unknown structure containing glucosamine
[a]*DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside
[b]*Ara* Arabinose, *Gal* galactose, *Glc* glucose, *GlcN* glucosamine, *Man* mannose, *UK* unknown sugar, *Rib* ribose

Isolation Procedures

The isolation protocol for the type species is shown in *Table 37.29*.

Taxonomy, Historical, and Current

The genus *Labedaea* is composed of a single species, *Labedaea rhizosphaerae*, represented by a single strain RS-49^T (=KCTC 19662^T = DSM 45361^T) isolated from soil from the rhizosphere of *Peucedanum japonicum* Thunb. (Japanese common name botan-bofu) on Mara Island, Jeju, Republic of Korea. The strain exhibits fairly unique morphological characteristics of swollen mycelia tips (*Labedaea* *Fig. 37.23*) similar to that observed in *Crossiella* species. Single spores seen arising from the substrate mycelium of this strain (*Labedaea* *Fig. 37.24*) are also similar to that observed in *Actinophytocola* and *Kibdelosporangium* species. *Labedaea rhizosphaerae* is phylogenetically closer to the genus *Actinomycetospora* but is chemotaxonomically distinct from these three genera on the basis of a unique phospholipid profile.

The morphological and physiological characteristics of the type strain are summarized in *Table 37.30*.

Lechevalieria Labeda, Hatano, Kroppenstedt, and Tamura 2001, 1049^{VP}

Le. che. val. i. er´ i. a. M. L. fem. n. *Lechevalieria*, named for Hubert and Mary Lechevalier, American microbiologists who contributed substantially to the field of actinomycete biology during their careers at the Waksman Institute of Microbiology.

Phenotypic Analyses

Lechevalieria strains produce branching substrate mycelium (approximately 0.5 μm in diameter) and scant to extensive aerial mycelium depending upon the species and growth medium. The substrate mycelium fragments into coccoid to coccoidal-rod-shaped elements and sporangia, coremia, or motile spores are not produced. Species are Gram-stain positive, aerobic, catalase positive, and resistant to lysozyme. As can be seen in *Table 37.31*, the whole-cell hydrolysates *Lechevalieria* species contain galactose, mannose, and traces of rhamnose. The phospholipid present in all species is phosphatidylethanolamine with diphosphatidlglycerol also fund for the majority of species. The major menaquinone is MK-9(H_4), which is typical of most genera within the order. The morphological and physiological characteristics of *Lechevalieria* species are

Table 37.27

Chemotaxonomic characteristics and method of isolation of species of the genus *Kibdelosporangium*

	Kibdelosporangium aridum subsp. *aridum* ATCC 39323^T	*Kibdelosporangium aridum* subsp. *largum* ATCC 39922^T	*Kibdelosporangium philippinense* ATCC 49844^T	*Kibdelosporangium phytohabitans* CCTCC AA 2010001^T
Fatty acids (%)				
iso-$C_{14:0}$	2.7	1.8	10.1	6.6
iso-$C_{15:0}$	13.0	13.3	4.9	9.4
ante*iso*-$C_{15:0}$	14.7	6.9	3.5	10.8
iso-$C_{16:0}$	24.7	20.7	33.9	28.8
$C_{16:0}$	4.7	5.4	4.1	12.9
ante*iso*-$C_{17:0}$	12.3	7.9	1.7	6.8
$C_{17:1}$ ω6c	8.0	9.5	14.1	4.8
Sum in feature 3[a]	6.5	12.1	3.1	6.8
Phospholipids[b]	PE, PME, DPG, PI, PIM	PE, PME, DPG, PI, PIM	PE, PME, PI	PE, PME, PG, PI, PL
Menaquinones	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-9(H_2)
Whole-cell sugars[c]	Ara, Gal	Ara, Gal	Ara, Gal	Ara, Gal
DNA G + C content (mol %)	66	nr	nr	67.2
Origin of isolate	Desert soil sample; Pima County, Arizona	Desert soil sample; Pima County, Arizona	Soil sample; Philippines	Root of the oilseed plant *Jatropha curcas* L; Sichuan province, China.
Method of isolation	nr	nr	Isolated using selective isolation procedures	Root samples were air dried for 48 h and subsequently washed ultrasonically to remove the surface soils. Samples were then surface sterilized according to the procedures of Qin et al. (2008a). Subsequently, the surface-sterilized samples were aseptically crumbled into smaller fragments using a commercial blender (Joyoung), spread onto glycerol–asparagine (ISP 5) agar medium (Shirling and Gottlieb 1966) and incubated at 28 °C for 2–6 weeks

Data from: Shearer et al. (1986a, b), Mertz and Yao (1988), Xing et al. (2012)

PL unknown phospholipids, *PME* phosphatidylmethylethanolamine

[a]Summed features represent groups of two or three fatty acids that cannot be separated by GC with the MIDI system. Summed features 3, comprised 16:1 ω7c/16:1 ω6

[b]*DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside

[c]*Ara* Arabinose, *Gal* galactose

nr not reported

summarized in *Table 37.32* and permits differentiation between species. Although gross morphology can be useful for distinguishing between the species, many of them appear quite similar when growing on agar media.

The G+C content of the DNA of members of this genus ranges from 68 to 70.5 (mol%).

The type species is *Lechevalieria aerocolonigenes* ATCC 23870^T = BCRC 13661^T = CIP 107109^T = DSM 40034^T = NBRC 13195^T = ISP 5034^T = JCM 4150^T = JCM 4614^T = NRRL B-3298^T = NRRL ISP-5034^T (Labeda et al. 2001).

Isolation Procedures

The isolation protocols for the species of this genus are shown in *Table 37.31*.

Taxonomy, Historical, and Current

The phylogenetic position of the genus, based on 16S rRNA gene sequences, has become uncertain with the addition of several

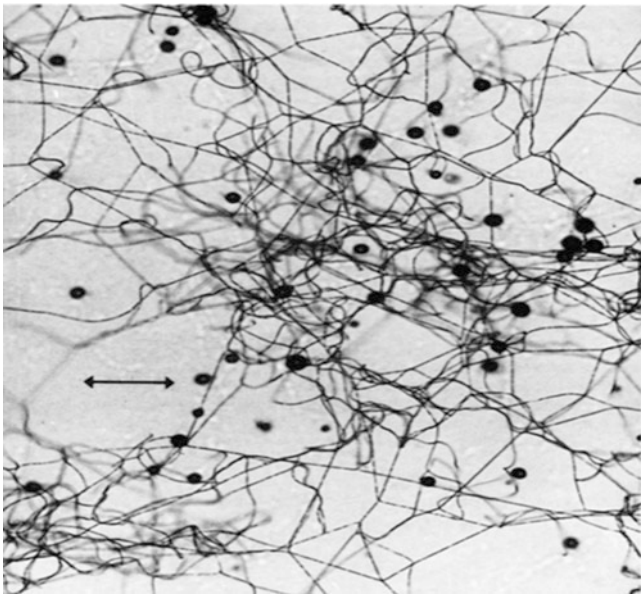

Fig. 37.18
Micrograph of aerial mycelium with long, irregularly curved chains of spores and, nearer the agar surface, sporangium-like structures (*K. aridum*; 26-day culture on water agar). Bar equals 55 μm

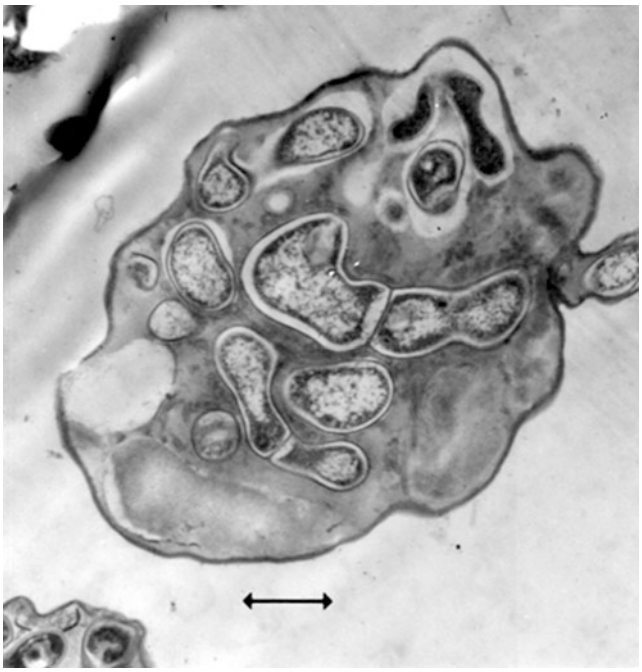

Fig. 37.20
Transmission electron micrograph; thin section of sporangium-like structure (2.5-week-old culture on thin potato-carrot agar). Bar equals 1.2 μm (Reproduced with permission from Shearer et al. (1986a))

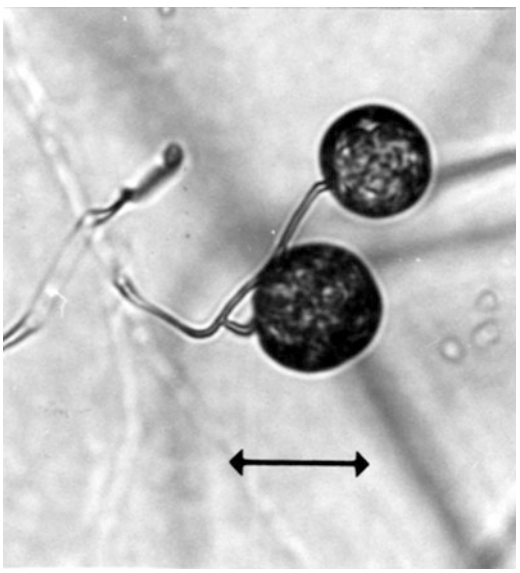

Fig. 37.19
Micrograph of sporangium-like structures (8-week old culture on water agar). Bar equals 14 μm (Reproduced with permission from Shearer et al. (1986a))

recent species which results in polyphyletic distribution of the taxa (◉ *Fig. 37.3*) but the original species comprising *Lechevalieria* sensu *strictu*, including *Lechevalieria aerocolonigenes* and *Lechevalieria flava* as well as the later species *Lechevalieria xinjingensis*, appear to be closely related to the genera *Allokutzneria* and *Kutzneria*. The more recently described species, including *Lechevalieria atacamensis*, *Lechevalieria deserti*, *Lechevalieria roselyniae*, and *Lechevalieria fradiae*, appear to be more closely related to species of the genus *Lentzea*, with the later species being observed within the radiation of *Lentzea* species (◉ *Fig. 37.3*). The taxonomic status of these 4 species is uncertain, but it is interesting to note that the 16S rRNA gene sequences of all of the *Lechevalieria* species contain the genus-specific diagnostic nucleotide signature pattern of CCG (618–620) along with TT (844–845) as can be seen in Figure *Actinosynnema*, *Lechevalieria*, *Lentzea*, *Saccharothrix*, and *Umezawaea* nucleotide signatures.

The genus *Lechevalieria* was described by Labeda et al. (2001) to contain two species observed to be incorrectly classified as members of the genus *Saccharothrix* on the basis of phylogenetic analysis of 16S rRNA gene sequences. These species, *Lechevalieria aerocolonigenes* and *Lechevalieria flava*, had been previously reclassified from the genera *Streptomyces* and *Nocardiopsis*, respectively, on the basis of chemotaxonomic properties (Labeda 1986; Grund and Kroppenstedt 1989). The phylogeny of *Saccharothrix* species and related taxa based on 16S rRNA sequences elucidated the significance of previously unrecognized chemotaxonomic markers which are essential for distinguishing *Lechevalieria* species from neighboring taxa as can be seen in ◉ *Table 37.1*.

Species of the genus *Lechevalieria* can be distinguished from the genera *Actinosynnema*, *Allokutzneria*, and *Saccharothrix* by their lack of hydroxy-substituted fatty acids in the phosphatidylethanolamine component of the diagnostic phospholipids,

Table 37.28

Physiological characteristics of species of the genus *Kibdelosporangium*

	Kibdelosporangium aridum subsp. *aridum* ATCC 39323^T	*Kibdelosporangium aridum* subsp. *largum* ATCC39922^T	*Kibdelosporangium philippinense* ATCC49844^T	*Kibdelosporangium phytohabitans* CCTCC AA 2010001^T
Color of substrate mycelium	Off-white to grayish yellow-brown	Off-white to yellow-brown	Pale yellow to orange-yellow	White to brown
Color of aerial mycelium	White	White to light gray	White	White or gray white
Soluble pigments	–	Yellow-brown	Light brown to light reddish-brown	Black
Crystals produced in agar	+	+	–	nr
Antibiotics produced	Aridicins A, B, and C	Aridicins A, B, and C Kibdelins A, B, C, and D	Ristocetin-like glycopeptide	nr
Decomposition/ hydrolysis of				
Adenine	–	–	–	nr
Allantoin	+	w	–	nr
Casein	+	–	+	nr
Cellulose	–	–	nr	nr
Esculin	+	+	+	nr
Gelatin	+	+	+	nr
Hippurate	+	+	+	nr
Hypoxanthine	+	+	+	nr
Starch	–	–	–	nr
Tyrosine	+	+	+	nr
Urea	+	+	+	nr
Xanthine	–	–	–	nr
Acid production/ carbon utilization	**Acid from**	**Acid from**	**Acid from**	**Carbon utilization**
Adonitol	–	nr	–	nr
L-Arabinose	+	+	–	nr
D-Cellobiose	+	+	+	+
Dextrin	+	+	–	–
Dulcitol	–	–	–	–
i-Erythritol	–	–	–	–
D-Fructose	+	+	+	+
D-Galactose	+	+	+	+
Glucose	+	+	+	+
Glycerol	+	+	+	+
i-Inositol	+	+	+	–
Inulin	–	–	–	nr
Lactose	+	–	+	+
Maltose	+	+	+	nr
D-Mannitol	+	+	+	+
D-Mannose	+	+	+	+
D-Melezitose	–	+	+	nr
Melibiose	+	+	+	nr
α-Methyl-D-glucoside	+	+	+	nr
β-Methyl-D-xyloside	+	nr	nr	nr

Table 37.28 (continued)

	Kibdelosporangium aridum subsp. *aridum* ATCC 39323^T	*Kibdelosporangium aridum* subsp. *largum* ATCC39922^T	*Kibdelosporangium philippinense* ATCC49844^T	*Kibdelosporangium phytohabitans* CCTCC AA 2010001^T
Raffinose	+	+	–	+
Rhamnose	+	+	+	–
D-Ribose	+	+	+	–
Salicin	v	v	–	nr
D-Sorbitol	–	–	–	+
L-Sorbose	–	–	–	+
Sucrose	+	+	–	–
Trehalose	+	+	+	+
D-Xylose	+	+	+	+
Utilization of				
Acetate	+	+	+	nr
Benzoate	–	–	–	nr
Citrate	+	+	+	nr
Formate	+	+	+	nr
Lactate	+	+	+	nr
Malate	+	+	+	nr
Oxalate	+	+	+	nr
Propionate	+	+	+	nr
Pyruvate	+	+	+	nr
Succinate	+	+	+	nr
Tartrate	–	–	–	nr
Production of:				
Nitrate reductase	–	–	+	nr
Phosphatase	+	+	+	nr
Hydrogen sulfide	+	+	+	nr
Melanin	+	+	+	nr
Growth in:				
Lysozyme broth	–	–	–	nr
2 % NaCl	+	+	+	+
4 % NaCl	+	v	–	–
5–7 % NaCl	v	v	–	–
8 % NaCl	–	–	–	–
Survival at 50 °C/8 h	+	nr	nr	nr
Growth at:				
10 °C	v	–	–	+
15 °C	+	+	–	+
42 °C	+	+	–	–
45 °C	tr	tr	–	–

Data from: Shearer et al. (1986a, b), Mertz and Yao (1988), Xing et al. (2012)
Symbols: + positive, *w* weakly positive, – negative, *nr* not reported, *v* variable, *tr* traces

similar to the species of the genera *Kutzneria* and *Lentzea*. *Lechevalieria*, *Saccharothrix*, and *Umezawaea* strains tend to contain varying amounts of rhamnose in whole-cell hydrolysates, while *Lentzea* strains are observed to totally lack or contain only trace amounts of rhamnose and may also contain ribose. *Actinosynnema* species have only galactose and mannose as their diagnostic whole-cell sugar pattern while *Allokutzneria* contains arabinose, galactose, and mannose.

The seven species of the genus *Lechevalieria* are *Lechevalieria aerocolonigenes* ATCC 23870^T = BCRC (formerly CCRC) 13661^T = CIP 107109^T = DSM 40034^T = NRBC 13195^T = ISP 5034^T = JCM 4150^T = JCM 4614^T = NRRL B-3298^T = NRRL ISP-5034^T (Labeda et al. 2001); *Lechevalieria atacamensis* CGMCC 4.5536^T = JCM 17492^T = NRRL B-24706^T; *Lechevalieria deserti* CGMCC 4.5535^T = JCM 17493^T = NRRL B-24707^T (Okoro et al. 2010); *Lechevalieria flava* ATCC 29533^T = BCRC (formerly CCRC) 13328^T = CIP 107110^T = DSM 43885^T = NRBC 14521^T = INA 2171^T = JCM 3296^T = NRRL B-16131^T (Labeda et al. 2001); *Lechevalieria fradiae* CGMCC 4.3506^T = JCM 14205^T (Zhang et al. 2007); *Lechevalieria roselyniae* CGMCC 4.5537^T = JCM 17494^T = NRRL B-24708^T (Okoro et al. 2010); and *Lechevalieria xinjiangensis* CGMCC 4.3525^T = DSM 45081^T = JCM 15473^T (Wang et al. 2007).

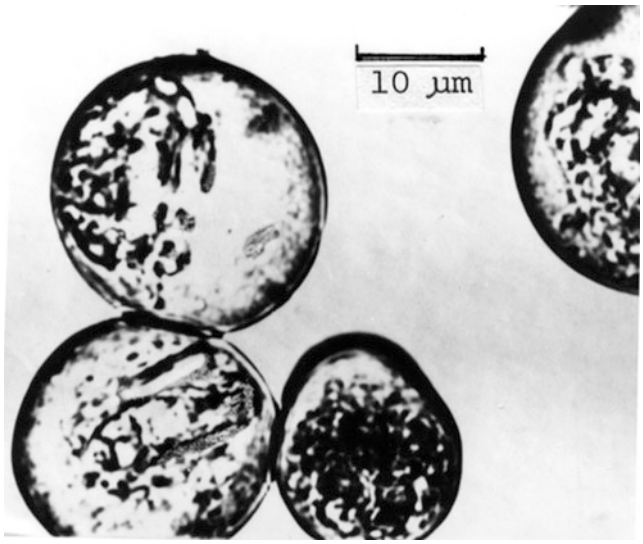

Fig. 37.21
***Kutzneria kofuensis* sporangia. Note that sporangial walls are thick. Light micrograph stained with methylene blue (Reproduced by permission from Nonomura and Ohara 1969)**

Lentzea Yassin, Rainey, Brzezinka, Jahnke, Weissbrodt, Budzikiewicz, Stackebrandt, and Schaal 1995, 362^{VP} emended Labeda, Hatano, Kroppenstedt, and Tamura 2001, 1049

Lent´ ze.a. M. L.fem. n. *Lentzea*, named after Friedrich A. Lentze, a German microbiologist who devoted a considerable part of his life to studying pathogenic actinomycetes.

Phenotypic Analyses

Lentzea strains produce branching vegetative mycelia (approximately 0.5–0.7 µmin diameter) as well as aerial mycelium that fragments into rod-shaped elements. Members of the genus are aerobic, Gram-stain positive, catalase positive, and resistant to lysozyme. Typical of all genera within the *Pseudonocardiales* as can be seen in ❯ *Table 37.33*, the whole-cell hydrolysates contain galactose, but mannose is also present in the majority of species. The diagnostic phospholipids present are significant amounts of phosphatidylethanolamine along with diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol, and MK-9(H_4) is the principal menaquinone.

Phylogenetically, the genus *Lentzea* represents a line of descent adjacent to the genus *Actinosynnema* and close to the genera *Lechevalieria*, *Saccharothrix*, and *Umezawaea*. Sequence of the 16S rRNA gene contains genus-specific diagnostic nucleotide signature patterns of TCAA (617–620) and GCC (843–845) as can be seen in Figure *Actinosynnema*, *Lechevalieria*, *Lentzea*, *Saccharothrix*, and *Umezawaea*.

The G+C content of the DNA of the members of this genus ranges from 64.1 to 79.6 (mol%).

The type species is *Lentzea albidocapillata* ATCC 51859 = CCUG 48294^T = CIP 104842^T = DSM 44073^T = NBRC 15855^T = IMMIB D-958^T = IMSNU 21253^T = JCM 9732^T = NBRC 100372^T = NRRL B-24057^T (Yassin et al. 1995).

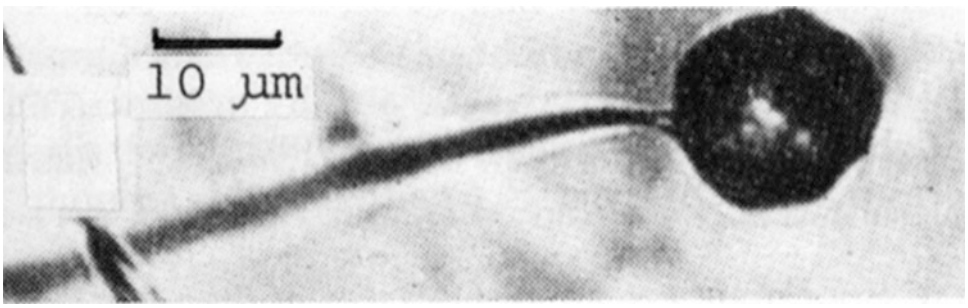

Fig. 37.22
***Kutzneria kofuensis* sporangiophores are long. Light micrograph (Reproduced by permission from Nonomura and Ohara 1969)**

Table 37.29
Chemotaxonomic characteristics and methods of isolation of *Labedaea* species

Species	Whole-cell sugars[a]	Phospholipids[b]	Major menaquinone (s)	Major fatty acids (>5 %)	DNA G+C (mol %)	Origin of isolate	Method of isolation
Labedaea rhizosphaerae KCTC 19662^T	Glc, Rha, Gal, Rib, Man, Ara, Xyl	DPG, PG, PI, PL, PDE	MK-9(H_4)	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-C_{161} H	64.2	Rhizosphere soil of *Peucedanum japonicum* Thunb; Mara Island, Jeju, Republic of Korea	Serially diluted rhizosphere soil samples (10-fold dilutions) were inoculated on starch–casein agar and incubated at 30 °C for 14 days, as described by Lee (2009).

Data from: Lee (2012)
[a]*Ara* arabinose, *Gal* galactose, *Glc* glucose, *Man* mannose, *Ri* ribose, *Rha* rhamnose, *Xyl* xylose
[b]*DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PDE* phosphatidyldimethylethanolamine, *PI* phosphatidylinositol, *PL* unknown phospholipid

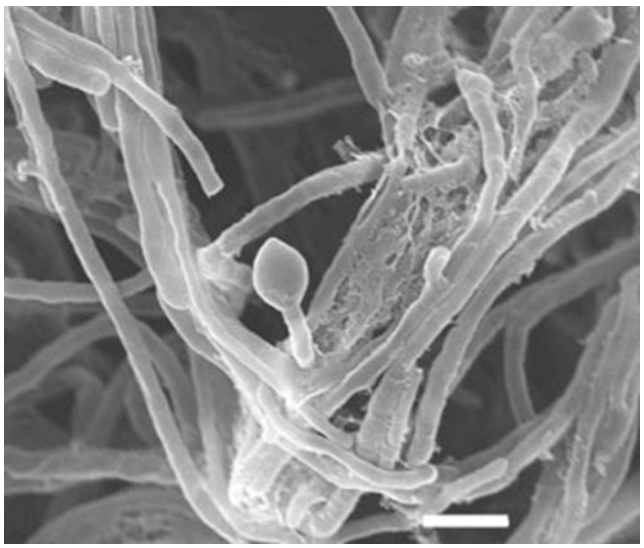

Fig. 37.23
Scanning electron micrograph of *Labedaea rhizosphaera* KCTC 19662^T illustrating the swelling of a hyphal tip. Bar represents 1 μm

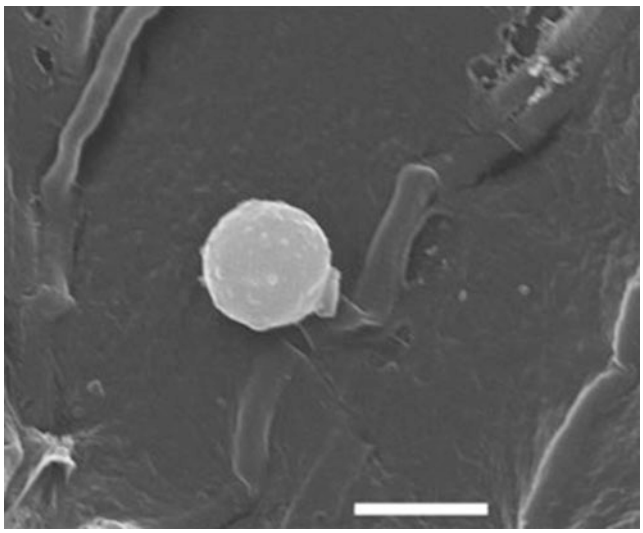

Fig. 37.24
Scanning electron micrograph of *Labedaea rhizosphaera* KCTC 19662^T illustrating a single spore formed on the substrate mycelium (soil extract agar, 6 weeks). Bar represents 1 μm

Isolation Procedures

The isolation protocol for some species is shown in *Table 37.33*.

Taxonomy, Historical, and Current

The genus *Lentzea* was originally proposed by Yassin et al. (1995) based on a single isolate from a tissue sample taken from an abdominal mass of a patient with peritoneal carcinomatosis. The proposal was based on the phylogenetic position of the strain based on its 16S rRNA gene sequence as well as chemotaxonomic properties that distinguished it from the most closely related genus *Saccharothrix*. A later proposal to consider *Lentzea* as a member of the genus *Saccharothrix* was made by Lee et al. (2000) based on their chemotaxonomic and phylogenetic studies. A subsequent study by Labeda et al. (2001) demonstrated that the genus *Lentzea* was valid and could be differentiated from *Saccharothrix* and *Lechevalieria* on the basis of phylogenetic position (*Fig. 37.3*), diagnostic signature nucleotides TCAA (617–620) and GCC (843–845) in 16S rRNA gene sequences

Table 37.30
Physiological properties of *Labedaea* species

	Labedaea rhizosphaerae KCTC 19662^T
Gross morphology	
Substrate mycelium	White to pale yellow, strong yellow
Aerial mycelium	White to yellowish-white
Soluble pigments	None
Hydrolysis of	
Casein	+
Carboxymethyl-cellulose	–
DNA	+
Esculin	+
Gelatin	+
Starch	–
Urea	–
Decomposition of	
Hypoxanthine	+
Tyrosine	+
Xanthine	–
Production of	
Hydrogen sulfide	–
Nitrate reductase	–
Growth on	
Adonitol	–
Arabinose	–
Cellobiose	+
Dextran	+
Dulcitol	–
Erythritol	–
Fructose	+
Galactose	–
Glucose	+
Glycerol	–
Inositol	–
Lactose	+
Maltose	+
Mannitol	+
Mannose	–
Melezitose	+
Melibiose	+
Methyl-α-D-glucoside	–
Methyl-α-D-mannoside	–
Raffinose	+
Rhamnose	+
Ribose	–
Salicin	–
Sorbitol	–
Sucrose	+
Trehalose	+
Xylitol	–
Xylose	+
Assimilation of	
Acetate	–
Benzoate	–
Citrate	+
Formate	–
Malate	–
Succinate	–
Tartrate	–
Growth in the presence of	
3 % (w/v) NaCl	+
4 % (w/v) NaCl	–
Growth at	
20 °C	–
25 °C	+
42 °C	+
45 °C	–

Data from: Lee (2012)
Symbols: + positive, – negative

(Figure *Actinosynnema*, *Lechevalieria*, *Lentzea*, *Saccharothrix*, and *Umezawaea* nucleotide signatures), and distinct chemotaxonomic characteristics. *Lentzea kentuckyensis* and *Lentzea jangxiensis* are not observed to have the characteristic nucleotide signatures and cluster closely with a strain identified as *Lechevalieria fradiae* in the phylogenetic tree, raising some questions regarding the correct generic assignment of these three species. *Lentzea* species lack phosphatidylethanolamine containing 2-hydroxy-fatty acids among their polar lipids, thus differentiating them from species of the genus *Saccharothrix*, and their whole-cell sugar pattern, consisting of galactose, mannose, and small quantities of ribose, is distinct from *Lechevalieria* and *Saccharothrix* species. *Lentzea* species are morphologically quite similar to those in the genera *Lechevalieria* and *Saccharothrix* and produce aerial mycelium which may exhibit a "zig-zag" morphology (*Lentzea* ◉ *Fig. 37.25a*) and fragments into coccoidal-rod-shaped elements (*Lentzea* ◉ *Fig. 37.25b*), thus making it difficult to differentiate members of these genera on the basis of gross morphology alone.

The eight species of the genus *Lentzea* are *Lentzea albidocapillata* ATCC 51859^T = CCUG 48294^T = CIP 104842^T = DSM 44073^T = NRBC 15855^T = IMMIB D-958^T = IMSNU 21253^T = JCM 9732^T = NBRC 100372^T = NRRL B-24057^T (Yassin et al. 1995); *Lentzea albida* DSM 44437^T = NRBC 16102^T = JCM 10670^T = NRRL B-24073^T; *Lentzea californiensis* DSM 43393^T = IMRU 550^T = JCM

Table 37.31
Chemotaxonomic properties and method of isolation of *Lechevalieria* species

Species	Phospholipids[a]	Major fatty acids	Whole-cell sugars[b]	Major menaquinone(s)	DNA G+C content (mol%)	Origin of isolation sample	Method of isolation
Lechevalieria aerocolonigenes	PE	nr	Gal, Man, Rha	MK-9(H_4)	70.5	nr	nr
Lechevalieria atacamensis	PE, DPG	ante*iso*-$C_{15:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{14:0}$, *iso*-$C_{17:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$	Gal, Man, Rha	MK-9(H_4)	nr	Hyperarid soil sample; Atacama Desert, Chile	Strain was isolated from SM2 agar plates (Tan et al. 2006b) that had been inoculated with soil suspensions and incubated at 28 °C for 21 days, as described by Okoro et al. (2009).
Lechevalieria deserti	PE, DPG	ante*iso*-$C_{15:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{14:0}$, *iso*-$C_{17:0}$, $C_{16:1}$, $C_{16:0}$	Gal, Man, Rha	MK-9(H_4)	nr	Hyperarid soil sample; Atacama Desert, Chile	Strain was isolated from SM2 agar plates (Tan et al. 2006b) that had been inoculated with soil suspensions and incubated at 28 °C for 21 days, as described by Okoro et al. (2009).
Lechevalieria flava	PE	nr	Gal, Man, Rha	MK-9(H_4)	nr	nr	nr
Lechevalieria fradiae	PE, DPG	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{14:0}$, *iso*-$C_{17:0}$, *iso*-$C_{16:1}$ H, $C_{17:1}$ ω6*c*, $C_{15:1}$ ω6*c*	Gal, Man, Rha	MK-9(H_4)	68.0	Soil sample; Wutaishan mountain, China	Strain was isolated on a glucose–yeast extract and malt extract agar plate, which had been seeded with a soil suspension and incubated at 28 °C for 2 weeks.
Lechevalieria nigeriaca	DPG, PE, PG, PI	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{14:0}$, $C_{16:0}$, ante*iso*-$C_{17:0}$	Gal, Man, Rha, (Rib, Glc)	MK-9(H_4)	68.4	Arid soil sample; Abuja, Nigeria	Soil suspension was inoculated on SM2 agar plates (Tan et al. 2006), supplemented with (+) Dmelezitose (1 %, w/v), cycloheximide (50 μg ml^{-1}), neomycin sulfate (4 μg ml^{-1}) and nystatin (50 μg ml^{-1}), incubated at 28 °C for 21 days.
Lechevalieria roselyniae	PE, DPG	ante*iso*-$C_{15:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{14:0}$, *iso*-$C_{17:0}$, $C_{16:1}$, $C_{16:0}$	Gal, Man, Rha	MK-9(H_4)	nr	Hyperarid soil sample; Atacama Desert, Chile	Strain was isolated from SM2 agar plates (Tan et al. 2006) that had been inoculated with soil suspensions and incubated at 28 °C for 21 days, as described by Okoro et al. (2009).
Lechevalieria xinjiangensis	PE, PME	ante*iso*-$C_{15:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$, $C_{16:1}$, $C_{18:0}$	Gal, Man, Rha, (Rib, Glc)	MK-9(H_4)	68.6	Radiation contaminated soil sample; Xinjiang, China	Strain was isolated from a plate containing modified Bennett's agar (Jones 1949) that had been inoculated with a soil suspension and incubated at 28 °C for 14 days.

Data from: Camas et al. (2013b), Labeda et al. (2001), Okoro et al. (2010), Zhang et al. (2007), Wang et al. (2007)
Symbols: *nr* not reported
[a]*PE* phosphatidylethanolamine, *DPG* diphosphatidylglycerol, *PME* phosphatidylmethyethanolamine
[b]*Gal* galacatose, *Man* mannose, *Glc* glucose, *Rha* rhamnose, *Rib* ribose

Table 37.32

Physiological characteristics of species of the genus *Lechevalieria*

	Lechevalieria aerocolonigenes NRRL B-3298 T	Lechevaleiria atacamensis CGMCC 4.5536 T	Lechevalieria deserti CGMCC 4.5535 T	Lechevalieria flava NRRL B-16131 T	Lechevalieria fradiae CGMCC 4.3506 T	Lechevalieria nigeriaca NJ2035T	Lechevalieria roselynia CGMCC 4.5537 T	Lechevalieria xinjiangensis CGMCC 4.3525 T
Color of substrate mycelium	Yellowish to brownish	Grayish white to white	Grayish white to white	Yellow	Yellow	nr	Grayish white to white to light brown	Yellow to orange
Color of aerial mycelium	White	Light yellowish white	Light yellowish white	White	None	Gray or light yellow	White to light brown	White to yellow; brownish white
Color of soluble pigments	Yellowish to brownish	None	None	None	None	None	None	None
Decomposition/ hydrolysis of								
Adenine	–	nr	nr	–	–	nr	+	–
Allantoin	–	+	+	+	–	–	+	+
Casein	+	+	+	–	+	nr	+	+
Esculin	+	+	+	+	+	+	–	–
Hypoxanthine	+	+	+	+	–	+	+	–
Tyrosine	+	+	+	+	+	–	+	–
Urea	w	–	–	+	+	–	–	–
Acid production/ carbon utilization								
Adonitol	–	–	+	–	–	–	+	+
Arabinose	+	nr	nr	+	+	+	nr	nr
Cellobiose	+	+	nr	+	–	+	nr	nr
Dextrin	+	nr	nr	+	–	+	nr	nr
Erythritol	–	–	–	+	+	nr	–	–
Fructose	+	nr	+	+	–	nr	nr	nr
Galactose	+	nr	+	nr	–	+	nr	nr
Glucose	+	+	nr	+	–	nr	nr	nr
Inositol	+	nr	+	+	–	nr	nr	–
Inulin	–	+	+	–	–	+	+	–
Lactose	+	+	+	+	–	+	+	–
Mannitol	+	+	+.	+	–	+	+	+
Mannose	+	+	+	+	–	+	+	+

Table 37.32 (continued)

	Lechevalieria aerocolonigenes NRRL B-3298 T	Lechevaleiria atacamensis CGMCC 4.5536 T	Lechevalieria deserti CGMCC 4.5535 T	Lechevalieria flava NRRL B-16131 T	Lechevalieria fradiae CGMCC 4.3506 T	Lechevalieria nigeriaca NJ2035T	Lechevalieria roselynia CGMCC 4.5537 T	Lechevalieria xinjiangensis CGMCC 4.3525 T
Melezitose	−	−	−		+	−	−	−
Melibiose	+	nr	nr	+	−	nr	nr	nr
α-Methyl-D-glucoside	w	−	−	+	+	nr	−	−
Raffinose	+	nr	nr	+	+	nr	nr	nr
Rhamnose	+	+	+	+	−	+	+	+
Salicin	+	+	+	+	−	nr	−	−
Sorbitol	−	+	nr	−	−	−	nr	nr
Sucrose	+	nr	nr	+	−	+	nr	nr
Trehalose	+	+	+	+	−	nr	+	−
Utilization of								
Acetate	+	nr	nr	−	+	nr	nr	+
Citrate	+	+	+	+	−	nr	+	+
Lactate	+	nr	nr	−	+	nr	+	nr
Oxalate	+	+	+	−	−	nr	+	+
Propionate	+	nr	+	nr	nr	nr	nr	+
Succinate	+	+	+	−	+	nr	+	+
Tartrate	+	nr	+	nr	−	nr	+	nr
Production of								
Nitrate reductase	+	−	−	+	+	−	−	+
Growth in the presence of								
4 % NaCl	+	−	+	+	+	+	+	+
5 % NaCl	w	−	+	−	+	+	+	+
Growth at								
10 °C	+	−	−	+	−	−	−	+
37 °C	+	+	+	+	+	+	+	+
42 °C	w	−	−	+	+	+	−	+
45 °C	−	−	−	−	+	+	−	+

Data from: (Camas et al. 2013b), Labeda et al. (2001), Okoro et al. (2010), Zhang et al. (2007), Wang et al. (2007)
Symbols: + positive, − negative, *w* weak positive, *nr* not reported

Table 37.33

Chemotaxonomic properties and methods of isolation of *Lentzea* species

Species	Phospholipids[a]	Major fatty acids (>5 %)	Whole-cell sugars[b]	Major menaquinones	Mol% G+C of DNA	Origin of isolation sample	Method of isolation
Lentzea albidocapillata	PE, DPG, PG, PI	ante*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{15:0}$, $C_{16:1}$	Gal, Man, Rha	MK-9(H_4)	68.6	Tissue specimen taken from an abdominal mass in a patient suffering from peritoneal carcinomatosis	Isolated on a Columbia blood agar plate and subcultured on brain–heart infusion agar (Difco) and glucose–yeast extract and malt extract (GYM) agar
Lentzea albida	PE, DPG, PG, PI	ante*iso*-$C_{15:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$, $C_{15:0}$	Gal, Man, Rib, Rha	MK-9(H_4)	nr	Soil sample; Jiangxi Province, China	nr
Lentzea californiensis	PE, DPG, PG, PI	ante*iso*-$C_{15:0}$, *iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$	Gal, Rha	MK-9(H_4)	nr	Soil sample, California	nr
Lentzea flaviverrucosa	PE, DPG, PIMs	$C_{16:0}$10-methyl, ante*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{15:0}$, $C_{16:0}$, $C_{16:1}$	Gal, Man, Rib, Glc	MK-9(H_4), MK-9(H_2)	64.1	Soil sample; Shanxi province, China	nr
Lentzea jiangxiensis	DPG, PE, OH-PE, PI	ante*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{15:0}$, *iso*-$C_{14:0}$, $C_{17:1}\omega6c$, $C_{16:1}\omega7c$	Gal, Man, Rib	MK-9(H_4)	69.6	Acidic soil sample; Jiangxi Province, China	Serial dilution of soil sample followed by inoculation on modified GTV agar (Busti et al. 2006) containing 500 ml L^{-1} soil extract, 25.0 g L^{-1} agar, 3 mM $CaCl_2$, supplemented with 0.1 % (v/v) B-vitamin solution, 50 mg L^{-1} cycloheximide, 50 mg L^{-1} nystatin and 20 mg L^{-1} nalidixic acid. Incubated at 28 °C for 2 weeks
Lentzea kentuckyensis	PE, OH-PE, DPG, PI	ante*iso*-$C_{15:0}$, *iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$	Gal, Rib	MK-9(H_4)	nr	Equine placenta; Kentucky, USA	nr
Lentzea violacea	PE, DPG, PG, PI	$C_{16:0}$10-methyl, *iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:1}$	Gal, Man, Rha	MK-9(H_4)	79.6	Soil sample from gold mine cave; Kongju, Korea	Isolated on tap water agar and oligotrophic medium (M5) by using dilution plating method
Lentzea waywayandensis	PE, DPG, PG, PI	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{16:1}$, $C_{16:1}$ *cis*-9	Gal, Man	MK-9(H_4)	79.1	Soil sample; Lake Waywayanda, New Jersey	nr

Data from: Labeda et al. (2001), Yassin et al. (1995), Xie et al. (2002), Li et al. (2012b), Labeda et al. (2007), Labeda and Lyons (1989b), Lee et al. (2000)

Symbols: *nr* not reported

[a]*PE* phosphatidylethanolamine, *OH-PE* hydroxy phosphatidylethanolamine, *DPG* diphosphatidylglycerol, *PI* phosphatidylinositol, *PIMs* phosphatidylinositolmannosides

[b]*Gal* galacatose, *Man* mannose, *Glc* glucose, *Rha* rhamnose, *Rib* ribose

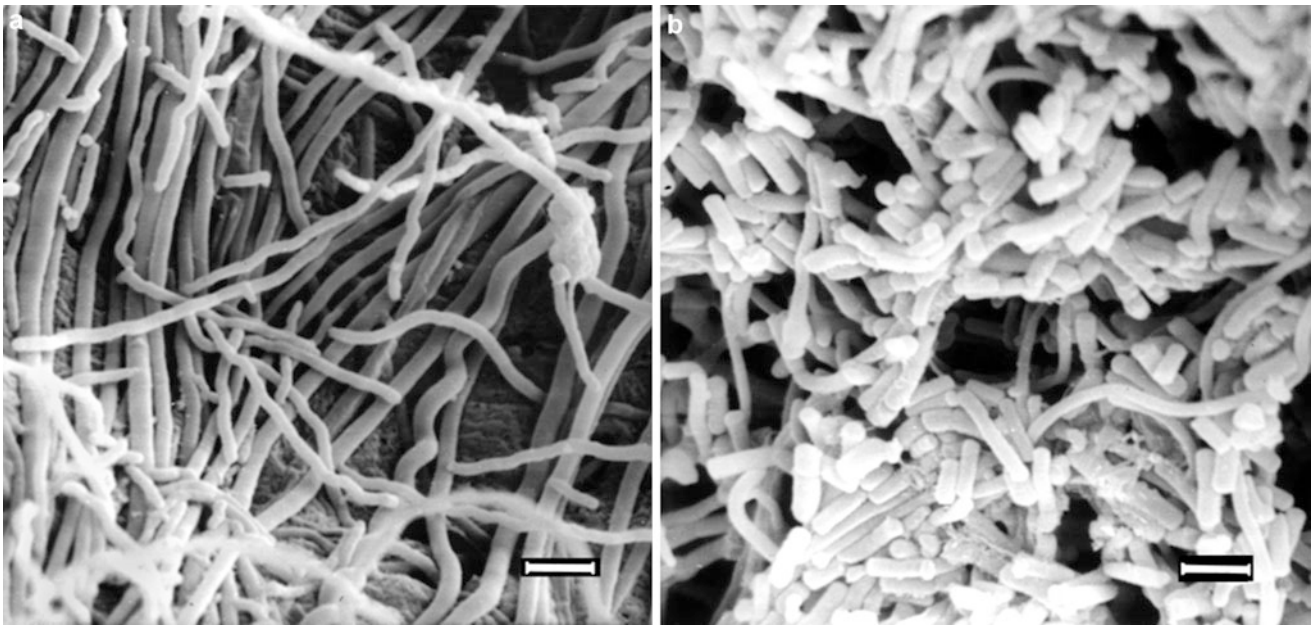

Fig. 37.25
Scanning electron micrographs of *Lentzea albidocapillata* IMMIB D-958^T grown for 14 days on yeast extract-malt extract agar illustrating "zig-zag" aerial mycelium (a) and coccoidal-rod shaped fragmentation of the aerial mycelium (b). Bar markers = 2 μm (Micrographs courtesy of Dr. A. F. Yassin, University of Bonn)

11305^T = NRRL B-16137^T (Labeda et al. 2001); *Lentzea flaviverrucosa* DSM 44664^T = JCM 11373^T = NBRC 100042^T (Xie et al. 2002); *Lentzea jiangxiensis* CGMCC 4.6609^T = NBRC 106680^T (Li et al. 2012); *Lentzea kentuckyensis* DSM 44909^T = JCM 14913^T = NRRL B-24416^T (Labeda et al. 2007); *Lentzea violacea* IMSNU 50388^T = JCM 10975^T (Lee et al. 2000); and *Lentzea waywayandensis* ATCC 51594^T = DSM 44232^T = NRBC 14970^T = JCM 9114^T = NRRL B-16159^T (Labeda et al. 2001).

The physiological characteristics of *Lentzea* species are summarized in ❯ *Table 37.34* and can be used to differentiate between species, although a common set of physiological tests has not been used in the description of all species. Gross colonial morphology provides some additional information of the species based on the color of the substrate mycelium, production and color of aerial mycelium, and production and color of soluble pigments when growing on agar media. The distinct fatty-acid profiles of each *Lentzea* species, as shown in ❯ *Table 37.33*, can also be used to differentiate between species.

Prauserella Kim and Goodfellow 1999, 510^{VP} emended Li, Xu, Tang, Xu, Kroppenstedt, Stackebrandt, and Jiang 2003C, 1547

Prau.se.rel'la.N.L. fem. dim. n. *Prauserella* named after Helmut Prauser, a German microbiologist who made many contributions to actinomycete systematics.

Phenotypic Analyses

Prauserella cultures consist of extensively branched substrate mycelium of approximately 0.6–0.8 μm in diameter. Fragmentation into irregular rod-shaped elements occurs within 24–48 h on rich media. Some strains form aerial hyphae that may be branched short or long chains that are straight or flexuous. Growth is limited to temperatures of 10–45 °C. The optimal temperature for growth is between 28 °C and 34 °C with an optimal pH range of 6.8–7.2. Optimal growth occurs in the presence of 8–15 % NaCl (w/v). Most strains are halophilic or halotolerant. *Prauserella* species are aerobic, Gram-stain positive, nonacid or alcohol fast, nonmotile, with motile spores. As shown in ❯ *Table 37.35* the cell wall contains *meso*-diaminopimelic acid and whole-cell hydrolysates contain arabinose, galactose, and ribose (Lechevalier et al. 1986; Li et al. 2009; Mertz and Yao 1993). The predominant menaquinone is MK-9(H_4), however, *Prauserella rugosa* also contains MK-9(H_2). Diagnostic phospholipids include diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylinositol. Mycolic acids are not present. Branched chain and saturated fatty acids present include iso-$C_{16:0}$ in all species (Li et al. 2009; Schäfer et al. 2010; Wang et al. 2010).

The G+C content of the DNA for members of the genus *Prauserella* ranges between 65.8 and 70.1 (mol%).

The type species is *Prauserella rugosa* is DSM 43194^T = ATCC 43014^T = NCIMB 89263^T (Kim and Goodfellow 1999).

Table 37.34

Physiological properties of *Lentzea* species

	Lentzea albidocapillata DSM 44073 T	Lentzea albida NBRC 16102 T	Lentzea californiensis NRRL 16137 T	Lentzea flaviverrucosa CGMCC 4.0578 T	Lentzea jiangxiensis CGMCC 4.6609 T	Lentzea kentuckyensis NRRL B-24416 T	Lentzea violacea IMSNU 50388 T	Lentzea waywayandensis NRRL B-16159 T
Color of substrate mycelia	Yellow, yellowish brown	Yellowish orange	Yellow to orange-brown	Pale yellow to yellowish brown	Yellowish white to yellow	Yellow to strong yellow	Violet	Pale yellow to dark yellow
Color of aerial mycelia	White to yellowish white	White	White	White to yellowish white	Yellowish white	White to yellowish white	White	White
Soluble pigments	None	None	Orange	None	None	Faint brown	Reddish brown	None
Hydrolysis of:								
Adenine	−	−	−	−	−	−	−	−
Allantoin	nr	−	−	−	+	−	nr	−
Casein	+	+	+	−	+	+	+	+
Esculin	+	+	+	−	+	+	+	+
Gelatin	+	nr	nr	+	−	+	−	+
Hypoxanthine	+	+	+	+	−	+	+	+
Starch	+	+	+	+	+	+	+	+
Tyrosine	+	+	+	+	+	+	+	+
Urea	±	±	+	−	−	+	+	+
Xanthine	−	−	−	−	−	+	−	−
Production of:								
Nitrate reductase	−	−	+	+	+	−	−	+
Phosphatase	+	nr	nr	+	nr	−	nr	+
Acid from:								
Adonitol	+	+	−	−	−	−	−	+
Arabinose	+	+	+	nr	+	+	+	+
Cellobiose	+	+	+	+	+	+	−	+
Dextrin	nr	+	+	+	+	nr	nr	+
Dulcitol	nr	−	−	nr	nr	−	nr	−
Erythritol	nr	−	−	+	−	−	nr	+
Fructose	+	+	+	nr	+	+	+	+
Galactose	+	+	+	nr	+	+	+	+
Glucose	+	+	+	nr	+	+	+	+
Glycerol	nr	+	+	+	+	+	nr	+
Inositol	+	+	+	+	+	+	−	+
Lactose	+	w	+	−	+	+	+	+
Maltose	+	+	+	+	w	+	−	+

Table 37.34 (continued)

	Lentzea albidocapillata DSM 44073 T	Lentzea albida NBRC 16102 T	Lentzea californiensis NRRL 16137 T	Lentzea flaviverrucosa CGMCC 4.0578 T	Lentzea jiangxiensis CGMCC 4.6609 T	Lentzea kentuckyensis NRRL B-24416 T	Lentzea violacea IMSNU 50388 T	Lentzea waywayandensis NRRL B-16159 T
Mannitol	+	+	+	+	+	+	–	+
Mannose	nr	+	+	+	+	+	–	+
Melibiose	nr	+	+	+	nr	+	–	+
β-Methyl-xyloside	nr	–	–	nr	nr	±	nr	–
Raffinose	±	–	+	+	+	+	+	+
Rhamnose	+	+	+	–	w	+	–	+
Salicin				+	nr	+	–	+
Sucrose	+	+	+	+	+	+	–	+
Trehalose	+	+	+	–	nr	+	–	+
Xylose	+	+	+	–	+	+	–	+
Assimilation of:								
Acetate	–	+	+	+	nr	–	+	+
Benzoate	–	–	–	–	nr	–	–	–
Citrate	–	+	+	–	nr	±	–	+
Lactate	–	–	–	–	nr	±	+	+
Malate	+	+	+	+	nr	+	–	+
Mucate	nr	–	–	nr	nr	–	nr	–
Oxalate	nr	–	–	–	nr	+	+	+
Propionate	nr	+	+	+	nr	–	+	+
Succinate	nr	+	+	–	nr	+	+	+
Tartrate	nr	–	–	–	nr	–	–	–
Growth in the presence of:								
4 %, w/v NaCl	+	+	+	–	+	+	+	+
5 %, w/v NaCl	nr	+	+	–	+	+	nr	+
Growth at:								
10 °C	+	–	+	nr	+	+	+	+
37 °C	+	+	+	+	+	+	+	w
42 °C	–	+	–	+	–	–	–	–
45 °C	–	+	–	–	–	–	–	–

Data from: Labeda et al. (2001), Yassin et al. (1995), Xie et al. (2002), Li et al. (2012), Labeda et al. (2007), Labeda and Lyons (1989b), Lee et al. (2000)

Symbols: + positive, ± doubtful/moderate response, – negative, *w* weak reaction, *nr* not reported

Table 37.35
Chemotaxonomic characteristics of *Prauserella* species[a]

Species	Characteristic				
	Cell-wall sugars[b]	Major menaquinones	Phospholipids[c]	DNA G + C content (mol%)	Predominant fatty acids
P. rugosa	nr	MK-9(H_4), MK-9(H_2)	PE, DPG, PI, PG	67.0–68.9	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$ H, $C_{16:1}\omega9c$
P. aidingensis	Ara, Gal, Rib	MK-9(H_4)	PC, PME, PI, PG, DPG, PL	70.1	*iso*-$C_{16:0}$, $C_{16:1}\omega9c$, *iso*-$C_{16:0}$ 2-OH
P. alba	Ara, Gal, Rib	MK-9(H_4)	PI, PG, DPG, PE, PME	66.7	*iso*-$C_{16:0}$
P. flava	Ara, Gal, Rib	MK-9(H_4)	PC, PME, PI, PG, DPG, PL	69.9	*iso*-$C_{16:0}$, ante*iso*-$C_{17:1}$, *iso*-$C_{16:0}$2-OH
P. halophila	Ara, Gal, Rib	MK-9(H_4)	PI, PG, DPG, PE, PME	65.8	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:1}$cis-9, $C_{16:1}$
P. marina	nr	MK-9(H_4)	PE, PC, PI, PG, DPG, PME	66.1	*iso*-$C_{16:0}$, $C_{16:0}$, *iso*-$C_{15:0}$
P. muralis	Ara, Gal, Glc	MK-9(H_4)	PE, PS, DPG, PG, PI, PL	nr	*iso*-$C_{16:0}$, $C_{16:0}$, $C_{17:1}\omega6c$, $C_{17:0}$
P. salsuginis	Ara, Gal, Rib	MK-9(H_4)	PC, PME, PI, PG, DPG, PL	69.1	*iso*-$C_{16:0}$, ante*iso*-$C_{17:1}$, *iso*-$C_{16:0}$ 2-OH
P. sediminis	Ara, Gal, Rib	MK-9(H_4)	PC, PME, PI, PG, DPG, PL	69.1	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$ 2-OH, ante*iso*-$C_{17:1}$

Symbols: *nr* not reported
PI phosphatidylinositol, *PL* unknown phospholipids, *PME* phosphatidylmethylethanolamine
[a]Data from: Kim and Goodfellow (1999), Li et al. (2003b, 2009), Schäfer et al. (2010), Wang et al. (2010)
[b]*Ara* arabinose, *Gal* galactose, *Rib* ribose, *Glc* glucose
[c]*PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PS* phosphatidylserine, *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol

Isolation and Maintenance Procedures

Details of the source samples and methods of isolation of the 9 species of *Prauserella* are shown in *Table 37.36*.

Prauserella can be maintained on agar media commonly used for actinobacteria supplemented with 10 % (w/v) NaCl for halophilic and halotolerant strains.

Taxonomy, Historical, and Current

Sequencing of 16S rRNA places the genus *Prauserella* in the order *Pseudonocardiales*. Within the order, *Prauserella* is most closely related to the genus *Saccharomonospora* (Labeda et al. 2010a). *Prauserella halophila* and *Prauserella muralis* share 95.8 % 16S rRNA sequence similarity. *Prauserella flava* and *Prauserella salsuginis* share 100 % 16S rRNA sequence similarity and 56.9 % DNA–DNA relatedness (Li et al. 2009). *Prauserella* can be distinguished from other members of the order *Pseudonocardiales* by chemotaxonomic and morphological features and by comparative 16S rRNA gene sequence analyses (Cook and Meyers 2003; Kim and Goodfellow 1999; Labeda 2010a).

The genus *Prauserella* was proposed by Kim and Goodfellow (1999). The type species *Prauserella rugosa* was isolated from the rumen of a cow and originally designated "*Nocardia rugosa*" (di Marco and Spalla 1957). The type species was later moved to the genus *Amycolatopsis* as *Amycolatopsis rugosa* (Lechevalier et al. 1986), then removed due to differences in fatty acid composition (Henssen et al. 1987; Mertz and Yao 1993), DNA–DNA relatedness (Labeda 1995) and phenotype (De Boer et al. 1990). There are currently 9 species in the genus *Prauserella*. The genus description was emended by (Li et al. 2003b). Members of the genus can be differentiated by the chemotaxonomic features, as well as DNA–DNA relatedness (Li et al. 2003b; Li et al. 2009) and fatty-acid profiles (Li et al. 2009; Schäfer et al. 2010; Wang et al. 2010).

The nine different species of the genus *Prauserella* are *Prauserella rugosa* ATCC 43014^T = CIP 106520^T = DSM 43194^T = NRBC 14506^T = IMRU 3760^T = JCM 9736^T = NCIMB 8926^T = NRRL B-2295^T = VKM Ac-1243^T (Kim and Goodfellow 1999); *Prauserella aidingensis* YIM 90636^T = CCTCC AA 208053^T = DSM 45266^T (Li et al. 2009); *Prauserella alba* YIM 90005^T = CCTCC AA 001016^T = DSM 44590^T = JCM 13022^T (Li et al. 2003b); *Prauserella flava* YIM 90630^T = CCTCC AA 208052^T = DSM 45265^T (Li et al. 2009); *Prauserella halophila* YIM 90001^T = CCTCC AA 001015^T = DSM 44617^T = JCM 13023^T (Li et al. 2003b); *Prauserella marina* MS498^T = CCTCC AA 208056^T = DSM 45268^T (Wang et al. 2010); *Prauserella muralis* CCM 7635^T = CCUG 57426^T = DSM 45305^T = JCM

Table 37.36

Cultural characteristics and methods of isolation of *Prauserella* species[a, b]

Species	Characteristics							
	Aerial mycelium	Aerial mycelium colour	Substrate mycelium colour				Origin of isolation sample	Isolation method
			ISP medium 2	ISP medium 3	ISP medium 4	ISP medium 5		
P. rugosa	–	nr	nr	nr	nr	nr	Rumen of a cow	nr
P. aidingensis	+	White	Brilliant yellow	Slightly gray white	nr	Light gray white	Water from salt lake Xinjiang Province, China	Cellulose–casein multi-salt medium, (Tang et al. 2008); incubated at 37 °C for 3 weeks
P. alba	+	White	Orange yellow	Gray white	Yellow white to light yellow	Yellow white to light yellow	Hypersaline soil, Xinjiang Province, China	Starch–casein media [20 % (w/v) NaCl, pH 7.0] incubated at 28 °C for about 4 weeks
P. flava	–	nr	Brilliant yellow	Brown	nr	Light yellow	Water from salt lake Xinjiang Province, China	Cellulose–casein multi-salt medium, (Tang et al. 2008); incubated at 37 °C for 3 weeks
P. halophila	+	White to yellow	Light yellow	Deep gray white	Light yellow	Light gray white	Hypersaline soil, Xinjiang Province, China	Starch–casein media, pH 7.0, incubated at 28 °C for about 4 weeks
P. marina	+	White	Moderate reddish brown	Pale pink	nr	Light gray white	Ocean sediment, South China sea	Isolated on MOPS-proline agar medium, incubated at 22 °C for 4 weeks
P. muralis	+	White	nr	nr	nr	nr	Wall of a house colonized with moulds, Germany	Agar plates containing mineral agar according to Gauze et al. (1983), incubated at 28 °C for 2 weeks
P. salsuginis	+	White	Brilliant yellow	nr	nr	Light gray white	Water from salt lake Xinjiang Province, China	Cellulose–casein multi-salt medium, (Tang et al. 2008); incubated at 37 °C for 3 weeks
P. sediminis	+	White	Orange yellow	Pale yellow	nr	Deep gray white	Water from salt lake Xinjiang Province, China	Cellulose–casein multi- salt medium, (Tang et al. 2008); incubated at 37 °C for 3 weeks

[a]Symbols: + positive, – negative, *nr* not reported

[b]Data from: Kim and Goodfellow (1999), Li et al. (2003b, 2009), Schäfer et al. (2010), Wang et al. (2010)

17974^T = NRRL B-24780^T (Schäfer et al. 2010); *Prauserella salsuginis* YIM 90625^T = CCTCC AA 208051^T = DSM 45264^T; and *Prauserella sediminis* YIM 90694^T = CCTCC AA 208054^T = DSM 45267^T (Li et al. 2009)

The species of the genus *Prauserella* can be distinguished from each other on the basis of their cultural and physiological characteristics as shown in *Tables 37.36* and *37.37*, respectively.

Table 37.37

Physiological characteristics of *Prauserella* species[a, b]

Species	Characteristic																											
	Temperature		Growth in NaCl:		Degradation of:		Carbon source utilization:																	Nitrogen Utilization					
	Range (°C)	Optimum (°C)	Range (%)	Optimum (%)	Gelatin	Urea	L — Arabinose	Cellobiose	D — Fructose	D — Galactose	myo — Inositol	Lactose	Maltose	D — Mannitol	D — Mannose	Raffinose	L — Rhamnose	D — Ribose	Trehalose	D — Xylitol	D — Xylose	L — Arginine	L — Glycine	L — Hydroxyproline	L — Lysine	L — Serine	L — Threonine
P. rugosa	10–45	34	0–20	5–10	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–
P. aidingensis	15–45	28–37	5–15	8–10	+	–	–	–	+	+	–	+	–	–	–	–	+	+	+	–	+	–	–	+	–	–	+
P. alba	10–45	28	0–25	10–15	+	–	+	+	+	+	+	nr	+	+	nr	nr	+	+	–	+	+	+	+	+	+	+	+
P. flava	15–45	28–37	5–15	8–10	+	–	–	–	+	–	+	+	–	–	–	–	–	+	–	–	+	–	–	+	+	–	+
P. halophila	10–45	28	5–25	10–15	+	+	–	+	+	–	+	nr	–	+	nr	nr	+	+	+	+	+	+	+	+	+	+	+
P. marina	15–45	28–37	0–10	0–5	+	–	+	–	–	+	–	–	+	+	+	–	+	+	+	–	+	+	nr	+	–	+	+
P. muralis	nr	25–30	nr	nr	nr	nr	+	+	+	+	–	nr	+	+	+	nr	+	+	+	nr	+	nr	nr	nr	nr	nr	nr
P. salsuginis	15–45	28–37	5–15	8–10	+	–	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	–	–	+	+	–	+
P. sediminis	15–45	28–37	5–20	10	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	+	+	–	+

[a]Data from: Kim and Goodfellow (1999), Li et al. (2003b, 2009), Schäfer et al. (2010), Wang et al. (2010)

[b]Symbols: + positive, – negative, nr not reported

Saccharomonospora Nonomura and Ohara 1971, 899[AL]

Sac.cha.ro.mon.o.spo'ra. Gr. n. *sakchâr* sugar; Gr. adj. *monos* single, solitary; Gr. fem. n. *spora* seed; N.L. fem. n. *spora* a spore; N.L. fem. n. *Saccharomonospora* the sugar (−containing) single-spored (organism).

Phenotypic Analyses

Saccharomonospora cultures consist of rarely fragmented substrate mycelia with various pigmentation as shown in Table 37.38. Aerial mycelium is white, yellow white, green and dark to light blue. Both substrate and aerial mycelia are irregularly branched. Spores are single or paired and occasionally in short chains and are ovoid, ellipsoidal, or round (0.7–1.1 × 1.0–1.8 μm) in shape. Spore surfaces are smooth, warty, or wrinkled. *Saccharomonospora* species are Gram-stain positive, aerobic, chemoorganotrophic, and thermotolerant or mesophilic. As can be seen in Table 37.39, the cell wall contains *meso*-diaminopimelic acid, arabinose, and galactose. The diagnostic phospholipid is phosphatidylinositol, but some species may also contain diphosphatidylglycerol. The predominant menaquinone is MK-9(H_4), while MK-8(H_4), MK-9(H_2), and MK-7(H_4) are variably present. Mycolic acids are not present. The temperature range for growth is 24–60 °C and sodium chloride is required for growth of some species (Table 37.39).

The G+C content of the DNA of members of this genus is 68.1–71.8 (mol%).

The type species is *Saccharomonospora viridis* ATCC 15386^T = CCUG 5913^T = DSM 43017^T = IFO NBRC 12207^T = JCM 3036^T = NRRL B-3044^T = VKM Ac-681^T (Schuurmans et al. 1956; Nonomura and Ohara 1971).

Isolation and Maintenance Procedures

Details of the source samples and methods of isolation of species of this genus are shown in Table 37.38.

Taxonomy, Historical, and Current

Saccharomonospora viridis was published as "*Thermoactinomyces viridis*" (Schuurmans et al. 1956) and later reclassified as "*Thermomonospora viridis*" (Küster and Locci 1963). Nonomura and Ohara (1971) proposed the genus *Saccharomonospora* based on morphological and chemotaxonomic criteria, later supported by phenetic data (Goodfellow and Pirouz 1982; McCarthy and Cross 1984).

The genus *Saccharomonospora* currently contains nine species. Recently, the sequenced genome (4.8 Mb) of *Saccharomonospora azurea* type strain (NA-128^T) was published and predicted to contain 4,472 protein coding and 58 RNA genes (Klenk et al. 2012).

The nine different species of the genus *Saccharomonospora* are *Saccharomonospora viridis* ATCC 15386^T = CCUG 5913^T = DSM 43017^T = NRBC 12207^T = JCM 3036^T = NRRL B-3044^T = VKM Ac-681^T (Schuurmans et al. 1956; Nonomura and Ohara 1971); *Saccharomonospora azurea* NA-128^T = SIIA 86128^T = ATCC 43670^T = DSM 44631^T = NRBC 14651^T = JCM 7551^T (Runmao 1987); *Saccharomonospora cyanea* NA-134^T = SIIA 86134^T = ATCC 43724^T = DSM 44106^T = NRBC 14841^T = JCM 7552^T (Runmao et al. 1988); *Saccharomonospora glauca* K62^T = DSM 43769^T = NRBC 14831^T = JCM 7444^T (Greiner-Mai et al. 1988); *Saccharomonospora halophila* DSM 44411^T = JCM 11761^T = NRRL B-24125^T (Al-Zarban et al. 2002); *Saccharomonospora marina* CCTCC AA 209048^T = KCTC 19701^T (Liu et al. 2010); *Saccharomonospora paurometabolica* BCRC 16315^T = CCTCC AA 00101^T = DSM 44619^T = JCM 13241^T = YIM 90007^T (Li et al. 2003a); *Saccharomonospora saliphila* YIM 90502^T = DSM 45087^T = JCM 15627^T = KCTC 19234^T (Syed et al. 2008); and *Saccharomonospora xinjiangensis* CCTCC AA 97021^T = DSM 44391^T = JCM 11270^T (Jin et al. 1998).

The genera most closely related to *Saccharomonospora* are *Prauserella* and *Thermocrispum* (Kim and Goodfellow 1999). The type strains share 95.2–98.5 % 16S rRNA gene sequence similarity. The DNA–DNA relatedness of *Saccharomonospora saliphila* to *Saccharomonospora azurea*, *Saccharomonospora halophila*, and *Saccharomonospora paurometabolica* is 46.0 %, 41.0 %, and 42.5 %, respectively (Syed et al. 2008). The DNA–DNA relatedness of *Saccharomonospora paurometabolica* and *Saccharomonospora halophila* is 53.8 % (Li et al. 2003a).

The production of single spores at the end of aerial hyphae allows *Saccharomonospora* to be distinguished from other members of the family *Pseudonocardiaceae*. The absence of fragmented substrate hyphae differentiates the genus from *Prauserella*, *Saccharopolyspora*, and *Thermoscrispum*. The absence of sporangia-like structures differentiates the genus from *Crossiella*, *Kibdelosporangium*, *Kutzneria*, *Streptoalloteichus*, and *Thermocrispum*. Phospholipid patterns type PII and type PIV distinguish *Saccharomonospora* from *Saccharopolyspora* (type PIII).

Saccharomonospora isolates can be detected by a PCR-based method (Salazar et al. 2000) and by fluorescence in situ hybridization (FISH) (Neef et al. 2003).

Isolation, Enrichment and Maintenance Procedures

Saccharomonosporae have been isolated from a wide range of habitats as shown in Table 37.39. *Saccharomonospora* have been isolated on modified glycerol–asparagine agar, half-strength tryptone–soy agar, HV agar (Hayakawa 1990) and R8 agar (Amner et al. 1989). Cycloheximide (50 μg/ml) can prevent bacterial growth. *Saccharomonospora* can be maintained on yeast extract and malt extract (ISP 2) agar, inorganic salts–starch (ISP 4) agar, CzapekDox yeast extract–Casamino acids (CYC)

Table 37.38

Cultural characteristics and methods of isolation of *Saccharomonospora*[a, b] species

Species	Characteristics					Origin of isolation sample	Method of isolation
	Aerial mycelium colour	Substrate mycelium colour	Colour of diffusible pigment	Spores in pairs	Spore ornamentation		
S. viridis	Green	Green	Green	–	Warty	Manure, compost, overheated fodder, soil, lake sediments, peat	nr
S. amisosensis	White	Yellowish to pale orange	Moderate reddish orange	+	nr	Sediment sample; Southern back sea coast, Turkey	Isolated from the sediment sample by using SM3 medium (Tan et al. 2006), supplemented with filter sterilized cycloheximide (50 μg ml^{-1}), nalidixic acid (10 μg ml^{-1}), novobiocin (10 μg ml^{-1}) and nystatin (50 μg ml^{-1}); incubated at 28 °C for 30 days.
S. azurea	Azure	nr	Absent	–	Smooth	Soil, Sichuan, China	Oatmeal agar (ISP 3); incubated at 28 °C for 2–4 weeks
S. cyanea	Light to dark blue	nr	Absent	–	Warty	Soil, Sichuan, China	Procedure of Shirling and Gottlieb (1966) with a basal mineral salts agar (CM medium).
S. glauca	Light blue to greenish	Dark green	Dark green	–	Warty	Mouldy hay, soil, compost, and manure	nr
S. halophila	Light blue to greenish	nr	Absent	+	Warty	Marsh soil, Kuwait	nr
S. marina	White, gray to orange-white	nr	nr	+	Smooth/ wrinkled	Ocean sediment, East China sea	PLA emulsified agar (Pranamuda and Tokiwa 1999) at 28 °C for 4 weeks
S. paurometabolica	White	Orange to yellow, light yellow brown, light yellow orange, white	Absent	–	Smooth/ wrinkled	Soil sample, Xinjiang Province, China	Modified glycerol/asparagine agar (Shirling and Gottlieb 1966) [ISP5 medium containing 20 % (w/v) NaCl] and incubated at 28 °C for about 4 weeks
S. saliphila	Gray red	Grayish red, dark red, blackish red	Absent	+	Smooth/ wrinkled	Muddy soil, Karnataka, India	Modified glycerol/asparagine agar (Shirling and Gottlieb 1966) [ISP5 medium containing 20 % (w/v) NaCl] and incubated at 28 °C for about 4 weeks
S. xinjiangensis	Yellow-white, light green gray	Light yellow	Light yellow-brown	+	Smooth	Soil, Xingiang Province, China	Glycerol–asparagine agar and HV agar

[a]Data from: Schuurmans et al. (1956), Runmao (1987), Runmao et al. (1988), Greiner-Mai et al. (1988), Al-Zarban et al. (2002), Liu et al. (2010), Li et al. (2003a), Syed et al. (2008), Jin et al. (1998), and Veyisoglu et al. (2013)

[b]Symbols: + positive, – negative, *nr* not reported

Table 37.39

Physiological and chemotaxonomic characteristics of *Saccharomonospora*[a] species

Species	Growth on sole carbon source (1 %, w/v)									Growth in NaCl, %, (w/v)										
	L – Arabinose	Galactose	Glucose	Mannitol	Mannose	Melibiose	Rhamnose	Ribose	Xylose	0	5	7	10	20	30	Growth temperature, °C	Major menaquinone(s)	Phospholipids[b]	Whole-cell sugars[c]	DNA G+C content (mol%)	Predominant fatty acids
S. viridis	–	–	Dbt	Dbt	–	nr	–	nr	–	+	–	–	–	–	–	35–50	MK-9(H_4)	PI, PIM, DPG, acyl-PG	Ara, Gal	69	nr
S. azurea	–	–	+	–	+	+	–	+	+	+	+	+	–	–	–	24–40	nr	nr	Ara, Gal	nr	nr
S. amisosensis	nr	+	–	+	+	nr	+	nr	–	+	+	+	+	–	–	28–45	MK-9(H_4) (62 %); MK8(H_4) (22 %)	DPG, PE, PI, PIM	Ara, Gal, (Glc, Xyl)	68.9	*iso*-$C_{16:0}$, *iso*-$C_{16:0}$ 2-OH, $C_{16:1}$*cis* 9
S. cyanea	nr	+	–	–	+	–	nr	+	Dbt	+	–	–	–	–	–	24–40 (28–37)	nr	nr	Ara, Gal	nr	nr
S. glauca	+	nr	+	+	nr	nr	–	nr	+	+	–	–	–	–	–	37–60 (50)	MK-9(H_4) (60 %), MK8(H_4) (20–30 %)	OH-PE, lyso-PE	Ara, Gal	nr	nr
S. halophila	+	+	+	+	+	+	+	Dbt	–	–	–	–	+	+	+	(28–30)	MK-9(H_4) (88 %), MK 8(H_4) (12 %)	PI, DPG, OH-PE, lyso-PE	Ara, Gal	nr	*iso*-$C_{16:0}$, $C_{16:0}$, $C_{16:1}$, *iso*-$C_{16:0}$ 2-OH
S. marina	+	+	+	+	+	+	+	–	+	+	+	–	–	–	–	(28–37)	MK-9(H_4) (90 %), MK8(H_4) (10 %)	PI, PG, DPG	Ara, Gal	68.1	*iso*-$C_{16:0}$, $C_{17:1}$ ω6c, $C_{15:0}$, $C_{16:0}$, $C_{17:1}$ ω8c, *iso*-$C_{16:1}$ H
S. paurometabolica	–	–	–	–	nr	–	–	–	–	–	+	+	+	+	+	(35–37)	MK-9(H_4) (90 %), MK-9(H_2) (10 %)	PI, PG, DPG, OH-PE	Ara, Gal, Rib	71	$C_{18:1}$, $C_{16:0}$, *iso*-$C_{16:0}$
S. saliphila	–	+	–	+	–	+	+	nr	+	+	+	+	+	+	–	(28–30)	MK-9(H_4) (90 %), MK8(H_4) (10 %)	PI, PG, DPG	Ara, Gal	71.8	*iso*-$C_{16:0}$, $C_{17:1}$ ω6c, $C_{15:0}$, *iso*-$C_{16:1}$ OH
S. xinjiangensis	nr	nr	nr	nr	+	nr	+	nr	+	+	–	–	–	–	–	45–50	MK-9(H_2), MK-9(H_4), MK- 7(H_4)	PC, GluNU	Ara, Gal	nr	$C_{17:1}$ ω8c, $C_{17:1}$ ω6c, $C_{16:0}$, $C_{17:0}$, $C_{15:0}$

Symbols: + positive, – negative, *nr* not reported, *Dbt* doubtful

[a]Data from: Schuurmans et al. (1956), Runmao (1987), Runmao et al. (1988), Greiner-Mai et al. (1988), Al-Zarban et al. (2002), Liu et al. (2010), Li et al. (2003a), Syed et al. (2008), Jin et al. (1998) and Veyisoglu et al. (2013)

bAll species contain phosphatidylethanolamine (*PE*); *OH-PE* hydroxyphosphatidylethanolamine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PC* phosphatidylcholine, *GluNU* glucosamine-containing phospholipid

[c]*Ara* arabinose, *Gal* galactose, *Glc* glucose, *Rib* ribose, xylose

agar (Cross and Attwell 1974), starch–nitrate agar with 10 % (w/v) NaCl (Al-Zarban et al. 2002), and tryptic soy agar (TSA).

Pathogenicity and Clinical Relevance

Saccharomonospora viridis may be one of the causative agents of hypersensitivity pneumonitis including farmer's lung disease (Greene et al. 1981; Harvey et al. 2001; Roberts et al. 1976; Treuhaft et al. 1980; Wenzel et al. 1974).

Application

Several strains produce antibiotics effective against Gram-positive bacteria, including *Saccharomonospora viridis*, *Saccharomonospora glauca*, "*Saccharomonospora caesia*," and "*Saccharomonospora internatus*" (Greiner-Mai et al. 1988). *Saccharomonospora viridis* produces thermoviridin (Schuurmans et al. 1956).

Several *Saccharomonospora* produce enzymes that degrade proteins, starch, mushroom compost, rice straw, synthetic food waste compost, and polyester (Abdulla and El-Shatoury 2007; Collins et al. 1992; Dolashka et al. 1998; Song et al. 2001; Tseng et al. 2007).

Saccharopolyspora Lacey and Goodfellow 1975, 76^{AL} emended Korn-Wendisch, Kempf, Grund, Kroppenstedt and Kutzner 1989, 438

Sac.cha.ro.po.ly.spo'ra.N.L. n. *Saccharum* generic name of sugar cane; Gr. adj. *polus* many; Gr. n. *spora* a seed, and in biology a spore; N.L. fem. n. *Saccharopolyspora* the many spored (organism) from sugar cane.

Phenotypic Analyses

Cultures of *Saccharopolyspora* consist of branched substrate mycelium that may remain intact or fragment into rod-shaped and/or coccoid structures. Fragmentation occurs in older parts of cultures. Colonies have a wrinkled surface and are thin, raised, or convex. Substrate hyphae are 0.4–0.6 μm in diameter, buff, brownish red, orange, or yellow and mucoid or gelatinous. Aerial hyphae segment into bead-like chains of spores inside a smooth sheath. Aerial hyphae are 0.5–0.7 μm in diameter, white to gray or pinkish white. Spore chains are straight, hooked, loops, or loose spirals. Spore shape is round to oval, 0.7–1.3 × 0.5–0.7 μm in size, with a hairy, smooth, spiny, rough, or warty surface. The cultural characteristics distinguishing each *Saccharopolyspora* species is shown in ➤ *Table 37.40*.

As shown in ➤ *Table 37.41*, the cell wall contains *meso*-diaminopimelic acid and whole-cell hydrolysates contain arabinose and galactose. Diagnostic phospholipids are diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylcholine. The predominant menaquinone is MK-9(H_4), but *S. thermophila* contains MK-9(H_6). Predominant fatty acids are iso-$C_{16:0}$ and anteiso-$C_{17:0}$. No mycolic acids are present.

The G+C content of the DNA of species of *Saccharopolyspora* ranges from 66.3 to 76.9 (mol%).

The utilization of sole carbon sources is shown in ➤ *Table 37.41*.

The type species is *Saccharopolyspora hirsuta* (Lacey and Goodfellow 1975) ATCC 27875^T = DSM 43463^T = NBRC 13919^T = JCM 3170^T = NRRL B-5792^T = NRRL B-16205^T = VKM Ac-666^T.

Isolation Procedures

Details of the source samples and methods of isolation of species of this genus are shown in ➤ *Table 37.40*.

Taxonomy, Historical, and Current

The genus *Saccharopolyspora* was originally proposed in 1975 and can be distinguished by the production of aerial mycelia with bead-like chains of spores in a hairy sheath (Lacey and Goodfellow 1975). Sequence analysis of 16S rRNA genes places *Saccharopolyspora* in the family *Pseudonocardiaceae* (Labeda et al. 2010a; Lu et al. 2001). The genus currently has 21 species, ten of which were identified only in the last 10 years. The most closely related *Saccharopolyspora* species are *Saccharopolyspora hirsuta* subsp. *kobensis* and *Saccharopolyspora jiangxiensis* with 16S rRNA gene sequence similarity of 99 %. The most distantly related are *Saccharopolyspora erythraea* and *Saccharopolyspora thermoflava* with a sequence similarity of 92 %.

Saccharopolyspora and *Streptomyces* strains (Embley et al. 1988) are phylogenetically distant, evident in the poor expression of erythromycin biosynthesis genes in *Streptomyceslividans* (Stanzak et al. 1986; Thompson et al. 1982). Similarly, *Streptomyces* phages may be unstable in *Saccharopolyspora* strains (Gayer-Herkert et al. 1989; Yamamoto et al. 1986). Both *Saccharopolyspora erythraea* and *Saccharopolyspora rectivirgula* have been successfully transformed (Gayer-Herkert et al. 1989; Yamamoto et al. 1986).

Isolation, Enrichment, and Maintenance Procedures

Saccharopolyspora have been isolated from a variety of sources (see ➤ *Table 37.40*) on various media, including half-strength nutrient agar (Lacey 1971, 1974), starch–casein–arginine agar (Iwasaki et al. 1979), R8 agar (Amner et al. 1989), half-strength tryptone–soy agar supplemented with casein hydrolysates (Lacey 1989), yeast extract and malt extract agar (Yuan et al. 2008), oatmealagar (Lu et al. 2001), cellulose–casein multi-salts agar (Tang et al. 2009a), and starch–casein agar supplemented with ketoconazole and nalidixic acid (Duangmal et al. 2010). To inhibit fungal growth, medium can be supplemented with

Table 37.40

Cultural characteristics and methods of isolation of the type strains of species belonging to the genus *Saccharopolyspora*

Species	Characteristics					Origin of isolation sample	Method of isolation	References
	Spore chains	Spore surfaces	Aerial mycelium colour	Substrate mycelium colour	Diffusible pigment colour			
Saccharopolyspora hirsuta	Straight to loose spirals	Hairy	White	Colourless to buff	Yellow	Soil	nr	Korn-Wendisch et al. (1989)
Saccharopolyspora hirsute subsp. *kobensis*	nr	nr	White	Yellow to pink	Yellow to red	Soil	nr	Kim and Goodfellow (2012)
Saccharopolyspora antimicrobica	Straight	Rough	White	White to buff to pink	Buff, pink to brown	Soil, Beijing and Sichuan, China	Yeast extract and malt extract agar medium (ISP 2; Shirling and Gottlieb 1966), incubated at 28 °C for 21 days	Yuan et al. (2008)
Saccharopolyspora cebuensis	Straight	Smooth	White	White	Brown	Marine sponge (*Haliclona* sp.); Cebu, Philippines	Isolated on M1 agar	Pimentel-Elardo et al. (2008)
Saccharopolyspora erythraea	Open spirals	Smooth	Pink to Brownish gray to white	Orangish yellow to reddish brown	Faint yellow to pinkish orangish brown	Soil	nr	Korn-Wendisch et al. (1989)
Saccharopolyspora flava	Straight	Smooth	White	Yellow	–	Garden soil; Xishan, China	Oatmeal agar plate (Shirling and Gottlieb 1966) seeded with a soil suspension; incubated at 28 °C for 5 days	Lu et al. (2001)
Saccharopolyspora gloriosae	Hooks/ curved	Smooth	White	Pale orange - yellow	–	Stem of *Gloriosa superba* L; Yunnan, China	Tap water–yeast extract agar (Crawford et al. 1993), incubated at 28 °C for 4 weeks	Qin et al. (2010a)
Saccharopolyspora gregorii	Hooks/ flexuous	Smooth	White to yellow	Colourless to buff	Pale yellowish to buff	Grass, hay, straw, barley grain, and soil	Half-strength nutrient agar supplemented with cycloheximide and a wind tunnel/ Andersen sampler method (Gregory and Lacey 1963; Lacey 1971)	Goodfellow et al. (1989)
Saccharopolyspora halophila	Straight	Smooth	White to yellow	Yellow to orangish yellow	–	Saline lake; Xinjiang, Northwest China	Cellulose-casein multi-salt (CCMS) medium; incubated at 37 °C for 3 weeks	Tang et al. (2009a)
Saccharopolyspora hordei	Hooks/ spirals	Smooth	White to yellow	Colourless to buff	Colourless to light buff	Barley and oat grains, grass hay, straw, and sugar cane bagasse	Half-strength nutrient agar supplemented with cycloheximide and a wind tunnel/ Andersen sampler method (Gregory and Lacey 1963; Lacey 1971)	Goodfellow et al. (1989)
Saccharopolyspora jiangxiensis	Straight to flexuous	Smooth or irregularly rough	White-buff	Colourless to buff	–	Grass field soil; Jiangxi, China	Glucose–yeast extract and malt extract medium (GYM), incubated at 28 °C for 14 days	Zhang et al. (2009)

Saccharopolyspora lacisalsi	Bead-like chains	Smooth	White, yellowish gray	Yellow, yellowish bluff	–	Sediment of Lop Nur salt lake; Xinjiang, North-west China	Isolated on YC medium containing (g l^{-1}): yeast extract, 3.0 g; casein hydrolysate acid, 0.5 g; KNO_3, 0.5 g; $CaCO_3$, 0.1 g; NaCl, 200 g; KCl, 20 g; $MgCl_2$, 2 g; 1.2 ml trace element solution (Slobodkin et al. 1997); agar, 20.0 g; pH 7.5	Guan et al. (2011)
Saccharopolyspora phatthalungensis	Hooks/ open loops	Spiny	White	Yellowish to yellowish brown	Brownish black	Rhizosphere soil of rubber trees(*Hevea brasiliensis*); Phatthalung, Thailand	Starch casein agar plates (Küster and Williams 1964) supplemented with ketoconazole and nalidixic acid as antifungal and antibacterial agents; incubated at 28 °C for 14 days	Duangmal et al. (2010), Kim and Goodfellow (2012)
Saccharopolyspora qijiaojingensis	Straight	Smooth	White to yellow	White to yellow	–	Salt lake; Xinjiang, China	Cellulose-casein-multisalts (CCMS) medium, described by Tang et al. (2008), incubated at 28 °C	Tang et al. (2009b)
Saccharopolyspora rectivirgula	Straight	Smooth or irregularly rough	White to light pink	Yellow to orange	–	Mouldy hay, soil, compost, and manure	nr	Korn-Wendisch et al. (1989)
Saccharopolyspora rosea	Straight	Smooth	Yellowish to white	Yellowish to brownish yellow	Pink	Bronchial lavage sample	Löwenstein–Jensen medium and Columbia agar supplemented with 5 % sheep blood agar and brain–heart infusion agar	Yassin (2009)
Saccharopolyspora shandongensis	Spiral	Spiny	White	Colourless to buff	Brown	Wheat-field soil; Shandong Province, China	Glucose–yeast extract and malt extract (GYM), incubated at 28 °C for 2 weeks	Zhang et al. (2008)
Saccharopolyspora spinosa	Hooks/ open loops	Spiny	White to pink	Gray to orangish yellow to brown	Brown	soil collected in a sugar mill rum still; Virgin Islands	Medium containing 30 g of tryptic soy broth, 3 g of yeast extract, 2 g of $MgS0_4 \cdot 7H_2O$, 5 g of glucose, and 4 g of maltose in 1 L of deionized water with novobiocin as inhibitor, incubated at 30 °C for 72 h	Mertz and Yao (1990)
Saccharopolyspora spinosporotrichia	Spiral	Warty	White-grey	Brown to red	Brown	Soil; China	nr	Zhou et al. (1998)
Saccharopolyspora taberi	–	–	–	Colourless - yellow	–	Soil	nr	Korn-Wendisch et al. (1989)
Saccharopolyspora thermophila	Hooks/ flexuous	Smooth	White	Colourless to buff	–	Garden soil; Xishan, China	Oatmeal agar plate (Shirling and Gottlieb 1966), 28 °C for 5 days	Lu et al. (2001)
Saccharopolyspora tripterygii	Straight	Smooth	White	Orange to yellow	–	Stem sample of *Tripterygium hypoglaucum*; Yunnan, China	Isolation procedures were performed as described by Li et al. (2009)	Li et al. (2009)

Symbols: – negative/absent, *nr* not reported

Table 37.41

Physiological and chemotaxonomic properties of the type strains of species belonging to the genus *Saccharopolyspora*[a, b]

Species	Characteristics																			
	Degradation of:													*Growth on carbon sources:*						
	Adenine	Casein	Chitin	Esculin	Elastin	Hypoxanthine	Starch	Tyrosine	Urea	Xanthine	Nitratereduction	NaCltolerance(w/v)	Temperature range (°C)	L – Arabinose	D – Galactose	D – Lactose	Maltose	Mannitol	Raffinose	L – Rhamnose
Saccharopolyspora hirsuta	+	+	–	+	+	+	+	+	+	+	–	<7	25–50	–	+	+	+	+	+	+
Saccharopolyspora hirsute subsp. *kobensis*	+	nr	nr	+	nr	nr	+	nr	nr	+	nr	12	20–42	nr	nr	nr	nr	nr	nr	nr
Saccharopolyspora antimicrobica	+	+	–	+	–	+	+	+	+	–	+	≤7	20–45	+	+	+	+	+	+	+
Saccharopolyspora cebuensis	–	–	–	+	nr	–	+	+	nr	nr	–	2.5–12.5	15–37	+	+	+	+	–	+	+
Saccharopolyspora erythraea	+	–	+	+	+	+	+	+	nr	+	+	<5	20–42	+	+	–	+	+	+	+
Saccharopolyspora flava	+	–	–	+	–	+	+	–	+	+	+	7	28–37	–	+	+	+	+	+	+
Saccharopolyspora gloriosae	+	–	–	+	nr	+	+	+	nr	+	–	≤11	10–32	+	–	–	+	+	–	+
Saccharopolyspora gregorii	–	+	–	+	+	+	+	+	_	+	_	13	10–35	+	+	–	+	+	+	+
Saccharopolyspora halophila	–	+	–	+	nr	+	–	+	+	nr	+	3–20	10–45	+	+	+	+	+	+	+
Saccharopolyspora hordei	+	+	+	+	+	–	+	+	–	+	–	<13	20–60	+	+	+	+	+	+	+
Saccharopolyspora jiangxiensis	+	–	–	+	+	–	+	+	+	+	+	<11	15–45	+	+	+	+	+	+	+
Saccharopolyspora lacisalsi	–	–	nr	nr	nr	–	–	–	–	–	+	5–25	25–42	–	+	–	+	nr	+	–
Saccharopolyspora phatthalungensis	–	+	–	–		+	–	+	+	–	–	<7	18–42	–[e]	+[e]	nr	–[e]	+[e]	–[e]	–[e]
Saccharopolyspora qijiaojingensis	–	–	–	–	nr	+	–	–	–	+	–	6–22	20–40	–	+	+	+	+	nr	+
Saccharopolyspora rectivirgula	–	–	–	+	–	+	+	+	+	+	+	<10	37–63	–	+	+	+	+	+	+
Saccharopolyspora rosea	+	+	nr	+	–	+	nr	+	+	–	–	nr	22–42	+	+	–	+	+	–	–
Saccharopolyspora shandongensis	+	+	–	+	+	+	+	+	+	+	+	<7	15–38	+	+	–	+	+	+	+
Saccharopolyspora spinosa	–	–	–	+	–	+	–	+	+	–	+	<11	15–37	+	–	–	–	+	–	–
Saccharopolyspora spinosporotrichia	–	+	–	+	+	nr	+	–	+	–	–	2–3	28–37	–	+	–	+	+	+	+
Saccharopolyspora taberi	+	+	+	+	+	+	+	+		+	+	7	20–4	–	+	+	+	+	+	+
Saccharopolyspora thermophila	+	–	–	+	–	+	+	+		–	–	7	45–55	–	+	+	+	+	+	+
Saccharopolyspora tripterygii	nr	nr	nr	+	nr	nr	–	nr	+	nr	–	≤12	10–37	+	+	+	+	+	+	–

PL unknown phospholipid, *PME* phosphatidylmethylethanolamine, *GL* unknown glycolipid, *PIM* phosphatidylinositol mannosides

[a]Data for type strains. All strains were positive for the utilization of fructose, glucose, and mannose as the sole carbon sources for energy and growth

[b] + positive, – negative, *nr* not reported

[c]*Gal* galactose, *Glc* glucose, *Ara* arabinose, *Rib* ribose, *Xyl* xylose

[d]Determined by acid production from substrate

[e]*PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol

Sucrose	d − Xylose	DNAG + Ccontent(mol%)	Phospholipids[c]	Major menaquinones	Whole-cell sugars[d]	Predominant fatty acids	References
+	+	nr	PG, DPG, PI, PE, PC, PME, *lyso*-PE, OH-PE	MK-9(H_4), MK-9(H_2)	Ara, Gal	nr	Korn-Wendisch et al. (1989)
nr	nr	nr	nr	nr	nr	nr	Kim and Goodfellow (2012)
+	+	69.3	DPG, PC, PG, PI	MK-9(H_4)	Ara, Gal	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$	Yuan et al. (2008)
+	+	72.6	PC, PE, PME, DPG, PG, PI, PL	MK-9(H_4)	Ara, Gal, Glc, Rib	nr	Pimentel-Elardo et al. (2008)
+	+	76.9	PG, DPG, PI, PE, PC, PME, *lyso*-PE	MK-9(H_4), MK-10 (H_4)	Ara, Gal	nr	Korn-Wendisch et al. (1989)
+	+	67	PC	MK-9(H_4)	Ara, Gal	nr	Lu et al. (2001)
−	+	71.6	PC, PE, PME, DPG, PG, PI, PIM, PL	MK-9(H_4)	Ara, Gal, Glc, Rib	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:1}$*cis*9	Qin et al. (2010a)
+	+	74	DPG, PG, PI, PL, GL, PC, PE	MK-9(H_4)	Ara, Gal	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{17:0}$	Goodfellow et al. (1989)
+	+	66.3	DPG, PC, PI	MK-9(H_4)	Glc, Ara, Rib	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$	Tang et al. (2009a)
+	+	72	DPG, PG, PI, PL, GL, PC, PE	MK-9(H_4)	Ara, Gal	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{17:0}$	Goodfellow et al. (1989)
+	+	70.3	PC, PE, PG	MK-9(H_4)	Ara, Gal	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{17:0}$	Zhang et al. (2009)
+	−	68.2	PG, PC, PI, PL	MK-9(H_4), MK-9(H_6)	Ara, Glc, Rib, Xyl	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$	Guan et al. (2011)
−[e]	+[e]	70.3	PC, PG, PI	MK-9(H_4)	Ara, Gal	$C_{16:0}$, $C_{17:0}$10-methyl	Duangmal et al. (2010), Kim and Goodfellow (2012)
−	−	70.1	DPG, PC, PI, PE, PIM, PL	MK-9(H_4)	Ara, Gal	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{17:0}$	Tang et al. (2009b)
+	+	70.4	PG, DPG, PI, PE, PC, PME, *lyso*-PE	MK-9(H_4), MK-10(H_4)	Ara, Gal	*iso*-$C_{16/18}$, *iso*-$C_{15/17}$, ante*iso*-$C_{15/17}$	Korn-Wendisch et al. (1989)
+	+	nr	DPG, PG, PC, PI	MK-9(H_4), MK-10(H_4)	Ara, Gal	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{17:0}$	Yassin (2009)
+	+	70.1	PC	MK-9(H_4)	Ara, Gal	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{17:0}$, *iso*-$C_{18:0}$, $C_{17:1}$ ω8c	Zhang et al. (2008)
−	−	nr	PC	MK-9(H_4)	Ara, Gal	*iso*-$C_{16:0}$, *iso*-$C_{17:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{15:0}$	Mertz and Yao (1990)
+	+	70.4	PC	MK-9(H_4)	Ara, Gal	nr	Zhou et al. (1998)
+	+	70.8	PG, DPG, PI, PE, PC, PME, *lyso*-PE	MK-9(H_4), MK-10(H_4)	Ara, Gal	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{17:0}$	Korn-Wendisch et al. (1989)
+	−	73.1	nr	MK-9(H_6), MK-9(H_8)	nr	nr	Lu et al. (2001)
−	+	70.5	DPG, PG, PME, PE, PC	MK-9(H_4)	Ara, Glc, Gal, Rib	*iso*-$C_{15:0}$, *iso*-$C_{18:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{17:0}$	Li et al. (2009)

50 µg/ml cycloheximide (Cross et al. 1968). The ideal pH range for most strains is 5.0–9.0 (Qin et al. 2008a; Tang et al. 2009b; Zhang et al. 2009) with growth occurring at temperatures from 28 °C to 40 °C.

Application

Saccharopolyspora erythraea produces erythromycin A, erythronolide B (Martin and Rosenbrook 1967), a trypsin-like protease (Yoshida et al. 1971), a rennin-like enzyme (Sternberg 1976), and an *N*-acetylmuramidase (Morita et al. 1978). *Saccharopolyspora spinosa* produces the glycosylated polyketide-derived macrolide insecticides, spinosyn A and D (Hong et al. 2006, 2008; Huang et al. 2009).

Saccharopolyspora aurantica produces pesticidal compounds designated CL307-24 (Etienne et al. 1993). *Saccharopolyspora hirsuta* produces a cyclic polyketide, nodusmicin (Whaley et al. 1980); the macrolide, nargenicin A (Ikeda et al. 1985); and the aminoglycoside complex, apramycin (Kamiya et al. 1983; O'Connor et al. 1976).

Saccharopolyspora hirsuta subsp. *kobensis* produces sporaricin and related compounds (Deushi et al. 1979; Umezawa et al. 1987). *Saccharopolyspora* sp. strain AC 3440 synthesizes 4-deamino-4-hydroxyapromycin (Awata et al. 1983).

Bacteriophages

Saccharopolyspora phages have an icosahedron capsid enclosing a double-stranded genome with *cos* termini and variable tail length (Grund and Hutchinson 1987; Katz et al. 1988; Kurup and Heinzen 1978; Schneider et al. 1987; Schneider and Kutzner 1989). The use of *Saccharopolyspora* phages as cloning vectors (Katz et al. 1988; Schneider and Kutzner 1989) has mostly focused on those isolated from *Saccharopolyspora erythraea* and *Saccharopolyspora rectivirgula* (Donadio et al. 1986; Grund and Hutchinson 1987; Katz et al. 1988; Kempf et al. 1987; Schneider et al. 1987). Methods were adapted from *Streptomyces* species (Hopwood et al. 1985; Lanning and Williams 1982), with modifications to baiting and indicator strains (Greiner-Mai et al. 1987; Grund and Hutchinson 1987; Kurup and Heinzen 1978).

Saccharopolyspora erythraea and *Saccharopolyspora rectivirgula* phages are able to infect either host (Korn-Wendisch et al. 1989; Smorawinska et al. 1988), and some *Saccharopolyspora erythraea* phages are also able to infect *Saccharopolyspora hirsuta* and *Saccharopolyspora taberi* (Grund and Hutchinson 1987; Korn-Wendisch et al. 1989).

Molecular Analyses

The erythromycin biosynthesis and resistance genes from *Saccharopolyspora erythraea* have been mapped and cloned and found to be located together on the same chromosome (Baltz et al. 1986; Stanzak et al. 1986; Tuan et al. 1986; Vanden Boom 2000; Weber et al. 1985). The *Saccharopolyspora spinosa* biosynthetic gene cluster is 74 kb in size and contains genes that may be involved in synthesising the tetracyclic polyketide aglycone nucleus of spinosyns (Hong et al. 2008; Matsushima and Baltz 1994; Waldron et al. 2001). The cluster contains five large open reading frames which encode a type 1 polyketide synthase (Waldron et al. 2001). Recently, genes involved in the spinosyn biosynthesis pathway were characterized, specifically those involved in the synthesis of D-forosamine [(4-dimethylamino)-2,3,4,6-tetrade-oxy-b-D-threo-hexopyranose] (Hong et al. 2008).

Mobile genetic elements can encode proteins with important functions. The integrated and conjugative element (AICE), PSE102, identified in *Saccharopolyspora erythraea* encodes a putative aminoglycoside phosphotransferase which may confer antibiotic resistance (Brown et al. 1988, 1994; te Poele et al. 2008).

Both *Saccharopolyspora erythraea*, *Amycolatopsis mediterranei*, and *Amycolatopsis methanolica* contain AICEs with a highly conserved structure of four functional modules enabling conjugative transfer, excision/integration, regulation, and replication.

The sequenced genome (8.2 kb) of *Saccharopolyspora erythraea* NRRL 2338 has been published (Oliynyk et al. 2007) and shows considerable divergence from the *Streptomyces* species, being circular, rather than linear. A core region covering half of the chromosome contains genes for erythromycin biosynthesis, primary metabolism, cell division, information transfer, and sporulation (Katz and Khosla 2007). The remainder of the chromosome contains genes involved in secondary metabolism, polyketide, ribosomal peptide, and terpenoid biosynthesis. Recent transcriptional profiling using a DNA microarray identified genes involved in distinct growth phases, as well as gene clusters for secondary metabolism (Peano et al. 2007). Recently, the sequenced genome (8.6 kb) of *Saccharopolyspora spinosa* NRRL 18395 was published (Pan et al. 2011). It has a G+C content of 67.94 % and is predicted to contain 8,302 coding sequences (CDSs), including a gene cluster involved in the synthesis of spinosyns.

Pathogenicity, Clinical Relevance

Saccharopolyspora rectivirgula is a known cause of extrinsic allergic alveolitis condition known as farmer's lung (Campbell 1932; Pepys et al. 1963). The condition is caused by the inhalation of large numbers of spores released from the disturbance of mouldy vegetative material (Lacey 1981). The disease is chronic (Lacey 1988), affecting up to up to 8.6 % of farm workers in western Scotland and the Orkneys (Grant et al. 1972) and 8.4 % of Wisconsin dairy farmers (Roberts et al. 1976). Infection is detected using an enzyme-linked immunosorbent assay (Ramasamy et al. 1987), to identify antibodies in the sera of patients (Brummund et al. 1988; Mäntyjärvi and Kurup 1988). Up to 75 individual antigenic components have been identified in *Saccharopolyspora* (Lacey 1989).

Saccharothrix Labeda, Testa, Lechevalier and Lechevalier 1984, 429^{VP} emended Labeda and Lechevalier 1989, 422

Sac´char.o.thrix. Gr. neut. n. *sakchâr*, sugar: Gr. fem. n. *thrix* hair; *Saccharothrix*, sugar-containing hair.

Phenotypic Analyses

Saccharothrix species are aerobic actinomycetes that produce branching vegetative mycelia (approximately 0.5–0.7 µm in diameter) as well as aerial mycelia on many growth media. Both the vegetative and aerial hyphae fragment into coccoid to coccoid-rod, nonmotile elements, and a "zig-zag" morphology of the aerial hyphae is typically observed during sporulation of most species. Cells are Gram-stain positive, resistant to lysozyme, and catalase positive. The cell walls of *Saccharothrix* strains contain *meso*-diaminopimelic acid, galactose, rhamnose, and a trace of mannose, as can be seen in the *Table 37.42*. The presence of phosphatidylethanolamine is diagnostic for this genus. The principal menaquinone is MK-9(H_4); and in addition some species also contain MK-9(H_6), MK-9(H_8) and MK-10(H_4). The typical fatty-acid profile for *Saccharothrix* species consists predominantly of iso-$C_{15:0}$, iso-$C_{16:0}$, and anteiso-$C_{17:0}$.

The G+C content of the DNA of members of this genus ranges from 67 to 74 (mol%).

The type species is *Saccharothrix australiensis* NRRL 11239^T = ATCC 31497^T = DSM 43800^T = NRBC 14444^T = JCM 3370^T = VKM Ac-894^T (Labeda et al. 1984).

Isolation Procedures

Details of the source samples and methods of isolation of some species of this genus are shown in *Table 37.42*.

Taxonomy, Historical, and Current

Phylogenetically, the genus *Saccharothrix* represents a line of descent closest to the genera *Actinosynnema*, *Lentzea*, and *Lechevalieria* as can be seen in the *Fig. 37.3*. The 16S rRNA gene sequences for all described species contains the genus-specific diagnostic nucleotide signature pattern of CAC (607–609) and GTG (617–619) as can be seen in Figure *Actinosynnema*, *Lechevalieria*, *Lentzea*, *Saccharothrix*, and *Umezawaea* nucleotide signatures.

Following the description of the genus *Saccharothrix* by Labeda et al. (1984), predominantly based on chemotaxonomic and morphological properties, a number of species were subsequently transferred into this genus from other actinomycete genera, including *Actinomadura* and *Nocardiopsis*. Evaluation of the phylogenetic relationships among the species of the genus based on 16S rRNA gene sequences and reassessment of chemotaxonomic properties resulted in transfer of many of the species into other genera, including *Crossiella cryophila*, *Goodfellowiella coeruleoviolacea*, *Lechevalieria aerocolonigenes*, *Lechevalieria flava*, *Lentzea waywayandensis*, and *Umezawaea tangerina* that were all validly described as species within the genus *Saccharothrix* (Labeda 2001; Labeda et al. 2001; Labeda and Kroppenstedt 2006, 2007).

The aligned sequences of the 16S rRNA gene for *Saccharothrix*, *Actinosynnema*, *Lechevalieria*, *Lentzea*, and *Umezawaea* species illustrate that *Saccharothrix* strains can easily be distinguished from the other genera on the basis of the diagnostic nucleotide signatures CAC (607–609) and GTG (617–619). Salazar et al. (2002) also reported a set of specific PCR primers for the genus *Saccharothrix*, Stx2 (5′-AAGGCCCTTCGGGGTACACGAG-3′) and Stx1 (5′-TCGACCGCAGGCTCCACG-3′), that with a PCR annealing temperature of 66 °C permitted the rapid detection of all species except *Saccharothrix texasensis*.

The phylogenetically near genera *Lechevalieria* and *Lentzea* can be chemotaxonomically differentiated from *Saccharothrix* because they lack phosphatidylethanolamine containing 2-hydroxy fatty acids in their phospholipid profiles (Labeda et al. 2001). Although the species of the genus *Actinosynnema* contain this phospholipid, they can be easily be differentiated because of their unique colonial morphology. *Umezawaea* also contains 2-hydroxy fatty acid containing phospholipids but uniquely also contains *lyso*-phosphatidylethanolamine.

The species of the genus are *Saccharothrix australiensis* NRRL 11239^T = ATCC 31497^T = DSM 43800^T = NRBC 14444^T = JCM 3370^T = VKM Ac-894^T (Labeda et al. 1984); *Saccharothrix algeriensis* NRRL B-24137^{T}DSM 44581^T = JCM 13242^T = NBRC 101915^T (Zitouni et al. 2004); *Saccharothrix coeruleofusus* DSM 43679^T = ATCC 35108^T = NBRC 14520^T = INA 1335^T = JCM 3313^T = NRRL B-16115^T = VKM Ac-855^T (Grund and Kroppenstedt 1989); *Saccharothrix espanaensis* NRRL 15764^T = ATCC 51144^T = DSM 44229^T = JCM 9112^T = VKMAc-1969^T (Labeda and Lechevalier 1989); *Saccharothrix longispora* ATCC 35109^T = DSM 43749^T = NRBC 14522^T = INA 10222^T = JCM 3314^T = NRRL B-16116^T = VKM Ac-907^T; *Saccharothrix mutabilis* subsp. *capreolus* ATCC 23892^T = DSM 40225^T = NRBC 12847^T = JCM 4248^T = JCM 4630^T = NRRL 2773^T = VKM Ac-1848^T; *Saccharothrix mutabilis* subsp. *mutabilis* NRRL B-16077^T = ATCC 31520^T = DSM 43853^T = NBRC 14310^T = JCM 3380^T = VKM Ac-2023; *Saccharothrix syringae* DSM 43886^T = ATCC 51364^T = NBRC 14523^T = INA 2240^T = JCM 6844^T = NRRL B-16468^T = VKM Ac-1858^T (Grund and Kroppenstedt 1989); *Saccharothrix texasensis* NRRL B-16134^T = ATCC 51593^T = DSM 44231^T = NBRC 14971^T = JCM 9113^T = VKM Ac-1968^T (Labeda and Lyons 1989b); *Saccharothrix variisporea* NRRL B-16296^T = ATCC 31203^T = DSM 43911^T = JCM 3273^T = NBRC 14104^T (Kim et al. 2011); *Saccharothrix violaceirubra* NBRC 102064^T = JCM 16955^T = KCTC 19326^T (Otoguro et al. 2009); and *Saccharothrix xinjiangensis* JCM 12329^T = NBRC 101911^T (Hu et al. 2004).

Table 37.42
Chemotaxonomic characteristics and methods of isolation of species of the genus *Saccharothrix* and *Umezawaea*

Species	Whole-cell sugars[a]	Phospholipids[b]	Major menaquinone(s)	Major fatty acids (>3 %)	DNA G+C (mol %)	Origin of isolate	Method of isolation
Saccharothrix algeriensis NRRL B-24137^T	Gal, Man, Rha, Glc, Rib	PE, OH-PE, GluNu	nr	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, *iso*-$C_{16:1}$H, *iso*-$C_{16:0}$, $C_{16:1}$*cis*-9, $C_{16:0}$9-methyl, ante*iso*-$C_{17:0}$, $C_{17:1}$*cis*-9, *iso*-$C_{16:0}$2-OH	nr	Saharan soils; Adrar, Algeria	Isolated by a dilution agarplating method using humic acid/B vitamin agar medium (Hayakawa and Nonomura 1987) supplemented with streptomycin sulfate (10 μg ml^{-1}) and actidione (50 μg ml^{-1})
Saccharothrix australiensis NRRL 11239^T	Gal, Man, Rha	PE, OH-PE, PME, DPG, PI	MK-9(H_4), MK-10(H_4)	nr	73	Soil sample; Australia	Isolated on yeast extract and malt extract agar (ISP 2) and ATCC medium 172
Saccharothrix coeruleofusus DSM 43679^T	Gal, Rha	PE	MK-9(H_4)	nr	67.0	nr	nr
Saccharothrix espanaensis NRRL 15764^T	Gal, Rha	PE	MK-9(H_4)	nr	72.2	Soil sample; Spain	nr
Saccharothrix hoggarensis NRRL 45457^T	Gal, Rha	PE, PME, PI, PIM, DPG	MK-9(H_4)	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$9-methyl, ante*iso*-$C_{17:0}$	nr	Saharan soil sample; South Algeria (Hoggar)	Isolated by a dilution plating method using humic acid vitamin agar (Hayakawa and Nonomura 1987) supplemented with 50 μg ml^{-1} actidione
Saccharothrix longispora ATCC 35109^T	Gal, Rha	PE	MK-9(H_4)	nr	68.0	nr	nr
Saccharothrix mutabilis subsp. *capreolus* ATCC 23892^T	Gal, Rha	PE	MK-9(H_4)	nr	nr	nr	nr
Saccharothrix mutabilis subsp. *mutabilis* NRRL B-16077^T	Gal, Rha	PE	MK-9(H_4)	nr	73.1	Soil	
Saccharothrix saharensis DSM 45456^T	Gal, Man, Rha, Rib	PE, PME, PI, PIM, DPG	MK-9(H_4), MK-7(H_4)	*iso*-$C_{16:0}$, *iso*-$C_{15:0}$	nr	Soil sample; Adrar palm grove, South Algeria	Isolated by a dilution plating method using humic acid vitamin agar (Hayakawa and Nonomura 1987) supplemented with 50 μg ml^{-1} cycloheximide

Saccharothrix syringae DSM 43886^T	Gal, Rha	PE	MK-9(H_4)	nr	nr	nr	nr
Saccharothrix texasensis NRRL B-16134^T	Gal, Rha	PE	MK-9(H_4)	nr	72.5	Soil sample; Flower mountain, Texas, USA	nr
Saccharothrix variisporea NRRL B-16296^T	Gal, Man, Rha	DPG, PI, PE, PG	MK-9(H_4)	*iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, *iso*-$C_{15:0}$, $C_{16:0}$9-methyl, *iso*-$C_{17:0}$, *iso*-$C_{16:1}$H, $C_{17:1}\omega$6c, $C_{16:1}$2-OH, $C_{16:1}\omega$7c, ante*iso*-$C_{15:0}$, $C_{17:1}\omega$8c, ante*iso*-$C_{17:1}$	74	Soil sample; India	nr
Saccharothrix violaceirubra NBRC 102064^T	Gal, Man, Rha	PE	MK-9(H_4), MK-9(H_6), MK-9(H_8)	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, *iso*-$C_{16:1}$H, *iso*-$C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:1}\omega$6c	70.8	Soil and plant litter sample; Yamanashi, Japan	Isolated on humic acid/vitamin (HV) agar (Hayakawa and Nonomura 1987) supplemented with cycloheximide (50 $\mu g\ l^{-1}$) and nalidixic acid (20 $\mu g\ l^{-1}$)
Saccharothrix xinjiangensis CGMCC 4.1731^T	Gal, Man	PE, DPG	MK-9(H_4), MK-10(H_4)	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$, ante*iso*-$C_{17:0}$, $C_{17:1}\omega$8c	70.4	Water samples; Tianchi Lake, Xinjiang, China	Isolated by plating 0.1 ml filtered (pore size = 0.22 μm) lake water onto agar plates with benzene, anthracene, phenathrene or pyrene as the sole carbon source
Umezawaea tangerina NRRL B-24463^T	Gal, Man, Rib, Rha (trace)	PE, PI, OH-PE, lyso-PE, DPG, PIM	MK-9(H_4)	*iso*-$C_{14:0}$, *iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, $C_{15:0}$, *iso*-$C_{16:1}$, *iso*-$C_{16:0}$, $C_{16:1}$ (c9), $C_{16:0}$, $C_{16:0}$ 10-methyl, $C_{17:1}$ (c9), *iso*-$C_{16:0}$ 2-OH	74.0	Soil sample	nr

Data from: Boubetra et al. (2013a), Boubetra et al. (2013b), Zitouni et al. (2004b), Labeda et al. (1984), Grund and Kroppenstedt (1989), Labeda and Lechevalier (1989a), Preobrazhenskaya and Sveshnikova (1974), Labeda and Lyons (1989), Kim et al. (2011), Otoguro et al. (2009), Hu et al. (2004), Labeda and Kroppenstedt (2007), Kinoshita et al. (1999)

Symbols: *nr* not reported

[a]*DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PE* phosphatidylethanolamine, *OH-PE* hydroxyphosphatidylethanolamine, *PI* phosphatidylinositol, *PIM* phosphatidylinositolmannosides, *PME* phosphatidylmethylethanolamine

[b]*Gal* galactose, *Rha* rhamnose, *Man* mannose, *Ri* ribose, *Glc* glucose

Table 37.43

Morphological and physiological properties of *Saccharothrix* and *Umezawaea* species

	Saccharothrix algeriensis NRRL B-24137 T	*Saccharothrix australiensis* NRRL 11239 T	*Saccharothrix coeruleofusus* DSM 43679 T	*Saccharothrix espanaensis* NRRL 15764 T	*Saccharothrix hoggarensis* NRRL 45457 T	*Saccharothrix longispora* ATCC 35109 T	*Saccharothrix mutabilis* subsp. *capreolus* ATCC 23892 T
Color of substrate mycelium	Vivid yellow, orange-yellow, or yellowish-brown	Brownish to grayish-yellow	Yellowish	Grayish-yellow to yellowish-brown	Pale to light yellow (nutrient agar), yellow to deep yellowish (ISP 2, ISP 4), light brown (Bennett's agar), dark brown (ISP 3)	Yellow or red	Yellowish to brownish
Color of aerial mycelium	Yellow-orange	White to yellowish-gray	Blue to dark blue on defined media	Sparse	Light grayish blue (ISP 2), yellowish white (nutrient agar), pinkish brown (Bennett's agar)	None or blue on glycerol–nitrate agar	Sparse white
Soluble pigments	Bright yellow	Brownish	None	Yellow	None	None	None
Hydrolysis of:							
Adenine	–	–	–	–	–	+	–
Casein	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+
Hippurate	nr	–	–	+	nr	–	+
Hypoxanthine	–	–	–	+	+	–	+
Starch	–	–	+	–	+	+	+
Tyrosine	+	+	–	–	+	+	+
Urea	nr	–	–	–	+	+	–
Xanthine	–	–	–	–	–	–	–
Production of:							
Nitrate reductase	+	+	–	w	–	+	–
Phosphatase	nr	–	nr	+	nr	nr	nr
Acid from:							
Adonitol	–	–	–	–	–	–	–
Arabinose	–	–	+	–	+	+	+
Cellobiose	–	+	+	+	+	+	+
Dextrin	–	+	+	–	nr	+	+
Dulcitol	–	–	nr	–	nr	nr	nr
Erythritol	–	+	nr	–	nr	nr	nr
Fructose	+	+	+	+	+	+	+
Galactose	+	+	nr	nr	+	nr	nr
Glucose	+	+	+	+	+	+	+
Glycerol	+	+	nr	+	+	+	nr
Inositol	–	–	–	–	–	–	+
Lactose	–	–	+	–	–	+	–
Maltose	+	+	nr	v	+	nr	nr
Mannitol	–	nr	nr	–	–	nr	nr
Mannose	–	+	nr	+	–	nr	nr
Melibiose	–	–	–	–	+	–	+
Methyl- α-D-glucoside	–	–	+	–	–	–	–
β-Methyl-xyloside	nr	–	nr	–	nr	nr	nr
Raffinose	–	–	+	–	–	–	–
Rhamnose	–	–	+	–	+	+	–
Salicin	nr	–	nr	–	–	nr	nr
Sorbitol	–	+	+	–	+	–	–
Sucrose	–	–	+	+	+	+	–
Trehalose	nr	+	nr	+	–	nr	nr
Xylose	–	–	+	v	+	+	+

Saccharothrix mutabilis subsp. *mutabilis* NRRL B-16077 T	*Saccharothrix saharensis* DSM 45456 T	*Saccharothrix syringae* DSM 43886 T	*Saccharothrix texasensis* NRRL B-16134 T	*Saccharothrix variiisporea* NRRL B-16296 T	*Saccharothrix violaceirubra* NBRC 102064 T	*Saccharothrix xinjiangensis* CGMCC 4.1731 T	*Umezawaea tangerina* NRRL B-24463 T
Yellow to yellowish-brown	Brown (nutrient agar), deep yellowish brown (ISP 3), Strong brown (ISP 2), greyish (Bennett's agar), bluish (ISP 4)	Yellowish to brownish	Dark yellow to brownish-yellow	Light orange	Purple to dark red	Pinkish-buff to pale orange to pale brown	Pale yellow, pale yellow-orange, or pale yellowish-brown
White	Bluish white (ISP 2, nutrient agar), light-bluish gray (ISP 3), white (Bennett's agar)	Sparse white; lilac on glycerol nitrate agar	Sparse white	Sparse white	White	Grayish-white	White to brownish-white
Light yellow to yellowish-brown	Deep blue (ISP 2, ISP 4), light reddish brown (nutrient agar)	None	Brown to reddish-brown	Black melanin	None	Pale brown	None to faint brown
−	+	−	−	−	−	+	−
+	+	+	+	+	nr	nr	+
+	+	+	+	+	nr	nr	nr
+	+	+	+	+	nr	nr	nr
+	nr	+	+	nr	+	nr	nr
+	+	−	−	+	−	nr	+
+	+	+	+	+	−	−	+
+	+	+	+	+	+	−	+
−	nr	−	−	+	nr	nr	−
−	−	−	−	−	nr	nr	nr
+	+	−	−	−	+	−	−
+	nr	nr	+	nr	nr	nr	+
−	−	−	−	−	nr	nr	−
+	+	+	+	+	−	+	+
+	+	+	+	+	nr	nr	+
+	nr	+	+	+	−	nr	+
−	nr	nr	−	−	nr	nr	nr
−	nr	nr	−	nr	nr	nr	−
+	+	+	v	nr	nr	nr	+
+	+	nr	+	+	nr	nr	nr
+	+	+	+	+	+	nr	+
+	+	nr	+	+	nr	nr	+
+	+	−	+	+	−	nr	+
+	−	+	+	+	−	+	nr
nr	+	nr	nr	+	+	nr	nr
nr	+	nr	+	+	−	nr	nr
+	+	nr	+	+	+	nr	nr
+	−	+	+	+	−	+	nr
+	+	−	+	+	−	+	nr
nr	nr	nr	v	nr	nr	nr	nr
+	+	+	−	+	−	+	nr
−	+	+	+	+	−	+	nr
+	nr	nr	+	+	−	nr	nr
−	−	−	−	−	−	−	nr
+	−	+	+	+	+	+	nr
+	+	nr	+	+	+	nr	nr
+	+	+	+	+	−	+	+

Table 37.43 (continued)

	Saccharothrix algeriensis NRRL B-24137 ^{T}	*Saccharothrix australiensis* NRRL 11239 ^{T}	*Saccharothrix coeruleofusus* DSM 43679 ^{T}	*Saccharothrix espanaensis* NRRL 15764 ^{T}	*Saccharothrix hoggarensis* NRRL 45457 ^{T}	*Saccharothrix longispora* ATCC 35109 ^{T}	*Saccharothrix mutabilis* subsp. *capreolus* ATCC 23892 ^{T}
Assimilation of:							
Acetate	+	+	nr	+	+	nr	nr
Benzoate	−	−	nr	−	−	−	nr
Citrate	+	−	−	v	−	+	−
Lactate	nr	v	−	+	+	+	−
Malate	nr	+	−	+	nr	+	+
Mucate	nr	−	nr	−	nr	nr	nr
Oxalate	−	−	nr	−	−	nr	nr
Propionate	−	+	nr	v	+	nr	nr
Succinate	+	+	nr	+	+	nr	nr
Tartrate	−	−	nr	−	−	nr	nr
Growth in the presence of:							
4 % NaCl	+	+	+	+	+	+	+
5 % NaCl:	−	−	+	−	+	+	+
Growth at:							
10 °C	+	+	+	+	−	+	+
37 °C	+	+	+	+	+	+	+
45 °C	+	+	+	−	+	−	+
50 °C	nr	nr	nr	nr	nr	nr	nr

Data from: Boubetra et al. (2013a), Boubetra et al. (2013b), Zitouni et al. (2004b), Labeda et al. (1984), Grund and Kroppenstedt (1989), Labeda and Lechevalier (1989), Preobrazhenskaya and Sveshnikova (1974), Labeda and Lyons (1989), Kim et al. (2011), Otoguro et al. (2009), Hu et al. (2004), Labeda and Kroppenstedt (2007), Kinoshita et al. (1999)

Symbols: + positive, − negative, *v* variable positive reaction, *w* weak growth, *nr* not reported

Saccharothrix mutabilis subsp. *mutabilis* NRRL B-16077 T	*Saccharothrix saharensis* DSM 45456 T	*Saccharothrix syringae* DSM 43886 T	*Saccharothrix texasensis* NRRL B-16134 T	*Saccharothrix variiisporea* NRRL B-16296 T	*Saccharothrix violaceirubra* NBRC 102064 T	*Saccharothrix xinjiangensis* CGMCC 4.1731 T	*Umezawaea tangerina* NRRL B-24463 T
+	+	nr	+	nr	nr	nr	nr
–	–	nr	–	nr	nr	–	nr
+	–	–	–	–	–	–	+
+	+	+	+	–	–	–	nr
+	nr	+	+	+	–	nr	nr
–	nr	–	–	nr	nr	nr	nr
–	–	nr	–	–	nr	nr	+
+	+	nr	v	+	nr	nr	nr
+	+	nr	+	nr	nr	nr	nr
–	–	nr	–	+	nr	–	–
–	–	+	–	+	–	–	+
–	–	+	–	–	–	–	nr
+	–	+	+	+	+	+	–
+	+	+	+	+	–	+	–
+	+	+	–	W	–	+	–
nr	–	nr	–	–	–	+	–

The species of the genus *Saccharothrix* can be readily differentiated from each other on the basis of their morphological and physiological characteristics, which are summarized in ❯ *Table 37.43*.

Sciscionella Tian, Zhi, Qiu, Yun-Qi, Zhang, Tang, Xu, Zhang, and Li 2009, 222[VP]

Sci.sci.o.nel'la.N.L. fem. dim. n. Sciscionella arbitrary name formed from the acronym of the South China Sea Institute of Oceanology, SCISCIO, which isolated and characterized this taxon.

Phenotypic Analyses

Sciscionella species are aerobic, Gram-stain-positive, nonacid-fast, nonmotile actinobacteria which form yellow colonies. Substrate mycelium fragments into rod-shaped elements, 2.5–3.5 mm long. Sparse aerial mycelium forms on some media containing natural seawater.

The type IV cell wall contains *meso*-diaminopimelic acid and the whole-cell sugars present are galactose, arabinose, and glucose. Phospholipids are type III, including diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and unknown phosphoglycolipids. The predominant menaquinone is MK-9(H_4). The major cellular fatty acids are iso-$C_{16:0}$ and iso-$C_{16:0}$ 2-OH.

The G+C content of the DNA of the single type species is 69 (mol%).

The type strain of this genus is *Sciscionella marina* SCSIO 00231^T =5KCTC 19433^T = 5CCTCC AA208009^T, which was isolated from a sediment sample collected from the northern South China Sea (Tian et al. 2009).

Isolation Procedures

Strain SCSIO 00231 T was isolated on Gauze No. 1 medium prepared with seawater instead of distilled water, and incubated at 28 °C for 3 weeks. Enrichment and isolation procedures are described in ❯ *Table 37.26*.

Taxonomy, Historical, and Current

Phylogenetic analysis showed that *Sciscionella* belongs to the order *Pseudonocardiales*, in which it forms a distinct lineage and is associated with the genus *Thermocrispum*, which showed a 93 % sequence (16S rRNA gene) similarity. The other closest phylogenetic genera are *Saccharopolyspora* and *Amycolatopsis* with 92.6 % and 93.1 % sequence similarities, respectively.

Physiological and chemotaxonomic characteristics of *Sciscionella marina* SCSIO 00231^T and its closest phylogenetic neighbors are compared in ❯ *Tables 37.25* and ❯ *37.26*, respectively.

Streptoalloteichus Tomita, Nakakita, Hoshino, Numata, and Kawaguchi 1987, 211[VP] emended Tamura, Ishida, Otoguro, Hatano, and Suzuki 2008, 689

Strep.to.al.lo.tei'chus. Gr. adj. *streptos* bent; Gr. adj. *allos* different; Gr. n. *teichos* wall; N.L. masc. n. *Streptoalloteichus* intended to mean streptomycete with different wall.

Phenotypic Analyses

Streptoalloteichus cells are aerobic, Gram-stain positive, and nonacid fast. Cultures form vegetative hyphae (0.5 µm diameter), which are well branched and non-fragmenting. Vegetative and aerial mycelium color varies (❯ *Table 37.44*), with chains of five to 50 spores (0.5–1.2 µm diameter) occurring at the tips of aerial hyphae. *Streptoalloteichus* species form sporangia-like structures and produce motile or nonmotile spores that have a smooth surface. The cell wall contains *meso*-diaminopimelic acid and the diagnostic phospholipid is phosphatidylethanolamine (pattern type PII). Menaquinones MK-9(H_6) and MK-10(H_6) are found in both species, but *S. tenebrarius* also contains MK-9(H_4) and MK-10(H_4). Predominant whole-cell hydrolysates are galactose and mannose but may also contain L-rhamnose and D-glucose. Predominant fatty acids are iso-$C_{15:0}$, and anteiso-$C_{17:0}$ (❯ *Table 37.45*).

The G+C content of the DNA of the type species was not reported; for *Streptoalloteichus tenebrarius* it is 71.6 (mol. %).

The type strains of the genus are *Streptoalloteichus hindustanus* ATCC 31217^T = IFO 15115^T (Tomita et al. 1987) and *Streptoalloteichus tenebrarius* NBRC 16177^T = ATCC 17920^T = DSM 40477^T = JCM 4838^T = NRRL B-12390^T = ISP 5477^T (Tamura et al. 2008b).

Taxonomy, Historical, and Current

The genus *Streptoalloteichus* was originally proposed in Tomita et al. (1978), and the type and species *Streptoalloteichus hindustanus* was first published in Tomita et al. (1987). *Streptoalloteichus tenebrarius* was originally published as *Streptomyces tenebrarius* (Higgins and Kastner 1967).

The two type strains share 99.5 % 16S rRNA gene sequence similarity, but only 24.3–37.9 % DNA–DNA relatedness (Tamura et al. 2008b), (Lechevalier et al. 1977), (Ohkuma et al. 1988).

Table 37.44

Cultural characteristics that distinguish the two species of the genus *Streptoalloteichus*[a, b]

Species	Characteristic																
	Morphology		Aerial mycelium colour					Vegetative mycelium colour					Light sensitivity for formation of aerial mycelium	Diffusible pigment	Growth		
	Sporagia –like vessels	Motile spores	ISP 2 medium	ISP 4 medium	ISP 5 medium	ISP 6 medium	Bennett's agar	ISP 2 medium	ISP 4 medium	ISP 5 medium	ISP 6 medium	Bennett's agar		ISP 4 medium	ISP 5 medium	Antibiotic production
S. hindustanus ATCC 31217^T	+	+	Thick pale yellowish pink	Pale pinkish yellow	Patches, white, turning yellowish gray later	Scant, white	Velvety, light yellowish beige	Light yellowish brown	Thin, colorless to grayish yellow	Thin, colorless to grayish yellow	Moderate brown	Pale olivaceous yellow to light brown	–	None	Restricted	Tallysomycins A, B, and C; nebramycin factors II, IV, and V
S. tenebrarius ATCC 17920^T	–	–	nr	Light grayish - yellow with white areas	Pale yellow with white areas	nr	White	Grayish pink	nr	Pale yellow	nr	Pale yellow	+	Grayish pink	Moderate	Nebramycin factors I to XIII

[a]Data from: Tomita et al. (1987), Tamura et al. (2008a)

[b]Symbols: + positive/present, - negative/absent, *nr* not reported

Table 37.45

Physiological, Chemotaxonomic and methods of isolation of the two species of the genus *Streptoalloteichus*[a]

Species	Characteristic													
	Utilization of:						Tolerance to 7 %(w/v) NaCl[b]	Cell chemistry					Origin of isolation	Method of isolation
	Maltose	L — Arabinose	D — Xylose	Salicin	myo — Inositol	Lactose		Whole-cell sugars [c]	Menaquinones	DNA G+C content (mol %)	Phospholipids[d]	Major fatty acids		
S. hindustanus ATCC 31217^T	—	—	—	—	—	w	—	Gal, Man, Rha	MK-9(H_6), MK-10(H_6)	nr	PE, PI	nr	Soil, Gujarat, India	Bacto-nutrient agar (Difco Laboratories) supplemented with butirosin at 50 μg/ml, incubated at 43 °C for 3 weeks
S. tenebrarius ATCC 17920^T	+	+	+	+	+	—	+	Gal, Man, Glc	MK-10(H_6), MK-10(H_4), MK-9(H_6), MK-9(H_4)	71.6	PE	ante*iso*-$C_{17:0}$, *iso*-$C_{15:0}$, ante*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, $C_{16:0}$ 9-methyl, *iso*-$C_{17:0}$	Soil	nr

Symbols: + positive/present, —, negative/absent, *nr* not reported, *w* weak

[a]Data from: Tomita et al. (1987), Tamura et al. (2008a)

[b]Both grow at 5 %, neither grows at 10 %

[c]*Gal* galactose, *Man* mannose, *Rha* rhamnose, *Glc* glucose

[d]*PI* phospatidylinositol, *PE* phosphatidylethanolamine

Thermocrispum Korn-Wendisch, Rainey, Kroppenstedt, Kempf, Majazza, Kutzner, and Stackebrandt 1995, 73[VP]

Ther.mo.crispum. Gr. adj. *thermos* warm, hot; L. neut. adj. *crispum* tightly curled; N.L. neut. n. *Thermocrispum* a heat-loving, tightly curled organism.

Phenotypic Analyses

Cultures of *Thermocrispum* consist of yellow to light brown branched substrate mycelium (Table 37.46). Aerial mycelium is white and straight to flexuous and aggregates into pseudosporangia that contain septate hyphae and fragment into rodlike structures. No soluble pigments are produced. Cells are aerobic, Gram-stain positive, catalase positive, nonacid fast, lysozyme sensitive, and thermophilic.

As shown in Table 37.47, *Thermocrispum* cell walls contain *meso*-diaminopimelic acid. Whole-cell hydrolysates contain arabinose, mannose, and glucose. The sugar pattern is type C. The phospholipids are phosphatidylethanolamine, phosphatidylinositol, and hydroxyphosphatidylethanolamine. No mycolic acids are present. The predominant menaquinone is MK-9(H_4). Fatty-acid profiles consist mainly of iso-$C_{16:0}$ and $C_{17:1}$.

Thermocrispum has a DNA G+C content 69–73 (mol%).

The type species is *Thermocrispum municipale* ATCC 51796^T = DSM 44069^T = NRBC 15806^T = JCM 9704^T (Korn-Wendisch et al. 1995).

Isolation Procedures

The source material and isolation protocol is shown in Table 37.47.

Thermocrispum can be maintained on CYC agar, GYM agar, Hickey Tresner agar (Hickey and Tresner 1952), oatmeal agar, potato–carrot agar, PM agar, R2A agar, R8 agar (Amner et al. 1989), and TSA.

Taxonomy, Historical, and Current

Sequence analysis of the 16S rRNA gene places the genus *Thermocrispum* in the family *Pseudonocardiaceae* (Figs. 37.1, 37.2, 37.3, 37.4, and 37.5). Chemotaxonomic profiles can be used to distinguish *Thermocrispum* from other genera in the family *Pseudonocardiaceae*. *Thermocrispum* has a type III cell-wall chemotype, whereas *Actinopolyspora*, *Amycolatopsis*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharomonospora*, and *Saccharopolyspora* have a type IV chemotype. *Thermocrispum* has a type PII phospholipid pattern, which is different from that of *Actinopolyspora*, *Pseudonocardia*, and *Saccharopolyspora*. *Thermocrispum* contains themenaquinone MK-9(H_4), but not MK-8(H_4) found in *Pseudonocardia* and *Saccharomonospora*. Sequence similarity of the 16S rRNA gene shows neighboring genera as *Amycolatopsis*, *Kibdelosporangium*, *Prauserella*, *Saccharomonospora*, and *Saccharopolyspora*. Restriction fragment patterns of the 16S rRNA gene can be used to distinguish between related genera (Cook and Meyers 2003).

The two species are *Thermocrispum agreste* and *Thermocrispum municipale*, with a 98.2 % 16S rRNA gene sequence similarity between these strains (Korn-Wendisch et al. 1995). Possible heterogeneity exists within strains of *Thermocrispum municipale*, evident in differences in phage sensitivity and fatty-acid profile (Korn-Wendisch et al. 1995).

Thermocrispum is resistant to phages that infect the genera *Amycolatopsis*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora*, and *Saccharothrix* and is sensitive to genus-specific phages. The two species of *Thermocrispum* can be distinguished by band patterns of esterases (Korn-Wendisch et al. 1995) and phage sensitivity.

Thermocrispum agreste CHB77T can be lyzed by its corresponding phage, f77, but is sensitive to only two of the five phages isolated for *Thermocrispum municipale* strains MKD8, MKD10, MKD19, MKD35T, and MKD38, even if high phage titres are used. In contrast, only four of the nine *Thermocrispum municipale* strains can be lyzed by a high titre of f77. The strains of *Thermocrispum municipale* form three subgroups: group 1, strains MKD8, TMK2, and TMD78, can be lyzed by all five phages isolated for strains MKD8, MKD10, MKD19, MKD35T, and MKD38; group 2, strains TMS14 and MKD38, are not lyzed by f10 even if high phage titres are used, and strain MKD35T can be lyzed by this phage only if a high phage titre is used; group 3, strains MKD10, MKD19, and MKD57, are sensitive to phages f35 and f38 only if high phage titres are used.

Activity

Thermocrispum agreste shows slight activity against *Micrococcus luteus* and *Rhodococcus rhodochrous* and also inhibits *Azotobacter chroococcum*, *Candida albicans*, *Geotrichum candidum*, and *Saccharomyces cerevisiae*, but not against *Escherichia coli*, *Corynebacterium glutamicum*, *Bacillus subtilis*, or *Staphylococcus aureus*. *Thermocrispum municipale* does not display any antimicrobial activity.

Umezawaea Labeda and Kroppenstedt 2007, 2761[VP]

Um.e.za'wa.e.a N.L, fem. n. *Umezawaea* named for the late Hamao Umezawa, of the Institute of Microbial Chemistry, Tokyo, in recognition of his leadership and contributions to the study of the biology and natural products of actinomycetes.

Table 37.46

Cultural and physiological characteristics of the two species of the genus *Thermocrispum*[a, b]

Species	Characteristics																														
	Aerial mycelium colour	Vegetative mycelium colour	Growth at:				Growth in the presence of NaCl			Resistance to 25 μg/ml kanamycin	Resistance to lysosyme	Degradation of:							Utilization of:									Hemolysis	Egg yolk reaction
			20 (°C)	28 (°C)	62.5 (°C)	65 (°C)	7 (%)	10 (%)	13 (%)			Elastin	Guanine	Casein	Tyrosine	Gelatin	Urea	Allantonin	Fructose	Maltose	Melezitose	Mannitol	Sorbitol (= glucitol)	Salicin	Sodium acetate	Sodium citrate		
T. muncipale DSM 44070^T	White	Yellow to light brown	w	+++	(+)	–	+++	++	–	(+)	–	–	(+)	+	+	+	+	–	–	+	+	v	(+)	v	(+)	+	v	–	–
T. agreste DSM 44069^T	White	Yellow to light brown	tr	++	++	–	++	–	–	+	–	+	–	+	+	+	–	–	tr	(+)	tr	tr	tr	+	tr	–	+	+

[a]Data from: Korn-Wendisch et al. (1995)

[b]Symbols: +++ good growth with abunrant aerial mycelium, ++ good growth with moderate aerial mycelium, + positive growth or reaction, (+) poor growth or moderate reaction, – negative, *w* weak, *v* variable, *tr* traces of growth

Table 37.47

Chemotaxonomic characteristics and methods of isolation of the two species of the genus *Thermocrispum*[a]

Species	Characteristics					Origin of isolation sample	Method of isolation
	DNA G+C content (mol %)	Phospholipids[b]	Whole-cell sugars[c]	Major menaquinone	Predominant fatty acids		
T. muncipale DSM 44070^T	69–73	PE, PI, OH-PE	Ara, Man, Glc	MK-9(H_4)	*iso*-$C_{16:0}$, $C_{17:1}$	Mushroom compost; Germany	Isolated using dilution plate techniques and Andersen sampler method. Peptone-maize agar (PM agar), R8 agar, and trypticase soy agar (TSA) were used as the basal media, supplemented with antibiotics, dyes, cycloheximide and nystatin (50 μg ml^{-1} each); incubated at 50 °C.
T. agreste DSM 44069^T	69–73	PE, PI, OH-PE	Ara, Man, Glc	MK-9(H_4)	*iso*-$C_{16:0}$, $C_{17:1}$	Municipal waste compost, air of compost plants, and air of a refuse incineration plant; South Germany	As above

[a]Data from: Korn-Wendisch et al. (1995)
[b]*PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *OH-PE* hydroxyphosphatidylethanolamine
[c]*Ara* arabinose, *Man* mannose, *Glc* glucose

Phenotypic Analysis

Umezawaea are aerobic, Gram-stain-positive, nonacid-fast, and nonmotile actinomycetes that produce branched substrate mycelium (approximately 0.3–0.5 μm in diameter) and, on some media, aerial mycelia. The substrate mycelium fragments into ovoid or cylindrical conidia (0.3–0.5 μm by 1.1–1.9 μm) and pseudosporangia are produced on some media. The chemotaxonomic profile of *Umezawaea* is quite similar to that of *Saccharothrix* species (See *Table 37.42*), but *lyso*-phosphatidylethanolamine is uniquely found in the phospholipid pattern. The phylogenetically nearest neighboring genus is *Saccharothrix* (*Fig. 37.3*) but the nucleotide signatures for *Umezawaea tangerina* are distinctly different, as can be seen in Figure nucleotide signatures.

The G+C content of the DNA of the type species is 74 (mol%).

The type species is *Umezawaea tangerine* NRRL B-24463^T = DSM 44720^T = FERM P-16053^T = JCM 10302^T = NBRC 16184^T (Labeda and Kroppenstedt 2007).

Taxonomy, Historical, and Current

Originally described as a species *of Saccharothrix* (Kinoshita et al. 1999), *Umezawaea tangerina* had been observed to produce pseudosporangia on the aerial mycelium in the initial description. Phylogenetic analyses of all of the species of the genus *Saccharothrix* showed that this strain was consistently phylogenetically distant from *Saccharothrix* sensu *strictu.* Reevaluation of the chemotaxonomic characteristics of this strain determined that it was significantly different from *Saccharothrix* and other genera in the family *Pseudonocardineae*, primarily in the presence of significant quantities of *lyso*-phosphatidylethanolamine

in the phospholipid profile which supported the creation of the new genus (Labeda and Kroppenstedt 2007). Morphological and physiological characteristics of the type species *Umezawaea tangerina* in comparison to those of the species from the genus *Saccharothrix* are seen in Table 37.43.

Yuhushiella Mao, Wang Dai, Zhang, Tang, Ren, Yang, Goodfellow, Zhang, and Liu 2011, 621[VP]

Yu.hu.shi.el'la.N.L. dim.ending -ella; N.L. fem. n. Yuhushiella named after Professor Yuhu Shi, a Chinese microbiologist.

Phenotypic Analyses

Yuhushiella species are aerobic, Gram-stain-positive, nonacid-fast, nonmotile, thermotolerant actinobacteria. Substrate mycelium is straight to flexuous, smooth, and branched (Table 37.25). Swelling and aggregation of the hyphae occur but fragmentation is seldom observed. The cell wall (type IV) contains *meso*-diaminopimelic acid (Table 37.26). Whole-cell hydrolysates contain ribose, arabinose, glucose, and galactose. The diagnostic phospholipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylinositol mannosides, phospholipids of unknown structure, and glucosamine-containing phospholipids of unknown structure. Mycolic acids are absent and *N*-acetylated-type muramic acid is present. The predominant menaquinone is MK-9(H_4) with minor amounts of MK-9, MK-9(H_2), MK-10(H_2), MK-8(H_2), and MK-8. The predominant fatty acid is iso-$C_{16:0}$.

The G+C content of the genomic DNA of the type strain is 69.9 (mol%).

The type species is *Yuhushiella deserti* RA45^T = CGMCC 4.5579^T = JCM 16584^T, which was isolated from a desert region in Xinjiang Uighur Autonomous Region, China (Mao et al. 2011).

Isolation Procedures

The isolation protocol is shown in Table 37.26.

Taxonomy, Historical, and Current

Comparative analysis of the 16S rRNA gene sequence and phenotypic characterization revealed that strain RA45^T belonged to the order *Pseudonocardiales* with more than 5 % 16S rRNA gene sequence divergence from recognized species of the genera in the order, forming a distinct lineage within the evolutionary radiation occupied by the genera *Amycolatopsis*, *Prauserella*, *Thermocrispum*, *Saccharomonospora*, *Saccharopolyspora*, and *Sciscionella*, but distinct from each of them. The affiliation to the family was supported by the presence of suborder- and family-specific 16S rRNA signature nucleotides. *Yuhushiella* differed from known genera of the family in its polar lipid composition (phospholipid type IV). The study observed no aerial mycelium under any of the culture conditions tested.

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38 The Family *Rubrobacteraceae*

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Abstract
Rubrobacteraceae, a family of the order *Rubrobacterales*, class *Actinobacteria*, embraces the genus *Rubrobacter* with three species: *Rubrobacter xylanophilus*, *R. taiwanensis*, and *R. radiotolerans* (Carreto et al. Int J Syst Bacteriol 46: 460–465, 1996; Chen et al. Int J Syst Evol Microbiol 54: 1849–1855, 2004; Suzuki et al. FEMS Microbiol Lett 52: 33–40, 1988). These three species tolerate extremely high levels of ionizing radiation and possess unique straight-chain internally branched fatty acids. Moreover, the species are moderately thermophilic or thermophilic. These organisms have been primarily isolated from hot springs and deteriorated ancient walls or wall paintings. The species "*R. bracarensis*" has been effectively published, but the name has not yet been validated.

Taxonomy, Historical and Current

Short Description of the Family

The subclass *Rubrobacteridae*, the order *Rubrobacterales*, and the family *Rubrobacteraceae* were proposed by Stackebrandt et al. (1997), to comprise only the species of the genus *Rubrobacter*. After this publication, several deep lineages of the class *Actinobacteria* underwent a number of taxonomic assignments. Later, the families *Thermoleophilaceae*, *Conexibacteraceae*, and *Solirubrobacteraceae* were proposed to encompass the species of the genera *Thermoleophilum*, *Conexibacter*, and *Solirubrobacter*, respectively (Stackebrandt 2004, 2005a, b). The next year the family *Patulibacteraceae* was proposed for species of the genus *Patulibacter* (Takahashi et al. 2006). Therefore, the order *Rubrobacterales* comprised five families of deep lineages of the class *Actinobacteria*, namely, the families *Thermoleophilaceae*, *Conexibacteraceae*, *Solirubrobacteraceae*, *Patulibacteraceae*, and *Rubrobacteraceae*. However, more recently, the family *Thermoleophilaceae* was elevated in taxonomic rank to constitute the order *Thermoleophilales*, and the families *Conexibacteraceae*, *Patulibacteraceae*, and *Solirubrobacteraceae* were assigned to the order *Solirubrobacterales* (Reddy and Garcia-Pichel 2009) based on phenotypic and chemotaxonomic properties and 16S rRNA gene sequence signature nucleotides leaving the family *Rubrobacteraceae* as the sole family of the *Rubrobacterales*. This taxonomic reshuffle led the same authors to emend the description of the order *Rubrobacterales*. Later in the same volume of the International Journal of Systematic and Evolutionary Microbiology, Zhi et al. (2009) emended the subclass *Rubrobacteridae*, the order *Rubrobacterales*, and the family *Rubrobacteraceae* but maintained the families *Conexibacteraceae*, *Patulibacteraceae*, *Solirubrobacteraceae*, and *Thermoleophilaceae* within the order *Rubrobacteriales*. These taxa were also emended on the basis of 16S rRNA gene sequence signature nucleotides. In this chapter the classification of Reddy and Garcia-Pichel (2009) will be considered since, as shown later, it better reflects the characteristics of these taxa.

Recently, a new organism named *Gaiella occulta* was described and included in a separate family and order of the subclass *Rubrobacteridae*, namely, the family *Gaiellaceae* of the order *Gaiellales* because of unique phenotypic characteristics and phylogenetic analysis (Albuquerque et al. 2011). The proposal of the order *Gaiellales* also conforms to the classification of Reddy and Garcia-Pichel (2009) for the subclass *Rubrobacteridae*.

Rubrobacteraceae Rainey et al. 1997; emend. Stackebrandt 2004; emend. Zhi et al. 2009

Rubrobacteraceae (Ru.bro.bac.te.ra' ce.ae. N.L. masc. n. *Rubrobacter*, type genus of the family; suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Rubrobacteraceae*, the *Rubrobacter* family).

The members of the family *Rubrobacteraceae* of the order *Rubrobacterales* stain Gram-positive. The organisms form irregular cells but do not form endospores. Strictly aerobic and chemoorganotrophic. Members contain L-Lys as the diamino acid at position 3 of the peptidoglycan and menaquinone 8 (MK-8) as the predominant respiratory lipoquinone. Straight-chain internally branched fatty acids are major

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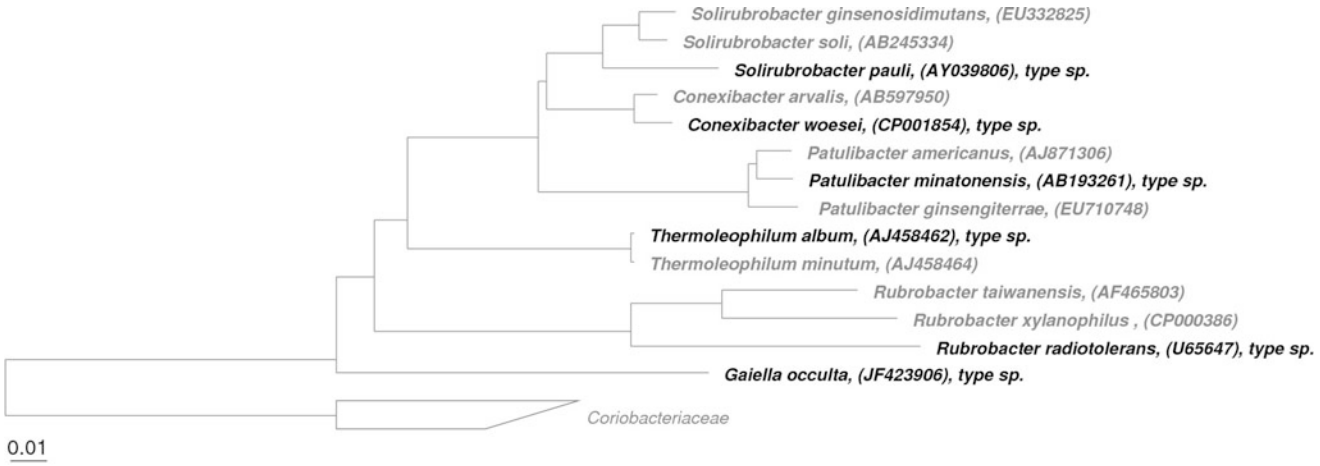

Fig. 38.1

Phylogenetic reconstruction of the family *Rubrobacteraceae* 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

constituents. The pattern of 16S rRNA signatures consists of nucleotides at positions: 52: 359 (G-C), 63: 104 (C-G), 70: 98 (A-U), 127: 234 (G-C), 139: 224 (U-A), 144: 178 (G-C), 145:177 (G-C), 291:309 (U-A), 293: 304 (G-U), 370: 391 (C-G), 377: 386 (G-C), 408: 434 (G-C), 409:433 (G-C), 580: 776 (U-A), 590: 649 (C-G), 600: 638 (U-G), 657: 749 (G-C), 670: 736 (A-U), 681: 709 (C-G), 722: 733 (G-C), 823: 877 (G-C), 906 (A), 941: 1342 (A-U), 953: 1228 (U-A), 954: 1226 (C-G), 955: 1225 (U-A), 999:1041 (U-A), 1051: 1207 (C-G), 1115: 1185 (C-G), 1311: 1326 (A-U), 1313: 1324 (U-A), and 1410: 1490 (U-A) (Reddy and Garcia-Pichel 2009; Zhi et al. 2009). The family comprises the type and only genus *Rubrobacter*.

Phylogenetic Structure of the Family and Its Genus

The family *Rubrobacteraceae* (order *Rubrobacterales*) comprises the genus *Rubrobacter* with three validly named species. The type species of the family is *Rubrobacter radiotolerans* (Suzuki et al. 1988); the two additional species being *R. xylanophilus* and *R. taiwanensis* (Carreto et al. 1996; Chen et al. 2004). This family represents one deep-branching lineage of the phylum Actinobacteria, which is most closely related to the species of the families *Thermoleophilaceae*, *Conexibacteraceae*, *Solirubrobacteraceae*, and *Patulibacteraceae*. The 16S rRNA sequence analysis of the type species of the family *Rubrobacteraceae* (*R. radiotolerans* DSM 46359^T, X87134) with the type strains of the families *Solirubrobacteraceae* (*S. pauli* B33D1^T, AY039806), *Patulibacteraceae* (*P. minatonensis* KV-614^T, AB193261), *Conexibacteraceae* (*C. woesei* DSM 14684^T, CP001854), *Thermoleophilaceae* (*T. minutum* ATCC 35268^T, AJ458464), and *Gaiellaceae* (*G. occulta* F2-233^T, JF423906) shows very low similarities of between about 83 % to 84 % (*Fig. 38.1*).

Phenotypic Analyses

The main features of *Rubrobacter radiotolerans, Rubrobacter xylanophilus, and Rubrobacter taiwanensis* are listed in *Table 38.1*.

Rubrobacter Susuki et al. 1989

Rubrobacter (Ru. bro. bac'ter. L. adj *ruber -bra -brum*, red; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Rubrobacter*, red rod).

Rubrobacter forms non-motile pleomorphic cells (rods or coccoid) that stain Gram-positive. Moderately thermophilic or thermophilic. Catalase and oxidase positive. Nitrate is reduced to nitrite. The principal amino acid of the cell wall peptidoglycan is L-lysine. The peptidoglycan type is A3α′. The peptidoglycan of strain PRD-1^T and strain P-1^T contained lysine, glutamic acid, and alanine at a molar ratio of 1:1:3. The major respiratory lipoquinone is MK-8. Mycolic acids are absent. Polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, one unidentified phosphoglycolipid, one unidentified glycolipid, and unidentified phospholipids. Major fatty acids are internally branched straight-chain saturated fatty acids, such as $C_{16:0}$ 12-methyl and $C_{18:0}$ 14-methyl. The G + C content of the DNA is in the range 64.9–68.5 mol%. The type species is *Rubrobacter radiotolerans*. The type strain P-1^T (=IAM 12072^T = JCM 2153^T)

Table 38.1

Phenotypic and chemotaxonomic characteristics of members of the genus *Rubrobacter*

	R. radiotolerans[a, b, c]	*R. xylanophilus*[b, c]	*R. taiwanensis*[c]
	P-1^T	PRD-1^T	LS-293^T
Morphology	Irregular rods	Pleomorphic (rods or coccoid)	Pleomorphic (rods or coccoid)
Cell size (μm)	0.8–1.0 × 1.0–4.0	0.9–1.0 × 1.0–3.0	0.9–1.0 × 1.0–3.0
Colony morphology	Circular, convex, smooth, and opaque	Circular, convex, smooth, and opaque	Circular, convex, smooth, and opaque
Pigmentation	Bright pink	Light pink	Light pink
Temperature for growth (°C)			
Range	30–55	40–70	30–70
Optimum	46–48	60	60
pH for growth			
Range	6.0–10.0	6.0–10.0	6.0–11.0
Optimum	7.0–7.4	7.5–8.0	8.0
NaCl for growth (%)			
Range	0–6[a, b]	0–6[b]	0–5
	0–5[c]	0–5[c]	
Optimum	0–1	0	0
Metabolism	Aerobic	Aerobic	Aerobic
Indole production	−	nd	nd
Methyl red	−	nd	nd
Presence of			
DNAse	−	−	+
Hydrolysis of			
Gelatin	−[a], +[b, c]	+	+
Esculin	+	+	−
Arbutin	+	+	nd
Xylan	−	+	−
Hide powder azure	+	+	nd
Hippurate	+	+	nd
Elastin	−	−	nd
Fibrin	−	−	nd
Tyrosine	−	−	nd
Olive oil	−	−	nd
Tween 80	−	−	nd
Assimilation of			
D-galactose	−	+	+
D-xylose	+[a],−[b, c]	+	+
D-melibiose	–	+	+
L-rhamnose	+	+	–
Maltose	+	nd	+
Sucrose	−[a],+[b]	+	nd
Lactose	−[a],+[b, c]	+	+
Salicin	+	+	nd
Glycerol	+	–	–
Erythritol	+	+	nd
Galactitol	–	w	nd
D-mannitol	+	–	–

Table 38.1 (continued)

	R. radiotolerans[a, b, c] P-1^T	*R. xylanophilus*[b, c] PRD-1^T	*R. taiwanensis*[c] LS-293^T
myo-inositol	–	+	+
Ribitol	+	–	–
Citrate	–	–	nd
Malate	+	+	–
Acetate	–	w	nd
Succinate	–	+	–
Acetamide	–	+	nd
L-arginine	nd	nd	+
L-glutamine	–	w	+
L-serine	–	–	+
Acid production from carbohydrates	–	nd	nd
Peptidoglycan type	A3α′	A3α′	nd
Diagnostic peptidoglycan amino acids[d]	L-Lys	L-Lys	nd
Mycolic acids	–	–	nd
Major fatty acid	$C_{16:0}$ 12-methyl	$C_{18:0}$ 14-methyl	$C_{18:0}$ 14-methyl
Polar lipids[e]	DPG, PG, PGL, GL, PL1, PL2, PL3	DPG, PG, PGL, GL, PL1, PL3, PL4	nd
G + C content (mol%)	67.9[a], 64.9[c]	67.6	68.5

All of the organisms are oxidase, catalase, β-galactosidase, and β-glucosidase positive. D-glucose, D-fructose, D-cellobiose, D-trehalose, D-arabinose, L-arabinose, D-raffinose, D-mannose, pyruvate, L-glutamate, L-asparagine, and L-proline are utilized by all of the strains. All of the organisms are α-galactosidase and α-glucosidase negative. None of the strains utilize sorbitol or hydrolyze starch, casein, cellulose, and tributyrin

Symbols: + positive, – negative, *w* weakly positive, *nd* not determined

[a]Suzuki et al. 1988

[b]Carreto et al. 1996

[c]Chen et al. 2004

[d]L-Lys, L-lysine

[e]*DGP* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PGL* unknown phosphoglycolipid, *GL* unknown glycolipid, *PL1,2,3,4* unknown phospholipids 1,2,3,4

was isolated from a radioactive hot spring at Misasa, Japan (Suzuki et al. 1988; Yoshinaka et al. 1973). The type strain of *Rubrobacter xylanophilus* PRD-1^T (=DSM 9941^T) was isolated from a thermally polluted runoff from a carpet factory in Wilton, Wiltshire, United Kingdom (Carreto et al. 1996). A third species is *Rubrobacter taiwanensis*: the type strain is LS-293^T (=ATCC BAA-406^T = BCRC 17173^T) isolated from Lu-shan hot springs in Taiwan. Strain LS-286 (=ATCC BAA-452 = BCRC 17198), isolated from the same source, is a reference strain (Chen et al. 2004).

The species of the genus *Rubrobacter*, namely, *R. radiotolerans*, *R. xylanophilus*, and *R. taiwanensis*, are some of the most extremely radiation-resistant organisms known; the dose required to reduce the number of viable units to 37 % (the mean dose required to reduce a single colony-forming unit of the irradiated population) is in the range of 7.6–9.0 kGy (kGray) for the type strain of *R. radiotolerans* and the closely related isolate RSPS-4 and 4.6–5.2 kGy for the type strain of *R. xylanophilus* and closely related RSPS-21 (Ferreira et al. 1999). Comparable results were obtained by Chen et al. (2004) for the type strain of *R. taiwanensis* with a reduction to 37 % similar to that of the type strain of *R. radiotolerans*. The ionizing-radiation resistance of these three organisms is comparable and in some cases appears to be slightly higher than those of the most radiation resistant species of the genus *Deinococcus*. *R. xylanophilus* and *R. taiwanensis* have much higher growth temperature than *Deinococcus* spp. (Carreto et al. 1996; Chen et al. 2004; Ferreira et al. 1997, 1999).

A species assigned to the genus *Rubrobacter*, and named "*R. bracarensis*," was isolated from a green discolored interior wall of a church (Jurado et al. 2012). This organism has a lower growth temperature range (10–45 °C) and a higher salinity range (0–30 % NaCl) than any of the three other known *Rubrobacter* spp. However, resistance to ultraviolet light or ionizing radiation was not examined. Moreover, the new organism does not possess the archetypal internally branched chain fatty acids present in large levels in the three validly named species (Carreto et al. 1996; Chen et al. 2004; Ferreira et al. 1999; Suzuki et al. 1988). Rather, this organism possesses large amounts of anteiso-$C_{17:1}$ ω9*c* (about 90 %). The incongruent fatty acid results cannot be explained at this time, but it should be noted that Suzuki et al. (1988) and Carreto et al. (1996) used mass spectrometry to

identify the internally branched fatty acids of *Rubrobacter* spp., while Jurado et al. (2012) did not. It should also be stated that iso- and anteiso-branched fatty acids have not been detected, or are detected in trace amounts, in the other species of the genus *Rubrobacter*.

Isolation, Enrichment, and Maintenance Procedures

Rubrobacter radiotolerans was isolated from a radioactive hot spring at Misasa, Japan (Yoshinaka et al. 1973). Water containing mud, fur, and moss was collected from the hot spring at Misasa, which is reported to have a very high radon content. Samples were poured into sterilized test tubes with cotton plugs and irradiated with cesium-137 γ-rays at a dose rate of 1.7×10^5 rad/h for 5 h at 25 °C in the controlled environment radiation facility. Immediately after irradiation, 0.1 ml of the sample was placed on the surface of solid B-agar (10 g beef extract, 10 g bacto-peptone, 5 g yeast extract, 2 g glucose, 1 L deionized water, pH 7.2) plate with the aid of a glass spreader. The plates were incubated at 37 °C for 3 weeks. After a week of incubation, one pink colony was selected, purified, and maintained on the same medium. *Rubrobacter radiotolerans* is routinely grown in *Thermus* medium (Williams and da Costa 1992).

Rubrobacter xylanophilus was isolated from thermally polluted runoff (temperature, 50 °C) from a carpet factory in Wilton, Wiltshire, United Kingdom (Carreto et al. 1996). This strain was isolated by spreading a biofilm sample with a glass rod on tryptone soya agar. After incubation at 50 °C for 5 days, one pink-pigmented colony appeared on a culture plate and was purified on the same medium. *Rubrobacter xylanophilus* is routinely grown in *Thermus* medium.

Rubrobacter taiwanensis was isolated from samples of water, thermally heated soil, and mud from natural hot springs in the Lu-shan area, Nantou, Taiwan (Chen et al. 2004). Aliquots (100 ml) of untreated water samples were spread directly onto *Thermus* agar plates (Williams and da Costa 1992), which were subsequently sealed in plastic bags and incubated at 50 °C for 7 days. Pink-pigmented colonies were picked from the plates and subcultured for purification. *Rubrobacter taiwanensis* is routinely grown in *Thermus* medium.

Members of this family do not require special procedures for maintenance and long-term storage. Generally strains are maintained on *Thermus* medium at 4 °C for a few days and can be stored frozen at −70 °C in *Thermus* medium containing 15 % glycerol without loss of viability. Long-term preservation is by lyophilization.

Ecology

Habitat

The type strains of *R. radiotolerans*, *R. xylanophilus*, and *R. taiwanensis* were isolated from hot springs or a thermally polluted stream. Other strains with very high 16S rRNA sequence similarity with the type strains of *R. radiotolerans* and *R. xylanophilus* have been isolated, after gamma-irradiation of the samples, from hot spring water at the S. Pedro do Sul and Alcafache hot springs in Central Portugal (Ferreira et al. 1999). Other unclassified 16S rRNA clone sequences have been detected, and strains of the genus *Rubrobacter* have also been isolated from discolored and deteriorated ancient walls and wall paintings (Imperi et al. 2007; Laiz et al. 2009; Schabereiter-Gurtner et al. 2001), as well as arid soils (Holmes et al. 2000). The type strains of the species of *R. radiotolerans*, *R. taiwanensis*, and *R. xylanophilus* are among the most ionizing radiation-resistant organisms known (Chen et al. 2004; Ferreira et al. 1997, 1999; Suzuki et al. 1988). These organisms are also halotolerant which may give them selective advantage in colonizing dry environments and saline environments. To achieve colonizing saline environments, *R. xylanophilus* and *R. radiotolerans* accumulate the compatible solutes trehalose and mannosylglycerate. However, it should be noted that the high levels of trehalose and mannosylglycerate accumulate constitutively, changing very little when the concentrations of salt are added to the growth medium, with the growth temperature and upon the addition of oxidative stress agents to the medium (Empadinhas et al. 2007).

Many 16S rRNA clone sequences have been retrieved from a variety of habitats. Some derive from desert soils (JF706680, HQ910287), coastal (saline) soils (HQ397509, JX240777), and dry, discolored, and sun-exposed walls (AM746686, JN020173), indicating that these organisms are desiccation resistant, are able to grow in environments with moderate salinities, and are probably radiation resistant, as well.

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39 The Families *Sanguibacteraceae* and *Rarobacteraceae*

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Abstract
The monogeneric family *Sanguibacteraceae*, defined on the basis of 16S rRNA gene sequence comparison and signature nucleotides, is a member of the order *Micrococcales*. Its precise 16S rRNA gene sequence-based phylogenetic position among the other 14 families of the order has not yet been determined with confidence as this lineage branches with varying neighbors depending upon algorithms and selection of sequences used in the analyses. The monogeneric family *Rarobacteraceae* was included in this chapter as it branches adjacent to *Sanguibacteraceae* in several ML and NJ trees. The six species of *Sanguibacter* possess a DNA mol% G+C between 69 and 73 mol%, an A4α peptidoglycan type, the predominant menaquinone MK-9(H_4), straight-chain saturated, as well as iso- and anteiso-methyl-branched fatty acids and resemble certain members of *Cellulomonadaceae* and *Promicromonosporaceae*. Some type strains were isolated from blood of apparently healthy cows while others were found in soil, sand, and sediment. An extended range of habitats was revealed by non-culture studies and encompasses terrestrial and aquatic ecosystems. Strains are medically nonrelevant and have limited application potential. The genus *Rarobacter* embraces two species with yeast-lysing abilities. The ornithine-containing peptidoglycan type is A4β, and menaquinones are fully unsaturated and of the MK-9 type. Members of the genus must be considered rare actinobacteria as the literature mentions almost no additional cultures or 16S rRNA gene clones.

Taxonomy: Historical and Current

The history of the family began with a polyphasic study on six strains isolated from venous blood of apparently healthy dairy cows in Western Spain (Fernández-Garayzábal et al. 1995). The motile, facultatively anaerobic, catalase positive and oxidase negative strains formed two physiologically separate groups, each being highly related by 16S rRNA gene sequence among, and closely related between each other (98.6 % similarity). The nearest neighbors of the organisms were members of the *Cellulomonas/Oerskovia* clade (93–96 % similarity) and *Terrabacter tumescens* (95.7 %). An unrooted dendrogram showed the milk isolates and *T. tumescens* form one lineage, adjacent to cellulomonads and oerskoviae. Based upon chemotaxonomic evidence, the authors expressed their surprise about the phylogenetic clustering as peptidoglycan structure as well as fatty acid compositions differed markedly among the organisms of this group: While the cow blood isolates possessed, like oerskoviae, a type A4α peptidoglycan (L-Lys–L-Ser–D-Glu vs. L-Lys–L-Thr–D-Glu, respectively), it differed from those of cellulomonads (A4β) and *Terrabacter* (A3γ). Also with respect to fatty acid composition, a higher similarity was detected between the blood isolates and cellulomonads/oerskoviae (mainly straight-chain and anteiso-methyl-branched, and lower amounts of iso-branched types), whereas those of *T. tumescens* were mainly unsaturated, iso-branched, and unsaturated methyl-branched acids. An artificially close genealogical relationship was given as an explanation for the position of the two groups of blood isolates within the *Cellulomonas/Oerskovia/Terrabacter* cluster which were described as members of a new genus *Sanguibacter*, containing the type species *S. keddieii* and *S. suarezii* (Fernández-Garayzábal et al. 1995).

The second half of the 1990s saw an enormous increase of 16S rRNA gene sequences, rapidly changing the closely branching lineages among spore-forming actinomycetes and their non-spore-forming relatives. The branching pattern available in 1997 was used by Stackebrandt and collaborators to outline a hierarchic system of the *Actinobacteria* in which

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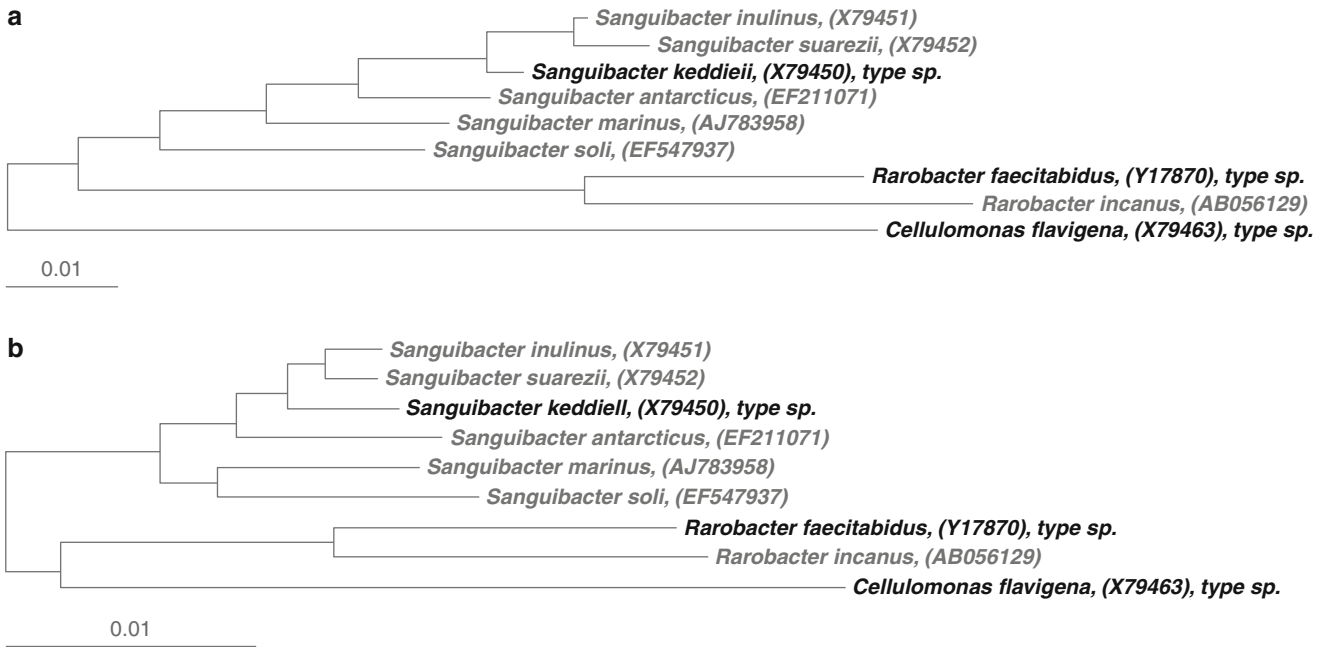

Fig. 39.1

Maximum likelihood based on the RAxML algorithm (Stamatakis 2006) (a) and Neighbor-Joining (b) genealogy reconstruction of the sequences of all members of the family *Sanguibacteraceae* present in the LTP_106 (Yarza et al. 2010). The trees were reconstructed by using a subset of sequences representative of close relative genera to stabilize the tree topology. In addition, a 60 % conservational filter for the whole bacterial domain was used to remove hypervariable positions. The bar indicates 1 % sequence divergence. Type strain numbers referring to the accession numbers are those indicated in Table 39.1

Sanguibacter, together with *Terrabacter* and *Intrasporangium*, defined the family *Intrasporangiaceae* within the suborder *Micrococcineae*, order *Actinomycetales* (Stackebrandt et al. 1997). The family *Intrasporangiaceae* was more closely related to *Dermabacteraceae*, *Jonesiaceae*, and *Brevibacteraceae* than to *Micrococcaceae* or *Cellulomonadaceae*. This sequence- and topology-based scheme did not take into account any physiological or chemotaxonomic data, and it did not take long that the membership of *Sanguibacter* within *Intrasporangiaceae* was questioned, e.g., on the basis of polyamine composition (Busse and Schumann 1999).

A few years later, the phylogenetic position supported the view that *Sanguibacter* species group with the genus *Rarobacter* but separate from both *Intrasporangiaceae* and *Cellulomonadaceae*. Based upon genealogy and supported by chemotaxonomic evidence, the family *Sanguibacteraceae*, among others, was created on the basis of a set of 16S rRNA signature nucleotides (Stackebrandt and Schumann 2000). In an update of the hierarchic classification system (Zhi et al. 2009), this set was revised and the family description emended accordingly (see below).

The phylogenetic dendrogram of *Actinobacteria* has not yet stabilized, and in the latest edition of *Bergey's Manual*, volume 5 (Actinobacteria), the family *Sanguibacteraceae* appears as a specific relative of *Oerskovia* (Ludwig et al. 2012). The same situation is seen in the maximum-likelihood dendrogram of Ivanova et al. (2009). The most recent RaXML version (http://www.arb-silva.de/fileadmin/silva_databases/living_tree/LTP_release_106/LTPs106_SSU_tree.pdf) clusters *Sanguibacteraceae* with *Rarobacteraceae* as a sister clade of both *Cellulomonadaceae* and *Promicromonosporaceae* (see Fig. 39.1).

Another six isolates, also recovered from blood of apparently healthy cows in Spain, constituted the species *Sanguibacter inulinus* (Pascual Ramos et al. 1996). It was phylogenetically close to *S. keddieii* and *S. suarezii* (99.2–99.4 % 16S rRNA gene sequence similarities) but could be distinguished from each other phenotypically and by DNA-DNA hybridization.

The genus *Rarobacter* with the type species *Rarobacter faecitabidus* was described by Yamamoto et al. (1988) for facultative anaerobic isolates capable of lysing yeast cells. Its physiological and chemotaxonomic properties differed from other actinobacterial genera with an L-ornithine-based peptidoglycan described in 1988, e.g., *Cellulomonas*, *Curtobacterium*, and *Aureobacterium*. The second species, *R. incanus*, was described by Goto-Yamamoto et al. (1993) for a different group of yeast-cell-lysing strains from Japan and Brazil which was separated from *R. faecitabidus* by low DNA hybridization values and, as determined for the type strain YLM-32^T, the lack of serine in the peptidoglycan.

Molecular Analyses

The DNA-DNA hybridization (DDH) method was used to relate strains at the intraspecies level (*S. inulinus* and *S. suarezii*)

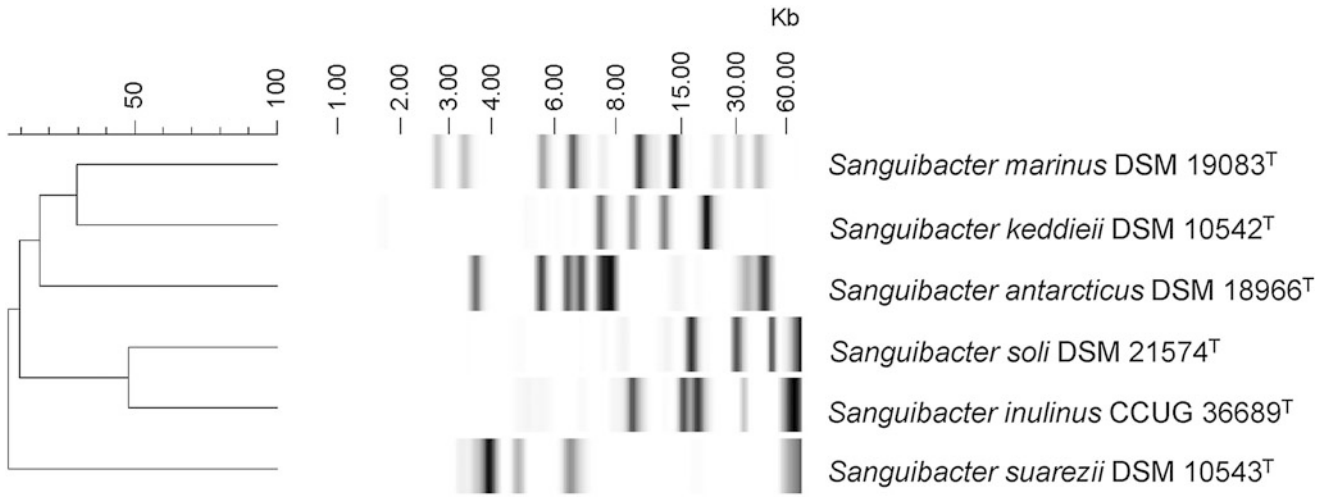

Fig. 39.2

Riboprint patterns of the type strains of the family *Sanguibacteraceae* generated by using the restriction enzyme *Pvu*II. The dendrogram has been calculated with the BioNumerics software (Applied Math, Kortrijk, Belgium)

as well as to confirm their species status and that of *S. keddieii* (Pascual Ramos et al. 1996). The molecular intraspecies similarities were also determined by comparative analysis of the 16S rRNA gene sequence 5′ terminus (Fernández-Garayzábal et al. 1995; Pascual Ramos et al. 1996). The other three species were defined by only the type strain which showed less than 98 % 16S rRNA gene sequence similarities among other type strains of the genus which made it unnecessary to perform DDH (Stackebrandt and Ebers 2006).

Extensive DDH was performed for *Rarobacter* strains of different origin, and five clusters with intracluster similarities >50 % were identified (Goto-Yamamoto et al. 1993). Two of these clusters corresponded to *R. faecitabidus* and *R. incanus*, while the other clusters were not formally described as new species.

Riboprinting

Multiband Riboprint patterns obtained by digestions of the DNA with *Pvu*II, the preferred restriction enzyme for the majority of members of the order *Micrococcales*, differentiate all six *Sanguibacter* type strains. Residual amounts of undigested DNA (band >60 kbp) remained for *S. soli*, *S. inulius* and *S. suarezii* (*Fig. 39.2*).

Mass Spectral Analyses (MALDI-TOF Mass Spectrometry)

S. inulinus ST 50^T shows a 16S rRNA gene sequence similarity as high as 98.92 % to both *S. suarezii* ST 26^T and *S. keddieii* DSM 10542^T (calculated by the EzTaxon-e server; Kim et al. 2012). While *S. inulinus* CCUG 36689^T and *S. suarezii* DSM 10543^T can easily be differentiated by their MALDI-TOF mass spectra, the spectra of *S. inulinus* CCUG 36689^T and *S. keddieii* DSM 10542^T are highly similar. Except for *S. keddieii* and *S. inulinus*, the species of the genus *Sanguibacter* can be identified on the basis of their dissimilar whole cell MALDI-TOF mass spectra.

The MALDI-TOF mass spectra of *Rarobacter faecitabidus* DSM 4813^T and *Rarobacter incanus* DSM 10596^T form a cluster distant from those of the type strains of the genus *Sanguibacter* and differ significantly from one another (*Fig. 39.3*).

Genome Sequence

The full genome of *Sanguibacter keddieii* DSM 10542^T has been sequenced (Ivanova et al. 2009) within the frame of the Tree of Life project (http://genome.jgi.doe.gov/programs/bacteria-archaea/index.jsf). The genome with a mol% G+C content of 71.9 % (original description 70.0 %) is 4.253 Mbp long. 3.805 genes were predicted, 3.735 of which were genes coding for proteins while 70 genes coded for RNAs. Most of the genes (74.4 %) could be annotated and assigned a putative function (71.1 %) to clusters of orthologous genes (COGs), while the remaining ones were annotated as hypothetical proteins. The majority of genes with COG functions were linked to carbohydrate and metabolism (354, 12 % of total), as well as transcription (317, 10 % of total), 237 genes (8 % of total) were associated with the functional category amino acid transport and metabolism, and 199 genes (7 % of total) were involved in inorganic iron transport and metabolism. The metabolic network statistics lists 205 metabolic pathways, 714 enzymes and 935 metabolic reactions. The comparative values for one of the nearest neighbors of *Sanguibacter keddieii* with a complete sequenced genome of a similar size, *Cellulomonas flavigena* DSM 20109^T, are generally a few percent lower.

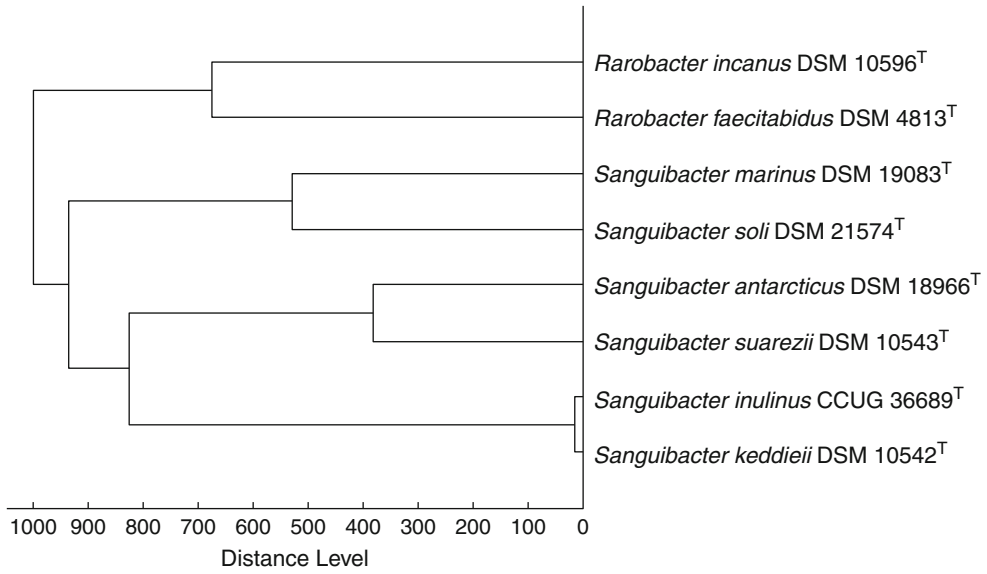

Fig. 39.3
Score-oriented dendrogram generated by the BioTyper software (version 3.0, Bruker Daltonics) showing the similarity of MALDI-TOF mass spectra of cell extracts of the type strains of the families *Sanguibacteraceae* and *Rarobacteraceae*

Phenotypic Analyses

Sanguibacteraceae Stackebrandt and Schumann 2000, 1284^{VP}; emend Zhi et al. 2009, 599

San.gui.bac.te.ra'ce.a.e. M.L. masc. n. *Sanguibacter* type genus of the family; -*aceae* ending to denote a family; M.L. fem. pl. n. *Sanguibacteraceae* the *Sanguibacter* family.

The family *Sanguibacteraceae* is monogeneric and contains six species. The original set of signatures given for the family by Stackebrandt and Schumann (2000) was revised by Zhi et al. (2009) on the basis of three species. It contains (position : composition) 120 : A; 131–231 : C-G; 196 : U; 342–347 : C-G; 444–490 : A-U; 580–761 : C-G; 602–636 : G-U; 670–736 : A-U; 822–878 : G-C; 823–877 : G-C; 826–874 : C-G; 827: U; 843 : C; 950–1231 : U-A; 1047–1210 : G-C; 1109 : C; 1145 : G; 1309–1328 : G-C; 1361 : G; 1383 : C. Note that the set of signatures given by Pascual Ramos and Fernández-Garayzábal (2012) is not the revised set of Zhi et al. (2009).

Phenotypically, the description of the family is as given for the genus. The description differs from that given by Fernandez-Garáyzabál et al. (1995) by the inclusion of properties of four additional species (see *Table 39.1*): Cells are Gram-positive and motile rods or irregular rods. Catalase positive, most strains are oxidase negative. Mesophilic and organotrophic. Facultative anaerobic (not determined for one type strain). Acid produced from a wide range of carbohydrates. Mycolic acids are not produced. The long-chain cellular fatty acids are primarily of the straight-chain saturated and anteiso(ai)-methyl-branched chain types; Iso(i)-methyl branched chain acids are present in small amounts. MK-9(H_4) is the major menaquinone. The cell wall murein is L-Lys–L-Ser–D-Glu (type A4α, according to Schleifer and Kandler 1972; A11.48 according to the revised scheme of Schumann (2011) and www.peptidoglycan-types.info). The few species investigated contain minor amounts of the polyamines putrescine and spermidine (Busse and Schumann 1999). Data on polar lipids and, except for *Sanguibacter suarezii* ATCC 51766 (galactose, mannose, ribose) (Brown et al. 2005), whole cell sugars were not reported. The DNA mol% G+C ranges between 69.0 and 73.5. The family contains the type genus *Sanguibacter* Fernández-Garayzábal et al. (1995).

The availability of the type strain of *Sanguibacter inulinus* from public resource centers appears to be a moot-point after a comparative study by Riboprinting, MALDI-TOF MS, and 16S rRNA gene sequencing on strains NCIMB 703024^T, DSM 17437^T (obtained as CIP 106681^T), and CCUG 36689^T. MALDI-TOF and Riboprinting dendrograms indicate the same pattern for strains NCIMB 703024^T and DSM 17437^T which are identical with those of *Sanguibacter suarezii* DSM 10543^T. This finding is in agreement with 99 % similarity of the partial 16S rRNA gene sequences of strains DSM 17437^T and DSM 10543^T, detected already in 2005 and the reason why strain DSM 17437^T has never been included in the DSMZ Catalogue of Strains. Considering the history documented in www.StrainInfo.net and a personal communication from Takuji Kudo (Japan Collection of Microorganisms), the type strain JCM 11442^T is also identical with the type strain of *S. suarezii*. As a consequence, CIP and JCM have removed the type strain of *S. inulinus* from their strain catalogs.

Table 39.1

Phenotypic differences between the type strains of *Sanguibacter* (*S.*) and *Rarobacter* (*R.*) species (Data compiled from Hong et al. (2008) and Kim et al. (2008), supplemented with data from the original species descriptions (*S. keddieii* and *S. suarezii*, Fernández-Garayzábal et al. 1995; *S. inulinus*, Pascual Ramos et al. 1996; *S. soli*, Kim et al. 2008; *S. marinus*, Huang et al. 2005; *S. antarcticus*, Hong et al. 2008). Data for *Rarobacter* type strains were from Yamamoto et al. (1988) and Goto-Yamamoto et al. (1993))

Properties	*S. keddieii* ST-74^T	*S. suarezii* ST-26^T	*S. inulinus* ST-50^T	*S. soli* DCY22^T	*S. marinus* 1-19^T	*S. antarcticus* KOPRI21702^T	*R. faecitabidus* YLM-1^T	*R. incanus* YLM-32^T
Colony color	Yellow	Pale yellow	Pale yellow	Yellow	Pale yellow	Yellow	Pale yellow	Pale grayish
Growth range (°C)	nd	nd	nd	25–42	15–37	4–30	20–39	20–39
Optimum growth temperature (°C)	25–30	25–30	25–30	37	25–30	23–26	30	
pH range	nd	nd	nd	5–9	5.5–9	4–9	6–8	6–9
Catalase	+	+	+	+	+	+	+	–
Oxidase	–	–	–	+	–	–	+	+
Nitrate reduction	–	v	w	–	+	+	–	–
Major fatty acids[b] (>10 %)	$C_{16:0}$, ai-$C_{15:0}$, $C_{18:0}$	$C_{16:0}$, ai-$C_{15:0}$, $C_{14:0}$	nd	Unknown ELC 13.961, ai-$C_{17:0}$, i-$C_{18:0}$	ai-$C_{15:0}$, ai-$C_{15:1}$A, $C_{16:0}$	ai-$C_{15:0}$	ai-$C_{15:0}$[d], i-$C_{16:0}$[e], $C_{16:0}$[f]	ai-$C_{15:0}$[d], i-$C_{14:0}$[e], i-$C_{16:0}$[d], $C_{14:0}$[e], $C_{16:0}$[d]
Mol% G+C of DNA	70.0	69.5	70.5	69.8	73.4	69.5	66	65
Gelatin hydrolysis	+	–	nd	–	+	–	+	+
Acid from[c]								
Gluconate	v	–	+	–	–	–	nd	nd
5-keto-gluconate	–	+	–	–	–	–	nd	nd
Gentobiose	+	+	+	+	–	+	nd	nd
D-Lyxose	+	+	+	–	–	–	nd	nd
Melibiose	+	+	+	–	–	–	nd	nd
Methyl-α-D-glucoside	+	–	+	+	w	–	–	–
Methyl-α-D-mannoside	+	–	+	–	–	–	nd	nd
Methyl-β-D-xyloside	+	+	v	–	–	–	nd	nd
D-Raffinose	+	v	+	–	–	–	–	–
L-Rhamnose	v	+	+	–	–	–	–	–
D-Ribose	+	+	+	+	–	+	nd	nd
D-Lactose	+	+	+	+	+	–	–	–
D-Mannose	+	+	+	+	+	–	+	+
Salicin	V	+	+		+	–	+	+
L-Sorbose	nd	–	–	–	–	–	–	–
Turanose	+	+	+	w	–	+	nd	nd
L-Xylose	nd	–	–	–	–	–	nd	nd
Glycerol	+	+	+	+	–	–	–	–
D-Mannitol	–	–	v	–	–	+	–	–
D-Sorbitol	+	–	–	–	–	–	–	–
N-acetyl glucosamine	+	–	+	+	–	–	nd	nd
Amygdalin	+	w	+	–	–	–		
Arbutin	+	+	+	+	+	–	+	+

Table 39.1 (continued)

Properties	*S. keddieii* ST-74^T	*S. suarezii* ST-26^T	*S. inulinus* ST-50^T	*S. soli* DCY22^T	*S. marinus* 1-19^T	*S. antarcticus* KOPRI21702^T	*R. faecitabidus* YLM-1^T	*R. incanus* YLM-32^T
D-Galactose	+	+	+	+	+	–	–	–
Inulin	–	–	+	–	–	–	–	–

+ positive, – negative, *v* variable, *w* weak, *nd* not determined
[a]ai, anteiso; i, iso
[b]Data from Kim et al. (2008) and Hong et al. (2008). Some deviations are present in the table by Pascual Ramos and Fernández-Garayzábal (2012)
[c]Formed under aerobic and anaerobic conditions
[d]Formed under aerobic conditions
[e]Formed under anaerobic conditions

The original deposition of the type strain *Sanguibacter inulinus* ST-50^T (Pascual Ramos et al. 1996) was with the National Collection of Food Bacteria, Reading, UK (incorporated into NCIMB), as NCFB 3024^T and according to www.StrainInfo.net, the CCUG (Culture Collection, University of Göteborg) obtained the type strain directly from there, assigning to it the number CCUG 36689^T. Riboprint and MALDI-TOF data show that the type strains *S. inulinus* CCUG 36689^T and *S. suarezii* DSM 10543^T are indeed different. The sequence of the 5′ terminal 460 nucleotides of the 16S rRNA sequence of strain CCUG 36689^T displays 100 % identity with the sequence X79451 originally deposited for *Sanguibacter inulinus* ST-50^T (attention, the species designations are sometime in error in the description fields of the 16S rRNA gene sequences of both type strains ST-26^T and ST-50^T deposited in public sequence databases). Our study revealed that CCUG 36689^T is the authentic type strain of *Sanguibacter inulinus*, while the authenticity of *Sanguibacter inulinus* strains deposited in other collections needs to be re-examined.

Rarobacteraceae Stackebrandt and Schumann 2000, 1284^{VP}, emend Zhi et al. 2009, 598

Ra.ro.bac.te.ra.ce'a.e. N. L. masc. n. *Rarobacter* type genus of the family; L. suff. *–aceae* ending to denote a family; N.L.fem.pl.n *Rarobacteraceae* the *Rarobacter* family.

The set of 16S rRNA signature oligonucleotides are (position : composition) 120 : A; 131–231 : C-G; 196 : A; 342–347 : C-G; 444–490 : A-U; 580–761 : C-G; 602–636 : G-U; 670–736 : A-U; 822–878 : G-C; 823–877 : G-C; 826–874 : C-G; 827: U; 843 : U; 950–1231 : U-A; 1047–1210 : G-C; 1109 : C; 1145 : G; 1309–1328 : G-C; 1361 : G; 1383 : C.

Except for two single base exchanges (pos. 196, 843), this set is similar to the one defining *Sanguibacteraceae*. Members of the family contain an A4β peptidoglycan type, the fully unsaturated menaquinone MK-9, anteiso-branched (major), iso-branched, and straight-chain fatty acids. Endospores are not formed, and cells are not acid-fast. Facultative anaerobic, both type strains require catalase, heme or hemoproteins as well as biotin and thiamine (*R. faecitabidus* only). Carbon dioxide is required for anaerobic growth. Major end product of aerobic glucose utilization is acetic acid, while formic acid, ethanol, and acetic acid are formed under anaerobic conditions. Phenotypically, the description of the family is as given for the genus (Yamamoto et al. 1988). Cells of both species are irregular rods, motile by multitricous flagella. The two species differ in the composition of the amino acid composition of peptidoglycan and in the percentage of fatty acid composition determined under aerobic and anaerobic conditions. Major fatty acids are of the ai-$C_{15:0}$ type (18–54 %), while i-$C_{16:0}$ (7–22 %) and $C_{16:0}$ (7–14 %) occur in lower amounts. Both strains are similar with respect to their ability to form acid from carbohydrates (Table 39.1). While *R. faecitabidus* forms acid from cellobiose, *R. incanus* does not. Other properties are indicated in the original species descriptions.

All type strains of *Sanguibacter* form acid from glucose, L-arabinose, fructose, maltose, sucrose, cellobiose, and D-xylose but not from melizitose, adonitol, and D-arabinose. Phenotypic properties, including minor fatty acid components (<10 %) not listed in Table 39.1, should be looked at in the original species description.

The two type strains of *Rarobacter* form acid from glucose, dextrin, D-fructose, and maltose but not from L-arabinose, trehalose, erythritol, adonitol, or dulcitol.

Isolation, Enrichment and Maintenance Procedures

As strains of *Sanguibacter keddieii*, *S. suarezii*, and *S. inulinus* were originally recovered from blood as presumptive members of the genus *Listeria*, a selective *Listeria* agar (Dominguez Rodriguez et al. 1984) was used for their isolation. The medium contained per liter: peptone 3 g, neopeptone 5 g, proteose peptone 3 g, esculin 1 g, NaCl 5 g, disodium phosphate 2-hydrate 12 g, ammonia-ferric citrate 40 mg, Acriflavin HCl 6 mg, defibrinated sheep blood 50 mL, and agar 15 g. Subcultures grew on tryptose soy agar (TSA, Difco).

S. marinus was isolated on Difco marine agar and maintained on nutrient agar or TSA agar. *S. soli* was isolated from serially diluted soil, spread onto 1/10-strength R2A agar (Difco), while *S. antarcticus* was isolated on ZoBell agar (Zobell 1946) supplemented with 0.4 % colloidal chitin.

DSMZ-kept strains are maintained in trypticase soy yeast extract medium No 92 (http://www.dsmz.de/?id=441, containing the following components per liter: trypticase soy broth 30.0 g, Yeast extract 3.0 g, and agar 15.0 g; pH 7.0—7.2), between 28 °C and 30 °C. The medium for *S. antarcticus* contains in addition *N*-acetyl glucosamine 1 g L^{-1}.

The type strain of *Rarobacter faecitabidus* YLM-1^T was isolated from activated sludge of the waste water treatment system of an alcoholic beverage factory. Bacto agar (Difco), supplemented with activated sludge extract, was cooled to 45 °C after sterilization at 120 °C for 10 min. Before pouring to a plate, 18 mL of the agar were mixed with 2 mL of a suspension of living yeast cells (*Hansenula anomala* J 45–1, 2×10^9 cells per mL) and 0.1 mL of the sample suspension. The yeast-lysing bacteria were isolated from plaques formed after incubation at 30 °C for 3–5 days. The procedure was repeated until no other bacteria could be detected. Strain YLM-1^T was separated from yeast cells in liquid media by centrifugation at 3,000 rpm for 10 min followed by filtration of the supernatant through membrane filters with pore sizes of 0.8 or 1.2 µm (Hasuo et al. 1984). Strain YLM-1^T was maintained on YM-catalase agar, which contained (per liter) 5 g of yeast extract (Oxoid Ltd., London, United Kingdom), 5 g of malt extract (Difco Laboratories, Detroit, Mich.), 5.74 g of K_2HPO_4, 205 mg of $MgSO_4 \cdot 7H_2O$, 1.15 g of $NH_4H_2PO_4$, 60 mg of catalase (C-10; Sigma Chemical Co., St. Louis, Mo.), and 15 g of agar. Catalase and $MgSO_4 \cdot 7H_2O$ were separately sterilized by filtration (Yamamoto et al. 1988).

Rarobacter incanus YLM-32^T was isolated from activated sludge mixed in soft agar-yeast plates containing 5.74 g of K_2HPO_4, 1.15 g of $NH_4H_2PO_4$, 0.205 g of $MgSO_4 \cdot 7H_2O$, 10^{10} viable cells of *Saccharomyces cerevisiae* K-701 and 7 g of agar per liter. The plates were incubated at 30 °C for 3–10 days, and plaque-forming colonies were isolated (Goto-Yamamoto et al. 1993).

Strain YLM-32^T was maintained on TYMC agar containing 5.0 g of trypticase peptone (BBL), 3.0 g of yeast extract (Difco), 3.0 g of malt extract (Difco), 5.8 g of K_2HPO_4, 1.15 g of $MgSO_4.7H_2O$, 0.12 g of catalase C-10 (Sigma) per liter (Goto-Yamamoto et al. 1993).

While DSMZ cultivates *R. faecitabidus* DSM 4813^T aerobically on YM-catalase agar as described by Yamamoto et al. (1988), anaerobic cultivation is preferred for *R. incanus* DSM 10596^T on medium No 104 (http://www.dsmz.de/?id=441) at 30 °C.

Strains of both families are maintained in glycerol (10 % v/v) at −80 °C on medium-term and preserved in liquid nitrogen or as freeze-dried cultures on long-term.

Habitat

The type strains and additional isolates of *S. keddieii*, *S. suarezii*, and *S. inulinus* originate from bovine blood of Western Spain, and single isolates came from samples of a coastal sediment of the Eastern China Sea (*S. marinus*), a ginseng field in South Korea (*S. soli*), and sea sand on King George Island, Antarctica (*S. antarctica*). The literature on additional *Sanguibacter* isolates is sparse. *S. keddieii* was found as a major taxon among airborne organisms recovered from bioaerosols of plant processing industries in Poland (herb and grain processing, flax threshing, grain storing, baking, and cereals production) (Góra et al. 2009). Two isolates with identical 16S rRNA gene sequence with that of *S. suarezii* were found as folate producers in Skandinavian cereal samples (Herranen et al. 2010), and one isolate of this species has been found in natural maple sap from Canada (Lagacé et al. 2004).

The range of isolation places appears to be even broader when entries of 16S rRNA gene sequences referring to named *Sanguibacter* species or *Sanguibacter* spp. are considered. Rarely clone sequences but a few additional cultures have been recovered from the cold environments such Arctic (Vardhan Reddy et al. 2009; Shiwaji et al. unpublished, JQ396627) and Antarctic environments (e.g., AF479343; Christner 2002), Mt. Everest glacial meltwater (e.g., EU584528; Liu, unpublished), but also temperate freshwater (FJ006928; Baiket al. unpublished), subsurface oil reservoir (AB126692; Nunoura et al. unpublished), Caribbean marine sponges (Tabares et al. 2011), and even in the gastrointestinal intestine of pigs (Leser et al. 2002). *Figure 39.4* shows a BLAST tree view for the present phylogenetic relatedness among cultures and uncultured *Sanguibacter* strains (May 2012).

The Japanese strains of *Rarobacter faecitabidus* were isolated from activated sludge of a wastewater treatment system or from discharge water, both of an alcohol beverage factory. Strains of *R. incanus* were isolated from various Japanese and Brazilian samples (e.g., soil, flowers, fermented foods). BLAST analysis using the 16S rRNA gene sequence of *R. faecitabidus* YLM-1 (accession number NR_026484) as source revealed, besides three isolates deposited in culture collections, only a single entry with 99 % identity which belong to a clone (nbw93e11c1, GQ065838.1) obtained from the occiput skin of a human (Grice et al. unpublished; NISC Comparative Sequencing Program).

Pathogenicity: Clinical Relevance

A comment that some of the isolates recovered from bovine blood and from milk in Western Spain have the ability to establish a low-grade bacteremia in cattle originates from the publication of Fernández-Garayzábal et al. (1995). However, as the isolates comprised members of *Oerskovia xanthineolytica* and *Sanguibacter* and no strain numbers were indicated, it is not obvious which taxa caused bacteremia. Funke et al. (1997) list *Sanguibacter* among other coryneforms as "medically nonrelevant."

Another study reports the pathogenicity of strain C4 (named CHPR4 for the deposited 16S rRNA sequence AY289118; see *Fig. 39.2*) for locusts. This nonmotile irregular rod produces chitinase and causes histopathologic changes in the midgut of the grassland locusts *Myrmeleotettix palpalis* (Zub). Its phylogenetic position, determined by

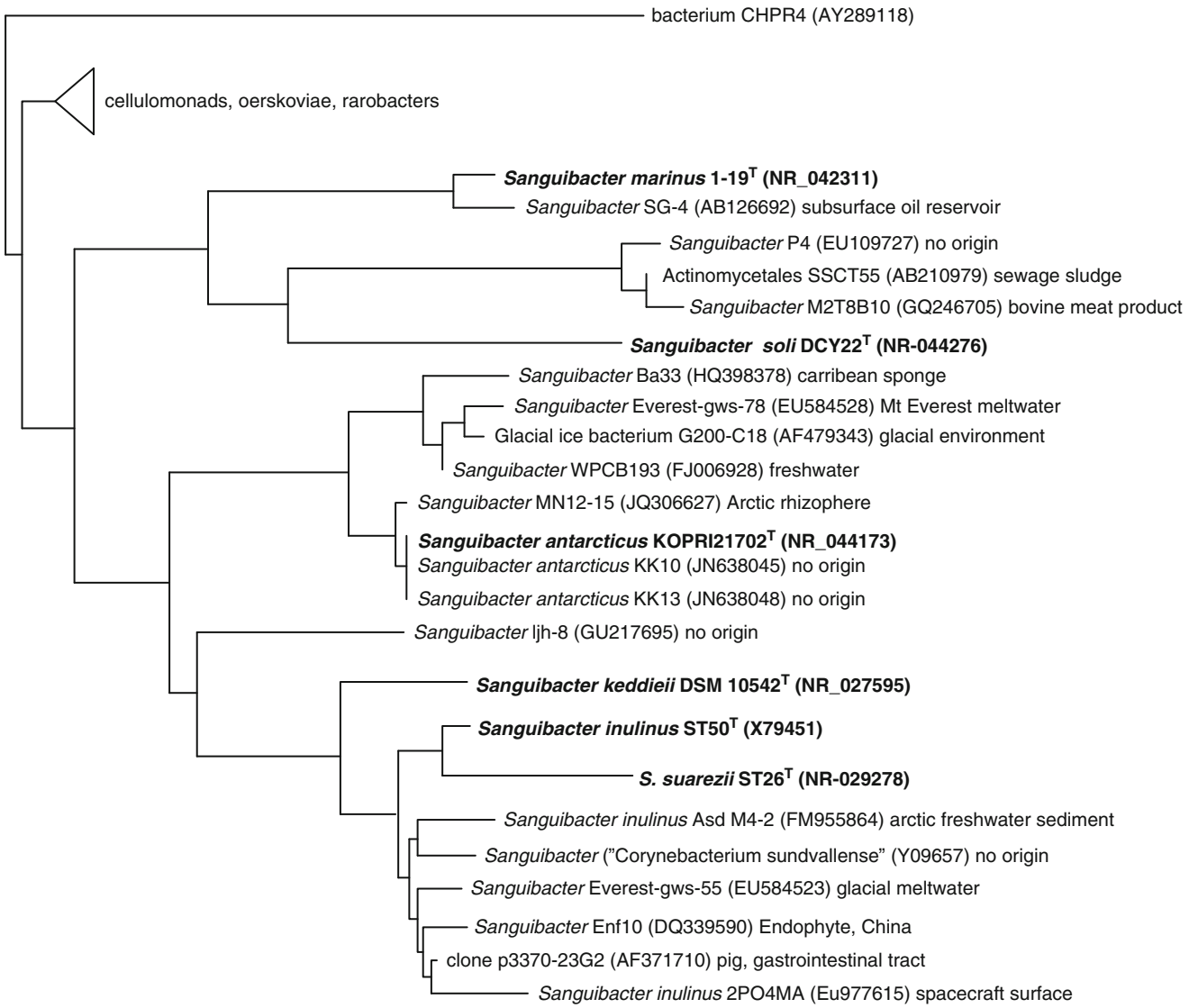

Fig. 39.4

Fast Minimum Evolutionary tree showing the relatedness of *Sanguibacter* type strains (in *bold*) and relatives reported to occur in various environments. Numbers in brackets are accession numbers (Source: NCIB, BLAST; http://blast.ncbi.nlm.nih.gov/)

BLAST similarity analysis, between *Sanguibacter*aceae, *Cellulomonadaceae*, and *Micrococcaceae* questions a specific phylogenetic closeness of strain C4 and members of *Sanguibacter* (Yong et al. 2005).

Application

As mentioned in the previous subchapter, strain C4 (CHPR4) is probably not an authentic member of *Sanguibacter*, but it appears under this genus designation in the literature (see *Fig. 39.2*, top entry). For characterization of its chitinase gene, see Tao et al. (2006). Due to its potential role in pest control, the open reading frame of the chitinase gene was cloned and expressed (Yong et al. 2005). The *Chi58* ORF was a modular enzyme composed of a signal peptide sequence, a polycystic kidney disease I domain, and a glycosyl hydrolase family 18 domain, showing a high level of amino acid similarity to the chitinase A of *Serratia marcescens* (95.5–99.6 %), *Burkholderia cepacia* (99.5 %), *Serratia plymuthica* (93.1 %), and *Enterobacter* (99.3 %), but a low sequence homology with chitinase A from *Bacillus* spp. (28.3–29.8 %).

Optimization parameters for the expression of a cold-active endochitinase of *Sanguibacter antarcticus* KOPRI 21702^T were determined (Han et al. 2011), resulting in 7.5-fold increase in Chi21702 production over unoptimized conditions. In order to express this enzyme in a methylotrophic *Pichia pastoris* strain, 25 amino acids of the putative signal peptide of the *S. antarcticus* gene were eliminated and the coding region of the mature chitinase gene optimized according to the nuclear

codon usage of *Pichia* (Lee et al. 2010). Optimal activity for the chitinase was observed at 37 °C, and a pH of 7.6. 63 % of the optimal activity was retained at 10 °C and 44 % activity at 0 °C.

The search for yeast-lysing (*Saccharomyces*, *Hansenula*, *Candida*) bacteria led to the isolation of 50 strains belonging to named and as-yet not named *Rarobacter* species. Subsequently, a serine protease with mannose-binding activity was characterized in *R. faecitabidus* (Shimoi and Tadenuma 1991) and its molecular structure determined (Shimoi et al. 1992). Its action on SDS-extracted cell wall of *Saccharomyces cerevisiae* resulted in the solubilization of a glycoprotein with a molecular weight of 40,000, named gp40 (Shimoi et al. 1995).

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40 The Family *Segniliparaceae*

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Abstract
Segniliparaceae, a family within the order *Actinomycetales* and suborder *Corynebacterineae*, comprises the sole genus *Segniliparus* with two species. The two type strains have been isolated from human sputum and are described to be opportunistic pathogens. They are aerobic, mesophilic, and chemoorganotroph. The most distinctive characteristic is the presence of ultralong C60–C100 mycolic acids. These are non-oxygenated α-mycolates with high levels of *cis* unsaturation, a feature solely present on *Segniliparus* species Hong (PLoS One 7: e39017, 2012). The type strain of *Segniliparus rotundus* is CDC 1076^T (=ATCC BAA-972^T = CIP 108378^T = DSM 44985^T) and of *Segniliparus rugosus* CDC 945^T (=ATCC BAA-974^T = CIP 108380^T = DSM 45245^T). Of each species, further strains have been isolated, mainly from the human habitat.

Taxonomy, Historical and Current

Short Description of the Family

Segniliparaceae Butler et al. 2005

Segniliparaceae (Seg'ni.li.par.a'ce.ae. N.L. masc. n. *Segniliparus* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Segniliparaceae*, the *Segniliparus* family).

Members of the family are non-spore forming and nonmotile. The genus *Segniliparus* is the only genus in the family with the two species *Segniliparus rotundus* and *S. rugosus* (Butler et al. 2005). The family belongs to the suborder *Corynebacterineae*, order *Actinomycetales*, subclass *Actinobacteridae*, class *Actinobacteria*, and phylum Actinobacteria (Garrity and Holt 2001; Ludwig et al. 2012; Stackebrandt et al. 1997; Zhi et al. 2009). The cells are rod-shaped without any branching. They are aerobic, strongly acid-fast, and produce multiple chemical functional groups of high-molecular-mass, nonpolar, mycolic acids.

Molecular Analyses

The DNA–DNA association value between the type strains of *Segniliparus rotundus* and *S. rugosus* was <28 % using the hydroxyapatite method with an optimum reassociation temperature of 70 °C (Butler et al. 2005). A complete genome sequence has been obtained for *Segniliparus rotundus* (Sikorski et al. 2010) and a high-quality draft genome sequence for *S. rugosus* has been published by Earl et al (2011), revealing genome sizes of 3.16 and 3.64 megabases and a DNA coding region of 92.3 % and 86.4 %, respectively. The number of genes associated with the general COG functional categories is similar in both strains; however, there is a larger proportion of genes not in COGs in *S. rugosus* (46.1 %) compared to *S. rotundus* (41.3 %) (Earl et al. 2011; Sikorski et al. 2010).

Phylogenetic Structure of the Family and its Genus

The 16S rRNA gene sequences of the two type strains of the genus *Segniliparus* differ by only 1.1 % (Sikorski et al. 2010) (*Fig. 40.1*). The next closest relatives of the genus *Segniliparus*, as based on 16S rRNA gene sequences, are the members of the genus *Rhodococcus*, family of *Nocardiaceae*, which share 93.3–94.8 % 16S rRNA genes sequence similarity with strain *Segniliparus rotundus* CDC 1076^T (Ludwig et al. 2012; Sikorski et al. 2010). A BLAST survey against the nucleotide database identified only very few entries at a similarity above 93 %, e.g., as obtained from wastewater (Del Casale et al. 2011), suggesting a rather limited ecological distribution of the genus *Segniliparus*.

Phenotypic Analyses

Segniliparus Butler et al. 2005

Segniliparus (Seg.ni.li.pa'rus. L. adj. segnis slow; Gr. adj. liparos fat/fatty; N.L. masc. n. *Segniliparus* the slow fatty one, the one

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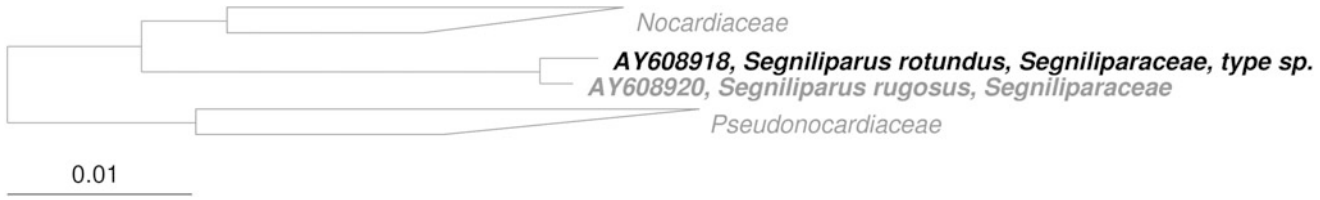

Fig. 40.1

Phylogenetic reconstruction of the family *Segniliparaceae* 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

Table 40.1

Phenotypic and chemotaxonomic characteristics of the type strains of *Segniliparus rotundus* and *S. rugosus*. Both strains are nonmotile. The range and optimum of NaCl and pH for growth, the presence of oxidase and polar lipids, the assimilation of aliphatic hydrocarbons, the resistance to radiation, and the peptidoglycan type were not determined

Properties	*Segniliparus rotundus* CDC 1076^{T} [a, b]	*Segniliparus rugosus* CDC 945^{T} [a, c]
Morphology	Similar-sized short rods	Irregular-sized rods
Pigmentation	Nonpigmented	Nonpigmented
Gram stain	nd, most probably negative	nd, most probably negative
Temperature for growth (°C)		
Range	28–37	22–42
Optimum	33	33
Metabolism	Aerobic	Aerobic
Reduction of nitrate	–	Variable
Presence of		
Catalase	+	+
Diagnostic peptidoglycan amino acids[d]	*meso*-dpm	*meso*-dpm
Major fatty acids	Saturated straight chain, tuberculostearic acid	Saturated straight chain, tuberculostearic acid
Major respiratory lipoquinone[e]	MK-8(H_4)/MK-8(H_2), few MK-8(H_6), traces of MK-9(H_2)	nd
G+C content (mol%)	66.8	68.1

Symbols: + positive; – negative; *nd* not determined
[a]Butler et al. 2005
[b]Sikorski et al. 2010
[c]Earl et al. 2011
[d]*meso*-dpm, *meso*-diaminopimelic acid
[e]MK, menaquinone

with slow fats, to indicate the possession of slowly reacting fatty acids, i.e., late-eluting mycolic acids detected with HPLC). The genus comprises two species, *Segniliparus rotundus* and *S. rugosus*.

The main features of the sole genus in the family *Segniliparaceae* are listed in *Table 40.1*. *Segniliparus* species form nonmotile rod-shaped cells with 0.4–0.9 μm width and 1.0–4.5 μm length. The cells are aerobic and acid–alcohol fast. Spores or aerial mycelium have not been observed, though the cells are occasionally V-shaped but not with true branching (Butler et al. 2005). The cells grow in 3–4 days on days on Löwenstein–Jensen (LJ), Middlebrook 7H10, and Middlebrook 7H11 agar at an optimal temperature of 33 °C and yield nonpigmented, non-photochromogenic colonies that do not produce a diagnostic odor. The growth on heart infusion (HI) agar is poor. The cells show arylsulfatase activity but are negative for niacin production. A semiquantitative catalase test produces bubbles of >45 mm (Butler et al. 2005). A definite range of salinity tolerance is not known, but growth tolerance with

sodium chloride on LJ and American Trudeau Society (ATS) media in 14 days is described to be positive (Butler et al. 2005). Urea is hydrolyzed but acetamide, adenine, casein, citrate, esculin, hypoxanthine, tyrosine, and xanthine are not. D-glucose, maltose, and trehalose are used as carbon sources and produce acid, whereas adonitol, L-arabinose, cellobiose, dulcitol, i-erythritol, galactose, i-myoinositol, lactose, mannose, melibiose, raffinose, L-rhamnose, salicin, and sodium citrate are not (Butler et al. 2005). Utilization of D-fructose, glycerol, D-mannitol, D-sorbitol, and sucrose is variable. The API CORYNE test kit numerical profile is 2040000 and revealed that the two species are positive for β-glucosidase and pyrazinamidase activities but negative for alkaline phosphatase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, and pyrrolidonyl arylamidase activity at 33 °C. The antimicrobial susceptibility patterns have been determined from several strains of both species using serial twofold broth microdilution assays and are listed in detail in *Table 40.2*. The species have the same fatty acid composition, with prominent fatty acids of $C_{10:0}$, $C_{14:0}$, $C_{16:0}$, and tuberculostearic acid (Butler et al. 2005). The quinones are mainly MK-8(H_4) and MK-8(H_2) with some MK-8(H_6) and traces of MK9(H_2) (Sikorski et al. 2010). The species do not produce a *Rhodococcus equi*-specific ChoE virulence factor (Butler et al. 2005). The gas–liquid chromatography thermal cleavage product of the mycolic acids is a $C_{24:0}$ acid–methyl ester. The high-performance liquid chromatography mycolic acid pattern consists of three late-emerging groups of peaks with the final peak co-eluting with the high-molecular-mass internal standard. Thin-layer chromatography demonstrates three nonpolar α^+ (C_{84}-C_{100}) -, α (C_{73}-C_{83}) -, and α' (C_{60}-C_{66}) – mycolic acid chemical functional groups. These ultralong non-oxygenated α-mycolates with high levels of *cis* unsaturation are a special and sole phenotypic characteristic of *Segniliparus* species. Overall 65 homologous mycolic acids have been observed in *Segniliparus*. The overall length of the mycolic acids, which is among the longest lipids known in cell biology, is distinctly atypical of rapid growing mycolata (Hong et al. 2012; Lanéelle et al. 2013).

Isolation, Enrichment, and Maintenance Procedures

The type strain of *S. rotundus* was isolated from human sputum in a public health laboratory in Tennessee, USA, in 2005 or before (Butler et al. 2005). The type strain of *S. rugosus* was isolated from human sputum in a public health laboratory in Alabama, USA, in 1998 (Earl et al. 2011). Information of case histories on the type strains is not available. Several other strains have been isolated from humans, for some of them also case histories have been published (Butler et al. 2007; Hansen et al. 2009; Koh et al. 2011).

Strains of the genus *Segniliparus* do not require special procedures for maintenance and long-term storage. Can be stored frozen at –24 °C in appropriate medium or water containing 43 % glycerol and in liquid nitrogen in appropriate medium or water containing 5 % dimethylsulfoxide without loss of viability. Long-term preservation is by lyophilization with 20 % skin milk.

Ecology

Habitat

The type strains of the genus *Segniliparus* have been isolated from human sputum in 2005 or before (Butler et al. 2005; Earl et al. 2011). Further strains have been isolated from patients with cystic fibrosis (*S. rugosus*, most probably USA, but also in Australia) and bronchiectasis (*S. rotundus*, South Korea), from sputum, bronchus, or nasal samples (Butler et al. 2007; Hansen et al. 2009; Koh et al. 2011). The presence of *S. rugosus* in *Ixodes ricinus* ticks was identified by denaturating gradient gel electrophoresis (DGGE) of 16S rRNA gene amplicons and subsequent sequencing of the DGGE band. The ticks were collected in Sunnmøre, Norway, in May/June/September 2010 both as host-seeking ticks and feeding ticks picked from cats and dogs (Tveten and Sjåstad 2011). This suggests that transmission of *S. rugosus* between mammalian hosts can take place via ticks (Tveten and Sjåstad 2011). Further isolates of *S. rugosus* have been obtained from a subadult female California sea lion (*Zalophus californianus*) stranded on the beach of San Onofre, California, USA, in April 2010 (Evans 2011). Though in environmental databases hardly any 16S sequences related to *Segniliparus* are present, this finding addresses the question of whether *S. rugosus* could be free-living in the oceans or part of the flora of any number of ocean-dwelling vertebrates or invertebrates (Evans 2011; Sikorski et al. 2010).

Pathogenicity, Clinical Relevance

Although both *S. rugosus* and *S. rotundus* are officially classified to belong to risk group 1 (TRBA 2010), occasionally members of the species are suspected to behave as opportunistic pathogens in immunocompromised humans. This appears to be specifically true for humans suffering from cystic fibrosis (CF) (Butler et al. 2007; Hansen et al. 2009) and lung diseases such as tuberculosis and bronchiectasis (Koh et al. 2011). Hence, it is supposed that *Segniliparus* species can cause pneumonia in patients with bronchiectasis (Koh et al. 2011). Clinically, the CF cases exhibited a marked and rapid decline in lung function and radiologic studies over a short period of time which was not characteristic of CF or infections usually associated with this disease (Butler et al. 2007). However, the public health significance of *Segniliparus* species is still unclear. Also other mammalians such as sea lions may be affected by *Segniliparus* species (Evans 2011). Potentially, members of *Segniliparus* may be transmitted via ticks (Tveten and Sjåstad 2011), but may also originate from an environmental source (Butler et al. 2007).

Table 40.2

Antimicrobial susceptibility patterns of *Segniliparus rotundus* and *S. rugosus* isolates. The numbers are minimal inhibitory concentrations in μg/ml determined by using a microbroth dilution assay

	S. rotundus ATCC BAA-972Ta (human sputum, USA)	*S. rotundus* CIP 108380$^{T\ b}$ (human sputum, USA)	*S. rotundus* ATCC BAA-973[a] (human nasal, USA)	*S. rotundus*[b] (human sputum, Korea)	*S. rugosus* ATCC BAA-974Ta (human sputum, USA)	*S. rugosus* CIP 108378^{Tb} (human sputum, USA)	*S. rugosus* ATCC BAA-975[a] (human bronchus, USA)	*S. rugosus* MO 1714[a] (human sputum, USA)	*S. rugosus* MB 549[a] (human BAL, USA)	*S. rugosus* AS 513[a] (human BAL, USA)	*S. rugosus*[c] (human sputum, Australia)
Amikacin (AMK)	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Amoxicillin clavulanic acid (AMC)	2/1	nd	4/2	nd	64/32	nd	>64/32	>64/32	> 64/32	32/16	64/32
Ceftriaxone (CEF)	4	nd	16	nd	64	nd	>64	>64	>64	64	>64
Ciprofloxacin (CIP)	≤0.25	≤0.125	≤0.12	1	16	>16	4	8	8	2	4
Clofazimine (CLO)	0.5	nd	1	nd	2	nd	2	>2	>2	>2	nd
Clarithromycin (CLR)	4	4	8	2	32	64	64	>64	>64	>64	16
Ethambutol (EMB)	>16	>32	>16	>32	>16	>32	>16	>16	>16	>16	nd
Cefoxitin (FOX)	16	2	32	>256	64	8	256	>256	>256	128	64
Gatifloxacin (GAT)	2	nd	0.5	nd	8	nd	8	2	8	1	0.5
Imipenem (IMP)	2	≤0.5	2	16	2	4	4	2	2	1	1
Linezolid (LZD)	2	nd	2	nd	64	nd	>64	64	32	32	64
Minocycline (MIN)	≤0.5	nd	≤0.5	nd	>32	nd	>32	>32	>32	>32	>32
Moxifloxacin (MOX)	≤0.12	≤0.125	≤0.5	1	0.5	≤0.125	8	8	8	2	nd
Rifabutin (RFB)	0.25	nd	1	nd	0.5	nd	1	2	2	1	nd
Rifampin (RIF)	≤0.25	≤0.125	0.5	>16	>16	>16	>16	>16	>16	>16	nd
Streptomycin (STR)	8	nd	16	nd	>128	nd	>128	128	>128	>128	nd
Sulfamethoxazole (SMX)	4	16	4	>128	4	>128	8	8	32	8	nd
Tigecycline (TIG)	nd	nd	1	nd	>2	nd	>2	>2	>2	>2	nd
Trimethoprim-sulfamethoxazole (SXT)	≤0.25/4.8	nd	≤0.25/4.8	nd	≤0.25/4.8	nd	0.5/9.5	0.5/9.5	2/38	≤0.25/4.8	2/38
Tobramycin (TOB)	>64	>32	64	>32	>64	>32	>64	>64	>64	>64	>64

nd not determined, *BAL* bronchoalveolar lavage

[a]Butler et al. 2007

[b]Koh et al. 2011

[c]Hansen et al. 2009

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41 The Family *Sporichthyaceae*

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Abstract
Sporichthyaceae, a family within the order "*Frankiales*," includes the genus *Sporichthya*. Members of this family are facultative anaerobes and have a cell wall containing a large amount of LL-diaminopimelic acid. *Sporichthya* aerial hyphae grow upright on the surface of the medium through holdfasts, which are outgrowths of the basal cell wall. No substrate mycelium forms.

Taxonomy: Historical and Current

Short Description of the Families

The family descriptions are based mainly on phylogenetic positions and patterns of 16S rRNA gene sequence signatures. However, the family *Sporichthyaceae* exhibits some diagnostic phenotypic characteristics differing from each other and from four neighboring families of the order "*Frankiales*" (◉ *Table 41.1*).

Sporichthyaceae Rainey, Ward-Rainey and Stackebrandt 1997, 487[VP] Emend. Zhi, Li and Stackebrandt 2009, 596[VP]

Spo.rich.thy.a'ce.ae. N.L. fem. n. *Sporichthya*, type genus of the family; L. suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Sporichthyaceae*, the *Sporichthya* family (Stackebrandt et al. 1997).

Phylogenetically, a member of the order *Frankiales* (Ludwig et al. 2012) in the phylum Actinobacteria.

The family currently only contains the genus *Sporichthya* (Lechevalier et al. 1968). Species *Sporichthya polymorpha* and *Sporichthya brevicatena* have been proposed to be members of the genus *Sporichthya*. This family was proposed by Stackebrandt et al. (1997), and emended by Zhi et al. (2009).

Short aerial hyphae made up of rod-shaped spores are present. No substrate mycelium. Motile. Cross-linkage of the peptidoglycan is by the A type; diagnostic diamino acids include LL-diaminopimelic acid. Methyl 14-methylpentadecanoate (iso-$C_{16:0}$), hexadecanoic acid ($C_{16:0}$), heptadecanoic acid ($C_{17:1}$), and/or 10-methylated octadecanoic acid may also be present as prominent fatty acids. Menaquinones MK-9(H_6) and MK-9(H_8) are the predominant isoprenoid quinones. The pattern of 16S rRNA signatures consists of nucleotides at positions 184:193 (A–G), 195 (C), 196 (A), 416:427 (G–C), 600:638 (U–G), 601:637 (G–U), 602:636 (C–G), 612:628 (U–A), 841 (U), 952:1229 (U–A), 986:1219 (A–U), 1042 (A), and 1059:1198 (U–A). Predominant polar lipids include phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, and several phospholipids and glycolipids may also be present. Mycolic acids and teichoic acids are absent. Galactose and mannose as whole cell sugars are detected. DNA G+C values are approximately 71 mol%. Members of the genus *Sporichthya* have been isolated from soil.

Phylogenetic Structure of the Family and Its Genus

At the present time, the family *Sporichthyaceae* includes only two validly described species of the genus *Sporichthya*. The phylogenetic tree indicates that these two species form a coherent clade (◉ *Fig. 41.1*). The family *Sporichthyaceae* was identified by Stackebrandt et al. (1997) based on phylogenetic position and the presence of a unique set of 16S rRNA gene

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Table 41.1

Diagnostic properties in which the family *Sporichthyaceae* differs from each other and four neighboring families of the order *Frankiales* (Carlsohn et al. 2008, amended)

Taxon	*Sporichthyaceae*	*Acidothermaceae*	*Cryptosporangiaceae*	*Frankiaceae*	*Geodermatophilaceae*	*Nakamurellaceae*
Cellular morphology	Short aerial hyphae; no substrate mycelium	Slender rods, filaments	Substrate and aerial hyphae or aerial mycelia and sporangia	Substrate hyphae; no aerial mycelium; multilocular sporangia	Thallus consisting of cuboid to oval cells; rudimentary hyphae; no aerial mycelium or Cocci, rods, vibrios; pairs, tetrads; clusters	Cocci; pairs; clusters
Spore/bud formation	Coccoid to rod-shaped spores	−	Sporangiospores or fragmentation of aerial hyphae	Sporangiospores	Zoospores, Buds	−
Motility	+	−	+ or −	−	+ or −	−
Cell-wall diamino acid(s)	LL-A_2pm	A_2pm, Ser, Ala	*meso*-A_2pm	*meso*-A_2pm	*meso*-A_2pm	*meso*-A_2pm
Major menaquinone(s)	MK-9(H_8), MK-9(H_6), MK-8(H_6)	ND	MK-9(H_6), MK-9(H_4), MK-9(H_8)	MK-9(H_4), MK-9(H_6), MK-9(H_8)	MK-9(H_4), MK-8(H_4), MK-9(H_6), MK-9	MK-8(H_4), MK-9(H_4)
Polar lipid(s)[a]	PI, PG, DPG, PL	ND	DPG, PE, PS, PI, PL, GL	PI, PIM, DPG	PE, PIM, PI, DPG, PG	DPG, PE, PE-dimethyl
Predominant fatty acid(s)	$C_{16:0}$, iso-$C_{16:0}$, $C_{17:1}$, $C_{17:0}$	ND	iso-$C_{16:0}$, $C_{17:1}$, $C_{18:1}$, 10-methyl $C_{17:0}$, $C_{17:1}$ cis9	iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{17:1}$	iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{16:1}$, $C_{18:1}$v9c, $C_{17:1}$v8c, $C_{17:0}$, anteiso-$C_{17:0}$	iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{18:1}$; anteiso-$C_{15:0}$, $C_{17:0}$
DNA G+C content (mol%)	71	61	65–76	66–71	68–75	68–73

Data for reference genera were taken from Lechevalier (1994) and Mirza et al. (1991) (*Frankiaceae*), Luedemann and Fonseca (1989), Kroppenstedt (1985), Collins et al. (1984), Urzì et al. (2004) and Mevs et al. (2000) (*Geodermatophilaceae*), Yoshimi et al. (1996) and Yoon et al. (2007) (*Nakamurellaceae*), Tamura et al. (1999) and Rainey et al. (1993) (*Sporichthya*), Mohagheghi et al. (1986) (*Acidothermaceae*), Tamura et al. (1998) (*Cryptosporangium*). +, Present; −, absent; ND, no data available.

[a]*DPG* Diphosphatidylglycerol, *GL* unknown glycolipid(s), *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PE*-dimethyl, phosphatidyldimethylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PL* unknown phospholipid(s).

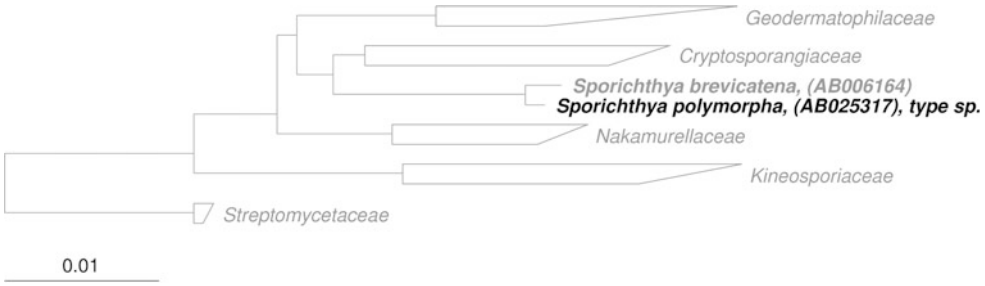

Fig. 41.1

Phylogenetic reconstruction of the family *Sporichthyaceae* based on 16S rRNA gene sequences and created using the neighbor-joining algorithm. 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

sequence signature nucleotides. On the basis of modifications in this set and due to newly described actinobacterial species and the availability of their 16S rRNA gene sequences, the description of the family *Sporichthyaceae* was amended (Zhi et al. 2009). Since only one genus and two validly described species have been reported for this family, the pattern of the 16S rRNA signatures is based solely on the 16S rRNA gene sequences of the two strains. The pattern may change when additional information regarding members of the family *Sporichthyaceae* is obtained.

Molecular Analyses

DNA-DNA Hybridization Studies

DNA-DNA hybridization (DDH) values between *Sporichthya polymorpha* and *Sporichthya brevicatena* range from 20 % to 28 % (Tamura et al. 1999).

Genome Analyses

The incomplete genome sequence of the type strain of *Sporichthya polymorpha* DSM 43042^T (GOLD ID Gi02549) has been released. The genome of the type strain of *S. polymorpha* is 5,500,153 bp, contains 5,279 orfs, and a mol% G+C content of 71 %.

Phages

A *Sporichthya* phage, which is assigned as Sp1 (= DSM 49146 = IMET7840), has been identified. This phage causes true lysis of *Sporichthya polymorpha* DSM 46113 and increases plaque size (3.0-mm diameter), as well as producing clear plaques with distinct margins. This phage is used for classification and identification (Prauser 1984).

Phenotypic Analyses

Sporichthya Lechevalier Lechevalier and Holbert 1968, AL

Gr. n. *spora*, a seed and, in biology, a spore; Gr. n. *ikhthus*, fish; N.L. fem. n. *Sporichthya*, an organism with fish-like spores.

Sporichthya strains form short chains of aerial mycelium, but not substrate mycelium. Aerial hyphae are maintained upright on the basal cell on the surface of the medium by holdfasts. Most spore chains are short, but some are long (15–20 μm). Ordinary cells are rod-shaped or oval (approximate 0.5–1.5 μm). Primary mycelium is not formed. The sparingly branched aerial mycelium contains rod-shaped to coccoid spores, which may become polarly flagellated and motile in the presence of water (Williams et al., 1989). Many spores showed motility after incubation at 28 °C for 30 min in distilled water. *Sporichthya* strains grow under strictly aerobic or facultatively anaerobic conditions. The physiological characteristics and cellular fatty acids compositions of the members of the genus *Sporichthya* are shown in *Tables 41.2* and *41.3*. The phylogenetic relationship and chemotaxonomical characteristics shown in the family description were reported by Rainey et al. (1993).

The mol% G+C content is 71 %.

The type species is *Sporichthya polymorpha* (Lechevalier et al. 1968). The type strain is ATCC 23823 = BCRC 13424 = DSM 43042 = IFM 1245 = IMSNU 21310 = JCM 3089 = KACC 20041 = KCTC 9797 = NBRC 12702 = NRRL B-3709 = VKM Ac-1863.

Isolation, Enrichment, and Maintenance Procedures

Sporichthya polymorpha was isolated from greenhouse soil collected in New Brunswick, NJ, USA (Lechevalier et al. 1968). Only five *Sporichthya* strains have been isolated in the laboratory of Lechevalier et al. over the past 20 years.

Sporichthya brevicatena (Tamura et al. 1999) was isolated from soil samples collected in Yamanashi Prefecture, Japan, on

Table 41.2
Physiological characteristics of *S. polymorpha* and *S. brevicatena* (Tamura et al. 1999).

Characteristic[a]	*S. polymorpha*	*S. brevicatena*
Utilization of:		
D-Glucose	+	+
Inositol	+	+
Raffinose	+	+
Saccharose	w	+
D-Fructose	–	–
D-Mannitol	–	w
L-Rhamnose	+	–
D-Xylose	–	+
Glycerol	–	+
Lactose	+	+
D-Galactose	+	+
Maltose	w	w
D-Mannose	+	w
Melibiose	–	+
Solubility of calcium malate	–	–
Starch hydrolysis	–	–
Nitrite from nitrate	+	+
Peptonization of milk	–	–
Gelatin liquefaction	–	–
Pigmentation in:		
ISP-6	–	–
ISP-7	–	w
Growth temperature (°C):		
10	–	–
15	–	w
20	+	+
25	+	+
30	+	+
37	+	w
45	–	–

[a] +, positive; w, weakly positive; –, negative.

Table 41.3
Cellular fatty acid compositions (%) of *S. polymorpha* and *S. brevicatena*

Fatty acid	*S. polymorpha*	*S. brevicatena*
Iso-branched fatty acids		
$C_{16:0}$	8.9	24.4
$C_{18:0}$	7.5	Trace
Saturated fatty acids		
$C_{14:0}$	4.5	
$C_{15:0}$	6.1	2.8
$C_{16:0}$	24.4	6.6
$C_{17:0}$		17.1
$C_{18:0}$		1.8
Unsaturated fatty acids		
$C_{16:1}$	9.9	2.5
$C_{17:1}$	20.9	18.5
$C_{18:1}$	5.3	2.5
10-methyl fatty acids		
$C_{18:0}$		17.5
$C_{19:0}$		3.4
Unidentified fatty acids		3.0

*Data from Rainey et al. (1993) and Tamura et al. (1999).

HV agar (Hayakawa and Nonomura 1987) using the capillary method (Hayakawa et al. 1991), which was improved to include vanillin as a chemoattractant. Agar plates were incubated at 28 °C for approximately 3 weeks. Subcultivation of the isolate was conducted using ISP 2 medium and yeast extract-starch agar.

Because *Sporichthya* also has motile zoospores, isolation of *Sporichthya* was reported using the RC method (Takeuchi et al. 1996; Hayakawa et al. 2000). This technique involves immersing the air-dried source material in 10 mM phosphate buffer containing 10 % soil extract, incubating the preparation at 30 °C for 90 min, followed by centrifugation of the fluid at 1,500 × g for 20 min. Portions of the supernatant containing actinomycete zoospores were plated on humic acid-vitamin agar supplemented with nalidixic acid and trimethoprim. The *Sporichthya* strain was isolated from a soil sample collected from a corn field. Suzuki et al. (1999) developed a selective isolation method for *Sporichthya* strains. This method involves the RC method using a flooding solution containing 0.1 % skim milk in 10 mM MOPS (morpholinepropanesulfonic acid) (pH 8.0), and then incubating the preparation at 27 °C for 60 min to obtain high yields of motile spores followed by centrifugation at 1,000 × g for 10 min, the use of gellan gum plus 2 mM $CaCl_2$ as selective medium for stimulated growth, and dry heat treatment of soil samples at 80 °C for 60 min to increase the ratio of *Sporichthya*. They isolated a number of *Sporichthya* strains from 21 soil samples, which were collected in Belgium, France, India, Japan, Papua New Guinea, Spain, Taiwan, the United Kingdom, and the United States.

Members of the family *Sporichthyaceae* grow in complex liquid or on solidified media, such as ISP-2 medium, Bennett's agar containing 1 g/L yeast extract, 1 g/L beef extract, 2 g/L NZ amine, 10 g/L glucose, and 20 g/L agar (pH 7.3), YS agar and yeast extract-glucose broth containing 10 g/L yeast extract, and 10 g/L glucose. Members of these families do not require special procedures for maintenance or preservation. Cultures can be

maintained by serial transfers onto the appropriate solid media. Growth on agar slants can be maintained at 4 °C for over 1 month. Medium-term preservation is in 12–15 % (v/v) glycerol suspensions at −80 °C. Long-term preservation of liquid cultures supplemented with 12–15 % (v/v) glycerol or 7 % (v/v) dimethylsulfoxide is recommended in the vapor phase of liquid nitrogen (−150 °C). Freeze-drying and L-drying methods can be also applied for long-term storage.

Ecology

The main habitat of *Sporichthyaceae* strains appears to be soil. *Sporichthya* strains are widely distributed throughout the world (Suzuki et al. 1999). *Sporichthya* sp. I10A-02001 (GenBank accession number JX273674) which has 93.3–93.8 % of 16S rRNA gene sequence similarities to previous known species, *Sporichthya* sp. KAR54 (EF451684) which has 93.3–92.6 %, and *Sporichthya* sp. 1252 (AB054908) which has 925–91.9 % were isolated from surface-sterilized root of a medicinal plant, *Perilla frutescens*, at suburb of Beijing, China, permafrost (Hansen et al. 2007), mudflow deposit of the Mt. Pinatubo, respectively. In addition, uncultured bacterium clone FFCH14579 (EU132514) which has 97.0–97.3 % similarities, uncultured bacterium clone 0502TCLN027 (AB695759) which has 95.6–96.3 % similarities, and uncultured *Sporichthya* sp. clone CH1-4 (JX079395) which has 96.0–95.5 % similarities were collected from soil of an undisturbed mixed grass prairie preserve at Kessler farm, Oklahoma, USA (Elshahed et al. 2008), soil at Tottori, Japan, and contaminated soil at India, respectively.

Pathogenicity and Clinical Relevance

Pathogenicity has not been reported for any member of the family *Sporichthyaceae*.

No information regarding antibiotic sensitivity and resistance is available for members of the genus *Sporichthya*.

Application

There have been several reports regarding the special use of members of the family *Sporichthyaceae*.

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42 The Family *Streptomycetaceae*

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Abstract
The family *Streptomycetaceae* comprises the genera *Streptomyces*, *Kitasatospora*, and *Streptacidiphilus* that are very difficult to differentiate both with genotypic and phenotypic characteristics. A separate generic status for *Kitasatospora* and *Streptacidiphilus* is questionable. Members of the family can be characterized as non-acid-alcohol-fast actinomycetes that generate most often an extensively branched substrate mycelium

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that rarely fragments. At maturity, the aerial mycelium forms chains of few to many spores. A large variety of pigments is produced, responsible for the color of the substrate and aerial mycelium. The organisms are chemoorganotrophic with an oxidative type of metabolism and grow within different pH ranges. *Streptomyces* are notable for their complex developmental cycle and production of bioactive secondary metabolites, producing more than a third of commercially available antibiotics. Antibacterial, antifungal, antiparasitic, and immunosuppressant compounds have been identified as products of *Streptomyces* secondary metabolism. *Streptomyces* can be distinguished from other filamentous actinomycetes on the basis of morphological characteristics, in particular by vegetative mycelium, aerial mycelium, and arthrospores. The genus comprises at the time of writing more than 600 species with validated names. 16S rRNA gene sequence-based analysis for species delineation within the *Streptomycetaceae* is of limited value. The variations within the 16S rRNA genes—even in the variable regions—are too small to resolve problems of species differentiation and to establish a taxonomic structure within the genus. Comprehensive comparative studies including protein-coding gene sequences with higher phylogenetic resolution and genome-based studies are needed to clarify the species delineation within the *Streptomycetaceae*.

Taxonomy, Historical and Current

Short Description of the Family

Family *Streptomycetaceae* Waksman and Henrici 1943, 339^{AL} emend. Stackebrandt, Rainey, and Ward-Rainey, 1997, 486, emend. Kim, Lonsdale, Seong and Goodfellow, 2003, 113

Strep.to.my.ce.ta'ce.ae. N.L. masc. n. *Streptomyces*, type genus of the family; L. suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Streptomycetaceae*, the *Streptomyces* family

The family *Streptomycetaceae* belongs to the *Actinomycetales* suborder *Streptomycinae* Rainey, Ward-Rainey, and Stackebrandt 1997 of the class *Actinobacteria* Stackebrandt, Rainey, and Ward-Rainey 1997. Type genus is *Streptomyces* Waksman and Henrici 1943, 107^{AL}. Organisms are Gram-positive, aerobic, non-acid-alcohol-fast actinomycetes that generate an extensively branched substrate mycelium that rarely fragments. At maturity, the aerial mycelium forms chains of three to many spores. Members of a few species produce short spore chains on the substrate mycelium. A large variety of pigments is produced, responsible for the color of the substrate and aerial mycelium. The organisms are chemoorganotrophic with an oxidative type of metabolism and grow within different pH ranges. Cell walls of the substrate mycelium contain either LL- or *meso*-diaminopimelic acid as the predominant diamino acid; aerial or submerged spores contain LL-diaminopimelic acid (peptidoglycan type A3γ). Whole-organism lipid profiles usually comprise complex mixtures of saturated *iso*- and *anteiso*-fatty acids and as major polar lipids hexa- and octa-hydrogenated menaquinones with nine isoprene units as the predominant isoprenologues, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. Mycolic acids are not present. Sugar profiles may contain major quantities of either galactose or galactose and rhamnose. The mol% G+C of the DNA ranges between 66 % and 74 %. The 16S rRNA gene sequence signature pattern consists of nucleotides at positions: 71 (G), 80–89 (G-C), 81–88 (C-G), 82–87 (U-G), 127–234 (G-C), 209 (C), 210 (C), 211 (G), 610 (G), 671–735 (U-A), 819 (G), 837–849 (C-G), 950–1231 (U-G), 955–1225 (C-G), 965 (C), 1254–1283 (A-U), and 1409–1491 (GG).

Phylogenetic Structure of the Family and Its Genera

The family *Streptomycetaceae* was established by Waksman and Henrici in 1943 to accommodate actinomycetes with branched slender mycelia and spores on aerial hyphae that show rarely or no presence of a septate and no fragmentation into oidia (Waksman and Henrici 1943). At that time, the description was primarily based on morphological characteristics. In the 8th edition of *Bergey's Manual of Determinative Bacteriology*, Pridham and Tresner (1974a) listed the genera *Streptomyces*, *Streptoverticillium*, *Sporichthya*, and *Microellobospora* as members of the family. Other genera, such as *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Kitasatoa*, and *Microellobospora* have been differentiated from *Streptomyces* by morphological criteria; however, they harbor many common phenotypic and genotypic features with *Streptomyces* and have therefore been proposed as synonyms of this genus (Goodfellow et al. 1986a, b, c, d, e). The genus *Sporichthya* is now classified within the family *Sporichthyaceae* of the suborder *Frankineae* (Stackebrandt et al. 1997).

The genus *Streptoverticillium* was also found to share many characteristics with streptomycetes but is distinguishable from *Streptomyces* by its verticillate sporophores. The proposal that the genus became a synonym of streptomycetes (Witt and Stackebrandt 1990) was further supported by numerical phenetic (Williams et al. 1983a; Kämpfer et al. 1991) and rRNA/DNA similarities (Gladek et al. 1985; Witt and Stackebrandt 1990). The unification of *Kitasatospora* with *Streptomyces* was proposed by Wellington et al. (1992) on the basis of morphological, biochemical, and 16S rRNA gene sequence similarities. Nevertheless, the genus was revived by Zhang et al. (1997) mainly on the basis of the ratio of *meso*-A_2pm to LL-DAP in whole-cell hydrolysates. The *meso*-A_2pm content in *Kitasatospora* strains is 49–89 % and 1–16 % in *Streptomyces* strains. In addition, galactose is only contained in whole-cell hydrolysates of *Kitasatospora* strains but not in hydrolysates of *Streptomyces* strains. The genus *Streptacidiphilus* was proposed by Kim et al. (2003) as an additional member of the family. These acidophilic actinomycetes grow over a pH range of 3.5–6.5 with an optimum of 4.5–5.5 and have been isolated from acidic soils and litter.

Table 42.1

Morphological, physiological, and chemotaxonomic characteristics of *Streptomyces*, *Kitasatospora*, *and Streptacidiphilus* strains[a] (According to Kim et al. 2003)

	Streptomyces	*Kitasatospora*	*Streptacidiphilus*
Long chains of spores formed on aerial hyphae	+	+	+
pH range for growth	5.0–11.5	5.5–9.0	3.5–6.0
Optimal pH range	6.5–8.0[b]	Not determined	4.5–5.5
Diagnostic sugars in whole-organism hydrolysates	None	Galactose[c]	Galactose, rhamnose
Isomer(s) of diaminopimelic acids in whole-organism hydrolysates	LL-A_{2pm}	LL-/*meso*[d] A_{2pm}	LL-A_{2pm}
Fatty acid pattern[e]	2c	2c	2c
Predominant phospholipids[f]	DPG,PE,PI,PIMs	DPG,PE,PI,PIMs	DPG,PE,PI,PIMs
Major menaquinones[g]	MK-9($H_{6'}$ H_8)	MK-9($H_{6'}$ H_8)	MK-9($H_{6'}$ H_8)
G + C content of DNA (mol%)	66–73	70–74	70–72

[a]Data obtained from Kim et al. (2003) and previous studies (Shirling and Gottlieb 1977; Ōmura et al. 1989; Lonsdale 1985; Williams et al. 1989; Nakagaito et al. 1992; Antony-Babu and Goodfellow 2008); [b]Alkalophilic strains, which grow between pH 5.0 and 11.0, have an optimum at pH 9 to 9.5 (Mikami et al. 1982; Antony-Babu and Goodfellow 2008); [c]Rhamnose was detected in whole-organism hydolysates of *Kitasatospora mediocidica* (Labeda 1988); [d]Aerial and submerged spores contain LL-A_{2pm} and vegetative mycelia *meso*-A_{2pm}; [e]Fatty acid group sensu Kroppenstedt (1985); [f]DPG, diphospatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides; [g]MK-9($H_{6'}$ H_8), hexa- and octa-hydrogenated menaquinones with nine isoprene units

The genera *Streptomyces*, *Kitasatospora*, and *Streptacidiphilus* are very difficult to differentiate on the basis of phenotypic characteristics (including the few distinguishing chemotaxonomic markers indicated above). The few distinctive features of the three genera are presented in *Table 42.1*. Even though 16S rRNA gene sequence analyses have offered a framework for prokaryotic classification, the current classification based on this molecule has not resolved the taxonomic problems within the family *Streptomycetaceae* (Kämpfer 2006). In phylogenetic analyses based on nearly full-length 16S rRNA gene sequences, *Kitasatospora* and *Streptacidiphilus* form stable, separate subbranches within the family *Streptomycetaceae*; however, they are grouped within the large *Streptomyces* tree (*Fig. 42.1*) and the 16S rRNA gene sequences similarities are equally high to many *Streptomyces* species groups. Multilocus sequence analysis (MLSA) based on concatenated partial sequences of five housekeeping genes showed the formation of distinct separate clusters with high bootstrap support for species of the genera *Kitasatospora* and *Streptacidiphilus*. However, only a selection of species was investigated and the distinct separation was not supported by all single gene-based phylogenies (Han et al. 2012). A very high agreement with the genome sequence data for *Streptomyces* and *Kitasatospora* was also found on the basis of DNA-DNA microarray hybridizations, when the genome content of *Streptomyces avermitilis*, *Streptomyces cattleya*, *Streptomyces maritimus*, and *Kitasatospora aureofaciens* was compared with that of *Streptomyces coelicolor* A3(2) (Hsiao and Kirby 2008).

Due to all these reasons, it remains uncertain, whether a separate generic status for *Kitasatospora* and *Streptacidiphilus* is justified. In the 2nd edition of *Bergey's Manual*, these genera are cited as genera incertae sedis (Kämpfer 2012).

Molecular Analyses

The genetics of streptomycetes is a rapidly developing area, and plenty of information has been published within the last years (for reviews and more information, see, e.g., Chen et al. 2002; Donadio et al. 2002; Paradkar et al. 2003; Schrempf 2006; Ventura et al. 2007; Hopwood 2003 2007; Hsiao and Kirby 2008; Dyson 2010, and references therein). Information on the complex structure of *Streptomyces* genomes is increasing, mainly because streptomycetes are very abundant and important as soil inhabitants, where they are regarded as major components in the cycling of organic carbon compounds. They are also capable of forming many and diverse hydrolytic exoenzymes, like cellulases and chitinases.

Streptomyces are notable for their complex developmental cycle and production of bioactive secondary metabolites, producing more than a third of commercially available antibiotics. Antibacterial, antifungal, antiparasitic, and immunosuppressant compounds have all been identified as products of *Streptomyces* secondary metabolism (Omura et al. 1992; Hopwood 2007). The agar liquefying strain designated *Streptomyces coelicolor* A3(2) (reviewed by Hopwood 1999) is widely considered the model organism. This strain exhibits the main features of its genus with several distinctive characteristics, most notably production of the red mycelial bound pigment undecylprodigiosin and the diffusible blue pigment actinorhodin.

The whole-genome sequences of several *Streptomyces* strains have been examined and are published or available online: e.g., *S. coelicolor* A3(2) (representing the model streptomycete; Bentley et al. 2002); *Streptomyces ambofaciens* strains ATCC 15154, DSM 40697, ETH 9247, and ETH 11317 (*S. ambofaciens* is known for its remarkable genetic instability); *S. avermitilis* MA-4680 (the producer of avermectin; Ikeda et al. 2003); *S. griseus* subsp. *griseus* NBRC 13350 (producer of bioactive

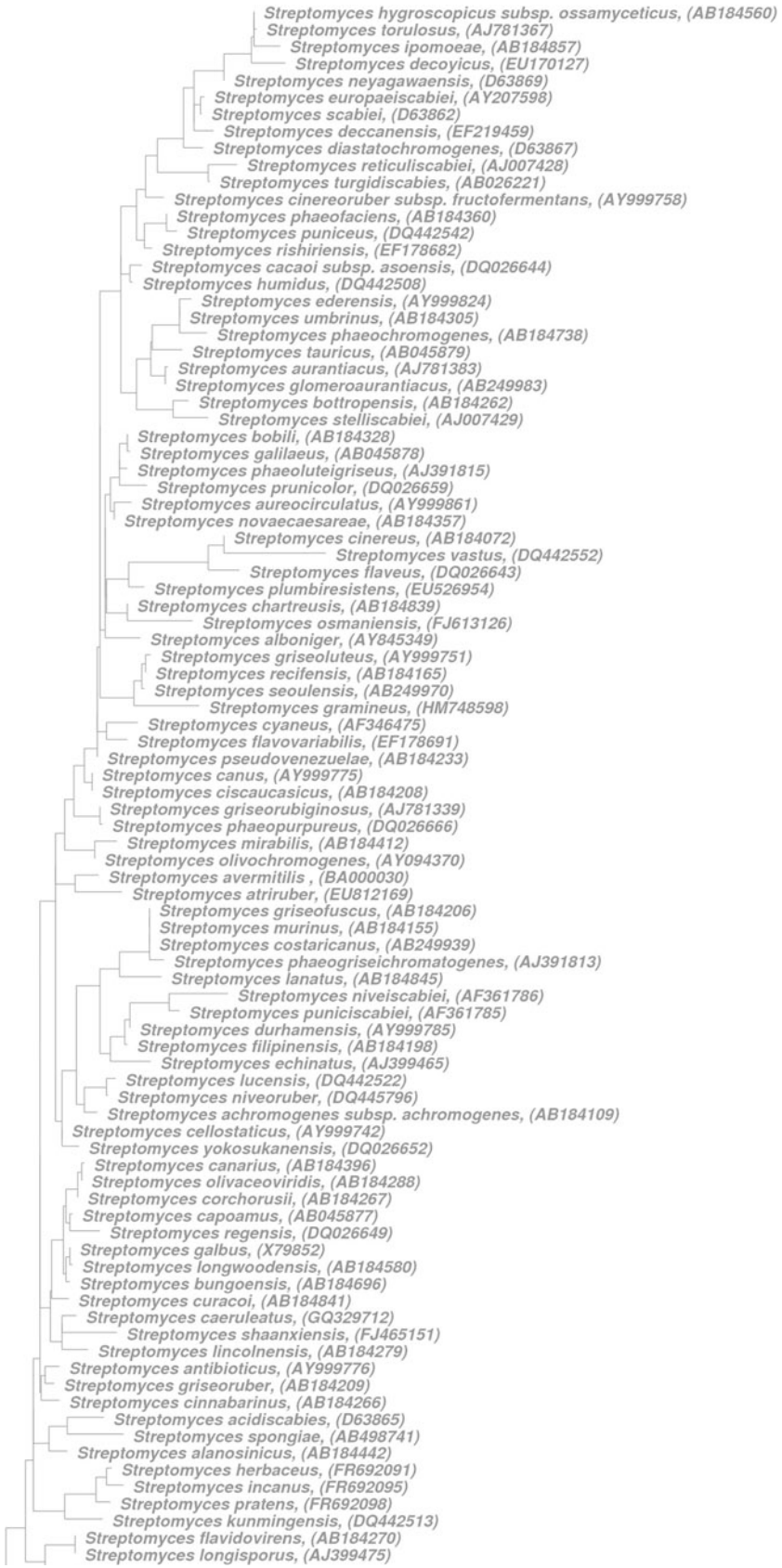

Fig. 42.1 (continued)

Fig. 42.1 (continued)

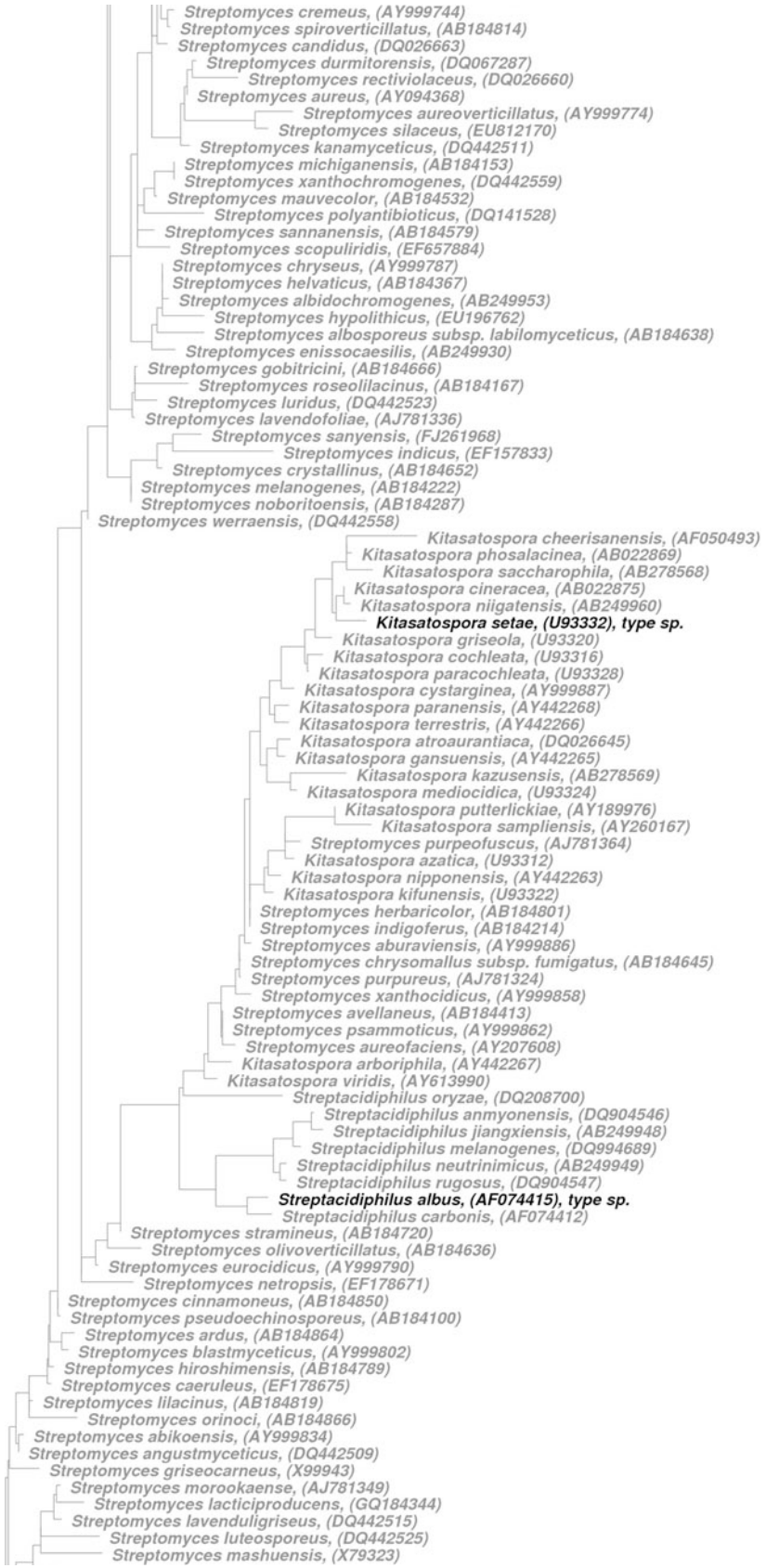

Fig. 42.1 (continued)

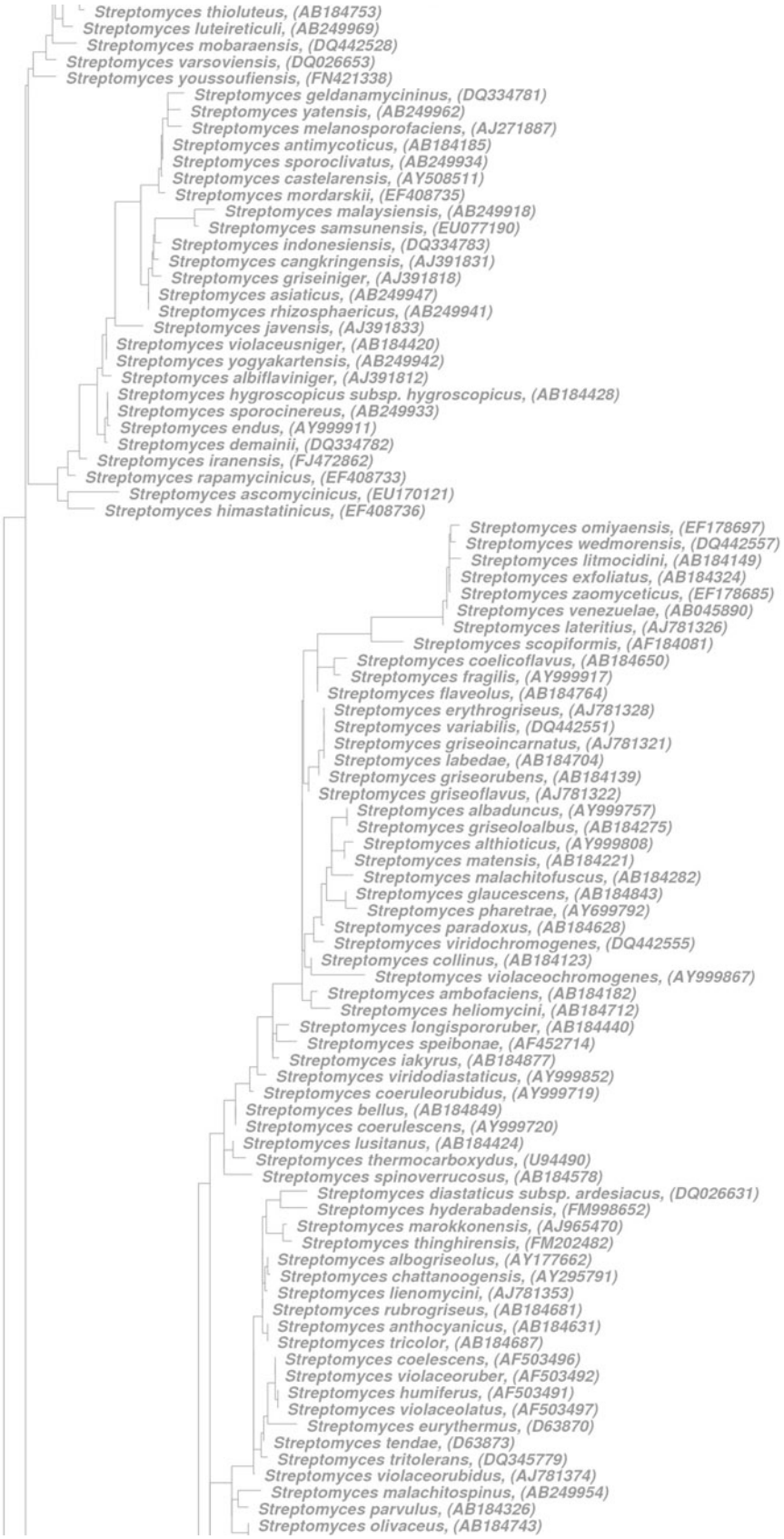

Fig. 42.1 (continued)

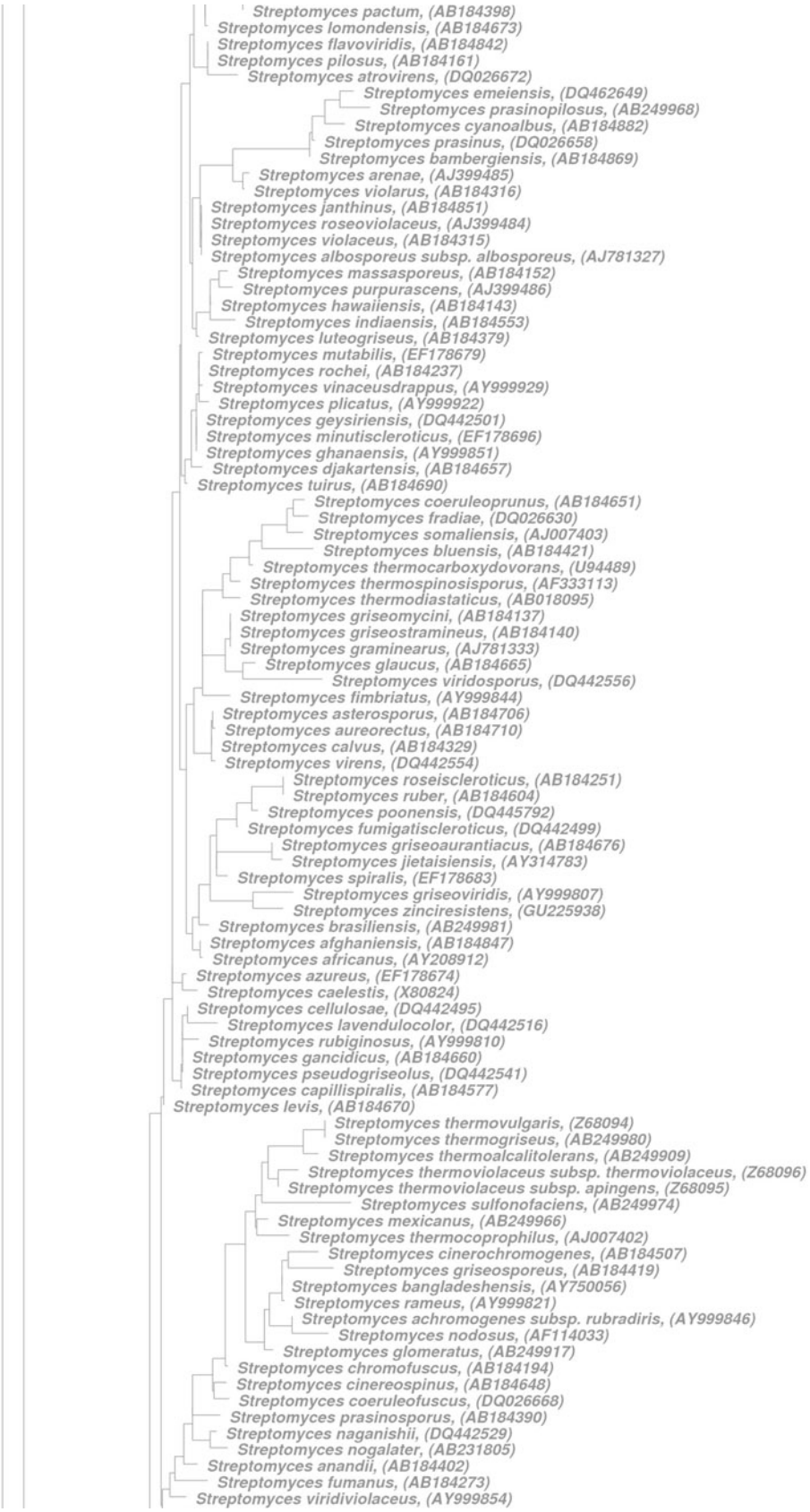

Fig. 42.1 (continued)

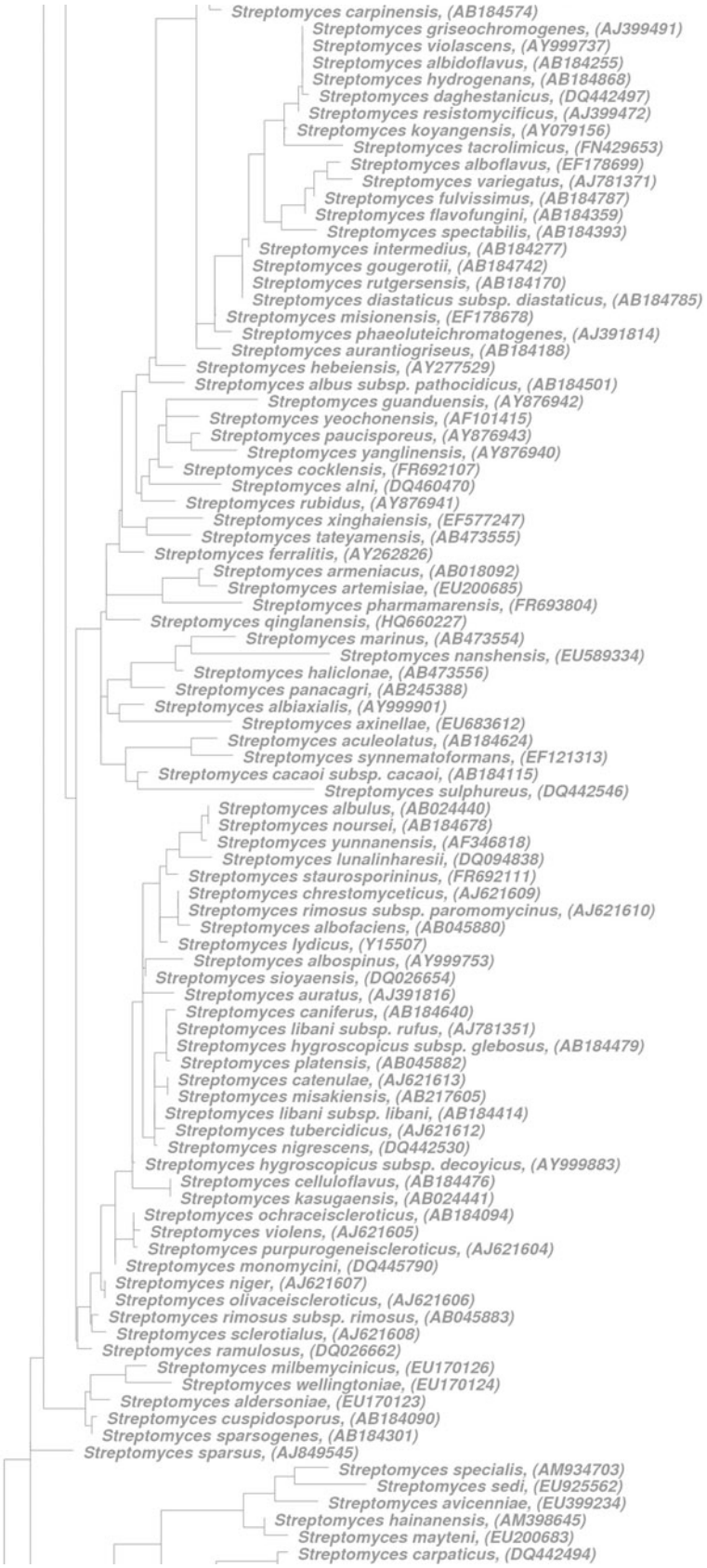

Fig. 42.1 (continued)

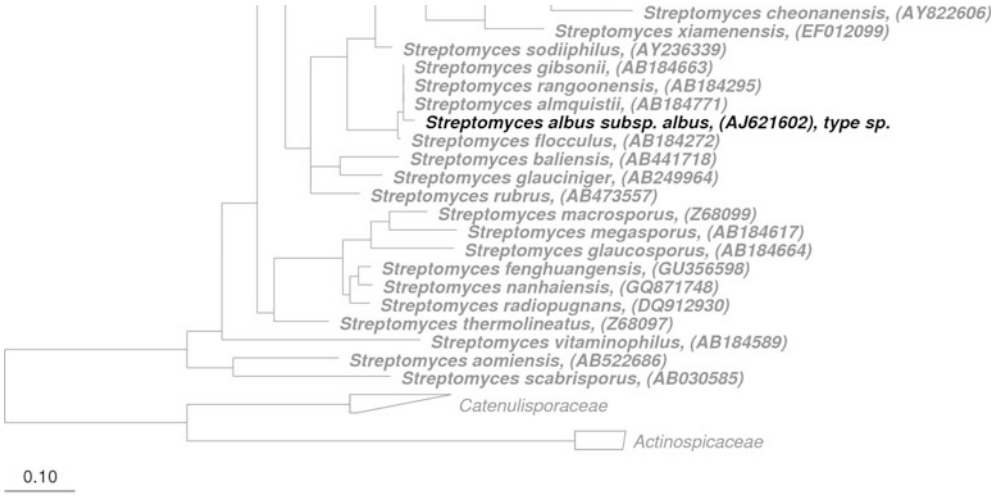

Fig. 42.1

(a–f) Phylogenetic reconstruction of the family *Streptomycetaceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Representative sequences from closely related taxa were used as outgroups. Scale bar indicates estimated sequence divergence

secondary metabolites, including streptomycin; Ohnishi et al. 2008); *S. scabies* 87.22 (causing potato scab); *S. bingchenggensis* BCW-1, producer of the anthelmintic macrolide milbemycins (Wang et al. 2010); the chloramphenicol producer *S. venezuelae* ATCC 10712 (EMBL accession number FR845719); and *S. cattleya* NRRL 8057, a rare synthesizer of fluorinated antibiotics (Barbe et al. 2011).

All *Streptomyces* strains studied so far contain a large genome, typically of linear topology. The chromosomes of sequenced *Streptomyces* range in size from 6,283,062 bp for *S. cattleya*, if the 1,809,491 bp linear plasmid comprising its second replicon is excluded from the comparison, to 11,936,683 bp predicted to code for 10,023 genes in *S. bingchenggensis*; the latter is thought to be the largest bacterial genome sequenced to date. The *S. coelicolor* A3(2) genome was originally considered to be circular, by analogy with other well-studied bacteria such as *Escherichia coli*. However, on the basis of analysis of cosmid libraries in combination with comparisons of physical maps from the wild-type and mutant strains of *S. coelicolor* A3(2) and *S. lividans*, it was established that the chromosome is present in a linear form in wild-type strains (Lin et al. 1993; Redenbach et al. 1996). The presence of a linear chromosome in other streptomycetes, including *S. ambofaciens* (Leblond et al. 1996), *S. antibioticus*, *S. lipmanii*, *S. moderatus*, *S. parvulus*, *S. rochei* (Lin et al. 1993), *S. griseus* (Lezhava et al. 1995), and *S. hygroscopicus* (Pang et al. 2002a, b) has been revealed by pulse-field gel electrophoresis (PFGE) studies. In addition, a linear arrangement of the chromosome has also been shown for a *Streptoverticillium* sp. (Redenbach et al. 1998).

The *S. coelicolor* A3(2) chromosome contains about 8,667 mega base pairs (Mbp) which correspond to 7,825 genes. Twenty-six gene clusters encode known or predicted secondary metabolites (Bentley et al. 2002). It is interesting to note that the *S. coelicolor* chromosome was shown to carry more genes (i.e., 7,825) than the eukaryote *Saccharomyces cerevisiae* (containing 6,203 genes). The genome size of *S. avermitilis* MA-4680 comprises about 9,025 Mbp (average G+C content 70.7 mol%), which corresponds to 7,574 potential open reading frames, 35 % of which constitute 721 paralogous families (Omura et al. 2001; Ventura et al. 2007). In total, 30 gene clusters encode for secondary metabolites. It has been determined that one large region of 6,500 Mbp is highly conserved in the *S. coelicolor* A3(2) and *S. avermitilis* MA-4680 genomes with respect to gene order and thus may contain essential genes. The terminal regions are not conserved and contain "nonessential genes" (Ikeda et al. 2003). Moreover, it is worth mentioning that an ancient synteny (conservation of gene order) has been uncovered between the central core of the *S. coelicolor* A3(2) chromosome and the whole chromosomes of *Corynebacterium diphtheriae* and *Mycobacterium tuberculosis* (Bentley et al. 2002).

Whole-genome synteny plots have revealed that common genes present in the genomes of *S. coelicolor* A3(2) and *S. avermitilis* MA-4680 display a high conservation in their overall position and orientation (Ventura et al. 2007). Ikeda et al. (2003) estimated by reciprocal BLAST analysis that about two-thirds (5,283) of the corresponding genes represent conserved orthologs. Similarly, it has been found that 4,837 genes are orthologues between these two strains (Ventura et al. 2007). When *S. scabies* ATCC 49173 is added to this comparison, the number of conserved genes among the three strains decreases to 4,190, and, as pointed out by Ventura et al. (2007), a four-way analysis, including the hitherto unpublished sequence of *S. venezuelae*, ATCC 10595 reduces the number to 3,566 genes. Presumably, that number will decrease further as more

Streptomyces genomes are available. In addition, Ventura et al. (2007) showed that only about 17 % of the 3,566 genes common to the four *Streptomyces* genomes are present in *E. coli* K-12 and *Bacillus subtilis* 168.

Hsiao and Kirby (2008) used DNA-DNA microarray hybridization to compare the genome content of *S. avermitilis* ATCC 31267, *S. cattleya* ATCC 35852, *S. maritimus* Yang-Ming, and *Kitasatospora aureofaciens* ATCC 10762 with that of *S. coelicolor* A3(2). Using this approach an accordance of about 93 % with the genome sequence data available for *S. avermitilis* ATCC 31267 was detected and a number of trends in the genome structure for *Streptomyces* and closely related *Kitasatospora* species could be shown. The central core region was found to be well conserved, and also a low degree of gene conservation within the terminal regions of the linear chromosome across all four strains was determined. Between these regions, two areas of intermediate gene conservation were identified by microarray analysis. Nevertheless some conserved genes were also detected in the terminal regions.

The replication process of the *Streptomyces* chromosome has been summarized by Schrempf (2006) and Ventura et al. (2007). The replication of linear *Streptomyces* chromosomes and plasmids proceeds bidirectionally towards the telomeres and is initiated from a fairly centrally located replication origin rich in DnaA box sequences. The chromosomal replication origin (*oriC*) region shows high conservation among *S. coelicolor* A3(2) (Calcutt and Schmidt 1992), *S. lividans* 66 (Zakrzewska-Czerwinska and Schrempf 1992), *S. lividans* TK21, *S. antibioticus* ETH 7451, and *S. chrysomallus* ATCC 11523 (Jakimowicz et al. 1998). Interestingly, it has been revealed that in contrast to the high overall G+C content (69–73 mol%) of *Streptomyces* DNA, the region of the origin (*oriC*) is rich in A + T (64 mol%). The chromosomes of *Streptomyces* species typically contain terminal inverted repeats (TIRs) at their ends, which are covalently bound to terminal proteins. TIR lengths among available sequenced *Streptomyces* chromosomes vary considerably with 174 bp for *S. avermitilis*, 18,488 bp for *S. scabies*, 21,653 bp for *S. coelicolor* M145, and approximately 198 kb for *S. ambofaciens* (Ventura et al. 2007). The chromosomal telomeres are replicated by a special mechanism, which is initiated by priming from the terminal protein covalently bound to the 5′-ends. These ends are made up of approximately 250–320 characteristic nucleotides and possess a complex secondary structure (Ventura et al. 2007).

The origin of the linearity of the *Streptomyces* chromosome is believed to have occurred by single-crossover recombination between an initially circular chromosome and a linear plasmid. Quite a few examples of exchange of ends between chromosomes and linear plasmids, resulting in hybrid molecules with different right and left ends, have been reported. Streptomycete genome architecture is extensively reviewed by Kirby and Chen (2011).

Several examples of apparent redundancy of metabolic genes have been determined in the genomes of streptomycetes, which are considered to be due to the complex morphological and physiological differentiation of streptomycetes. The metabolic genes include those that function in carbon storage transactions, genes coding for enzymes of the pentose phosphate pathway, genes specific for different hyphal cell types, and several *fabH*-like genes, which are essential for the first step in fatty acid biosynthesis (some of these are linked with secondary metabolism gene sets) (Ventura et al. 2007). It is worth mentioning that a number of genes are not present in streptomycetes genomes. For instance, two of the three subunits of exonuclease V (the *recB* and *recC* genes) are not found in streptomycetes; nevertheless they are present in other actinobacteria such as mycobacteria. The XerCD pathway, which is responsible for the resolution of circular chromosomes after replication, is absent from streptomycetes, consistent with the linearity of *Streptomyces* chromosomes. The conserved *ftsA* gene, which is involved in the complex cell division process and widely distributed in the domain Bacteria, is commonly not found in *Actinobacteria*. Moreover, the *minC* and *minE* genes, which are involved in the choice of division site in many unicellular bacteria, are not present in *Streptomyces* strains (Ventura et al. 2007).

Streptomyces colonies frequently display a high spontaneous variability in pigmentation, sporulation, and antibiotic biosynthesis. The various antibiotic resistances, A-factor formation, and synthesis of tyrosinase or arginosuccinate are encoded by unstable genes. This genetic instability can be stimulated by mutagens, such as ethidium bromide, mitomycin, and ultraviolet light, and by gyrase (topoisomerase II)-inhibiting antibiotics. Often, these variations are a result of large chromosomal deletions, preferentially taking place at the telomeric and subtelomeric regions and including up to 2 Mbp of DNA (Hütter and Eckhardt 1988; Schrempf et al. 1989; Leblond and Decaris 1994; Chen 1995). More information about these processes is published (Schrempf 2006). The variability of the chromosomal DNA is additionally enhanced by its interaction with linear and circular plasmids, phages, transposons, and insertion elements. Since streptomycetes inhabit rapidly changing environments, the high plasticity of the genome likely represents an effective prerequisite for quick adaptation.

Streptomyces Extrachromosomal Elements

Streptomyces can contain small, covalently closed circular (ccc) high copy-number plasmids, larger ccc low copy-number plasmids, ccc plasmids that arise by reversible site-specific recombination from the chromosome, and linear plasmids that share features of the chromosome: a centrally located origin of replication and terminal inverted repeats with bound terminal proteins to prime end patching (reviewed by Vogelmann et al. 2011). A property common to maybe all is their self-transmissibility and ability to mobilize chromosomal DNA, making them supremely important in promoting genetic exchange and streptomycete evolution. The frequency of plasmid transfer between strains can approach 100 % and is apparent in laboratory conditions by the formation of "pocks" on a lawn of recipient bacteria. These pocks are zones of (so far unexplained) retarded development surrounding the primary site of plasmid transfer: the retarded development is

a consequence of intrahyphal spread of copies of the newly transferred plasmid. The conjugation mechanism (reviewed by Vogelmann et al. 2011) is quite unlike that of enteric bacteria such as *E. coli*. For the latter, conjugation depends on the function of more than 30 genes (e.g., the transfer region of the F-plasmid). In contrast, a single *tra* gene is responsible for the transfer of circular streptomycete plasmids (e.g., pIJ101, pSN22, pSG5, SCP2, and pSAM2). In addition, "spread" genes (*spd*) promote subsequent intrahyphal spread, leading to the appearance of pocks. The F-plasmid *E. coli* paradigm involves transfer of a single DNA strand from donor to recipient after the two strains have been temporarily united via the sex pilus, processes specified by more than 30 F-plasmid *tra* genes. In *Streptomyces*, plasmid transfer is likely to be initiated by the fusion of donor and recipient hyphae growing together, perhaps promoted by plasmid functions: certain plasmids, such as the linear SLP2 of *S. lividans*, encode a muramidase-like function that could remodel the peptidoglycan of the fusing hyphae. For other plasmids, this may be unnecessary: the Tra protein of pSG5 localizes to growing hyphal tips, where peptidoglycan is constantly being remodeled as part of the growth process of hyphal extension. The streptomycete plasmid Tra proteins belong to the same family as FtsK, first characterized in *E. coli* as being involved in chromosomal DNA segregation at cell division. FtsK is assembled as a hexameric ring with a hole as an integral part of the septum formed during cytokinesis. Two DNA double helices are accommodated in the hole as FtsK pumps a looped circular double-stranded chromosomal DNA molecule between compartments in an ATP-dependent manner. The emerging evidence is that *Streptomyces* plasmid Tra proteins also hydrolyze ATP as they transfer double-stranded DNA between hyphae. Similar to FtsK, the Tra proteins also have a membrane-spanning domain that could promote the fusion of membranes necessary for interhyphal DNA transfer. The purified Tra protein of pSVH1 binds noncovalently, as a hexameric assembly, to a specific plasmid sequence, *clt*: this binding presumably permits specific transfer of the plasmid, but there is no DNA processing (e.g., nicking) during conjugal transfer as is necessary for single-stranded F-plasmid transfer between *E. coli* strains.

The mobilization of chromosomal DNA during the transfer of circular plasmids is believed to be a consequence of transient recombination between a plasmid and the chromosome, leading to transfer of the resulting cointegration as a loop through a Tra protein ring. It is unclear whether this normally leads to complete or partial transfer of the donor chromosome. A model for chromosome transfer by linear plasmids invokes mobilization due to interactions between the terminal proteins of both replicons, giving rise to "end-first" transfer of the chromosome. If the two ends of the plasmid are bound to the two ends of the chromosome, transfer of the entire quasi-circular loop is possible.

Plasmid-encoded functions include antibiotic production (Kinashi et al. 1991; Gravius et al. 1994) and mercury resistance (Ravel et al. 1998). The replication mechanism is best understood for pSLA2. It is initiated bidirectionally near the center of the plasmid and proceeds towards its telomeric ends, generating 3′ leading-strand overhangs. It should be mentioned that various other members of the order *Actinomycetales* contain genes on linear plasmids, among them those required for biphenyl degradation (*Rhodococcus erythropolis* and *Rhodococcus globerulus*; Kosono et al. 1997), hydrogen autotrophy (*Rhodococcus opacus*, formerly *Nocardia opaca*; Kalkus et al. 1993), isopropylbenzene and trichlorethylene catabolism (*Rhodococcus erythropolis*; Kebeler et al. 1996), and fasciation in plants (*Rhodococcus fascians*; Crespi et al. 1992). Linear plasmids have also been identified in *Mycobacteria* (*M. arium*, *M. branderi*, *M. celaturum*, and *M. xenopi*). Their termini correspond to those of linear plasmids from *Streptomyces* and *Rhodococcus* species (Picardeau and Vincent 1998); it remains an open question whether circular and linear plasmids are exchanged during conjugation among these other actinomycetes. There is only limited information about *Streptomyces* transposons; a summary of this topic is provided by Schrempf (2006). Phages with broad or narrow host ranges can be recovered from soil and several of them have been applied for classifying strains (for review, see Kutzner 1981). Nevertheless, in present taxonomic studies, phage host range studies are not carried out.

The use of *Streptomyces* plasmids for gene cloning is discussed in chapter 11 of the book by Kieser et al. (2000).

DNA Regions in Mycelial Actinobacterial Genomes Acquired by HGT

Regardless of the large synteny between the central regions of *Streptomyces* genomes, hundreds of insertion-deletion (indel) differences exist between *S. coelicolor* A3(2) and *S. avermitilis* MA-4680, most of them involving one or a few genes. This circumstance frequently complicates the recognition of synteny at the level of small groups of genes. Streptomycetes also contain several larger islands of species-specific DNA (Ventura et al. 2007). Prior to the publication of other *Streptomyces* genome sequences, 14 islands of likely laterally acquired DNA were identified by Bentley et al. (2002) in the *S. coelicolor* A3(2) genome on the basis of gene content, atypical GC content, and location next to a tRNA determinant. Approximately 50 % of these islands were shared with *S. ambofaciens*. This clearly illustrates that in pairwise synteny plots the genes in the "subtelomeric arms" of *Streptomyces* chromosomes display much less conservation between species than those in the central regions, or "cores," and that the cores contain most of the genes conserved with other actinobacteria (Bentley et al. 2002; Ikeda et al. 2003; Choulet et al. 2006).

DNA-DNA Hybridization

The percent DNA-DNA hybridization and the decrease in thermal stability of hybrids are currently used as the "gold standards" for species delineation in taxonomy (Wayne et al. 1987).

DNA-DNA hybridizations of total chromosomal DNA have been widely used in the classification of *Streptomyces* species. Strains of the *Streptomyces albidoflavus* cluster 1, which were earlier defined by Williams et al. (1983a) using numerical phenetic methods, were the subject of an initial DNA-DNA hybridization study performed by reassociation of labeled DNA on nitrocellulose filters (Mordarski et al. 1986). Good correlation was found between the results of the two approaches, and the homogeneity of the *Streptomyces albidoflavus* subcluster *albidoflavus* was confirmed. Nevertheless, two other subclusters obtained by DNA-DNA hybridization were not matching with the *S. anulatus* or *S. halstedii* subclusters established by Williams et al. (1983a), although some correlation was found with the groupings of Kämpfer et al. (1991). DNA-DNA hybridization data further supported the assignment of *Streptoverticillium* strains to the genus *Streptomyces* (reassociation of labeled DNA on filters; Witt and Stackebrandt 1990), which was later confirmed in the numerical phenetic study of Kämpfer et al. (1991).

The most extensive DNA-DNA hybridization studies on strains assigned to some of the major phenetic groups of Williams et al. (1983a) were performed by Labeda et al. (Labeda 1993, 1996, 1998 and Labeda and Lyons 1991a, b). Here only little correlation between the DNA-DNA pairing and numerical phenetic data was found with respect to the *S. cyaneus* (Labeda and Lyons 1991a), *Streptomyces violaceusniger* (Labeda and Lyons 1991b), *S. lavendulae* (Labeda 1993), the verticil-forming streptomycetes (formerly *Streptoverticillium* species; Labeda 1996; Hatano et al. 2003), and *S. fulvissimus* and *S. griseoviridis* phenotypic clusters (Labeda 1998); nevertheless, some concordance was found with phenotypic groups delimited by Kämpfer et al. (1991; ❯ *Table 42.2*). The fact that certain regions within the *Streptomyces* chromosome display substantial genetic instability supports the continued use of DNA-DNA hybridization (Redenbach et al. 1993). Yet, data obtained by DNA-DNA pairing can be influenced by the presence of large plasmids in *Streptomyces* strains.

Fingerprinting Techniques

In randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), single primers with arbitrary nucleotide sequences are used to amplify DNA at low annealing temperatures in order to detect polymorphisms. The method is used as a rapid screening method to identify similarities among streptomycetes. To obtain validated results, the reaction parameters of this technique have to be stringently standardized including annealing temperature, primer sequence, PCR-reagents, and concentration and quality of template DNA. RAPD-PCR produces a distinctive fingerprint of PCR products and allows the detection of chromosomal dissimilarities between individual isolates without having any prior information of chromosomal sequences.

Mehling et al. (1995) applied this method to identify different actinomycete species; however, they were only capable of detecting characteristic banding patterns for closely related species when a highly specific actinomycete primer was used. Similar results were obtained when the method was used to determine interspecific relationships among members of the *Streptomyces albidoflavus* cluster of Williams et al. (1983a), even so the resulting fingerprints contained only four bands (Huddleston et al. 1995). Anzai et al. (1994) studied 11 primers with different fragment patterns from zero to 20 bands and found that variations in fingerprint patterns can be achieved by substitution of a single base on the arbitrary primer, whereas alteration of the primer sequence at the 3′ end resulted in the most significant differences. Anzai and colleagues used an optimized procedure to investigate the relationship of *Streptomyces lavendulae* and *Streptomyces virginiae* strains; members of these taxa were assigned to the same numerically defined groups by Williams et al. (1983a) and Kämpfer et al. (1991). RAPD-PCR data showed good correlation with other methods such as low-frequency restriction fragment analysis (LFRFA), DNA-DNA hybridization, and cultural and physiological tests; nevertheless interspecific relationships of *S. lavendulae* and *S. virginiae* strains could not be clarified.

Restriction Digests of Total Chromosomal DNA

Low-frequency restriction fragment analysis (LFRFA) is based on the digestion of the entire bacterial chromosomal DNA with restriction endonucleases that cut infrequently and produce a distinctive band pattern after separation by pulsed-field gel electrophoresis (PFGE). Because of their high DNA G+C content, rare adenine-thymine cutting enzymes are used for streptomycetes. In the first study, Beyazova and Lechevalier (1993) studied 59 strains from eight species groups and discovered that the method was valuable since related strains were clustered together. Nevertheless, some discrepancies were found, such as for the strains grouped into the *Streptomyces cyaneus* cluster of Williams et al. (1983a). Like RAPD-PCR, the method seems to be useful for the identification of very closely related strains, but cannot be used to determine interspecific relationships. In addition, one has to be careful with the interpretation of fingerprints, as misinterpretation of banding patterns can result from large chromosomal amplifications or deletions (Rauland et al. 1995).

Nucleic Acid Sequence Comparisons of 16S rRNA and Other Genes

In an early review about the application of 16S rRNA gene sequence analysis for classifying streptomycetes, the importance of the gene region selected for comparison was highlighted (Stackebrandt et al. 1992). It was discovered that relationships between strains were influenced by the variable region (*a*, *b*, or *c*) used for comparison. By sequencing the c region, Kataoka et al. (1997) were able to determine inter- and intraspecies relationships between 89 streptomycete type strains, representing several clusters of Williams et al. (1983a). In total, 42 of the strains were

Table 42.2

***Streptomyces* species, *Kitasatospora* species and *Streptacidiphilus* species included in comprehensive taxonomic studies (for details see footnotes)**

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
Most closely to group *S.costaricanus* et rel.										
S.galbus	**203**	DSM 40089T	ATCC 23910	LMG 19879	ISP 5089		X79852	A 15	I 08	006 1-10
S.longwoodensis	**314**	DSM 41677T		LMG 20096		NBRC 14251	AB184580			
S.bungoensis	**81**	DSM 41781T	IFO 15711	LMG 20439		NBRC 15711	AB184696			
S.corchorusii	**140**	DSM 40340T	ATCC 25444	LMG 20488	ISP 5340	NBRC 13032	AB184267	A 20	I 13	009 1-19
S.canarius	**88**	DSM 40528T	ATCC 27423	LMG 20443	ISP 5528	NBRC 13431	AB184396	A 20	I 13	009 1-19
S.olivaceoviridis	**370**	DSM 40334T	ATCC 23630	LMG 19324	ISP 5334	NBRC 13066	AB184288	A 20	I 13	009 1-19
S.capoamus	**95**	DSM 40494T	ATCC 19006	LMG 20447	ISP 5494	JCM 4734	AB045877	C 45	II 13	1-7 1-15
S.regensis	**424**	DSM 40551T	ATCC 27461	LMG 20300	ISP 5551	NRRL B-11479	DQ026649	A 20	I 13	009 1-19
S.griseochromogenes	**232**	DSM 40499T	ATCC 14511	LMG 19891	ISP 5499	NBRC 13413	AB184387	A 18	I 11	1-5 011
S.cellostaticus	**102**	DSM 40189T	ATCC 23894	LMG 20452	ISP 5189	NBRC 12849	AB184192	A 06	I 05	007 003
S.yokosukanensis	**553**	DSM 40224T	ATCC 25520	LMG 21040	ISP 5224	NRRL B-3353	DQ026652	A 30	II 06	009 1-19
S.antibioticus	**38**	DSM 40234T	ATCC 8663	LMG 20412	ISP 5234	NRRL B-1701	AY999776	A 31	I 21	1-7 1-15
S.griseoruber	**242**	DSM 40281T	ATCC 23919	LMG 19325	ISP 5281	NBRC 12873	AB184209	A 21	I 14	018 023
S.cinnabarinus	**122**	DSM 40467T	ATCC 23617	LMG 20467	ISP 5467	NBRC 13028	AB184266	A 18	I 11	009 1-19
S.acidiscabies	**5**	DSM 41668T	ATCC 49003	LMG 19856			D63865			
S.alanosinicus	**10**	DSM 40606T	ATCC 15710	LMG 20391	ISP 5606	NBRC 13493	AB184442		IV 01 (gray series)	009 1-19
Group *S.costaricanus* et rel.										
S.griseofuscus	**234**	DSM 40191T	ATCC 23916	LMG 19885	ISP 5191	NBRC 12870	AB184206	A 12	I 07	1-6 1-16
S.murinus	**346**	DSM 40091T	ATCC 19788	LMG 10475	ISP 5091	NBRC 12799	AB184155	A 17	I 10	1-6 1-16
S.costaricanus	**141**	DSM 41827	ATCC 55274			NBRC 100773	AB249939			
S.phaeogriseichromatogenes	**388**	DSM 40710	NRRL 2834				AJ391813			
Most closely to group *S.costaricanus* et rel.										
S.lanatus	**293**	DSM 40090T	ATCC 19775	LMG 19380	ISP 5090	NBRC 12787	AB184845	A 18	I 11	016 1-19
S.durhamensis	**157**	DSM 40539T	ATCC 23194	LMG 20501	ISP 5539	NRRL B-3309	AY999785	A 30	II 06	009 1-19
S.filipinensis	**177**	DSM 40112T	ATCC 23905	LMG 19333	ISP 5112	NBRC 12860	AB184198	A 30	II 06	009 1-19
S.puniciscabiei	**411**		KACC 20253	LMG 21391		S77	AF361785			
S.niveiscabiei	**358**			LMG 21392		S78	AF361786			
S.echinatus	**159**	DSM 40013T	ATCC 19748	LMG 5972	ISP 5013		AJ399465	A 18	I 11	1-6 1-10
S.longisporus	**313**	DSM 40166T	ATCC 23931	LMG 20053	ISP 5166		AJ399475	A 18	I 11	009 1-19
S.avermitilis	**63**		ATCC 31267			MA-4680	BA000030			
S.kunmingensis	**288**	DSM 41681T		LMG 20521		NRRL B-16240	DQ442513			
S.mirabilis	**340**	DSM 40553T	ATCC 27447	LMG 20076	ISP 5553	NBRC 13450	AB184412	A 19	I 12	1-7 1-19
S.olivochromogenes	**372**	DSM 40451T	ATCC 3336	LMG 20071	ISP 5451		AY094370	A 19	I 12	009 1-19
Most closely to group *S.cyanoalbus* et rel.										
S.lucensis	**315**	DSM 40317T	ATCC 17804	LMG 20065	ISP 5317	NRRL B-5626	DQ442522	A 31	I 21	1-5 1-16
S.niveoruber	**359**	DSM 40638T	ATCC 14971	LMG 19379		NRRL B-2724	DQ445796		IV 08 (red series)	013 1-19
S.achromogenes ssp. achromogenes	**3**	DSM 40028T	ATCC 12767	LMG 20387	ISP 5028	NBRC 12735	AB184109	A 19	I 12	1-5 009
S.griseorubiginosus	**243**	DSM 40469T	ATCC 23627	LMG 19941	ISP 5469		AJ781339	A 18	I 11	009 1-19
S.phaeopurpureus	**391**	DSM 40125T	ATCC 23946	LMG 20051	ISP 5125	NRRL B-2260	DQ026666	A 09	II 02	009 1-19
S.curacoi	**144**	DSM 40107T	ATCC 13385	LMG 20491	ISP 5107	NRRL B-2901	EF626595	A 18	I 11	009 0-19
S.lincolnensis	**307**	DSM 40355T	ATCC 25466	LMG 20068	ISP 5355	NBRC 13054	AB184279	A 19	I 12	009 1-19
S.cyaneus	**147**	DSM 40108T	ATCC 14923	LMG 20494	ISP 5108	NRRL B-2296	AF346475	A 18		009 1-19
Group *S.cyanoalbus* et rel.										
S.cyanoalbus	**148**	DSM 40198T	ATCC 15859	LMG 19343	ISP 5198	NBRC 12857	AB184882	A 37	I 17	007 003

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.hirsutus	**262**	DSM 40095T		LMG 19927	ISP 5095	NBRC 12786	AB184844			
S.prasinus	**403**	DSM 40099T	ATCC 19800	LMG 20259	ISP 5099	NRRL B-2712	DQ026658	A 37	I 17	007 003
S.bambergiensis	**70**	DSM 40590T	ATCC 13879	LMG 19299	ISP 5590	NBRC 13479	AB184869	A Sm	III 10	075 1-25
S.emeiensis	**163**	DSM 41884				CGMCC 4.3504	DQ462649			
S.prasinopilosus	**401**	DSM 40098T	ATCC 19799	LMG 19345	ISP 5098	NRRL B-2711	EF626597	A 37	I 17	007 003
Most closely to group *S.cyanoalbus* et rel.										
S.flavovariabilis	**191**	DSM 41479T		LMG 19905		NRRL B-16367	EF178691			
S.aureocirculatus	**55**	DSM 40386T	ATCC 19823	LMG 21794	ISP 5386	NBRC 13018	AB184260	A 03	II 20	033 1-33
S.novaecaesareae	**366**	DSM 40358T	ATCC 27452	LMG 20069	ISP 5358	NBRC 13368	AB184357	J Sm	III 25	004 006
S.prunicolor	**404**	DSM 40335T	ATCC 25487	LMG 19311	ISP 5335	NRRL B-12281	DQ026659	A 11	III 01	1-1 1-1
S.phaeoluteigriseus	**390**	DSM 41896	NRRL 5182			NRRL ISP-5182	AJ391815			
S.bobili	**78**	DSM 40056T	ATCC 3310	LMG 20436	ISP 5056	NBRC 16166	AB249925		IV 02 (white series)	1-7 1-15
S.galilaeus	**204**	DSM 40481T	ATCC 14969	LMG 21790	ISP 5481	JCM 4757	AB045878	A 19	I 12	1-7 1-15
Most closely to groups *S.cyanoalbus* et rel. and *S.griseoluteus* et rel.										
S.chartreusis	**107**	DSM 40085T	ATCC 14922	LMG 20455	ISP 5085	NBRC 12753	AB184839	A 18	I 11	009 1-19
S.resistomycificus	**425**	DSM 40133T	ATCC 19804		ISP 5133	NBRC 12814	AB184166	A 18	I 11	009 1-19
Most closely to group *S.griseoluteus* et rel.										
S.griseoluteus	**238**	DSM 40392T	ATCC 12768	LMG 19356	ISP 5392	JCM 4765	AY999751	C 43	II 11	1-5 1-16
S.recifensis	**421**	DSM 40115T	ATCC 19803	LMG 20261	ISP 5115	NBRC 12813	AB184165	A 23	I 20	1-5 059
S.seoulensis	**458**	NBRC 16668=	NBRC 16255	JCM 10116		NBRC 16668	AB249970			
Most closely to groups *S.cyanoalbus* et rel. and *S.griseoluteus* et rel.										
S.canus	**93**	DSM 40017T	ATCC 12237	LMG 19329	ISP 5017	NRRL B-1989	AY999775	A 25	III 02	009 1-19
S.ciscaucasicus	**126**	DSM 40275T		LMG 20474	ISP 5275		AY508512			
S.pseudovenezuelae	**408**					NBRC 12904	AB184233			
S.alboniger	**21**	DSM 40043T	ATCC 12461	LMG 20397	ISP 5043		AY845349	A 1B	I 02	1-6 1-31
Most closely to group *S.scabiei* et rel.										
S.bottropensis	**79**	DSM 40262T	ATCC 25435	LMG 20437	ISP 5262		AB026217	A 19	I 12	009 1-19
S.stelliscabiei	**480**	DSM 41803	NCPPB 4040			CFBP 4521	AJ007429			
S.europaeiscabiei	**170**	DSM 41802				KACC 20186	AY207598			
S.scabiei	**454**	DSM 41658T	ATCC 49173	LMG 20323			D63862			
S.diastatochromogenes	**153**	DSM 40449T	ATCC 12309	LMG 20498	ISP 5449		D63867	A 19	I 12	009 1-19
S.hygroscopicus ssp. ossamyceticus	**270**	DSM 40824T	ATCC 15420	LMG 19951		NBRC 13983	AB184560		I 16	009 1-19
S.ipomoeae	**277**	DSM 40383T	ATCC 25462	LMG 20520	ISP 5383	NBRC 13050	AB184857		IV 02 (blue series)	077 074
S.torulosus	**505**	DSM 40894T	NRRL B-3889	LMG 20305			AJ781367		IV 31 (gray series)	009 1-19
S.neyagawaensis	**353**	DSM 40588T	ATCC 27449	LMG 20080	ISP 5588		D63869	A 18	I 11	009 1-19
Most closely to group *S.scabiei* et rel.										
S.reticuliscabiei	**426**	DSM 41804	CIP 107061			CFBP 4531	AJ007428			
S.turgidiscabies	**510**		ATCC 702348T			ATCC 700248	AB026221			
S.cacaoi ssp. asoensis	**83**	DSM 41440T	ATCC 19093	LMG 20440		NRRL B-16592	DQ026644			
S.humidus	**263**	DSM 40263T	ATCC 12760	LMG 19936	ISP 5263	NRRL B-3172		A 19	I 12	009 1-19
S.rishiriensis	**430**	DSM 40489T	ATCC 14812	LMG 20297	ISP 5489	NRRL B-3239	EF178682	A 19	I 12	1-7 1-15

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s.[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.cinereoruber ssp. fructofermentans	**118**	DSM 40692T	NRRL 2588	LMG 20463		JCM 4956	AY999758		I 04	006 1-18
S.phaeofaciens	**387**	DSM 40367T		LMG 20070	ISP 5367	NBRC 13372	AB184360			
S.puniceus	**410**	DSM 40083T	ATCC 19801	LMG 20258	ISP 5083	NRRL B-2895	DQ442542	A 09	II 02	005 029
Group *S.aurantiacus* et rel.										
S.aurantiacus	**52**	DSM 40412T	ATCC 19822	LMG 19358	ISP 5412		AJ781383	C 45	II 13	012 019
S.glomeroaurantiacus	**221**	DSM 41782T	IFO 15418			NBRC 15418	AB249983			
S.tauricus	**488**	DSM 40560T	ATCC 27470	LMG 20301	ISP 5560	JCM 4837	AB045879	A 19		012 019
S.ederensis	**161**	DSM 40741T	ATCC 15304	LMG 20504		NBRC 15410	AB184658		IV 14 (gray series)	013 1-19
S.phaeochromogenes	**386**	DSM 40073T	ATCC 3338	LMG 19348	ISP 5073	NBRC 3180	AB184738	A 40	I 18	009 0-19
S.umbrinus	**511**	DSM 40278T	ATCC 19929	LMG 20280	ISP 5278	NBRC 13091	AB184305	A 05	I 04	1-6 1-16
S.rectiviolaceus	**423**	DSM 41459T		LMG 20310		NRRL B-16374	DQ026660			
Group *S.aureus* et rel.										
S.kanamyceticus	**281**	DSM 40500T		LMG 19351	ISP 5500	NRRL B-2535	DQ442511			
S.durmitorensis	**158**	DSM 41863				MS405	DQ067287			
S.aureus	**60**	DSM 41785	NCIMB 13927			NBRC 100912	AB249976			
Group *S.cinereus* et rel.										
S.cinereus	**120**	DSM 43033T		LMG 21310		NBRC 12247	AB184072			
S.flaveus	**182**	DSM 43153T	ATCC 15332	LMG 19323		NRRL B-16074	DQ026643			
S.vastus	**515**	DSM 40309T		LMG 21043		NRRL B-12232	DQ442552			
Most closely to group *S.cinereus* et rel.										
S.laceyi	**291**	DSM 41788	NBRC 100783			NBRC 100783	AB249944			
Group *S.argenteolus* et rel.										
S.griseolus	**237**	DSM 40067T	ATCC 3325	LMG 19878	ISP 5067	NBRC 3415	AB184768	A 1C	I 03	1-2 015
S.halstedii	**255**	DSM 40068T	ATCC 10897		ISP 5068	NRRL B-1238	EF178695	A 1C	I 03	1-2 015
S.argenteolus	**44**	DSM 40226T	ATCC 11009	LMG 5967	ISP 5226	JCM 4623	AB045872	A 15	I 08	1-5 011
S.cinereorectus	**116**	DSM 41469T		LMG 20461		NBRC 15395	AB184646			
S.flavovirens	**192**	DSM 40062T	ATCC 3320	LMG 20516	ISP 5062	NRRL B-2685	DQ026635	A 1C		1-2 015
S.flavogriseus	**188**	DSM 40323T	ATCC 25452	LMG 19887	ISP 5323	CBS 101.34	AJ494864	A 1C	I 03	1-2 015
S.nitrosporeus	**357**	DSM 40023T		LMG 20044	ISP 5023	NRRL B-1316	EF178680			
Most closely to groups *S.argenteolus* et rel. and *S.atroolivaceus* et rel.										
S.luridiscabiei	**316**		KACC 20252	LMG 21390		S63	AF361784			
S.acrimycini	**6**	DSM 40135T	ATCC 19885	LMG 21798	ISP 5135	AS 4.1673	AY999889		IV 04 (green series)	1-3 010
S.griseoplanus	**240**	DSM 40009T	ATCC 19766	LMG 19923	ISP 5009	AS 4.1868	AY999894	A 29	I 15	078 060
S.baarnensis	**66**	DSM 40232T	ATCC 23885	LMG 20431	ISP 5232	NRRL B-1902	EF178688	A 1B	I 02	006 1-2
S.flavofuscus	**187**	DSM 41426T	ATCC 19908	LMG 19900		NBRC 100768	AB249935			
S.praecox	**400**	DSM 40393T	ATCC 3374	LMG 20290	ISP 5393	NBRC 13073	AB184293		IV 08 (yellow series)	1-3 1-2
S.fimicarius	**179**	DSM 40322T	ATCC 25449	LMG 21044	ISP 5322		AY999784	A 1B	I 02	1-3 1-2
S.anulatus	**40**	DSM 40361T	ATCC 27416	LMG 19301	ISP 5361	NRRL B-2000	DQ026637	A 1B	I 02	047 1-35
Group *S.atroolivaceus* et rel.										
S.mutomycini	**348**	DSM 41691T		LMG 20098		NBRC 100999	AB249951			
S.olivoviridis	**376**	DSM 40211T	ATCC 15882	LMG 20057	ISP 5211	NBRC 12897	AB184227	A 03	II 20	1-3 010
S.atroolivaceus	**50**	DSM 40137T	ATCC 19725	LMG 19306	ISP 5137		AJ781320	A 03	II 20	006 0-10

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.clavifer	**128**	DSM 40843T		LMG 20476		NRRL B-2557	DQ026670			
S.finlayi	**180**	DSM 40218T	ATCC 23340	LMG 19373	ISP 5218	NRRL B-12114	AY999788	I Sm	III 24	22-4 043
Most closely to groups *S.argenteolus* et rel. and *S.atroolivaceus* et rel.										
S.griseus ssp.griseus	**248**	DSM 40236T	ATCC 23345	LMG 19302	ISP 5236	KACC 20084	AY207604	A 1B	I 02	1-3 1-2
S.lavendulae ssp. lavendulae	**297**	DSM 40069T	ATCC 8664	LMG 19925	ISP 5069	NBRC 12343	AB184080	F 61	I 22	22-3 042
S.cavourensis ssp. washingtonensis	**101**	DSM 41423T		LMG 20451		NRRL B-8030	DQ026671			
S.cyaneofuscatus	**146**	DSM 40148T	ATCC 23619	LMG 20493	ISP 5148	NBRC 13190	AB184860	A 1B	I 02	1-3 1-2
Not closely related to one of the groups										
S.mediolani	**332**	DSM 41058T	(DSM 41647T)	LMG 20093		NBRC 15427	AB184674			
S.rubiginosohelvolus	**444**	DSM 40176T	ATCC 19926	LMG 20267	ISP 5176	NBRC 12912	AB184240		IV 12 (red series)	006 1-2
S.parvus	**383**	DSM 40348T	ATCC 12433	LMG 20524	ISP 5348	NRRL B-1455	DQ442537	A 1B	I 02	1-3 1-2
S.albovinaceus	**26**	DSM 40136T	ATCC 15823	LMG 20402	ISP 5136	NBRC 12739	AB249958	A 1B	I 02	1-3 008
S.bacillaris	**67**	DSM 40598	ATCC 15855	LMG 8585	ISP 5598	NBRC 13487	AB184439	A 1B	I 02	1-3 1-2
S.griseinus	**228**	DSM 40047T	ATCC 23915	LMG 19875	ISP 5047	NBRC 12869	AB184205	A 1B	I 02	1-3 1-2
S.sindenensis	**462**	DSM 40255T	ATCC 23963	LMG 21041	ISP 5255	NBRC 3399	AB184759	A 1B		1-3 1-2
S.pluricolorescens	**397**	DSM 40019T	ATCC 19798	LMG 8576	ISP 5019	NRRL B-2121	DQ442540	A 1B	I 02	1-3 1-2
S.globisporus ssp. globisporus	**216**	DSM 40199T	ATCC 15864	LMG 8578	ISP 5199	NRRL B-2872	EF178686	A 1B	I 02	1-3 1-2
S.badius	**68**	DSM 40139T	ATCC 19888	LMG 19353	ISP 5139	NRRL B-2567	AY999783	C Sm	III 15	1-1 1-1
S.californicus	**86**	DSM 40058T	ATCC 3312/ ATCC 19	LMG 19309	ISP 5058	NBRC 3386	AB184755	A 09	II 02	1-3 030
S.floridae	**195**	DSM 40938T	NCIB 9345	LMG 19899		NBRC 15405	AB184656		IV 04 (yellow series	)1-3 1-2
S.alboviridis	**27**	DSM 40326T	ATCC 25425	LMG 20403	ISP 5326	NBRC 13013	AB184256	A 1B	I 02	1-3 1-2
S.microflavus	**338**	DSM 40331T	ATCC 13231	LMG 19327	ISP 5331	NRRL B-2156	DQ445795	A 23	I 20	1-3 1-2
S.fulvorobeus	**200**	DSM 41455T		LMG 19901		NBRC 15897	AB184711			
S.lipmanii	**308**	DSM 40070T	ATCC 3331	LMG 20047	ISP 5070	NBRC 12791	AB184148	A 1B	I 02	1-3 1-2
Group *S.avidini* et rel.										
S.spororaveus	**478**	DSM 41462T		LMG 20313			AJ781370			
S.xanthophaeus	**546**	DSM 40134T	ATCC 19819	LMG 21039	ISP 5134	NRRL B-5414	DQ442560	F 61	I 22	084 067
S.nojiriensis	**364**	DSM 41655T		LMG 20094			AJ781355			
S.cirratus	**125**	DSM 40479T	ATCC 14699	LMG 20473	ISP 5479	NRRL B-3250	AY999794	F 62	II 14	22-3 042
S.vinaceus	**518**	DSM 40515T	ATCC 27476	LMG 20533	ISP 5515	NBRC 13425	AB184394	A 06	I 05	22-3 042
S.columbiensis	**139**	DSM 40558T	ATCC 27425	LMG 20487	ISP 5558	NRRL B-1990	DQ026646	F 61	I 22	22-3 042
S.lavendulae ssp grasserius	**298**	DSM 40385T		LMG 19938			AY999841			
S.goshikiensis	**223**	DSM 40190T	ATCC 23914	LMG 19884	ISP 5190	NRRL B-5428	EF178693	F 61	I 22	22-3 042
S.sporoverrucosus	**479**	DSM 41463T		LMG 20314		NRRL B-16379	DQ442544			
S.avidinii	**64**	DSM 40526T	ATCC 27419	LMG 20428	ISP 5526	NBRC 13429	AB184395	F 56		023 004
S.subrutilus	**482**	DSM 40445T	ATCC 27467	LMG 20294	ISP 5445		X80825	F 61		22-3 042
Group *S.cinnamonensis* et rel.										
S.globosus	**219**	DSM 40815T	ATCC 14979	LMG 19896			AJ781330		IV 19 (gray series)	22-3 042
S.toxytricini	**506**	DSM 40178T	ATCC 19813	LMG 20269	ISP 5178	NRRL B-5426	DQ442548	F 61		22-3 042
S.flavotricini	**190**	DSM 40152T	ATCC 23621	LMG 19880	ISP 5152	NBRC 12770	AB184132	F 61	I 22	22-3 042
S.polychromogenes	**398**	DSM 40316T	ATCC 12595	LMG 20287	ISP 5316	NBRC 13072	AB184292	F 61	I 22	22-3 042
S.racemochromogenes	**416**	DSM 40194T	ATCC 23954	LMG 20273	ISP 5194	NRRL B-5430	DQ026656	F 61	I 22	22-3 042
S.katrae	**284**	DSM 40550T	ATCC 27440	LMG 19945	ISP 5550	NBRC 13447	AB184409	F 61	I 22	22-3 042
S.cinnamonensis	**123**	DSM 40803T	ATCC 12308	LMG 20468		NBRC 15873	AB184707		IV 02 (red series)	22-3 042
S.virginiae	**532**	DSM 40094T	ATCC 19817	LMG 20534	ISP 5094	IFO 3729	D85119	F 61	I 22	22-3 042

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain S[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
Group *S.albolongus* et rel.										
S.cavourensis ssp. cavourensis	**100**	DSM 40300T	ATCC 14889	LMG 20450	ISP 5300	NRRL 2740	DQ445791	A 1B	I 02	1-3 1-2
S.celluloflavus	**103**	DSM 40839T	ATCC 29806	LMG 21796		NBRC 13780	AB184476		IV 01 (yellow series)	020 032
S.albolongus	**20**	DSM 40570T	ATCC 27414	LMG 20396	ISP 5570	NBRC 13465	AB184425	F 63	II 15	22-4 043
S.griseobrunneus	**230**	DSM 40066T	ATCC 19762	LMG 19877	ISP 5066	NBRC 12775	AB249912	A 1B	I 02	1-3 1-2
Group *S.crystallinus* et rel.										
S.melanogenes	**334**	DSM 40192T	ATCC 23937	LMG 20056	ISP 5192	NBRC 12890	AB184222	A 33	II 07	009 1-09
S.noboritoensis	**361**	DSM 40223T	ATCC 25477	LMG 19337	ISP 5223	NBRC 13065	AB184287	A 33	II 07	009 1-09
S.crystallinus	**143**	DSM 40945T		LMG 20490		NBRC 15401	AB184652		IV 03 (red series)	009 1-09
Group *S.mauvecolor* et rel.										
S.michiganensis	**337**	DSM 40015T	ATCC 14970	LMG 20042	ISP 5015	NBRC 12797	AB184153	A 06	I 05	005 029
S.xanthochromogenes	**543**	DSM 40111T	ATCC 19818	LMG 19366	ISP 5111	NRRL B-5410	DQ442559	F 63	II 15	005 029
S.mauvecolor	**331**	DSM 41702T		LMG 20100		NBRC 13854	AB184532			
Not closely related to one of the groups										
S.cremeus	**142**	DSM 40147T	ATCC 19897	LMG 20489	ISP 5147	NBRC 12760	AB184124	A 1B	I 02	002 1-7
S.spiroverticillatus	**474**	DSM 40036T	ATCC 19811	LMG 20254	ISP 5036	NBRC 3931	AB184814	A 06	I 05	002 1-7
S.candidus	**89**	DSM 40141T	ATCC 19891		ISP 5141		DQ026663	A 03		002 1-7
Group *S.exfoliatus* et rel.										
S.lateritius	**294**	DSM 40163T	ATCC 19913	LMG 19372	ISP 5163		AJ781326	H Sm	III 23	22-3 1-08
S.venezuelae	**516**	DSM 40230T	ATCC 10712	LMG 19308	ISP 5230	JCM 4526	AB045890	A 06	I 05	002 1-7
S.omiyaensis	**377**	DSM 40552T	ATCC 27454	LMG 20075	ISP 5552	NRRL B-1587	EF178697	A 05	I 04	002 1-7
S.wedmorensis	**540**	DSM 41676T	ATCC 21239	LMG 21050		NRRL 3426	DQ442557			
S.litmocidini	**309**	DSM 40164T	ATCC 19914	LMG 20052	ISP 5164	NBRC 12792	AB184149	A 05	I 04	002 1-7
S.yerevanensis	**551**	DSM 43167T		LMG 21053		NRRL B-16943	EF178684		III 18	080 066
S.zaomyceticus	**555**	DSM 40196T	ATCC 27482	LMG 19853	ISP 5196	NRRL B-2038	EF178685	A 05	I 04	002 1-7
S.exfoliatus	**172**	DSM 40060T	ATCC 12627	LMG 19307	ISP 5060	NBRC 13191	AB184324	A 05	I 04	002 1-7
S.narbonensis	**350**	DSM 40016T	ATCC 19790	LMG 20043	ISP 5016	NRRL B-1680	DQ445794	A 04	I 04	002 1-7
Most closely to group *S.exfoliatus* et rel.										
S.albidochromogenes	**13**	DSM 41800	NBRC 101003			NBRC 101003	AB249953			
S.flavidovirens	**184**	DSM 40150T	ATCC 19900	LMG 19387	ISP 5150	NBRC 13039	AB184270		IV 03 (yellow series)	026 033
S.enissocaesilis	**165**	DSM 41454T		LMG 20506		NBRC 100763	AB249930			
S.albosporeus ssp. labilomyceticus	**24**	DSM 41672T		LMG 20400		NBRC 15387	AB184638			
S.chryseus	**113**	DSM 40420T	ATCC 19829	LMG 20458	ISP 5420	NRRL B-12347	AY999787	A 17	I 10	22-3 1-08
S.helvaticus	**259**	DSM 40431T	ATCC 19841	LMG 19940	ISP 5431	NBRC 13382	AB184367	F 62	II 14	22-3 043
Not closely related to one of the groups										
S.beijiangensis	**72**	DSM 41794	NBRC 100044			YIM6	AF385681			
S.drozdowiczii	**156**		NRRL B-24297				EF654097			
S.yanii	**548**		AS 4.1146	JCM 3331		IFO 14669	AB006159			
Group *S.graminofaciens* et rel.										
S.peucetius	**385**	DSM 40754T	NCIB 10972	LMG 20084		JCM 9920	AB045887		IV 09 (red series)	035 1-33
S.xantholiticus	**545**	DSM 40244T	ATCC 27481	LMG 19402	ISP 5244	NBRC 13354	AB184349	C 24	II 05	062 024
S.kurssanovii	**289**	DSM 40162T	ATCC 15824	LMG 19933	ISP 5162	NBRC 13192	AB184325	F 60	IV 20 (gray series)	025 1-15
S.graminofaciens	**226**	DSM 40559T	ATCC 12705	LMG 19892	ISP 5559		AJ781329	A 26	III 03	004 1-23
Group *S.amakusaensis* et rel.										
S.amakusaensis	**33**	DSM 40219T	ATCC 23876	LMG 19350	ISP 5219	NRRL B-3351	AY999781	B Sm	III 12	079 063
S.inusitatus	**276**	DSM 41441T		LMG 19955		NBRC 13601	AB184445			
S.clavuligerus	**129**	DSM 40751T	ATCC 27064	LMG 20477	DSM 738T	NRRL 3585	AY999718		IV 10 (gray series)	22-5 036

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
Group *S.atratus* et rel.										
S.atratus	**48**	DSM 41673T		LMG 20420		NRRL B-16927	DQ026638			
S.sanglieri	**451**	DSM 41791	NBRC 100784			NBRC 100784	AB249945			
S.gelaticus	**207**	DSM 40065T	ATCC 3323	LMG 19376	ISP 5065	NRRL B-2928	DQ026636	A Sm	III 11	003 1-3
S.pulveraceus	**409**	DSM 41657T		LMG 20322		NBRC 3855	AB184806			
Not closely related to one of the groups										
S.sannanensis	**452**	DSM 41705T		LMG 20329		NBRC 14239	AB184579			
Most closely to group *S.laurentii* et rel.										
S.showdoensis	**461**	DSM 40504T	ATCC 15105	LMG 20298	ISP 5504	NBRC 13417	AB184389	A 06	I 05	22-2 037
S.viridobrunneus	**535**	DSM 41466T		LMG 20317			AJ781372			
S.roseoviridis	**441**	DSM 40175T	ATCC 23959	LMG 20266	ISP 5175	NBRC 12911	AB184239	A 05	I 04	22-2 037
S.vietnamensis	**517**		CCTCC M 205143	JCM 21785		GIMV4.0001	DQ311081			
S.nashvillensis	**351**	DSM 40314T	ATCC 25476	LMG 20064	ISP 5314	NBRC 13064	AB184286	A 05	I 04	002 1-7
S.tanashiensis	**487**	DSM 40195T	ATCC 23967	LMG 20274	ISP 5195		AJ781362		IV 30 (gray series)	002 1-7
S.roseolus	**437**	DSM 40174T	ATCC 23210	LMG 20265	ISP 5174	NBRC 12816	AB184168	A 05	I 04	002 1-7
S.bikiniensis	**74**	DSM 40581T	ATCC 11062	LMG 19367	ISP 5581		X79851	F 64	III 21	22-4 1-07
S.violaceorectus	**522**	DSM 40279T	ATCC 25514	LMG 20281	ISP 5279	NBRC 13102	AB184314	A 05	I 04	002 1-7
S.cinereoruber ssp. cinereoruber	**117**	DSM 40012T	ATCC 19740	LMG 20462	ISP 5012	NBRC 12756	AB184121	A 05	I 04	002 038
Group *S.laurentii* et rel.										
S.laurentii	**295**	DSM 41684T		LMG 19959			AJ781342			
S.termitum	**490**	DSM 40329T	ATCC 25499	LMG 20289	ISP 5329	NBRC 13087	AB184302	A 05	I 04	22-2 037
S.roseofulvus	**435**	DSM 40172T	ATCC 19921	LMG 20263	ISP 5172	NBRC 13194	AB184327	A 14	II 04	002 1-7
Most closely to group *S.laurentii* et rel.										
S.filamentosus	**176**	DSM 40022T	ATCC 19753	LMG 20512	ISP 5022	NBRC 12767	AB184130	A 05	I 04	002 1-7
Group *S.gobitricini* et rel.										
S.gobitricini	**222**	DSM 41701T		LMG 19910		NBRC 15419	AB184666			
S.lavendofoliae	**296**	DSM 40217T	ATCC 15872	LMG 19935	ISP 5217		AJ781336		IV 07 (red series)	22-3 1-08
S.luridus	**317**	DSM 40081T	ATCC 19782	LMG 19365	ISP 5081	NRRL B-5409	DQ442523	F 62	II 14	22-3 1-08
S.roseolilacinus	**436**	DSM 40173T	ATCC 19922	LMG 20264	ISP 5173	NBRC 12815	AB184167	G 68	II 18	22-5 039
Not closely related to one of the groups										
S.biverticillatus	**75**	DSM 40272T	ATCC 23615	LMG 20433	ISP 5272		AJ781381		Sv. 01	22-1 040
S.werraensis	**541**	DSM 40486T	ATCC 14424	LMG 21047	ISP 5486	NRRL B-5317	DQ442558	A 12	I 07	006 1-18
S.globisporus ssp. caucasicus	**217**	DSM 40814T	ATCC 19907	LMG 19895		NRRL B-2593	EF178676		I 02	1-1 1-1
S.albireticuli	**16**	DSM 40051T	ATCC 19721	LMG 20393	ISP 5051	NBRC 12737	AB184881	F SM	Sv. 11	076 069
S.eurocidicus	**169**	DSM 40604T	ATCC 27428	LMG 20509	ISP 5604	NRRL B-1676	AY999790	F 56	Sv. 02	22-1 040
S.stramineus	**481**	DSM 41783T	NBRC 16131			NBRC 16131	AB184720			
S.olivoverticillatus	**375**	DSM 40250T	NRRL B-1994T	LMG 20058		NBRC 15273	AB184636			
S.netropsis	**352**	DSM 40259T	ATCC 23940	LMG 5979	ISP 5259	NBRC 12893	AB184848	F 56	Sv. 01	22-1 040
Group *Kitasatospora* - *Streptacidiphilus* - *Streptomyces*										
Subgroup *Kitasatospora* - *Streptomyces*										
K.gansuensis	**7***	DSM 44786	NBRC 101835			HKI 0314	AY442265			
S.atroaurantiacus	**49**	DSM 41649T		LMG 20421		NRRL B-24282	DQ026645			
K.mediocidica	**10***	DSM 43929	IFO 14789			IFO 14789	U93324			
S.purpeofuscus	**412**	DSM 40283T	ATCC 23952	LMG 20283	ISP 5283		AJ781364		IV 26 (gray series)	22-3 043

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s.[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.chrysomallus ssp. fumigatus	**115**	DSM 41424T		LMG 21793		NBRC 15394	AB184645			
S.purpureus	**414**	DSM 43362T				LMG 19368	AJ781324		I 23	22-3 1-05
S.xanthocidicus	**544**	DSM 40575T	ATCC 27480	LMG 19370	ISP 5575	IFO 13469	AY999858	F 66	II 16	22-4 043
S.aburaviensis	**2**	DSM 40033T	ATCC 23869	LMG 19305	ISP 5033	NRRL B-2218	AY999779	A 02	II 01	22-3 043
S.herbaricolor	**260**	DSM 40123T	ATCC 23922	LMG 19929	ISP 5123	NBRC 3838	AB184801	A 02	II 01	22-4 043
S.indigoferus	**273**	DSM 40124T		LMG 19930	ISP 5124	NBRC 12878	AB184214			
S.avellaneus	**61**	DSM 40554T	ATCC 23730	LMG 20427	ISP 5554	NBRC 13451	AB184413		II 17	002 1-7
S.psammoticus	**405**	DSM 40341T	ATCC 25488	LMG 20525	ISP 5341	IFO 13971	AY999862	F 67	II 17	011 1-21
S.aureofaciens	**56**	DSM 40127T	ATCC 10762	LMG 5968	ISP 5127	KACC 20180	AY207608	A 14	II 04	22-4 043
K.sampliensis	**17***	DSM 44898	NBRC 102069			VT-36	AY260167			
K.putterlickiae	**16***	DSM 44665	NBRC 100917			F18-98	AY189976			
K.kifunensis	**9***	DSM 41654	IFO 15206				AB022874			
K.azatica	**2***	DSM 41650T		LMG 20429		IFO 13803	U93312			
K.nipponensis	**12***	DSM 44787	NBRC 101836			HKI 0315	AY442263			
K.cineracea	**4***		NRRL B-24134			SK-3255	AB022875			
K.niigatensis	**11***		IFO 16453			SK-3406	AB022876			
K.cheerisanensis	**3***		KCTC 2395			YC75	AF050493			
K.phosalacinea	**15***	DSM 43860T	NRRL B-16230	LMG 20102		KA-338	AB022869			
K.paracochleata	**13***	DSM 41656	IFO 14769			NBRC 14769	U93328			
K.cochleata	**5***	DSM 41652T	IFO 14768T				U93316			
K.griseola	**8***	DSM 43859	NRRL B-16229			AM-9660	AB022870			
K.setae	**18***	DSM 43861T	IFO (now NBRC) 142	LMG 20529		KM-6054	AB022868			
K.paranensis	**14***	DSM 44788	NBRC 101837			HKI 0190	AY442268			
K.cystarginea	**6***	DSM 41680	IFO 14836			JCM 7356	U93318			
K.terrestris	**19***	DSM 44789	NBRC 101838			HKI 0186	AY442266			
K.viridis	**20***	DSM 44826				52108a	AY613990			
K.arboriphila	**1***	DSM 44785	NBRC 101834			HKI 0189	AY442267			
S.alboverticillatus	**25**	DSM 41678T	(DSM 41500T)	LMG 20401		JCM 5010	AY999766			
Group *Kitasatospora* - *Streptacidiphilus* - *Streptomyces*										
Streptacidiphilus oryzae	**7†**		CGMCC 4.2012	JCM 13271		TH49	DQ208700			
Subgroup *Streptacidiphilus albus* et rel.										
Streptacidiphilus albus	**1†**	DSM 41753				JL 83	AF074415			
Streptacidiphilus carbonis	**3†**	DSM 41754				JL 415	AF074412			
Streptacidiphilus neutrinimicus	**6†**	DSM 41755	NBRC 100921			JL 206	AF074410			
Subgroup *Streptacidiphilus anmyonensis* et rel.										
Streptacidiphilus jiangxiensis	**4†**		NBRC 100920	JCM 12277			AB249948			
Streptacidiphilus anmyonensis	**2†**		NBRC 103185			AM-11	DQ904546			
Streptacidiphilus melanogenes	**5†**		NBRC 103184			SB-B34	DQ994689			
Streptacidiphilus rugosus	**8†**		NBRC 103186			AM-16	DQ904547			
Not closely related to one of the groups										
S.ardus	**42**	DSM 40527T	ATCC 27417	LMG 20415	ISP 5527	NBRC 13430	AB184864		Sv. 03	22-1 040
S.blastmyceticus	**76**	DSM 40029T	ATCC 19731	LMG 20434	ISP 5029	NRRL B-5480	AY999802	F 58	Sv. 02	22-1 040
S.caeruleus	**85**	DSM 40103T	ATCC 27421	LMG 19399	ISP 5103	NRRL B-2194	EF178675		IV 07 (gray series)	058 050
S.hiroshimensis	**261**	DSM 40037T	ATCC 19772	LMG 19924	ISP 5037	NBRC 3720	AB184789	F 57	Sv. 01	22-1 040
S.cinnamoneus ssp. cinnamoneus	**124**	DSM 40005T	ATCC 11874	LMG 8602	ISP 5005	NBRC 12852	AB184850	F 55	Sv. 02	22-1 040
S.pseudoechinosporeus	**406**	DSM 43035T		LMG 21052		NBRC 12518	AB184100			
S.lilacinus	**305**	DSM 40254T	ATCC 23930	LMG 20059	ISP 5254	NBRC 3944	AB184819		Sv. 16	22-1 040

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.sapporonensis	**453**	DSM 41675T		LMG 20324		NBRC 13823	AB184508			
S.varsoviensis	**514**	DSM 40346T	ATCC 25505	LMG 20083	ISP 5346	NRRL B-3589	DQ026653	C 46	III 13	037 028
S.abikoensis	**1**	DSM 40831T	NRRL B-2113T	LMG 20386		NBRC 13860	AB184537			
S.lavenduligriseus	**299**	DSM 40487T	ATCC 13306	LMG 19943	ISP 5487	NRRL B-3173	DQ442515	A 34	Sv. 02	1-5 009
S.morookaensis	**345**	DSM 40503T	ATCC 19166	LMG 20074	ISP 5503		AJ781349	F 59	Sv. 08	22-1 040
S.thioluteus	**504**	DSM 40027T	ATCC 12310	LMG 21037	ISP 5027	NBRC 3364	AB184753	F Sm	Sv. 21	22-1 040
S.luteireticuli	**319**	DSM 40509	ATCC 27446		ISP 5509	NBRC 13422	AB249969		Sv.	1-8 1-17
S.ehimensis	**162**	DSM 40253T	ATCC 23903	LMG 20505	ISP 5253	KCTC 9727	AY999834		Sv. 09	22-1 040
S.hygroscopicus ssp. angustmyceticus	**267**	DSM 41683T		LMG 19958		NRRL B-2347	DQ442509			
Group *S.ochraceiscleroticus* et rel.										
S.ochraceiscleroticus	**367**	DSM 40594T/ DSM 43	ATCC 15814	LMG 19349		NBRC 12394	AB184094		III 08	069 1-26
S.purpurogeneiscleroticus	**415**	DSM 40271T=	DSM 43156T	LMG 20331			AJ621604	A 40		069 1-26
S.violens	**530**	DSM 40597T	ATCC 15898	LMG 20303	ISP 5597		AJ621605	A 40	I 18	069 1-26
S.monomycini	**344**	DSM 41801T				NRRL B-24309	DQ445790			
S.niger	**354**	DSM 40302T	= DSM 43049T	LMG 20101			AJ621607	A 40	I 18	069 1-26
S.olivaceiscleroticus	**369**	DSM 40595T	ATCC 15722	LMG 20081	ISP 5595		AJ621606		IV 24 (gray series)	069 1-26
Most closely to groups *S.ochraceiscleroticuset* rel. and *S.albofaciens* et rel.										
S.auratus	**54**	DSM 41897				NRRL 8097	AJ391816			
Group *S.albofaciens* et rel.										
S.chrestomyceticus	**111**	DSM 40545T	ATCC 14947	LMG 20457	ISP 5545		AJ621609	B 42	I 19	035 1-33
S.rimosus ssp. paromomycinus	**429**	DSM 41429T		LMG 20308			AJ621610			
S.albofaciens	**17**	DSM 40268T	ATCC 25184	LMG 20394	ISP 5268	JCM 4342	AB045880	B 42	I 19	035 1-33
Most closely to groups *S.ochraceiscleroticuset* rel. and *S.albofaciens* et rel.										
S.erumpens	**166**	DSM 40941T	ATCC 23266	LMG 20507			AJ621603		IV 15 (gray series)	035 1-33
S.rimosus ssp. rimosus	**428**	DSM 40260T	ATCC 10970	LMG 19352	ISP 5260	JCM 4667	AB045883	B 42	I 19	035 1-33
S.sclerotialus	**456**	DSM 40269T=	DSM 43032T	LMG 20528			AJ621608		I 18	069 1-26
Group *S.albulus* et rel.										
S.albulus	**28**	DSM 40492T	ATCC 12757	LMG 20404	ISP 5492	IMC S-0802	AB024440	A 29	I 15	025 109
S.noursei	**365**	DSM 40635T	ATCC 11455	LMG 5982		NBRC 15452	AB184678		IV 23 (gray series)	025 1-09
S.yunnanensis	**554**	DSM 41793	CGMCC 4.1004	JCM 12115		YIM 41004	AF346818			
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel. and *S.albulus* et rel.										
S.kasugaensis	**283**	DSM 40819T		LMG 19949	ISP 5819	M338-M1	AB024441			
S.chattanoogensis	**108**	DSM 40002T	ATCC 19739	LMG 19339	ISP 5002		AJ621611			
S.lydicus	**323**	DSM 40461T	ATCC 25470	LMG 19331	ISP 5461		Y15507	A 29	I 15	025 005
S.albospinus	**22**	DSM 41674T		LMG 20398		NBRC 13846	AB184527			
S.sioyaensis	**463**	DSM 40032T	ATCC 13989	LMG 20531	ISP 5032	NRRL B-5408	DQ026654	A 29	I 15	025 005
S. hygroscopicus ssp. decoyicus	**268**	DSM 41427T		LMG 19954		AS 4.1861	AY999883			
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel., *S.albulus* et rel. and *S.caniferus* et rel.										
S.catenulae	**98**	DSM 40258T	ATCC 12476	LMG 20449	ISP 5258		AJ621613	C 43	II 11	035 041
S.misakiensis	**341**	DSM 40222T	ATCC 23938	LMG 19369	ISP 5222	IFO 12891	AB217605	F 66	II 16	22-4 043

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.ramulosus	**419**	DSM 40100T	ATCC 19802	LMG 19354	ISP 5100	NRRL B-2714	DQ026662	C Sm	III 16	035 041
Group *S.caniferus* et rel.										
S.hygroscopicus ssp. glebosus	**269**	DSM 40823T		LMG 19950		NBRC 13786	AB184479			
S.libani ssp. rufus	**303**	DSM 41230T		LMG 20087			AJ781351			
S.platensis	**395**	DSM 40041T	ATCC 13865	LMG 20046	ISP 5041	JCM 4662	AB045882	A 29	I 15	025 005
S.caniferus	**92**	DSM 41453T		LMG 20446		NBRC 15389	AB184640			
Most closely to group *S.caniferus* et rel.										
S.libani ssp. libani	**302**	DSM 40555T	ATCC 23732	LMG 20077	ISP 5555	NBRC 13452	AB184414	A 29	I 15	025 005
S.tubercidicus	**508**	DSM 40261T	ATCC 25502	LMG 19361	ISP 5261		AJ621612	C 47	III 14	025 005
S.nigrescens	**355**	DSM 40276T	ATCC 23941	LMG 19332	ISP 5276	NRRL B-12176	DQ442530	A 29	I 15	025 005
Group *S.albiflaviniger* et rel.										
S.antimycoticus	**39**	DSM 40284T	ATCC 23880	LMG 20413	ISP 5284	NBRC 12839	AB184185		IV 05 (gray series)	051 018
S.geldanamycininus	**208**	DSM 41894	NRRL 3602T			NRRL B-3602	DQ334781			
S.melanosporofaciens	**335**	DSM 40318T	ATCC 25473	LMG 20066	ISP 5318	NRRL B-12234	AJ271887	A 32	I 16	051 018
S.sporoclivatus	**477**	DSM 41461T		LMG 20312		NBRC 100767	AB249934			
S.yatensis	**549**	DSM 41771				NBRC 101000	AB249962			
S.rutgersensis ssp. castelarensis	**448**	DSM 40830T	ATCC 15191	LMG 20304			AY508511		I 01	055 018
S.indoniensis	**274**	DSM 41759T				A4R2	DQ334783			
S.griseiniger	**227**	DSM 41895	NRRL B-1865T				AJ391818			
S.rhizosphaericus	**427**	DSM 41760T				NBRC 100778	AB249941			
S.asiaticus	**46**	DSM 41761T				NBRC 100774	AB249947			
S.cangkringensis	**91**	DSM 41769T				D13P3	AJ391831			
S.malaysiensis	**327**	DSM 41697T		LMG 20099		NBRC 16446	AB249918			
S.javensis	**279**	DSM 41764T				B22P3	AJ391833			
S.endus	**164**	DSM 40187T	NRRL 2339	LMG 19393			AY999911			
S.sporocinereus	**476**	DSM 41460T		LMG 20311		NBRC 100766	AB249933			
S.hygroscopicus ssp. hygroscopicus	**266**	DSM 40578T	ATCC 27438	LMG 19335	ISP 5578	NBRC 13472	AB184428	A 32	I 16	085 012
S.demainii	**150**	DSM 41600	NRRL B-1478				DQ334782			
S.violaceusniger	**526**	DSM 40563T	ATCC 27477	LMG 19336	ISP 5563		AJ391823	A 32	I 16	051 018
S.yogyakartensis	**552**	DSM 41766T				NBRC 100779	AB249942			
S.albiflaviniger	**15**	DSM 41598T	NRRL B-1356T				AJ391812			
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel., *S.albulus* et rel., *S.caniferus* et rel. and *S.albiflaviniger* et rel.										
S.orinoci	**378**	DSM 40571T	ATCC 23202	LMG 20079	ISP 5571	NBRC 13466	AB184866	F 58	Sv. 17	22-1 040
S.mashuensis	**328**	DSM 40221T	ATCC 23934	LMG 8603	ISP 5221		X79323	F 55	Sv. 03	22-1 040
S.mobaraensis	**343**	DSM 40847T	ATCC 29032	LMG 20086		NRRL B-3729	DQ442528		Sv. 07	22-1 040
S.luteosporeus	**321**	DSM 40833T		LMG 20085		NNRL 2401	DQ442525			
S.aureoversilis	**58**	DSM 40387T	ATCC 15853	LMG 20425	ISP 5387	NBRC 13021	AB184855		Sv. 05	22-1 040
S.griseocarneus	**231**	DSM 40004T	ATCC 12628	LMG 19383	ISP 5004		X99943	F 55	Sv. 03	22-1 040
Group *S.albus* et rel.										
S.almquistii	**31**	DSM 40447T	ATCC 618	LMG 21307	ISP 5447	NBRC 13015	AB184258	A 16	I 09	030 1-34
S.rangoonensis	**420**	DSM 40452T	ATCC 6860	LMG 20295	ISP 5452	NBRC 13078	AB184295		IV 07 (white series)	030 1-34
S.gibsonii	**211**	DSM 43284T	ATCC 6852	LMG 19912		NBRC 15415	AB184663		IV 05 (white series)	030 1-34
S.albus ssp. albus	**29**	DSM 40313T	ATCC 3004		ISP 5313		AJ621602	A 16	I 09	032 027
S.flocculus	**194**	DSM 40327T	ATCC 25453	LMG 19889	ISP 5327	NBRC 13041	AB184272	A 16	I 09	030 1-34

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
Most closely to group *S.albus* et rel.										
S.cacaoi ssp. cacaoi	**82**	DSM 40057T	ATCC 3082	LMG 19320	ISP 5057	NBRC 12748	AB184115	A 16	I 09	031 1-34
S.sulphureus	**484**	DSM 40104T	ATCC 27468	LMG 19355	ISP 5104	NRRL B-1627	DQ442546	C Sm	III 17	068 002
S.rubidus	**443**		CGMCC 4.2026			13C15	AY876941			
S.yeochonensis	**550**		NBRC 100782	JCM 12366		CN 732	AF101415			
S.albus ssp. pathocidicus	**30**	DSM 40799T		LMG 20406		NBRC 13812	AB184501			
S.glauciniger	**213**			LMG 22082		NBRC 100913	AB249964			
S.guanduensis	**252**		CGMCC 4.2022			701	AY876942			
Most closely to groups *S.albus* et rel. and *S.glaucosporus* et rel.										
S.ferralitis	**174**	DSM 41836				SFOp68	AY262826			
S.vitaminophilus	**539**	DSM 41686T		LMG 21051		NBRC 14294	AB184589			
S.thermolineatus	**498**	DSM 41451T		LMG 20309			Z68097			
S.yanglinensis	**547**		CGMCC 4.2023	JCM 13275		1307	AY876940			
S.paucisporeus	**384**		CGMCC 4.2025			1413	AY876943			
Group *S.glaucosporus* et rel.										
S.macrosporus	**324**	DSM 41449T					Z68099			
S.megasporus	**333**	DSM 41476T		LMG 20092		NBRC 14749	AB184617			
S.glaucosporus	**214**	DSM 41689T		LMG 19907		NBRC 15416	AB184664			
S.radiopugnans	**417**	DSM 41901	CGMCC 4.3519			R97	DQ912930			
Most closely to group *S.glaucosporus* et rel.										
S.albiaxialis	**12**	DSM 41799	NBRC 101002			NRRL B-24327	AY999901			
S.armeniacus	**45**	DSM 43125T		LMG 20418		JCM 3070	AB018092			
Most closely to groups *S.albus* et rel. and *S.glaucosporus* et rel.										
S.cuspidosporus	**145**	DSM 41425T		LMG 20492		NBRC 12378	AB184090		IV 11 (gray series)	22-4 1-06
S.sparsogenes	**466**	DSM 40356T	ATCC 25498	LMG 19378	ISP 5356	NBRC 13086	AB184301	A 32	I 16	010 1-19
Most closely to group *S.geysiriensis* et rel.										
S.janthinus	**278**	DSM 40206T	ATCC 15870	LMG 8591	ISP 5206	NBRC 12879	AB184851	A 18	I 11	009 1-19
S.roseoviolaceus	**440**	DSM 40277T	ATCC 25493	LMG 8594	ISP 5277		AJ399484	A 18	I 11	009 1-19
S.violaceus	**525**	DSM 40082T	ATCC 15888	LMG 20257	ISP 5082	NBRC 13103	AB184315	A 06	I 05	009 0-19
S.albosporeus ssp. albosporeus	**23**	DSM 40795T	ATCC 15394	LMG 19403			AJ781327		IV 01 (red series)	063 049
S.arenae	**43**	DSM 40293T	ATCC 25428	LMG 20416	ISP 5293	NBRC 13016	AB249977	A 18	I 11	009 1-19
S.luteogriseus	**320**	DSM 40483T	ATCC 15072	LMG 20073	ISP 5483	NBRC 13402	AB184379	A 18	I 11	009 1-19
S.hawaiiensis	**256**	DSM 40042T	ATCC 12236	LMG 5975	ISP 5042	NBRC 12784	AB184143	A 18	I 11	009 1-19
S.cellulosae	**105**	DSM 40362T	ATCC 25439	LMG 19315	ISP 5362	NRRL B-2889	DQ442495	A 13	II 03	006 1-18
S.pseudogriseolus	**407**	DSM 40026T	ATCC 12770		ISP 5026	NRRL B-3288	DQ442541	A 12	I 07	006 1-18
S.gancidicus	**205**	DSM 40935T	NRRL B-1872	LMG 19898		NBRC 15412	AB184660		IV 17 (gray series)	006 1-18
S.rubiginosus	**445**	DSM 40177T	ATCC 19927	LMG 20268	ISP 5177	KCTC 9042	AY999810	A 12	I 07	006 1-18
S.capillispiralis	**94**	DSM 41695T		LMG 19909		NBRC 14222	AB184577			
S.lavendulocolor	**300**	DSM 40216T	ATCC 15871	LMG 19934	ISP 5216	NRRL B-3367	DQ442516	F 61	I 22	22-3 1-08
S.azureus	**65**	DSM 40106T	ATCC 14921	LMG 20430	ISP 5106	NRRL B-2655	EF178674	A 18	I 11	009 1-19
S.flavoviridis	**193**	DSM 40153T	ATCC 19903	LMG 19881	ISP 5153	NBRC 12772	AB184842	A 28		006 1-10
S.pilosus	**394**	DSM 40097T	ATCC 19797	LMG 20049	ISP 5097	NBRC 12807	AB184161	A 37	I 17	006 1-10
S.djakartensis	**155**	DSM 40743T	ATCC 13441	LMG 21795		NBRC 15409	AB184657		IV 12 (gray series)	035 1-33
Group *S.geysiriensis* et rel.										

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s.[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.ghanaensis	**210**	DSM 40746T	ATCC 14672	LMG 19894		KCTC 9882	AY999851		IV 05 (green series)	1-7 1-21
S.minutiscleroticus	**339**	DSM 40301T	ATCC 17757	LMG 20062	ISP 5301	NRRL B-12202	EF178696	A 15	I 08	006 1-18
S.geysiriensis	**209**	DSM 40742T	ATCC 15303	LMG 19893		NRRL B-12102	DQ442501		IV 18 (gray series)	006 1-18
S.plicatus	**396**	DSM 40319T	ATCC 25483	LMG 20288	ISP 5319	NBRC 13071	AB184291	A 12	I 07	006 1-18
S.rochei	**431**	DSM 40231T	ATCC 10739	LMG 19313	ISP 5231	NBRC 12908	AB184237	A 12	I 07	006 1-18
S.vinaceusdrappus	**519**	DSM 40470T	ATCC 25511	LMG 20296	ISP 5470	NRRL 2363	AY999929	A 12	I 07	006 1-18
S.mutabilis	**347**	DSM 40169T	ATCC 19919	LMG 20054	ISP 5169		EF178679	A 12	I 07	006 1-18
Most closely to group *S.geysiriensis* et rel.										
S.tuirus	**509**	DSM 40505T		LMG 20299		NBRC 15617	AB184690	A 21	I 14	006 1-18
S.afghaniensis	**8**	DSM 40228T	ATCC 23871	LMG 20390	ISP 5228		AJ399483	A 18	I 11	009 1-19
S.africanus	**9**	DSM 41829	NBRC 101005			CPJVR-H	AY208912			
Group *S.brasiliensis* et rel.										
S.roseiscleroticus	**432**	DSM 40303T	ATCC 17755	LMG 20284	ISP 5303	NBRC 13002	AB184251		II 19	049 022
S.ruber	**442**	DSM 40304T		LMG 20285		NBRC 14600	AB184604		IV 11 (red series)	049 022
S.spiralis	**473**	DSM 43836T		LMG 20332		NRRL B-16922	EF178683			
S.fumigatiscleroticus	**202**	DSM 43154T		LMG 19911		NRRL B-3856	DQ442499			
S.poonensis	**399**	DSM 40596T	ATCC 15723	LMG 19326	ISP 5596	NRRL B-2319	DQ445792	A 22	II 19	071 1-19
S.brasiliensis	**80**	DSM 43159T	ATCC 23727	LMG 20438		NBRC 101283	AB249981			
Group *S.atrovirens* et rel.										
S.atrovirens	**51**	DSM 41467T		LMG 20422		NRRL B-16357	DQ026672			
S.caelestis	**84**	DSM 40084T	ATCC 15084	LMG 20441	ISP 5084	NRRL 2418	X80824	A 18	I 11	009 1-19
S.fumanus	**201**	DSM 40154T	ATCC 19904	LMG 19882	ISP 5154	NBRC 13042	AB184273	A 18	I 11	1-7 1-19
S.fimbriatus	**178**	DSM 40942T	ATCC 15051	LMG 20513			AY999844		IV 16 (gray series)	006 1-18
Group *S.glaucus* et rel.										
S.griseostramineus	**245**	DSM 40161T	ATCC 23628	LMG 19932	ISP 5161	NBRC 12781	AB184140	F 60	IV 06 (green series)	006 1-10
S.griseomycini	**239**	DSM 40159T	ATCC 23625	LMG 19883	ISP 5159	NBRC 12778	AB184137	A 12	I 07	006 1-10
S.graminearus	**225**	DSM 41747T		LMG 19904			AJ781333			
S.viridiviolaceus	**534**	DSM 40280T	ATCC 27478	LMG 20282	ISP 5280	IFO 13359	AY999854		IV 35 (gray series)	006 1-18
S.glaucus	**215**	DSM 41456T		LMG 19902		NBRC 15417	AB184665			
Group *S.aureorectus* et rel.										
S.aureorectus	**57**	DSM 41692T	IFO 15896	LMG 19908		NBRC 15896	AB184710			
S.virens	**531**	DSM 41465T		LMG 20316		NRRL B-24331	DQ442554			
S.asterosporus	**47**	DSM 41452T		LMG 20419		NBRC 15872	AB184706			
S.calvus	**87**	DSM 40010T	ATCC 13382	LMG 20442	ISP 5010	NBRC 13200	AB184329	A 12	I 07	006 1-18
Most closely to groups *S.geysiriensis* et rel., *S.brasiliensis* et rel., *S.atrovirens* et rel., *S.glaucus* et rel. and *S.aureorectus* et rel.										
S.naganishii	**349**	DSM 40282T	ATCC 23939	LMG 21042	ISP 5282	NRRL B-1816	DQ442529	A 31	I 21	1-6 1-15
S.prasinosporus	**402**	DSM 40506T	ATCC 17918	LMG 19346	ISP 5506	NBRC 13419	AB184390	A 38	III 07	22-2 1-15
S.anandii	**36**	DSM 40535T	ATCC 19388	LMG 8600	ISP 5535	NBRC 13438	AB184402	B 42	I 19	021 1-05
S.carpinensis	**97**	DSM 43835T		LMG 19913		NBRC 14214	AB184574			
S.levis	**301**	DSM 41458T		LMG 20090		NBRC 15423	AB184670			
S.cinerochromogenes	**121**	DSM 41651T		LMG 20466		NBRC 13822	AB184507			
S.koyangensis	**287**		NBRC 100598			VK-A60	AY079156			
S.violarus	**527**	DSM 40205T	ATCC 15891	LMG 20275	ISP 5205	NBRC 13104	AB184316	A 18	I 11	009 1-19
Not closely related to one of the groups										

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.daghestanicus	**149**	DSM 40149T	ATCC 23620	LMG 20496	ISP 5149	NRRL B-5418	DQ442497	A 17		006 010
S.limosus	**306**	DSM 40131T	ATCC 19778	LMG 8570	ISP 5131	NBRC 12790	AB184147	A 1A	I 01	1-1 1-1
S.canescens	**90**	DSM 40001T	ATCC 19736	LMG 20445	ISP 5001	NBRC 12751	AB184117	A 1A	I 01	1-1 1-1
S.felleus	**173**	DSM 40130T	ATCC 19752	LMG 20511	ISP 5130	NBRC 12766	AB184129	A 1A	I 01	1-1 1-1
S.griseus ssp. solvifaciens	**251**	DSM 40933T	NRRL B-1561	LMG 19952		NBRC 13689	AB249915		I 02	1-1 1-1
S.violascens	**528**	DSM 40183T	ATCC 23968	LMG 20272	ISP 5183		AY999737	A 06	I 05	002 1-7
S.hydrogenans	**265**	DSM 40586T	ATCC 19631	LMG 19948	ISP 5586	NBRC 13475	AB184868	A 05	I 04	002 1-7
S.odorifer	**368**	DSM 40347T	ATCC 6246	LMG 8572	ISP 5347		Z76682	A 1A	I 01	1-1 1-1
S.albidoflavus	**14**	DSM 40455T	ATCC 25422	LMG 21791	ISP 5455	NBRC 13010	AB184255	A 1A	I 01	1-1 1-1
S.champavatii	**106**	DSM 40841T	NRRL B-5682	LMG 20454			DQ026642		IV 02 (yellow series)	1-1 1-1
S.sampsonii	**450**	DSM 40394T	ATCC 25495	LMG 8574	ISP 5394		D63871	A 1A	I 01	1-1 1-1
S.diastaticus ssp. diastaticus	**151**	DSM 40496T	ATCC 3315	LMG 19322	ISP 5496	NBRC 3714	AB184785	A 19	I 12	1-1 1-1
S.gougerotii	**224**	DSM 40324T	ATCC 10975	LMG 19888	ISP 5324	NBRC 3198	AB184742	A 1A	I 01	1-1 1-1
S.rutgersensis ssp.rutgersensis	**447**	DSM 40077T	ATCC 3350	LMG 8568	ISP 5077	NBRC 12819	AB184170	A 1A	I 01	1-1 1-1
S.intermedius	**275**	DSM 40372T	ATCC 3329	LMG 19304	ISP 5372	NBRC 13049	AB184277	A 1A	I 01	1-1 1-1
S.indiaensis	**272**	DSM 43803T		LMG 19961		NBRC 13964	AB184553			
S.thermocarboxydus	**494**	DSM 44293T					U94490			
S.massasporeus	**329**	DSM 40035T	ATCC 19785	LMG 19362	ISP 5035	NBRC 12796	AB184152	D SM	III 19	015 1-19
S.misionensis	**342**	DSM 40306T	ATCC 14991	LMG 20063	ISP 5306	NRRL B-3230	EF178678	A 31	I 21	1-6 1-16
S.phaeoluteichromatogenes	**389**					NRRL B-5799	AJ391814			
S.spectabilis	**468**	DSM 40512T	NRRL 2792T	LMG 5986	ISP 5512	NBRC 13424	AB184393			
S.cinereospinus	**119**	DSM 41470T		LMG 20464		NBRC 15397	AB184648			
S.coeruleofuscus	**134**	DSM 40144T	ATCC 23618	LMG 20482	ISP 5144	NRRL B-5417	DQ026668	A 18	I 11	009 1-19
S.chromofuscus	**112**	DSM 40273T	ATCC 23896	LMG 19317	ISP 5273	NBRC 12851	AB184194	A 15	I 08	006 1-18
S.scopiformis	**457**	DSM 41825	NBRC 200244	LMG 20251		NBRC 100244	AB249927			
S.spinoverrucosus	**472**	DSM 41648T		LMG 20321		NBRC 14228	AB184578			
Most closely to group *S.mexicanus* et rel.										
S.thermospinosisporus	**500**	DSM 41779	NBRC 100043	JCM 11756		AT10	AF333113			
S.thermodiastaticus	**496**	DSM 40573T	ATCC 27472	LMG 20302	ISP 5573	JCM 4840	AB018095	A 1C	I 03	006 1-18
S.thermocarboxydovorans	**493**	DSM 44296T		LMG 19860			U94489			
S.thermoviolaceus ssp. apingens	**502**	DSM 41392T		LMG 20307			Z68095			
S.thermoviolaceus ssp. thermoviolaceus	**501**	DSM 40443T	ATCC 19283	LMG 19359	ISP 5443		Z68096	C 45	II 13	004 006
S.nodosus	**362**	DSM 40109T	ATCC 14899	LMG 19430	ISP 5109		AF114033	A 35	II 08	006 1-11
S.viridosporus	**538**	DSM 40243T	ATCC 27479	LMG 20278	ISP 5243	NRRL 2414	DQ442556	A 15	I 08	006 1-18
Group *S.mexicanus* et rel.										
S.thermogriseus	**497**	DSM 41756T		LMG 20532		NBRC 100772	AB249980			
S.thermovulgaris	**503**	DSM 40444T	ATCC 19284	LMG 19342	ISP 5444		Z68094	A 36	II 09	021 002
S.thermoalcalitolerans	**491**	DSM 41741T		LMG 19858		NBRC 16322	AB249909			
S.mexicanus	**336**	DSM 41796				NBRC 100915	AB249966			
S.thermocoprophilus	**495**	DSM 41700T		LMG 19857		B19	AJ007402			
Most closely to group *S.mexicanus* et rel.										
S.bangladeshensis	**71**		NRRL B-24326	LMG 22738		AAB-4	AY750056			
S.rameus	**418**	DSM 41685T		LMG 20326		KCTC 9767	AY999821			
S.griseosporeus	**244**	DSM 40562T	ATCC 27435	LMG 19947	ISP 5562	NBRC 13458	AB184419	A 23	I 20	1-7 1-19
S.achromogenes ssp. rubradiris	**4**	DSM 40789T	NRRL 3061	LMG 20388		KCTC 9742	AY999846		I 12	028 009
S.glomeratus	**220**	DSM 41457T		LMG 19903		NBRC 15898	AB249917			
S.eurythermus	**171**	DSM 40014T	ATCC 14975	LMG 20510	ISP 5014		D63870	A 23	I 20	1-5 009
S.nogalater	**363**	DSM 40546T	ATCC 27451	LMG 19338	ISP 5546	JCM 4799	AB045886	A 34	III 06	1-5 009
S.fragilis	**198**	DSM 40044T	ATCC 23908	LMG 19874	ISP 5044	NRRL 2424	AY999917	G SM	III 22	078 058

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain S[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
Group *S.erythrogriseus* et rel.										
S.erythrogriseus	**168**	DSM 40116T	ATCC 27427	LMG 19406	ISP 5116		AJ781328		IV 04 (red series)	074 1-27
S.labedae	**290**	DSM 41446T		LMG 19956		NBRC 15864	AB184704			
S.griseoincarnatus	**235**	DSM 40274T	ATCC 23623	LMG 19316	ISP 5274		AJ781321	A 13	II 03	006 1-18
S.variabilis	**512**	DSM 40179T	ATCC 19930	LMG 20270	ISP 5179	NRRL B-3984	DQ442551	A 12	I 07	006 1-18
Most closely to group *S.erythrogriseus* et rel.										
S.althioticus	**32**	DSM 40092T	ATCC 19724	LMG 20408	ISP 5092	KCTC 9752	AY999808	A 12	I 07	006 1-18
S.matensis	**330**	DSM 40188T	ATCC 23935	LMG 20055	ISP 5188	NBRC 12889	AB184221	A 12	I 07	006 1-18
S.griseorubens	**241**	DSM 40160T	ATCC 19909	LMG 19931	ISP 5160	NBRC 12780	AB184139	A 12	I 07	006 1-18
S.viridochromogenes	**536**	DSM 40110T	ATCC 14920	LMG 20260	ISP 5110	NRRL B-1511	DQ442555	A 27	III 04	009 1-19
S.iakyrus	**271**	DSM 40482T	ATCC 15375	LMG 19942	ISP 5482	NBRC 13401	AB184877	A 18	I 11	009 1-19
S.violaceochromogenes	**520**	DSM 40181		LMG 20271		IFO 13100	AY999867			
S.collinus	**138**	DSM 40129T	ATCC 19743	LMG 20486	ISP 5129	NBRC 12759	AB184123	A 18	I 11	009 1-19
S.malachitofuscus	**325**	DSM 40332T	ATCC 25471	LMG 20067	ISP 5332	NBRC 13059	AB184282			006 1-18
S.paradoxus	**380**	DSM 43350T		LMG 20523		NBRC 14887	AB184628			
S.griseoflavus	**233**	DSM 40456T	ATCC 25456	LMG 19344	ISP 5456		AJ781322	A 37	I 17	006 1-18
S.flaveolus	**181**	DSM 40061T	ATCC 3319	LMG 19328	ISP 5061	NBRC 3408	AB184764	A 24	II 05	1-6 1-13
S.glaucescens	**212**	DSM 40155T	ATCC 23622	LMG 19330	ISP 5155	NBRC 12774	AB184843	A 28	III 05	006 1-10
S.pharetrae	**393**	DSM 41856	NRRL B-24333			CZA14	AY699792			
S.malachitospinus	**326**		IFO 101004			NBRC 101004	AB249954			
S.parvulus	**382**	DSM 40048T	ATCC 12434	LMG 21789	ISP 5048	NBRC 13193	AB184326	A 12		006 1-18
S.tendae	**489**	DSM 40101T	ATCC 19812	LMG 19314	ISP 5101		D63873	A 12	I 07	006 1-18
S.violaceorubidus	**524**	DSM 41478T		LMG 20319			AJ781374			
S.albaduncus	**11**	DSM 40478T	ATCC 14698	LMG 20392	ISP 5478	JCM 4715	AY999757		IV 02 (gray series)	006 1-10
S.griseoloalbus	**236**	DSM 40468T	ATCC 23624	LMG 21308	ISP 5468	NBRC 13046	AB184275		IV 05 (yellow series)	017 007
S.heliomycini	**258**	DSM 41690T	IFO 15899	LMG 19960		NBRC 15899	AB184712			
S.ambofaciens	**34**	DSM 40053T	ATCC 23877	LMG 20409	ISP 5053		M27245	A 23	I 20	006 1-18
Most closely to group *S.coelescens* et rel.										
S.rubrogriseus	**446**	DSM 41477T		LMG 20318		NBRC 15455	AB184681			
S.tricolor	**507**	DSM 41704T		LMG 20328		NBRC 15461	AB184687			
S.lienomycini	**304**	DSM 41475T		LMG 20091			AJ781353			
S.anthocyanicus	**37**	DSM 41422T		LMG 20411		NBRC 14892	AB184631		IV 03 (gray series)	013 1-19
S.olivaceus	**371**	DSM 40072T	ATCC 3335	LMG 19394	ISP 5072	NBRC 3200	AB184743	A 1C	I 03	042 014
S.pactum	**379**	DSM 40530T	ATCC 27456	LMG 19357	ISP 5530	NBRC 13433	AB184398	C 44	II 12	22-4 035
Group *S.coelescens* et rel.										
S.coelescens	**130**	DSM 40421T	ATCC 19830	LMG 20479	ISP 5421	ICSSB 1021	AF503496	A 21	I 14	006 1-18
S.humiferus	**264**	DSM 43030T		LMG 20519			AF503491			
S.violaceolatus	**521**	DSM 40438T	ATCC 19847	LMG 20293	ISP 5438	ICSSB 1022	AF503497	A 21	I 14	006 1-18
S.violaceoruber	**523**	DSM 40049T	ATCC 14980	LMG 20256	ISP 5049	ICSSB 1016	AF503492		IV 34 (gray series)	069 1-26
Most closely to group *S.coelescens* et rel.										
S.coelicoflavus	**131**	DSM 41471T		LMG 20480		NBRC 15399	AB184650			
S.diastaticus ssp. ardesiacus	**152**	DSM 40934	IFO 15402	LMG 20497T		NRRL B-1773	DQ026631			
Most closely to group *S.coeruleorubidus* et rel.										
S.lomondensis	**310**	DSM 41428T		LMG 20088		NBRC 15426	AB184673		IV 03 (blue series)	009 1-19

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.lusitanus	**318**	DSM 40568T	ATCC 15842	LMG 20078	ISP 5568	NBRC 13464	AB184424	C 44	II 12	006 1-18
S.purpurascens	**413**	DSM 40310T	ATCC 25489	LMG 20526	ISP 5310		AK399486	A 18	I 11	009 1-19
S.bellus	**73**	DSM 40185T	ATCC 14925	LMG 19401	ISP 5185	NBRC 12844	AB184849	A 18	I 11	061 1-22
S.coerulescens	**137**	DSM 40146T	ATCC 19896	LMG 8590	ISP 5146		AY999720	A 18	I 11	009 1-19
S.speibonae	**469**	DSM 41797T	ATCC BAA-411			PK-Blue	AF452714			
S.longispororuber	**312**	DSM 40599T	ATCC 27443	LMG 20082	ISP 5599	NBRC 13488	AB184440	A 10	I 06	033 1-33
Group *S.coeruleorubidus* et rel.										
S.albogriseolus	**19**	DSM 40003T	ATCC 23875	LMG 20395	ISP 5003	NRRL B-1305	AJ494865	A 12	I 07	006 1-18
S.viridodiastaticus	**537**	DSM 40249T	ATCC 25518	LMG 20279	ISP 5249	IFO 13106	AY999852		IV 36 (gray series)	006 1-18
S.coeruleorubidus	**136**	DSM 40145T	ATCC 13740	LMG 20484	ISP 5145		AY999719	A 18	I 11	009 1-19
Group *S.aurantiogriseus* et rel.										
S.coelicolor	**132**	DSM 40233T	ATCC 23899	LMG 8571	ISP 5233	NRRL B-2812	DQ442496	A 1A	I 01	1-1 1-1
S.griseoviridis	**247**	DSM 40229T	ATCC 23920	LMG 19321	ISP 5229	KCTC 9780	AY999807	A 17	I 10	006 010
S.aurantiogriseus	**53**	DSM 40138	ATCC 23883	LMG 19298	NRRL-ISP 5138	NRRL B-5416	AY999793			
Most closely to group *S.aurantiogriseus* et rel.										
S.griseoaurantiacus	**229**	DSM 40430T	ATCC 19840	LMG 21045	ISP 5430	NBRC 15440	AB184676	A 12	I 07	1-7 1-15
S.jietajiensis	**280**		AS 4.1859	JCM 12279		FXJ46	AY314783			
Group *S.coeruleoprunus* et rel.										
S.coeruleoprunus	**135**	DSM 41472T		LMG 20483		NBRC 15400	AB184651			
S.somaliensis	**465**	DSM 40738T					AJ007403			
S.fradiae	**197**	DSM 40063T	ATCC 10745	LMG 19371	ISP 5063	NRRL B-1195	DQ026630	G 68	II 18	22-5 039
Most closely to group *S.coeruleoprunus* et rel.										
S.bluensis	**77**	DSM 40564T	ATCC 27420	LMG 5969	ISP 5564		X79324	A 39	II 10	052 017
Not closely related to one of the groups										
S.variegatus	**513**	DSM 41464T		LMG 20315			AJ781371			
S.fulvissimus	**199**	DSM 40593T	ATCC 27431	LMG 19310	ISP 5593	NBRC 13482	AB184434	A 10		034 1-33
S.aureoverticillatus	**59**	DSM 40080T	ATCC 15854	LMG 20426	ISP 5080	NRRL B-3326	AY999774	A 10	I 06	033 1-33
S.flavofungini	**186**	DSM 40366T	ATCC 27430	LMG 21799	ISP 5366	NBRC 13371	AB184359	B 42		033 1-33
S.alboflavus	**18**	DSM 40045T	ATCC 12626	LMG 21038	ISP 5045	NRRL B-2373	EF178699	E 54	III 20	033 1-33
S.aculeolatus	**7**	DSM 41644T		LMG 19906		NBRC 14824	AB184624			
S.synnematoformans	**485**	DSM 41902	CGMCC 4.2055			S155	EF121313			
S.hebeiensis	**257**	DSM 41837	CCTCC AA 203005			YIM 001	AY277529			
Group *S.carpaticus* et rel.										
S.hainanensis	**254**	DSM 41900	CCTCC AA 205017			YIM 47672	AM398645			
S.specialis	**467**	DSM 41924	CCM 7499			GW 41-1564	AM934703			
S.carpaticus	**96**	DSM 41468T	ATCC 43678	LMG 20448		NRRL B-16359	DQ442494			
S.cheonanensis	**109**		NBRC 100940			VC−A46	AY822606			
Most closely to group *S.carpaticus* et rel.										
S.sulfonofaciens	**483**	DSM 41679T	ATCC 31892	LMG 20325		NBRC 14260	AB249974			
S.sodiiphilus	**464**		CCTCC AA 203015	JCM 13581		YIM 80305	AY236339			
Not closely related to one of the groups										
S.scabrisporus	**455**		NBRC 100760			KM-4927	AB030585			
S.gardneri	**206**	DSM 40064T	ATCC 9604	LMG 19876	ISP 5064	NBRC 3385	AB184754	A 04		002 1-07
S.flavidofuscus	**183**	DSM 41473T	ATCC 43683			NRRL B-16366	AY999914			

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain S[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
Regarded as later heterotypic synonym of *Streptomyces abikoensis* (for references, see list of type strains)										
S.luteoverticillatus	**322**	DSM 40038T	ATCC 23933	LMG 20045	ISP 5038		AB184803	F 55	Sv. 03	22-1 040
S.olivoreticuli ssp. olivoreticuli	**374**	DSM 40105T		LMG 20050	ISP 5105		AB184853			
S.parvisporogenes	**381**	DSM 40473T	ATCC 12568	LMG 20072	ISP 5473		AB249913		Sv. 02	22-1 040
Regarded as later heterotypic synonym of *Streptomyces anulatus* (for references, see list of type strains)										
S.chrysomallus ssp. chrysomallus	**114**	DSM 40128T	ATCC 11523	LMG 20459	ISP 5128		AB184644	A 1B		1-3 1-2
S.citreofluorescens	**127**	DSM 40265T	ATCC 15858	LMG 20475	ISP 5265		AB184195	A 1B	I 02	1-3 1-2
S.fluorescens	**196**	DSM 40203T	ATCC 15860	LMG 8579	ISP 5203		AB184199	A 1B	I 02	1-3 1-2
Regarded as later heterotypic synonym of *Streptomyces avermitilis* (for references, see list of type strains)										
S.avermectinius	**62**									
Regarded as later heterotypic synonym of *Streptomyces cacaoi* (for references, see list of type strains)										
S.aminophilus	**35**	DSM 40186T	ATCC 14961 L	MG 19319	ISP 5186		AB184183	A 16	I 09	031 1-34
Regarded as later heterotypic synonym of *Streptomyces caeruleus* (for references, see list of type strains)										
S.niveus	**360**	DSM 40088T	ATCC 19793	LMG 19395	ISP 5088		AB184160	A 1B	I 02	043 013
S.spheroides	**471**	DSM 40292T	ATCC 23965	LMG 19392	ISP 5292		EF178698	A 1B	I 02	040 048
Regarded as later heterotypic synonym of *Streptomyces cinnamoneus* (for references, see list of type strains)										
S.griseoverticillatus	**246**	DSM 40507T	ATCC 27436	LMG 19944	ISP 5507		AB184862	F 58		22-1 040
S.hachijoensis	**253**	DSM 40114T	ATCC 19769	LMG 19928	ISP 5114		AB184141	F 55	Sv. 04	22-1 040
Regarded as later heterotypic synonym of *Streptomyces chibaensis* (for references, see list of type strains)										
S.chibaensis	**110**	DSM 40220T	ATCC 23895	LMG 20456	ISP 5220		AB184193	A 24	II 05	009 1-19
Regarded as later heterotypic synonym of *Streptomyces filamentosus* (for references, see list of type strains)										
S.roseosporus	**438**	DSM 40122T	ATCC 23958	LMG 20262	ISP 5122		AB184238	A 05	I 04	002 1-7
Regarded as later heterotypic synonym of *Streptomyces flavofuscus* (for references, see list of type strains)										
S.globisporus ssp. flavofuscus	**218**		ATCC 19908				DQ026648			
Regarded as later heterotypic synonym of *Streptomyces flavovirens* (for references, see list of type strains)										
S.nigrifaciens	**356**	DSM 40071T	ATCC 19791	LMG 20048	ISP 5071		AB184158	A 1C	I 03	1-2 015
Regarded as later heterotypic synonym of *Streptomyces fradiae* (for references, see list of type strains)										

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S.roseoflavus	**434**	DSM 40536T	ATCC 13167	LMG 20535	ISP 5536				IV 10 (red series)	22-5 105
Regarded as later heterotypic synonym of *Streptomyces griseocarneus* (for references, see list of type strains)										
S.septatus	**459**	DSM 40577T	ATCC 27464	LMG 8604	ISP 5577		AB184883	F 55	Sv. 02	22-1 040
Regarded as later heterotypic synonym of *Streptomyces griseus* (for references, see list of type strains)										
S.setonii	**460**	DSM 40395T	ATCC 25497	LMG 20291	ISP 5395		D63872	A 1B	I 02	1-3 1-2
Regarded as later heterotypic synonym of *Streptomyces hiroshimensis* (for references, see list of type strains)										
S.rectiverticillatus	**422**	DSM 40436T	ATCC 19845	LMG 20292	ISP 5436		AB184296	F 57	Sv. 18	22-1 040
S.roseoverticillatus	**439**	DSM 40039T	ATCC 19807	LMG 20255	ISP 5039		AB184169		Sv. 01	22-1 040
S.salmonis	**449**	DSM 40895T	NRRL B-1472	LMG 20306			X53169		Sv. 05	22-1 040
S.spitsbergensis	**475**		ATCC 51269	JCM 8881			AB184700			
S.fervens ssp. fervens	**175**	DSM 40086T	ATCC 27429		ISP 5086		AB184871		Sv. 01	22-1 040
Regarded as later heterotypic synonym of *Streptomyces lilacinus* (for references, see list of type strains)										
S.kashmirensis	**282**	DSM 40336T		LMG 19937	ISP 5336		AB184546			
Regarded as later heterotypic synonym of *Streptomyces mashuensis* (for references, see list of type strains)										
S.kishiwadensis	**286**	DSM 40397T	ATCC 25464	LMG 19939	ISP 5397		AB184858		Sv. 15	22-1 040
Regarded as later heterotypic synonym of *Streptomyces microflavus* (for references, see list of type strains)										
S.griseus ssp. alpha	**249**	DSM 40937T	NRRL B-2249	LMG 19953			AB184668		I 02	1-3 1-2
S.griseus ssp. cretosus	**250**	DSM 40561T			ISP 5561		AB184418			
S.willmorei	**542**	DSM 40459T	ATCC 6867	LMG 21046	ISP 5459		AB184374	A 1B	I 02	1-3 1-2
Regarded as later heterotypic synonym of *Streptomyces minutiscleroticus* (for references, see list of type strains)										
S.flaviscleroticus	**185**	DSM 40270T	ATCC 19347	LMG 19886	ISP 5270		AB184634		I 08	017 007
Regarded as later heterotypic synonym of *Streptomyces mobaraensis* (for references, see list of type strains)										
S.ladakanum	**292**	DSM 40587T	NRRL 3191T				AB184430			
Regarded as later heterotypic synonym of *Streptomyces netropsis* (for references, see list of type strains)										
S.distallicus	**154**	DSM 40846T	NCIB 8936	LMG 20499			AB184703		Sv. 01	22-1 040
S.flavopersicus	**189**	DSM 40093T	ATCC 19756		ISP 5093		AB249911	F 56		22-1 040
S.kentuckensis	**285**	DSM 40052T	ATCC 12691		ISP 5052		AB184215	F SM	Sv. 11	22-1 040
S.syringium	**486**	DSM 41480T		LMG 20320			AJ781375			

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain S[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
Regarded as later heterotypic synonym of *Streptomyces phaeopurpureus* (for references, see list of type strains)										
S.phaeoviridis	**392**	DSM 40285T	ATCC 23947	LMG 20061	ISP 5285		AB184230	A 19	I 12	009 1-19
Regarded as later heterotypic synonym of *Stre ptomyces thermovulgaris* (for references, see list of type strains)										
S.thermonitrificans	**499**	DSM 40579T	ATCC 23385		ISP 5579		Z68098	A 36	II 09	021 002
Regarded as later heterotypic synonym of *Streptomyces tricolor* (for references, see list of type strains)										
S.roseodiastaticus	**433**	DSM 41703T		LMG 20327			AB184683			
Regarded as later heterotypic synonym of *Streptomyces olioverticillatus* (for references, see list of type strains)										
S.viridiflavus	**533**			LMG 20277T			AB184702			
Regarded as later heterotypic synonym of *Streptomyces violaceus* (for references, see list of type strains)										
S.violatus	**529**	DSM 40209T	ATCC 15892	LMG 19397	ISP 5209		AJ399480	A 18	I 11	050 019
No detailed sequence information available										
S.caviscabies	**99**	DSM 41811	ATCC 51928				AF112160			
***S.coeruleoflavus* (keine Seq. gefunden)**	**133**									
S.arabicus	**41**	DSM 40252T	ATCC 23881	LMG 20414	ISP 5225		D44271	A 12	I 07	006 1-18
S.baldaccii	**69**	DSM 40845T	ATCC 23654				X53164		Sv. 01	22-1 040
S.cellulolyticus	**104**									
S.echinoruber	**160**	DSM 41696T	IFO 14238							
S.erythraeus	**167**	DSM 40517T		LMG 20508						
S.longisporoflavus	**311**	DSM 40165T	ATCC 19915	LMG 19347	ISP 5165		AB184220	A 39	II 10	005 010
S.olivomycini	**373**									
S.speleomycini	**470**									
S.thermoautotrophicus	**492**	DSM 41605								
Not in tree										
S. aldersoniae	**556**	DSM 41909				NRRL 18513	EU170123			
S. alni	**557**	D65			CGMCC 4.3510	NRRL B-24611	DQ460470			
S. angustmycinicus	**558**	DSM 41683				NRRL B-2347	EU170119			
S. ascomycinicus	**559**	DSM 40822				NBRC 13987	EU170121			
S. atriruber	**560**	DSM 41860			LDDC6330-99	NRRL B-24165	EU812169			
S. avicenniae	**561**	DSM 41943			MCCC 1A01535	CGMCC 4.5510	EU399234			
S. axinellae	**562**	DSM 41948			Pol001	CIP 109838	EU683612			
S. baliensis	**563**	ID03-0915			BTCC B-608	NBRC 104276	AB441718			
S. castelarensis	**564**	DSM 40830	ATCC 15191				EF408732			
S. deccanensis	**565**	DAS-139			KCTC 19214	CCTCC AA 207004	EF219459			
S. decoyininicus	**566**	DSM 41427				NRRL 2666	EU170127			
S. gulbargensis	**567**	DAS 131			KCTC 19179	CCTCC AA-206001	DQ317411			
S. haliclonae	**568**	DSM 41970			Sp080513SC−31	NBRC 105049	AB473556			
S. himastatinicus	**568**	DSM 41914	ATCC 53653				EF408736			

Table 42.2 (continued)

Species names and groups[1]	No. in list of type strain s[2]	Type strain					Accession	Wil 83	Wil 89[4]	Käm 91[5]
S. hypolithicus	**569**	DSM 41950			HSM10	NRRL B-24669	EU196762			
S. iranensis	**570**	DSM 41954			HM 35	CCUG 57623	FJ472862			
S. lunalinharesii	**571**	DSM 41876	ATCC BAA-1231		RCQ1071	CIP 108852	DQ094838			
S. marinus	**572**	DSM 41968			Sp080513GE-26	NBRC 105047	AB473554			
S. marokkonensis	**573**	DSM 41918		LMG 23016	R-22003	Ap1	AJ965470			
S. mayteni	**574**	YIM 60475			KCTC 19383	CCTCC AA 207005	EU200683			
S. milbemycinicus	**575**	DSM 41911				NRRL 5739	EU170126			
S. modarskii	**576**	DSM 40771				NRRL B-1346	EF408735			
S. nanshensis	**577**	SCSIO 01066			KCTC 19400	CCTCC AA 208005	EU589334			
S. osmaniensis	**578**	OU-63			PCM 2690	CCTCC AA 209025	FJ613126			
S. plumbiresistens	**579**	CCNWHX 13-160			ACCC 41207	HAMBI 2991	EU526954			
S. polyantibioticus	**580**	DSM 44925			SPR	NRRL B-24448	DQ141528			
S. rapamycinicus	**581**		ATCC 29253			NRRL 5491	EF408733			
S. ruanii	**582**	DSM 40276				ISP 5276	EF408737			
S. sedi	**583**	DSM 41942			YIM 65188	CCTCC AA 208020	EU925562			
S. silaceus	**584**	DSM 41861			LDDC 6638-99	NRRL B-24166	EU812170			
S. tateyamensis	**585**	DSM 41969			Sp080513SC−30	NBRC 105048	AB473555			
S. thinghirensis	**586**	DSM 41919			S10	CCMM B35	FM202482			
S. tritolerans	**587**				DAS 165		DQ345779			
S. wellingtoniae	**588**	DSM 40632				NRRL B-1503	EU170124			
S. xiamenensis	**589**	DSM 41903			MCCC 1A01550	CGMCC 4.3534	EF012099			
S. xinghaiensis	**590**	S187		KCTC 19546	CCTCC AA 208049	NRRL B-24674	EF577247			
K. kazusensis	**21**	SK 60			KCTC 19565	JCM 14560	AB278569			
K. saccharophila	**22**	SK 15			KCTC 19566	JCM 14559	AB278568			

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
Most closely to group *S.costaricanus* et rel.																
S.galbus							Sch00	Lan2-00	BENP	+		+			cl22	Clade 1
S.longwoodensis							Sch00	Lan2-00	BENP	+		+			cl22	Clade 1
S.bungoensis								Lan2-00	BENP	+		+			cl22	–
S.corchorusii							Sch04	Lan2-26	BENP	+	(a)	+			cl52	Clade 2
S.canarius							Sch00	Lan2-00	BENP	+		+			cl22	Clade 2
S.olivaceoviridis		La-21		OC−III			Sch04	Lan2-00	BENP +	+		+		+	cl53	Clade 2
S.capoamus							Sch00		BENP	+		+		+	+	–
S.regensis							Sch00	Lan2-00	BENP	+		+			cl52	–
S.griseochromogenes						L2	Sch00	Lan2-00	BENP	+		+		+	cl22	–
S.cellostaticus							Sch15	Lan2-00	BENP	+		+			cl55	–
S.yokosukanensis							Sch00	Lan2-00	BENP	+		+			cl22	–
S.antibioticus				OC−IV			Sch00	Lan2-00	BENP	+		+			cl13	Clade 4
S.griseoruber		La-00		OC−I			Sch00	Lan2-00	BENP +	+		+	cl15	+	+	Clade 4
S.cinnabarinus						L2	Sch00	Lan2-00	BENP	+		+		+	+	–
S.acidiscabies							Sch00	Lan2-00	BENP	+		+		+	+	Clade 5
S.alanosinicus							Sch34	Lan2-00	BENP	+		+			cl12	Clade 5
Group *S.costaricanus* et rel.																
S.griseofuscus			FU-6		KA-G		Sch00	Lan2-00	BENP	+		+		+	cl06	Clade 12
S.murinus						L3	Sch00	Lan2-00	BENP	+		+		+	cl59	Clade 12
S.costaricanus																Clade 12
S.phaeogriseichromatogenes																Clade 12

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
Most closely to group *S.costaricanus* et rel.																
S.lanatus		La-21				L2	Sch00	Lan2-00	BENP +	+		+	cl09		cl22	Clade 11
S.durhamensis							Sch00	Lan2-00	BENP	+		+			cl22	Clade 10
S.filipinensis		La-10		OC–III			Sch00	Lan2-00	BENP +	+		+	cl09	+	cl23	Clade 10
S.puniciscabiei														+		Clade 10
S.niveiscabiei														+		–
S.echinatus			FU-1			L2	Sch00	Lan2-00	BENP	+		+			+	–
S.longisporus						L2	Sch00	Lan2-00	BENP	+		+		+	cl22	–
S.avermitilis																–
S.kunmingensis							Sch00	Lan2-00	BENP	+		+			cl22	–
S.mirabilis							Sch00	Lan2-00	BENP	+		+		+	cl20	Clade 20
S.olivochromogenes							Sch00	Lan2-00	BENP	+		+			cl20	Clade 20
Most closely to group *S.cyanoalbus* et rel.																
S.lucensis							Sch00	Lan2-00	BENP	+		+		+	+	Clade 9
S.niveoruber		La-01					Sch00	Lan2-00	BENP +	+		+	cl04		+	Clade 9
S.achromogenes ssp. achromogenes			FU-1		KA-B	L2	Sch00	Lan2-00	BENP	+		+		+	cl22	–
S.griseorubiginosus						L2	Sch23	Lan2-00	BENP	+		+		+	cl19	Clade 7
S.phaeopurpureus							Sch23	Lan2-02	BENP	+	(b)	+			cl51	Clade 7
S.curacoi						L2	Sch00	Lan2-00	BENP	+		+		+	cl55	–
S.lincolnensis							Sch00	Lan2-00	BENP	+		+		+	cl13	Clade 3
S.cyaneus						L2	Sch00	Lan2-00	BENP	+	(g)	+		+	+	–
Group *S.cyanoalbus* et rel.																
S.cyanoalbus		La-17				Sch15	Lan2-00	MG-016	BENBO			+	cl14	+	+	Clade 6
S.hirsutus							Sch00	Lan2-00	BENP	+		+		+	cl49	Clade 6
S.prasinus							Sch00		BENP	+		+	cl14	+	cl49	Clade 6
S.bambergiensis		La-20		OC–non			Sch00		BENP +	+		+	cl14		+	Clade 6
S.emeiensis																Clade 6
S.prasinopilosus		La-20					Sch00	Lan2-00	BENP +	+		+	cl14	+	cl49	Clade 6
Most closely to group *S.cyanoalbus* et rel.																
S.flavovariabilis							Sch00	Lan2-00	BENP	+		+		+	+	–
S.aureocirculatus							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 15
S.novaecaesareae				OC–IV			Sch00	Lan2-00	BENP	+		+		+	cl22	–
S.prunicolor		La-00		OC–II			Sch09		BENP +	+		+		+	+	–
S.phaeoluteigriseus														+		–
S.bobili							Sch37	Lan2-00	BENP	+		+		+	cl22	Clade 13
S.galilaeus							Sch37	Lan2-00	BENP	+		+		+	cl22	Clade 13
Most closely to groups *S.cyanoalbus* et rel. and *S.griseoluteus* et rel.																
S.chartreusis						L2	Sch30	Lan2-00	BENP	+		+		+	+	–
S.resistomycificus			FU-12a			L2	Sch00	Lan2-00	BENP	+		+		+	+	–
Most closely to group *S.griseoluteus* et rel.																
S.griseoluteus		La-24		OC–III			Sch00		BENP +	+		+		+	cl41	Clade 17
S.recifensis							Sch00		BENP	+		+		+	cl41	Clade 17
S.seoulensis																
Most closely to groups *S.cyanoalbus* et rel. and *S.griseoluteus* et rel.																
S.canus		La-21		OC–IV			Sch00	Lan2-00	BENP +	+		+	cl09	+	+	Clade 8
S.ciscaucasicus							Sch00	Lan2-05	BENP	+		+		+	cl19	Clade 8
S.pseudovenezuelae																–
S.alboniger							Sch00	Lan2-00	BENP	+		+		+	+	Clade 14

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
Most closely to group *S.scabiei* et rel.																
S.bottropensis							Sch00	Lan2-00	BENP	+		+		+	+	Clade 24
S.stelliscabiei																Clade 24
S.europaeiscabiei														+		Clade 25
S.scabiei							Sch00	Lan2-00	BENP	+		+			cl25	Clade 25
S.diastatochromogenes							Sch00	Lan2-00	BENP	+		+			cl25	Clade 25
S.hygroscopicus ssp. ossamyceticus							Sch20	Lan2-00	BENP	+		+			cl26	Clade 23
S.ipomoeae						L2	Sch00	Lan2-00	BENP	+		+		+	cl26	Clade 23
S.torulosus							Sch00	Lan2-00	BENP	+		+		+	cl26	Clade 23
S.neyagawaensis			FU-24			L2	Sch00	Lan2-00	BENP	+		+		+	cl26	Clade 23
Most closely to group *S.scabiei* et rel.																
S.reticuliscabiei																Clade 22
S.turgidiscabies														+		Clade 22
S.cacaoi ssp. asoensis							Sch00	Lan2-00	BENP	+		+		+	+	Clade 21
S.humidus							Sch00	Lan2-00	BENP	+		+			cl22	Clade 21
S.rishiriensis			FU-12a				Sch00	Lan2-00	BENP	+		+			+	–
S.cinereoruber ssp. fructofermentans							Sch00	Lan2-00	BENP	+		+		+	cl20	–
S.phaeofaciens							Sch00	Lan2-00	BENP	+		+		+	+	Clade 33
S.puniceus							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 33
Group *S.aurantiacus* et rel.																
S.aurantiacus		La-01		OC–non			Sch00	Lan2-00	BENP cl	+	(c)	+	cl05	+	cl24	Clade 19
S.glomeroaurantiacus																Clade 15
S.tauricus							Sch00	Lan2-00	BENP	+		+		+	cl24	Clade 19
S.ederensis							Sch00	Lan2-00	BENP	+		+			cl19	Clade 18
S.phaeochromogenes		La-01		OC–II			Sch00	Lan2-00	BENP +	+		+	cl06		–	Clade 18
S.umbrinus							Sch00	Lan2-15	BENP	+		+			cl54	Clade 18
S.rectiviolaceus							Sch00	Lan2-00	BENP	+		+		+	–	–
Group *S.aureus* et rel.																
S.kanamyceticus		La-11					Sch00	Lan2-00	BENP +	+		+	cl02		cl23	–
S.durmitorensis																Clade 26
S.aureus																Clade 26
Group *S.cinereus* et rel.																
S.cinereus							Sch00	Lan2-00	BENP	+		+		+	+	Clade 16
S.flaveus		La-21							BENP +	+		+			–	Clade 16
S.vastus							Sch21	Lan2-00	BENP	+		+		+	+	Clade 16
Most closely to group *S.cinereus* et rel.																
S.laceyi														+		Clade 27
Group *S.argenteolus* et rel.																
S.griseolus			FU-24		KA-B		Sch06		BENP	+		+		+	cl53	Clade 36
S.halstedii			FU-24	OC–I	KA-B		Sch06							+		Clade 36
S.argenteolus					KA-B		Sch00	Lan2-00	BENP	+		+		+	cl23	–
S.cinereorectus							Sch00	Lan2-30	BENP	+	(b)	+		+	cl28	Clade 61
S.flavovirens							Sch05	Lan2-09	BENP	+	(a)	+		+	cl53	Clade 37
S.flavogriseus			FU-19b		KA-B		Sch00/0	Lan2-09	BENP	+		+			cl53	Clade 37
S.nitrosporeus							Sch26	Lan2-00	BENP	+		+		+	+	–
Most closely to groups *S.argenteolus* et rel. and *S.atroolivaceus* et rel.																
S.luridiscabiei														+		–
S.acrimycini							Sch00	Lan2-00	BENP	+		+			cl05	–
S.griseoplanus							Sch00	Lan2-00	BENP	+		+		+	cl28	Clade 54
S.baarnensis					KA-B		Sch01		BENP	+		+		+	cl23	–
S.flavofuscus							Sch00	Lan2-19	BENP	+		+		+	cl23	–

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.praecox							Sch01		BENP	+		+		+	cl23	–
S.fimicarius			FU-9				Sch01		BENP	+		+		+	cl23	–
S.anulatus		La-22		OC–I	KA-B		Sch01	Lan2-18	BENP +	+	(a)(e)	+	cl02	+	cl23	–
Group *S.atroolivaceus* et rel.																
S.mutomycini							Sch00	Lan2-00	BENP	+		+		+	cl23	Clade 34
S.olivoviridis							Sch16		BENP	+		+		+	cl23	Clade 34
S.atroolivaceus		La-23		OC–I			Sch16		BENP +	+		+	cl02		cl23	Clade 34
S.clavifer							Sch00	Lan2-05	BENP	+		+			cl19	Clade 34
S.finlayi		La-00		OC–I			Sch00	Lan2-00	BENP +	+		+	cl02	+	cl23	Clade 34
Most closely to groups *S.argenteolus* et rel. and *S.atroolivaceus* et rel.																
S.griseus ssp.griseus		La-22	FU-19		KA-B		Sch00	Lan2-00	BENP +	+		+	cl02	+	cl23	–
S.lavendulae ssp. lavendulae			FU-12b	OC–I		L3/L	Sch00	Lan2-00	BENP	+	(e)(l)	+			–	Clade 39
S.cavourensis ssp. washingtonensis							Sch00	Lan2-00	BENP	+		+			cl23	–
S.cyaneofuscatus							Sch00	Lan2-00	BENP	+		+		+	cl23	–
Not closely related to one of the groups																
S.mediolani							Sch20	Lan2-16	BENP	+		+			cl23	–
S.rubiginosohelvolus							Sch03		BENP	+		+			cl23	Clade 42
S.parvus			FU-6		KA-B		Sch00	Lan2-00	BENP	+		+	cl02		cl23	–
S.albovinaceus					KA-B		Sch03	Lan2-16	BENP	+		+		+	cl23	–
S.bacillaris					KA-B		Sch00	Lan2-00	BENP	+		+		+	cl50	–
S.griseinus			FU-6		KA-B		Sch03	Lan2-00	BENP	+		+			–	–
S.sindenensis					KA-B		Sch00		BENP	+		+		+	cl23	–
S.pluricolorescens					KA-B		Sch03		BENP	+		+		+	–	–
S.globisporus ssp. globisporus					KA-B		Sch20	Lan2-00	BENP	+		+			cl23	–
S.badius		La-00		OC–I			Sch00		BENP +	+		+	cl02	+	cl23	–
S.californicus		La-22	FU-6	OC–I			Sch32	Lan2-00	BENP +	+		+	cl02		cl23	Clade 33
S.floridae							Sch32	Lan2-00	BENP	+		+		+	cl23	Clade 33
S.alboviridis					KA-B		Sch02	Lan2-00	BENP	+		+		+	cl23	–
S.microflavus		La-22		OC–I			Sch02	Lan2-12	BENP +	+	(a)	+	cl02	+	cl23	–
S.fulvorobeus							Sch00	Lan2-00	BENP	+		+		+	cl23	–
S.lipmanii			FU-9		KA-B		Sch02	Lan2-12	BENP	+	(a)	+			cl23	–
Group *S.avidini* et rel.																
S.spororaveus							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 39
S.xanthophaeus						L5	Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 39
S.nojiriensis							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 39
S.cirratus							Sch00	Lan2-00	BENP	+		+			cl22	Clade 39
S.vinaceus					KA-A		Sch00	Lan2-23	BENP	+	(b)	+			cl08	Clade 39
S.columbiensis			FU-12b			L5	Sch00	Lan2-13	BENP	+	(b)(l)	+		+	–	Clade 39
S.lavendulae ssp grasserius								Lan2-00	BENP	+		+			–	Clade 39
S.goshikiensis						L3/L	Sch00		BENP	+		+			cl22	Clade 39
S.sporoverrucosus							Sch00		BENP	+		+		+	cl22	Clade 39
S.avidinii							Sch00	Lan2-00	BENP	+		+			cl22	Clade 39
S.subrutilus						L5	Sch00	Lan2-00	BENP	+		+			cl22	Clade 39
Group *S.cinnamonensis* et rel.																
S.globosus							Sch00	Lan2-00	BENP	+		+		+	cl31	Clade 38
S.toxytricini						L3/L	Sch00	Lan2-00	BENP	+		+		+	cl31	Clade 38
S.flavotricini			FU-1			L3/L	Sch00	Lan2-00	BENP	+		+			cl22	Clade 38
S.polychromogenes						L3/L	Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 38
S.racemochromogenes						L5	Sch00	Lan2-00	BENP	+		+			cl22	Clade 38
S.katrae						L5	Sch26	Lan2-00	BENP	+		+		+	cl22	Clade 38
S.cinnamonensis							Sch00	Lan2-00	BENP	+		+			cl22	Clade 39
S.virginiae			FU-12b			L3/L	Sch00	Lan2-00	BENP	+		+			cl22	Clade 39
Group *S.albolongus* et rel.																
S.cavourensis ssp. cavourensis			FU-6		KA-A		Sch00	Lan2-00	BENP	+		+		+	cl22	

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.celluloflavus							Sch00	Lan2-00	BENP	+		+		+	cl42	Clade 31
S.albolongus						L4	Sch00	Lan2-00	BENP	+		+			+	–
S.griseobrunneus			FU-6		KA-A		Sch00	Lan2-00	BENP	+		+			cl50	Clade 31
Group *S.crystallinus* et rel.																
S.melanogenes							Sch00	Lan2-00	BENP	+		+		+	cl13	
S.noboritoensis		La-19		OC–I			Sch00	Lan2-00	BENP +	+		+	cl06		cl34	Clade 30
S.crystallinus							Sch00	Lan2-00	BENP	+		+		+	cl34	–
Group *S.mauvecolor* et rel.																
S.michiganensis							Sch00	Lan2-00	BENP	+		+			cl14	Clade 29
S.xanthochromogenes		La-23		OC–I			Sch00	Lan2-00	BENP +	+		+	cl02	+	+	Clade 29
S.mauvecolor							Sch00	Lan2-00	BENP	+		+		+	cl34	Clade 29
Not closely related to one of the groups																
S.cremeus			FU-21				Sch00	Lan2-00	BENP	+		+			cl22	Clade 32
S.spiroverticillatus					KA-A		Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 32
S.candidus							Sch00							+		–
Group *S.exfoliatus* et rel.																
S.lateritius		La-00		OC–II			Sch00	Lan2-00	BENP +	+		+	cl07	+	cl22	Clade 40
S.venezuelae		La-00			KA-C		Sch00	Lan2-00	BENP +	+		+	cl07		cl22	Clade 40
S.omiyaensis					KA-C		Sch00	Lan2-00	BENP	+		+		+	cl23	Clade 40
S.wedmorensis							Sch00	Lan2-00	BENP	+		+			cl23	Clade 40
S.litmocidini					KA-C		Sch00		BENP	+		+			cl22	Clade 40
S.yerevanensis				OC–1					BENP	+		+			+	
S.zaomyceticus					KA-C		Sch00	Lan2-00	BENP	+		+			cl23	Clade 40
S.exfoliatus		La-00		OC–II	KA-C		Sch00	Lan2-00	BENP +	+		+	cl01	+	cl23	Clade 40
S.narbonensis					KA-C		Sch00	Lan2-00	BENP	+		+			cl44	Clade 40
Most closely to group *S.exfoliatus* et rel.																
S.albidochromogenes																Clade 41
S.flavidovirens		La-22					Sch00	Lan2-00	BENP +	+		+		+	+	Clade 41
S.enissocaesilis							Sch00	Lan2-06	BENP	+		+		+	cl58	Clade 41
S.albosporeus ssp. labilomyceticus							Sch00	Lan2-00	BENP	+		+		+	cl23	–
S.chryseus						L3	Sch28	Lan2-00	BENP	+		+			cl23	Clade 41
S.helvaticus							Sch28	Lan2-00	BENP	+		+		+	cl23	Clade 41
Not closely related to one of the groups																
S.beijiangensis														+		–
S.drozdowiczii														+		–
S.yanii																Clade 35
Group *S.graminofaciens* et rel.																
S.peucetius							Sch00	Lan2-00	BENP	+		+			cl21	Clade 28
S.xantholiticus		La-21					Sch00	Lan2-00	BENP +	+		+	cl04	+	cl21	Clade 28
S.kurssanovii							Sch00	Lan2-00	BENP	+		+		+	cl21	Clade 28
S.graminofaciens				OC–I			Sch00	Lan2-00	BENP	+		+		+	+	Clade 28
Group *S.amakusaensis* et rel.																
S.amakusaensis		La-00		OC–I		L2	Sch00	Lan2-00	BENP +	+		+	cl14	+	+	Clade 47
S.inusitatus							Sch00	Lan2-00	BENP	+		+		+	cl13	Clade 47
S.clavuligerus							Sch00	Lan2-00	BENP	+		+		+	+	–
Group *S.atratus* et rel.																
S.atratus							Sch00	Lan2-00	BENP	+		+		+	cl23	Clade 35
S.sanglieri														+		Clade 35
S.gelaticus		La-00					Sch00	Lan2-00	BENP +	+		+		+	+	Clade 35
S.pulveraceus							Sch00	Lan2-00	BENP	+		+			cl23	Clade 35
Not closely related to one of the groups																
S.sannanensis							Sch07	Lan2-04	BENP	+		+			cl22	Clade 103

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
Most closely to group *S.laurentii* et rel.																
S.showdoensis							Sch00	Lan2-00	BENP	+		+		+	cl23	Clade 45
S.viridobrunneus							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 45
S.roseoviridis							Sch00	Lan2-00	BENP	+		+		+	cl44	–
S.vietnamensis																Clade 46
S.nashvillensis							Sch00	Lan2-00	BENP	+		+		+	cl23	Clade 42
S.tanashiensis							Sch00	Lan2-00	BENP	+		+			cl22	Clade 42
S.roseolus							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 43
S.bikiniensis		La-00		OC–II			Sch00	Lan2-00	BENP +	+		+	cl01		cl23	Clade 46
S.violaceorectus							Sch00		BENP	+		+		+	cl23	Clade 46
S.cinereoruber ssp. cinereoruber			FU-6				Sch00		BENP	+		+		+	cl23	–
Group *S.laurentii* et rel.																
S.laurentii							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 44
S.termitum							Sch00	Lan2-00	BENP	+		+			cl22	Clade 41
S.roseofulvus							Sch00	Lan2-00	BENP	+		+			cl22	Clade 44
Most closely to group *S.laurentii* et rel.																
S.filamentosus							Sch00	Lan2-24	BENP	+	(b)	+		+	cl23	–
Group *S.gobitricini* et rel.																
S.gobitricini							Sch00	Lan2-00	BENP	+		+			cl14	Clade 48
S.lavendofoliae							Sch00	Lan2-00	BENP	+		+		+	cl14	Clade 48
S.luridus		La-17		OC–II			Sch00	Lan2-00	BENP +	+		+			–	Clade 48
S.roseolilacinus							Sch00	Lan2-00	BENP	+		+			cl12	–
Not closely related to one of the groups																
S.biverticillatus	Ha7					L4		Lan2-00	BENP	+	(j)	+		+	cl13	–
S.werraensis					KA-G		Sch00	Lan2-00	BENP	+		+		+	cl04	–
S.globisporus ssp. caucasicus							Sch10		BENP	+		+		+	cl08	Clade 112
S.albireticuli	Ha5						Sch00	Lan2-00	BENP	+	(j)	+			cl13	Clade 51
S.eurocidicus	Ha5					L4	Sch00	Lan2-00	BENP	+	(j)	+			cl13	Clade 51
S.stramineus	Ha16															–
S.olivoverticillatus	Ha18					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl13	Clade 49
S.netropsis	Ha14		FU-21			L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl13	Clade 52
Group *Kitasatospora - Streptacidiphilus - Streptomyces*																
Subgroup *Kitasatospora - Streptomyces*																
K.gansuensis																Clade 59
S.atroaurantiacus							Sch00	Lan2-00	BENP	+		+		+	cl30	Clade 59
K.mediocidica																Clade 58
S.purpeofuscus							Sch00	Lan2-00	BENP	+		+			cl30	Clade 57
S.chrysomallus ssp. fumigatus							Sch00	Lan2-00	BENP	+		+		+	+	–
S.purpureus		La-18		OC–I			Sch00	Lan2-00						+		Clade 40
S.xanthocidicus		La-18					Sch00	Lan2-00	BENP +	+		+	cl03	+	cl29	–
S.aburaviensis		La-00		OC–I			Sch00	Lan2-00	BENP +	+		+	cl03	+	+	–
S.herbaricolor							Sch00	Lan2-00	BENP	+		+		+	cl30	Clade 38
S.indigoferus							Sch00	Lan2-00	BENP	+		+			cl30	–
S.avellaneus							Sch00	Lan2-28	BENP	+		+			cl29	Clade 55
S.psammoticus				OC–I			Sch09	Lan2-28	BENP	+		+		+	cl29	Clade 11
S.aureofaciens				OC–I			Sch00	Lan2-28	BENP	+		+		+	cl29	Clade 55
K.sampliensis																–
K.putterlickiae														+		–
K.kifunensis																–
K.azatica								Lan2-00	BENP	+		+		+	cl30	Clade 57
K.nipponensis																–
K.cineracea																Clade 60
K.niigatensis																Clade 60

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
K.cheerisanensis																–
K.phosalacinea								Lan2-00	BENP	+		+			cl27	–
K.paracochleata									BENP	+		+			cl28	Clade 61
K.cochleata								Lan2-30	BENP	+	(b)	+		+	cl28	Clade 61
K.griseola																–
K.setae								Lan2-00	BENP	+		+			cl27	–
K.paranensis																–
K.cystarginea																–
K.terrestris																–
K.viridis																–
K.arboriphila																–
S.alboverticillatus	Ha6						Sch00	Lan2-00	BENP	+	(j)	+		+	cl17	–
Group *Kitasatospora - Streptacidiphilus - Streptomyces*																
Streptacidiphilus oryzae																Clade 54
Subgroup *Streptacidiphilus albus* et rel.																
Streptacidiphilus albus														+		Clade 53
Streptacidiphilus carbonis																Clade 53
Streptacidiphilus neutrinimicus																Clade 53
Subgroup *Streptacidiphilus anmyonensis* et rel.																
Streptacidiphilus jiangxiensis																Clade 53
Streptacidiphilus anmyonensis																Clade 53
Streptacidiphilus melanogenes																Clade 53
Streptacidiphilus rugosus																Clade 53
Not closely related to one of the groups																
S.ardus	Ha2					L4	Sch00	Lan2-00	BENP	+	(j)	+			cl17	Clade 83
S.blastmyceticus	Ha3					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl17	Clade 83
S.caeruleus		La-19					Sch00	Lan2-14	BENP cl	+	(c)	+	cl09	+	cl47	
S.hiroshimensis	Ha7		FU-NC			L4	Sch00	Lan2-00	BENP	+	(j)	+			cl12	Clade 84
S.cinnamoneus ssp. cinnamoneus	Ha4					L4	Sch00		BENP	+	(j)	+			cl17	–
S.pseudoechinosporeus							Sch00	Lan2-00	BENP	+		+		+	+	Clade 84
S.lilacinus	Ha8						Sch00	Lan2-00	BENP	+	(j)	+		+	cl12	–
S.sapporonensis	Ha4						Sch17	Lan2-00	BENP	+	(j)	+		+	cl17	Clade 62
S.varsoviensis		La-12		OC–II			Sch00	Lan2-00	BENP cl	+	(c)	+		+	cl13	Clade 62
S.abikoensis	Ha1					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl12	–
S.lavenduligriseus						L4	Sch00	Lan2-00	BENP	+		+		+	cl59	Clade 115
S.morookaensis	Ha13						Sch00	Lan2-00	BENP	+	(j)	+			+	–
S.thioluteus	Ha17						Sch24	Lan2-00	BENP	+	(j)	+		+	cl12	
S.luteireticuli	Ha9					L4										Clade 64
S.ehimensis	Hal						Sch00	Lan2-00	BENP	+	(j)	+		+	cl12	Clade 63
S.hygroscopicus ssp. angustmyceticus							Sch00	Lan2-00	BENP	+		+			cl23	
Group *S.ochraceiscleroticus* et rel.																
S.ochraceiscleroticus				OC–non			Sch00	Lan2-00	BENP +	+		+	cl08	+	+	Clade 74
S.purpurogeneiscleroticus							Sch00	Lan2-00	BENP	+		+		+	+	Clade 74
S.violens							Sch00	Lan2-00	BENP	+		+		+	+	Clade 74
S.monomycini																Clade 73
S.niger							Sch00	Lan2-00	BENP	+		+		+	+	Clade 73
S.olivaceiscleroticus							Sch00	Lan2-00	BENP	+		+			+	Clade 73
Most closely to groups *S.ochraceiscleroticuset* rel. and *S.albofaciens* et rel.																
S.auratus																–
Group *S.albofaciens* et rel.																
S.chrestomyceticus							Sch00	Lan2-00	BENP	+		+		+	cl23	Clade 71

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.rimosus ssp. paromomycinus							Sch00	Lan2-00	BENP	+		+		+	cl23	Clade 71
S.albofaciens							Sch00	Lan2-00	BENP	+		+		+	cl10	Clade 71
Most closely to groups *S.ochraceiscleroticuset* rel. and *S.albofaciens* et rel.																
S.erumpens							Sch21	Lan2-00	BENP	+		+		+	cl23	Clade 72
S.rimosus ssp. rimosus		La-09		OC−non			Sch00	Lan2-00	BENP +	+		+	cl13	+	cl10	Clade 72
S.sclerotialus							Sch00	Lan2-00	BENP	+		+			cl02	–
Group *S.albulus* et rel.																
S.albulus							Sch00	Lan2-00	BENP	+		+		+	+	Clade 67
S.noursei								Lan2-00	BENP	+		+		+	+	Clade 67
S.yunnanensis														+		Clade 67
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel. and *S.albulus* et rel.																
S.kasugaensis							Sch00	Lan2-00	BENP	+		+		+	cl42	–
S.chattanoogensis		La-00		OC−non			Sch00	Lan2-00	BENP +	+		+	cl12	+	cl23	Clade 68
S.lydicus		La-09	FU-21	OC−non			Sch00	Lan2-00	BENP +	+		+	cl12	+	cl23	Clade 68
S.albospinus							Sch00	Lan2-00	BENP	+		+			+	–
S.sioyaensis							Sch00	Lan2-06	BENP	+		+		+	cl58	–
S. hygroscopicus ssp. decoyicus							Sch00	Lan2-00	BENP	+		+		+	cl23	
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel., *S.albulus* et rel. and *S.caniferus* et rel.																
S.catenulae							Sch00	Lan2-00	BENP	+		+		+	cl23	–
S.misakiensis		La-18		OC−non			Sch00	Lan2-00	BENP +	+		+		+	cl29	–
S.ramulosus		La-00		OC−non			Sch00	Lan2-00	BENP +	+		+	cl12	+	cl23	–
Group *S.caniferus* et rel.																
S.hygroscopicus ssp. glebosus							Sch22	Lan2-00	BENP	+		+			cl23	Clade 69
S.libani ssp. rufus							Sch22	Lan2-00	BENP	+		+		+	cl23	Clade 69
S.platensis			FU-21				Sch22	Lan2-00	BENP	+		+		+	cl23	Clade 69
S.caniferus							Sch00	Lan2-00	BENP	+		+			cl23	Clade 21
Most closely to group *S.caniferus* et rel.																
S.libani ssp. libani							Sch00		BENP	+		+			cl23	Clade 70
S.tubercidicus		La-02		OC−non			Sch00	Lan2-00	BENP +	+		+	cl12		cl23	Clade 70
S.nigrescens		La-02					Sch00		BENP +	+		+	cl12	+	cl23	Clade 70
Group *S.albiflaviniger* et rel.																
S.antimycoticus							Sch27		BENP	+		+		+	cl16	Clade 80
S.geldanamycininus														+		Clade 80
S.melanosporofaciens						L1	Sch00	Lan2-00	BENP	+		+		+	cl16	Clade 80
S.sporoclivatus							Sch27		BENP	+		+		+	cl16	Clade 80
S.yatensis														+		Clade 80
S.rutgersensis ssp. castelarensis							Sch00	Lan2-00	BENP	+		+			cl16	
S.indoniensis														+		Clade 78
S.griseiniger														+		Clade 78
S.rhizosphaericus																Clade 78
S.asiaticus																Clade 78
S.cangkringensis														+		Clade 78
S.malaysiensis							Sch00	Lan2-00	BENP	+		+			l15	Clade 79
S.javensis														+		Clade 76
S.endus		La-08				L1	Sch36	Lan2-29	BENP cl	+	(f)	+	cl08	+	cl16	Clade 75
S.sporocinereus							Sch36	Lan2-00	BENP	+		+			cl16	Clade 75
S.hygroscopicus ssp. hygroscopicus		La-08	FU-6			L1	Sch00	Lan2-29	BENP cl	+	(f)	+	cl08		cl16	Clade 75
S.demainii																Clade 75
S.violaceusniger		La-07		OC−I		L1	Sch00	Lan2-00	BENP +	+	(f)	+	cl09		cl15	Clade 76

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.yogyakartensis														+		Clade 76
S.albiflaviniger																Clade 76
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel., *S.albulus* et rel., *S.caniferus* et rel. and *S.albiflaviniger* et rel.																
S.orinoci	Ha15						Sch00	Lan2-00	BENP	+	(j)	+		+	cl15	–
S.mashuensis	Ha11					L4	Sch31		BENP	+	(j)	+			cl11	Clade 66
S.mobaraensis	Ha12		FU-12b			L4	Sch00		BENP	+	(j)	+			cl56	–
S.luteosporeus	Ha10						Sch00	Lan2-00	BENP	+	(j)	+			+	–
S.aureoversilis	Ha7					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl48	Clade 82
S.griseocarneus	Ha6	La-00	FU-12b			L4	Sch00	Lan2-00	BENP +	+	(j) €	+		+	cl17	–
Group *S.albus* et rel.																
S.almquistii							Sch24	Lan2-20	BENP	+		+		+	cl18	Clade 126
S.rangoonensis							Sch24	Lan2-20	BENP	+		+		+	cl18	Clade 126
S.gibsonii							Sch24	Lan2-20	BENP	+		+		+	cl18	Clade 126
S.albus ssp. albus			FU-6	OC–non			Sch24	Lan2-20						+		Clade 126
S.flocculus							Sch24	Lan2-00	BENP	+		+			cl18	Clade 126
Most closely to group *S.albus* et rel.																
S.cacaoi ssp. cacaoi		La-05					Sch00	Lan2-17	BENP cl	+	(c)	+	cl08	+	cl36	–
S.sulphureus		La-00		OC–non			Sch00		BENP +	+		+		+	–	–
S.rubidus																Clade 122
S.yeochonensis																Clade 122
S.albus ssp. pathocidicus							Sch00	Lan2-00	BENP	+		+			cl07	Clade 120
S.glauciniger																–
S.guanduensis																–
Most closely to groups *S.albus* et rel. and *S.glaucosporus* et rel.																
S.ferralitis														+		–
S.vitaminophilus							Sch00	Lan2-00	BENP	+		+		+	+	–
S.thermolineatus							Sch00	Lan2-00	BENP	+		+		+	+	–
S.yanglinensis																Clade 121
S.paucisporeus																Clade 121
Group *S.glaucosporus* et rel.																
S.macrosporus							Sch00	Lan2-00						+		Clade 127
S.megasporus							Sch00	Lan2-00	BENP	+		+			–	Clade 127
S.glaucosporus							Sch00	Lan2-00	BENP	+		+			cl44	Clade 127
S.radiopugnans																Clade 127
Most closely to group *S.glaucosporus* et rel.																
S.albiaxialis														+		–
S.armeniacus							Sch00	Lan2-00	BENP	+		+			+	Clade 124
Most closely to groups *S.albus* et rel. and *S.glaucosporus* et rel.																
S.cuspidosporus							Sch08	Lan2-00	BENP	+		+		+	cl56	Clade 81
S.sparsogenes		La-07				L1	Sch00		BENP +	+		+			–	Clade 81
Most closely to group *S.geysiriensis* et rel.																
S.janthinus						L2	Sch00		BENP	+		+			cl01	Clade 106
S.roseoviolaceus			FU-1			L2	Sch00		BENP	+		+			cl01	Clade 106
S.violaceus				OC–III			Sch00	Lan2-00	BENP	+		+		+	cl01	Clade 106
S.albosporeus ssp. albosporeus		La-01					Sch00	Lan2-00	BENP cl	+	(c)	+	cl05	+	cl24	
S.arenae						L2	Sch00	Lan2-00	BENP	+		+		+	cl01	Clade 93
S.luteogriseus						L2	Sch25	Lan2-00	BENP	+		+		+	cl01	–
S.hawaiiensis						L2	Sch34	Lan2-00	BENP	+		+			cl01	Clade 107
S.cellulosae		La-15		OC–non			Sch00	Lan2-00	BENP +	+		+	cl17		cl04	–
S.pseudogriseolus					KA-G		Sch13	Lan2-00						+		Clade 86

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.gancidicus							Sch13	Lan2-00	BENP	+		+		+	cl04	Clade 86
S.rubiginosus							Sch13	Lan2-00	BENP	+		+		+	cl04	Clade 86
S.capillispiralis							Sch00	Lan2-00	BENP	+		+		+	+	Clade 86
S.lavendulocolor						L5	Sch00	Lan2-00	BENP	+		+		+	cl12	Clade 48
S.azureus			FU-1			L2	Sch00	Lan2-00	BENP	+		+		+	cl01	Clade 88
S.flavoviridis							Sch00		BENP	+		+		+	cl35	Clade 111
S.pilosus							Sch00		BENP	+		+		+	cl35	Clade 111
S.djakartensis							Sch00	Lan2-00	BENP	+		+		+	cl04	–
Group *S.geysiriensis* et rel.																
S.ghanaensis							Sch00		BENP	+		+		+	+	
S.minutiscleroticus					KA-G		Sch00	Lan2-03	BENP	+	(a)	+			cl46	Clade 87
S.geysiriensis							Sch14	Lan2-00	BENP	+		+			cl39	Clade 119
S.plicatus					A-E		Sch14	Lan2-00	BENP	+		+		+	cl39	Clade 119
S.rochei		La.13		OC–III	A-E		Sch00	Lan2-00	BENP +	+		+	cl17	+	cl39	Clade 119
S.vinaceusdrappus					A-E		Sch14	Lan2-00	BENP	+		+			cl39	Clade 119
S.mutabilis					A-E		Sch00	Lan2-00	BENP	+		+		+	+	Clade 119
Most closely to group *S.geysiriensis* et rel.																
S.tuirus							Sch00	Lan2-00	BENP	+		+			cl01	–
S.afghaniensis			FU-1			L2	Sch00	Lan2-00	BENP	+		+		+	cl01	Clade 89
S.africanus														+		Clade 89
Group *S.brasiliensis* et rel.																
S.roseiscleroticus							Sch29	Lan2-01	BENP	+		+			cl38	Clade 92
S.ruber							Sch29	Lan2-01	BENP	+		+			cl38	Clade 92
S.spiralis							Sch00	Lan2-00	BENP	+		+		+	+	–
S.fumigatiscleroticus							Sch00	Lan2-00	BENP	+		+		+	+	Clade 91
S.poonensis		La-04		OC–III			Sch00	Lan2-00	BENP +	+		+	cl14	+	+	Clade 91
S.brasiliensis							Sch38	Lan2-00	BENP	+		+		+	+	–
Group *S.atrovirens* et rel.																
S.atrovirens							Sch00	Lan2-00	BENP	+		+		+	+	–
S.caelestis						L2	Sch00	Lan2-00	BENP	+		+		+	+	Clade 88
S.fumanus							Sch00	Lan2-00	BENP	+		+			cl12	–
S.fimbriatus							Sch00	Lan2-00	BENP	+		+		+	+	–
Group *S.glaucus* et rel.																
S.griseostramineus							Sch00	Lan2-10	BENP	+		+		+	cl04	Clade 104
S.griseomycini							Sch00	Lan2-10	BENP	+		+		+	cl04	Clade 104
S.graminearus							Sch00	Lan2-10	BENP	+		+		+	cl04	Clade 12
S.viridiviolaceus							Sch00	Lan2-00	BENP +	+		+			–	
S.glaucus							Sch00	Lan2-00	BENP	+		+		+	+	
Group *S.aureorectus* et rel.																
S.aureorectus							Sch19	Lan2-08	BENP	+		+			cl40	Clade 118
S.virens							Sch00	Lan2-08	BENP	+		+			cl40	Clade 118
S.asterosporus							Sch00	Lan2-08	BENP	+		+			cl40	Clade 118
S.calvus							Sch19	Lan2-08	BENP	+		+		+	cl40	Clade 118
Most closely to groups S. *geysiriensis* et rel., S.*brasiliensis* et rel., S.*atrovirens* et rel., S.*glaucus* et rel. and S.*aureorectus* et rel.																
S.naganishii							Sch00	Lan2-00	BENP	+		+		+	+	–
S.prasinosporus		L.10		OC–III			Sch25	Lan2-00	BENP +	+		+	cl10	+	cl54	–
S.anandii							Sch00	Lan2-00	BENP	+		+		+	cl08	–
S.carpinensis							Sch00	Lan2-00	BENP	+		+		+	+	–
S.levis							Sch00	Lan2-00	BENP	+		+			cl01	–
S.cinerochromogenes							Sch00	Lan2-00	BENP	+		+		+	cl57	–
S.koyangensis																Clade 112
S.violarus						L2	Sch00		BENP	+		+			cl01	Clade 93

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
Not closely related to one of the groups																
S.daghestanicus						L3	Sch40	Lan2-11	BENP	+		+			cl37	
S.limosus			FU-1		KA-D		Sch00	Lan2-22	BENP	+	(k)	+		+	cl08	Clade 112
S.canescens					KA-D		Sch10	Lan2-22	BENP	+	(k)	+			cl08	Clade 112
S.felleus					KA-D		Sch00	Lan2-22	BENP	+	(k)	+			cl08	Clade 112
S.griseus ssp. solvifaciens							Sch10	Lan2-00	BENP	+		+			cl08	Clade 112
S.violascens							Sch00	Lan2-00	BENP	+		+		+	cl23	
S.hydrogenans							Sch00	Lan2-00	BENP	+		+		+	–	
S.odorifer					KA-D		Sch00		BENP	+	(k)	+			cl08	Clade 112
S.albidoflavus		La-00	FU-1	OC–non	KA-D		Sch10		BENP +	+	(e)(k)	+	cl06		cl08	Clade 112
S.champavatii							Sch00		BENP	+		+			cl08	Clade 112
S.sampsonii					KA-D		Sch00	Lan2-22	BENP	+	(k)	+		+	cl08	Clade 112
S.diastaticus ssp. diastaticus		La-00	FU-1	OC–non			Sch00		BENP +	+		+		+	cl09	Clade 113
S.gougerotii					KA-D		Sch00		BENP	+	(k)	+		+	cl09	Clade 113
S.rutgersensis ssp.rutgersensis					KA-D		Sch00		BENP	+	(k)	+		+	cl09	Clade 113
S.intermedius		La-03			KA-D		Sch10	Lan2-00	BENP +	+		+	cl14		cl08	Clade 113
S.indiaensis							Sch00	Lan2-00	BENP	+		+		+	cl04	–
S.thermocarboxydus							Sch00							+		Clade 109
S.massasporeus		La-12		OC–III			Sch00	Lan2-00	BENP +	+		+	cl17	+	cl01	Clade 107
S.misionensis							Sch00	Lan2-15	BENP	+		+		+	cl54	Clade 97
S.phaeoluteichromatogenes																Clade 97
S.spectabilis						L3	Sch00	Lan2-00	BENP	+		+		+	+	–
S.cinereospinus							Sch00	Lan2-00	BENP	+		+		+	cl22	
S.coeruleofuscus						L2	Sch00	Lan2-00	BENP	+		+			cl01	Clade 114
S.chromofuscus		La-06		OC–III			Sch00	Lan2-00	BENP +	+		+	cl10	+	cl09	Clade 114
S.scopiformis														+		
S.spinoverrucosus							Sch00	Lan2-00	BENP	+		+		+	+	–
Most closely to group *S.mexicanus* et rel.																
S.thermospinosisporus																–
S.thermodiastaticus							Sch00	Lan2-00	BENP	+		+		+	cl04	–
S.thermocarboxydovorans							Sch00		BENP	+		+			cl04	–
S.thermoviolaceus ssp. apingens							Sch00		BENP	+		+		+	cl03	
S.thermoviolaceus ssp. thermoviolaceus		La-13					Sch00		BENP +	+		+	cl11	+	cl03	–
S.nodosus							Sch00	Lan2-00	BENP +	+		+	cl11	+	cl04	Clade 117
S.viridosporus							Sch00		BENP	+		+			+	–
Group *S.mexicanus* et rel.																
S.thermogriseus							Sch00	Lan2-07	BENP	+		+			cl32	Clade 105
S.thermovulgaris		La-00		OC–non			Sch00	Lan2-07	BENP +	+		+	cl10	+	cl32	Clade 105
S.thermoalcalitolerans							Sch00	Lan2-00	BENP	+		+			+	Clade 105
S.mexicanus																–
S.thermocoprophilus							Sch00	Lan2-00	BENP	+		+		+	+	–
Most closely to group *S.mexicanus* et rel.																
S.bangladeshensis																Clade 116
S.rameus							Sch33		BENP	+		+		+	cl02	Clade 116
S.griseosporeus							Sch00	Lan2-00	BENP	+		+		+	+	–
S.achromogenes ssp. rubradiris							Sch00	Lan2-00	BENP	+		+		+	+	Clade 117
S.glomeratus								Lan2-00	BENP	+		+		+	cl09	Clade 116
S.eurythermus							Sch00	Lan2-00	BENP	+		+			cl08	Clade 115
S.nogalater		La-14		OC–III			Sch00	Lan2-00	BENP +	+		+	cl14	+	cl04	–
S.fragilis				OC–III			Sch38	Lan2-00	BENP	+		+		+	+	–
Group *S.erythrogriseus* et rel.																
S.erythrogriseus		La-15					Sch35	Lan2-25	BENP +	+		+	cl17	+	cl01	Clade 100
S.labedae							Sch35	Lan2-25	BENP	+		+		+	cl01	Clade 100
S.griseoincarnatus		La-15					Sch35	Lan2-25	BENP +	+		+	cl17	+	cl01	Clade 100

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.variabilis					KA-F		Sch35	Lan2-25	BENP	+		+			cl01	Clade 100
Most closely to group *S.erythrogriseus* et rel.																
S.althioticus							Sch00	Lan2-00	BENP	+		+			+	Clade 99
S.matensis			FU-1				Sch00	Lan2-00	BENP	+		+		+	cl01	Clade 100
S.griseorubens					KA-F		Sch30	Lan2-00	BENP	+		+			cl01	Clade 100
S.viridochromogenes				OC−III		L2	Sch00	Lan2-00	BENP	+		+		+	cl54	–
S.iakyrus						L2	Sch00	Lan2-00	BENP	+		+		+	cl01	Clade 96
S.violaceochromogenes									BENP	+		+		+	cl01	Clade 96
S.collinus			FU-1			L2	Sch39	Lan2-00	BENP	+		+		+	+	–
S.malachitofuscus							Sch00	Lan2-00	BENP +	+		+		+	cl04	–
S.paradoxus							Sch00	Lan2-00	BENP	+		+		+	cl01	–
S.griseoflavus		La-04		OC−non			Sch00	Lan2-00	BENP +	+		+			cl01	
S.flaveolus		La-12		OC−III			Sch00	Lan2-00	BENP +	+		+	cl17	+	cl59	–
S.glaucescens		La-16		OC−III		L2	Sch00	Lan2-00	BENP +	+		+		+	cl57	Clade 98
S.pharetrae																Clade 98
S.malachitospinus																–
S.parvulus		La-24					Sch00	Lan2-00	BENP +	+		+	cl17		cl06	–
S.tendae		La-14			KA-E		Sch00	Lan2-00	BENP +	+		+	+	+	cl06	Clade 103
S.violaceorubidus							Sch00	Lan2-00	BENP	+		+			cl08	Clade 103
S.albaduncus							Sch00	Lan2-00	BENP	+		+		+	cl05	Clade 101
S.griseoloalbus							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 101
S.heliomycini							Sch00	Lan2-00	BENP	+		+			cl05	–
S.ambofaciens			FU-6				Sch00	Lan2-00	BENP	+		+		+	+	–
Most closely to group *S. coelescens* et rel.																
S.rubrogriseus							Sch00	Lan2-00	BENP	+		+			cl22	Clade 103
S.tricolor							Sch33	Lan2-21	BENP	+	(b)	+			cl02	Clade 116
S.lienomycini							Sch00	Lan2-00	BENP	+		+		+	cl22	Clade 103
S.anthocyanicus							Sch00	Lan2-04	BENP	+		+		+	cl22	Clade 103
S.olivaceus		La-23	FU-1				Sch00	Lan2-00	BENP +	+		+	cl16	+	cl13	–
S.pactum		La-11		OC−II			Sch00	Lan2-00	BENP +	+		+			+	–
Group *S.coelescens* et rel.																
S.coelescens							Sch07		BENP	+	(k)	+		+	cl22	Clade 103
S.humiferus							Sch08		BENP	+	(k)	+			cl22	Clade 103
S.violaceolatus							Sch08		BENP	+	(k)	+		+	cl22	Clade 103
S.violaceoruber							Sch07		BENP	+	(d)(k)	+		+	cl22	Clade 103
Most closely to group *S.coelescens* et rel.																
S.coelicoflavus							Sch00	Lan2-00	BENP	+		+			cl23	–
S.diastaticus ssp. ardesiacus									BENP	+		+		+	+	
Most closely to group *S.coeruleorubidus* et rel.																
S.lomondensis							Sch00	Lan2-00	BENP	+		+			cl04	–
S.lusitanus							Sch00	Lan2-00	BENP	+		+		+	–	Clade 109
S.purpurascens						L2	Sch00	Lan2-00	BENP	+	(g)	+		+	cl01	–
S.bellus		La1-02				L2	Sch00		BENP +	+		+	cl17	+	cl01	Clade 108
S.coerulescens						L2	Sch00	Lan2-00	BENP	+		+		+	cl01	Clade 114
S.speibonae														+		–
S.longispororuber					KA-F		Sch00	Lan2-00	BENP	+		+			cl01	–
Group *S.coeruleorubidus* et rel.																
S.albogriseolus					KA-F		Sch00	Lan2-00	BENP	+		+		+	+	Clade 110
S.viridodiastaticus							Sch00	Lan2-00	BENP	+		+			+	Clade 110
S.coeruleorubidus						L2	Sch00		BENP	+	(g)	+			cl01	Clade 108
Group *S.aurantiogriseus* et rel.																
S.coelicolor			FU-1		KA-D		Sch09	Lan2-00	BENP	+	(d)(k)	+		+	cl08	Clade 112
S.griseoviridis		La-06		OC−III		L3	Sch40	Lan2-11	BENP +	+	(h)	+		+	cl37	Clade 90
S.aurantiogriseus																–
Most closely to group *S.aurantiogriseus* et rel.																

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.griseoaurantiacus							Sch00	Lan2-00	BENP	+		+		+	+	Clade 94
S.jietajiensis																Clade 94
Group *S.coeruleoprunus* et rel.																
S.coeruleoprunus							Sch00	Lan2-00	BENP	+		+		+	cl33	Clade 85
S.somaliensis							Sch00	Lan2-00						+		Clade 85
S.fradiae		La-00		OC–I			Sch12	Lan2-27	BENP +	+	(b)	+	cl09	+	cl45	Clade 85
Most closely to group *S.coeruleoprunus* et rel.																
S.bluensis						L2	Sch00	Lan2-00	BENP	+		+		+	cl07	–
Not closely related to one of the groups																
S.variegatus							Sch00	Lan2-00	BENP	+		+		+	+	–
S.fulvissimus		La-00		OC–IV		L3	Sch00	Lan2-00	BENP +	+	(h)	+	cl14		cl43	Clade 95
S.aureoverticillatus						L3	Sch00	Lan2-00	BENP	+		+		+	+	–
S.flavofungini							Sch00	Lan2-00	BENP	+		+			+	Clade 95
S.alboflavus		La-00		OC–IV			Sch00	Lan2-00	BENP +	+		+			cl43	Clade 95
S.aculeolatus							Sch00	Lan2-00	BENP	+		+			+	Clade 123
S.synnematoformans																Clade 123
S.hebeiensis														+		Clade 120
Group *S.carpaticus* et rel.																
S.hainanensis																Clade 129
S.specialis																Clade 129
S.carpaticus							Sch00	Lan2-00	BENP	+		+			+	Clade 128
S.cheonanensis																Clade 128
Most closely to group *S.carpaticus* et rel.																
S.sulfonofaciens							Sch00	Lan2-00	BENP	+		+		+	+	–
S.sodiiphilus																–
Not closely related to one of the groups																
S.scabrisporus																Clade 130
S.gardneri			FU-23		KA-C		Sch00	Lan2-00	BENP	+		+		+	cl44	Clade 40
S.flavidofuscus							Sch00	Lan2-00	BENP	+		+		+	–	
Regarded as later heterotypic synonym of *Streptomyces abikoensis* (for references, see list of type strains)																
S.luteoverticillatus	Ha1		FU-12b				Sch00	Lan2-00	BENP	+	(j)	+		+	cl12	Clade 63
S.olivoreticuli ssp. olivoreticuli	Ha1						Sch00	Lan2-00	BENP	+	(j)	+		+	cl12	
S.parvisporogenes	Ha1						Sch00	Lan2-00	BENP	+	(j)	+			cl12	–
Regarded as later heterotypic synonym of *Streptomyces anulatus* (for references, see list of type strains)																
S.chrysomallus ssp. chrysomallus			FU-22		KA-B		Sch01	Lan2-18	BENP	+	(a)	+			cl23	–
S.citreofluorescens			FU-19b		KA-B		Sch01	Lan2-18	BENP	+	(a)	+		+	cl23	
S.fluorescens					KA-B		Sch01	Lab2-18	BENP	+	(a)	+		+	cl23	–
Regarded as later heterotypic synonym of *Streptomyces avermitilis* (for references, see list of type strains)																
S.avermectinius																
Regarded as later heterotypic synonym of *Streptomyces cacaoi* (for references, see list of type strains)																
S.aminophilus		La-05					Sch00	Lan2-17	BENP cl	+	(c)	+	cl08	+	cl36	
Regarded as later heterotypic synonym of *Streptomyces caeruleus* (for references, see list of type strains)																

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.niveus		La-19					Sch11	Lan2-14	BENP cl	+	(c)	+	cl09	+	cl47	Clade 27
S.spheroides		La-19					Sch11	Lan2-00	BENP cl	+	(c)	+	cl09		cl47	Clade 27
Regarded as later heterotypic synonym of *Streptomyces cinnamoneus* (for references, see list of type strains)																
S.griseoverticillatus	Ha4						Sch17	Lan2-00	BENP	+	(j)	+		+	cl17	Clade 62
S.hachijoensis	Ha4		FU-NC			L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl13	–
Regarded as later heterotypic synonym of *Streptomyces chibaensis* (for references, see list of type strains)																
S.chibaensis							Sch04	Lan2-26	BENP	+	(a)	+		+	cl52	Clade 2
Regarded as later heterotypic synonym of *Streptomyces filamentosus* (for references, see list of type strains)																
S.roseosporus							Sch00	Lan2-24	BENP	+	(b)	+			cl23	Clade 43
Regarded as later heterotypic synonym of *Streptomyces flavofuscus* (for references, see list of type strains)																
S.globisporus ssp. flavofuscus														+		–
Regarded as later heterotypic synonym of *Streptomyces flavovirens* (for references, see list of type strains)																
S.nigrifaciens					KA-B		Sch05	Lan2-09	BENP	+	(a)	+		+	cl53	Clade 37
Regarded as later heterotypic synonym of *Streptomyces fradiae* (for references, see list of type strains)																
S.roseoflavus							Sch12	Lan2-27	BENP	+	(b)	+			cl45	Clade 85
Regarded as later heterotypic synonym of *Streptomyces griseocarneus* (for references, see list of type strains)																
S.septatus	Ha6						Sch00	Lan2-00	BENP	+	(j)	+			+	–
Regarded as later heterotypic synonym of *Streptomyces griseus* (for references, see list of type strains)																
S.setonii					KA-B		Sch00	Lan2-19	BENP	+		+		+	cl23	–
Regarded as later heterotypic synonym of *Streptomyces hiroshimensis* (for references, see list of type strains)																
S.rectiverticillatus	Ha7						Sch00	Lan2-00	BENP	+	(j)	+		+	cl48	Clade 82
S.roseoverticillatus	Ha7					L4	Sch00	Lan2-00	BENP	+	(j)	+			cl13	Clade 65
S.salmonis	Ha7					L4	Sch00	Lan2-00	BENP	+	(j)	+		+	cl13	Clade 65
S.spitsbergensis	Ha7													+		Clade 50
S.fervens ssp. fervens						L4	Sch00									
Regarded as later heterotypic synonym of *Streptomyces lilacinus* (for references, see list of type strains)																
S.kashmirensis	Ha8						Sch00	Lan2-00	BENP	+	(j)	+			cl12	–
Regarded as later heterotypic synonym of *Streptomyces mashuensis* (for references, see list of type strains)																
S.kishiwadensis	Hal1						Sch31		BENP	+	(j)	+		+	cl11	Clade 66

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
Regarded as later heterotypic synonym of *Streptomyces microflavus* (for references, see list of type strains)																
S.griseus ssp. alpha							Sch02	Lan2-12	BENP	+	(a)	+			cl23	–
S.griseus ssp. cretosus							Sch02	Lan2-12						+		–
S.willmorei					KA-B		Sch02	Lan2-12	BENP	+	(a)	+		+	cl23	–
Regarded as later heterotypic synonym of *Streptomyces minutiscleroticus* (for references, see list of type strains)																
S.flaviscleroticus					KA-G		Sch00	Lan2-03	BENP	+	(a)	+		+	cl46	Clade 87
Regarded as later heterotypic synonym of *Streptomyces mobaraensis* (for references, see list of type strains)																
S.ladakanum	Ha12					L4										
Regarded as later heterotypic synonym of *Streptomyces netropsis* (for references, see list of type strains)																
S.distallicus	Hal4					L4		Lan2-13	BENP	+	(b)(j)	+		+	cl13	Clade 52
S.flavopersicus	Ha14					L4	Sch00							+		Clade 52
S.kentuckensis	Hal4					L4	Sch00							+		Clade 52
S.syringium	Ha14						Sch00	Lan2-00	BENP	+	(j)	+		+	cl13	Clade 52
Regarded as later heterotypic synonym of *Streptomyces phaeopurpureus* (for references, see list of type strains)																
S.phaeoviridis							Sch23	Lan2-02	BENP	+	(b)	+		+	cl51	Clade 129
Regarded as later heterotypic synonym of *Stre ptomyces thermovulgaris* (for references, see list of type strains)																
S.thermonitrificans														+		Clade 105
Regarded as later heterotypic synonym of *Streptomyces tricolor* (for references, see list of type strains)																
S.roseodiastaticus							Sch33	Lan2-21	BENP	+	(b)	+			cl02	Clade 116
Regarded as later heterotypic synonym of *Streptomyces olioverticillatus* (for references, see list of type strains)																
S.viridiflavus									BENP	+	(j)	+			cl13	Clade 49
Regarded as later heterotypic synonym of *Streptomyces violaceus* (for references, see list of type strains)																
S.violatus		La-12					Sch00		BENP cl	+	(c)	+	cl14	+	cl01	
No detailed sequence information available																
S.caviscabies																–
***S.coeruleoflavus* (keine Seq. gefunden)**																
S.arabicus							Sch00	Lan2-23	BENP	+	(b)	+		+	cl08	Clade 100
S.baldaccii	Ha7		FU-12b			L4								+		Clade 50
S.cellulolyticus														+		
S.echinoruber							Sch00							+		
S.erythraeus								Lan2-00	BENP	+		+		+	+	
S.longisporoflavus		La-00		OC–non			Sch00	Lan2-00	BENP +	+		+	cl06	+	cl19	Clade 26
S.olivomycini																
S.speleomycini																

Table 42.2 (continued)

Species names and groups[1]	Hat 03[6]	Lan 02[7]	Ful 95[8]	Och 95[9]	Kat 97[10]	Lab[11]	Sch[12]	Lan 04[13]	Lan 02[14]	Lan 04[15]	Lan 04[16]	Lan 04[17]	Lan 04[18]	Lan 04[19]	Lan 04[20]	LAB Clade[21]
S.thermoautotrophicus														+		
Not in tree																
S. aldersoniae																Clade 81
S. alni																–
S. angustmycinicus																–
S. ascomycinicus																–
S. atriruber																–
S. avicenniae																Clade 129
S. axinellae																–
S. baliensis																–
S. castelarensis																Clade 80
S. deccanensis																Clade 25
S. decoyininicus																–
S. gulbargensis																–
S. haliclonae																Clade 125
S. himastatinicus																–
S. hypolithicus																–
S. iranensis																Clade 77
S. lunalinharesii																Clade 67
S. marinus																Clade 125
S. marokkonensis																Clade 102
S. mayteni																Clade 129
S. milbemycinicus																–
S. modarskii																Clade 80
S. nanshensis																–
S. osmaniensis																–
S. plumbiresistens																–
S. polyantibioticus																–
S. rapamycinicus																Clade 77
S. ruanii																Clade 75
S. sedi																Clade 129
S. silaceus																–
S. tateyamensis																–
S. thinghirensis																Clade 102
S. tritolerans																Clade 103
S. wellingtoniae																–
S. xiamenensis																Clade 128
S. xinghaiensis																–
K. kazusensis																Clade 58
K. saccharophila																–

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08[23] MLSA	Rong Huan g 10[25] 16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12[29]	Morphological characters[30]				Physiological tests[31]										
													I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Most closely to group *S.costaricanus* et rel.																											
S.galbus													Gy	S	C+	SM	+	+	+	–	+	n	–	+	+	n	–
S.longwoodensis													Gy	S	C–	SM	+	+	+	–	+	+	±	+	+	+	–
S.bungoensis													Gy	S	C+	SPY	+	+	+	–	+	+	n	+	–	–	n
S.corchorusii	+												Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	+
S.canarius													Y	S	C–	SM	+	+	+	+	+	+	+	+	+	–	+
S.olivaceoviridis	+												Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	+
S.capoamus													R	RF	C+	SM	+	+	+	–	+	+	n	+	+	+	+
S.regensis													Gy	S	C+	n	+	+	+	–	+	–	n	+	+	n	+

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S *r*RNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S.griseochromogenes	+												Gy	S	C+	SPY	+	+	+	–	+	+	+	+	+	–	+
S.cellostaticus													Gy/ R	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
S.yokosukanensis													R	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
S.antibioticus	Group A18									Str	Str		Gy	RF	C+	SM	+	+	+	+	+	+	–	+	+	–	–
S.griseoruber													Gy	S	C+	SM	+	+	+	+	+	+	–	–	+	+	–
S.cinnabarinus													R	RF	C+	SM	+	+	+	+	+	n	+	+	+	n	+
S.acidiscabies												Clade-5	R	RF	C–	SM	+	+	+	+	+	n	–	+	n	n	+
S.alanosinicus												+	Gy	S	C+	SPY	+	+	+	–	+	+	+	+	+	+	n
Group *S.costaricanus* et rel.																											
S.griseofuscus													Gy	S	C–	SM	+	+	+	–	+	n	–	+	–	n	–
S.murinus													Gy	S	C–	SM	+	+	–	–	+	n	–	+	n	n	–
S.costaricanus													Gy	S	C–	SM	+	+	–	–	+	+	–	n	n	+	–
S.phaeogriseichromatogenes																											
Most closely to group *S.costaricanus* et rel.																											
S.lanatus													B	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
S.durhamensis													Gy	S	C+	SPY	+	+	+	–	+	+	+	+	+	–	n
S.filipinensis													Gy	S	C+	SPY	+	+	+	–	+	+	+	+	+	–	+
S.puniciscabiei												Clade-7		RF	C +/ C–	SPY	+	+	+	+	+	n	+	+	+		+
S.niveiscabiei												Clade-5	W/ Gy	RF	C–	SM	+	+	+	+	+	n	+	+	+	n	+
S.echinatus													Gy	S	C+	SPY	+	+	+	+	+	+	+	+	+	–	–
S.longisporus													W	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
S.avermitilis										Str	Str		GY	S	C+	SM	+	+	+	+	+	n	n	n	+	n	–
S.kunmingensis													W	S	C–	n	n	+	+	+	n	n	+	+	–	n	–
S.mirabilis													Gy	S	C+	SM	+	+	+	+	n	n	n	n	n	n	n
S.olivochromogenes	+												Gy	S	C+	SM	+	+	+	–	+	+	+	+	+	+	n
Most closely to group *S.cyanoalbus* et rel.																											
S.lucensis													Gy	S	C+	SPY	+	+	+	–	+	n	–	+	–	n	+
S.niveoruber													R	S	C–	SM	+	+	+	+	n	n	n	n	n	n	n
S.achromogenes ssp. achromogenes	+												Gy	RF	C+	SM	+	+	+	+	+	+	–	+	+	+	–
S.griseorubiginosus				+	+							Clade-4	Gy	RF	C+	SM	+	+	+	+	+	n	+	+	+	n	+
S.phaeopurpureus													Gy	RF	C+	SM	+	+	+	+	+	+	+	+	+	–	n
S.curacoi													B	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
S.lincolnensis	+												R	RF	C+	SM	+	+	+	+	+	+	+	+	+	+	+
S.cyaneus	+	24	+	+	+							+	B	S	C+	SPY	+	+	+	+	n	n	n	n	n	n	n
Group *S.cyanoalbus* et rel.																											
S.cyanoalbus													Gy	S	C–	H	+	+	+	+	+	+	+	+	–	–	+
S.hirsutus													G	S	C–	SPY	+	+	+	+	+	n	+	+	+	n	+
S.prasinus													G	S	C–	SPY	+	+	+	+	+	+	–	+	+	–	+
S.bambergiensis	+												G	S	C–	H	n	n	n	n	n	n	n	n	n	n	n
S.emeiensis													Gy	RF	C–	SPY	+	+	+	+	+	+	+	n	+	n	+
S.prasinopilosus													G	S	C–	H	+	+	+	+	+	+	–	+	+	–	n
Most closely to group *S.cyanoalbus* et rel.																											
S.flavovariabilis													R	S	C+	SPY	+	+	+	+	+	+	+	+	n	n	+
S.aureocirculatus													W	RF	C–	SM	+	n	–	–	+	+	–	+	+	n	–

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25]16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27]16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28]5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]									
S.novaecaesareae													n	n	C–	n	+	+	+	+	+	n	+	+	+	n	+
S.prunicolor													R	RF	C–	SM	+	+	+	+	+	n	+	+	+	n	n
S.phaeoluteigriseus																											
S.bobili		+	+	+	+							+	W	S	C+	SM	+	+	+	+	+	+	+	–	+	–	+
S.galilaeus	Asn(AAC) 442			XII	XII	I	III						Gy	S	C+	SM	+	+	+	+	n	n	n	n	n	n	n
Most closely to groups *S.cyanoalbus* et rel. and *S.griseoluteus* et rel.																											
S.chartreusis													B	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
S.resistomycificus													Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
Most closely to group *S.griseoluteus* et rel.																											
S.griseoluteus													Gy	RF	C+	SM	+	+	+	–	+	n	–	+	–	n	–
S.recifensis										Kit	Kit		Gy	S	C–	SM	+	+	+	–	+	+	+	+	–	+	+
S.seoulensis													Gy	RF	C–	SM	+		–		+		+	+	–	+	+
Most closely to groups *S.cyanoalbus* et rel. and *S.griseoluteus* et rel.																											
S.canus													Gy	S	C–	SPY	+	+	+	+	+	+	±	+	+	n	+
S.ciscaucasicus										Str	Str		Gy	S	C–	SPY	+	+	+	+	+	n	+	n	n	n	n
S.pseudovenezuelae																											
S.alboniger	+												W	RF	C–	SM	+	+	+	–	+	+	–	+	+	+	–
Most closely to group *S.scabiei* et rel.																											
S.bottropensis													Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
S.stelliscabiei												Clade-1	Gy	S	C+		+	+	+	+	+	n	+	+	+	n	+
S.europaeiscabiei												Clade-3	Gy	S	C+	n	+	+	+	+	+	n	+	+	+	n	+
S.scabiei										Str	Str	Clade-2	Gy	RF	C +/ C–	SM	+	+	+	+	+	n	–	+	–	n	–
S.diastatochromogenes												+	Gy	S/ RA	C+	SM	+	+	+	+	+	n	+	+	+	n	+
S.hygroscopicus ssp. ossamyceticus								XIII	XIII			+	Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	–	+
S.ipomoeae								XIII	XIII			Clade-7	B	S	C–	SPY	+	+	+	+	+	n	+	+	+	n	+
S.torulosus								XIII	XIII			+	Gy	S	C+	WTY	+	+	+	+	+	+	+	+	+	–	n
S.neyagawaensis								XIII	XIII			+	Gy	S	C+	SM	+	+	+	–	+	+	+	+	+	+	+
Most closely to group *S.scabiei* et rel.																											
S.reticuliscabiei												Clade-6	Gy	RF	C–		+	+		+	+		+	+	+		+
S.turgidiscabies												Clade-6	Gy	RF	C–	SM	+	+	+	+	+	n	+	+	+	n	+
S.cacaoi ssp. asoensis													Gy	RF	C+	SM	+	+	+	n	+	+	+	n	+	n	+
S.humidus													Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	+	–
S.rishiriensis													Gy	S	C+	SM	+	+	+	+	+	+	+	–	+	+	+
S.cinereoruber ssp. fructofermentans	+											+	Gy	RF	C+	SM	+	+	+	+	+	+	–	–	–	+	n
S.phaeofaciens								XIII	XIII				Gy	S	C+	SM	+	+	+	+	n	n	n	n	n	n	n
S.puniceus				VI	VI								Y	RF	C–	SM	+	+	+	–	+	+	–	+	–	+	±
Group *S.aurantiacus* et rel.																											
S.aurantiacus												+	R	S	C–	SM	+	±	+	+	+	+	±	+	+	n	±

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S.glomeroaurantiacus													R	S	C–	SM	+	+	–	–	+	+	–	+	+	–	–
S.tauricus												+	R	S	C–	SM	n	+	+	+	+	n	+	–	n	n	n
S.ederensis												+	Gy	RF	C+	SM	n	n	n	n	n	n	n	n	n	n	n
S.phaeochromogenes	+											+	R	RF	C+	SM	+	+	+	+	+	+	+	+	+	+	n
S.umbrinus												+	R	RF	C+	SM	+	+	+	+	+	+	+	+	+	–	n
S.rectiviolaceus													V	RF	C–	SM	+	+	+	+	+	+	+	+	+	n	+
Group *S.aureus* et rel.																											
S.kanamyceticus		+	+	+	+							+	Y	RF	C–	SM	+	+	+	–	+	+	+	+	–	+	n
S.durmitorensis													Y	RF	C–	SM	+	+	–	–	+	+	+	+	–	n	+
S.aureus		+	+	+	+							+	Gy	S	C+	SM	n	n	n	n	n	n	n	+	+	n	n
Group *S.cinereus* et rel.																											
S.cinereus													W	RF	C–	SM	+	+	+	+	+	+	+	+	+	+	+
S.flaveus													Gy	RF	C–	SM	+	+	+	+	+	+	+	+	+	+	+
S.vastus													Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	+
Most closely to group *S.cinereus* et rel.																											
S.laceyi		+	+	+	+								Gy/ Y/R	S		SM							+	–			
Group *S.argenteolus* et rel.																											
S.griseolus	+	+	+	VIII	VIII								Gy	RF	C–	SM	+	+	+	–	+	+	+	–	–	–	n
S.halstedii				X	X							+	Gy	RF	C–	SM	+	+	+	–	+	n	–	–	–	n	–
S.argenteolus	+	+	+	XII	XII	+	+					+	Gy	S	C–	SM	+	+	+	+	+	+	–	+	–	+	–
S.cinereorectus				+	+							+	Gy	RF	C–	SM	+	–	n	–	+	n	+	+	–	n	n
S.flavovirens				VIII	VIII								Gy	RF	C–	SM	+	+	+	+	+	+	+	+	–	–	–
S.flavogriseus		+	+	VIII	VIII					Str	Str		Gy	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
S.nitrosporeus				VIII	VIII								Gy	RF	C–	SM	+	+	+	+	–	+	–	–	–	–	–
Most closely to groups *S.argenteolus* et rel. and *S.atroolivaceus* et rel.																											
S.luridiscabiei		+	+	IV	IV							Clade-8	Y/ W	RF	C+	SM		+	+	+	+		+	+	+		+
S.acrimycini		Group	yes	I	Ia								G	S	C–	H	+	+	–	+	+	n	–	+	+	n	–
S.griseoplanus		Group	no	I	VII								Gy	S	C–	WTY	+	+	+	–	+	+	+	–	–	–	–
S.baarnensis				I	Ia								W	RF	C–	SM	+	+	+	+	+	+	–	+	+	n	n
S.flavofuscus				I	Ia								Y	RF	C–	SM											
S.praecox		Group	no	I	Ib								Y	RF	C–	SM	+	+	+	+	+	+	+	+	–	+	n
S.fimicarius		Group	yes	I	Ia							+	Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	–	n
S.anulatus		Group	no	I	Ib							+	Y	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
Group *S.atroolivaceus* et rel.																											
S.mutomycini		+	+	VII	VII								Gy	S	C–	SPY	+	+	+		+			+			
S.olivoviridis				VII	VII								Gy	S	C–	SPY	+	+	+	+	+	+	–	+	–	–	–
S.atroolivaceus		+	+	VII	VII							+	Gy	S	C–	WTY	+	+	+	+	+	n	n	n	n	n	n
S.clavifer				+	+							+	W	RF	C–	SM	+	+	–	+	+	+	–	+	–	–	n
S.finlayi		+	+	VII	VII								Gy	S	C–	H	+	+	+	+	–	n	–	–	–	n	±
Most closely to groups *S.argenteolus* et rel. and *S.atroolivaceus* et rel.																											
S.griseus ssp.griseus	Group A1B	Group	yes	II	II	+	+			Str	Str		Y	RF	C–	SM	+	+	–	–	+	+	–	+	–	+	–
S.lavendulae ssp. lavendulae													R	S	C+	SM	+	–	–	–	–	+	+	–	–	+	–
S.cavourensis ssp. washingtonensis				III	III								Y	RF	C+	SM	+	+	+	–	+	n	–	+	–	n	–
S.cyaneofuscatus		+	+	III	III							+	Y	RF	C+	SM	+	+	–	+	+	+	–	+	–	+	+

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25]16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27]16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28]5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
Not closely related to one of the groups																											
S.mediolani		Group	yes	V	Va								Y	RF													
S.rubiginosohelvolus				N	N					Str	Str		R	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
S.parvus				V	Vb								Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	–	n
S.albovinaceus	+	Group	yes	V	Va							+	W	RF	C–	SM	+	+	+	+	+	+	–	+	–	+	–
S.bacillaris				N	N							+	Y	RF	C+	SM	+	+	–	–	+	+	–	+	+	+	n
S.griseinus		Group	yes	V	Va								Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	–	–
S.sindenensis		Group	no	V	Vb					Str	Str	+	Y	RF	C–	SM	+	+	+	–	+	+	–	+	–	+	n
S.pluricolorescens													Y	RF	C–	SM	+	+	–	+	+	n	–	+	–	n	–
S.globisporus ssp. globisporus	Group A1B												Y	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
S.badius		Group	no	V	Vb							+	Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	–
S.californicus		+	+	VI	VI								Y	RF	C–	SM	+	+	–	–	+	+	–	+	–	–	–
S.floridae		+	+	VI	VI								Y	RF	C–	SM	+	+	–	–	+	+	–	+	–	–	n
S.alboviridis	Group A1B	Group	yes	IV	IV								Y	RF	C–	SM	+	+	–	+	+	n	–	+	–	n	–
S.microflavus		Group	yes	IV	IV							Clade-8	Y	RF	C–	SM	+	+	–	+	+	+	–	+	–	+	–
S.fulvorobeus		Group	no	IV									R-Y	S	C–	SM	+	–	+	–	n	n	n	–	–	n	n
S.lipmanii													Y	RF	C–	SM	+	+	–	+	+	+	+	+	–	+	±
Group *S.avidini* et rel.																											
S.spororaveus		+	+	XI	XI							+	Gy	S/ RA	C+	WTY/ SM	+	–	–	–	–	–	–	n	n	n	n
S.xanthophaeus	+												R	RF	C+	SM	+	–	–	–	–	n	–	–	–	n	–
S.nojiriensis		+	+	XI	XI							+	Gy	S	C+	SM	+	–	–	–	–	–	–	–	–	+	–
S.cirratus	Group F	+	+	XI	XI							+	Gy	S	C+	SM	+	+	+	–	+	+	–	–	–	–	+
S.vinaceus				XII	XII	I	III					+	R	S	C+	SM	+	–	–	–	n	n	n	n	n	n	n
S.columbiensis													R	S	C+	SM	+	–	–	–	n	n	n	n	n	n	n
S.lavendulae ssp grasserius													R	S	C+	SM	+	–	–	–	–	+	–	–	–	+	–
S.goshikiensis													R	S	C+	SM	+	–	–	–	+	n	–	–	–	n	–
S.sporoverrucosus													Gy/ Y	S	C+	WTY											
S.avidinii	Asn(AAC) 442												Gy	S	C+		n	–	–	n	n	n	–	n	n	n	n
S.subrutilus		+	+	XI	XI							+	R	RF	C+	SM	+	–	–	–	+	+	–	–	–	–	+
Group *S.cinnamonensis* et rel.																											
S.globosus				N	N								Gy	RF	C+	SM	+	+	+	–	n	n	n	n	n	n	n
S.toxytricini													R	S	C+	SM	+	–	–	–	±	n	–	–	–	n	–
S.flavotricini													R	RF	C+	SM	+	–	–	–	±	n	–	–	–	n	–
S.polychromogenes													B	RF	C+	SM	+	+	+	–	+	+	–	–	–	+	n
S.racemochromogenes													R	S	C+	SM	+	–	+	–	–	n	–	–	–	n	+
S.katrae													R	S	C+	SM	+	–	–	–	+	+	+	–	–	–	n
S.cinnamonensis													R	S	C+	SM	+	–	–	–	+	n	–	–	–	+	n
S.virginiae	Group F	+	+	XI	XI							+	R	S	C+	SM	+	–	–	–	+	n	–	–	+	+	n
Group *S.albolongus* et rel.																											
S.cavourensis ssp. cavourensis													Y/R	RF	C+	SM	+	+	–	–	+	n	–	+	–	n	n
S.celluloflavus													Y	RF	C–	SM	+	–	–	–	n	n	n	n	n	n	n
S.albolongus	+												W	RF	C–	SM	+	+	+	–	+	+	–	+	+	+	–
S.griseobrunneus		+	+	N	N								Y	RF	C+	SM	+	+	–	–	+	+	+	+	–	+	+
Group *S.crystallinus* et rel.																											
S.melanogenes													R	RF	C+	SM	+	+	+	–	+	n	+	+	+	n	±
S.noboritoensis													Gy	RF	C+	SM	+	+	+	–	+	n	+	+	+	+	±
S.crystallinus													R	RF	C+	SM	+	n	n	n	n	n	n	n	n	n	–

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
Group *S.mauvecolor* et rel.																											
S.michiganensis													Y	RF	C+	SM	+	+	–	–	+	n	±	+	+	n	–
S.xanthochromogenes													Y	RF	C+	SM	+	+	±	±	+	n	±	+	±	n	±
S.mauvecolor		+	+	N	N							+	Vi	S	C+	SPY	+	–	+	–	–	+	+	–	–	+	–
Not closely related to one of the groups																											
S.cremeus		+	+									+	R	S	C–	SM	+	+	+	–	+	+	–	–	–	–	–
S.spiroverticillatus		+	+	N	N							+	W	S	C–	SM	+	+	+	–	+	n	–	–	–	n	n
S.candidus													W	RF	C–	SM	+	+	+	+	n	n	–	+	–	n	–
Group *S.exfoliatus* et rel.																											
S.lateritius													R	S	C+	WTY	+	+	+	+	+	n	–	–	±	n	–
S.venezuelae		+	+	N	N								R	RF	C+	SM	+	–	–	–	–	n	–	–	–	n	–
S.omiyaensis													Gy	RF	C–	SM	+	+	–	+	–	+	–	–	–	–	n
S.wedmorensis													Gy	RF	C–	SM	+	+	+	+	+			+			
S.litmocidini													Gy	RF	C+	SM	+	±	+	–	±	n	–	–	–	n	–
S.yerevanensis													Gy		C–	SM	+	+	+	+	+	–	+	+	+	+	+
S.zaomyceticus													Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	+	+
S.exfoliatus		+	+	N	N							+	R	RF	C–	SM	+	+	+	+	+	+	+	–	–	+	+
S.narbonensis													Gy	RF	C+	SM	+	+	+	+	+	n	+	–	–	n	+
Most closely to group *S.exfoliatus* et rel.																											
S.albidochromogenes													W	S	C+	SM	+	+	+	–	+	n	–	+	–	n	n
S.flavidovirens										Str	Str		Y/ W	RF/ RA/	C+	SM	+	+	+	+/ –	+/ –	n	–	+/ –	+/ –	n	+/ –
S.enissocaesilis													n	S	C–	SM	+	+	+	–	n	n	–	+	n	n	n
S.albosporeus ssp. labilomyceticus													W	RF	C–	SM	+	+	–	–	–	+	+	n	–	+	+
S.chryseus													Y	S	C–	SM	+	n	+	–	n	+	–	–	–	n	–
S.helvaticus													Y	S	C–	SM	+	n	+	–	n	+	–	–	–	n	–
Not closely related to one of the groups																											
S.beijiangensis													n	RF-RA	C–	n	+	+	–	–	–	+	n	–	n	n	–
S.drozdowiczii													Gy	S	C+												
S.yanii		+	+	IX	IX							+	Gy	RF	C–	SM	+	+	n	n	+	n	+	+	n	n	n
Group *S.graminofaciens* et rel.																											
S.peucetius		+	+	X	X							+	R	S	C–	SM	+	+	–	–	+	n	+	+	n	n	+
S.xantholiticus													W	S	C–	SM	+	–	–	–	n	+	–	n	n	n	–
S.kurssanovii													Gy	S	C+	SM	+	+	+	–	+	+	+	–	–	–	+
S.graminofaciens		+	+	X	X								Gy	S	C–	WTY	+	+	+	+	n	n	n	n	n	n	n
Group *S.amakusaensis* et rel.																											
S.amakusaensis													B	S	C+	SM	+	–	±	–	–	–	–	–	–	–	±
S.inusitatus													B/ Gy	S	C–	SM	+					+					
S.clavuligerus													Gy	RF		SM											
Group *S.atratus* et rel.																											
S.atratus				IX	IX								Gy	S	C–	SM	+	+	–	+	+	+	+	–	–	+	n
S.sanglieri				IX	IX								Gy	S	C+	SM	n	+	+	+	+	+	+	n	n	n	+
S.gelaticus				IX	IX								Gy	RF	C–	SM	+	+	–	+	–	+	+	–	–	+	+
S.pulveraceus		+	+	IX	IX							+	Gy	S	C+	SM	n	+	–	+	+	+	+	–	–	+	–
Not closely related to one of the groups																											
S.sannanensis													Gy	S	C–	SM	±	±	–	–	–	–	–	–	–	–	–
Most closely to group *S.laurentii* et rel.																											

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25]16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27]16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28]5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S.showdoensis													Gy	RF	C+	SM	+	+	±	–	+	+	–	–	–	+	±
S.viridobrunneus													Gy	RF	C+	SM	+		–	–			+	–	–		
S.roseoviridis													R	RF	C+	SM	+	+	+	–	–	+	–	–	–	–	n
S.vietnamensis													W	RF	C+	n	+	+	+	+	+	+	n	n	n	n	+
S.nashvillensis													Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	+	±
S.tanashiensis		+	+	+	+					Str	Str		Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	+	–
S.roseolus													R	RF	C–	SM	+	+	+	+	±	n	–	–	–	n	–
S.bikiniensis	+												Gy	RF	C+	SM	+	+	–	–	–	+	–	–	–	±	±
S.violaceorectus													Gy	RF	C+	SM	+	+	+	–	+	n	–	–	–	n	+
S.cinereoruber ssp. cinereoruber													Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	+	–
Group *S.laurentii* et rel.																											
S.laurentii														RF	C–	SM	+	+	–	–	–	+	–	–	–	n	+
S.termitum													R	RF	C–	SM	+	+	–	n	–	n	–	–	–	n	n
S.roseofulvus													R	RF	C–	SM	+	+	+	+	+	+	+	–	–	+	+
Most closely to group *S.laurentii* et rel.																											
S.filamentosus													R	RF	C–	SM	+	+	+	–	–	+	–	–	–	–	+
Group *S.gobitricini* et rel.																											
S.gobitricini												+	R	RF	C+	SM	+	+	+	+	+	+	–	–	+	–	n
S.lavendofoliae												+	R	S	C+	SM	+	+	+	–	–	+	–	–	+	–	–
S.luridus												+	R	S	C+	SM	+	+	+	–	±	n	–	–	+	n	–
S.roseolilacinus												+	R	S	C–	SM	+	–	+	–	±	n	–	–	–	n	–
Not closely related to one of the groups																											
S.biverticillatus													Bi	VE	C+	SM	+	±	–	±	±	n	±	–	±	n	±
S.werraensis													Gy	S	C–	n	n	n	n	n	n	n	n	n	n	n	n
S.globisporus ssp. caucasicus							I	II					Y	RF	C–	SM	+	+	+	–	n	n	n	n	n	n	n
S.albireticuli	Group F									Str	Str		Ar	VE	C+	SM	+	±	±	±	±	n	±	±	+	n	+
S.eurocidicus													Ar	VE	C+	SM	+	–	–	–	±	n	–	–	±	n	+
S.stramineus													Y	VER	C+	SM	+	–	n	–	+	–	–	+	+	–	n
S.olivoverticillatus													Ar	VE	C+	SM	+	–	±	–	–	n	±	–	±	n	+
S.netropsis	Group F									Str	Str		Ke	VE	C–	SM	+	±	–	±	–	n	–	–	+	n	–
Group *Kitasatospora - Streptacidiphilus - Streptomyces*																											
Subgroup *Kitasatospora - Streptomyces*																											
K.gansuensis																											
S.atroaurantiacus													W	RF	C+	SM	+	+	+	–	+	n	–	–	–	n	+
K.mediocidica										Kit	Kit																
S.purpeofuscus													Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	–	n
S.chrysomallus ssp. fumigatus													Gy	RF	C–	SM	+	+	+	–	–	+	–	–	–	–	n
S.purpureus	+												Gy/ R	RF	C+	SM	+	–	+	–	+	n	–	–	+	+	–
S.xanthocidicus										Kit	Kit		Gy	RF	C–	SM	+	+	+	–	+	+	–	–	–	–	+
S.aburaviensis													Gy	RF	C–	SM	+	±	–	–	±	–	–	–	–	–	–
S.herbaricolor													Gy	RF	C+	SM	+	+	+	–	+	+	+	–	–	–	+
S.indigoferus													Gy	RF	C+	SM	+	+	+	–	–	+	–	–	–	–	n
S.avellaneus													Gy	S	C–	SM	+	±	–	–	+	n	–	n	–	n	+
S.psammoticus													Gy	S	C–	SM	+	–	–	–	+	n	–	–	–	–	+
S.aureofaciens													Gy	S	C–	SM	+	±	+	–	+	+	–	–	–	–	+
K.sampliensis																											
K.putterlickiae																											
K.kifunensis										Kit	Kit																

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
K.azatica	Kitasatospora									Kit	Kit																
K.nipponensis																											
K.cineracea										Kit	Kit																
K.niigatensis										Kit	Kit																
K.cheerisanensis	Kitasatospora																										
K.phosalacinea	Kitasatospora									Kit	Kit																
K.paracochleata																											
K.cochleata													Gy	S	C+	SM	+	–	+	–	–	n	n	n	–	n	–
K.griseola	Kitasatospora									Kit	Kit																
K.setae	Kitasatospora									Kit	Kit																
K.paranensis																											
K.cystarginea										Kit	Kit																
K.terrestris																											
K.viridis																											
K.arboriphila																											
S.alboverticillatus													W		C–								–	–			
Group *Kitasatospora - Streptacidiphilus - Streptomyces*																											
Streptacidiphilus oryzae										N	Sta																
Subgroup *Streptacidiphilus albus* et rel.																											
Streptacidiphilus albus										Sta	Sta																
Streptacidiphilus carbonis										Sta	Sta																
Streptacidiphilus neutrinimicus										Sta	Sta																
Subgroup *Streptacidiphilus anmyonensis* et rel.																											
Streptacidiphilus jiangxiensis										Sta	Sta																
Streptacidiphilus anmyonensis										Sta	Sta																
Streptacidiphilus melanogenes										Sta	Sta																
Streptacidiphilus rugosus										Sta	Sta																
Not closely related to one of the groups																											
S.ardus													Ar	VE	C+	SM	+	–	–	–	+	n	±	–	+	n	+
S.blastmyceticus													Mo	VE	C+	SM	+	–	–	±	–	n	±	–	±	n	+
S.caeruleus								XIII	XIII				Gy	RF	C–	SM	+	–	–	–	n	n	n	n	n	n	n
S.hiroshimensis	Group F							XII	XIIa	Str	Str		Hi	VE	C+	SM	+	–	–	–	–	n	–	–	+	n	±
S.cinnamoneus ssp. cinnamoneus								XII	XIIb				Ci	VE	C–	SM	+	±	–	±	±	n	±	±	+	n	+
S.pseudoechinosporeus													W/ Gy		C+	SM	+	+	+	+	+	+	+	+			+
S.lilacinus	Group F												Li	VE	C+	SM	+	–	–	–	–	n	–	–	±	n	±
S.sapporonensis													R		C–								–	–			
S.varsoviensis													W	S	C–	SM	+	–	–	–	+	+	–	+	+	+	–
S.abikoensis	Group F							XII	XI	Str	Str		Ar	VE	C+	SM	–	–	–	–	–	n	–	–	–	n	–
S.lavenduligriseus													Mo	VE	C–	SM	+	±	±	±	+	n	+	±	+	n	+
S.morookaensis													Y	VE	C–	SM	+	–	–	?	+	n	+	+	+	n	–
S.thioluteus										Str	Str		Th	VE	C–	SM	+	–	–	–	–	n	–	–	±	n	±
S.luteireticuli													Y/ Gy	VE	C+	SM	+	?	?	?	?	n	?	?	+	n	?
S.ehimensis	Group F							XI	XI				Ke	VE	C+	SM	+	±	–	±	±	n	–	±	±	n	±
S.hygroscopicus ssp. angustmyceticus								IX	IXb				Gy	S	C–	SM	+	–	–	–	n	±	±	+	–	–	+
Group *S.ochraceiscleroticus* et rel.																											
S.ochraceiscleroticus								VIII	VIII				W	S	C–	SM	+	+	+	+	+	+	+	+	+	+	n

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25]16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27]16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28]5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]									
S.purpurogeneiscleroticus								VIII	VIII				Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	n
S.violens								VIII	VIII				n	n	C–	n	+	+	+	+	+	n	+	+	n	+
S.monomycini								VII	VI				W/ Gy	S	C–	SM	+	+	–	–	+	n	–	+	–	n	n
S.niger								VII	VII				W/ Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	+
S.olivaceiscleroticus								VII	VII	Str	Str		Gy	S	C–	SM	+	+	+	+	n	n	n	n	n	n	n
Most closely to groups *S.ochraceiscleroticuset* rel. and *S.albofaciens* et rel.																										
S.auratus													Gy	S	C+	SM	+	n	n	+	+	+	+	+	n	+	+
Group *S.albofaciens* et rel.																										
S.chrestomyceticus								VI	VI				W	S	C–	SM	+	–	–	–	+	+	–	+	–	–	n
S.rimosus ssp. paromomycinus								VI	VI				W	S	C–	SM	+	–	–	–	+	+	+	+	+	–	–
S.albofaciens	+							VI	VI				W	S	C–	SM	+	±	+	–	+	n	+	+	+	n	±
Most closely to groups *S.ochraceiscleroticuset* rel. and *S.albofaciens* et rel.																										
S.erumpens		Group	yes	II	II								Gy	S	C–	SM	+	–	+	–	+	+	+	+	+	–	–
S.rimosus ssp. rimosus								N	VI	Str	Str		W	S	C–	SM	+	–	+	–	+	+	+	+	+	–	n
S.sclerotialus								N	XIII				W	S	C–	SM	+	+	+	+	+	n	+	+	+	n	+
Group *S.albulus* et rel.																										
S.albulus	+							V	V				Gy	S	C–	SPY	+	–	–	–	+	+	–	+	+	+	n
S.noursei													Gy	S	C–	SPY	+	–	–	–	+	+	–	+	+	–	+
S.yunnanensis														S	C–	RU	+	–	+	+	+	n	+	+	+	n	–
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel. and *S.albulus* et rel.																										
S.kasugaensis													Gy	S	C–	SM	+	–	–	–	+		+	–	+		–
S.chattanoogensis	+							V	V				Gy	S	C–	SPY	+	–	–	–	+	n	+	+	+	n	+
S.lydicus								V	V				Gy	S	C–	SM	+	+	+	–	+	+	+	+	–	n	+
S.albospinus								N	N				Gy	S	C–	SPY	+	±	–	–	+	+	+	+	+	+	–
S.sioyaensis	+						I	III					Gy	S	C–	SM	+	+	–	–	+	+	+	+	+	–	+
S. hygroscopicus ssp. decoyicus								X	IXa				Gy	S	C–	SM	+	+	–	–	+	+	–	+	+	–	n
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel., *S.albulus* et rel. and *S.caniferus* et rel.																										
S.catenulae	+							IX	IXb				Gy	RF	C–	SM	+	–	–	–	+	+	–	+	–	–	n
S.misakiensis													Gy	RF	C–	SM	+	–	–	–	+	n	+	+	+	n	+
S.ramulosus								X	N				Gy	RF	C–	SM	+	–	–	–	±	+	+	+	–	–	–
Group *S.caniferus* et rel.																										
S.hygroscopicus ssp. glebosus								X	X				Gy	S	C–	SM	+	+	–	–	+	+	+	+	+	–	+
S.libani ssp. rufus								X	X				Gy	S	C–	SM	+	+	+	–	+	n	+	n	+	n	+
S.platensis								X	X				Gy	S	C–	SM	+	–	–	–	+	n	+	+	+	n	n
S.caniferus								X	IXb			+	Gy	S	C–	SM	+	–	–	+	–	–	–	+	+	n	n
Most closely to group *S.caniferus* et rel.																										
S.libani ssp. libani	+							IX	IXa				Gy	S	C–	SM	+	+	–	–	+	n	+	n	+	n	+
S.tubercidicus	+							IX	X				Gy	S	C–	SM	+	–	–	–	+	n	+	+	+	n	+
S.nigrescens								IX	IXa				Gy	S	C–	SM	+	+	–	–	+	n	+	+	+	n	+
Group *S.albiflaviniger* et rel.																										
S.antimycoticus								II	II				Gy	S	C–	SM	+	+	+	+	+	n	–	+	+	+	+
S.geldanamycininus													Gy	S	C–	RU	n	n	+	n	–	n	n	n	n	–	n

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S *r*RNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S.melanosporofaciens								II	II				Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	–
S.sporoclivatus								II	II				Gy	S	C–	WTY	+	+	–	–	+	+	+	+	n	n	n
S.yatensis													Gy	S	C–	RU	+	+	+	+	+	+	+	+	+	+	+
S.rutgersensis ssp. castelarensis								II	II				Gy	S	C–	SM	+	+	+	+	+	+	+	+	–	+	–
S.indoniensis								I	I				Gy	S	C+	RU	n	n	n	n	n	n	n	n	n	n	+
S.griseiniger													Gy	S	C–	RU	n	n	–	n	+	n	n	n	n	–	n
S.rhizosphaericus								I	I				Gy	S	C–	RU											+
S.asiaticus								I	I				Gy	S	C–	RU	n	n	n	n	n	n	n	n	n	n	+
S.cangkringensis								I	I				Gy	S	C–	RU	n	n	n	n	n	n	n	n	n	n	+
S.malaysiensis													W/ Gy	S	C+	RU	+	+	+	+	+	+	+	+	n	–
S.javensis								III	III				Gy	S	C–	RU											+
S.endus								IV	IV				Gy	S	C–	SM	+	+	+	+	+	+	–	+	–	+	–
S.sporocinereus								IV	IV				Gy	S	C–	WTY	+	n	n	n	–	+	n	n	n	n	n
S.hygroscopicus ssp. hygroscopicus								IV	IV				Gy	S	C–	SM	+	+	+	+	+	n	–	n	–	+	n
S.demainii								IV	IV				Gy-Y	S	C–	RU	n	n	+	n	–	n	n	n	n	–	n
S.violaceusniger								III	III				Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	+	n
S.yogyakartensis								III	III				Gy	S	C–	RU	n	n	n	n	n	n	n	n	n	n	+
S.albiflaviniger													W	S	C–	RU	n	n	+	n	+	n	n	n	n	–	n
Most closely to groups *S.ochraceiscleroticucset* rel., *S.albofaciens* et rel., *S.albulus* et rel., *S.caniferus* et rel. and *S.albiflaviniger* et rel.																											
S.orinoci													Ar	VE	C–	SM	+	–	–	–	±	n	±	–	–	n	±
S.mashuensis								+	+				Ar	VE	C+	SM	+	–	–	–	+	n	±	–	+	n	+
S.mobaraensis													Mo	VE	C–	SM	+	±	±	–	+	n	–	±	±	n	+
S.luteosporeus													W	S	C–	SM	+	+	–	–	±	+	–	+	–	+	n
S.aureoversilis								XII	XIIa				Bi	VE	C+	SM	+	–	–	±	–	n	±	–	+	n	+
S.griseocarneus													Gr	VE	C+	SM	+	–	–	–	±	n	–	–	+	n	±
Group *S.albus* et rel.																											
S.almquistii	Group A16												W	S	C–	SM	+	+	–	–	+	n	–	+	–	+	–
S.rangoonensis										Str	Str		W	S	C–	SM	+	+	±	–	+	n	–	+	–	n	–
S.gibsonii													W	S	C–	SM	+	+	+	–	–	n	–	+	–	+	n
S.albus ssp. albus	Group A16												W	S	C–	SM	+	+	–	–	±	+	–	+	–	+	n
S.flocculus													W	S	C–	SM	+	+	+	–	+	+	+	+	+	+	n
Most closely to group *S.albus* et rel.																											
S.cacaoi ssp. cacaoi													W	S	C–	SM	+	+	+	–	+	n	±	+	–	n	±
S.sulphureus													Y	RF	C–	SM	+	+	+	–	+	n	+	n	–	+	n
S.rubidus														RF	C–	SM	+					+		+		+	
S.yeochonensis													Gy	RF	n	SM	n	n	n		n	n	n	n	n	+	n
S.albus ssp. pathocidicus													W	S	C–	SM	+	+	+	+	–	+	–	–	+	–	n
S.glauciniger													Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	n	+
S.guanduensis													Gy/ W	RF	C–	SM	+					+		+		+	
Most closely to groups *S.albus* et rel. and *S.glaucosporus* et rel.																											
S.ferralitis													W	S	C–	SM	+	n	–	n	n	+	n	+	n	n	n
S.vitaminophilus															C–	SM	+	+	–	+	–	n	–	–	–	n	–

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25]16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27]16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28]5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S.thermolineatus													G	RF	C–	SM	n	n	n	n	n	n	n	+	n	n	n
S.yanglinensis													W/ Gy	RF	C–	SM	+	n	n	n	n	n	n	+	n	+	n
S.paucisporeus													W/ Gy	RF	C +/ C–	SM	+					+		+			
Group *S.glaucosporus* et rel.																											
S.macrosporus								+	+				Gy	S	C–	SPY	+	+	+	+	+	n	–	+	+	n	–
S.megasporus													G	S	C–	SPY/ WT	+	+	+	+							
S.glaucosporus													G	S	C–	WTY	+	+	n	–	n	n	n	n	–	n	n
S.radiopugnans													W	S	C–	WTY	n	+	n	+	n	n	n	+	–	n	+
Most closely to group *S.glaucosporus* et rel.																											
S.albiaxialis													W	S	C–	SM	+	+	+	+	n	n	+	–	–	n	+
S.armeniacus													W	S	C–	n	+	+	+	+	+	+	+	–	n	+	+
Most closely to groups *S.albus* et rel. and *S.glaucosporus* et rel.																											
S.cuspidosporus													Gy	S	C–	SPY	+	+	+	+	+	+	+	+	+	+	+
S.sparsogenes													Gy	S	C–	SPY	+	+	+	+	+	±	+	+	±	–	+
Most closely to group *S.geysiriensis* et rel.																											
S.janthinus													R	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
S.roseoviolaceus													R	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
S.violaceus													R	S	C+	SY	+	+	+	+	+	+	+	n	+	n	+
S.albosporeus ssp. albosporeus													R	S	C–	SM	+	+	+	+	+	+	+	+	+	–	n
S.arenae													Gy	S	C+	SPY	+	+	+	+	+	+	+	+	+	n	+
S.luteogriseus													Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	+	+
S.hawaiiensis													W	S/ RA	C+	SPY	+	?	+	+	+	n	+	+	+	n	+
S.cellulosae													Y	RF	C–	SM	+	+	+	+	+	n	–	+	+	n	n
S.pseudogriseolus													Gy	S	C–	SPY	+	+	+	+	+	+	–	+	+	+	n
S.gancidicus													Gy	S	C–	SPY	+	+	+	+	+	+	–	+	+	–	–
S.rubiginosus													Gy	S	C–	SPY	+	+	±	+	+	n	–	+	+	n	+
S.capillispiralis													Gy	S	C–	H	n	n	–	n	n	n	–	n	n	–	–
S.lavendulocolor													+	R	S	C+	SM	+	+	+	–	–	+	–	–	+	–	–
S.azureus														B	S	C+	WTY	+	+	+	+	+	+	+	+	+	n	+
S.flavoviridis														Gy/ G	S	C+	H	+	+	+	+	+	n	–	+	+	n	–
S.pilosus														Gy	S	C+	H	+	+	+	+	+	n	–	+	+	n	–
S.djakartensis														Gy	S	C+	n	n	n	n	n	n	n	n	n	n	n	n
Group *S.geysiriensis* et rel.																											
S.ghanaensis														G	S	C–	SPY	n	n	n	n	n	n	n	n	n	n	n
S.minutiscleroticus														Gy	S	C–	n	+	n	+	+	+	+	–	+	n	+	–
S.geysiriensis														Gy	S	C–	H	n	n	n	n	n	n	n	n	n	n	n
S.plicatus														Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	n	–
S.rochei														Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	+	–
S.vinaceusdrappus														R	S	C–	SM	+	+	+	+	+	n	+	+	+	+	n
S.mutabilis														Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	–	±
Most closely to group *S.geysiriensis* et rel.																											
S.tuirus														R	S	C+	SM	+	+	+	+	+	+	+	+	+	–	+
S.afghaniensis														Gy	S	C+	SPY	+	+	+	+	+	+	+	±	±	n	+
S.africanus														B	S	C–	SPY	n	n	+	+	+	+	+	+	+	+	–

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S *r*RNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
Group *S.brasiliensis* et rel.																											
S.roseiscleroticus													R	S	C–	SM	+	+	+	+	+	+	–	+	–	–	n
S.ruber													W/R	S	C–	SM	+	+	+	+	+		–	+			
S.spiralis													Y-Gy	S	C–	SM	+	–	+	+	+	+	+	+	+	+	+
S.fumigatiscleroticus														S	C–		+	+	+	–	n	–	n	+	–	–	?
S.poonensis													Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	n
S.brasiliensis													Gy	S	C–	SM	+	n	+	+	+	+	+	+	+	+	+
Group *S.atrovirens* et rel.																											
S.atrovirens													Gy	S	C–	H	n	+	–	+	+	n	+	+	+	n	n
S.caelestis													B	S	C+	SM	+	+	+	+	+	+	+	–	+	–	+
S.fumanus													R	S	C–	SM	+	+	+	+	+	n	+	+	–	n	–
S.fimbriatus													Gy	S	C+	SPY	+	+	+	+	+	+	+	+	+	–	n
Group *S.glaucus* et rel.																											
S.griseostramineus													G	S	C+	H	+	+	+	+	+	n	n	+	+	n	–
S.griseomycini													G	S/RA	C+	H/SM	+	+	+	+	+	n	–	+	+	n	–
S.graminearus													Gy	S	C–	SM	+		+	+	+		+	+	+		
S.viridiviolaceus										Str	Str		Gy	S	C–	SPY	n	n	n	n	n	n	n	n	n	n	n
S.glaucus													B/G	S	C–	H	+	+	+	+	n	+	n	+	+	n	n
Group *S.aureorectus* et rel.																											
S.aureorectus													W	RF	C–	SM	n	+	n	n	n	+	+	n	n	n	n
S.virens													Gy	S	C–	SPY/WT	n	–	+	–	+	n	–	+	n	n	n
S.asterosporus													Gy	S	C–	SPY	+	–	+	+	+	+	–	+	n	n	n
S.calvus													Gy	S	C–	H	+	+	+	+	+	+	+	+	+	+	+
Most closely to groups S. *geysiriensis* et rel., S.*brasiliensis* et rel., S.*atrovirens* et rel., S.*glaucus* et rel. and S.*aureorectus* et rel.																											
S.naganishii													Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	+	–
S.prasinosporus													G	S	C+	H	+	+	+	+	+	n	n	+	+	+	–
S.anandii													Gy	S	C+	SM	+	+	+	–	+	+	+	+	+	–	n
S.carpinensis													Gy	S	C–	SM	+	–	n	+	+	+	+	+	–	+	–
S.levis													V	S	C–	SM	+	+	+		+		+	+			
S.cinerochromogenes													Gy	S	C+	SM	n	–	+	+	n	n	–	–	–	+	+
S.koyangensis													W/Gy	RF	C–	SM		+	+	–	+		–	+	–		–
S.violarus													R	S	C+	SPY	+	n	+	+	+	+	+	n	+	n	+
Not closely related to one of the groups																											
S.daghestanicus													R	S	C–	SM	+	+	+	+	+	n	–	+	–	n	–
S.limosus	+					I	I						Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	–
S.canescens						I	N						Y	RF	C–	SM	+	–	+	–	+	n	–	–	–	–	–
S.felleus	Asn(AAC) 442					I	I						Y	RF	C–	SM	+	+	+	+	n	n	–	+	–	+	n
S.griseus ssp. solvifaciens		+	+	XII	XII	I	N						Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	+	n
S.violascens	+												V	S	C+	SPY	+	+	+	–	+	n	+	–	±	n	±
S.hydrogenans													W/Y/Gy	RF	C–	SM	+	+	+	+	–	n	–	–	–	n	–
S.odorifer						I	II						Y	RF	C–	SM	+	+	+	–	+	n	–	+	+	+	n
S.albidoflavus						I	II					+	Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	–

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25]16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27]16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28]5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S.champavatii						I	N						Y	RF	C–	SM	+	+	+	–	+	+	–	+	n	n	–
S.sampsonii						I	I						Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	–
S.diastaticus ssp. diastaticus													Gy/ Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	+
S.gougerotii													Y	RF	C–	SM	+	–	+	–	n	n	n	n	n	n	n
S.rutgersensis ssp.rutgersensis													Y	RF	C–	SM	+	+	+	–	+	+	+	+	–	+	–
S.intermedius													Y	RF	C–	SM	+	+	+	–	+	+	+	+	–	+	+
S.indiaensis													Gy	S	C+	SM	+	+	+	–	+	n	–	+	+	–	+
S.thermocarboxydus													Gy	RA	C–	WTY	+	–	n	n	+	n	–	+	+	n	–
S.massasporeus													Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	+	+
S.misionensis													Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	–	–
S.phaeoluteichromatogenes																											
S.spectabilis	Asn(AAC) 442												R	RF	C+	SM	+	+	–	–	+	+	+	+	+	–	–
S.cinereospinus													Gy	S	C–	SPY	+	–	–	+	+	n	+	n	–	n	n
S.coeruleofuscus													B	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
S.chromofuscus													Gy	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	–
S.scopiformis													Gy	RF		SPY	+	+	+	+	+	+	–	–	+	n	+
S.spinoverrucosus													G	S	C+	SPY/ WT	+	+	+	+	+	n	+	+	+	n	+
Most closely to group *S.mexicanus* et rel.																											
S.thermospinosisporus													Gy	RF	C–	SPY							+	+	+		+
S.thermodiastaticus													Gy	S	C–	WTY/ SP	+	+	+	+	+	n	+	+	+	n	–
S.thermocarboxydovorans													Gy	RF	C–	SM	n	n	n	n	+	n	n	n	n	n	n
S.thermoviolaceus ssp. apingens													Gy	S	??	WTY	n	n	n	n	n	n	n	n	n	n	n
S.thermoviolaceus ssp. thermoviolaceus													Gy	S	C+	SM	n	n	n	n	n	n	n	n	n	n	n
S.nodosus	+									Str	Str		Gy	S	C–	SM	+	+	–	+	+	n	–	+	+	n	–
S.viridosporus													G	S	C–	SPY	+	+	+	+	+	+	–	+	+	–	±
Group *S.mexicanus* et rel.																											
S.thermogriseus															C–	SM	±	±		+	±		±	+	–		
S.thermovulgaris													Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	+
S.thermoalcalitolerans													Gy	n	C–	WTY	+	+	+	+	+	+	–	+	+	n	+
S.mexicanus													Gy	RF	C–	SM	+	+	+	–	+	+	+	+	–	n	–
S.thermocoprophilus													Gy	RF	C+	SM	+	+	+	n	+	n	–	+	n	n	–
Most closely to group *S.mexicanus* et rel.																											
S.bangladeshensis													Y-G	RF	C+	SM	+	–	+	+	+	n	+	+	+	+	n
S.rameus	+												Gy	S	C+	SM	n	+	+	–	+	+	+	+	–	+	+
S.griseosporeus													Gy	S	C+	SM	+	+	+	+	+	+	+	+	+	+	+
S.achromogenes ssp. rubradiris													Gy	S	C+	SM	+	+	+	+	+	+	+	+	±	±	+
S.glomeratus													Gy	S	C+	SM		+	+	+	+		+	+			
S.eurythermus													Gy	S	C+	SM	+	+	+	–	+	+	+	+	–	–	+
S.nogalater													Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	–	–
S.fragilis													R	S	C–	SM	+	+	+	–	–	+	–	–	–	–	±
Group *S.erythrogriseus* et rel.																											
S.erythrogriseus										Str	Str		Gy/ R/ W	S	C–	SPY/ SM	+	+	+	+	+	n	–	+	+	n	–
S.labedae										Str	Str		Gy	S	C–	SPY	+	+	–	+	+	+	–	+	+	–	–

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S.griseoincarnatus													Gy	S	C–	SPY	+	+	+	+	+	n	–	+	±	n	+
S.variabilis													Gy/R	S/RA	C–	SPY	+	+	+	+	+	n	–	+	+	n	–
Most closely to group *S.erythrogriseus* et rel.																											
S.althioticus													Gy	S	C–	SPY	+	+	+	+	+	+	–	+	+	n	±
S.matensis													Gy	S	C–	SPY	+	+	+	+	+	+	–	+	+	n	–
S.griseorubens													Gy	S	C–	SPY	+	+	±	+	+	n	–	+	±	n	–
S.viridochromogenes													B	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
S.iakyrus													Gy	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	n
S.violaceochromogenes										Str	Str		Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
S.collinus	Group A18												Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
S.malachitofuscus													Gy	S	C+	SPY	+	+	+	+	+	n	–	+	+	n	+
S.paradoxus													Gy	RA	C+	SM	+	+	+	+	+	+	+	+	+	n	+
S.griseoflavus													Gy	S	C–	SPY	+	+	+	+	+	n	–	+	+	n	–
S.flaveolus													Gy	S	C–	H	+	+	+	+	+	+	+	+	+	+	+
S.glaucescens													B/G	S	C+	H	+	+	+	+	+	n	–	+	+	n	–
S.pharetrae													Gy		C+	H			+	+				+			
S.malachitospinus													Gy	S	C–	SPY	+		+			+					
S.parvulus													Gy	S	C–	SM	+	+	+	+	+	n	–	+	+	+	+
S.tendae													Gy	S	C–	SM	+	+	+	+	+	n	–	+	+	n	+
S.violaceorubidus													Gy/W	S	C–	SM	+	+	+	+	+		+	+	+		
S.albaduncus													Gy	S	C–	SPY	+	+	+	+	+	+	+	+	+	+	±
S.griseoloalbus													Y	RF	C–	SM	+	+	+	+	+	n	n	+	+	n	+
S.heliomycini													Gy	S	C–	WTY/SP	+	+		+	+			+	+		
S.ambofaciens													Gy	S	C–	SM	+	+	+	+	+	n	–	+	+	n	+
Most closely to group *S.coelescens* et rel.																											
S.rubrogriseus													Gy/R	S	C–	SM											
S.tricolor													Gy	S	C–	SM	n	n	n	n	n	n	n	n	n	n	n
S.lienomycini													Gy	S	C+	SM		+	+	+	+		+	+	+		
S.anthocyanicus													Gy	S	C–	SM	+	n	+	+	+	+	–	–	+	n	–
S.olivaceus													Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	–	–
S.pactum													Gy	S	C–	H	+	–	–	–	–	+	–	–	–	–	–
Group *S.coelescens* et rel.																											
S.coelescens													Gy	S	C–	SM	+	n	+	n	n	n	n	–	n	n	n
S.humiferus													Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	n	–
S.violaceolatus													Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	+
S.violaceoruber													Gy	S	C–	SM	+	+	+	+	+	+	–	+	+	+	–
Most closely to group *S.coelescens* et rel.																											
S.coelicoflavus													n	S	C–	SM	+	+	+	+	+	n	n	+	+	n	n
S.diastaticus ssp. ardesiacus													Gy	S	C–	SM	+	+	+	+	n	n	n	n	n	n	n
Most closely to group *S.coeruleorubidus* et rel.																											
S.lomondensis													R/B	RF/S	C+	WTY/SP	+	+	+	+	+	+	+	+	+	n	+
S.lusitanus													Gy	S	C–	SM	+	–	±	–	+	n	–	–	±	–	+
S.purpurascens													R	S	C+	SPY	+	+	+	+	+	n	+	+	+	+	+
S.bellus													B	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25]16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27]16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28]5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S.coerulescens								N	XIII				B	S	C+	SPY	+	+	+	+	+	n	+	+	+	n	+
S.speibonae										Str	Str		Gy	S	C+	H		+	+	+	+	+	–	+	+	–	+
S.longispororuber													W	S	C+	SM	n	n	n	n	n	n	n	n	n	n	n
Group *S.coeruleorubidus* et rel.																											
S.albogriseolus	Asn(AAC) 442												Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	+	+
S.viridodiastaticus										Str	Str		Gy	S	C–	SPY	+	+	+	+	+	n	n	+	+	n	n
S.coeruleorubidus	+												B	S	C+	SPY	+	+	+	+	+	+	+	+	+	+	+
Group *S.aurantiogriseus* et rel.																											
S.coelicolor						I	II						Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	n
S.griseoviridis	+												R	S	C–	SM	+	+	+	+	+	+	–	+	–	n	–
S.aurantiogriseus													Gy	S	C+	SM	+	+	+	+	+	n	+	+	+	n	+
Most closely to group *S.aurantiogriseus* et rel.																											
S.griseoaurantiacus													Gy	S	C–	WTY	+	+	+	+	n	n	n	n	n	n	n
S.jietajiensis														RF	C–	SM	+	+				+		+		+	
Group *S.coeruleoprunus* et rel.																											
S.coeruleoprunus													B	RF	C–	SM	+	+	+	+	+	–	–	n	+	n	n
S.somaliensis	+												Y	RF	C–	SM	+	+	+	–	+	n	–	+	–	n	+
S.fradiae	+												R	S	C–	SM	+	+	+	–	–	+	+	–	–	–	–
Most closely to group *S.coeruleoprunus* et rel.																											
S.bluensis													B	S	C–	SPY	+	+	+	+	+	+	+	+	+	+	+
Not closely related to one of the groups																											
S.variegatus													W/ R	S	C–	SM		+	+	–	+		–				
S.fulvissimus													Y	RF	C+	SM	+	+	+	–	+	n	–	+	+	+	n
S.aureoverticillatus													R	S	C–	SM	+	+	+	–	+	+	+	+	+	+	n
S.flavofungini													W/ Y	RF	C–	SM	+	+	+	?	+	n	+	+	+	n	–
S.alboflavus	+												Y	RF	C+	SM	+	+	+	–	+	+	+	+	+	–	+
S.aculeolatus													W, Y,R	S	n	WTY –SP	+	+	+	+	+	n	+	+	–	n	–
S.synnematoformans													Gy-R	RF	C+	SM	+	n	n	n	+	+	n	n	n	–	–
S.hebeiensis														RF	C+	WTY		+				+					
Group *S.carpaticus* et rel.																											
S.hainanensis													W	S	C+	SM	+	–	–	–	–	–	–	–	–	–	–
S.specialis													W	S	C+	n	+	–	n	–	–	–	n	n	+	–	+
S.carpaticus													Gy	S	C–	SM	+	+	+	+	+	n	+	+	+	n	n
S.cheonanensis													Gy	RF	C+	SM	n	+	+	+	+	n	+	+	+	–	+
Most closely to group *S.carpaticus* et rel.																											
S.sulfonofaciens													R/V	RF		SM	+	+	+	+	+	n	–	+	–	n	+
S.sodiiphilus														RF	C–		–	–	–	+	–	–	–	–	–	n	–
Not closely related to one of the groups																											
S.scabrisporus													Gy	S	C–	RU	+	+	–	+	+	n	–	–	+	–	–
S.gardneri													Gy	RF	C+	SM	+	+	+	+	+	+	+	–	–	+	n
S.flavidofuscus		+	+										Y	S	C+	SM	+	n	+	+	+	n	+	+	+	n	n
Regarded as later heterotypic synonym of *Streptomyces abikoensis* (for references, see list of type strains)																											

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]									
S.luteoverticillatus								XI	XI				Lu	VE	C+	SM	+	–	–	–	+	n	±	+	–	n	+
S.olivoreticuli ssp. olivoreticuli													Lu	VE	C+	SM	+	–	–	–	–	n	±	–	±	n	+
S.parvisporogenes										Str	Str		Ar	VE	C+	SM	+	–	–	–	±	n	–	–	±	n	±
Regarded as later heterotypic synonym of *Streptomyces anulatus* (for references, see list of type strains)																											
S.chrysomallus ssp. chrysomallus													Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	+	–
S.citreofluorescens													Y	RF	C–	SM	+	+	+	+	+	+	–	+	–	–	–
S.fluorescens													Y	RF	C–	SM	+	+	+	–	+	+	–	+	–	+	–
Regarded as later heterotypic synonym of *Streptomyces avermitilis* (for references, see list of type strains)																											
S.avermectinius																											
Regarded as later heterotypic synonym of *Streptomyces cacaoi* (for references, see list of type strains)																											
S.aminophilus													W	S	C–	SM	+	+	+	–	+	+	–	+	–	+	n
Regarded as later heterotypic synonym of *Streptomyces caeruleus* (for references, see list of type strains)																											
S.niveus												+	Y	S	C–	SM	+	+	+	+	+	+	–	+	–	–	n
S.spheroides													Y	S	C–	SM	+	+	–	+	+	n	–	+	–	n	n
Regarded as later heterotypic synonym of *Streptomyces cinnamoneus* (for references, see list of type strains)																											
S.griseoverticillatus								XI	XIIb				Ke	VE	C–	SM	+	–	–	–	–	n	±	–	+	n	+
S.hachijoensis	Group F												Ci	VE	C–	SM	+	±	–	±	±	n	±	±	+	n	±
Regarded as later heterotypic synonym of *Streptomyces chibaensis* (for references, see list of type strains)																											
S.chibaensis													Gy	S	C–	SM	+	+	+	+	+	+	+	+	+	–	+
Regarded as later heterotypic synonym of *Streptomyces filamentosus* (for references, see list of type strains)																											
S.roseosporus													R	RF	C–	SM	+	+	+	+	–	n	–	–	–	+	–
Regarded as later heterotypic synonym of *Streptomyces flavofuscus* (for references, see list of type strains)																											
S.globisporus ssp. flavofuscus													Y	RF	C–	SM	+	+	+	+	n	n	n	n	n	n	n
Regarded as later heterotypic synonym of *Streptomyces flavovirens* (for references, see list of type strains)																											
S.nigrifaciens													Gy	RF	C–	SM	+	+	+	+	+	+	+	+	+	–	n
Regarded as later heterotypic synonym of *Streptomyces fradiae* (for references, see list of type strains)																											
S.roseoflavus													R	S	C–	SM	+	+	+	–	–	–	–	–	–	n	–
Regarded as later heterotypic synonym of *Streptomyces*																											

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25]16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27]16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28]5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
griseocarneus **(for references, see list of type strains)**																											
S.septatus													Y/R	VE	C+	SM	+	–	–	–	?	n	–	–	+	n	–
Regarded as later heterotypic synonym of ***Streptomyces griseus*** **(for references, see list of type strains)**																											
S.setonii	+	Group	no	I	II								Y	RF	C–	SM	+	+	+	+	+	n	–	+	–	n	–
Regarded as later heterotypic synonym of ***Streptomyces hiroshimensis*** **(for references, see list of type strains)**																											
S.rectiverticillatus								XII	XIIa				Ke	VE	C+	SM	+	±	–	±	+	n	±	+	+	n	±
S.roseoverticillatus													Bi	VE	C+	SM	+	–	–	–	–	n	–	–	–	n	+
S.salmonis													Sa	VE	C+	SM	+	–	–	–	–	n	–	–	±	n	±
S.spitsbergensis													R/V	RF		SM											
S.fervens ssp. fervens													Ba	VE	C+	SM	+	±	–	±	±	n	±	±	±	n	+
Regarded as later heterotypic synonym of ***Streptomyces lilacinus*** **(for references, see list of type strains)**																											
S.kashmirensis													Li	VE	C+	SM	–	–	–	–	–	n	–	–	–	n	–
Regarded as later heterotypic synonym of ***Streptomyces mashuensis*** **(for references, see list of type strains)**																											
S.kishiwadensis													Ar	VE	C+	SM	+	–	±	–	+	n	±	–	+	n	+
Regarded as later heterotypic synonym of ***Streptomyces microflavus*** **(for references, see list of type strains)**																											
S.griseus ssp. alpha		Group	yes	IV	IV								Y	RF	C–	SM	+	+	–	+	n	n	n	n	n	n	n
S.griseus ssp. cretosus		Group	yes	IV	IV								Y	RF	C–	SM	+	+	–	+	+	n	–	n	–	+	n
S.willmorei													Y	RF	C–	SM	+	+	–	+	n	n	n	n	n	n	n
Regarded as later heterotypic synonym of ***Streptomyces minutiscleroticus*** **(for references, see list of type strains)**																											
S.flaviscleroticus													n	n	C –/ C+	n	+	+	+	+	+	n	–	+	–	n	?
Regarded as later heterotypic synonym of ***Streptomyces mobaraensis*** **(for references, see list of type strains)**																											
S.ladakanum													W/ Y	VE	C–	SM	+	–	–	–	+	n	–	–	–	n	–
Regarded as later heterotypic synonym of ***Streptomyces netropsis*** **(for references, see list of type strains)**																											
S.distallicus													Ke	VE	C+	SM	+	±	–	±	±	n	±	–	+	n	±
S.flavopersicus													Ke	VE	C+	SM	+	–	–	–	±	n	–	–	+	n	±
S.kentuckensis													Ke	VE	C–	SM	+	±	–	–	±	n	±	±	+	n	+
S.syringium													R	S	C+	SM	+	–	–	–	+	–	–	–	–	n	–
Regarded as later heterotypic synonym of ***Streptomyces***																											

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S *r*RNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
phaeopurpureus **(for references, see list of type strains)**																											
S.phaeoviridis													R	S	C–	SM	+	+	+	+	+	n	+	+	n	n	+
Regarded as later heterotypic synonym of *Stre ptomyces thermovulgaris* (for references, see list of type strains)																											
S.thermonitrificans													Gy	RF	C+	SM	+	–	–	–	–	+	–	+	+	–	–
Regarded as later heterotypic synonym of *Streptomyces tricolor* (for references, see list of type strains)																											
S.roseodiastaticus					+	+							Gy	S	C–	SM	+	+	+	+	n	n	n	n	n	n	n
Regarded as later heterotypic synonym of *Streptomyces olioverticillatus* (for references, see list of type strains)																											
S.viridiflavus													Str	Str		W		C –								–	–
Regarded as later heterotypic synonym of *Streptomyces violaceus* (for references, see list of type strains)																											
S.violatus	Group A18												R	S	C+	SPY	+	+	+	+	+	+	+	n	+	n	+
No detailed sequence information available																											
S.caviscabies		Group	yes	I	Ia								W	RF	C–	SM	n	n	n	n	n	n	+	n	n	n	n
S.coeruleoflavus **(keine Seq. gefunden)**													B	S	C–	SPY	n	+	+	+	+	n	+	n	+	n	n
S.arabicus													Gy	S	C–	SPY	+	+	+	+	+	n	–	+	+	n	n
S.baldaccii													Ba	VE	C+	SM	+	–	–	–	–	n	±	±	±	n	+
S.cellulolyticus													W	RF	C–	WTY	+	+	+	–	+	n	n	n	+	n	+
S.echinoruber													Gy	S		SPY											
S.erythraeus													R	S	C–	SPY	+	+	+	+	+	+	+	+	+	+	n
S.longisporoflavus													Y	S	C–	SM	+	+	+	+	+	+	–	+	–	+	–
S.olivomycini														RF	C–	SM		+	+	+	+		+	+			
S.speleomycini													Y/ Gy	RF	C–	SM		+	+	+	+	+			+		
S.thermoautotrophicus													Gy														
Not in tree																											
S. aldersoniae													Gy/ W	L	C–	SM	n	+	+	–	+	+	+	n	n	+	n
S. alni													W/ Gy	RF	C–	SM	n	+	+	+	(+)	n	+	n	–	+	+
S. angustmycinicus													Gy/ W	S	C–	SM	n	–	–	–	+	+	+	n	n	–	n
S. ascomycinicus													Gy/ W	L	C–	SPY	n	+	+	+	+	+	+	n	n	+	n
S. atriruber													Gy	RF	C–	SM	+	+	+	+	+	+	+	+	+	+	±
S. avicenniae														S		SM	n	+	+	+	n	+	+	+	–	n	+
S. axinellae														S		SM	+	+	–	+	+	+	–	+	n	–	–
S. baliensis														RF	C+	WTY/ SM	+	+	+	+	+	+	+	+	n	n	+
S. castelarensis													Gy/ Bl	S	C–	RU	+	+	n	+	+	+	+	+	n	n	–
S. deccanensis													W	SC	C+	H	+	+	+	+	+	n	+	+	n	n	+

Table 42.2 (continued)

Species names and groups[1]	Kim 04[22] *rpoB*	Guo 08[23] 16S rRNA	Guo 08 [23] MLSA	Rong Huan g 10[25] 16S rRNA	Rong Huan g 10[25] MLS A	Rong 09[26] 16S rRNA	Rong 09[26] MLS A	Rong Huan g 12[27] 16S rRNA	Rong Huan g 12[27] MLS A	Han 12[28] 5S – 16S – 23S	Han 12[28] MLS A	Lab 12 [29]	Morphological characters [30]				Physiological tests [31]										
S. decoyininicus													Gy	S	C–	SM	n	+	–	–	+	+	–	n	n	–	n
S. gulbargensis														SC	C+	SM	+	+	+	+	n	+	+	+	n	n	+
S. haliclonae													W	S	C–	SM	+	–	–	–	+	n	+	+	n	n	+
S. himastatinicus													Gy/ Bl	S	C–	RU	+	+	n	+	+	+	+	+	n	n	+
S. hypolithicus														SC/ RF	C–	SM	+	–	–	n	–	+	–	–	n	±	–
S. iranensis													Y-Gy/ G	S		RU	n	+	+	+	n	+	+	+	+	n	+
S. lunalinharesii													Gy	S		SPY	n	–	+	+	n	n	–	n	n	n	+
S. marinus														S	C–	SM	+	+	+	–	+	n	–	+	n	n	+
S. marokkonensis													Gy	S	C–	SM	+	+	±	+	+	+	–	+	±	+	+
S. mayteni														S/L		SM	–	–	–	–	+	+	–	n	–	n	+
S. milbemycinicus													Gy	S/L	C–	WTY	n	+	+	+	–	–	–	n	n	–	n
S. modarskii													Gy/ Bl	S	C–	RU	+	+	n	+	+	+	+	n	n	n	+
S. nanshensis														S	C+	SM	+	+	n	+	+	+	+	+	+	n	+
S. osmaniensis													Gy-B	S	C+	SPY	n	+	+	+	+	+	+	+	n	n	+
S. plumbiresistens													G-W	RF	C–	SM	+	+	+	+	+	+	+	n	n	n	+
S. polyantibioticus														RF	C+	SM	+	+	–	–	+	+	+	–	n	+	–
S. rapamycinicus													Gy/ Bl	S	C–	RU	+	+	–	+	+	n	+	+	n	n	+
S. ruanii													Gy/ Bl	S	C–	RU	n	+	–	+	+	+	n	n	n	n	+
S. sedi														S		SM	n	–	n	–	+	–	–	–	–	n	n
S. silaceus													W/ Y-W	RF	C–	SM	+	+	–	–	+	+	+	+	+	+	–
S. tateyamensis													Gy	S	C–	SM	+	–	–	–	–	n	–	+	n	n	+
S. thinghirensis														S	C–	SM	+	–	n	+	+	+	–	+	n	n	±
S. tritolerans													W/ Gy	SC/ RF	C+	SM	+	+	+	+	+	+	+	+	–	n	+
S. wellingtoniae													R-Gy	S/L	C–	SM	n	+	+	+	+	+	+	n	n	–	n
S. xiamenensis														SC/ RF	C–	SM	n	–	+	+	n	–	–	+	n	n	+
S. xinghaiensis														SC/ RF		SM	+	n	–	+	+	+	–	+	n	n	+
K. kazusensis													W		C–	SM	+	+	+	–	–	+	–	–	–	n	–
K. saccharophila													Gy	SC/ RF	C–	SM	+	+	+	+	+	+	+	–	–	n	+

[1]Species are grouped according to the maximum-likelihood tree in *Fig. 42.1*

[2]Species list nummer of Kämpfer (2011); Without symbols: list of type strains of *Streptomyces*; [†] list of type strains of *Streptacidiphilus*; [a]list of type strains of *Kitasatospora*

[3]Groups as described in Williams et al. (1983a), grouping on the basis of numerical identification

[4]Groups as described in Williams et al. (1989), grouping mainly on the basis of numerical identification according to Williams et al. (1983a)

[5]Groups as described in Kämpfer et al. (1991), grouping on the basis of numerical identification

[6]Groups as described in Hatano et al. (2003), grouping on the basis of phenotypes, DNA-DNA hybridization and sequences of *gyrB*

[7]Groups as described in Lanoot et al. (2002), grouping on the basis of protein profiles

[8]Groups as described in Fulton et al. (1995), grouping on the basis of fingerprints of the rRNA operon

[9]Groups as described in Ochi (1995), grouping on the basis of the ribosomal AT-L30 protein

[10]Groups as described in Kataoka et al. (1997), grouping on the basis of partial 16S rRNA gene sequences containing a variable α region

[11]Groups as described in Labeda and Lyons (1991b) = L1, Labeda and Lyons (1991a) = L2, Labeda (1998) = L3, Labeda (1996) = L4, Labeda (1993) = L5, grouping on the basis of DNA relatedness

[12]Groups as described in Schumann (unpublished), grouping on the basis of ribotyping

[13]Groups as described in Lanoot et al. (2004), grouping on the basis of Box-PCR

[14]Groups as described in Lanoot et al. (2002), grouping on the basis of protein profiles

[15]Groups as described in Lanoot (2004), grouping on the basis of DNA-DNA hybridization

[16]Groups as cited in Lanoot (2004), grouping on the basis of DNA-DNA hybridization

[17]Groups as described in Lanoot (2004), grouping on the basis of ARDRA

[18]Groups as described in Lanoot (2004), grouping on the basis of ARDRA

[19]Groups as described in Lanoot (2004), grouping on the basis of analysis of the ITS region

[20]Groups as described in Lanoot (2004), grouping on the basis of 16S rRNA-ITS RFLP

[21]Groups as described by Labeda et al. (2012), clades represnet species that cluster together (bootstrap values >60% value) in Neighbor-joining tree based on 16S rRNA gene sequences

[22]Groups as described in Kim et al. (2004), grouping on the basis of *rpoB* gene sequences; + = strains that were used in this study; Group A16, group A18, group A1B, group F, Kitasatospora and Asn(AAC) = names of species groups based on the rpoB gene according to Kim et al. (2004)

[23]Groups as described in Guo et al. (2008), grouping on the basis of multilocus phylogeny calculated with the sequences of five housekeeping genes (*atpD*, *gyrB*, *recA*, *rpoB*, *trpB*) and the 16S rRNA gene

[24]+ = strains that were used in the study of Guo et al. (2008) but did not belong to one of the four detected groups; yes = strains that show the same grouping (I–IV) as in the 16 S rRNA gene sequence tree; no = strains do not show the same grouping (I–IV) as in the 16S rRNA gene sequence tree

[25]Groups as described by Rong and Huang (2010), grouping on basis on 16S rRNA gene or multilocus phylogeny using the scheme of Guo et al. (2008)

[26]Groups as described by Rong et al. (2009), grouping on basis on 16S rRNA gene or multilocus phylogeny using the scheme of Guo et al. (2008)

[27]Groups as described by Rong and Huang (2012), grouping on basis on 16S rRNA gene or multilocus phylogeny using the scheme of Guo et al. (2008)

[28]Groups as described by Han et al. (2012), grouping on basis on multilocus phylogeny using the scheme of Guo et al. (2008) and concatenated 5S-16S-23S rRNA gene sequence phylogeny

[29]Groups as described by Labeda et al. (2012), grouping on basis on multilocus phylogeny using the scheme of Guo et al. (2008)

[30]Morphological characters of species described before 1974 according to Pridham and Tresner (1974) and Baldacci and Locci (1974). n = not determined

I: Spore color en masse indicated as W (White), GY (Gray), Y (Yellow), R (Red), B (Blue), G (Green), V (Violet), Bl (Black), Ba (substrate mycelium pink-red to orange-red, aerial mycelium pink, gray-pink and violet-pink), Bi (substrate mycelium colorless, reddish and orange, yellow to brick red, aerial mycelium pinkish white), Hi (substrate mycelium brick red, aerial mycelium beige to pink-beige), Sa (substrate mycelium brick-red to orange, aerial mycelium white with pink and yellow shades), Lu (substrate mycelium yellow, yellowish to brown, aerial mycelium light yellow, yellowish to beige), Gr (substrate mycelium brownish yellow, aerial mycelium pinkish beige with lilac shades), Ci (substrate mycelium yellow to greenish, yellow and brown-yellow, aerial mycelium pinkish with beige and lilac shades), Ar (substrate mycelium light yellow to yellowish to pinkish yellow, aerial mycelium basically white with yellow, pink and gray), Ke (substrate mycelium yellow, yellowish to hazel-nut yellow, aerial mycelium beige with shades toward yellow, pink and cinnamon), Mo (substrate mycelium yellow to greenish yellow, aerial mycelium grayish green), Li (substrate mycelium brown, aerial mycelium pinkish white), Th (substrate mycelium brown-yellow to greenish, aerial mycelium light yellow)

II: Spore chain morphology indicated as RF (Rectus Fexibilis), S (Spira), VE (Verticil), RA (Retinaculum-Apertum), SC (straight chains). L (loop)

III: Melanoid pigments produced C+, not produced C−.

IV: Spore wall ornamentation indicated as SM (smooth), SPY (spiny), H (hairy), WTY (warty), RU (rugose)

[31]Physiological tests of species described before 1974 according to Pridham and Tresner (1974) and Baldacci and Locci (1974). + = utilization of carbon compounds positive, − = utilization of carbon compounds negative, n = not determined

I: D-Glucose

II: D-Xylose

III: L-Arbinose

IV: L-Rhamnose

IV: L-Rhamnose

VI: D-Galactose

VII: Raffinose

VIII: D-Mannitol

IX: i-Inositol

X: Salicin

XI: Sucrose

found to possess unique sequences, while the remaining strains were assigned to 15 groups. In a more extensive study, the c region sequences of 485 *Streptomyces* strains were deposited in GenBank, which was the first large set of streptomycete 16S rDNA sequence data that was publicly available (Kataoka et al. 1997). Anderson and Wellington (2001) published a phylogenetic tree based on the comparison of the *c* regions of representatives of the major cluster groups defined by Williams et al. (1983a). In this study the taxonomic status of the phenotypic groups was confirmed, apart from the *S. olivaceoviridis* and *S. griseoruber* strains which displayed identical c regions; these strains were present in clusters 20 and 21 of Williams et al. (1983a), respectively, and however formed cluster 9 by Kämpfer et al. (1991). The sequence data also displayed that the 60 strains, earlier assigned to three species groups in the *Streptomyces albidoflavus* group (Williams et al. 1983a), could be sorted into six groups (Kataoka et al. 1997); the three phenotypic subgroups of Williams et al. (1983a) were maintained, but did not cluster together.

Hain et al. (1997) designed 16S rRNA oligonucleotide probes to identify intraspecific relationships within the *Streptomyces albidoflavus* group and discovered that the resulting sequences were helpful for species differentiation but not for delineating strains. The intergenic 16S-23S rRNA spacer regions were found to be more suitable for the delineation of intraspecific relationships within that cluster. In addition, genus-specific probes have also been developed based on the 5S rRNA gene (Park et al. 1991) and the 23S rRNA gene (Mehling et al. 1995) sequences. By using 5S rRNA sequence data, it was possible to confirm the reclassification of the genera *Chainia*, *Elytrosporangium*, *Kitasatoa*, *Microellobosporia*, and *Streptoverticillium* into the genus *Streptomyces* (Park et al. 1991).

By now, for nearly all *Streptomyces* type strains complete 16S rRNA gene sequences are available from public databases. In Kämpfer (2012) a phylogenetic tree based on nearly full-length 16S rRNA gene sequences was calculated including type strains of all species of the genera *Streptomyces*, *Kitasatospora*, and *Streptacidiphilus*. Grouping in that tree was the basic for species arrangement given in ❯ *Table 42.2*. A phylogenetic 16S rRNA gene sequence-based tree containing all current *Streptomyces* species is shown in ❯ *Fig. 42.1* showing a similar clustering to the previously published tree (Kämpfer 2012). A comprehensive study based on nearly full-length 16S rRNA gene sequences was currently also performed by Labeda et al. (2012). Within this study 130 clades were defined based on clustering of species in a phylogenetic trees calculated with the neighbor-joining method. Clades defined by Labeda et al. are also listed in ❯ *Table 42.2*. Each clade represented a cluster of species, which was supported by high bootstrap value (>60 %). A comparison with phylogenetic trees calculated with different treeing methods showed that 106 of the clades were confirmed in phylogenetic trees generated with the maximum-parsimony and maximum-likelihood treeing methods but the relationship among the defined clades changed dependent on the treeing methods used. This illustrated the limited resolution of the 16S rRNA gene sequence-based analysis for species delineation within the *Streptomycetaceae*. The variations within the 16S rRNA genes—even in the variable regions—are too small to

resolve problems of species differentiation and to establish taxonomic structure within the genus (Witt and Stackebrandt 1990; Stackebrandt et al. 1991, 1992; Andersen and Wellington 2001). The fact that *Streptomyces* species may harbor different 16S rRNA gene sets causes further difficulties. For example, *S. coelicolor* A3(2), *S. lividans*, and several other *Streptomyces* species contain six ribosomal rRNA gene sets, whereby each set of rRNA genes includes one gene copy for 5S, 16S, and 23S rRNA (van Wezel et al. 1991) and lacks tRNA genes. So far, phylogenetic analysis based on 16S rRNA genes and resulting clustering may be the basis for further studies of highly similar species to reexamine or evaluate species delineation within the *Streptomycetaceae*. Comprehensive comparative studies of closely related species including DNA-DNA hybridization studies, investigation of protein-coding gene sequences with higher phylogenetic resolution, or even comparison based on core genomes are needed to clarify the species delineation within the *Streptomycetaceae*.

Next to rRNA genes, single protein-coding genes with a higher phylogenetic resolution were used to establish inter- and intraspecies level relationships within the genus. Hatano et al. (2003) studied the partial sequences of the *gyrB* gene of 64 whorl-forming streptomycetes. The *gyrB* gene encodes the B-subunit of DNA gyrase. Most members of the 46 species, eight subspecies, and 13 invalidly published species (including 10 strains studied by the International *Streptomyces* Project [ISP]) examined were assigned to two major groups. The larger group, which contained typical whorl-forming species (59 strains), was further subdivided into six major clusters of three or more species, seven minor clusters of two species, and five single-member clusters at the 97 % *gyrB* sequence similarity level. The major clusters included *S. abikoensis*, *S. cinnamoneus*, *S. distallicus*, *S. griseocarneus*, *S. hiroshimensis*, and *S. netropsis* strains, results that were consistent with previous data from phenotypic analyses. Members within each major and minor cluster proved to be very similar, with the exception of the *S. netropsis* cluster (which was phenotypically divided into the *S. netropsis* and *S. eurocidicus* subclusters) and the *S. distallicus* cluster (which was divided into the *S. distallicus* and *S. stramineus* subclusters). In addition to the cluster assignment, Hatano et al. classified these 59 strains of typical whorl-forming *Streptomyces* species into the following 18 species (including subjective synonym(s)): *S. abikoensis*, *S. ardus*, *S. blastmyceticus*, *S. cinnamoneus*, *S. eurocidicus*, *S. griseocarneus*, *S. hiroshimensis*, *Streptomyces lilacinus*, *S. luteoreticuli*, *S. luteosporeus*, *S. mashuensis*, *S. mobaraensis*, *S. morookaense*, *S. netropsis*, *S. orinoci*, *S. stramineus*, *S. thioluteus*, and *S. viridiflavus* (Table 42.2). All of the strains, which displayed 98.5–100 % *gyrB* sequence similarities, had also high DNA-DNA similarities (70–100 %), suggesting that *gyrB* sequences give a better resolution than corresponding 16S rRNA gene sequences.

By sequencing the RNA polymerase ß-subunit genes (*rpoB*) and 16S rRNA genes of 57 *Streptomyces* strains, five *Kitasatospora* strains, and a single *Micromonospora* strain, Kim et al. (2004) found that the resulting phylogenetic trees had similar topologies. Moreover, they found good concordance between the *rpoB* sequence and corresponding numerical phenetic data of Williams et al. (1983a). In the *rpoB* gene tree, the five *Kitasatospora* strains were clearly separated from the *Streptomyces* strains. These results illustrate that sequence analysis of additional genes (i.e., other housekeeping genes) will help to give a better insight into the intraspecific structure of the genus *Streptomyces* (Stackebrandt et al. 2002).

Beside 16S rRNA gene or single protein-coding gene phylogenies, multilocus sequence analysis (MLSA) was introduced in prokaryotic taxonomy because multilocus protein-coding gene sequence studies enable a higher phylogenetic resolution among species within genera or among closely related genera (Stackebrandt et al. 2002; Tindall et al. 2010). In MLSA studies approximately six to ten partial sequences of protein-coding genes, mainly conserved housekeeping genes, are used for phylogenetic studies. Single gene tree phylogenies and mainly concatenated sequence phylogenies are analyzed and, compared to the 16S rRNA gene-based phylogeny, reveal most often a higher and more stable phylogenetic resolution within a genus or closely related genera. MLSA schemes can be applied for the differentiation of prokaryotic species but have to be validated by DNA-DNA hybridization experiments (Tindall et al. 2010). Guo et al. (2008) established an MLSA scheme for the genus *Streptomyces* including partial sequences of the 16S rRNA gene and five housekeeping genes, the ATP-synthase F1 b-subunit (*atpD*), the DNA gyraseB subunit (*gyrB*), the recombinase A (*recA*), the RNA polymerase b-subunit (*rpoB*), and the tryptophan synthase b-subunit (*trpB*). With exception of the primers for *gyrB* (Hatano et al. 2003), all primers for amplification and sequencing of partial gene sequences were designed by Guo et al. based on full-length gene sequences of genome sequenced *Streptomyces* strains. The MLSA scheme was first supplied to study 45 valid species and subspecies of the *Streptomyces griseus* 16S rRNA gene clade. Guo et al. showed that all protein-coding gene sequences had a higher number of variable sides among the investigated strains (up to 48 % for the partial *gyrB* sequence) compared to the nearly full-length 16S rRNA gene (15.5 %). Several of the investigated species were assigned to four clusters, which included type strains with identical 16S rRNA gene sequences. Some of the strains with identical 16S rRNA gene sequences were assigned to different clusters, when phylogenetic trees were constructed based on the housekeeping gene sequences (clusters shown in Table 42.2). The trees based on individual gene sequences gave a slightly different resolution between strains compared to the tree based on concatenated sequences. It was concluded by the authors that phylogenetic trees generated on more than one gene sequence are more reliable and have a higher resolution power and topological stability. The study of the *S. griseus* 16S rRNA gene clade was enlarged by the inclusion of 18 more species in a subsequent study performed by Rong and Huang (2010). Again type strains that shared identical 16S rRNA gene sequences were grouped together, and single and concatenated protein-coding gene sequence phylogenies showed a higher resolution with differences in cluster formation (see Table 42.2). Including DNA-DNA hybridization values and phenotypic data, Rong and Huang (2010)

proposed the combination of 29 species and three subspecies of the *S. griseus* 16S rRNA gene clade as 11 genomic species; *S. griseinus* and *S. mediolani* were reclassified as later heterotypic synonyms of *S. albovinaceus*; *S. praecox* as a later heterotypic synonym of *S. anulatus*; *S. olivoviridis* as a later synonym of *S. atroolivaceus*; *S. griseobrunneus* as a later heterotypic synonym *of S. bacillaris*; *S.cavourensis* subsp. *washingtonensis* as a later heterotypic synonym of *S. cyaneofuscatus*; *S.acrimycini*, *S. baarnensis*, *S. caviscabies*, and *S. flavofuscus* as later heterotypic synonyms of *S. fimicarius*; *S. flavogriseus* as a later heterotypic synonym of *S. flavovirens*; *S. erumpens*, *S. ornatus*, and *S. setonii* as later heterotypic synonyms of *S. griseus*; *S. graminofaciens* as a heterotypic synonym of *S. halstedii*; *S. alboviridis*, *S. griseus* subsp. *alpha*, *S. griseus* subsp. *cretosus*, and *S. luridiscabiei* as later heterotypic synonyms of *S. microflavus*; and *S. californicus* and *S. floridae* as later heterotypic synonyms of *S. puniceus*.

Similar to the studies of Rong and Huang (2010), the MLSA scheme of Guo et al. was used in combination with DNA-DNA hybridization studies to reclassify the *Streptomyces albidoflavus* 16S rRNA gene clade (Rong et al. 2009). Within that study ten species and subspecies were reclassified as heterotypic synonyms of *S. albidoflavus* including *S. canescens*, *S. champavatii*, *S. coelicolor*, *S. felleus*, *S. globisporus* subsp. *caucasicus*, *S. griseus* subsp. *solvifaciens*, *S. limosus*, *S. odorifer*, and *S. sampsonii*.

Rong and Huang (2012) used the MLSA scheme to investigate the *S. hygroscopicus* 16S rRNA gene clade including *S. violaceusniger* and related species as well as five valid subspecies of the species *S. hydroscopicus*. In all, 58 species and subspecies were included in the study. Based on 16S rRNA gene sequence phylogenetic analysis, the authors grouped the investigated species into 13 clusters (I–XIII; see *Table 42.2*), which were supported by high bootstrap values. Similar to the previous studies, the 16S rRNA gene clusters were compared to clusters obtained by phylogenetic analysis based on concatenated protein-coding gene analysis. Again MLSA showed differences in clustering with higher phylogenetic resolution compared to that obtained by 16S rRNA gene-based analysis. Based on MLSA, DDH, and phenotypic data, 18 species and two subspecies were proposed to be heterotypic synonyms of eight genomic species; *S. luteoverticillatus* was reclassified as later heterotypic synonym of *S. ehimensis*; *S. demainii*, *S. endus*, and *S. sporocinereus* as heterotypic synonyms of *S. hygroscopicus* subsp. *hygroscopicus*; *S. yogyakartensis* as later heterotypic synonym of *S. javensis*; *S. libani* subsp. *libani* as later heterotypic synonym of *S. nigrescens*; *S. olivaceiscleroticus* as later heterotypic synonym of *S. niger*; *S. aureoversilis* as later heterotypic synonym of *S. rectiverticillatus*; *S. asiaticus*, *S. cangkringensis*, and *S. indonesiensis* as later heterotypic synonyms of *S. rhizosphaericus*; and *S. antimycoticus* and *S. sporoclivatus* as later heterotypic synonyms of *S. castelarensis*. In addition the two *S. hydroscopicus* subspecies *S. hydroscopicus* subsp. *globosus* and *S. hydroscopicus* subsp. *oassamyceticus* were reclassified to two separate species, *S. globosus* and *S. oassamyceticus*. Both clustered in different clusters determined by the MLSA phylogenetic analysis.

Based on the correlation of MLSA and DNA-DNA hybridization studies including data for the *S. griseus* (Rong and Huang 2010), the *S. albidoflavus* (Rong et al. 2009), and the *S. hydroscopicus* 16S rRNA gene clade (Rong and Huang 2012), Rong and Huang (2012) proposed an MLSA-based species cut-off value for the species *Streptomyces*. A five gene MLSA evolutionary distance of 0.007 showed a good correlation with the 70 % DDH values as species threshold. The authors illustrated that in contrast to the concatenated data, none of the single gene-based evolutionary distances showed a good correlation to the DDH values indicating the single gene tree phylogenies may not be sufficiently reliable to resolve *Streptomyces* species and do not have the power for species delineation.

The five-gene-based MLSA scheme of Guo et al. (2008) was furthermore applied by Labeda (2011) for the investigation of 10 phytopathogenic *Streptomyces* species including *S. scabiei*, *S. acidiscabies*, *S. europaeiscabiei*, *S. luridiscabiei*, *S. niveiscabiei*, *S. puniciscabiei*, *S. reticuliscabiei*, *S. stelliscabiei*, *S. turgidiscabies*, and *S. ipomoeae*, and an additional 52 species of which 19 species were closely related to the phytopathogenic species based on 16S rRNA gene analysis. The application of the MLSA scheme enabled a better and more reliable phylogenetic resolution of the investigated species. Reclassifications were not performed within that study.

Labeda et al. (2012) gave some examples illustrating the application of the MLSA scheme developed by Guo et al. 2008 where the uncertain affiliation of *Streptomyces* species obtained by 16S rRNA gene sequence-based analysis was resolved more clearly. For example, the stable 16S rRNA clade 112 contained species, which were proposed as heterotypic synonyms of *Streptomyces albidoflavus* by Rong et al. (2009), including *S. canescens*, *S. champavatii*, *S. coelicolor*, *Streptomyces felleus*, *S. globisporus* subsp. *caucasicus*, *S. griseus* subsp. *solvifaciens*, *S. limosus*, *Streptomyces odorifer*, and *S. sampsonii*. Here, the MLSA studies confirmed the high phylogenetic similarity obtained by 16S rRNA gene sequence analysis and supported the reclassification of species as heterotypic synonyms. In contrast, MLSA data confirmed the clustering of *S. reticuliscabiei* and *S. turbiscabiei* (Labeda clade 22), but based on single housekeeping gene sequence-based analysis (*atpD* and *trpB*), the species could clearly be distinguished as it was proposed earlier (Bouchek-Mechiche et al. 2006). With those and further examples, Labeda et al. clearly demonstrated that MLSA data could help to resolve the phylogenetic resolution within the genus *Streptomyces*.

Furthermore the study of Han et al. (2012) also showed that the application of the MLSA scheme of Guo et al. (2008) helped to distinguish species of the genera *Streptomyces*, *Kitasatospora*, and *Streptacidiphilus*. The phylogenetic tree based on concatenated protein-coding gene sequences revealed the formation of two distinct separate clusters including *Kitasatospora* and *Streptacidiphilus* species, respectively. However, single-gene-based phylogenies did not fully support the separation of the three genera. In all, 33 *Streptomyces*, 10 *Kitasatospora*, and 9 *Streptacidiphilus* type strains were investigated in that study. One result obtained was that *S. xanthocidcus* clustered within the *Kitasatospora* cluster, which was also confirmed by the

phylogenetic analysis performed in parallel based on concatenated sequence of ribosomal genes including the 5S, 16S, and 23S rRNA gene. Based on those data Han et al. proposed the transfer of this *Streptomyces* species to the genus *Kitasatospora*.

All current data indicated that the application of the MLSA scheme of Guo et al. (2008) enables a better phylogenetic resolution within the *Streptomycetaceae* compared to the 16S rRNA gene sequence phylogeny and is promising to replace DDH studies. However, MLSA studies should be treated with care because the resolution of the concatenated sequence base tree is strongly affected by single genes included in the analysis. MLSA studies are performed based on nucleotide sequences, and differences among closely related *Streptomyces* species are often based on synonymous substitutions of the third codon position, which may lead to an overestimation of the phylogenetic resolution. Therefore it is recommended to include a comparison of respective protein sequences because the amino acid sequences are considered to be a more reliable and stable indicator of phylogenetic relationships, since the function of proteins is closely tied to their primary sequences, and the pressure of selection only works at the protein level (Kämpfer and Glaeser 2012). Furthermore interpretations of MLSA studies based on different single gene-based affiliations within the *Streptomycetaceae* need to acknowledge that *Streptomyces* species are strongly affected by homologous recombination events and horizontal gene transfer, which may negatively impact on MLSA results (Doroghazi and Buckley 2010). In the current era of high throughput sequencing, the comparison of (partial) genome sequence data, e.g., by calculating and comparing average nucleotide identity (ANI) values, will be more promising to resolve the phylogenetic relationships of closely related *Streptomyces* species in future studies.

Rapid Methods for Gene Analysis in *Streptomycete* Taxonomy

Various alternative methods for gene analysis have been described which do not require sequencing. These methods involve either restriction analysis (Clarke et al. 1993; Fulton et al. 1995) or specialized gel electrophoresis techniques, which are used to examine the mobility of products (Hain et al. 1997; Heuer et al. 1997). Clarke et al. (1993) used a combination of several restriction enzymes, including *Bgl*I, *Eco*RI, *Pst*I, and *Pvu*II, to obtain restriction fragment length polymorphism (RFLP) patterns of purified rRNA extracted from members of the *Streptomyces albidoflavus* cluster (subgroups 1A and 1B of Williams et al. (1983a)). By using this approach they were able to differentiate between phenotypically similar strains, even though profiles differed considerably between *S. albidoflavus* species groups. Fulton et al. (1995) used *Mse*I fingerprints of rRNA operons (RiDiTS) for ribosomal restriction analysis of 98 named streptomycete strains, including members of cluster groups A (comprising clusters 1–41) and F (comprising clusters 55–67) of Williams et al. (1983a) as well as some other strains, which resulted in 11 pattern types with varying degrees of similarity to the Williams subclusters. Cluster groups A and F were discriminated although at a low resolution (70 % similarity), but individual clusters could not be.

Other techniques used to evaluate genotypic variation between streptomycetes include denaturing gradient gel electrophoresis (DGGE; Muyzer et al. 1993) with or without DNA-binding agents (Hain et al. 1997). Anderson and Wellington (2001) recommended DGGE in combination with other techniques. By using variable 16S rRNA regions, this method enables the differentiation of genus and species groups. Isolates ASB33, ASB37, and ASSF22 were assigned to *S. albidoflavus*, *S. griseoruber*, and *S. albidoflavus*, respectively, by using a combination of methods, including sequence comparisons, PFGE, and numerical taxonomy (Huddleston et al. 1995, 1997).

Phage Typing

Phage typing can be used for host identification at the genus and the species levels (Welsch et al. 1957; Kutzner 1961a, b; Korn et al. 1978; Wellington and Williams 1981a). For streptomycetes, two different groups of phages exist, in particular polyvalent phages (e.g., ϕC31; Chater et al. 1986) and species-specific phages (Andersen and Wellington 2001; ❯ *Table 42.3*). Several actinophages, mainly virulent, have been used for phage typing and are specific at the genus level (e.g., Wellington and Williams 1981a; Korn-Wendisch 1982; Prauser 1984). Actinophage host range studies helped to substantiate the transfer of the genera *Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Microellobosporia*, *Kitasatoa*, and *Streptoverticillium* to the genus *Streptomyces* (Goodfellow et al. 1986a, b, c, d; Witt and Stackebrandt 1990). Likewise, other studies supported the transfer of *Actinoplanes armeniacus* to the genus *Streptomyces* (Kroppenstedt et al. 1981; Wellington and Williams 1981b) and *Streptomyces erythraeus* to the genus *Saccharopolyspora* (Labeda 1987). Phage typing has been shown to be less useful for species or group identification of *Streptomyces*; nevertheless, there are a few exceptions (❯ *Table 42.3*).

Phages are widely used in genetic studies (see Chater 1986) and in industrial microbiology (Carvajal 1953; Ogata 1980), as illustrated in chapter 12 of Kieser et al. (2000). The temperate phage, ϕC31, has a broad host range within the genus *Streptomyces* and is one of the best-investigated temperate actinophages (Lomovskaya et al. 1980). This phage has been employed for various purposes (e.g., transfection, transduction, detection of transposon-like elements of host DNA, and cloning); details can be found in chapter 12 of Kieser et al. (2000).

Protein Profiling

Total protein extracts can be analyzed by polyacrylamide gel electrophoresis (PAGE), resulting in more or less complex banding patterns. These patterns have been used to elucidate relationships between species and subspecies level of various bacterial genera. By using one-dimensional (1-D) and

Table 42.3

Species-specific actinophages of the genus *Streptomyces* (Modified according to Andersen and Wellington 2001)

Actinophage	Host reference strain	Host species group	Host cluster No.[a]	Cluster No.[b]	References
33	*S. scabies* ATCC 23962	*S. atroolivaceus*	3	1–3	Wellington and Williams (1981a)
41	*S. matensis* ATCC 23935^T	*S. rochei*	12	6	Wellington and Williams (1981a)
89, DP 9	*S. griseus* ATCC 23345^T	*S. albidoflavus*	1B	1–3	Wellington and Williams (1981a)
90	*S. griseinus* ATCC 23915^T	*S. albidoflavus*	1B	1–3	Wellington and Williams (1981a)
98	*S. coelicolor* Müller ATCC 23899^T	*S. albidoflavus*	1A	1–1	Wellington and Williams (1981a)
100	*S. caesius* ATCC 19828	*S. griseoruber*	21	6	Wellington and Williams (1981a)
SV1, SV2	*S. venezuelae* ATCC 10712^T	*S. violaceus*	6	2	Stuttard (1982)
SAt1	*S. azureus* ATCC 14921^T	*S. cyaneus*	18	9	Ogata et al. (1985)
S3	*S. albus* DSM 40313^T	*S. albus*	16	32	Korn-Wendisch and Schneider (1992)
4, 5a, 5b, 49	*S. violaceoruber* DSM 40049^T	*S. violaceoruber*	SMC*	69	Korn-Wendisch and Schneider (1992)
14, 24, 233	*S. coelicolor* Müller ATCC 23899^T	*S. albidoflavus*	1A	1–1	Korn-Wendisch and Schneider (1992)

[a]Clusters according to Williams et al. (1983 a, b); SMC, single-member cluster (Williams et al. 1989)
[b]Clusters according to Kämpfer et al. (1991)

two-dimensional (2-D) protein electrophoresis, it is possible to determine such protein patterns. Manchester et al. (1990) used one-dimensional protein electrophoresis to analyze 37 *Streptomyces* strains, including 5 streptoverticillia, and observed some taxonomic correlations between the resulting profiles, groups established on phenotypes (Williams et al. 1983a; Kämpfer et al. 1991), and DNA hybridization data (*Table 42.2*). Nevertheless, only few of these correlations were confirmed by Lanoot et al. (2002).

A combination of PAGE and DNA-DNA hybridization was used to clarify the taxonomy of *Streptomyces* isolates that caused common potato scab (Paradis et al. 1994). By sodium dodecylsulfate (SDS)-PAGE analysis, the isolates could be assigned to two groups with a correlation coefficient of 0.75. The same groups were recovered by DNA-DNA hybridization, but not in the corresponding fatty acid analysis. This lack of correlation is due to the influence of growth conditions on fatty acid profiles (Saddler et al. 1986, 1987). Protein profiling does not allow the discrimination of pathogenic and nonpathogenic *Streptomyces* strains. Lanoot et al. (2002) used SDS-PAGE of whole-cell proteins in a study of 93 *Streptomyces* reference strains. Twenty-four clusters were established by subsequent computer-assisted numerical analysis and included strains with very similar protein profiles. Several type strains were assigned to five clusters, which had visually identical patterns. DNA-DNA hybridizations of these type strains displayed similarities higher than 70 %. Based on these results, *Streptomyces albosporeus* subsp. *albosporeus* LMG 19403^T was considered to be a subjective synonym of *Streptomyces aurantiacus* LMG 19358^T, *Streptomyces aminophilus* LMG 19319^T a subjective synonym of *Streptomyces cacaoi* subsp. *cacaoi* LMG 19320^T, *Streptomyces niveus* LMG 19395^T and *Streptomyces spheroides* LMG 19392^T subjective synonyms of *Streptomyces caeruleus* LMG 19399^T, and *Streptomyces violatus* LMG 19397^T a subjective synonym of *Streptomyces violaceus* LMG 19360^T (*Table 42.2*).

Two-dimensional PAGE of the total cellular proteins provides greater resolution than one-dimensional analysis. Very complex patterns can be obtained with 2-D PAGE, yet this method seems to be too sensitive to discriminate between proteins with high rates of evolution (Hori and Osawa 1987). 2-D PAGE studies designed to differentiate between ribosomal proteins of streptomycetes were first described by Mikulik et al. (1982) and later Ochi (1989). Hence, ribosomal AT-L30 proteins were found to give genus-specific profiles (Ochi 1992), whereas analyses of the N-terminal sequences of the AT-L30 protein allowed the assignment of streptomycete strains to different taxonomic groups (Ochi 1995). These groups were classified to phylogenetic groupings which suggested that the genus *Streptomyces* was well described. However, no correlation was found between Ochi's groupings and earlier established numerical phenetic groups (Williams et al. 1983a; Kämpfer et al. 1991). Details of these groupings are presented in *Table 42.2*.

Multilocus enzyme electrophoresis (MLEE) is based on the relative mobilities of cellular enzymes in a gel matrix. MLEE produces more specific patterns when compared to protein profiling. For instance, inter- and intraspecific characterization of 24 *Streptomyces* strains was achieved in a small study, when the appropriate enzymes were used (Oh et al. 1996).

The isolation and sequencing of specific proteins resulted in more detailed taxonomic studies of some *Streptomyces*. For example, the *Streptomyces* subtilisin inhibitor protein (SSI),

which plays a role in development (reviewed by Chater et al. (2010)), was used by Taguchi et al. (1996) to clarify the taxonomic status of *S. lividans* 66, *S. coelicolor* Müller ISP 5233^T, and *S. coelicolor* A3(2). Alignments of the SSI sequences supported the results of ribosomal sequence comparisons indicating that *S. coelicolor* A3(2) is more closely related to *S. lividans* 66 (cluster 21 of Williams et al. (1983a)) than to the type strain, *S. coelicolor* Müller ISP 5233^T (cluster 1).

Phenotypic Analyses

Genus *Streptomyces* Waksman and Henrici 1943, 339^{AL} emend. Witt and Stackebrandt 1990, 370 emend. Wellington, Stackebrandt, Sanders, Wolstrup and Jorgensen, 1992, 159

Strep.to.my'ces. Gr. adj. *streptos*, pliant, bent; Gr. n. *myces*, fungus; M.L. masc. n. fungus. *Streptomyces*, pliant or bent fungus[1].

The type species is *Streptomyces albus* (Rossi-Doria 1891) Waksman and Henrici 1943, 339. *Streptomyces* are Gram-positive, aerobic, non-acid-fast bacteria, which form extensively branched substrate and aerial mycelia. They are chemoorganotrophic with an oxidative type of metabolism. The vegetative hyphae, with a diameter of 0.5–2.0 μm, rarely fragment. At maturity the aerial mycelium generates chains of three to many spores. Some species form short chains of spores on the substrate mycelium and others have pycnidial-, sclerotia-, sporangia-, and synnemata-like structures. The spores are nonmotile. Frequently, colonies initially have a smooth surface but later develop a weft of aerial mycelium that may appear floccose, granular, powdery, or velvety. Colonies are discrete and lichenoid, leathery, or butyrous. *Streptomyces* produce a wide range of pigments responsible for the color of the vegetative and aerial mycelia. Additionally, colored diffusible pigments may be generated. Many strains are able to produce one or more antibiotic substances.

The optimal growth temperature for most species lies in the range of 25–35 °C. However, some species can grow at temperatures within the psychrophilic and thermophilic range. The optimum pH range for growth is between 6.5 and 8.0. They are widely distributed and abundant in soil, including composts. A few species are phytopathogens; a few others are pathogenic for humans and animals. *Streptomyces* are catalase positive. They usually degrade polymeric substrates such as casein, gelatin, hypoxanthine, and starch in addition to adenine and L-tyrosine and reduce nitrates to nitrites. Most species utilize a variety of organic compounds as sole carbon sources for energy and growth. The cell wall peptidoglycan contains major quantities of LL-diaminopimelic acid (L-A_2pm). Sometimes, low amounts of *meso*-diaminopimelic acid (*meso*-A_2pm) are present. The lipid profile contains major amounts of saturated, *iso*- and *anteiso*-fatty acids but lacks mycolic acids. In addition, they typically possess either hexa- or octa-hydrogenated menaquinones with nine isoprene units as the predominant isoprenolog, but menaquinones with eight and ten isoprene units are also found. A complex polar lipid pattern normally contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The mol% G+C content of the DNA lies between 69 and 78 (T_m).

[1] AL denotes the inclusion of this name on the Approved Lists of Bacterial Names (1980).

Table 42.4
Spore colors of aerial mycelium for grouping of streptomycetes and representative species of each color group (According to Korn-Wendisch and Kutzner 1992)

Color of aerial mycelium	Representative species (DSM no.)[a]
Blue: "azureus"	*S. viridochromogenes* (40110); *S. cyaneus* (40108)
Blue-green: "glaucus"	*S. glaucescens* (40155)
Brown (plus gray or red)	*S. eurythermus* (40014); *S. fragilis* (40044)
Gray: "cinereus"	*S. violaceoruber* (40049); *S. echinatus* (40013)
Gray pink/lavender: "cinnamomeus"	*S. lavendulae* (40069); *flavotricini* (40152)
Green: "prasinus"	*S. prasinus* (40099); *S. hirsutus* (40095)
Pink/light violet	*S. fradiae* (40063); *S. toxytricini* (40178)
White; "niveus"	*S. albus* (40313); *S. longisporus* (40166)
Yellow gray: "griseus"	*S. griseus* (40236); *S. coelicolor* (40233)
Not definable: white plus various light-colored shades	*S. alboniger* (40043); *S. rimosus* (40260)

[a]DSM no. 40XXX = ISP no. 5XXX; e.g., 40236 = ISP 5236

Differentiation of the Genus *Streptomyces* from Other Genera

Unknown actinomycetes are generally assigned to genera based on 16S rRNA gene sequence analyses. However, it may turn out to be problematic, especially in the case of *streptomyces*, to distinguish between species by using this approach (e.g., Stackebrandt et al. 1991, 1992; Kumar & Goodfellow 2002).

Members of the genus *Streptomyces* can usually be differentiated from other filamentous actinomycetes by their colony morphology (*Tables 42.4* and *42.5*), notably by substrate mycelium, aerial spore mass, and soluble pigment colors. Traditional methods highly recommended for this purpose are described by Korn-Wendisch and Kutzner (1992). A computer-assisted numerical analysis on the basis of the color-grouping method of Williams et al. (1969) was used by Antony-Babu et al. (2010) to group 321 alkaliphilic streptomycetes grown on

Table 42.5
Colors of substrate mycelium and soluble pigment occurring in streptomycetes (According to Korn-Wendisch and Kutzner 1992)

Color of substrate mycelium	Representative species (DSM no.)[a]
Gray brown to black (mainly endopigment)	*S. alboniger* (40043); *S. hygroscopicus* (40578)
	S. purpeofuscus (40283); *S. mirabilis* (40553)
Green (endopigment)	*S. malachiticus* (40167); *S. malachitorectus* (40333)
Green to gray olive (endo- and exopigment)	*S. flavoviridis* (40210); *S. olivoviridis* (40211)
	S. viridochromogenes (40110); *S. nigrifaciens* (40071)
Red brown to dark brown (endo- and exopigment)	*S. badius* (40139); *S. eurythermus* (40014)
	S. phaeochromogenes (40073); *S. ramulosus* (40100)
Red to blue/violet (mainly endopigment)	*S. californicus* (40058); *S. cinereoruber* (40012)
	S. violaceus (40082); *S. purpurascens* (40310)
Red violet to blue (endo- and/or exopigment)	*S. coelicolor* (40233); *S. cyaneus* (40108)
	S. violaceoruber (40049); *S. lateritius* (40163)
Orange to dark red (mainly endopigment)	*S. aurantiacus* (40412); *S. griseoruber* (40275)
	S. longispororuber (40599); *S. spectabilis* (40512)
Yellow orange/greenish yellow (endo- and exopigment)	*S. atroolivaceus* (40137); *S. canarius* (40528)
	S. galbus (40089); *S. tendae* (40101)

[a]DSM no. 40XXX = ISP no. 5 XXX

oatmeal agar (ISP 3) and peptone-yeast extract-iron agar (ISP medium 6). Using this approach, large numbers of streptomycetes can be assigned without using polyphasic taxonomic approaches.

Furthermore, *streptomyces* can often be distinguished from other filamentous actinomycetes on the basis of morphological characteristics, in particular by vegetative mycelium, aerial mycelium, and arthrospores (◗ *Figs. 42.2*–◗ *42.6*). Detailed information on the procedure used to determine such properties can be found in Korn-Wendisch and Kutzner (1992) and chapter 3 of Kieser et al. (2000). Members of the genus *Streptomyces* can also be differentiated from related taxa using chemotaxonomic methods (Lechevalier and Lechevalier 1970b). Streptomycetes typically produce major quantities of *iso*- and *anteiso*-methyl-branched fatty acids (Kroppenstedt 1985); they lack mycolic acids and contain LL-A_2pm in cell wall or whole-cell hydrolysates (Lechevalier and Lechevalier 1970b, c) (◗ *Table 42.1*). Major menaquinones are hexa- and octa-hydrogenated menaquinones with nine isoprene units (Kim et al. 2003). A major chemotaxonomic feature for the differentiation of *Streptomyces* from *Kitasatospora* is the presence of *meso*-A_2pm in whole-cell hydrolysates (◗ *Table 42.1*). In *Streptomyces* strains, the *meso*-A_2pm content is 1–16 %, while in *Kitasatospora* strains it is 49–89 % (Zhang et al. 1997). The predominant diamino acid of strains belonging to the genus *Streptacidophilus* is (like in *Streptomyces*) LL-diaminopimelic acid (Kim et al. 2003).

Genus *Kitasatospora* Ōmura Takahashi, Iwai, and Tanaka 1983, 672[VP]: Genera Incertae Sedis

(Ki.ta.sa.to.spo'ra. Jpn. n. Kitasato, a Japanese bacteriologist (1852–1931); M. L. fem. n. *spora* spore; M. L. fem. n. *Kitasatospora*, Kitasato spore).

The type species of the genus is *Kitasatospora setae* Ōmura, Takahashi, Iwai, and Tanaka 1982. They are Gram-positive, aerobic, non-acid-alcohol-fast actinomycetes which form an intensively branched, non-fragmenting mycelium. They form a stable substrate mycelium similar to that of *Streptomyces*, and the aerial mycelium generates long spore chains of more than 20 spores. They do not form sporangia. Organisms are chemoorganotrophic and grow from 15 °C to 42 °C and within the pH range of 5.5 to 9.0. Depending on the type of cells analyzed, the major constituents of the cell wall are galactose, glycine, and *meso*-A_2pm or LL-A_2pm. When cells are grown in liquid media, submerged spores which contain LL-A_2pm are formed, while the filamentous mycelia contain *meso*-A_2pm. When cells are grown on agar media, the substrate mycelium contains *meso*-A_2pm, whereas aerial spores contain LL-A_2pm. Whole-cell hydrolysates contain galactose but lack arabinose, madurose, and xylose. Cells generate large amounts of saturated, *iso*- and *anteiso*-fatty acids and have complex polar lipid patterns, which contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The predominant isoprenologues are hexa- and octa-hydrogenated menaquinones with nine isoprene units are. The glycolate test is negative. The mol% G+C content is between 66 and 73 (T_m).

Differentiation of the Genus *Kitasatosporia* from Other Genera

Despite some differences, members of the family *Streptomycetaceae* are morphologically and chemically homogeneous (◗ *Table 42.1*). Identification of most species is problematic, because in many cases only one (the type) or a few strains have been examined. *Kitasatospora* species may be differentiated from other genera in the family by slight differences in cell wall sugars and the presence of *meso*-A_2pm in the peptidoglycan (◗ *Table 42.1*). ◗ *Table 42.6* lists some phenotypic features, which can be used to distinguish between *Kitasatospora* species.

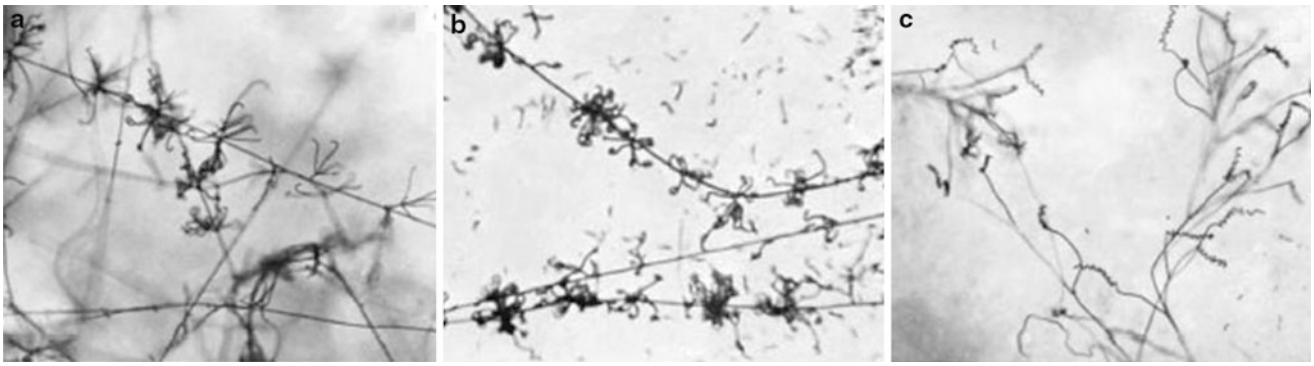

Fig. 42.2
Morphology of the aerial mycelium of three streptomycetes. (a) A *Streptoverticillium* species: spore chains arranged in typical verticils along straight, long aerial hyphae; the end of the spore chain is sometimes hook-like or forms one to two turns. (b) "*Streptomyces pallidus*": despite the verticil-like arrangement of spore chains, this organism was described as *Streptomyces* by Shirling and Gottlieb (1972). (c) A *Streptomyces* species: sympodially branched aerial hyphae; spore chains form spirals with up to 10 turns. All photos: ×250

Genus *Streptacidiphilus* Kim, Lonsdale, Seong, Goodfellow 2003, 1219^{VP}: Genera Incertae Sedis

Streptacidiphilus (strept.a.ci.di'phi.lus. Gr. adj. *streptos* pliant, twisted; L. neut. n. *acidum* acid; Gr. adj. *philos* loving; N.L. masc. n. *Streptacidiphilus* twisted, acid-loving).

The type strain of the genus is *Streptacidiphilus albus* (Kim et al. 2003). They are Gram-positive, aerobic, non-acid-alcohol-fast actinomycetes that form an intensively branched, non-fragmenting mycelium. At maturity the aerial mycelium bears long spore chains. Discrete leathery or lichenoid colonies are formed. A range of pigments is produced that are responsible for the color of the substrate and aerial mycelium. Colored diffusible pigments may be formed. Organisms are chemoorganotrophic with an oxidative type of metabolism. They utilize a wide range of carbon compounds as sole carbon sources for energy and growth. Most strains grow at temperatures between 15 °C and 30 °C and between pH 3.5 and 6.0 with an optimum around pH 5. They are widely distributed in acidic soil and litter. The major diamino acid in the peptidoglycan layer is LL-diaminopimelic acid. Whole-cell hydrolysates contain major amounts of galactose and rhamnose. Hexa- and octahydrogenated menaquinones with nine isoprene units are the predominant isoprenologues. Cells are rich in saturated, *iso*- and *anteiso*-fatty acids and produce complex polar lipid patterns that contain diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides. The mol% G+C of the DNA is 70–72. Phenotypic characteristics that differentiated *Streptacidiphilus* species among each other are listed in ❯ *Table 42.7*.

Differentiation of the Genus *Streptacidiphilus* from Other Genera

Members of the family *Streptomycetaceae* are morphologically and chemically homogeneous (❯ *Table 42.1*). In addition, identification of most species is difficult because in many instances only one (the type) or a few strains have been studied. *Streptacidiphilus* may be distinguished from the other genera in the family *Streptomycetacae* by slight differences in cell wall peptidoglycan composition and by growth at low pH values, as well as by 16S rRNA gene sequencing and MLSA analysis (❯ *Table 42.1*).

Further Descriptive Information on Streptomycetes

Morphology, Fine Structure, and Life Cycle

Early analyses of streptomycetes were ruled by a strong emphasis on morphology. The complex life cycle of streptomycetes (see below) presents three phenotypic traits for detailed microscopic characterization: (a) vegetative (substrate) mycelium (on solid and in liquid medium), (b) aerial mycelium bearing chains of arthrospores (sometimes called "sporophores") and (c) the arthrospores themselves (Kutzner 1981). Most diagnostic information for taxonomists has been provided by the last two characteristics.

It was pointed out by some early studies that streptomycetes formed spore chains on the vegetative mycelium in both solid and liquid culture (e.g., Carvajal 1947; Glauert and Hopwood 1960; Tresner et al. 1967). At least for one species, *S. venezuelae*, the spores produced in submerged culture have similar characteristics as aerial spores (Glazebrook et al. 1990).

The fine structure and development of the aerial arthrospores have been studied extensively (Locci and Sharples 1984, add recent refs). Aerial arthrospores are formed within a thin fibrous sheath by septation and disarticulation of preexisting hyphal elements. The spore wall is generated, at least in part, from wall layers of the parent hypha. This process is termed *holothallic development* (Locci and Sharples 1984) and was found to be characteristic for many other sporoactinomycetes

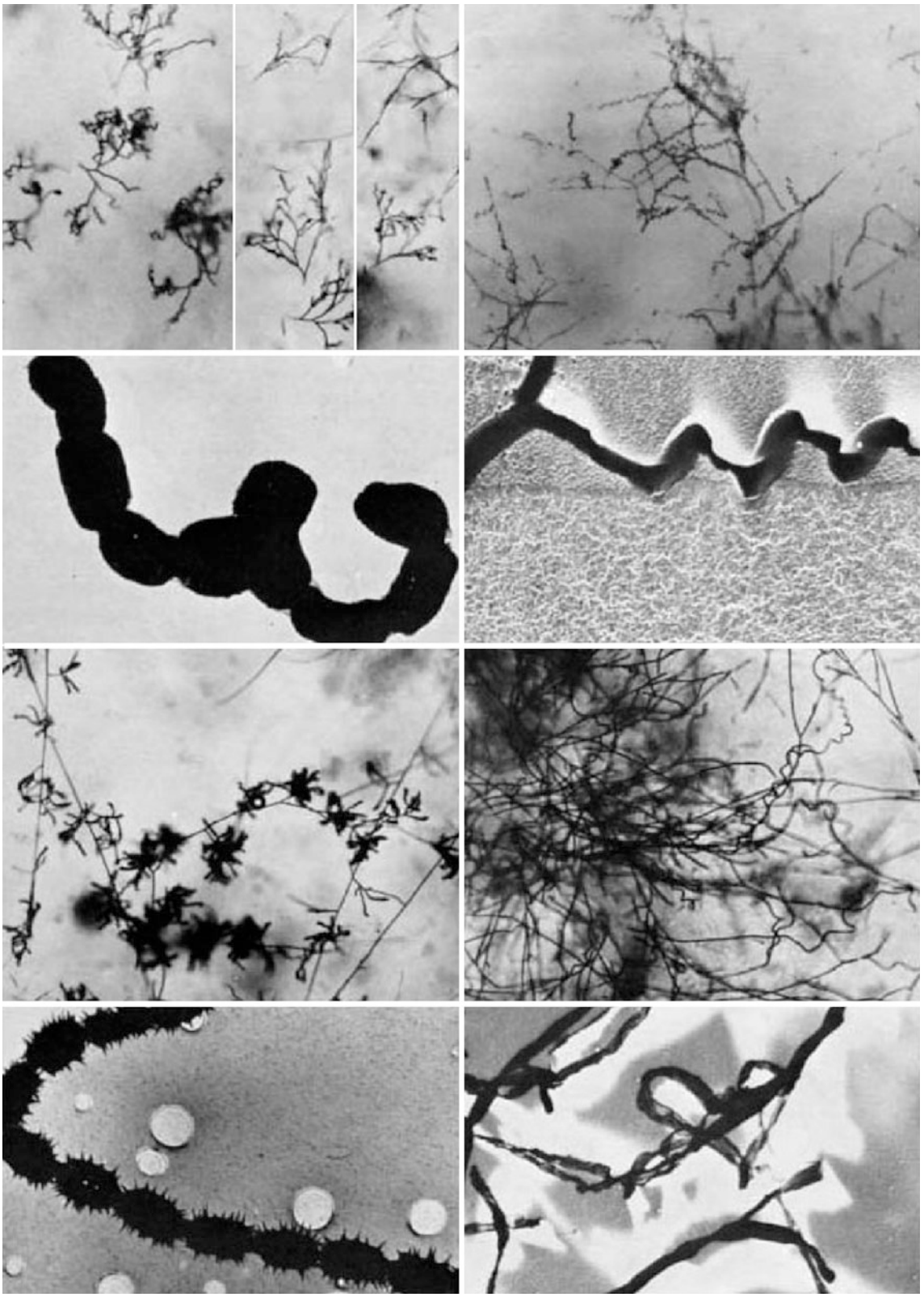

Fig. 42.3
Aerial mycelium of the fertile (*left*) and the sterile (*right*) strain of two streptomycetes. *First and third lines*, light microscopy (@ 250); *second and fourth lines*, electron microscopy (×15,000). (From Kutzner [1956], with permission.)

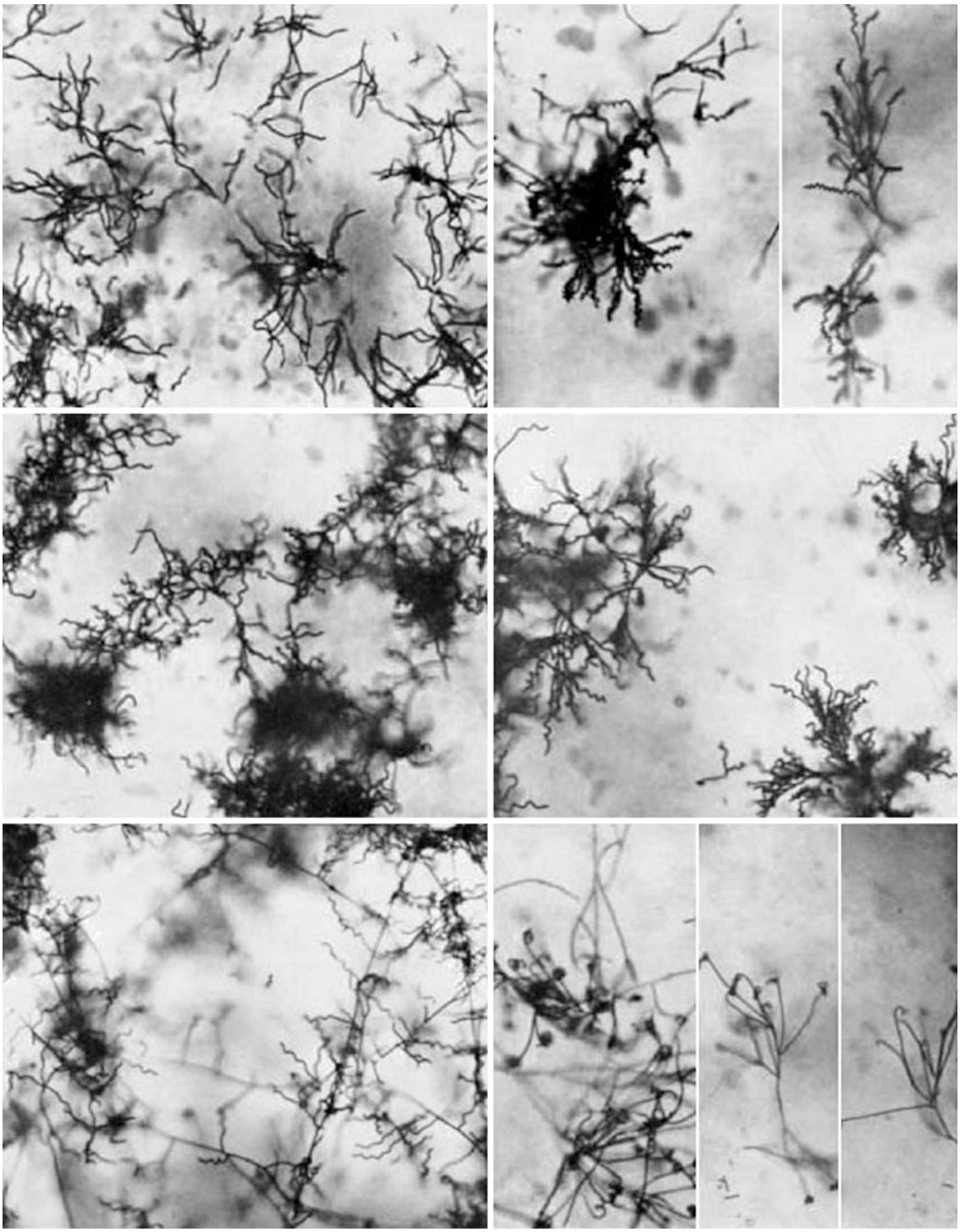

Fig. 42.4
Morphology of the aerial mycelium of some strains of *Streptomyces* (×250). (From Flaig and Kutzner [1960b], with permission.)

(Williams et al. 1973). The organization of the spore chains (or sporophores) has played a very important role in species descriptions for many years. Frequently, the chains are long and include more than 50 arthrospores. The variety of spore chain morphologies is enormous, and many categories have been established by some scientist: for instance, Ettlinger et al. (1958a) classified strains into 15 morphological types. A simpler and more practical scheme was proposed by Pridham et al. (1958) and adopted for the International *Streptomyces* Project (ISP; Shirling and Gottlieb 1966). There were three categories recognized:

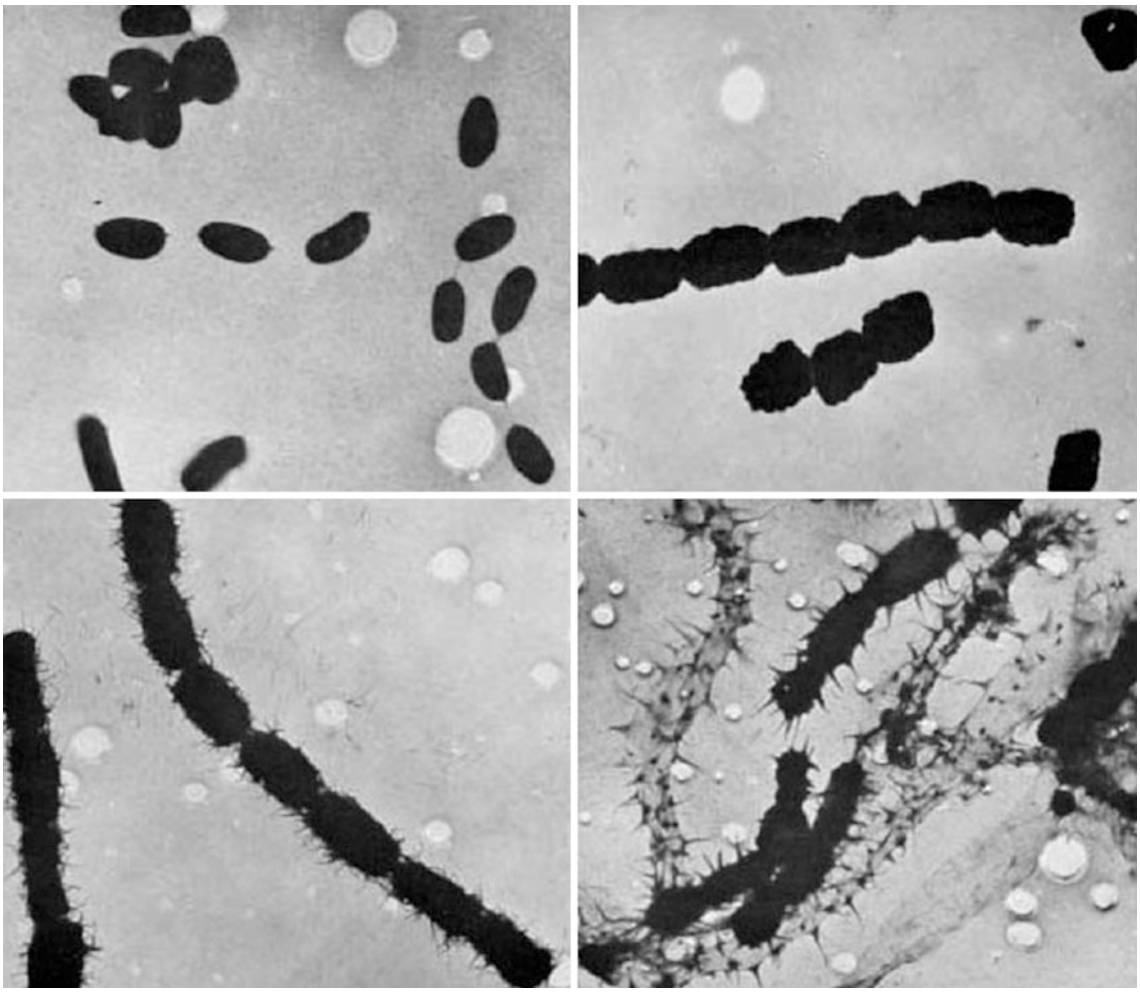

Fig. 42.5
Electron micrographs of four types of arthrospores of streptomycetes: smooth, warty, hairy and spiny. The spores are about 1 m long. (From Kutzner (1956), with permission.)

(a) straight to flexuous (*Rectiflexibiles*); (b) hooks, loops, or spirals with one to two turns (*Retinaculiaperti*) (Fig. 42.2a, b); and (c) spirals (*Spirales*) (Fig. 42.2c). However, it should be considered that even this simple system might cause problems, because it is not unusual that more than one category can be observed in the same culture, and the distinction between *Retinaculiaperti* and *Spirales* is not always clear (Shirling and Gottlieb 1977; Williams and Wellington 1980). Aerial filaments can also differentiate into verticils (Fig. 42.7), which is common for most of the species formerly grouped into the genus *Streptoverticillium* (Locci and Schofield 1989). An overview of different morphological forms of the aerial mycelium of *Streptomyces* and *Streptoverticillium* spp. is depicted in Figs. 42.2–42.4 and Fig. 42.7.

In addition, spore surface ornamentation has also been adopted as a characteristic for taxonomic description. The ornaments, which are in fact borne on the spore sheath, can be clustered into the categories hairy, smooth, warty, hairy, and spiny. A further type, rugose, was proposed by Dietz and Mathews (1971) (Figs. 42.5 and 42.6). Spore surface ornamentation is a stable character, but the differences between smooth, warty, and rugose types can be difficult to distinguish. Nevertheless, these problems can be resolved by using scanning electron microscopy.

Morphological Development

Unusually for a bacterium, *Streptomyces* exhibit a complex multicellular cycle of development and reproduction. Differentiation of the organism into distinct tissues from vegetative mycelium to aerial hyphae bearing spores progresses is depicted in Fig. 42.8 for the model organism *S. coelicolor* A3(2). Induction of germination may be induced by many factors including water, carbon dioxide, amino acids, and vitamins. Under favorable conditions germination begins with swelling of the spore. Spore polarity is established followed by emergence of one or more germ tubes. These grow by tip extension (Gray et al. 1990)

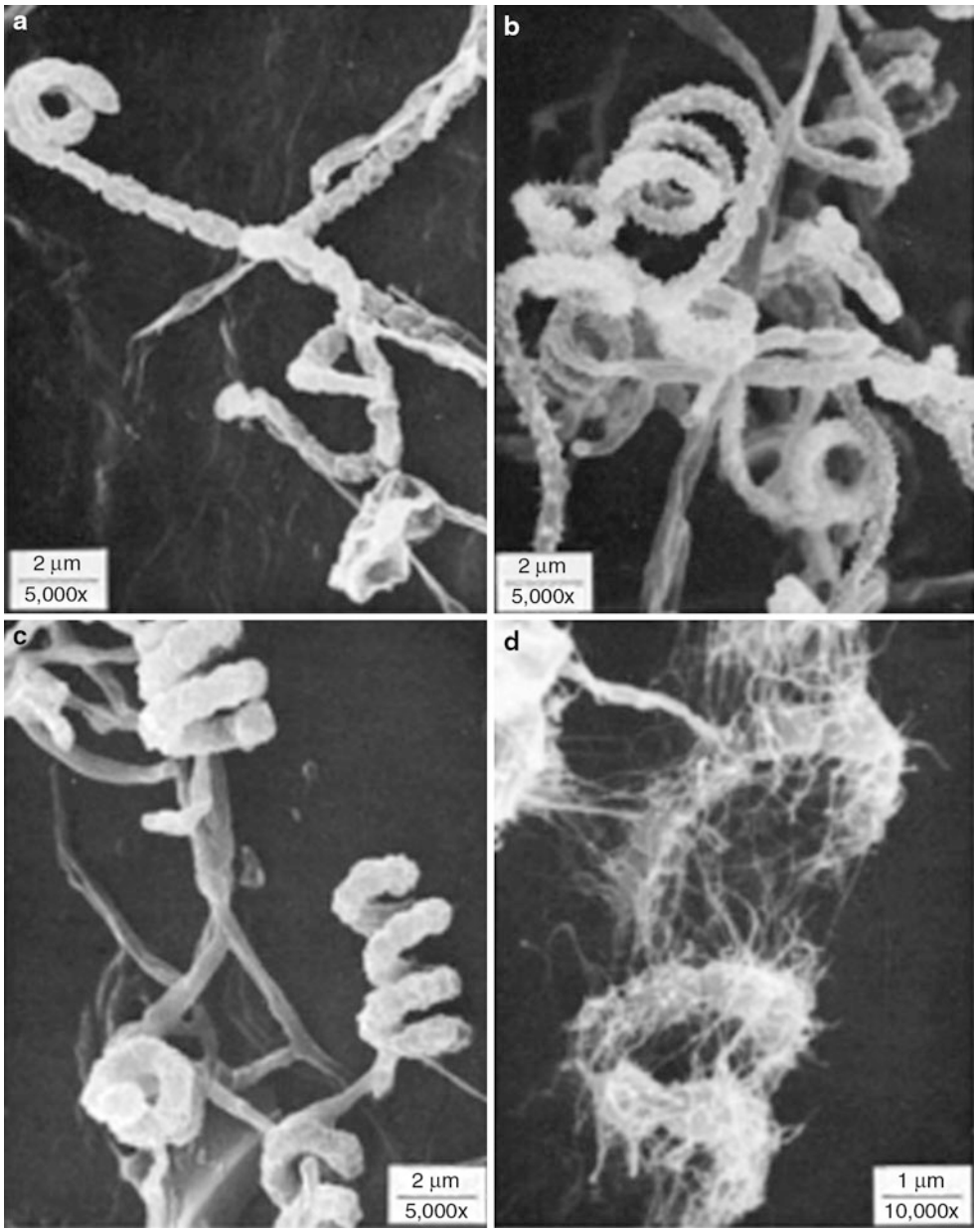

Fig. 42.6
Scanning electron microscopy of arthrospore chains. (a) *Streptomyces torulosus* (knobby); (b) *S. bluensis* (spiny); (c) *S. antimycoticus* (rugose); and (d) "*S. karnatakensis*" (hairy). (Courtesy of A. Dietz.)

and branching, forming branched hyphal filaments comprising a multi-nucleoid, irregularly septated substrate mycelium. Tip extension and the initiation of new branches are directed by a polarisome-like complex involving the essential polarity protein DivIVA (reviewed by Flardh et al. 2012). Phosphorylation of DivIVA by the serine/threonine kinase AfsK (that also regulates antibiotic production; see below) regulates the function of the polarisome and, hence, apical growth and branching (Hempel et al. 2012). Differentiation of the mycelia is initiated by a complex regulatory cascade in response to physiological stress, primarily nutrient depletion, resulting in formation of aerial hyphae coupled with production of extracellular proteins (Chater et al. 2010) and production of secondary metabolites to protect the nutrient source (*Fig. 42.8*). Formation of the aerial hyphae is controlled by the *bld* genes, so called because the inability of mutants defective in these genes to produce aerial hyphae gives them a "bald" appearance on certain media. Lysis is initiated in the compartments of much of the substrate mycelium in an ordered manner regarded as programmed cell death. As a result valuable nutrients may be channeled to fuel the extending aerial hyphae (Manteca et al. 2010). Sporulation begins with condensation and multiple replication of the chromosome in the apical compartments of the extended aerial hyphae, which begin to form multiple septa. Glycogen

Table 42.6

Phenotypic characteristics that distinguish *Kitasatospora* species (Modified from Groth et al. 2004)

Phenotypic test	1	2	3	4	5	6	7	8	9	10	11	12
Spore chain morphology*	RF, RA, S	RF	S[a]	RF	RF, S	RF, S	RF, RA	RF, RA, S	RF	RF	RF	RF, RA, S
Formation of melanoid pigment	+	–	–[a]	+	+	+	+	-	+	–	+	+
Growth temperature												
6 °C	–	–	–[a]	(+)	–	–	(+)	–	–	–	–	–
10 °C	–	(+)	–[a]	+	(+)	+	+	+	(+)	+	(+)	–
35 °C	+	(+)	+[a]	–	–	+	–	(+)	+	+	+	+
37 °C	+	–	+[a]	–	–	+	–	–	+	+	+	+
40 °C	(+)	–	+[a]	–	–	–	–	–	–	+	–	+
42 °C	–	–	–[a]	–	–	–	–	–	–	+[b]	–	–
Growth at pH												
8.0	+	+	+	+	+	+	–	+	+	+	+	+
9.0	–	–	+	+	+	–	–	–	+	+	+	+
9.5	–	–	–	+	–	–	–	–	–	+	+	–
Growth in the presence of NaCl (%)												
2.0	+	–	+	+	+	+	+	+	+	+	+	+
2.5	–	–	+	+	+	+	+	(+)	+	+	+	+
3.0	–	–	–	–	–	+	–	–	+	–	+	(+)
3.5	–	–	–	–	–	+	–	–	+	–	+	–
Liquefaction of gelatin	+	+	–[a]	+	–	–	–	+	+	+	+	+
Peptonization of milk	+	+	+[a]	+	–	–	–	+	+	+	+	+
Degradation of casein	+	+	ND	+	+	–	–	+	+	+	+	+
Nitrate reduction	+	+	–[a]	+	–	–	–	–	+	+	+	+
Hydrolysis of potato starch	+	+	+[a]	+	+	+	+	+	+	+	–	+

Phenotypic test	1	2	3	4	5	6	7	8	9	10	11	12
Growth on sole carbon sources												
(+)-L-Arabinose	+	+	±[a]	+	+	–	+	–	+	+[b]	–	+
(+)-D-Fructose	+	+	±[a]	+	–	+	+	(+)	–	+[b]	(+)	+
i-Inositol	–	–	+[a]	–	–	–	–	–	–	–[b]	–	–
(+)-D-Mannitol	–	–	–[a]	–	+	+	–	–	–	–[b]	–	–
(+)-D-Raffinose	+	–	–[a]	–	–	–	–	–	–	+[b]	–	+
(+)-L-Rhamnose	–	–	–[a]	–	–	–	–	–	+	+[b]	–	(+)
(–)-D-Sucrose	–	+	–[a]	+	+	+	+	(+)	–	+[b]	(+)	(+)
(+)-D-Xylose	+	+	–[a]	+	+	–	+	–	+	+[b]	–	+
Enzyme assay (API ZYM)												
N-Acetyl-β-glucosamidase	–	–	–	(+)	–	–	–	+	–	–	–	–
β-Galactosidase	(+)	+	+	+	+	+	–	+	+	+	+	+
α-Glucosidase	+	(+)	+	+	+	(+)	–	–	+	+	–	(+)
β-Glucosidase	–	–	–	+	–	–	–	–	–	–	+	–
α-Mannosidase	–	–	–	–	(+)	+	–	–	–	–	–	–
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	+	+	+	+	+	+	–	+
Antibiotic susceptibility												
Ampicillin (10 μg)	–	+	ND	(+)	(+)	–	–	–	(+)	+	+	–
Lincomycin hydrochloride (2 μg)	–	–	ND	–	–	–	(+)	–	(+)	–	–	–
Methicillin (5 μg)	–	+	ND	–	(+)	–	–	(+)	(+)	–	(+)	–

Table 42.6 (continued)

Phenotypic test	1	2	3	4	5	6	7	8	9	10	11	12
Norfloxacin (10 μg)	−	(+)	ND	−	−	+	(+)	−	−	−	−	(+)
Novobiocin (5 μg mL^{-1})	+	+	ND	+	+	+	+	+	+	−	+	+
Penicillin G (10 IU)	−	+	ND	−	−	−	−	−	−	+	+	−
Polymyxin B (300 IU)	+	+	ND	−	−	−	(+)	(+)	+	(+)	(+)	+
Sulfonamide (200 μg)	−	+	ND	+	(+)	−	−	(+)	(+)	−	−	−

Taxa: 1, *K. arboriphila* sp. nov. HKI 0189^T; 2, *K. azatica* DSM 41650^T; 3, *K. cystarginea* DSM 41680^T; 4, *K. gansuensis* sp. nov. HKI 0314^T; 5, *K. kifunensis* DSM 41654^T; 6, *K. kifunensis* HKI 0316; 7, *K. mediocidica* DSM 43929^T; 8, *K. nipponensis* sp. nov. HKI 0315^T; 9, *K. paranensis* sp. nov. HKI 0190^T; 10, *K. phosalacinea* DSM 43860^T; 11, *K. putterlickiae* DSM 44665^T; 12, *K. terrestris* sp. nov. HKI 0186^T

For the following properties, tests for which strain DSM 41680^{T}was not tested are indicated by "*a*." Spores of all of the tested strains are cylindrical with smooth surface. All strains were positive for the production of H_2S^a and growth on (+)-D-glucose and produced acid phosphatise, alkaline phosphatise, esterase (C4), esterase lipase (C8), and leucine arylamidase (API ZYM tests). They did not utilize cellulose^a as a sole carbon source; did not produce α-chymotrypsin, cystine arylamidase, α-galactosidase, β-glucuronidase, α-fucosidase, lipase (C14), trypsin, or valine arylamidase (API ZYM tests); and did not grow in the presence of NaCl (4 %, w/v) or at 42 °C and ph 4.0 or pH 10. Good growth occurred at temperatures of 15–32 °C and pH 5.0–7.0. All strains were sensitive to chloramphenicol (30 μg)^a, ciprofloxacin (5 μg)^a, imipenem (10 μg)^a, kanamycin sulfate (30 μg)^a, nalidixic acid (50 μg mL^{-1} agar)^a, oxytetracycline (30 μg)^a, rifampicin (30 μg)^a, streptomycin sulfate (10 μg)^a, and vancomycin (30 μg)^a

[a]Data from Kusakabe and Isono (1988)

[b]Data from Takahashi et al. (1984)

+ positive, − negative, (+) weakly positive, ± doubtful, *ND* not determined, *RF Rectiflexibiles*, *RA Retinaculiaperti*, *S Spirales*

Table 42.7

Phenotypic characteristics that distinguish *Streptacidiphilus* species (data from Cho et al. 2008)

Phenotypic characteristic	1	2	3	4	5	6	7	8
Growth at								
pH 3	+	+	+	−	+	−	+	−
Growth on sole nitrogen source at 0,1 % (w/v)								
L-Isoleucine	−	+	+	+	+	+	+	+
Growth on sole carbon source at 0,1 % (w/v)								
L-Arginine	+	−	+	+	+	−	+	+
L-Aspartic acid	+	+	−	+	−	+	+	−
Sodium oxalate	−	+	−	+	+	+	+	+
Growth on sole carbon source at 1 % (w/v)								
D-Gluconic acid	−	+	+	+	+	−	+	+
D-Glucosamine hydrochloride	+	+	+	+	+	+	+	−
Myo-inositol	−	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	−
D-Sorbitol	−	+	+	+	+	+	+	+
D-Xylose	−	+	+	−	+	+	+	−
Degradation of								
Starch	+	+	+	+	+	+	−	+
Tween20	−	−	−	−	−	−	+	−
Tween40	+	+	−	−	+	−	+	−
Tween80	−	−	+	−	+	−	+	+
Xanthine	+	+	−	+	+	−	−	−

1, *Streptacidiphilus albus* JL83^T; 2, *Streptacidiphilus anmyonensis* strain AM-11^T; 3, *Streptacidiphilus carbonis* JL415^T; 4, *Streptacidiphilus jiangxiensis* 33214^T; 5, *Streptacidiphilus melanogenes* strain SB-B34^T; 6, *Streptacidiphilus neutrinimicus* JL206^T; 7, *Streptacidiphilus oryzae* TH49^T; 8, *Streptacidiphilus rugosus* strain AM-16^T

All taxa were positive for the utilization of glycerol and sucrose and also for the growth at pH 4, 5, 6, 7, and 8

+ positive, − negative

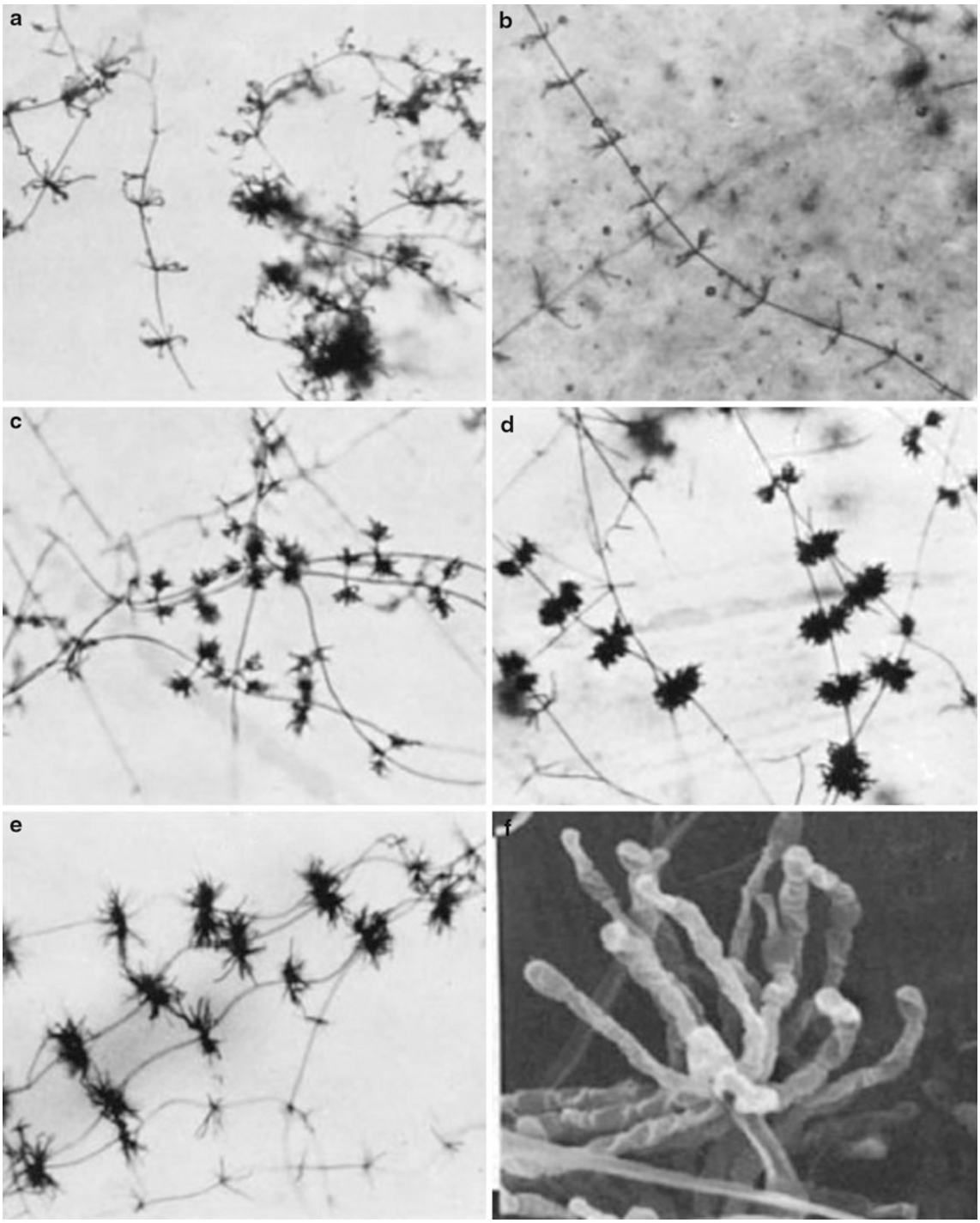

Fig. 42.7
Morphology of the aerial mycelium of some species of *Streptoverticillium*. (a)–(e) Light microscopy (×250). (Courtesy of C. Mütze.) (a) *Sv. netropsis* (DSM 40259). (b) "*Sv. reticulum*" (DSM 40893). (c) "*Sv. cinnamomeum subsp. azacolutum*" (DSM 40646). (d) *Sv. septatum* (DSM 40577). (e) *Sv. mobaraense* (DSM 40847). (f) Scanning electron microscopy: a *Streptoverticillium* species (×6,200).

reserves accumulated in the substrate mycelium are degraded or converted to trehalose which may play in role in protecting aerial hyphae and spores from desiccation (Braña et al. 1986). Synchronous cell division occurs as septation proceeds resulting in prespore compartments containing a single copy of the chromosome. Maturation of spores is regulated by the *whi* genes. Compartment walls thicken, change shape, and acquire their characteristic gray-brown pigment specified by the *whiE* cluster (Davis and Chater 1990; Shen et al. 1999). The cycle producing millions of spores, which will remain in a quiescent state until they encounter a suitable environment for germination, from a small biomass of substrate is therefore complete.

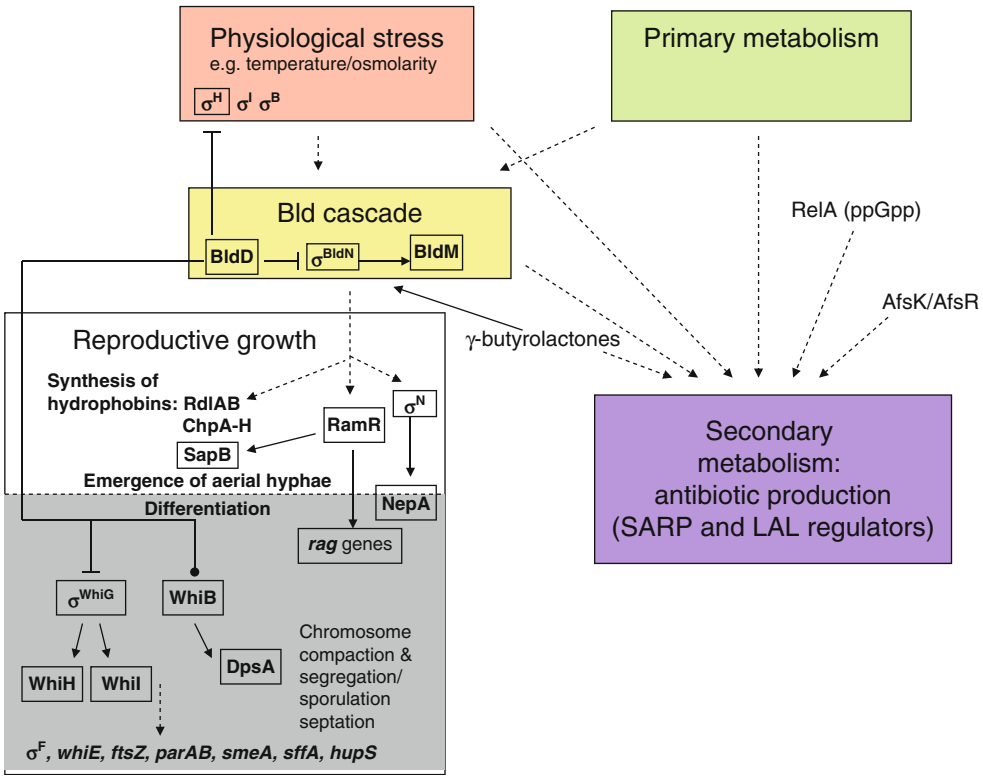

Fig. 42.8

Overview of regulation of morphological development and antibiotic production. Large boxes represent different processes of growth and metabolism, connected by arrows representing influences and dependencies of one process on another. A few of the key individual genes or proteins implicated in each process referred to in the text are indicated. *Dashed arrows* indicate that the precise mechanism of the interaction or dependency is unknown. *Solid arrows* joining boxed genes/proteins indicate a positive effect of one protein on expression of the dependent gene/protein. Perpendicular lines represent a negative or repressing effect. A line ending in a bullet indicates that the effect on gene expression is unclear

Mechanism and Regulation of Morphological Differentiation in the Model Organism *S. coelicolor*

The *bld* Genes

The formation of aerial hyphae and therefore spores is the result of extensive cell-cell signaling directed by the *bld* genes (Willey et al. 1993). Only a handful of genes involved in this process are thought to have been identified. With the exception of *bldB*, which is unconditionally blocked in aerial hyphae and antibiotic production, mutants disrupted in these genes have a carbon-source-dependent phenotype (Pope et al. 1996).

The *bldA* gene was identified by genetic complementation restoring aerial growth and actinorhodin production in *bldA* mutants (Piret and Chater 1985). Mutants defective in this gene are unable to form aerial hyphae and the diffusible blue pigment in a carbon-source-dependent manner. *bldA* encodes a tRNA that adds leucine to growing polypeptides in response to the UUA codon in mRNA (Lawlor et al. 1987), one of six different leucyl tRNA codons. Due to the high GC ratio content in Streptomycetes, UUA codons, corresponding to TTA in DNA, are very rare with only 145 of the predicted genes in *S. coelicolor* containing this codon (Chater 2006). This, coupled with the unusual nature of the UUA codon in that it cannot be translated efficiently by any other tRNA, mediates *bldA*'s translational regulatory effect. This effect has been demonstrated in regulation of undecylprodigiosin production by the *bldA*-dependent translation of the *redZ* TTA codon-containing mRNA. The target of the RedZ transcription factor is *redD*, encoding a pathway-specific regulator of antibiotic production (White and Bibb 1997).

bldB encodes a small peptide with DNA-binding activity. *bldB* mutants are unable to sporulate or produce antibiotics regardless of the carbon source indicating BldB, which forms oligomers and likely operates by interaction with another protein, may be a global regulator of morphogenesis, catabolite control, and antibiotic production (Pope et al. 1998; Eccleston et al. 2002).

BldC is required for normal and sustained transcription of the actinorhodin pathway-specific regulator gene *actII-4* and may be involved indirectly in *redD* transcription (Hunt et al. 2005). *bldC* encodes a DNA-binding protein; mutants present a carbon-source-dependent bald phenotype and are unable to produce actinorhodin (Merrick 1976; Hunt et al. 2005).

BldD belongs to a family of XRE transcription factors. It possesses a helix-turn-helix binding motif for DNA (Elliot and Leskiw 1999; Kim et al. 2006). *bldD* mutants are phenotypically

similar to *bldA* and *bldH* in that they and are blocked in morphological and physiological development on rich media, but development is at least partially restored on minimal media containing mannitol (Merrick 1976; Champness 1988). A BldD recognition sequence was identified in the promoter region of several known developmental regulatory genes and the transcription factor was found to repress expression of a number of these including *sigH* (Kelemen et al. 2001), *bldN*, *bldM*, and *whiG* (Elliot et al. 2001), discussed later in this chapter (Fig. 42.8). Subsequent transcriptomic analysis comparing a *bldD* mutant and the wild type demonstrated a large regulon controlled by BldD with at least 167 putative targets, including *bldC*, *bldB*, *ftsZ*, *sti1*, *ssgA*, and *ssgB*, around 80 % of which contain a readily recognized 15 bp palindromic binding motif (den Hengst et al. 2010). Transcription of many BldD target promoters is increased in *bldD* mutants (Elliot et al. 1998, 2001; Elliot and Leskiw 1999). BldD is capable of specifically recognizing its own promoter (Elliot and Leskiw 1999), but apart from this autoregulatory effect, the mechanism of transcriptional control of *bldD* is not yet understood (Elliot and Leskiw 1999; Kelemen et al. 2001).

bldG mutants are deficient in aerial hyphae formation and unable to produce pigmented antibiotics (Champness 1988). The *bldG* gene was predicted to encode an anti-anti-sigma factor involved in morphological and physiological differentiation (Bignell et al. 2000). Its antagonist was initially identified as the product of the cotranscribed *apgA* (antagonist partner of BldG) gene with interaction of the two proteins indicating their involvement in a partner-switching mechanism (Parashar et al. 2009). The regulatory target of the BldG-ApgA complex has not yet been identified but BldG has been shown to physically interact with this protein and the anti-sigma factor UshX (also known as PrsH), the antagonist of SigH, an alternative sigma factor involved in morphological development (Sevcikova et al. 2010).

bldH encodes the *S. coelicolor* ortholog of *S. griseus* AdpA which is autoregulated (Wolanski et al. 2011), dependent on *bldA* for translation of a TTA codon, and directly controlled by BldD (Nguyen et al. 2003; Takano et al. 2003). *bldH* mutants are phenotypically similar to *bldA* and *bldG* mutants (Champness 1988). σ^{AdsA}, part of the AdpA regulon in *S. griseus*, is orthologous with σ^{BldN} in *S. coelicolor* (Yamazaki et al. 2000). *bldN* encodes an extracytoplasmic function (ECF) family sigma factor which is unconditionally required for formation of aerial mycelium, and expression from the *bldN* promoter in *S. coelicolor* requires *bldH* (Bibb et al. 2000). Transcriptomic comparison between *bldN* mutants and the wild type showed all eight of the chaplin genes failed to be upregulated in the mutant strain, although it is unclear how this effect is mediated (Elliot et al. 2003). The only confirmed target of σ^{BldN} is *bldM* (Bibb, Molle and Buttner 2000). The product of *bldM*, an atypical response regulator BldM, may not require phosphorylation for its biological function (Molle and Buttner 2000), and its targets are as yet unknown.

BldJ is an oligopeptide proposed to be one of the first signals in the *bld* cascade and response to this factor is dependent on the presence of the oligopeptide import system encoded by the *bldK* gene cluster (Nodwell and Losick 1998). This cluster encodes the five subunits of an ABC membrane-spanning transporter which may be responsible for the import of an extracellular signal required for initiation of morphological differentiation (Nodwell et al. 1996). The *bldK* locus is well conserved among Streptomycetes; recent characterization of an orthologous cluster in *S. griseus* found mutation of *bldKB* in this cluster leads to impaired sporulation and secondary metabolite production reflective of *bldKB* and *bldKC* mutation in *S. coelicolor* (Akanuma et al. 2011).

The characterized *bld* mutants are all defective in production of SapB, a small morphogenic surfactant lantibiotic-like peptide implicated in aerial hyphae production, and wild-type phenotypes can be restored by exogenous supply of the modified peptide (Willey et al. 1991). It is the product of the *ramS* gene, itself part of the *ram* gene cluster whose expression is controlled by the response regulator RamR. SapB and morphological differentiation can be restored by growth of different *bld* mutants in close proximity by a process known as "cross-feeding" or extracellular complementation (Willey et al. 1993). Complementation is unidirectional and hierarchical; mutants can restore the phenotypes of all those mutants to the left but not to the right; bldJ → bldK/L → bldA/H → bldG → bldC → bldD/M, culminating in restoration of SapB production and aerial hyphae formation (Kelemen and Buttner 1998; Nodwell et al. 1999; Bibb et al. 2000; Willey et al. 2006; Chater 2006). The "donor" produces a signal the "recipient" is not competent to produce but is able to respond to, thus demonstrating the putative and, to date, incomplete signaling cascade mediated by these genes. This simple linear hierarchy is complicated by the fact that several *bld* mutants, including *bldB*, *bldI*, and *bldN*, do not fit into the cascade model (Nodwell et al. 1999), but many putative additional signals which may help unravel some of this complexity are yet to be identified. The *bld* gene cascade activates expression of *ramR* that is needed, in turn, for expression of *ramS* (Fig. 42.8). The SapB lantibiotic-like peptide is derived from proteolytic cleavage and posttranslational processing of the C-terminal half of the *ramS* gene product (Kodani et al. 2004). Unusually for this class of proteins, SapB does not have antibiotic activity but rather provides a structural role on the colony surface, tightly associated with the spore surface and in the medium around the colony. The putative operon within which RamS is encoded comprises four genes; *ramA*, *ramB*, *ramC*, and *ramS*. RamC is thought to be responsible for some of the posttranslational modification of SapB, and RamAB are considered components of an ABC transporter required for its export (Willey et al. 2006). The RamR response regulator also activates expression of the *rag* operon whose products are important in subsequent development of the aerial hyphae (San Paolo et al. 2006).

The Hydrophobic Coating of Aerial Hyphae and Mature Spores

Streptomyces forms a hydrophobic sheath that coats its aerial (but not vegetative) mycelium and mature spores (Hopwood

et al. 1970). The sheath, comprising of two classes of proteins, lowers the surface tension of the aqueous substrate enabling extension of the hyphae up from the colony surface and into the air. One class of secreted proteins involved in formation of the hydrophobic sheath is the chaplins (chap is the abbreviated form of *Coelicolor Hydrophobic Aerial Protein*) (Claessen et al. 2003; Elliot et al. 2003). Unlike SapB, which is required for aerial hyphae formation only on rich media (Willey et al. 1991), the chaplins (Chp) are synthesized under all the tested growth conditions and are required for SapB-independent formation of aerial mycelium on soy flour mannitol or minimal media (Capstick et al. 2007), so providing alternative mechanisms for aerial hyphae to emerge from an aqueous environment based on nutritional availability in the soil environment. There are eight known chaplins which have been divided into two groups; ChpA–C, the long chaplins, and ChpD–H, the short chaplins. The long chaplins are around 225 amino acids in length and contain two copies of the hydrophobic chaplin domain that defines this class. ChpA–C have a C-terminal sortase signal indicative of cell wall attachment where, along with the rodlins, anchored fibrils organize the short chaplins which are polymerized into a paired rodlet ultrastructure (Elliot et al. 2003). Short chaplins have only one chaplin domain and are approximately 55 amino acids long. ChpC, E, and H are the minimal requirements for normal aerial hyphal formation (Di Berardo et al. 2008). The mechanism of polymerization into amyloid fibers is not known, but recently two amyloid domains were identified in the model chaplin, ChpH, and in vitro studies indicated these contribute to their assembly (Capstick et al. 2011).

The second class of proteins implicated so far in formation of the hydrophobic hyphal and spore coating is the rodlin proteins, encoded by *rodA* and *rodB*, which influence the organization of the chaplins but are not required for formation of hydrophobic aerial hyphae (Claessen et al. 2002). RodA and RodB are present at the surface of aerial structures but mutation in their genes does not affect aerial hyphae formation, though the rodlet layer is absent in these mutants (Claessen et al. 2002, 2004).

NepA, a small secreted protein, may be involved in this process. *nepA* is the only known target for the alternative sigma factor σ^N, apart from its autoregulation (Fig. 42.8). *sigN* null mutants are delayed in development in a carbon-source-dependent manner and transcription of the gene is absent in *bld* but not *whi* mutants, indicating a role in aerial hyphae formation (Dalton et al. 2007). Interestingly, transcription of *nepA* is absent in mutants where all eight chaplins have been deleted (de Jong et al. 2009) indicating its expression is coupled to aerial hyphae formation. Its role is as yet undefined due to conflicting reports on its localization within *S. coelicolor*. Confinement of expression of NepA in the "subapical stem," a compartment just below the apical sporogenic cell, was observed by Dalton et al. (2007). However, by transcriptomic and localization analysis of EGFP-tagged, NepA, expression was also detected in mature spore chains where it is proposed that NepA, minus its export signal peptide, is incorporated as a highly insoluble spore wall protein with a role in maintaining spore dormancy until conditions become favorable for germination (de Jong et al. 2009).

The *whi* Genes and Sporulation

While emergence of hyphae from the colony surface of the aerial mycelium is controlled by the *bld* genes, the subsequent development of the apical hyphal compartment and multiple synchronous cell division into unigenomic spores is controlled, in part, by the *whi* genes. Mutants defective in *whi* gene products have a characteristic white appearance on the colony surface due to their inability to form mature spores and their gray-brown polyketide coating. The "early" *whi* genes are *whiA*, *whiB*, *whiG*, *whiH*, *whiI*, and *whiJ*, and these are strictly and non-conditionally required for the early stages of conversion from aerial hyphae to spores (reviewed by McCormick and Flärdh 2012).

whiG encodes σ^{WhiG}, an RNA polymerase sigma factor highly similar to the motility sigma factor in *Bacillus subtilis* which is required at the earliest known stage of differentiation from hyphae to spores (Chater et al. 1989) and directly controls *whiH* and *whiI* (Fig. 42.8). *whiI* encodes an atypical orphan response regulator lacking conserved residues in the conventional phosphorylation pocket. Transcription from its promoter requires σ^{WhiG}, and it is implicated in autoregulation and regulation of expression of *whiH* (Aínsa et al. 1999). The product of *whiH* shares most similarity with a large family of bacterial repressor proteins involved in carbon metabolism and is believed to negatively control its own expression (Ryding et al. 1998).

A role for WhiA and WhiB has been predicted in cessation of aerial hyphae formation as microscopically *whiA* and *whiB* mutants have been observed to have very long, tightly coiled, poorly septated hyphae (Chater 1972; Aínsa et al. 2000). Since transcription of these genes does not require σ^{WhiG}, proposed models assume that WhiA and WhiB act via a parallel pathway that converges with the σ^{WhiG} -mediated pathway involving WhiH and WhiI (Aínsa et al. 2000). A link between the two pathways governing aerial hyphae and sporulation, *bld* and *whi*, exists in BldD. A binding site for BldD has been identified in the *whiB* promoter sequence (den Hengst et al. 2010) and since WhiB is capable of upregulation of *whiA* transcription (Jakimowicz et al. 2006), BldD may play a role, albeit indirect in the case of *whiA*, in regulating the expression of these two genes. BldD also directly represses the first *whi* gene in the cascade, *whiG*, preventing σ^{WhiG} -mediated transcription of *whiH* and *whiI* (Elliot et al. 2001), thus regulating both arms of the putative signaling cascade. As yet, the only confirmed target of the transcription factor WhiB is the *dpsA* gene whose product is one of several nucleoid-associated proteins involved in chromosome compaction during sporulation (Facey et al. 2011).

whiJ encodes a transcription factor belonging to the same family as BldD. *whiJ* is unusual among the characterized *whi* genes in that it is not required for sporulation—a complete deletion of the gene has no phenotypic effect. The morphological deficiencies resulting in the white phenotype were the result of mutant alleles allowing synthesis of a truncated protein with an intact N-terminal DNA-binding domain (Aìnsa et al. 2010). Aìnsa et al. (2010) proposed a model for WhiJ as a repressor of developmental genes which is released from the operator sequences by binding to the product of its adjacent gene,

SCO4542, since deletion of the latter gene results in a medium-dependent white colony phenotype which can be completely suppressed by deletion of *whiJ* in the mutants.

The eight genes comprising the *whiE* cluster are predicted to encode components of type II polyketide synthase and are responsible for production of the spore wall-associated gray polyketide pigment that coats mature spores (Davis and Chater 1990). Two promoters have been identified for *whiE* transcription with transcription of both *whiEP1* and *whiEP2* dependent on *whiA*, *whiB*, *whiG*, *whiH*, *whiI*, and *whiJ* and mutation of *sigF* blocked transcription from *whiEP2* (Kelemen et al. 1998).

There are several homologues of *whiB* in *S. coelicolor* and these form part of a family known as the *whiB*-like (*wbl*) genes. Orthologs of these genes have been found in many actinomycetes, include some of those with a simpler lifecycle, but not in any other organisms (Soliveri et al. 2000). Two *wbl* genes are required for normal sporulation in *S. coelicolor*; *whiD* and *wblA*. Transcriptional analysis indicates that *whiD* is required at the time of sporulation septa formation (Molle et al. 2000). Disruption of *wblA* has a wide-ranging effect on gene regulation, affecting primary and secondary metabolism in addition to morphological differentiation. Mutants exhibit aerial hyphae that are almost entirely deficient in spore chains and appear red due to ectopic production of the pigmented antibiotics (Fowler-Goldsworthy et al. 2011).

whiA, *whiB*, *whiG*, and *whiI* have been implicated in transcriptional regulation of the sporulation-specific sigma factor, σ^F, which is required for wild-type spore formation (Kelemen et al. 1996). This sigma factor is expressed in developing and mature spore chains (Sun et al. 1999). Mutation of the gene leads to irregular, thin-walled, poorly pigmented spores with reduced chromosomal DNA condensation (Kelemen et al. 1996).

SsgA and the SsgA-like proteins (SALPs) are small proteins found as multiple paralogues only in sporulating or mycelial actinobacteria. In addition to SsgA, *S. coelicolor* possesses six SALPs including SsgB (Noens et al. 2005). Each of the SALPs is thought to act at different stages of spore formation performing a function related to septa and spore wall peptidoglycan buildup and degradation, with SsgA and SsgB carrying out an essential function in sporulation-specific cell division (Noens et al. 2005). Deletion of *ssgA* or *ssgB* critically affects aerial hyphal septation and therefore spore formation (van Wezel et al. 2000; Sevcikova and Kormanec 2003). Transcription of *ssgA* is linked to the adjacent upstream gene *ssgR* with the transcription of *ssgA* absent in *ssgR* mutants (Traag et al. 2004; Wolanski et al. 2011). *ssgR*-independent overexpression of SsgA induces irregular, unusually thick, septation of hyphae which are wider than in the wild type (van Wezel et al. 2000). Transcription of *ssgA* and *ssgR* is independent of the *whi* genes, despite the white colony phenotype of mutants, but both genes are targets for BldD (den Hengst et al. 2010). *ssgB* is also regulated by the stress response sigma factor, σ^H, which is repressed by BldD (Kelemen et al. 2001; Sevcikova and Kormanec 2003). Thus, BldD provides another link between aerial hyphae formation and sporulation.

Formation of spores requires tightly regulated cell division to ensure only one copy of the genome is inherited by each of the progeny. A number of proteins have been implicated in this process in *S. coelicolor* including FtsZ, CrgA, ParA, ParB, and SmeA and the spore nucleoid-associated proteins DpsA, DpsB, DpsC, and HupS. FtsZ has been demonstrated to be necessary for the formation of both crosswalls in vegetative mycelium and sporulation septa (McCormick et al. 1994). Division is directed by FtsZ which forms polymeric spiral structures in the synctial hyphae which are subsequently modified to form regularly spaced Z-rings along the sporogenic cell (Grantcharova et al. 2005). These tubulin-like proteins form a cytoskeleton which provides the force for constriction of the cell at sporulation septa and provide a base for sequestered proteins involved in cell division (Erickson et al. 2010). A BldD binding site has been identified in the *ftsZ* promoter region; however, the promoter to which it is proposed to bind is presumed to be constitutively rather than developmentally expressed (den Hengst et al. 2010). To date the developmental control of Z-ring assembly is not well understood with few proteins involved in FtsZ polymerization and cellular organization identified. CrgA, a small transmembrane protein, may have a role in cell division. Mutants disrupted in this gene erect aerial hyphae and produce antibiotics precociously and overexpression leads to aerial hyphae lacking sporulation septa (Del Sol et al. 2003). Overexpression of CrgA prevents FtsZ localization, and therefore it may have a role in Z-ring formation (Del Sol et al. 2006).

The *parAB* operon is developmentally controlled and involved in partitioning of DNA during multiple septation of the apical compartment; mutation of *parB* leads to incorrect allocation of DNA to around a tenth of the resultant spores (Kim et al. 2000). The small membrane protein SmeA is expressed specifically in sporogenic cells and influences septal placement and chromosomal segregation and condensation (Ausmees et al. 2007). The three *dps* genes of *S. coelicolor* influence sporulation, septal positioning, and spore nucleoids with developmental control of *dpsA* involving binding of WhiB to the *dpsA* promoter region (Facey et al. 2009, 2011). HupS is nucleoid associated in spores, developmentally upregulated in the apical compartment of aerial hyphae and dependent on WhiA, WhiG, and WhiI for transcription (Salerno et al. 2009).

γ-Butyrolactones and *bldH*

It has long been known that bacteria communicate within their environment. Quorum sensing involves secretion of signaling molecules that act as inducers and affect gene expression of the recipients after binding to their receptors. In some bacteria the signaling molecules are *N*-acyl-L-homoserine lactones (Swift et al. 1996). By this mechanism colonies can coordinate gene expression and therefore development. The first signaling molecules in *Streptomyces*, the γ-butyrolactones, were identified in the 1960s and have been extensively studied since. These hormone-like regulators are important for antibiotic production and morphological differentiation though their role and the current understanding of regulation varies within the genus. The well-characterized A-factor

(2-isocapryloyl-3R-hydroxymethyl- γ-butyrolactone) of *S. griseus* is involved in induction of antibiotic biosynthesis and onset of morphological differentiation (Ohnishi et al. 1999). The A-factor dependent protein AdpA mediates the response to this signaling molecule. A-factor binds to ArpA, the *adpA* repressor, and releases the ArpA dimer from the *adpA* promoter region thus allowing transcription of *adpA*. AdpA then transcriptionally activates a large regulon including the pathway-specific regulator of streptomycin biosynthesis, the *S. griseus* SapB homolog, and *ssgA* and is therefore a positive regulator of streptomycin biosynthesis and aerial mycelium formation in this organism (Ohnishi et al. 1999).

A-factor homologues have been found in many Streptomycetes. The role of γ-butyrolactones in *S. coelicolor* is less well defined than in *S. griseus*. Three have so far been identified in *S. coelicolor* and are known as the *S. coelicolor* butanolides (SCBs). SCB1, the most abundant of these, stimulates actinorhodin and undecylprodigiosin production (Takano 2006). The AdpA ortholog in *S. coelicolor* is BldH which also has a key role in development but the mechanism is not yet well understood (Nguyen et al. 2003; Takano et al. 2003). Transcription of several sporulation genes including the *ramR* cluster, *bldN*, *bldM*, and *whiI*, is dramatically decreased in *bldH* mutants (Xu et al. 2010). The presence of a TTA codon in the coding sequence of this gene may explain many of the affects of *bldA* mutation on morphogenesis as this codon cannot be translated by the *bldA* tRNA in this mutant (Nguyen et al. 2003; Takano et al. 2003).

Although many of the genes involved in aerial hyphae formation and sporulation have been identified along with some of their regulators, the pathways through which the signals are mediated still require a great deal of elucidation.

A link exists between alternative sigma factors, stress, and morphological development in *S. coelicolor* (Fig. 42.8). The *S. coelicolor* genome encodes around 66 sigma factors, at least nine of which are *B. subtilis* σ^B paralogues (Bentley et al. 2002). The stress response in *B. subtilis* is mediated by σ^B, the first alternative sigma factor identified in bacteria, isolated from the RNA polymerase holoenzyme following physiological stress (Haldenwang and Losick 1979). In descending order of homology to *B. subtilis* σ^B, *S. coelicolor* σ^B (formerly σ^J), σ^L, σ^I, σ^N, σ^F, σ^H, σ^K, σ^M, and σ^G are involved in stress responses and development. In addition to these "SigB-like" sigma factors numerous anti- and anti-anti-sigma factors similar to the antagonists RsbW and RsbV (regulators of SigB) are found within the genome. Following global analysis of gene expression following salt, heat, and ethanol stress, it was demonstrated that different sets of proteins are simulated in response to different types of stress coordinated with morphological development in this organism (Vohradsky et al. 2000).

sigF encodes a sigma factor important for spore maturation (Kelemen et al. 2001). This developmental role does not appear to be linked to a stress response. Mutants disrupted in *sigF* exhibit an unconditional *whi* phenotype, with smaller thin-walled spores (Potúcková et al. 1995). A lack of σ^F expression has been identified in a number of spore compartments of *ftsK* mutants (Wang et al. 2007). Autonomous expression of σ^F in spore compartments may play a role as a checkpoint ensuring that FtsK-coupled chromosome segregation and cell division occurs properly (Wang et al. 2007). *sigF* is adjacent to *sigN*, but the genes are expressed independently (Dalton et al. 2007). σ^N is thought to play a role in the formation of aerial hyphae, controlling expression of *nepA*. Mutants have a conditionally *bld* phenotype in a carbon-source-dependent manner (Dalton et al. 2007). The role of σ^G, which shows the most homology with σ^F, is as yet unknown. Disruption of this gene does not result in an obvious phenotype, with no observable difference in growth, morphology, differentiation, or pigmented antibiotic production (Kormanec et al. 1999). σ^K has a role in the correct timing of aerial hyphae formation. Deletion of σ^K leads to increased expression of the key chaplins, ChpE and ChpH, with an earlier switch from vegetative to aerial growth and increased production of actinorhodin and undecylprodigiosin (Mao et al. 2009).

σ^B, σ^H, σ^I, σ^L, and σ^M have all been implicated in the osmotic stress response, underlining the complexity of the stress response in *S. coelicolor* which may involve multiple sigma factors regulating single types of stress. A potential hierarchy exists between σ^B, σ^L, and σ^M. Transcription of these three sigma factors is induced by osmotic stress. *sigL* mutants are unable to sporulate or produce actinorhodin following salt stress, and *sigM* mutants sporulate poorly (Lee et al. 2005). *sigL* transcription is absent in *sigB* mutants and *sigM* transcription is absent in *sigB* or *sigL* mutants, indicating a hierarchical relationship where σ^B controls σ^L and σ^M expression and σ^L controls σ^M expression (Lee et al. 2005).

σ^B, σ^H, and σ^I are specifically induced by osmotic stress (Cho et al. 2001; Kelemen et al. 2001) each with their own induction profile (Viollier et al. 2003). Transcription of *sigI* has been observed following osmotic stress only, and it is not present under normal growth conditions nor induced by heat or ethanol stress (Viollier et al. 2003). This indicates that σ^I is not required for differentiation of *S. coelicolor* with further evidence provided by lack of a distinguishable phenotype in mutants disrupted in this gene (Lee et al. 2005; Homerova et al. 2012). σ^I is regulated by a partner-switching mechanism reminiscent of the RsbW/RsbU system in *B. subtilis*. Transcription of *sigI* is directed from a single promoter induced by osmotic stress and the activity of σ^I is regulated posttranslationally by its antagonist PrsI and the anti-sigma factor antagonist ArsI (Homerova et al. 2012).

sigH is involved in morphological development and the response to osmotic stress (Kelemen et al. 2001). Along with its putative anti-sigma factor PrsH (putative regulator of *sigH* also known as UshX), σ^H is cotranscribed from two developmentally regulated promoters, *sigHp1* and *sigHp2*, as part of a two-gene operon (Kelemen et al. 2001). Contradictory phenotypes have been reported in *sigH* mutants; no significant phenotypic difference could be observed between the wild type and mutants by Viollier et al. (2003), contrasting with the previously reported osmosensitivity of *sigH* mutants which were poorly septated and rarely possessed spore chains when grown in conditions of high external osmolarity (Sevciková et al. 2001). PrsH regulates σ^H by direct interaction of the two proteins. The anti-anti-sigma factor BldG is the antagonist of PrsH and the two

proteins have also been shown to interact directly (Sevcikova et al. 2010). Under normal growth conditions σ^H forms the σ^H:PrsH complex with its antagonist and σ^H-mediated transcription is prevented. In response to unknown signals including conditions of high external osmolarity, BldG is activated, likely by dephosphorylation if this system draws parallels the Rsb system in *B. subtilis*, and is able to interact with PrsH. Formation of this BldG:PrsH complex releases σ^H which is now free to associate with RNA polymerase and direct σ^H-dependent transcription of genes containing the recognition sequence for this form of the holoenzyme. In addition, *sigH* is transcriptionally regulated by the *bldD* gene product. The transcription factor BldD has been found to bind the σ^H promoter *sigHp2*, developmentally expressed exclusively in sporulating aerial hyphae, negatively regulating its transcription (Kelemen et al. 2001). Interestingly, simultaneous deletion of *sigH* and *prsH* also results in a developmental block indicating the latter may also have an antagonist role for additional sigma factors (Viollier et al. 2003), which had previously been observed with the σ^U antagonist, RsuA (Gehring et al. 2001). Among targets of σ^H are the sporulation-specific *ssgB* (Keijser et al. 2003; Sevcikova and Kormanec 2003) and *sigJ*, encoding an ECF sigma factor, the expression of which is induced during sporulation but mutation results in no observable morphological defect (Mazurakova et al. 2006).

σ^B is a key regulator of sporulation and the osmotic stress response (Cho et al. 2001; Kelemen et al. 2001; Viollier et al. 2003; Lee et al. 2005). Mutants precociously produce pigmented antibiotics and are unable to produce aerial hyphae when high concentrations of osmolyte are present in the growth media. *sigB* forms part of an operon which includes the upstream genes, *rsbB* and *rsbA*, under the control of the constitutive *sigBp2* promoter (Cho et al. 2001). A second inducible promoter, *sigBp1*, is locating directly upstream of the coding region of *sigB* which is induced during differentiation and following osmotic stress in a σ^B-dependent manner (Cho et al. 2001). σ^B has been proposed as a master regulator of morphogenesis in addition to osmotic and oxidative stress. More than 280 genes were found to be expressed in a σ^B-dependent manner in response to osmotic stress, including *sigB* itself, and its antagonist *rsbV* (Lee et al. 2005).

The genes immediately preceding *sigB*, *rsbA* and *rsbB*, encode an anti- and anti-anti-sigma factor, respectively. The C-terminal domain of RsbA shows a high level of homology with RsbW, the *B. subtilis* σ^B anti-sigma factor. RsbA coelutes from *S. coelicolor* total protein with σ^B and prevents σ^B-mediated transcription in vitro indicating it is indeed the antagonist for this sigma factor (Lee et al. 2004). Despite the homology of *rsbB* with *rsbV* of *B. subtilis*, the σ^B anti-anti-sigma factor, the former lacks a highly conserved serine residue, which is phosphorylated in the latter to prevent its association with RsbW. This coupled with the lack of observable phenotype in these mutants makes it unlikely that RsbB is the RsbA antagonist. A putative six-gene operon lies separately on the chromosome from *sigB*, *rsbA*, and *rsbB*. σ^B-mediated transcription of *rsbV*, *rsbR*, *rsbS*, *rsbT*, *rsbU1*, and *rsbU* is induced following osmotic stress and RsbV is able to phosphorylate and inhibit the activity of RsbA in vitro providing evidence of a role for RsbA as the σ^B anti-anti-sigma factor (Lee et al. 2004). It is proposed that these antagonists along with RsbU, and other as yet uncharacterized members of the cascade, regulate σ^B activity in a partner-switching mechanism similar to that observed in *B. subtilis*. Following osmotic induction of *sigB* and its regulon, permitting physiological adaptation, SigB activity is subsequently antagonized by an anti-sigma factor domain of the product of the osmoadaptation gene *osaC* (Fernandez-Martinez et al. 2009).

Cell division in *Streptomyces* is initiated, as in other bacteria, by the polymerization of the FtsZ protein on the inner surface of the cytoplasmic membrane to form the Z-ring structure at the future division site (Lutkenhaus 1997; Margolin 2003). Transcription studies of *S. griseus* genes suggest that *ftsZ* is expressed during both sporulation and vegetative growth (Dharmatilake and Kendrick 1994). For *S. coelicolor* A3(2), FtsZ is necessary for septation within the vegetative substrate mycelium as well as for the synchronous formation of septae within the developing aerial hyphae prior to detectable separation of nucleoids (Grantcharova et al. 2003). However, in other ways these two types of cell division are different both in terms of their outcome and underlying mechanisms. The highly regular formation of multiple sporulation septa is dependent on components of the typical bacterial "divisome" such as FtsW, FtsQ, and FtsI and leads to unigenomic spores that separate from each other. In contrast, irregular and infrequent cross-wall formation in the vegetative hyphae occurs independent of the majority of divisome components, with the exception of FtsZ, and the hyphal compartments remain joined after this type of cell division (Mistry et al. 2008). The cycle of differentiation is typically observed on solid media; however, for some strains (i.e., *S. griseus*; McCue et al. 1996; *S. venezuelae*, Glazebrook et al. 1990) sporulation in liquid culture has also been reported. *S. coelicolor* A2(3) also sporulates at the air interface of standing liquid cultures (van Keulen et al. 2003). The basis for hyphal buoyancy in standing liquid cultures is not clearly understood: although *S. coelicolor* A2(3) has two *gvp* gene clusters encoding proteins that resemble gas vesicle proteins of cyanobacteria and their homologues within halophilic archaea, the hyphae of *gvp* mutants exhibit similar buoyancy to the wild type (van Keulen et al. 2005). Interestingly, all sequenced *Streptomyces* genomes, and many other actinomycetes, have *gvp* gene clusters. The structure and role of "gas vesicles" in actinomycetes remains enigmatic. For various *Streptomyces* strains, a transient slow down during growth in liquid culture is reported before entering the stationary phase. In this transition phase, a decrease in GTP and a *relA*-dependent increase in ppGpp (guanosine 3′, 5′-bispyrophosphate) as well as the activation of genes essential for secondary metabolism is detected (Chakraburrty et al. 1997). The synthesis of two ribosomal proteins is drastically reduced when the culture approaches the stationary phase (Blanco et al. 1994).

Peptidoglycans

The chemical composition and ultrastructure of the cell walls of streptomycetes is characteristic for Gram-positive bacteria

(Schleifer and Kandler 1972). Under the electron microscope, cell walls which are composed of multilayered peptidoglycan strands appear as homogeneous electron dense layers with a diameter of about 16–35 nm. The heteropolymer peptidoglycan is made of heteropolysaccharide chains (the so-called sugar backbone), which are connected by peptide cross-links. The sugar backbone is composed of alternating β-1,4-linked units of the sugar derivatives *N*-acetylglucosamine and *N*-acetylmuramic acid. The carboxyl group of the muramic acid is further substituted by an oligopeptide of alternating D- and L-amino acids (Schleifer and Kandler 1972). In *Streptomyces*, the substitution is a tetrapeptide L-Ala–D-Glu–LL-A_2pm–D-Ala being crosslinked by a pentaglycine bridge extending from the C-terminal D-alanine of the peptide unit to the amino group located on the D carbon of LL-A_2pm. The resulting macromolecular structure forms the cell envelope. This LL-A_2pm-Gly_5 is also termed A3γ peptidoglycan type (Schleifer and Kandler 1972) and is typical for streptomycetes as well as some other combined-wall chemotype I actinomycetes (Lechevalier and Lechevalier 1970a, b, c).

In a study by Lechevalier et al. specific amino acids in purified cell walls were used to group aerobic actinomycetes into four so-called wall chemotypes. Cell walls with *meso*-A_2pm and LL-A_2pm were the first to be detected. Cell wall composition may vary with the developmental stage of streptomycetes. Takahashi and coworkers (1984) reported that submerged mycelium of strains having a cell wall with *meso*-A_2pm and LL-A_2pm consists of LL-DAP and glycine (wall chemotype I), while in the spores only *meso*-A_2pm could be detected (wall chemotype III according to Lechevalier and Lechevalier (1970a, b, c)). The quantitative distribution of cell wall amino acids and cell wall sugars differed in the cell wall composition of aerial, substrate, and submerged mycelium of 11 streptomycetes. *N*-acetylmuramic acid is present as the glycolyl type in the cell walls of *Streptomyces*, as in all other actinomycetes (Uchida and Aida 1977).

Muramic acid phosphate residues are necessary as attachment points to teichoic acids, which are polymeric substances containing repeating phosphodiester groups. They are composed of *N*-acetylamino sugars or polyols (i.e., the sugar alcohols glycerol and ribitol) or both and are helpful for the identification of Gram-positive bacteria. The structure of teichoic acids does not differ between streptomycetes and other Gram-positive bacteria. The polymers are made of either ribitol phosphate or glycerol phosphate. Important for the teichoic acids of actinomycetes is the absence of ester-bound D-alanine; instead, ester-linked acetic acid and sometimes succinic acid residues are present (Naumova et al. 1980).

The synthesis of either ribitol phosphate (e.g., *S. streptomycinii* and *S. violaceus*) or glycerol phosphate polymers (e.g., *S. antibioticus*, *S. levoris*, *S. rimosus*, and *S. thermovulgaris*) in streptomycetes has been reported (Naumova et al. 1980). In ribitol teichoic acids, positions 1 and 5 of ribitol are connected to the phosphates but in glycerol teichoic acids, position 1 is commonly connected to 3, and in other types, links to 2 (as in *S. antibioticus*) are uncommon. Polyol phosphates can be substituted with diverse combinations of sugars or amino sugars or even both. The sugars or amino sugars are linked to ribitol or glycerol via glycosidic bonds. The role of teichoic acid in the taxonomy of *Streptomyces* is unclear, since only a few strains have been analyzed in detail (Naumova et al. 1980).

Cell Wall Polysaccharides

Cell wall polysaccharides seem to have no diagnostic value (Lechevalier et al. 1971) for strains, which contain LL-DAP in their cell wall. Occasionally, diagnostic sugars present in actinomycetes (i.e., arabinose, galactose, and xylose) have been reported in streptomycetes. The presence of diagnostic sugars in streptomycetes was extensively examined by Kroppenstedt (1977) who analyzed hundreds of strains. Glucose, mannose, and ribose are usually detected in small amounts.

Phospholipids and Glycolipids

The lipids of streptomycetes consist mainly of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylinositol mannosides (PIMs). A summary of the lipid composition of actinomycetes can be found in Lechevalier et al. (1977). Glycolipids are not useful for the identification of streptomycetes, because they do not occur consistently. In addition, culture conditions largely determine their qualitative and quantitative lipid composition. For example, the glycolipid content increases significantly under phosphate limiting conditions.

Polar lipids have an important taxonomic value in actinomycetes, as demonstrated by Lechevalier et al. (1977). The phospholipids of 97 actinomycete strains, representing 20 genera, were analyzed and assigned to five phospholipid types by Lechevalier et al. (1977). These phospholipid groups are characterized by the presence or absence of certain nitrogenous phospholipids. Members of the family *Streptomycetaceae* have a phospholipid type II. The marker lipids of this type are phosphatidylethanolamine (PE), hydroxy-PE, lyso-PE, and methyl-PE. However, differentiation can be achieved by using additional lipids (e.g., phosphomonoester [PME] and hydroxyphosphatidylethanolamine [OH-PE]) and the presence or absence of PG and PI (*Fig. 42.9*). In *S. coelicolor*, the anionic phospholipid cardiolipin is enriched at hyphal tips, branch points, and anucleate regions; overexpression of cardiolipin synthase results in weakened hyphal tips, misshaped aerial hyphae, and anucleate spores and demonstrates that synthesis of this phospholipid is a requirement for morphogenesis in *Streptomyces* (Jyothikumar et al. 2012).

Menaquinones

Streptomycetes contain only menaquinones (Collins and Jones 1981). The synthesized quinones include a partly saturated

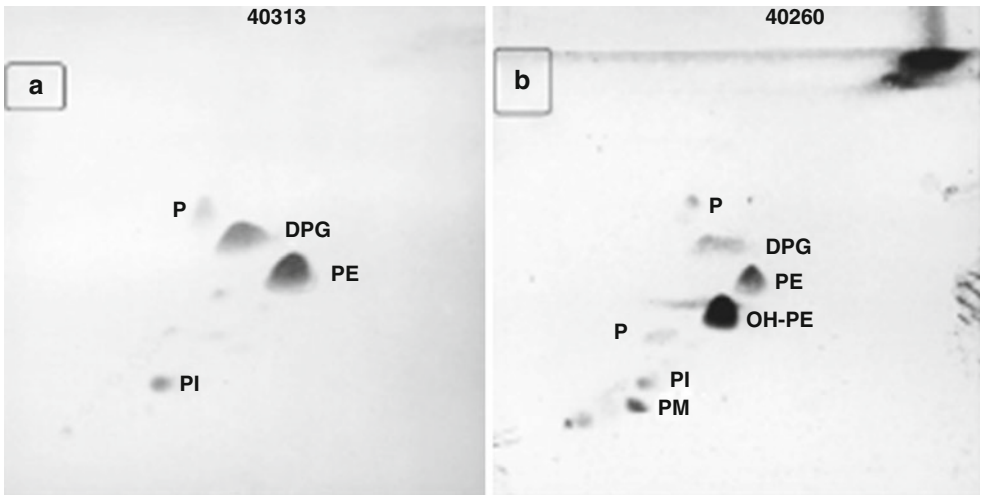

Fig. 42.9

Two-dimensional thin layer chromatograms of polar lipids of (a) *Streptomyces albus* (DSM 40313) and (b) *Streptomyces rimosus* (DSM 40260). Abbreviations: DPG, diphosphatidyglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PE, phosphatidylethanolamine; OH-PE, hydroxy-phosphatidylethanolamine; and P, phospholipids of unknown structure. (Courtesy of R. M. Kroppenstedt.)

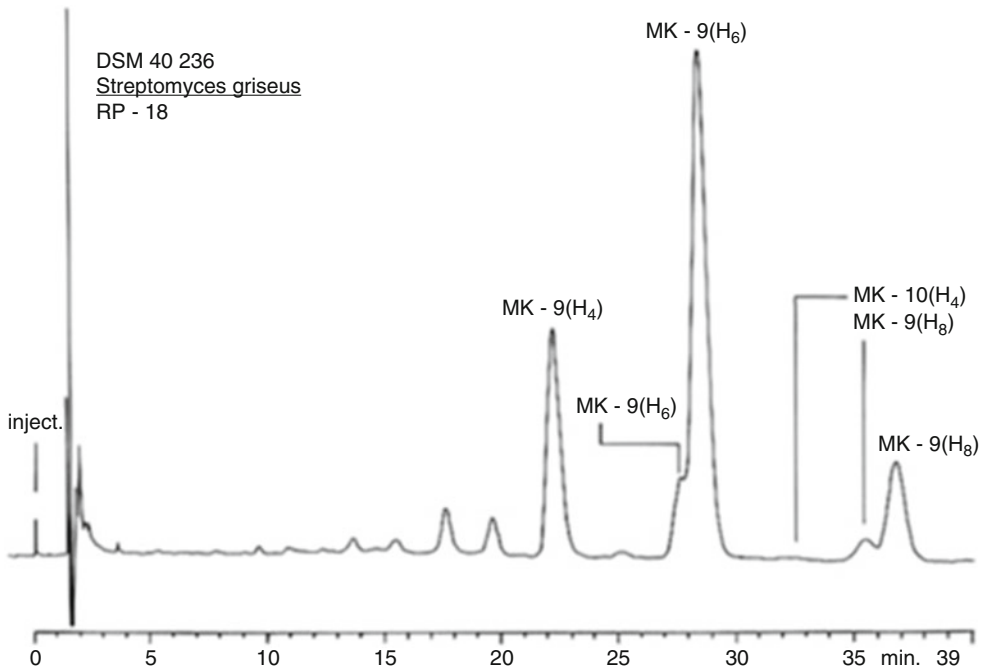

Fig. 42.10

Menaquinone profile of *Streptomyces griseus* (DSM 40236). The extent of hydrogenation of the isoprene units is shown by the subscript of the abbreviation. For instance, MK-9 (H_8) is a menaquinone with four hydrogenated isoprene units. (Courtesy of R. M. Kroppenstedt.)

isoprenoid side chain at position 3 of the naphthoquinone ring. In this, streptomycetes resemble the majority of actinomycetes. The composition of menaquinones provides a great taxonomic value for the identification of actinomycetes. The following three variations are useful for identification and classification: (1) the different numbers of isoprene units, (2) the different degrees of hydrogenation, and (3) the position of hydrogenated isoprene units (*Fig. 42.10*). The menaquinones of streptomycetes have three to four (rarely five) saturated isoprene units with a highly hydrogenated isoprenoid chain. The actinomycetes, which belong to this type, can be differentiated by a different degree of saturation.

Fatty Acid Analysis

Hofheinz and Grisebach (1965) studied selected *Saccharopolyspora erythraeus* (formerly *Streptomyces erythraeus*) and *Streptomyces halstedii* strains to clarify the biosynthetic pathway of branched chain fatty acid synthesis. It was found that *Streptomyces* species synthesize terminally branched fatty acids. From 2-methylbutyrate, as starting compound, *anteiso*-branched fatty acids with an odd number of carbon atoms are formed. In contrast, isobutyrate and isovalerate as starting compounds lead to the formation of *iso*-branched fatty acids with odd and even numbers of C-atoms, respectively. Therefore *iso*- and *anteiso*-branched fatty acids appear in pairs with odd numbers of C-atoms only.

Hofheinz and Grisebach also identified individual *iso*- and *anteiso*-branched fatty acids with chain lengths of 15 and 17 carbon atoms in both *Saccharopolyspora erythraeus* and *S. halstedii*. In addition, high amounts of 14-methyl pentadecanoic acid (*iso*-C16:0) were detected, while small amounts of unbranched fatty acids, tuberculostearic acid, and their homologues were only detected in the *Saccharopolyspora erythraea* strains, but not in *S. halstedii*.

A few streptomycetes produce minor quantities of hydroxy fatty acids in the presence of optimal amounts of oxygen. These fatty acids are easily destroyed in a non-deactivated injection port of capillary gas chromatography systems and are therefore not always detected. Nevertheless, some streptomycetes synthesize hydroxy fatty acids which are of high diagnostic value, when strains are grown under reproducible culture conditions. Hydroxy fatty acids have been found in all strains of *S. coelicolor* (30), in 20 of 27 *S. hygroscopicus* strains, in *S. rimosus* (14), and *S. violaceusniger* (18), but not in *S. albus* (33), *S. fradiae* (25), *S. glaucescens* (8), *S. griseus* (22), *S. lavendulae* (18), *S. violaceoruber* (16), and *S. viridochromogenes* (25; Kroppenstedt 1992; R. M. Kroppenstedt, unpublished observations).

Saddler and coworkers (1986) studied the fatty acid profiles of *Streptomyces cyaneus* strains and associated soil isolates, which formed a blue aerial spore mass; 13 of 19 blue-spored strains belonged to the *S. cyaneus* cluster (Hütter 1962; Pridham and Tresner 1974; Korn et al. 1978). It was shown that 8 of 10 blue-spored isolates were grouped together based on their fatty acid profile, while 17 of 34 *S. cyaneus* strains were assigned to a separate cluster. Saddler and his colleagues were able to demonstrate that the *Streptomyces cyaneus* taxon as defined by Williams et al. (1983a) was heterogeneous. They also concluded that conventional characteristics, such as color, ornamentation, and chain morphology of spores, were not reliable for the classification of streptomycetes but would be helpful for presumptive identification, a point also made by Williams et al. (1983a).

Generally, fatty acid patterns cannot be used to classify *Streptomyces* species (Phillips 1992; R. M. Kroppenstedt, unpublished observation); however, when used under standardized conditions, they are still helpful for the rapid characterization (independent of the taxonomic status) of large quantities of wild-type streptomycetes isolated from the environment (Saddler et al. 1987).

Curie-Point Pyrolysis Mass Spectrometry

A different method that has been used for the identification and classification of actinomycetes is pyrolysis mass spectrometry (PyMS; Sanglier et al. 1992; Ferguson et al. 1997). The exposure of whole cells to high temperatures causes their degradation in a nonoxidative environment leading to the generation of pyrolysate which can be analyzed by mass spectrometry. This method results in the generation of a fingerprint for each organism; however, it needs to be rigorously standardized.

Sanglier et al. (1992) applied this method to strains belonging to *Streptomyces albidoflavus* species group defined by Williams et al. (1983a) and was able to recover members of the *S. albidoflavus* and *S. anulatus* strains in distinct groups. The six *S. halstedii* strains (the third subgroup) were divided to three groups. *Streptomyces albidoflavus* strains and *S. anulatus* strains were also reassigned in different groups by Kämpfer et al. (1991). They also determined that *S. anulatus* ISP 5361^T, the strain used to name the *Streptomyces anulatus* cluster, formed a single-membered cluster (Kämpfer et al. 1991).

Serology

Serological methods have infrequently been used in *Streptomyces* taxonomy. Ridell et al. (1986) used antisera against the mycelia of streptomycetes, streptoverticillia and *Nocardiopsis* species to verify the high similarity between *S. lavendulae* and streptoverticillia (Witt and Stackebrandt 1990; Kämpfer et al. 1991). The antisera used by Kirby and Rybick (1986) proved to be genus specific and to a certain degree also group specific when tested against *S. cattleya* (cluster 47 of Williams et al. 1983a) and *S. griseus* (*S. anulatus*, cluster 1B of Williams et al. (1983a)). Wipat et al. (1994) generated a monoclonal antibody to *Streptomyces lividans* 1326, which was specific for *Streptomyces lividans* strain 1326 and for strains assigned to cluster 21 by Williams et al. (1983a).

Colonial Characteristics

Streptomycetes possess various colonial features, such as substrate mycelium, pigmentation of spores, and diffusible exopigments, together with the morphology of colonies and the texture of the aerial mycelium (❯ *Figs. 42.11* and ❯ *42.12*). The formation of different pigments has been widely used for identification and classification; however, it is important to mention that colony morphology is too variable for use as a taxonomic character.

One feature, generally used in streptomycete taxonomy, is spore mass color. Strains showing different spore colors have been assigned to "sections," "series," and "species groups" (Burkholder et al. 1954; Flaig and Kutzner 1954, 1960a; Hesseltine et al. 1954; Gauze et al. 1957; Ettlinger et al. 1958; Pridham et al. 1958; Krasil'nikov 1960). In the 8th edition of *Bergey's Manual* (Pridham and Tresner 1974b), *Streptomyces*

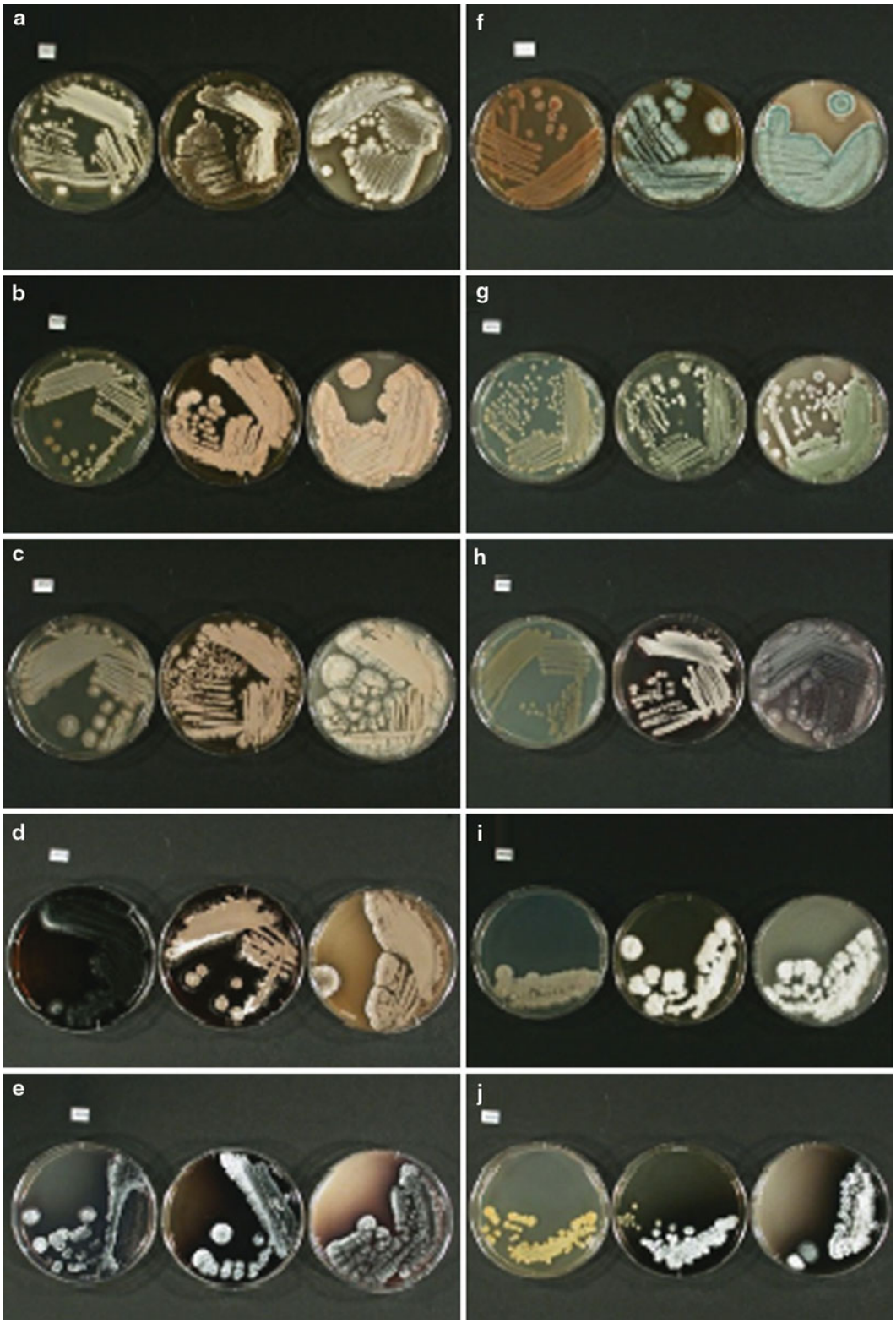

Fig. 42.11
a–j: Color of the aerial mycelium of *Streptomyces* strains grown on different agar media after 3 weeks of incubation at 28 °C. *Left*: starch-casein-nitrate agar; *middle*: GYM agar; *right*: oatmeal agar (for compositions, see *Table 42.8* and *42.9*). Species names and strain numbers are given in *Table 42.4*

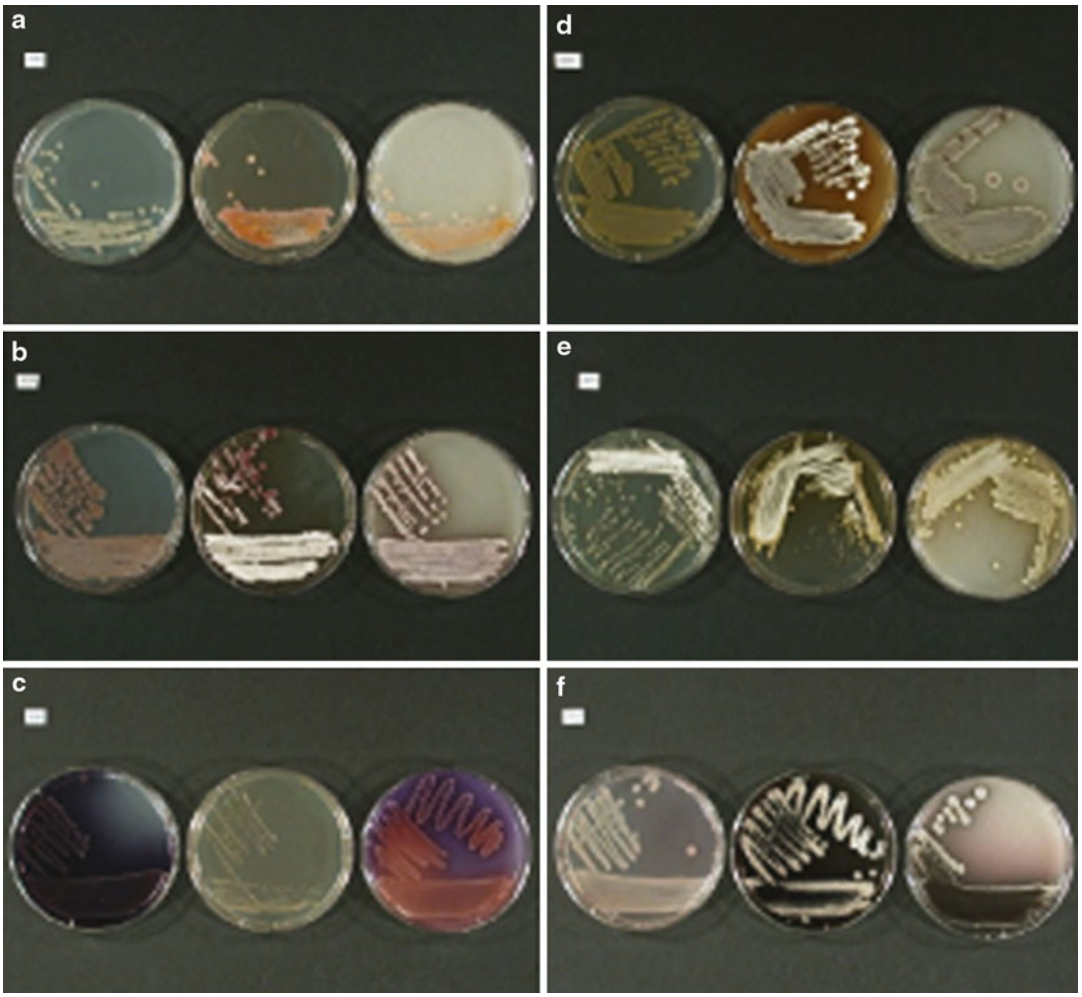

Fig. 42.12
a–f: Color of the substrate mycelium and soluble pigments of *Streptomyces* strains grown on different agar media after 7 days of incubation at 28 °C. *Left*: starch-caseinnitrate agar; *middle*: GYM agar; *right*: oatmeal agar (for compositions, see *Table 42.8* and *42.9*). Species names and strain numbers are given in *Table 42.5*

species were assigned to seven color series: blue, gray, green, red, violet, white, and yellow. In a later study, the series were extended to accommodate additional colors (Kutzner 1981). The color of the spore mass is still a useful criteria, but its determination may be difficult, since the color can be influenced by different factors such as the growth conditions, the medium, and age of the culture. Sometimes the color cannot clearly be attributed to any established category.

The color of the soluble pigment and the substrate mycelium are of high taxonomic value when they are evident, e.g., blue, dark green, red, and violet (*Fig. 42.12*). The color of the substrate mycelium has also been used in a preliminary approach to group streptomycetes (Baldacci et al. 1954; Baldacci 1958; Krasil'nikov et al. 1961b). Nevertheless, the expression of the various pigments is again often influenced by the medium composition, pH, temperature, and age of the culture (Kutzner 1981). In addition, diffusible pigments and their pH sensitivity have been used as taxonomic characters (Waksman and Curtis 1916; Jensen 1930; Shirling and Gottlieb 1970), and chemically different pigments can exhibit the same color (Krasil'nikov 1970; Kutzner 1981). Some *Streptomyces* strains produce anthracycline glycoside, diazaindophenol, naphthoquinone, phenoxazinone, and prodigiosin pigments (Kutzner 1981).

Production of Extracellular Enzymes

Streptomycetes are widely distributed in soil and play an important role in the recycling of organic matter. Consequently, *Streptomyces* genomes encode high numbers of predicted secreted proteins. *S. coelicolor* A3(2) expresses ca. 800 predicted secreted proteins including 60 proteases, 13 chitinases/chitosanases, eight cellulases/endoglucanases, three amylases, and two pectate lyases (Bentley et al. 2002; Ventura et al. 2007; Chater et al. 2010). Most of the *Streptomyces* strains studied so far can utilize chitin not only as a carbon but also as a nitrogen source (Blaak and Schrempf 1995), and, often, several chitinases are produced

(Robbins et al. 1988; Miyashita et al. 1991). For more information, see Schrempf (2006) and Chater et al. (2010). Amylases and their inhibitors are also commonly present in streptomycetes. An α-amylase gene (*aml*) of *S. limosus* ATCC 19778 has been cloned by Virolle and Bibb (1988). From sequence information it can be concluded that the genome of *S. coelicolor* A3(2) contains many genes which code for glucosyltransferases. Furthermore, xylanases and their genes have been identified from several streptomycetes, i.e., *S. lividans* 10–164 (Pagé et al. 1996), *S. halstedii* JM8 (Ruiz-Arribas et al. 1998), and the thermophilic *S. thermoviolaceus* OPC-520 (Tsujibo et al. 1997). Laccases, including those produced by *S. cyameus* CECT 3335, can be efficiently applied for biobleaching of kraft pulps (Arias et al. 2003).

Extracellular proteases are widely distributed among streptomycetes, and several corresponding genes have been characterized (Kim and Lee 1995). Streptomycetes also possess many genes encoding for protease inhibitors (Taguchi et al. 1996), including leupeptin and subtilisin (Hiraga et al. 2000). Chater et al. (2010) proposed a general model for an extracellular protease cascade that contributes to *Streptomyces* development. Moreover, keratinases are frequently detected (for review, see Kutzner 1981). A small number of extracellular lipases and their genes have also been examined from different *Streptomyces* strains (Servín-González et al. 1997; Sommer et al. 1997), among them lipolytic enzymes expressed by *S. rimosus* R6-554 W (Vujaklija et al. 2002).

Streptomyces encode at least three different protein secretion systems: the general secretory (Sec) pathway, the twin arginine translocation (Tat) pathway, and the ESX-1/type VII secretion pathway (reviewed by Palmer and Hutchings 2011). In addition, they encode numerous ABC transporters, often linked to the export of lantibiotics (modified bioactive peptides). The most remarkable aspect of protein secretion concerns the large number of non-cofactor-containing proteins exported by the Tat machinery in *Streptomyces*; in other bacteria this is generally not the case.

Primary Metabolism

In contrast to secondary metabolism of streptomycetes, relatively few studies have been published on the primary metabolism. Some genes have been identified; among them those encoding key pathway enzymes like glucose kinase, phosphofructokinase, and glucose-6-phosphate dehydrogenases (reviewed in Van Keulen et al. 2011). Butler et al. (2002) identified one gene (*devB*) encoding a 6-phosphogluconolactonase and two *zwf* genes determining isozymes of glucose-6-phosphate dehydrogenases, the first enzyme in the oxidative pentose phosphate pathway [PPP]) in *S. lividans* 66. The pentose phosphate pathway and the tricarboxylic cycle relative to glucose uptake have been examined in *S. noursei* ATCC 11455 (Jonsbu et al. 2001). *S. tenebrarrius* was shown to distribute carbon almost evenly over glycolysis and the Entner-Doudoroff pathway during exponential growth on glucose (Borodina et al. 2005).

Analogous to enteric bacteria, glutamine synthetase I (GSI) in *S. coelicolor* A3(2) is posttranslationally controlled by adenylyltransferase (Hesketh et al. 2002b). In *S. clavuligerus* NRRL-3585 *a* novel class of glutamate dehydrogenases (GDH) has been found (Minambres et al. 2000). Furthermore, *S. coelicolor* A3(2) is capable of using fatty acids (C4–C18) as sole carbon sources (Banchio and Gramajo 1997), and the glyoxylate cycle also seems to be present, at least in *S. clavuligerus* NRRL-3585 (Soh et al. 2001). The genes *matB* and *matC* were used to generate strain variants of *Streptomyces* for the production of antibiotics (Kim and Goodfellow 2002).

In order to improve knowledge on metabolic fluxes (Obanye et al. 1996) as well as the biotechnological production of pharmacologically active compounds, the genes and pathways required for the biosynthesis of primary compounds, including their regulation pattern (Rodríguez-García et al. 1997), should be studied in more detail. Detailed analyses of proteins involved in primary and secondary metabolism are available (Hesketh et al. 2002a; Huang et al. 2001). Proteomic and metabolomic data are currently being studied (e.g., Novotna et al. 2003). Borodina et al. (2008) used an integrated approach of genome-scale modeling of the metabolic network of *S. coelicolor* A3(2) with metabolic flux analyses coupled with genome-wide transcriptomic analyses of wild type and a phosphofructokinase (*pfkA2*) mutant strain to demonstrate extensive remodeling of metabolism at the glucose-6-phosphate node at the transition stage from primary to secondary metabolism. This study showed a successful rational approach for metabolic engineering of central carbon metabolic pathways to improve secondary metabolite production.

Secondary Metabolism

Streptomycetes have been the most important source of antibiotics since the discovery of actinomycin D, streptomycin, and streptothricin in the 1940s by Waksman and coworkers (for a review, see Hopwood 2007). Streptomycetes produce an enormous range of chemically diverse substances, many of them acting as antibiotics, cytostatics, fungicides, or as modulators of immune responses (see, e.g., Horinouchi 2002; Bérdy 2005; Challis and Hopwood 2003; Van Wezel and Vijgenboom 2004, and Hopwood 2007, for more detailed information). Subsequently, the study of *Streptomyces* genomes has been of great interest and has led to the discovery of 30 gene sets that code for these compounds in the *S. avermitilis* MA-4,680 genome and 26 in that of *S. coelicolor* A3(2) (Ventura et al. 2007). Interestingly, many of these gene sets are present in one genome but not in others. Also the same position in different chromosomes can be occupied by different secondary metabolism clusters, for instance, the *pks1* cluster of *S. avermitilis* MA-4680 is substituted in *S. ambofaciens* ATCC 23877 by a different secondary metabolism cluster of 28 genes, and in *S. coelicolor* A3(2) by a 31-gene insertion (Choulet et al. 2006).

Gene clusters for secondary metabolism, especially those that are species specific, are often located in the subtelomeric

chromosome arms. The more abundant genes for secondary metabolism, like the ones responsible for the production of the odor compound geosmin, different siderophores, and pentalenolactone, are typically harbored in syntenous locations within the central core region (Bentley et al. 2002; Ikeda et al. 2003). Furthermore, certain linear plasmids may also carry such gene clusters, thereby explaining the impact of lateral gene transfer between chromosomes present in different streptomycetes (Ventura et al. 2007).

Various important pharmacologically active compounds are encoded by genes, which are located within DNA stretches of 20 kb to more than 100 kb. Successful cloning has been achieved by screening total genomic DNA or gene libraries with homologous or heterologous gene probes generated by cloning, by complementing mutants, or with the help of polymerase chain reactions (PCR), as well as by transposon mutagenesis (Schrempf 2006). It is interesting that the genes responsible for antibiotic biosynthesis are often located near one or more genes mediating resistance to the corresponding antibiotic. The following listing has been adapted from Schrempf (2006): By complementation of mutants, the gene cluster for the biosynthesis of the polyketide actinorhodin was achieved (Malpartida and Hopwood 1984). Additional gene clusters for polyketides were cloned using a gene probe for the predicted key step for polyketide synthesis. These polyketides include daunorubicin (Stutzman-Engwall and Hutchinson 1989), frenolicin (Bibb et al. 1994), granaticin (Sherman et al. 1989), griseusin B (Yu et al. 1994), jadomycin B (Han et al. 1994), mithramycin (Lombo et al. 1996), tetracyclines (Binnie et al. 1989), tetracenomycin C (Motamedi and Hutchinson 1987), tetrangomycin (Hong et al. 1997), and urdamycin A (Decker and Haag 1995).

Several gene clusters encoding for macrolides have been identified, including the genes for carbomycin (Epp et al. 1987), oleandomycin (Swan et al. 1994), rapamycin (Schwecke et al. 1995), and tylosin (Fishman et al. 1987). Genes for peptide antibiotics, such as actinomycin (Stindl and Keller 1994; Hsieh and Jones 1995) and bialaphos (Murakami et al. 1986), and cyclopentenoid antibiotics (such as methylenomycin; Chater and Bruton 1985) have also been detected. Additionally, genes have been found for the production of aminoglycosides such as puromycin (Lacalle et al. 1992), ansamycins such as rubradirin (Sohng et al. 1997), carbapenems (Nakata et al. 1989), cephamycin (Aharonowitz and Cohen 1992; Paradkar et al. 1996) and cyclophilins (Pahl et al. 1997), nikkomycin (a nucleoside peptide; Bormann et al. 1996) and nosiheptide (a thiopeptide; Dosch et al. 1988), rifamycin (August et al. 1998), streptomycin (Ohnuki et al. 1985; Distler et al. 1987), and undecylprodigiosin (a pyrrole; Feitelson and Hopwood 1983; Malpartida et al. 1990).

Many secondary metabolite biosynthetic gene clusters contain at least one pathway-specific regulatory gene, encoding a *Streptomyces* antibiotic regulatory protein (SARP) or a transcriptional regulator of the LAL family (large ATP-binding regulators of the LuxR family). The former are transcriptional activators containing a winged helix-turn-helix motif towards their N-termini that is also found in the OmpR family of proteins. The SARP family of proteins has been found only in actinomycetes, and mostly within the streptomycetes. A very complex set of regulatory cascades link physiological, environmental, and developmental signals to the expression of pleiotropic regulatory genes and ultimately these pathway-specific regulators, thereby controlling secondary metabolism after the main growth phase of the bacterium (◉ *Fig. 42.8*; reviewed by van Wezel and McDowall 2011). Examples of the pleiotropic regulators are the products of the *bld* genes that also regulate the growth of aerial hyphae. The gamma-butyrolactone signaling molecules also impact on antibiotic production (reviewed by Gottelt et al. 2011). They are effective in nanomolar concentrations, and the best characterized is A-factor produced by *S. griseus*. Unusually, A-factor is required for both secondary metabolism (streptomycin and grixazone biosynthesis) and morphological development. A-factor, specified by the *afsA* gene, binds to its cytoplasmic receptor protein ArpA, thereby preventing the latter from binding and repressing the *adpA* promoter. AdpA is required for activation of transcription of both *strR*, the pathway-specific regulatory gene for streptomycin production, and other members of the *adpA* regulon, some of which are required for morphological differentiation. Gamma-butyrolactones produced by other species specifically regulate secondary metabolism. Examples are the virginiae butanolides controlling virginiamycin biosynthesis in *Streptomyces virginiae* and SCB1 that regulates production (possibly indirectly) of the pigmented antibiotics of *S. coelicolor*. A pleiotropic regulator of antibiotic production in *S. coelicolor*, AfsR, shares in its N-terminal region significant amino acid homology with the SARP family of proteins while containing ATP-binding sequences in its central region. AfsR plays a role in a signal transduction phosphorelay that regulates synthesis of the pigmented antibiotics Act and Red and also the CDA specified by the *cda* cluster (Horinouchi 2003). A membrane-associated serine/threonine protein kinase, AfsK, when activated by an as yet unknown environmental cue, autophosphorylates and in turn phosphorylates cytoplasmic AfsR. AfsR-P then activates transcription of *afsS*, encoding a 63-amino acid protein that functions in an unknown way to enhance the production of Act, Red, and CDA. Interestingly, AfsK serves to link growth and antibiotic production as another of its targets is DivIVA, a component of the polarisome that directs apical growth and branching of hyphae (Hempel et al. 2012).

The analysis of complete genome sequences has revealed an impressive number of cryptic gene clusters potentially encoding as yet unidentified secondary metabolites. As these are a potential source of new antibiotics, it is of interest that at least for a few examples their activation has been accompanied by synthesis of new metabolites. This is exemplified in *S. ambofaciens*, where genetic manipulation leading to ectopic expression of a pathway-specific regulatory gene triggered expression of previously silent genes and led to isolation of a new macrolide antibiotic, stambomycin (Laureti et al. 2011).

More detailed information on the secondary metabolism of streptomycetes, including the generation of new compounds by

combinatorial biosynthesis, is covered in text books and/or reviews (e.g., Bérdy 2005; Challis and Hopwood 2003; Van Wezel and Vijgenboom 2004; Hopwood 2007; Dyson 2010; Olano et al. 2011; van Wezel and McDowall 2011).

Isolation, Enrichment, and Maintenance Procedures

The procedures used to isolate streptomycetes are extensively summarized by Korn-Wendisch and Kutzner (1992). These procedures are briefly described here. Further information about isolation for special purposes, growth, and preservation of streptomycetes can be found in the excellent textbook *Practical Streptomyces Genetics* (Kieser et al. 2000).

In general, isolation procedures for microorganisms are dependent on the number of individuals relative to the number of other microbes within the habitat as well as on the nature of the microorganism to be isolated (Stolp and Starr 1981). Direct plating of a serial dilution on a nutrient agar medium can readily lead to a pure culture, if the chosen organism is best adapted to the selected isolation conditions. Nevertheless, this procedure does not work well for isolation of streptomycetes. Instead enrichment cultures or selective media and/or specific isolation conditions are typically used.

The following selective criteria can be applied to isolate members of the family *Streptomycetaceae* (Korn-Wendisch and Kutzner 1992; Williams and Wellington 1982a, b; Williams et al. 1984a): (1) choice of sample material containing the microorganisms of interest; (2) pretreatment of the sample and, in some cases, enrichment of the chosen microbial groups; (3) use of selective media or selective incubation conditions or both; and (4) colony selection on the basis of colony morphology.

Streptomycetes exist in and can be isolated from a wide range of habitats. In most cases, the organisms are extracted from soil or other environmental samples, followed by dilution of cells (cell aggregates) to allow cultivation on solid media.

Isolation from Soil

Vegetative mycelia and spore chains of streptomycetes are often closely associated with soil mineral and organic particles. For isolation, vigorous shaking of the sample with the diluent is often necessary to suspend the spores or mycelial fragments. In addition, it can be helpful to use glass beads and agitate the sample on a shaker. Other isolation methods are described in the literature, including the use of mechanical devices such as the Turmix blender, Ultra-Turrax homogenizer, Ultrasonics sonicator-disrupter, Waring blender, or a mortar and pestle. Nevertheless, the efficiency of these pretreatments has not been compared in detail. Additional procedures described comprise the use of chemical disruption methods to separate mycelia from spores. For instance, Herron and Wellington (1990) gently agitated soil samples with an ion-exchange resin Chelex-100 (Biorad) followed by differential centrifugation and filtration. The dispersion and differential centrifugation (DDC) technique, which is a multistep procedure that combines several physicochemical treatments, was introduced by Hopkins et al. (1991). It can also be used to increase the yield and diversity of actinobacteria from natural habitats (Goodfellow and Fiedler 2010).

Subsequent handling of samples (i.e., preparing dilutions and plating) differs little from standard microbiological practice. Before dilutions are prepared, coarse particles of the soil suspensions should be allowed to settle. Another option is to utilize the soil particles for the incubation of "soil plates" (Warcup 1950); this method is also used to isolate fungi. For streptomycetes, the addition of lime to soil can be a helpful enrichment factor (see chapter 2 of Kieser et al. (2000) and references therein). Distribution of diluted samples on isolation plates may be carried out with a sterile glass rod (or Drigalski spatula).

To avoid the spread of motile bacteria via water films, plates can be dried at 45 °C before incubation (Vickers and Williams 1987). A 100-fold increase of streptomycete colonies on isolation plates can be accomplished by the addition of $CaCO_3$ to air-dried soil samples (10:1 w/w) and subsequent incubation at 26 °C for 7–9 days in a water-saturated atmosphere (Tsao et al. 1960; El-Nakeeb and Lechevalier 1963). Another highly recommended procedure is to mix the soil suspension with the molten agar (Korn-Wendisch and Kutzner 1992).

Jensen (1930) enriched streptomycetes by supplementing soil with keratin; it has also been described that adding chitin to soil increases the growth of streptomycetes (Williams and Mayfield 1971). In addition, enrichment of acidophilic and neutrophilic streptomycetes in acidic soil and litter can be achieved adding fungal chitin (Williams and Robinson 1981). Chitin in the form of insect wings has also been used as an isolation strategy (Jagnow 1957; Okafor 1966; Veldkamp 1955). Other selective isolation methods studied by Porter and Wilhelm (1961) include the use of various other organic materials, such as cottonseed meal, dried blood flour (15 mg/g of soil), peanut meal, and salmon viscera meal. Additionally, the number of streptomycetes were enhanced up to 1000fold, when the enrichment cultures were incubated under moist conditions (Porter and Wilhelm 1961).

Arginine glycerol agar is frequently used for the selective isolation of streptomycetes (El-Nakeeb and Lechevalier 1963), as well as colloidal chitin agar (Hsu and Lockwood 1975), HV agar (Hayakawa and Nonomura 1987a, b), and reduced arginine starch salts agar.

To reduce or inhibit other microbes, several biological, chemical, and physical methods have been examined (see reviews by Goodfellow and Williams 1986, and Goodfellow 2010). The centrifugation of soil suspensions for 20 min at 1600 × g has been described to separate streptomycetes spores (in the supernatant) from other bacteria and fungal spores (in the sediment) (Nüesch 1965). However, this method has not been very successful. El-Nakeeb and Lechevalier (1963) applied a similar approach, but obtained a significantly lower number of streptomycete colonies as compared to the control. A simple sedimentation method was reported by Voelskow (1988/1989),

who suspended 1 g of soil in 15 mL of salt solution and mixed the preparation by vigorously shaking followed by ultrasonic treatment. Samples were taken from different levels of this solution after 1, 2, and 4 h of sedimentation, further diluted, and plated onto agar surfaces.

Arthrospores display relatively high resistance to low moisture tension; therefore initial heating and drying procedures can be applied to environmental samples to reduce the number of unwanted bacteria. A relative increase in streptomycete concentrations can be achieved by drying samples or by prolonged storage at room temperature for mesophiles and at 50–60 °C for thermophiles. The vegetative bacterial proportion in a soil sample can be reduced without affecting the colony counts of streptomycetes by heat treatment (40–50 °C, 2–16 h), as reported by Williams et al. (1972b).

Membrane filtration has been used for the enrichment of streptomycetes from water (Burman et al. 1969) and from seawater and mud (Okami and Okazaki 1972) samples. This method was also applied as a first step in the isolation of streptomycetes from soil. Trolldenier (1966) filtered 1 mL aliquots of a tenfold dilution series through membranes (0.3-µm pore size) and subsequently placed them upside down on a suitable agar medium supplemented with 10 % compost soil. This procedure allowed streptomycetes to grow through the pores and develop colonies between the agar surface and the membrane filter, whereas other bacteria and fungi were unable to grow through the pores. In total a 3–5-fold increase in the number of streptomycete colonies was noted when compared with poured plates without supplemented soil.

The use of cellulose ester membrane filters (pore size 0.01–3.0 µm) was introduced by Hirsch and Christensen (1983). The membrane filters were placed onto nutrient agar containing antifungal antibiotics (cycloheximide and candicidin) and samples of soil, water, and vegetable material were used as inoculum. The hyphae of actinomycetes were capable of penetrating the pores of the membrane filters and grow on the underlying agar medium after 4 days, whereas the growth of the other bacteria was restricted to the surface of the filters. Afterwards, the membrane filters were removed and the plates reincubated to allow further development of actinomycete colonies. Filters (0.22–0.45 µm) can also be used for the exclusive recovery of actinomycetes, as described by Polsinelli and Mazza (1984) and Hanka et al. (1985).

To improve the isolation efficiency of streptomycetes, the addition of chemicals to environmental samples has also been described by several authors. One recommended method to reduce unwanted bacteria and fungi involves phenol treatment of a dense soil suspension (1.4 % for 10 min). However El-Nakeeb and Lechevalier (1963) obtained less favorable results with this method. Burman et al. (1969) found that streptomycetes and other actinomycetes were somewhat more resistant to ammonia, chloramine, and sodium hypochlorite than other bacteria. Thus they used these agents for the treatment of water samples.

Isolation from Aquatic Habitats

The media listed in *Table 42.8* can be used to isolate streptomycetes from aquatic habitats. Hsu and Lockwood (1975) found that chitin agar was more effective for the incubation of actinomycetes from aquatic habitats than egg albumin, glycerol-arginine, starch-casein, and *Actinomyces* isolation agars (see also *Table 42.8*).

Following dilution, it is possible to streak water samples directly onto solid medium. If low quantities of actinomycetes are expected, they can be concentrated by membrane filtration of the sample [for details, see Burman et al. (1969)].

The selective isolation of streptomycetes from marine habitats can be increased, when media are supplemented with seawater or an equivalent. Media containing 25 or 75 % seawater (Weyland 1981a, b), artificial seawater (Goodfellow and Haynes 1984), and deionized water supplemented with 3.0 % NaCl (Okami and Okazaki 1978) have all proved to be effective. See Weyland (1981a) and Goodfellow and Haynes (1984) for further details.

Isolation of Airborne Spores

Streptomyces spores from self-heating material such as hay or compost can be agitated in a sedimentation chamber (see below; Lacey and Dutkiewicz 1976b) or a wind tunnel (Lacey and Dutkiewicz 1976a). The resulting aerosol can be further used in an Andersen sampler to inoculate agar plates (Goodfellow and Williams 1986). This method is widely employed for the isolation of thermophilic actinomycetes; however, it can also be used for the isolation of mesophilic streptomycetes from soil. Other devices such as filtration samplers (e.g., Sartorius MD 8) can also be applied for the sampling of airborne streptomycetes.

Isolation of Thermophilic Streptomycetes

Most thermophilic actinomycetes derive from samples taken from high temperature environments (e.g., compost materials, fodders, and manure heaps). Therefore, it is recommended to use high temperatures (45–60 °C) for the selective isolation of such organisms (Festenstein et al. 1965).

It is also important to culture thermophilic streptomycetes in a humid atmosphere (Greiner-Mai et al. 1987) by incubating plates in large jars with water at the bottom; another effective method is to seal Petri dishes with masking tape.

Interestingly, the media recommended for the isolation of thermophilic actinomycetes, including streptomycetes, contain higher nutrient concentrations than those used for mesophilic strains. Sometimes, such media are also supplemented with antibacterial and antifungal agents (Lacey and Dutkiewicz 1976a; Goodfellow et al. 1987). Special procedures recommended for the isolation of thermophilic actinomycetes are available (Uridil and Tetrault 1959; Fergus 1964; Gregory and Lacey 1963; Cross 1968; Kim et al. 1996, 1998, 2000).

Table 42.8

Some media recommended for the selective isolation of streptomycetes

Reference[a]	1	2	3	4	5
Ingredients (g/L)	Starch-casein-KNO_3 agar	Raffinose-histidine agar	Glycerol-arginine agar	Chitin agar	*Actinomyces* isolation agar
Casein	0.3	–	–	–	–
Sodium caseinate	–	–	–	–	2.0
Sodium propionate	–	–	–	–	4.0
Starch	10.0[b]	–	–	–	–
Raffinose	–	10.0	–	–	–
KNO_3	2.0	–	–	–	–
Glycerol	–	–	12.5	–	5.0[c]
Chitin (colloidal)	–	–	–	4.0	–
Arginine	–	–	1.0	–	–
Asparagine	–	–	0.1	–	–
Histidine	–	1.0	–	–	–
$CaCO_3$	0.02	–	–	–	–
$CuSO_4.5H_2O$	–	–	0.001	–	–
$Fe_2(SO_4)_3.6H_2O$	–	–	0.01	–	–
$FeSO_4.7H_2O$	0.01	0.01	–	0.01	0.001
K_2 H PO_4	2.0	1.0	1.0	0.7	0.5
KH_2 PO_4	–	–	–	0.3	–
$MgSO_4.7H_2O$	0.05	0.5	0.5	0.5	0.1
$MnCl_2.4H_2O$	–	–	–	0.001	–
$MnSO_4.H_2O$	–	–	0.001	–	–
NaCl	2.0	1.0	–	–	–
$ZnSO_4.7H_2O$	–	–	0.001	0.001	–
Agar[d]	18.0	12.0	15.0	20.0	15.0
pH				Adjusted to 7.0–7.5 or lower or higher depending on the flora to be isolated	

[a]Reference: (1) according to Küster and Williams (1964b); (2) Vickers et al. (1984); (3) El-Nakeeb and Lechevalier (1963); (4) Hsu and Lockwood (1975); and (5) Difco Laboratories

[b]Alternatively, glycerol at 10 g/L can be used

[c]Not contained in the dehydrated medium; added at the time of preparation

[d]The different amounts of the agar are due to the varying quality used by the individual authors

Isolation of Antibiotic-Producing Actinomycetes

Antibiotic-producing streptomycetes can be isolated following the same procedures as stated above. The antibiotic-producing activity of streptomycetes is normally tested after the isolation of pure cultures; nevertheless, procedures are available to detect them directly on isolation plates. Antibiotic-producing strains can, for instance, be detected on initial dilution plates by flooding or spraying them with appropriate indicator organisms; the plates are then incubated until zones of inhibition are detected (Lindner and Wallhäusser 1955; Wilde 1964). Alternatively, antibiotic activity of the colonies against selected sensitive organisms can be examined by using a simple replication procedure (Lechevalier and Corke 1953). Additional information about protocols which can be applied for the selective isolation and screening of antibiotic-producing actinomycetes can be found in Nolan and Cross (1988). Procedures for the selective isolation of streptomycetes with the generation of spore suspensions and for more sophisticated experimental techniques are described by Kieser et al. (2000).

Table 42.9

Nutrients and selective agents recommended for isolation of streptomycetes from soil (According to Korn-Wendisch and Kutzner 1992)

Nutrients and selective agents in the medium			
Antibiotic	Preferred C and N source	Others	Reference[a]
Rifampicin	Starch, casein, KNO_3		1
Cycloheximide, nystatin, penicillin, polymyxin	Starch, casein, KNO_3		2
Cycloheximide	Starch, casein, KNO_3	Rose Bengal	3
Cycloheximide, pimaricin, nystatin	Glycerol, arginine		4
Cycloheximide	Dextrose, asparagine		5
	Asparagine	Propionate	6
	Chitin		7
	Starch, KNO_3		8
	Starch, casein, KNO_3		9
	Glycerol, casein, KNO_3		9
	Glycerol, arginine		10
	Raffinose, histidine		1

[a](1) Vickers et al. 1984; (2) Williams and Davies 1965; (3) Ottow 1972; (4) Porter et al. 1960; (5) Corke and Chase 1956; (6) Crook et al. 1950; (7) Lingappa and Lockwood 1962; (8) Flaig and Kutzner 1960b; (9) Küster and Williams 1964b; (4) El-Nakeeb and Lechevalier 1963

Isolation from Diseased Plants

Three general steps have been recommended for the isolation of streptomycetes from diseased plant material such as scabby potatoes or beet surface layers (see also Korn-Wendisch and Kutzner 1992): (1) sterilization of the surfaces of tubers, beets, or roots; (2) maceration of plant tissues; and (3) use of appropriate isolation media. Several authors have described detailed methods for the isolation of *Streptomyces scabies* from potatoes (Taylor 1936; KenKnight and Munzie 1939; Menzies and Dade 1959; Adams and Lapwood 1978; or Archuleta and Easton 1981).

Optimal Growth Conditions and Use of Selective Media for Enrichment

For the isolation of streptomycetes, various media have been empirically formulated. In *Table 42.9*, selected carbon and nitrogen compounds are listed, which are especially suitable for the isolation of these organisms. The most frequently used media with their formulas are listed in *Tables 42.8* and *42.10*. Nevertheless, streptomycetes can also be grown on very poor media such as water agar.

Early on, it was recognized that streptomycetes are capable of breaking down chitin (Veldkamp 1955; Jagnow 1957). Based on this observation, Lingappa and Lockwood (1962) described a chitin medium for selective isolation. However, this chitin medium proved to be only slightly better than water agar, which was recognized by the authors and later also by El-Nakeeb and Lechevalier (1963). Hsu and Lockwood (1975) developed a useful medium for the isolation of actinomycetes (*Streptomyces*, *Nocardia*, and *Micromonospora*) from water samples by adding mineral salts to the chitin medium (*Table 42.8*). However, the medium was only poorly effective for isolation of actinomycetes from soil. It has to be considered that chitinolytic activity is not a genus-specific feature for *Streptomyces*. From over 300 strains tested, only 25 % were strongly chitinolytic (Williams et al. 1983a). As a result, this commonly used medium selects only for chitinolytic streptomycete strains which might not be the most abundant in soil. The vast majority of streptomycetes degrade starch, and therefore it represents a suitable selective carbon source for enrichment of streptomycetes. The combination of starch and nitrate is utilized by numerous streptomycetes in contrast to other bacteria (Flaig and Kutzner 1960b). Küster and Williams (1964 a, b), who improved this medium, stated: "The three best media, allowing good development of streptomycetes while suppressing bacterial growth, were those containing starch or glycerol as the carbon source with casein, arginine or nitrate as the nitrogen source." Additionally, streptomycete isolation is favored by a combination of glycerol and arginine (Benedict et al. 1955). Further studies by El-Nakeeb and Lechevalier (1963) revealed that this medium (*Tables 42.8* and *42.10*) was superior to nine other media, resulting in higher numbers and proportions of streptomycete colonies.

Moreover, other compounds have been successfully used for the selective isolation of streptomycetes such as cholesterol (Brown and Peterson 1966), elemental sulfur (Wieringa 1966), pectin (Wieringa 1955), poly-ß-hydroxybutyrate (Delafield et al. 1965), rubber (Nette et al. 1959), and natural and artificial humic acids (Hayakawa and Nonomura 1987a, b). The selective mechanisms of most of these compounds are based on the production of visible clearing zones or other changes in the medium.

To suppress fungal growth, agents with antifungal activity (antibiotics), such as cycloheximide (actidione, 50–100 µg/mL), are commonly used to supplement isolation media (*Table 42.9*),

Table 42.10

Composition of some media suitable for the cultivation of streptomycetes[a] (According to Korn-Wendisch and Kutzner 1992)

	Ingredients		Comments
1.	**Inorganic salts-starch agar**		
	Starch (soluble)	10.0 g	Make a paste of the starch with a small amount of cold distilled water and bring to a volume of 1 L; then add the other ingredients. The pH should be between 7.0 and 7.4. Do not adjust it if it is within this range
	$CaCO_3$	2.0 g	
	K_2HPO_4 (anhydrous basis)	1.0 g	
	NaCl	1.0 g	
	$(NH_4)_2SO_4$	2.0 g	
	$MgSO_4 \cdot 7H_2O$	1.0 g	
	Trace salts solution (see no. 5)	1.0 mL	
	Agar	12.0 g	
	Distilled water	1 L	
2.	**Glucose-yeast extract-malt extract (GYM) agar**		
	Glucose	4.0 g	Addition of $CaCO_3$ (2.0 g/L) is advantageous for many streptomycetes. Adjust medium to pH 7.2
	Malt extract	10.0 g	
	Yeast extract	4.0 g	
	Agar	12.0 g	
	Distilled water	1 L	
3.	**Glycerol-asparagine agar**		
	Glycerol	10.0 g	The pH should be between 7.0 and 7.4. Do not adjust if it is within this range
	L-Asparagine (anhydrous basis)	1.0 g	
	K_2HPO_4	1.0 g	
	Trace salts solution (see no. 5)	1.0 mL	
	Agar	12.0 g	
	Distilled water	1 L	
4.	**Oatmeal agar**		
	Oatmeal	20.0 g	Cook 20.0 g oatmeal in 1 L distilled water for 20 min. Filter through cheesecloth. Add distilled water to restore volume of filtrate to 1 L, and then add trace salts solution and agar. Adjust to pH 7.2
	Trace salts solution (see no. 5)	1.0 mL	
	Agar	12.0 g	
	Distilled water	1 L	
5.	**Trace salts solution**		
	$FeSO_4 \, 7H_2O$	0.1 g	
	$MnCl_2 \, 4H_2O$	0.1 g	
	$ZnSO_4 \, 7H_2O$	0.1 g	
	Distilled water	100.0 mL	
6.	**Trace elements solution SPV-4**		
	$CaCl_2 \, 2H_2O$	4.0 g	SPV-4 is used as an alternative to (5). Add 5 mL of this stock solution to 1 L of medium
	$CoCl_2$	0.022 g	
	$CuSO_4 \, 5H_2O$	0.04 g	
	Fe (III) citrate	1.0 g	
	$MnSO_4$	0.2 g	
	$Na_2B_4O_7 \, 10H_2O$	0.1 g	
	$Na_2MoO_4 \, 2H_2O$	0.025 g	
	$ZnCl_2$	0.1 g	
	Distilled water	1 L	

[a]Recipes 1–5 from Shirling and Gottlieb (1966) and recipe 6 from Voelskow (1988/1989)

as described by Williams and Davies (1965). In addition, these authors found that nystatin and pimaricin (each 10–50 μg/mL) were even more effective.

Compounds with antibacterial activity need to be used with caution as some actinomycetes may also be sensitive to them. For example, penicillin (1 μg/mL) and polymyxin (5 μg/mL) inhibit the growth of many unwanted bacteria but also suppress some streptomycetes (Williams and Davies 1965). Actinobacterial genera, most notably streptomycetes, vary significantly in their sensitivity to antibacterial agents (Preobrazhenskaya et al. 1978). Hence, it may be recommended to use antibacterial compounds for the isolation of other actinobacterial genera (Cross 1982).

Nevertheless, the selective isolation of certain species or groups of *Streptomyces* can be facilitated by media containing antibiotics, as displayed by the use of starch-casein agar supplemented with rifampicin (50 μg/mL) for the selective isolation of members of the *Streptomyces diastaticus* cluster by Williams et al. (1983a) and Vickers et al. (1984). Wellington et al. (1987) described a similar effect, by using several media containing different C and N sources, as well as media supplemented with inhibitory compounds.

The isolation of streptoverticil-producing *Streptomyces* species was favored by a selective isolation medium supplemented with cycloheximide and nystatin (each 50 μg/mL) to suppress fungal growth and oxytetracycline (25 μg/mL) to inhibit growth of other actinomycete genera, including *Streptomyces* groups (Hanka et al. (1985)). The selectivity of this medium was further enhanced by the addition of lysozyme (1,000 μg/mL) (Hanka and Schaadt 1988). Starch-casein-nitrate agar (Ottow 1972) containing Rose Bengal (35 mg/L) inhibits the growth of most bacteria and controls spreading of fungi across isolation plates. Fungi can also be suppressed by the addition of sodium propionate (Crook et al. 1950; ❯ *Table 42.9*).

Soil stimulates growth, sporulation and pigmentation of actinomycetes/streptomycetes (Trolldenier 1966) and is therefore often supplemented to isolation media to increase the number of colonies. The addition of montmorillonite or Ca-humate to liquid media promotes the growth and metabolic activity of some actinomycetes (Martin et al. 1976). A similar effect has been observed for clay in dialysis tubes after a short lag period, an observation which may be explained by the adsorption of one or more inhibitory substances produced during growth. Adsorbing materials have a positive effect on the genetic stability of other bacteria and on fungi (Martin et al. 1976).

The environmental requirements and tolerances of streptomycetes have been described in detail by Kutzner (1981). Streptomycetes are usually regarded as obligate aerobes with a limited capacity for growth under microaerophilic conditions (Kutzner 1981, Van Keulen et al. 2003, 2007), and dissimilatory reduction of nitrate is common (Van Keulen et al. 2007; Fischer et al. 2010). Despite poor growth under anaerobic or microaerophilic conditions, many *Streptomycetes* are able to withstand long periods of anaerobic stress (Van Keulen et al. 2007). Whether streptomycetes grow aerobically or microaerophilically is mainly dependent on the nutritional composition of the medium. In a nutrient rich medium, streptomycetes grow aerobically at the surface of the semisolid agar column, but in poor media or in a medium with a nonutilizable carbon source, they grow microaerophilically in semisolid agar. In stationary liquid culture, streptomycetes grow as pellicles at the air-liquid and liquid–solid surface interfaces, whereas the medium itself stays completely clear (Van Keulen et al. 2003).

Most streptomycetes are neutrophilic organisms, which means they grow optimally between pH 5.0 and 9.0 with an optimum close to pH 7.0. Thus, most isolation media have pH values of 7.0–7.5. Only a few of the type strains studied by Williams et al. (1983a) were capable to grow at pH 4.3, although large populations of acidophilic and acidoduric strains have been isolated from acidic soils (Williams et al. 1971; Khan and Williams 1975; Hagedorn 1976). Acidophilic streptomycetes grow in a pH ranging from 3.5 to 6.5, and optimally between pH 4.5 and 5.5. However, a wide range of pH requirements are present among streptomycetes from acidic environments (Flowers and Williams 1977b). Acidophilic strains are capable to produce diastases (Williams and Flowers 1978) and chitinases which have pH optima below that of the corresponding enzymes from neutrophilic strains. Populations of alkaliphilic streptomycetes, with a growth optimum at pH 9.0–9.5, have been isolated from soils in Japan. Also six of the type strains tested were able to grow at pH 11.5 (Mikami et al. 1982). Moreover, large populations of alkaliphilic streptomycetes have been isolated from a beach and dune sand system at Ross Links in Northumberland, UK (Antony-Babu and Goodfellow 2008).

The majority of streptomycetes isolated from soils are mesophilic; hence, their optimal growth temperature lies between 22 °C and 37 °C (in most cases at 28 °C). In contrast, several thermotolerant and thermophilic strains grow well at higher temperatures (40, 45, 50, or 55 °C), while psychrophilic strains (e.g., from marine environments) grow best at 15–20 °C. A series of type strains studied by Williams et al. (1983a) grew at 10, 37, and 45 °C, although a few grew slowly at 4 °C (Williams et al. 1983a). Nevertheless, in many cases, the optimal temperature for maximal yield or rapid growth may not be the best option for studying the production of secondary metabolites (e.g., antibiotics and pigments). This means that culture conditions are dependent on the aims of the study. Mesophilic actinomycetes usually produce visible colonies within 7–14 days of incubation, whereas thermophilic strains tend to form colonies faster within 2–5 days. Marine and other psychrophilic actinomycetes grow much slower and may need several weeks (up to 10) for visible colonies to appear on isolation media.

In general, colonies of *Streptomyces* can be easily identified by their macroscopic and microscopic appearance. Purification is usually facilitated by transferring colonies from isolation plates into nonselective medium. Williams and Wellington (1982a) stated that purification is "undoubtedly the most time-consuming and often the most frustrating stage of the isolation procedure." Acidophilic streptomycetes can be readily isolated on acidified starch-casein agar supplemented with cycloheximide and nystatin (Kim et al. 2003).

Cultivation and Preparation of Inoculum

Most streptomycetes are nonfastidious organisms with a chemoorganotrophic metabolism. For optimal growth streptomycetes require an organic carbon source, such as starch, glucose, glycerol, and lactate, and usually the provision of a suitable inorganic nitrogen source, like ammonium or nitrate (Kutzner 1981). Nevertheless, different isolates can vary significantly in their carbon and nitrogen source utilization patterns, which are frequently used as taxonomic characteristics (e.g., Shirling and Gottlieb 1966; Pridham and Tresner 1974b; Williams et al. 1983a; Kämpfer et al. 1991). Commonly used carbon sources comprise cellobiose, glucose, glycerol, d-mannose, and trehalose; valuable nitrogen sources are ammonium, L-arginine, L-asparagine, and nitrate. The majority of strains can degrade casein, esculin, gelatin, and hypoxanthine, but only a small number utilize organic acids, inulin, l-methionine, nitrite, or xylitol. Streptomycetes grow well on many different media, but spore formation is usually most prolific on those with a high carbon-nitrogen ratio (Kutzner 1981). In general streptomycetes require a good supply of free water for growth but are unable to develop at high osmotic or matric potentials. Survival of streptomycetes in dry conditions is supported by the resistance of arthrospores to desiccation, as opposed to vegetative mycelia (Williams et al. 1972).

The need to supplement culture media with specific trace elements has not been examined in detail. Spicher (1955) described the positive effect of trace elements in soil on the growth of streptomycetes. Nevertheless, many of the early used media (even the "synthetic" media) were not supplemented with trace elements. In fact, recipes of many authors (*Tables 42.8* and *42.10*) included only a selected number of metal ions. A rather complete mixture (SPV-4; *Table 42.10*) has been found to be optimal for the growth of actinomycetes and other bacteria (Voelskow 1988/1989).

"Synthetic media" can be used for the cultivation of streptomycetes. However, the need for specific nutritional requirements with respect to vitamins and organic growth factors has not been addressed. Growth rates and biomass production can be improved by using complex organic substrates such as malt extract, oatmeal, or yeast extract. In addition, a combination of a complex organic carbon source with a single amino acid as nitrogen source (e.g., glutamic acid, arginine, or asparagine) is also suitable.

"General media" have been recommended for the cultivation of streptomycetes, as they allow the completion of the *Streptomyces*' life cycle, including germination of spores, growth of substrate and aerial mycelium, and visible formation of spores (visible, because of the typical color of the spores). Some of these media were used in the International *Streptomyces* Project (ISP), among them glucose-yeast extract-malt extract, glycerol-asparagine, inorganic salts-starch, and oatmeal agars (Shirling and Gottlieb 1966). Numerous general media have been proposed for the growth of streptomycetes, four of which are of considerable practical value (*Table 42.10*). For additional media formulations, see Waksman (1961) and Williams and Cross (1971).

$CaCO_3$ is added to some media, as Ca^{2+} supports growth and neutralizes acids produced by many streptomycetes; such media also allow good sporulation. Cultures should be examined microscopically to detect the extent of macroscopically heavy aerial mycelia as they may contain very few spores, whereas aerial mycelia which are hardly detectable by the naked eye may be a good source of spores.

A list of specialized media, especially for the study of streptomycetes genetics, is provided by Kieser et al. (2000).

Solid media in dishes or slants are recommended for the growth of streptomycetes for subcultivation and maintenance as well as for most diagnostic tests. Numerous strains produce aerial mycelia and spores on solid media when the entire surface is covered by confluent growth. In contrast to many molds, *Streptomyces* colonies only spread over a limited area; thus, a point inoculation will usually not result in confluent growth. However, if streaked onto an agar plate, some strains require empty spaces between the streaks (cross-hatch inoculation) to induce sporulation. Dry conditions are usually more suitable for sporulation. A horizontal incubation of slants for the first 2 days allows liquid to soak into the agar surface (Hopwood et al. 1985). A suspension of inoculum in liquid should be used as starting material for sporulation (Kieser et al. 2000).

For the generation of cultures, single colonies should be selected and streaked onto fresh media. When streptomycetes are cultivated on solid media, morphological heterogeneity is frequently observed. More details can be found in Kieser et al. (2000). Successive rounds of mass culture should be avoided, especially in genetic studies because this technique reduces the accumulation of revertants or increases the gradual loss of selected plasmids or both (Kieser et al. 2000).

For some diagnostic tests, precultivation of grown colonies in liquid media is necessary to obtain a homogeneous suspension (Kämpfer et al. 1991). Streptomycetes should be cultivated in liquid medium without agitation. This precultivation step is required for the provision of cell material for biochemical analysis, for certain physiological studies (e.g., degradation tests), and for the production of secondary metabolites (e.g., antibiotics) or enzymes. For many detailed studies, e.g., for preparation of protoplasts for fusion, transfection, or transformation, liquid cultures should also be started from an inoculum of spores.

The multicellular lifestyle of streptomycetes causes some problems in the study of metabolic properties, as not all cells of the initial suspension are in the same physiological state. Commonly, streptomycetes grow by mycelial elongation and branching. Nevertheless, physiological homogeneity cannot be maintained when nutrients become limited to central parts of the colony. Hence, spore germlings are used in physiological studies, although large quantities of spores are needed. To circumvent this problem, liquid cultures can be supplemented, e.g., with dispersants like agar, carboxymethylcellulose, Junlon®, polyethylene glycol, starch, and sucrose. A summary of the advantages and disadvantages of these methods can be found in chapter 2 of Kieser et al. (2000). Due to the highly aerobic nature of streptomycetes, it is necessary to shake cultures during

incubation. Recommended procedures include Erlenmeyer flasks with the use of indentations or stainless steel springs, but tubes in a slanted position on a shaker or roller also facilitate an excellent supply of oxygen for small quantities of broth, with 3–5 mL being sufficient for some physiological tests. However, it should be taken into account that a few secondary metabolites (e.g., antibiotics and pigments, which are formed on solid media) may not be produced under these culture conditions.

Two media recommended by Korn-Wendisch and Kutzner (1992) have been widely used for submerged cultivation of streptomycetes (g/L): (1) GPYB broth (glucose, 10.0; peptone from casein, 5.0; yeast extract, 5.0; beef extract, 5.0; $CaCl_2 \cdot 2H_2O$, 0.74; pH 7.2) and (2) soybean meal-mannitol nutrient medium (soybean meal, 20.0; mannitol, 20.0; pH 7.2). For subculturing streptomycetes, arthrospores and vegetative mycelium can be used as inoculation material; vegetative mycelium occasionally includes "submerged spores" (Wilkin and Rhodes 1955). Similar procedures are recommended by Kieser et al. (2000).

Spore suspensions stored at 4 °C can be used for several weeks. As spores tend to settle and clump, a few glass beads should be added to the screw-cap tubes, which help to resuspend spores before use. The preparation of mycelia for detailed DNA or RNA studies is described in chapters 8 and 9 of Kieser et al. (2000).

Maintenance Procedures

Several different procedures have been used (Kirsop and Snell 1984) for the short- and long-term preservation of microorganisms. Korn-Wendisch and Kutzner (1992) described three short-term preservation methods: (1) agar slope cultures may be stored at 4 °C for few months, (2) spore suspensions can be mixed with soft water agar and kept at 4 °C (Kutzner 1972), and (3) glycerol can be added to spore suspensions (final concentration, 10 %, v/v) and stored at −20 °C (Wellington and Williams 1978). After thawing, these cultures can serve as inocula for most diagnostic tests, except carbon utilization (Williams et al. 1983a).

For long-term preservation, Kieser et al. (2000) proposed the preparation of spore suspensions in 20 % glycerol which can be stored at −20 °C. In another method, strains are cultured in complex media (like trypticase soy broth [TSB] agar), then 20 % glycerol plus 10 % lactose are added, and the samples are stored in the vapor phase of liquid nitrogen. A third method employs drying on unglazed porcelain beads (Lange and Boyd 1968), followed by soil culture (Pridham et al. 1973), and lyophilization (Hopwood and Ferguson 1969).

Spore suspensions or homogenized mycelia are mixed with glycerol to give a final concentration of 25 %; this mixture can be kept at −25 °C for long-term preservation (Wellington and Williams 1978). Alternatively, spores and mycelia suspended in 10 % skim milk can be lyophilized. Liquid nitrogen cryopreservation is a very simple, reliable, and time-saving method. In this method, living cells are stored in small polyvinyl chloride (PVC) tubes ("straws") at −196 °C; this procedure has been tested for several actinomycetes. First, bacteria are harvested from well-sporulated cultures grown on suitable agar media in Petri dishes by a 2 × 25-mm piece of sterile PVC tubing that is pressed into the mycelial mat and agar, and then carefully raised to excise the agar plug. This procedure is repeated until the tube is filled with agar, latter is then placed in a sterile cryovial (the screw cap marked with the strain accession number). In total up to 13 tubes can be placed into a 1.8-mL vial. Two vials prepared for each strain are then fixed to a metal clamp for freezing in the gas phase of a liquid nitrogen container. After 10–15 min, when temperature falls below −130 °C, the clamp can be immersed in the liquid phase at −196 °C. A container with a capacity of 250 L will hold at least 8,000 vials or 4,000 strains. For viability testing, one tube is removed from the vial within the nitrogen gas atmosphere of the container and thawed directly on a suitable agar medium. The mycelium will be visible after a few days of incubation. Plugs may be pushed out of the tubes with a sterile needle when strains do not produce abundant mycelium.

Isolation, Enrichment, and Maintenance Procedures for the Genus *Kitasatospora*

Enrichment, isolation, and maintenance procedures are as described for *Streptomyces* species.

Isolation, Enrichment, and Maintenance Procedures for the Genus *Streptacidiphilus*

Kim et al. (2003) described the isolation of eighteen strains of streptoacidiphili on acidified starch agar plates supplemented with cycloheximide and nystatin (each 50 μg mL^{-1}) using the isolation procedure reported by Goodfellow and Dawson (1978). *S. jiangxiensis* was isolated by plating acidic rhizosphere soil suspensions, prepared using a differential centrifugation procedure (Wang et al. 2003), onto an acidified selective isolation medium containing aspartate, sucrose, and L-glutamate as carbon sources. Acidified starch-casein-nitrate agar (Küster and Williams 1964b) can also be applied (Wang et al. 2006) in combination with differential centrifugation, as described by Sembiring et al. (2000). Another method for the isolation of acidophilic actinobacteria from soil is described by Cho et al. (2006).

Kim et al. (2003) reported that short-term storage would be achieved by growing strains on acidified modified Bennett's agar (Jones 1949). Acidified oatmeal (ISP medium 3, Shirling and Gottlieb 1966) and acidified inorganic salts-starch (ISP medium 4, Shirling and Gottlieb 1966) agar can also be used for this purpose. For long-term preservation lyophilization, freezing in glycerol and storage in liquid nitrogen can be used. Glycerol suspensions are prepared by scraping aerial growth or substrate mycelium or both from heavily inoculated plates and making heavy suspensions in 3 mL of aqueous glycerol in small (e.g., bijoux) bottles, which are stored at −20 °C.

Ecology

Streptomycetes are highly abundant in soil, which is their major natural habitat. As stated above, most streptomycetes are able to degrade complex and fractious animal and plant materials, which are usually polymeric residues such as proteins (e.g., elastin and keratin), polysaccharides (e.g., cellulose, chitin, pectin, and starch), lignocellulose, and aromatic compounds. The biodegradative activities and complex extracellular biology of actinomycetes have been discussed in various reviews (Lechevalier 1988; Crawford 1988; Peczynska-Czoch and Mordarski 1988; Chater et al. 2010).

Streptomycetes are capable of breaking down lignin, which naturally occurs in a lignocellulose complex together with cellulose and xylan (hemicellulose). Using ^{14}C-labeled lignin, it has been shown that streptomycetes (Crawford 1978; Antai and Crawford 1981) as well as other genera of actinomycetes are involved in the process of lignin degradation (McCarthy and Broda 1984; McCarthy et al. 1984, 1986), although fungi play a more important role in this process (Crawford 1981, 1988; Janshekar and Fiechter 1983; Kirk and Farell 1987).

Ligniolytic streptomycetes can also decompose the cellulose of the lignocellulose complex. For detailed information, see Ramachandra et al. (1988), Wang et al. (1990), Crawford et al. (1993), Chamberlain and Crawford (2000), Kormanec et al. (2001), Gottschalk et al. (2003), and Kaneko et al. (2003). Additionally, mesophilic and thermophilic streptomycetes have been reported to possess multicomponent cellulases, which consist of several endo- and exoglucanases (Enger and Sleeper 1965; Crawford and McCoy 1972; MacKenzie et al. 1984; Harchand and Singh 1997; Marri et al. 1997; Ulrich and Wirth 1999; Wirth and Ulrich 2002). Chater et al. (2010) have reviewed the nonenzymatic proteins that *Streptomycetes* employ to target and bind to cellulose and its degradative processes which include a combined oxidative and cellulolytic attack using catalase/peroxidase enzymes and Avicelases.

Xylanases, which are involved in the degradation of the lignocellulose complex, seem to be common among thermophilic actinomycetes; however, they have also been detected in mesophilic streptomycetes (Kluepfel and Ishaque 1982; McCarthy et al. 1985; Kluepfel et al. 1986; Deobald and Crawford 1987; Godden et al. 1989; Schäfer et al. 1996; Morosoli et al. 1999).

Other polymer degrading enzymes found in streptomycetes include pectinolytic complexes (Sato and Kaji 1975, 1977, 1980a, b) and chitinolytic complexes, which consist of chitinase and chitobiase. These have been isolated from *S. griseus* (Berger and Reynolds 1958), *S. antibioticus* (Jeuniaux 1966), and other streptomycetes (Beyer and Diekmann 1985). Specialized chitin-binding (CHB) proteins and CHB domains in chitinases allow *Streptomycetes* to interact strongly with various types of α-chitin and β-chitin (reviewed in Chater et al. 2010). Genes for family 18 and 19 chitinases, which use different reaction mechanisms to hydrolyze the glycolytic bonds between saccharides in chitin and chitosan, have both been identified in various *Streptomyces* genome sequences. For example, the genome sequence of *S. coelicolor* A3(2) contains 11 deduced family 18 chitinases and two family 19 chitinases, with various modular arrangements comprising catalytic, substrate-binding, and linker domains (Chater et al. 2010). This potentially enables them to hydrolyze the natural diversity of chitin types.

Starch, which is primarily used as material in the food, paper, and textile industry, can be degraded by numerous fungi and bacteria. The enzymes involved are amylases, some of which have been found in several streptomycetes (Mordarski et al. 1970; Suganuma et al. 1980; Fairbairn et al. 1986; McKillop et al. 1986).

Actinomycetes are also capable of using keratin as the sole source of C, N, S, and energy (reviewed by Kornillowicz-Kowalska and Bohacz 2011). Native keratin in feathers, nails, horns, and hair are subjected to intensive degradation by keratinolytic actinomycetes, mostly *Streptomycetes*, e.g., *S. fradiae*, *S. pactum*, *S. thermoviolaceus*, or other genera, e.g., *Thermoactinomyces*.

In addition to degrading polymeric compounds, streptomycetes have the ability to degrade other organic materials, such as wool (Noval and Nickerson 1959), cotton and plant fibers (Khan et al. 1978; Lacey and Lacey 1987), plastics (Pommer and Lorenz 1986), and hydrocarbons in emulsions and jet fuel (Genner and Hill 1981). Lacey (1988) and Behal (2000) give a detailed review about the biodegradation of natural and synthetic substances. An important waste material accumulating worldwide is rubber due to difficulties with reusing natural and synthetic rubber materials. Actinomycetes of the CNM (Corynebacterium, Nocardia, Mycobacterium) group are the most potent rubber-degrading bacteria isolated so far, which require direct contact with the rubber substrates. Another group of rubber-degrading bacteria generally also belong to the actinomycetes (e.g., *Actinoplanes*, *Streptomyces*, and *Micromonospora*) (recently reviewed by Yikmis and Steinbüchel 2012). *Streptomyces* sp. K30 excretes via the Tat pathway a so-called Latex-clearing protein (Lcp) and heteromultimeric aldehyde dehydrogenases, which enable degradation of poly(*cis*-1,4-isoprene) to isoprenoid acids, which can be further metabolized via β-oxidation (reviewed by Yikmis and Steinbüchel 2012).

As well as efficient degraders of (recalcitrant) organic matter, *Streptomyces* spp. have also been isolated from polluted environments, e.g., natural heavy metal containing and polluted sites in Germany, Italy, and China (Amoroso et al. 2000; Schmidt et al. 2007; Lin et al. 2011a). One of the German isolates, *S. acidiscabies E13* could grow on media containing as high as 10 mM nickel chloride, whereas it was also tolerant for copper, cadmium, chromium, and iron (Schmidt et al. 2007). Furthermore, two *S. mirabilis* strains, isolated from an uranium mine, were able to grow on media containing as high as 100 mM nickel chloride, whereas they could also tolerate up to 100 mM zinc (Schmidt et al. 2009). The draft genome sequence of *S. zinciresistens* K42 (CCNWNQ 0016 T), isolated from a Chinese copper-zinc mine tailing with a maximum level of resistance to Zn^{2+} of 35 mM (Lin et al. 2011a), showed a large number of genes encoding proteins predicted to be involved in conferring metal resistance (Lin et al. 2011b). Many of these genes appear to have been acquired through horizontal gene transfer.

In soil, streptomycetes can show distinct mycelial growth. In this habitat, they are adapted to multiple and often rapidly changing physical conditions (e.g., frost, drought, shifts in aeration, hydrostatic pressure, moisture tension, pH, and anaerobic conditions) by the formation of spores, which are semidormant stages in the life cycle and allow the survival in soil for long periods (Mayfield et al. 1972; Ensign 1978). Viable cultures of cells have been reported by Morita (1985) from 70-year-old soil samples. Streptomycetes are mainly present in soil as inactive spores. One disadvantage of persisting as a spore is the very low germination efficiency, which may be caused by competition with other microorganisms. Spores, which pre-germinate, can grow for a short time and then re-sporulate (Lloyd 1969). A number of factors may cause the germination of spores. In addition to special signaling factors, the presence of exogenous nutrients, water, and Ca^{+2} seems to be necessary components (Ensign 1978). Beside germination, nutrients also influence the degree of hyphal growth and the time of differentiation into aerial hyphae. Fodder and other organic material, freshwater, and marine habitats as well as potable water systems can come into contact with soil (Korn-Wendisch and Kutzner 1992), e.g., by human and other activities. Natural materials (e.g., fodder, hay, grain, and wood) and synthetic products (e.g., cotton textiles, fabric, paper, rubber, plastics, and plasticizers), which can be found in or transported to soil, can be degraded with the help of mesophilic and especially thermophilic streptomycetes.

The Terrestrial Habitat

The soil pore network is contained within the soil matrix. Bacterial communities (in the form of single cells or microcolonies) are often found within the soil pores in close association with soil surfaces. Bacterial communities are often limited in movement to under a micrometer (Trevors et al. 1990) through the soil column without the aid of a transporting agent, such as water flow, growing roots, or burrowing organisms. The fate and activity of these organisms is therefore largely dependent on their site of origin. The soil matrix acts as a migratory barrier to non-hyphal bacteria. However hyphal bacteria may overcome this constraint by crossing air-filled spaces by hyphal/mycelial growth (Schäfer et al. 1998). Filamentous organisms also benefit from their ability to transfer carbonaceous compounds over the whole distance of the organism providing resources across the whole hyphal matrix, thus allowing them to cross nutrient-poor sites (Nazir et al. 2010). A tentative model has been proposed for *S. coelicolor* A3(2) incorporating the two previous observations (Yeo and Chater 2005). It is hypothesized that in tiny enclosed spaces in soil, nonreproductive "exploratory" aerial hyphae grow through the gas phase until they encounter a new, potentially colonizable surface with nutrients and/or water (Yeo and Chater 2005). It is proposed that the accumulation of signaling molecules in the vapor phase of the enclosed space might determine the choice of this nonreproductive developmental pathway, which is possibly linked to inhibition of sigma factor WhiG. Importantly, the exploratory aerial hyphae are thought to deposit and use "phase I" glycogen, which is normally found in the region of vegetative mycelium near aerial hypha branches. This is in contrast to deposition and use of "phase II" glycogen in apical compartments of reproductive aerial hyphae as they are undergoing sporulating septation (Yeo and Chater 2005).

Soil is one of the most diverse habitats known for microorganisms (Dance 2008), with a significantly higher diversity of prokaryotes compared to any other natural environment (Gans et al. 2005). The main factor driving the biodiversity of soil is its heterogeneity, providing a range of microhabitats which differentially select prokaryotic or fungal types (Standing and Killham 2007). The growth and metabolism of microorganisms in soil can lead to changes in pH, redox potential, and the ionic strength of soil (Haferburg and Kothe 2007). Soil microorganisms also have a major influence on particle aggregation as well as soil texture and the availability of nutrients for plants (Barto et al. 2010).

The dynamics of the composition of a microbial community in soil is therefore dependent on abiotic and biotic factors, especially vegetation, soil type, content and type of organic matter, pH, temperature, season and climate, and circulation of water and air. Actinobacteria have been identified as one of the predominant phylotypes in several metagenomic studies of soils (e.g., Buckley and Schmidt 2003; Smith et al. 2006) and phylogenetic analysis has revealed that actinobacterial populations may be cosmopolitan, with several dominant phylotypes being identified at different geographical locations. Culture-independent molecular ecological studies on soils usually group actinobacteria and often do not specify the (changes in) distribution of *Streptomyces* species (e.g., Sheik et al. 2011; Sagova-Mareckova et al. 2011). Nonetheless, many *Streptomyces* species are found distributed over a diverse range of soil habitats.

A culture-independent study on microbial community dynamics in petroleum hydrocarbon-contaminated wetlands microcosms showed that enhanced degradation after addition of inorganic nutrients was linked to an increase in the amount of *Streptomyces* (Bachoon et al. 2001), suggesting that *Streptomyces* could be used for bioremediation of oil pollution.

Jenkins et al. (2009) showed that soil pH is a major driver of change in actinobacterial communities in long-term managed grasslands. The relative abundance of *Streptomyces* spp. increased after additions of farm yard manure to relatively acidic plots (pH 5.6–5.8). A higher relative abundance of *Streptomycetes* was also correlated to soil water content (Jenkins et al. 2009). *Streptomyces* abundance was also higher in grassland soils compared to forest soils (Nacke et al. 2011). This study also confirmed that soil pH is a major determinant for bacterial community structure. *Streptomyces* was also in the top 20 of most abundant bacterial genera in soils of a grass monoculture pasture and a mixture of grasses at a conservation site in the Texas High Plains region (USA), but they were much less abundant in two managed agricultural soils (Acosta-Martinez et al. 2008).

Among the 48 genera of nonphototrophs detected in the bacterial communities in biological soil crusts from the

Colorado Plateau, actinobacteria (particularly *Streptomyces* spp.) were very common and diverse, with 18 genera and an average contribution to the total 16S rDNA amplificate of 11.8 % (Gundlapally et al. 2006). Many of the actinobacterial and *Streptomyces* isolates are mycelial and it is suggested that these abundant nonphototrophic crust bacteria may play a significant role in the physical process of crust formation and maintenance—a role typically assigned to filamentous cyanobacteria.

A comparison of the actinobacterial community composition in three alkaline and saline soils of the former lake Texcoco (Mexico) following flooding showed an inverse relationship between the abundance of actinobacteria and the salinity and alkalinity of the soil. *Streptomyces* 16S DNA sequences could be identified in the intermediate saline and alkaline soil, which had an electrolytic conductivity of 56 dS m^{-1} and a pH of 10.1 (Valenzuela-Encinas et al. 2009).

Laskaris et al. (2012) showed that neither streptomycin producers nor *S. griseus* was prevalent in the several fresh or chitin- and starch-amended soils examined (less than 0.1 % of soil actinobacteria) as determined by amplicon sequencing of *atpD*, a housekeeping gene coding for the ATP-synthase beta chain, and *S. griseus*-specific and "generic" *strA*, a streptomycin resistance gene coding for streptomycin and related phosphotransferases. This finding was unexpected as previous research from the same group showed that *S. griseus* was the prevailing species in a population of streptomycin-resistant isolates from all of the tested soils (Tolba et al. 2002). The latter study also showed that *str* gene distribution was prevalent in the streptomycin-treated soil. Other work by the same group showed that the relative abundance of the *Streptomyces* population and the number of *Streptomyces*-like chitinase gene sequences increased following chitin baiting of a sewage-sludge-amended upland grassland pasture (Metcalfe et al. 2002).

Antarctic Dry Valley desert soil harbors highly diverse actinobacterial communities (Babalola et al. 2009). Phylogenetic analysis of clones generated with actinobacterium and streptomycete-specific PCR primers revealed that the majority of the phylotypes were most closely related to uncultured *Pseudonocardia* and *Nocardioides* species. In contrast, the majority of the cultured isolates (>80 %) were *Streptomyces* species even though phylotypes affiliated to the genus *Streptomyces* were detected at a low frequency in the metagenomic study (Babalola et al. 2009).

It is not unusual for *Streptomyces* to represent the dominant actinobacterial or even microbial population in culture-dependent studies. In most cases they make up about 1–20 % of the total viable count, which is 10^4 to 10^7 colony-forming units (CFU) per g soil (Korn-Wendisch and Kutzner 1992; Watson and Williams 1974; Saadoun et al. 1998). Early culture-dependent studies by Williams et al. (1969) assigned soil streptomycetes to color groups, based on diffusible pigment colors produced on oatmeal agar and on their ability to form melanin pigments on peptone-yeast extract-iron agar. Subsequently, this classification into color groups was used as a tool by other researchers as well to study the diversity of streptomycetes in natural habitats (e.g., Goodfellow and Haynes 1984; Atalan et al. 2000; Sembiring et al. 2000). Nevertheless, this color grouping is based on subjective interpretation and comparison of data between different studies is difficult. A potential improvement of this method was recently presented by a computer-assisted numerical analysis of 321 alkaliphilic streptomycetes that were assigned to color groups (Antony-Babu et al. 2010). The authors argue that, with this method, distances between individual colors could be calculated more objectively and that the data can be compared with computer-assisted numerically defined color groups in future studies on streptomycete taxonomy in natural habitats.

While the use of colors for *Streptomyces* taxonomy is functional, most culture-dependent studies rely on molecular and metabolic methods to detect the presence, abundance, and activity of *Streptomyces*. D'Costa et al. (2006) isolated a collection of spore-forming bacteria resembling actinomycetes both morphologically and microscopically from soil samples originating from diverse locations (urban, agricultural, and forest). Amplification and sequencing of 16S ribosomal DNA from a subset of strains indicated that they belonged to the actinomycete genus *Streptomyces*. Without exception, every strain in the library was found to be multidrug resistant to seven or eight antibiotics on average, with two strains being resistant to 15 of 21 drugs. Several antibiotics, including the synthetic dihydrofolate reductase (DHFR) inhibitor trimethoprim and the new lipopeptide daptomycin, were almost universally ineffective against the library. This result was surprising as the authors had not expected to find such extensive daptomycin resistance (D'Costa et al. 2006). These findings suggest *Streptomyces* are an environmental reservoir of resistance determinants, which could possibly be mobilized into the microbial community. Surprisingly, the occurrence of the oxytetracycline resistance genes *otrA* and *otrB* genes was limited in tetracycline-resistant *Streptomyces* isolates from a range of European habitats, including bulk and rhizosphere soil and manure and seawater (Nikolakopoulou et al. 2005). This finding could imply that some of the isolates may carry tetracycline-resistance genes that were not screened for.

Phylogenetic analysis of actinomycete isolates of freshly excavated Miocene lacustrine sediment (17–19 Myr old, lacking recent organic carbon, but may contain some fossil carbon) and four sites of primary succession (initial, early, middle, and late stages; aged 1–44 years) on the same sediment after years of mining activity revealed four distinct actinomycete clusters corresponding to the stages of succession (Chronakova et al. 2010). The relative proportion of actinomycetes, specifically *Streptomyces*, among total bacterial and genetic diversity increased significantly with the age of the sampling site. Moreover, pioneer species, such as operational taxonomic units (OTU) related to *S. microflavus*, *S. flavofuscus*, *S. anulatus*, *S. atratus*, *S. exfoliatus*, and *S. avidinii*, were replaced by late succession species, such as OTUs related to *S. aureus*, *S. tauricus*, *S. prunicolor*, *S. phaeochromogenes*, and *S. griseochromogenes*, during successive colonization of early, mid, and late colliery heaps (Chronakova et al. 2010). A few isolates of *Kitasatospora* were also identified among strains from the middle and late stages. The observed shift in community structure is associated

with plant root development, increasing availability of organic matter, and possibly earthworm feeding activity. The fresh Miocene lacustrine cluster isolates showed close relationships with *S. gougerotii*, *S. champavatii*, and *Streptomyces* sp. 445. Interestingly, these "ancient" *Streptomyces* strains were present only in this soil horizon and were not retrieved from soils of the primary succession gradient, suggesting an important role during early colonization of *terra nova*. Astonishingly, >50 % of these "ancient" strains were able to produce antibiotics acting against Gram-positive and Gram-negative bacteria and yeast as shown by the agar plug method, with up to 100 % and 71 % of examined strains encoding for, respectively, non-ribosomal peptide synthases and polyketide synthases. Up to 37 % of these "ancient" strains were resistant to antibiotics such as amoxicillin. These findings suggest that antibiosis and antibiotic resistance are ancient microbial traits. Additional evidence for the coexistence and coevolution of antibiotic resistance and biosynthesis genes in soil bacteria was provided recently (Laskaris et al. 2010).

Moreover, a more recent study, combining experimental and theoretical observations, suggests that diverse *Streptomyces* communities do not represent a stable ecological state but form an intrinsically dynamic eco-evolutionary phenomenon (Vetsigian et al. 2011). All pairwise interactions were recorded among 64 *Streptomyces* strains isolated from several individual grains of soil. A rich set of "sender-receiver" interactions was observed, including inhibition and promotion of growth and aerial mycelium formation. The probability that two random isolates interact was balanced; it was neither close to zero nor one. The interactions were also not random: the distribution of the number of interactions per sender was bimodal and there was enrichment for reciprocity—if strain A inhibits or promotes B, it is likely that B also inhibits or promotes A. Such reciprocity was further enriched in strains derived from the same soil grain, suggesting that it may be a property of coexisting communities. Interactions appeared to evolve rapidly: isolates with identical 16S rRNA sequences could have very different interaction patterns. A simple eco-evolutionary model of bacteria interacting through antibiotic production showed how fast evolution of production and resistance could lead to the observed statistical properties of the network. In the model, communities were evolutionarily unstable—they were constantly being invaded by strains with new sets of interactions (Vetsigian et al. 2011).

Katsifas et al. (1999) showed that the rhizosphere was the dominant factor in determining the population structure of different Greek terrestrial ecosystems, including heavily disturbed agricultural soils, rhizospheres of rare indigenous plants, and secluded preserved areas. Cluster groups appearing in only one or two habitats were *S. griseoflavus*, *S. rimosus*, *Streptoverticillium blastmyceticum*, *Nocardia mediterranea*, and *S. fulvissimus*. The most common cluster groups were *S. cyaneus*, *S. albidoflavus*, *S. diastaticus*, and *S. exfoliatus*, which were isolated from at least six different habitats.

Unburned prairie soils amended with low- and high-complex carbon (and nitrogen) at different concentrations also affected the (relative) abundance of *Streptomyces* species and their antibiosis and substrate utilization profiles (Schlatter et al. 2009). Cellulose and lignin amendments resulted in mesocosms with the highest *Streptomyces* densities. Also, *Streptomyces* communities receiving high concentration amendments were more inhibiting, whereas those receiving low-concentration amendments used substrates more efficiently (Schlatter et al. 2009). Substrate use and inhibitory activity were positively correlated among isolates in some treatments, such as low-concentration lignin, but were negatively correlated in soils with amendments such as low glucose concentrations, whereas no significant correlation was found in high-concentration lignin or glucose communities (Schlatter et al. 2009). Williams et al. (1972b) have shown that streptomycetes resist desiccation because of their ability to form arthrospores. Besides, the water tension then needed for growth can be much lower than for other bacteria, but on the other hand, they may be very sensitive to water-logged conditions.

Most attention has been directed to neutrophilic streptomycetes which are commonly present in neutral to alkaline soils (e.g., Flaig and Kutzner 1960b). Acidotolerant and acidophilic streptomycetes are abundant in acidic soils. Since acidophilic streptomycetes produce specific and stable amylases (Williams and Flowers 1978; Williams and Robinson 1981), they can be isolated using starch-casein agar adjusted to pH 5.0 and supplemented with antifungal agents (Khan and Williams 1975; Hagedorn 1976). In contrast, alkalitolerant and alkaliphilic streptomycetes are mainly found in alkaline soils (Babu and Goddfellow 2008; Mikami et al. 1982, 1985; Taber 1959, 1960).

Streptomycetes, as well as other soil actinobacteria, have been isolated from the surface, internal organs, and the intestinal tract of terrestrial insects and arthropods, such as attine and *Allomerus* ants, beewolf wasps, bark and ambrosia beetles, stinkbugs, firebugs, and sweet potato whitefly biotype Q (Szabó et al. 1967; Bignell et al. 1980, 1981; Bignell 1984; Haeder et al. ; Barke et al. 2010; Kaltenpoth 2006; Scott et al. 2008; Grubbs et al. 2011; Zucchi et al. 2012; Sudakaran et al. 2012; Indiragandhi et al. 2010), the gut of earthworms and potworms and their surrounding soil (Brüsewitz 1959; Parle 1963a, b; Kristufek et al. 1993, 1995), and pellets formed by woodlice and millipedes (Márialigeti et al. 1984). While these *Streptomyces* species are able to grow independently, it is thought that these animal-bacterial associations are mostly mutualistic or symbiotic. The animal host provides easy access to nutrients for *Streptomyces*, who then supplies its host with beneficial and growth promoting compounds enabling protection against infection for themselves, for their offspring, or their food (reviewed recently by Seipke et al. 2012; Kaltenpoth 2009). These interactions between microbes and their symbiotic hosts based on antibiotics production are environmentally successful and have existed for a long time. This suggests that medical use of antibiotics could possibly be long-term successful as well.

Streptomycetes are also found in the rhizosphere and as plant endophytes (Goodfellow and Williams 1983; Sembiring et al. 2000; Schrey and Tarkka 2008), where they are thought to play an important role. *Streptomyces* can provide small

metabolites (i) as plant growth promoting factors, e.g., auxin (reviewed by Strap and Crawford 2006); (ii) for the promotion of hyphal elongation and growth of fungi and other mycorrhiza (Schrey et al. 2012; Schrey and Tarkka 2008); (iii) to biologically control plant diseases, e.g., faerifungin (reviewed by Seipke et al. 2012; Kinkel et al. 2012; Schrey and Tarkka 2008); and (iv) to modulate plant defense mechanisms (reviewed by Schrey and Tarkka 2008). It has been proposed ever since the discovery of antibiotics from *streptomyces* that antibiotic-producing organisms have a competitive advantage over nonproducing organisms. However, there is no clear proof for the in situ production of antibiotics in unsterilized soil (Williams 1982) except for phenazine production by pseudomonads, which can be recovered from wheat roots at concentrations of 27–43 ng/g of root within the adhering soil (Thomashow et al. 1990). Antibiotics are difficult to detect in soil as they are likely produced in low concentrations and may be instable (Brian 1957; Williams 1982). Moreover, they may be adsorbed onto soil colloids (Williams 1982) and may also be produced only at certain stages of the growth cycle (Williams and Khan 1974; Williams 1982). Further, almost all of the evidence for the involvement of antibiotics in biocontrol is indirect or circumstantial. Indirect antibiotic production in soil was reported by Rothrock and Gottlieb (1984) by co-inoculating *Streptomyces* species in soil with root rot fungi of pea and soybean. However, they could not establish a clear relationship between antibiotics activity or antagonism on agar media and reduction in disease activity. Later studies also showed that *Streptomyces* were indeed efficient biocontrol agents of root plant diseases (see above). Recently, Kinkel et al. (2012) showed that *Streptomyces* from naturally occurring disease suppressive soils displayed a significantly higher intensity of inhibition of plant-pathogenic *S. scabies* than *Streptomyces* from disease-conducive soils. The diversity of inhibitory interactions across pathogen isolates was also significantly higher among the former, antagonistic *Streptomyces* than those from disease-conducive communities. Other and recent studies have identified endophytic *Streptomyces* species as attractive biocontrol agents (reviewed in Seipke et al. 2012; Schrey and Tarkka 2008).

Antibiotic production (oxytetracycline) by *S. rimosus* in sterile soil microcosms was indirectly and qualitatively shown through co-inoculation of a genetically engineered GFP-containing tetracycline-sensitive biosensor (Hansen et al. 2001). Streptothricin biosynthesis by *S. rochei* F20 in sterile soil microcosms was shown by using reverse transcription-PCR (RT-PCR) of one of the streptothricin-biosynthetic genes *sttA*, but *sttA* expression could not be not detected in sterile and nonsterile rhizosphere or rhizoplane soil cosms with spring wheat seedlings (Anukool et al. 2004).

The Aquatic Habitat

Actinomycetes can easily be isolated from aquatic ecosystems and especially from sediments of rivers and lakes. In particular, rivers carry large amounts of various actinomycetes, including streptomycetes. In addition, actinomycetes are capable to survive as dormant spores in aquatic habitats for a long period (Al-Diwany and Cross 1978). "Soil" streptomycetes are often present in water bodies such as creeks, ponds, and rivers, especially after waterflow over land after heavy rainfalls, though they can also be redistributed through air in aerosols generated by wind flowing over dry soils. Streptomycetes also find their way into the sediments of the lakes, rivers, and, after transfer to the sea, into marine sediments. Burman (1973) found 59–200 streptomycetes and 10–20 micromonosporae per mL river water sampled from the river Thames. The streptomycetes grew on decaying vegetation on riverbanks and mud flats at low water or on floating mats of decaying algae or other vegetation.

Odorous substances formed by streptomycetes are washed into or produced in the water giving rise to "earthy tastes" in drinking water supplies and bottom-feeding freshwater fish. Geosmin and methyl-isoborneol are the two most frequently detected odorous, "off flavor-tasting" compounds in water (Gerber 1979a, b; Zaitlin and Watson 2006). Wood et al. (1983) noted that preventing the contamination of drinking water with these compounds, and thus the earthy tastes in water reservoirs and supply systems, depends on locating the production sites and identifying the distribution patterns of these substances (Silvey and Roach 1975; Lechevalier et al. 1980). Burman (1973) found that filtration processes help to reduce the quantity of streptomycetes in drinking water. He also identified a new, aquatic strain of *Streptomyces* in the distribution system (for details, see Burman 1973). It is these compounds that give moist soil its characteristic smell. A biodiversity study on the bottom sediments and water of Lake Baikal showed that 66 % of the water isolates belong to the genus *Streptomyces*, whereas 51 % of the sediment isolates belong to the genus *Micromonospora* (Terkina et al. 2002). Jiang and Xu (1996) found a similar prevalence for *Streptomyces* and *Micromonospora* species in 12 Yunnan Middle Plateau lakes in China. *Streptomyces* was also shown to be the most abundant species (17 %) of actinomycetes in water samples of different sites in Rankala Lake of Kolhapur City, India (Nakade 2012). Aquatic vegetation from three stream sites located within Savannah River Site, South Carolina (USA), was also sampled to determine the presence, distribution, and diversity of actinomycetes on submersed macrophytes. 34 % of the distinctly actinomycete isolates were *Streptomyces*, thereby forming the dominant species (Wohl and McArthur 1998).

It has been considered by several authors that *streptomyces* also occur in marine habitats, including sediments (Cross 1981a; Goodfellow and Haynes 1984; Okazaki and Okami 1976; Weyland 1981a, b;, Weyland and Helmke 1988). Streptomycetes have been found in the littoral and inshore zone and in deep-sea sediments. Even though they can be isolated from both sites, they are not necessarily part of the autochthonous microflora, but can possibly have a terrestrial origin. Streptomycetes isolated from sediments (Roach and Silvey 1959) and from decaying seaweed (Siebert and Schwartz 1956) in littoral zones were capable of growing on polymeric substances characteristic of these habitats, such as agar and chitin (Humm and Shepard 1946), alginate and laminarin (Chesters et al. 1956), and cellulose (Chandramohan et al. 1972).

In sediments, the ratio of different actinomycete taxa depends on the location and the depth of the sampling sites (Weyland 1981a; Weyland and Helmke 1988). In the open sea, only low numbers of actinomycetes are generally detected (viable counts about 100 CFU per mL of wet sediment). It is believed that the distribution of streptomycetes is correlated with the barotolerance (Helmke 1981), halotolerance, and psychrophilism (Weyland 1981b) (horizontal as well as vertical) of *Streptomyces*, *Micromonosporae*, and *Rhodococci*. In contrast, Goodfellow and Haynes (1984) did not find any correlation between depth, pH or salinity, and the number of actinomycetes recovered from marine sediments. In their study, 732 isolates were examined, of which 250 belonged to *Streptomyces*, 250 to *Micromonospora*, 140 to *Rhodococcus*, and 92 were assigned to the genus *Thermoactinomyces*. The isolated streptomycetes were subsequently identified using a computer-assisted approach (Williams et al. 1983b) and about half of them were assigned to a cluster equated with *Streptomyces albidoflavus* (Williams et al. 1983a).

Streptomycetes were primarily detected in sediments of shallow seas (70–520 m deep) with 300–1,270 colonies per cm^3, whereas *Micromonospora* prevailed in samples 700–1,600 m deep (Okami and Okazaki 1978). However, these authors did not detect actinomycetes from depths of 2,800 and 5,000 m in the Pacific Ocean. Yet, Pathomaree and coworkers (2006) were able to isolate actinomycetes, including streptomycetes, from a depth of 10,898 m in the Mariana Trench in the Pacific. Marine streptomycetes generally tolerate higher salt concentrations than their terrestrial counterparts, though salt tolerance among streptomycetes is widespread (Tresner et al. 1968). Some of the isolated marine streptomycetes were found to be obligate halophiles (Okazaki and Okami 1976).

Culture-dependent and culture-independent studies have shown that many indigenous marine actinomycetes exist in the oceans and are widely distributed in different marine ecosystems (reviewed by Lam 2006; Zotchev 2012). Interestingly, considerable new marine diversity is also being discovered within the genus *Streptomyces*, a taxonomically complex group of actinomycetes that commonly occur in soils and account for the vast majority of antibiotics discovered so far (reviewed in Ward and Bora 2006; Fenical and Jensen 2006). *Streptomyces* spp. are readily cultured from marine samples, especially when collected near shore, and many if not most of these strains are closely related or identical to strains previously reported from land. However, distinct marine phylotypes are beginning to be recognized, in addition to strains that occur both on land and in the sea. These marine actinomycetes include alkaliphilic *Streptomyces* spp. 38, the marine clade MAR4, and what seems to be a new genus within the family *Streptomycetaceae* for which the name "*Marinispora*" has been proposed, but not yet confirmed formally (Kwon et al. 2006; Fenical and Jensen 2006; Ward and Bora 2006). Several new species of "*Marinispora*" have been shown to produce marinisporolides, novel polyene-polyol macrolide compounds (Kwon et al. 2006, 2009). Numerous antibiotic-producing streptomycetes have been isolated from marine habitats (Goodfellow and Fiedler 2010; Hotta et al. 1980; Okami and Okazaki 1972; Okami et al. 1976) including seaweed (Nissen 1963). Recently, Goodfellow and Fielder (2010) provided a review on a bioprospecting strategy based upon the premise that new secondary metabolites can be found by screening relatively small numbers of dereplicated, novel actinomycetes isolated from marine sediments.

Furthermore, interactions between *Streptomyces* species and aquatic invertebrates have also been shown recently (recently reviewed in Seipke et al. 2012; Taylor et al. 2007), with similar relationships as discussed for terrestrial invertebrates. Stable *Streptomyces* associations and symbioses have been shown for marine sponges and cone snails. Antibiotic-producing bacteria associated with marine sponges offer protection against disease and biofouling. Interestingly, *Streptomyces* species isolated from cone snails have shown neurological bioactivity (Peraud et al. 2009). Results from the dorsal root ganglion (DRG) assay suggest that the compounds act directly on channels or receptors. The data support either a decrease in K^+ channel activation or an increase in Na^+ channel activation. More recently, Lin et al. (2010) showed that one cone snail associate, *Streptomyces* sp. CP32, produces a series of natural products that enhance or diminish whole-cell Ca^{2+} flux in DRG assays. These compounds include known thiazoline compounds and a series of new derivatives, pulicatins A–E.

Thermophilic Streptomycetes

The genus *Streptomyces* consists mainly of mesophilic species; however, a few streptomycetes are thermotolerant (growing up to 45 °C) and some are thermophilic. So far, all described thermophilic streptomycetes grow at temperatures between 28 °C and 55 °C and several grow at even higher temperatures. Kim et al. (1999) studied the taxonomy of thermophilic streptomycetes in detail. Additional thermophilic species (*S. thermocoprophilus* and *S. thermospinisporus*) were described by Kim et al. (2000) and Kim and Goodfellow (2002). Thermophilic actinomycetes are commonly widespread and can be isolated from diverse sources like soils (Tendler and Burkholder 1961; Craveri and Pagani 1962), pig feces (Ohta and Ikeda 1978), sewage-sludge compost (Millner 1982), freshwater habitats (Cross 1981a, b), hay (Roussel et al. 2005), and volcanic and desert steppe zone soils (Kurapova et al. 2012; Zenova et al. 2009).

As a part of their life cycle, thermophilic streptomycetes grow actively at sites of high temperatures, such as compost, manure, and self-heating hay or grain. After completion of the vegetative phase, the formation of large quantities of spores begins. The spores are returned with the compost or manure to the fields and pastures and can subsequently colonize plant material and hay directly or via soil dust (Korn-Wendisch and Kutzner 1992). Thus, the genus *Streptomyces* accounts for the majority of actinomycetes isolated from bioaerosols in the surroundings of composting facilities or in mushroom farming units that utilize compost (Kämpfer et al. unpublished observation, Lacey 1974).

Pathogenicity and Clinical Relevance

Streptomycetes as Plant Pathogens

Some of the many saprophytic *Streptomyces* species are plant pathogens and may be involved in the development of economically important diseases, including potato scab. *Streptomyces scabies* can still be considered as the dominant plant pathogen worldwide but is only one of many streptomycetes which cause very similar disease symptoms on plants. In addition, *S. scabiei* (Lambert and Loria 1989a), *S. acidiscabies* (Lambert and Loria 1989b), *S. turgidiscabies* (Miyajima et al. 1998), *S. europaeiscabiei*, *S. stelliscabiei* (Bouchek-Mechiche et al. 2000), *S. luridiscabiei*, *S. puniciscabiei*, and *S. niveiscabiei* (Park et al. 2003) have been shown to act as plant pathogens causing either common scab or netted scab, mostly in potatoes.

Streptomyces scabies (synonym *S. scabiei*) being the most important and oldest characterized potato scab pathogen has been isolated from various sources such as beets, carrot, peanut, and radish, among other crops (Loria et al. 2006). Strains of *S. scabies* are phenotypically similar to *S. bottropensis*, *S. diastatochromogenes*, or *S. neyagawaensis*, which is further underlined by 16S rRNA gene sequence analyses. *Streptomyces europaeiscabiei*, the most closely related species to *S. scabies*, has been isolated from various locations in Europe. *Streptomyces turgidiscabies* has been isolated from cases of potato scab in Finland, but also from Japan and Korea (Loria et al. 2006). Three species, *Streptomyces luridiscabiei*, *Streptomyces niveiscabiei*, and *Streptomyces puniciscabiei*, are the causal agents of potato scab in Korea. *Streptomyces acidiscabies* has been isolated from low pH soils in the northeastern United States, among other locations.

As revealed by DNA-DNA hybridization studies and 16S rRNA gene sequence analyses, the documented pathogenic strains fall outside of the described species, listed above (Loria et al. 2006). This is due to the existence of a transmissible pathogenicity island which seems to confer the pathogenic phenotype on some otherwise nonpathogenic species and to the polyphyletic nature of scab-causing species itself, as reviewed by Loria et al. (2006). The pathogenic mechanisms used by these species to manipulate their hosts have been extensively studied and summarized by Loria et al. (2006, 2008). For example, the nitrated dipeptide phytotoxin, thaxtomin, plays an important role in blocking cellulose biosynthesis of expanding plant tissues, inducing $Ca2^{+}$ spiking, and causing cell death. In addition, a secreted necrogenic protein, Nec1, contributes to virulence on different plant species. A detailed genetic analysis revealed that the genes coding for thaxtomin and *nec1* lie on a large mobilizable plasmid PAI, besides other putative virulence genes, including a cytokinin biosynthetic pathway and a saponinase homolog. During conjugation this PAI is mobilized and inserts itself site-specifically into the linear chromosome of recipient species, thereby accounting for the emergence of new pathogens in agricultural systems.

Streptomycetes as Human Pathogens

Only very few streptomycetes have been isolated from human pathological specimens so far. Some organisms cause actinomycetoma, which is a localized chronic, destructive, and progressive infection of skin, subcutaneous tissue, and eventually bone (McNeil and Brown 1994; Develoux et al. 1999). In certain tropical and subtropical regions, this disease is endemic and has a devastating effect on patients, as it frequently leads to deformities, disabilities, and eventually amputation of the affected organs. *Streptomyces somaliensis* is often implicated to cause the disease, particularly in parts of the Sudan. Nevertheless some of the main causal agents belong to other genera and species, i.e., *Actinomadura madurae*, *Actinomadura pelletieri*, *Nocardia brasiliensis*, *Nocardia otitidiscaviarum*, and *Nocardia transvalensis* (Trujillo and Goodfellow 2003). However, the identification of a second species, *Streptomyces sudanensis*, by Quintana et al. (2008) suggests that some strains identified as *S. somaliensis* (Gumaa and Mahgoub 1975; Taha 1983; Gumaa 1994; Fahal 2006) may have been misclassified. Indeed, there is evidence that streptomycetes associated with cases of actinomycetoma in the Sudan (Mahgoub 1985; Fahal and Hasan 1992; Fahal 2004, 2006) may be underspeciated (Trujillo and Goodfellow 2003; Quintana et al. 2008).

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43 The Family *Streptosporangiaceae*

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Abstract
The family *Streptosporangiaceae* encompasses the genera *Streptosporangium* as type genus, *Nonomuraea*, *Planomonospora*, *Planobispora*, *Microbispora*, *Planotetraspora*, *Herbidospora*, *Acrocarpospora*, *Thermopolyspora*. On the basis of phylogenetic analysis, the genus *Sinosporangium* branches deeply and membership of the family is not clear. Members of this family comprehend aerobic, Gram-positive, non-acid-alcohol-fast, chemoorganotrophic actinomycetes that form a branched, nonfragmenting substrate mycelium. Members are chemically homogeneous but morphologically diverse. However, strains that bear spore vesicles (*Acrocarpospora*, *Planobispora*, *Planomonospora*, *Planotetraspora* and *Streptosporangium*) are closely related to organisms that have two or more spores in spore chains (*Herbidospora*, *Microbispora*, *Microtetraspora* and *Nonomuraea*). Molecular analyses including DNA-DNA hybridization were performed on members of several genera, and the genome sequence of *Streptosporangium roseum* NI 9100[T] has been published. Several isolation strategies were developed using baiting, chemical, physical,and physicochemical techniques for the genera *Streptosporangium, Planobispora*, *Planomonospora, Herbidospora*, *Microbispora*, *Microtetraspora*, and *Nonomuraea*. Members of the family are mainly found in soil and a few species were isolated from seashore including sand or sediments, and plant materials including roots and leafs.

Taxonomy: Historical and Current

Short Description of the Family and Their Genera

▶ *Strep.to.spo.ran.gi.a.ce'a.e.* N.L. neut. n. *Streptosporangium* type genus of the family, *-aceae* ending to denote a family; N.L. fem. pl. n. *Streptosporangiaceae* the *Streptosporangium* family (Modified from *Bergey's Manual*).

Phylogenetically a member of the order *Streptosporangiales* (Goodfellow 2012), phylum Actinobacteria. The family contains the type genus *Streptosporangium* (Chouch 1955a; emended by Stackbrandt et al. 1994), *Microbispora* (Nonomura and Ohara 1957; emended by Zhang et al. 1998), *Planomonospora* (Thiemann et al. 1967), *Microtetraspora* (Thiemann et al. 1968), *Planobispora* (Thiemann and Beretta 1968), *Planotetraspora* (Runmao et al. 1993; emended by Tamura and Sakane 2004), *Herbidospora* (Kudo et al. 1993), *Nonomuraea* (Zhang et al. 1998), *Acrocarpospora* (Tamura et al. 2000), *Thermopolyspora* (Goodfellow et al. 2005), *Sphaerisporangium* (Ara and Kudo 2007; emended by Cao et al. 2009). *Sinosporangium* (Zhang et al. 2011) is included in the order *Streptosporangiales*, but its membership to the family is not clear. Aerobic, Gram-positive, non-acid-alcohol-fast,

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chemoorganotrophic actinomycetes that form a branched, stable non-fragmenting substrate mycelium which bears aerial hyphae that differentiate either into short chains of arthrospores or into spore vesicles containing one to many motile or nonmotile spores. Members of the family contain *meso*-A_2pm (A1γ type peptidoglycan according to Schleifer and Kandler (1972)) as diagnostic amino acid of peptidoglycan, muramic acid is in the *N*-acetylated form (Uchida et al. 1999), the cell wall contains madurose as the diagnostic sugar (wall chemotype III *sensu* Lechevalier and Lechevalier 1970a), contain major amounts of glucosamine-containing polar lipids (phospholipid type IV *sensu* Lechevalier et al. 1977), and partially tetrahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue (Kroppenstedt et al. 1990). The guanine-plus-cytosine content of the DNA lies within the range of 66–74 % mol. The type genus of the family is *Streptosporangium* (Couch 1955a).[AL]

Couch (1955a) classified the genus *Streptosporangium* in the family "*Actinosporangiaceae*" together with sporangiate actinomycetes belonging to the genus *Actinoplanes*. The family was subsequently renamed *Actinoplanaceae* by Couch (1955b). In addition to the type genus *Actinoplanes* and *Streptosporangium*, this taxon encompassed the genera *Amorphosporangium*, *Ampullariella*, *Dactylosporangium*, *Kitasatoa*, *Pilimelia*, *Planobispora*, *Planomonospora*, and *Spirillospora* (Couch and Bland 1974). Members of all of these genera were considered to form spore vesicles (sporangia).

It was shown subsequently that members of the genera *Planobispora*, *Planomonospora*, *Spirillospora*, and *Streptosporangium* formed a DNA homology group that was separated from a second aggregate DNA homology group which encompassed the genera *Actinoplanes*, *Ampullariella*, and *Dactylosporangium* (Farina and Bradley 1970). Organisms of the first group contained the sugar madurose and had a wall chemotype III, whereas those in the second taxon had a wall chemotype II, i.e., they contained *meso*- and/or hydroxy-A_2pm and glycine (Lechevalier et al. 1970a). The genera *Actinoplanes*, *Dactylosporangium*, *Micromonospora*, and *Pilimelia* are now known to have many properties in common and are classified in the family *Micromonosporaceae* (Krassilnikov 1938), emended by Goodfellow et al. (1990). The genus *Kitasatoa* has become a subjective synonym of the genus *Streptomyces* (Goodfellow et al. 1986), and the genera *Amorphosporangium* and *Ampullariella* have been reduced to subjective synonyms of the genus *Actinoplanes* (Stackebrandt and Kroppenstedt 1987).

Goodfellow and Cross (1984) assigned the oligosporic genera *Actinomadura* (Lechevalier and Lechevalier 1970a), *Microbispora* and *Microtetraspora* and the sporangiate genera *Planobispora*, *Planomonospora*, *Spirillospora* Couch (1963) and *Streptosporangium* to an aggregate group, the maduromycetes. Apart from representatives of the genus *Spirillospora*, these taxa formed a recognizable suprageneric group based on 16S rRNA gene cataloging and sequencing data (Stackebrandt 1986). The genus *Spirillospora* is currently classified in the family *Thermomonosporaceae*.

Phylogenetic Structure of the Family and Its Genera

The taxonomic status of genera assigned to the maduromycetes was formalized by the proposal that *Streptosporangium* be recognized as the type genus of a new suprageneric taxon, the family *Streptosporangiaceae*. This family was also seen as a taxonomic niche for the genera *Microbispora*, *Microtetraspora* (including the *Actinomadura pusilla* group *sensu* Kroppenstedt et al. (1990)), *Planobispora*, *Planomonospora*, and tentatively for the genus *Spirillospora*. Additional genera have been added to the family, namely, *Herbidospora*, *Planotetraspora*, *Nonomuraea*, *Acrocarpospora*, *Sphaerisporangium*, *Thermopolyspora*, and *Sinosporangium*. Representatives of the ten genera classified in the family *Streptosporangiaceae* form distinct, but related taxa based on 16S rRNA gene sequence data (*Fig. 43.1*). Because the genus *Sinosporangium* is branching slightly deeper in the suborder *Streptosporangineae*, the genus *Sinosporangium* could not be placed into the family *Streptosporangiaceae*. However, signature nucleotide analysis of 16S rRNA gene clearly characterized that the genus *Sinosprangium* contained the signature nucleotide pattern defined for the suborder *Streptosporangineae* (Zhang et al. 2011).

Based on the Neighbor-joining tree, all the genera including the family formed three clades. *Thermopolyspora flexuosa* and *Sinosporangium album* branched more deeply within this suborder.

Differentiation from Closely Related Taxa

Streptosporangiaceae strains can be distinguished from all other actinomycetes using a combination of chemotaxonomic and morphological features. Members of the family show a range of morphological properties but are relatively homogeneous from a chemotaxonomic perspective (*Table 43.1*). Simplified procedures are available for detecting chemical markers, notably, cell wall constituents (Staneck and Roberts 1974; Uchida and Aida 1977, Uchida et al. 1999; Hancock 1994), fatty acids (Suzuki and Komagata 1983; Kroppenstedt et al. 1990), menaquinones (Collins et al. 1977; Kroppenstedt 1982; Minnikin et al. 1984; Suzuki et al. 1993), and polar lipids (Minnikin et al. 1984; Suzuki et al. 1993). Procedures such as these are being progressively complemented or replaced by molecular systematic methods, including the use of oligonucleotide primers (Monciardini et al. 2002) and 16S rRNA gene sequences (Stackebrandt et al. 1997; Tamura et al. 2000; Stach et al. 2003).

Primary diagnostic chemotaxonomic data can be gained by examination of whole-organism hydrolysates (Lechevalier and Lechevalier 1980). One-dimensional thin layer chromatography will determine whether an organism contains diaminopimelic acid and whether the latter is in the *LL*- or *meso*- form. Sporoactinomycetes rich in *LL*-A_2pm can be provisionally assigned to the genus *Streptomyces*. The detection of meso-A_2pm

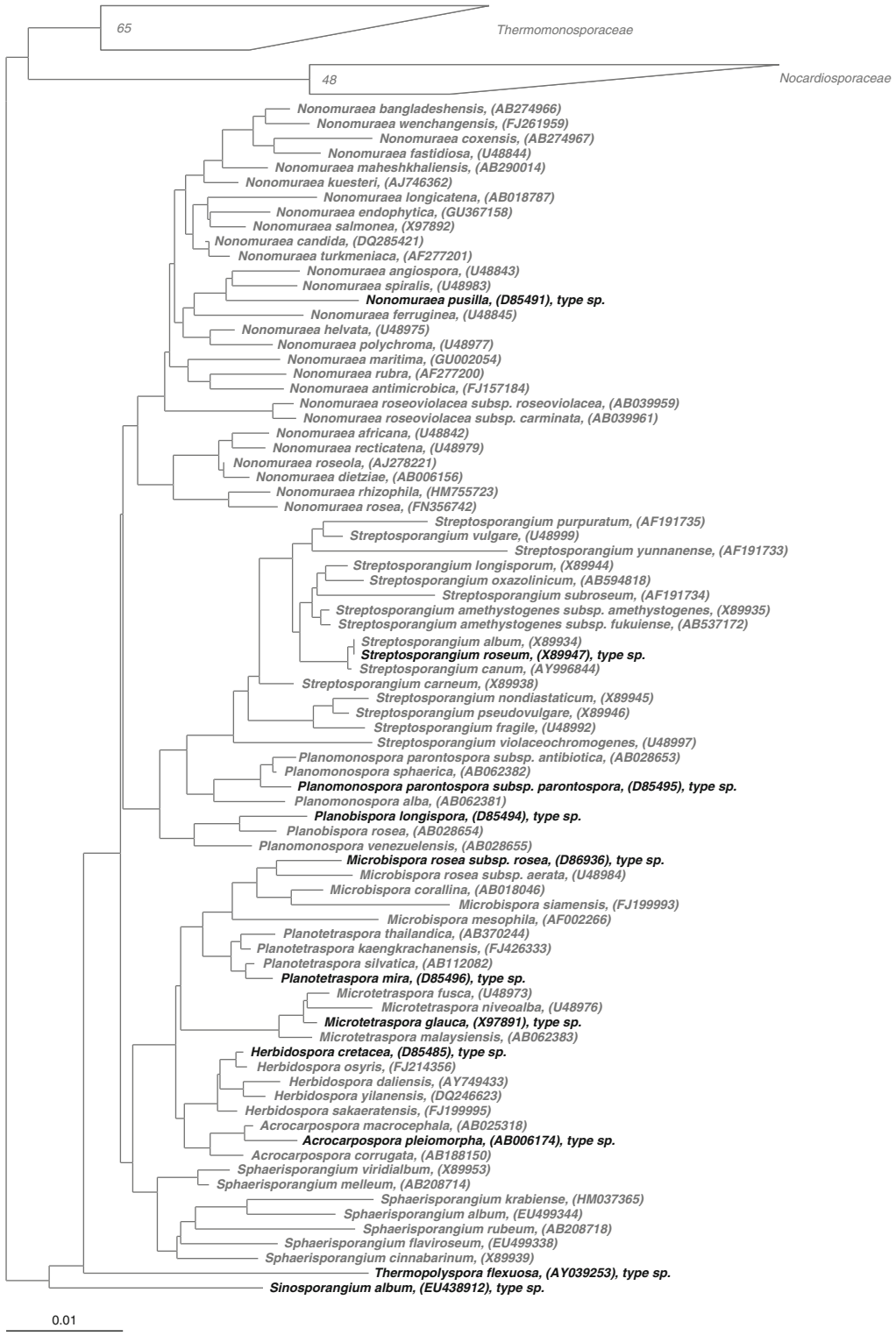

Fig. 43.1

Phylogenetic reconstruction of the family *Streptosporangiaceae* 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

Table 43.1

Morphological features and chemotaxonomic characteristics of members of the genera classified in the family *Streptosporangiaceae*[a]

Characteristics	Genus								
	Acrocarpospora	*Herbidospora*	*Microbispora*	*Microtetraspora*	*Nonomuraea*	*Planobispora*	*Planomonospora*	*Planotetraspora*	*Sphaerisporangium*
Vesicle formation	Club or globose spore vesicles on aerial hyphae	Spore chains on aerial hyphae	Spores in characteristic longitudinal pairs on aerial hyphae	Spore chains containing four or more spores on short aerial hyphae	Spore chains or pseudosporangia formed on aerial hyphae	Cylindrical to clavate spore vesicles containing longitudinal pairs of spores on aerial hyphae	Cylindrical to clavate spore vesicles containing single spores on aerial hyphae	Spore vesicles containing four spores on aerial hyphae	Globose spore vesicles on aerial hyphae
Cell-wall chemo-type[b]	III	III	III	III	III	III	III	III	III
Whole-organism sugar pattern[c]	B, C	B	B, C	B, C	B, C	B	B	D, A	B
Fatty-acid type[d]	3c	3c	3c	3c	3c	3c	3c	ND	3c
Major menaquinones (MK)[e]	$-9[H_2, H_4, H_6]$	$-10[H_4, H_6, H_8]$	$-9[H_0, H_2, H_4]$	$-9[H_2, H_4, H_6]$	$-9[H_0, H_2, H_4]$	$-9[H_2, H_4]$	$-9[H_2]$	ND	$-9[H_4, H_6]$
Phospholipid type[f]	IV, II	IV	IV	IV	IV	IV	IV	ND	IV

Characteristics	Genus		
	Streptosporangium	*Thermopolyspora*	*Sinosporangium*
Vesicle formation	Globose spore vesicles on aerial hyphae	Hooked or irregular spiral chains of four to ten warty to spiny ornamented spores on aerial hyphae	Globose sporangia on aerial hyphae
Cell-wall chemo-type[b]	III	III	III
Whole-organism sugar pattern[c]	B	C	A
Fatty-acid type[d]	3c	3c	3c
Major menaquinones (MK)[e]	$-9[H_4]$	$-9[H_2, H_4]$	$-9[H_2, H_4]$
Phospholipid type[f]	IV	IV	IV

[a]Data taken from Goodfellow (1989a, b, 1990, 2005); Kroppenstedt et al. (1990); Kudo et al. (1993); Tamura et al. (2000); Ara and Kudo (2007); and Zhang et al. (2011))

[b]Major constituents: alanine, glutamic acid, glucosamine, and *meso*-A_2pm (Lechevalier and Lechevalier 1970b); [c]A, cross-linkage between positions 3 and 4 of adjacent peptide subunits; 1, peptide bridge absent; γ, *meso*-A_2pm at position 3 of the tetrapeptide subunits (Schleifer and Kandler 1972); [d]saturated fatty acids, unsaturated fatty acids, *iso*-fatty acids (variable) and methyl-branched fatty acids (Kroppenstedt 1985); [e]*Herbidospora* strains contain tetrahydrogenated menaquinones with ten isoprene units (Kudo et al. 1993), organisms in the remaining taxa contain tetra-hydrogenated menaquinones with nine isoprene units (Kroppenstedt 1982); [f]phospholipid patterns: PI, phosphatidylglycerol (variable); PII, only phosphatidylethanolamine; PIV, phospholipids containing glucosamine (with phosphatidylmethylethanolamine variable) (Lechevalier et al. 1977)

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and madurose with the absence of characteristic sugars serves to separate strains of *Streptosporangiaceae* from those of *Actinoplanes* and related genera, *Nocardia* and related genera, *Pseudonocardia* and related genera, *Nocardiopsis* and related genera, and *Thermomonospora* and related genera, but not from the genera *Dermatophilus* and *Frankia*. The latter can be distinguished readily from *Streptosporangium* and allied taxa on morphological grounds. To date, the presence of madurose is associated with wall chemotype III actinomycetes, although there is an unconfirmed report of this sugar from a wall chemotype I actinomycete with streptomycete morphology (Weyland et al. 1982). The discovery of 3-*O*-methylgalactosyl (madurosyl) units in the structure of teichoic acids of a *Nonomuraea roseoviolaceae* subsp. *carminata* strain (previously *Actinomadura carminata*; Naumova et al. 1986) is also noteworthy, as madurose is not perceived to be a cell wall constituent according to Lechevalier and Lechevalier (1981a).

Wall chemotype III actinomycetes which form spore vesicles can be identified to the genus level using morphological features (*Table 43.1*), though care must be taken to distinguish between *Spirillospora* and *Streptosporangium* strains even though only the former produces motile spores (Vobis and Kothe 1989c). Note also that the sporogenic hyphae in the spore vesicles of spirillosporae are branched, whereas those in streptosporangiae are unbranched. Chemical analyses are required if the genera *Microbispora*, *Microtetraspora*, and *Nonomuraea* are to be reliably separated from the genus *Actinomadura* (Kroppenstedt et al. 1990). Actinomadurae contain mostly hexahydrogenated menaquinones with nine isoprene units (MK-9 [H_6]) and diphosphatidylglycerol and phosphatidylinositol as predominant polar lipids (phospholipid type I *sensu* Lechevalier et al. 1977) whereas *Microbispora*, *Microtetraspora*, and *Nonomuraea* strains have major amounts of MK-9 [H_4] saturated at positions III and IV, with major amounts of diphosphatidylglycerol, hydroxylated phosphatidylethanolamine, uncharacterized glycolipids, and a glucosamine-containing phospholipid (type IV phospholipid pattern *sensu* Lechevalier et al. 1977).

The separation of the genera *Microbispora*, *Microtetraspora*, and *Nonomuraea* solely on morphological criteria is questionable. *Microbispora* is distinguished from related genera (*Table 43.2*) primarily by the formation of paired spores on aerial hyphae. These are either sessile or borne on short sporophores. In *M. rosea*, the latter were found to be attached to the base of the spore by a ball and socket arrangement (Williams 1970). *Microbispora* species are currently separated by using a range of phenotypic properties (*Table 43.2*). Cultures of *M. aerata*, *M. amethystogenes*, and *M. parva* deposit crystals with a metallic sheen in the medium, particularly when grown on Pablum extract agar (Lechevalier and Lechevalier 1957) for about 10 days (Gerber and Lechevalier 1964). These crystals are composed of iodinin (1,6-phenazinediol-5,10-dioxide), a red, water-soluble pigment. In addition, *M. aerata* produces two brown-yellow pigments (2-aminophenoaxazine-3-one and 1,6-phenazinediol), a yellow pigment (2-acetamidophenoxazine-3-one; Gerber and Lechevalier 1964), and an orange pigment (1,6-phenazinediol-5-oxide; Gerber and Lechevalier 1965). Microtetrasporae can be recognized by their ability to produce short spore chains which typically contain four spores. The identification of species within the genus is still based on phenotypic properties, notably on the color of the aerial and substrate mycelium (*Table 43.3*).

Nonomuraea strains are distinguished from members of related genera by their ability to form chains of spores or pseudovesicles on aerial hyphae. The constituent species may be distinguished by means of spore chain morphology, spore wall ornamentation, color of mature sporulated aerial mycelium, and substrate mycelium pigmentation (*Table 43.4*). Nevertheless, identification of many of these species is difficult, because in most instances, only one (the type) strain or a few strains have been examined. Even when several strains have been studied, the results of biochemical and physiological tests have proved to be variable or inconsistent when data from the literature are compared. However, numerical taxonomic evidence indicates that most of the validly described taxa merit species status (Goodfellow et al. 1979; Goodfellow and Pirouz 1982).

Streptosporangium species can be distinguished from one another using a combination of phenotypic properties, notably morphological features (see "Genus *Streptosporangium*" *Fig. 43.1*; *Table 43.5*). They can be separated by spore vesicle size, sporangiophore length, spore shape, aerial spore mass color, and substrate mycelium pigmentation and subdivided according to the nature of the vesicular wall. At one extreme, the spore vesicular membrane of *S. fragile* is so thin that it cannot be detected by light microscopy (Shearer et al. 1983). This feature may lead to difficulty in differentiating *Streptosporangium* from *Nonomuraea* strains, as members of some species of the latter produce pseudovesicles covered by a slimy substance (Nonomura and Ohara 1971c). The remaining species of *Streptosporangium* form thin vesicular membranes that are readily disrupted in water. *Streptosporangium amethystogenes* produces violet crystals of iodine after a month's incubation at 30 °C on oatmeal-yeast extract agar.

Improved phenotypic tests are needed for the identification of unknown streptosporangiae. Clearly such tests should be based on a representative set of strains. Whitham (1988) generated a probability matrix on the basis of 26 diagnostic properties for the identification of unknown streptosporangiae to established and novel *Streptosporangium* species. In a continuation of these studies, Kim (1999) assigned 65 out of 70 marker *Streptosporangium* strains and 12 out of 131 putative streptosporangiae isolated from soil to known species of *Streptosporangium*. A further 19 of the soil isolates were identified to known species when less stringent cut-off points were adopted for a positive identification.

The genera *Planobispora*, *Planomonospora*, and *Planotetraspora* may be distinguished by the shape of their spore vesicles and by the number of encased spores. *Planobispora* and *Planomonospora* species can be separated using a judicious selection of phenotypic properties (*Table 43.6*). *Planobispora longispora* and *Planobispora rosea* have many properties in common (Goodfellow and Pirouz 1982) but can be separated by

Table 43.2
Characteristics differentiating validly described species of the genus *Microbispora*

Characteristics	*M. amethystogenes*	*M. corallina*	*M. mesophila*	*M. rosea*	*M. siamensis*
Morphology					
Aerial mycelium color	Pink	Pink	White	Pale pink	Pale pink
Substrate mycelium color	Light brown	Coral pink to reddish	Brown	Orange	Colorless to yellow
Soluble pigments	–	–	–	–	+
Iodinin production	+	–	ND	–	ND
Degradation of					
Hypoxanthine	–	–	+	+	ND
Starch	–	v	+	–	ND
Testosterone	–	ND	ND	+	ND
Xanthine	–	–	+	–	ND
Xylan	–	–	+	–	ND
Nitrate reduction	–	–	+	? Data missing	–
Growth on sole carbon sources (1 %, w/v)					
Arabinose	+	+	+	+	+
Glycerol	+	+	–	+	ND
Inositol	+	+	–	–	–
Rhamnose	–	–	–	+	–
Growth at					
25 °C	+	+	+	+	+
50 °C	–	–	–	–	+
55 °C	–	–	–	–	–
Requirement for					
Biotin	–	–	ND	+	ND
Thiamine	+	+	ND	+	ND

From McCarthy and Cross (1984),Rao et al. (1987), Nonomura (1989b), Nakajima et al. (1999) and Biindaeng et al. (2009)
Symbols and abbreviations: +, positive; negative; ND, not determined; and v, variable

using cultural characteristics (Thiemann 1974a). *Planobispora longispora* produces a hyaline- to creamish-colored substrate mycelium and a white aerial mycelium, whereas *P. rosea* has a rose-colored substrate mycelium and an aerial mycelium with a light rose tinge. The type strain of *P. mira*, unlike that of *P. silvatica*, produces acid from lactose, mannitol, mannose, and rhamnose and uses glucose, mannitol, and xylose as sole carbon sources. Conversely, the *P. silviatica* strain degrades xanthine and uses melibiose and raffinose as sole carbon sources. The two strains can also be distinguished using colonial characteristics.

Planomonospora parontospora and *P. venezuelensis* strains can be distinguished by the morphological arrangement of their spore vesicles, different menaquinone profiles (Collins et al. 1984), and the characteristic color of the mycelium (Thiemann 1974b). *Planomonospora sphaerica* can be distinguished from the other members of the genus by its ability to form large spherical bodies when grown on inorganic salts starch agar (Mertz 1994).

Members of the genera *Acrocarpospora* and *Herbidospora* can also be recognized on morphological grounds (*Table 43.1*). The identification of species of *Acrocarpospora* is based on the discontinuous distribution of a few phenotypic properties (*Table 43.7*).

Molecular Analyses

DNA-DNA hybridization (DDH) studies have been performed on *Acrocarpospora*, *Herbidospora*, *Microbispora*, *Microtetraspora*, *Nonomuraea*, *Planotetraspora*, *Sinosporangium*, *Sphaerisporangium*, and *Streptosporangium*. However, the results of DDH were not available on *Planobispora*, *Planomonospora*, and *Thermopolyspora*.

The description of *Acrocarpospora* species includes the results of DDH studies, and moderate DDH relatedness of 45–49 % between *A. pleiomorpha* and *A. macrocephala*. *A. corrugata* and other species were more distantly related (24–12 %). The relatedness between *Acrocarpospora* species and other genera was quite low (<9 %) (Tamura et al. 2000).

Table 43.3

Characteristics differentiating the type strains of *Microtetraspora* species

Characteristics	M *fusca* DSM 43841^T	M. *glauca* DSM 43311^T	M. *malaysiensis* DSM 44579^T	*M. niveoalba* DSM 43174^T
Morphology				
Aerial spore mass color				
Blue-gray	–	+	–	–
Gray	+	+	–	–
White	–	–	+	+
Substrate mycelium color				
Cream-yellow	–	–	+	–
Greenish-blue	–	+	–	–
Purplish	+	–	–	–
Branched spore chains	–	–	–	+
Requirement for biotin	–	+	–	–
Biochemical tests				
Reduction of nitrate	–	.+	ND	+
Urea hydrolysis	+	–	–	+
Degradation of				
Elastin	ND	–	–	+
Gelatin	–	+	ND	+
Hypoxanthine	–	+	–	+
Starch	–	+	ND	+
Testosterone	–	+	+	+
Xanthine	–	+	–	+
Xylan	–	+	–	–
Growth on sole carbon sources				
L(+) Arabinose	+	+	–	+
D(+) Fructose	–	+	+	+
D(+) Galactose	–	+	–	–
Glycerol	–	+	–	+
D(+) Mannitol	–	+	+	+
D(+) Mannose	+	+	–	+
meso-Inositol	–	+	–	+
L(+) Rhamnose	–	+	+	–
D(+) Trehalose	+	+	–	+
Xylitol	–	–	–	+
Citrate	+	+	+	–
Fumarate	–	+	–	+
Malate	–	+	–	+

Symbols and abbreviations: +, positive or present; negative or absent; ND, not determined; T, type strain; and DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg IB, D-38124 Braunschweig, Germany (From Nonomura (1989b) and Nakajima et al. (2003))

In an early DDH study of *Herbidospora*, *H. cretacea* strains fell into three groups with intergroup relatedness of 44–68 % (Kudo et al. 1993). The intragroup relatedness of the of *H. cretacea* isolates was higher than 70 % (Kudo et al. 1993). The type strains of *H. cretacea* and *H. osyris* were closely related (62.9 %) (Li et al. 2009a), and subsequently, *H. osyris* was classified as asynonyms of *H. cretacea* (Ara et al. 2012). Other DDH studies found *Hebidospora* species to be moderately related (>35 % similarity) (Tsenget al. 2010; Boondaeng et al. 2011; Ara et al. 2012). With more than 73 % DDH similarity, *Streptosporangium claviforme* and *H. osyris* strains were determined to be synonymous to *H. cretacea* (Boondaeng et al. 2011; Ara et al. 2012).

Table 43.4
Characteristics distinguishing the type strains of *Nonomuraea* species

Characteristics	*N. africana*	*N. angiospora*	*N. antimicrobica*	*N. bangladeshensis*	*N. candida*	*N. coxensis*	*N. dietziae*	*N. endophytica*	*N. fastidiosa*	*N. ferruginea*	*N. helvata*
Morphology											
Spore chains	str	sp	sp	sp	h	str, h	str, sp	str	s, sp	h, s	h, psp
Spore ornamentation	Smooth	Ridged	Smooth	Smooth	Smooth	Smooth		Warty		Folded	Smooth
No. of spores	4–10	4–15	15–37	8–12	ND	12–17	Up to 30	≥20	4–10	4–10	4–10
Growth on ISP medium 3											
Aerial mycelium	Grayish/blue	White	White/pink	Pale brown	White	Pink to white	Beige	White	White/pink	White/pink	White
Substrate mycelium	Yellow	White/ochre	Moderate brown	Pale brown	Yellow-white	Orange	Beige	Deep brown	Colorless	Pink	Yellow/brown
Soluble pigment	Yellowish/brown	None	Pink	None	None	None	Yellow	Orange-brown	None	None	None
Biochemical tests											
Esculin hydrolysis	+	+	–	ND	+	ND	ND	ND	+	–	+
Nitrate reductase	+	–	–	–	–	+	ND	–	+	+	+
Degradation tests											
Casein	+	+	+	ND	+	ND	ND	–	+	+	–
DNA	+	+	ND	ND	ND	ND	ND	ND	+	+	–
Elastin	–	+	ND	ND	ND	ND	ND	ND	+	+	ND
Gelatin	+	+	–	ND	(+)	ND	–	–	+	–	–
Hypoxanthine	+	+	–	ND	–	ND	ND	+	+	+	–
Starch	+	–	–	+	–	+	ND	–	–	+	–
Tyrosine	+	–	–	+	–	–	ND	+	–	+	–
Xanthine	–	+	ND	ND	–	ND	ND	–	–	–	–

Characteristics	*N. kuesteri*	*N longicatena*	*N. maheshkhaliensis*	*N. maritima*	*N. polychroma*	*N. pusilla*	*N. recticatena*	*N. rhizophila*	*N. rosea*	*N. roseola*	*N. roseoviolacea* subsp. *carminata*	*N. roseoviolacea* subsp. *roseoviolacea*
Morphology												
Spore chains	sp	str	sp	s, sp	ND	psp	str	sp	s	sp, str	sp. psp	psp
Spore ornamentation	ND	Smooth	Rough	Smooth to rough	ND	Smooth	Smooth	Rough	ND	Folded	Smooth	Smooth
No. of spores	ND	10–30	17–20	10–30	ND	>10	4–20	7–10	4–10	4–20	ND	4–20
Growth on ISP medium 3												
Aerial mycelium	Trace	White	White	White	Trace	White/ cream	White/cream	White	White	Pink	Pink	Pink/violet
Substrate mycelium	Yellow	Ochre	Light wheat	White	Colorless/ brown	Gray/brown	Dark yellow/brown	Brown-yellow	Pink to violet	Brown/ red	Old-wine	Violet
Soluble pigment	None	None	None	None	None	None	None	None	None	None	Wine-red	Violet

Biochemical tests												
Esculin hydrolysis	+	+	ND	−	+	+	+	ND	+	+	ND	+
Nitrate reductase	ND	−	ND	(+)	−	+	+	−	ND	+	−	+
Degradation tests												
Casein	−	+	ND	+	−	−	−	ND	+	−	−	−
DNA	ND	−	ND	ND	−	+	−	ND	ND	−	ND	+
Elastin	ND	+	ND	ND	+	−	+	ND	ND	−	ND	−
Gelatin	−	−	ND	−	+	+	+	−	+	+	−	+
Hypoxanthine	−	+	ND	−	+	+	+	+	+	+	ND	+
Starch	−	+	ND	−	−	−	+	−	+	−	+	−
Tyrosine	−	−	ND	−	−	+	−	+	+	+	ND	−
Xanthine	−	−	ND	ND	−	−	−	+	+	−	ND	−

Characteristics	*N. rubra*	*n. salmonea*	*N. spiralis*	*N. turkmeniaca*	*N. wenchangensis*
Morphology					
Spore chains	h, s, sp	h, s	sp	sp	s
Spore ornamentation	Smooth	Warty	Folded	Smooth	Rough
No. of spores	4–20	4–30	4–20	10–20	6–10
Growth on ISP medium 3					
Aerial mycelium	Trace	Pink	White/yellow	Trace	White
Substrate mycelium	Orange red	Red	Yellow/brown	Violet/red	Pale pink
Soluble pigment	Red	None	None	Pink/violet	None
Biochemical tests					
Esculin hydrolysis	−	+	+	+	+
Nitrate reductase	+	+	+	+	+
Degradation tests					
Casein	−	+	−	+	+
DNA	−	+	−	−	ND
Elastin	+	+	−	−	ND
Gelatin	+	+	+	+	−
Hypoxanthine	+	+	−	+	−
Starch	+	−	−	+	−
Tyrosine	+	+	+	−	+
Xanthine	−	−	−	−	−

From Meyer (1989), Chiba et al. (1999), Gyoubu and Miyadoh (2001), Stackebrandt et al. (2001), Kampfer et al. (2010), Li et al. (2011), Wang et al. (2011), Xi et al. (2011) and Zhao et al. (2011)

Symbols and abbreviations: +, positive; −, negative; *ND*, not determined; *h*, hooks, curled; *psp*, pseudovesicles; *s*, spirals of 1–2 turns; *sp.* spirals of 3–5 turns; *str*, straight

Table 43.5

Pretreatment procedures used for the selective isolation of members of the family *Streptosporangiaceae* from environmental samples

Treatment	Substrate	Media	Antibiotic(s)	Target genera	References
Baiting					
Paspalum grass	Soil	3 %, w/v agar	Cycloheximide, nystatin	*Streptosporangium*	Couch (1954, 1955a, 1963)
Pollen and hair	Soil and water	3 %, w/v agar	Cycloheximide nystatin	*Planomonospora*	Vobis (1989a, b)
Chemical					
Chloramine-T	Soil	Humic acid-vitamin agar	Cycloheximide nalidixic acid	*Herbidospora*, *Microbispora*, *Microtetraspora*, *Nonomuraea*, *Streptosporangium*	Hayakawa et al. (1997)
Physical					
Air-dried soil heated at 100 °C or 120 °C for 1 h	Soil	Arginine-vitamins agar	Cycloheximide, nystatin	*Microbispora*, *Streptosporangium*	Nonomura and Ohara (1969a, b)
Air-dried soil heated at 100 °C or 120 °C for 1 h	Soil	Glucose-asparagine with soil extract agar	Cycloheximide, nystatin	*Microtetraspora*	Nonomura and Ohara (1971a, b)
Air-dried soil heated at 120 °C for 1 h	Soil	SE agar/Soil extract agar	Cyclohexamide, nalidixic acid, polymivin B, penicillin	*Microbispora*, *Microtetraspora*, *Streptosporangium*	Nonomura and Ohara (1960a, b, 1969a, 1971a, b)
Desiccated soil at 28 °C for 1 week			Cycloheximide, nystatin	*Herbidospora*	Kudo et al. (1993)
Air-dried soil heated at 90 °C or 100 °C for 1 h, rehydration and centrifugation	Plant/soil	Yeast-extract agar/Humic acid-vitamin agar	Ampicillin, cycloheximide, enoxacin, nalidixic acid, nystatin, streptomycin, trimethoprim	*Planobispora, Planomonospora*	Suzuki et al. (2001a, b)
Physico-chemical					
Air-dried soil heated at 120 °C for 1 h, 10^{-1} dilution treated with 1.5 % phenol and 0.01 % chlorhexidine gluconate	Soil	Humic acid trace salts gellan gum agar	Cyclohexamide, nalidixic acid	*Microbispora*	Hayakawa and Nonomura (1991)
Air-dried soil heated at 120 °C for 1 h, 10^{-1} soil suspension treated with 0.01 % (w/v) benzethonium chloride	Soil	Humic acid-vitamin agar	Leucomycin, nalidixic acid	*Streptosporangium*	Hayakawa et al. (1991)
Air-dried soil heated at 110 °C for 1 h, 10^{-1} soil suspension treated with 0.05 % (w/v) benzethonium chloride	Soil	LSV-SE agar	Kanamycin, nalidixic acid, norfloxacin	*Microtetraspora*	Hayakawa et al. (1996)

The genus *Microbispora* was proposed by Nonomura and Ohara (1957) and currently encompasses four species. All species descriptions include DDH studies which found them to be distantly related (12–46 %), e.g., *M. siamensis* and other members of the genus *Microbispora* (19–46 %, Boondaeng et al. 2009), and between *M. hainanensis* and *M. corallina* (12.4–13.6 %, Xu et al. 2012). According to the results of DDH studies, ten species of the genus *Microbispora* were suggested to be combined into the type species *M. rosea* with two subspecies, *M. rosea* subsp. *rosea* and *M. rosea* subsp. *aerate* (Miyadoh et al. 1990).

Microtetraspora species encompasses four species, and their descriptions were also based upon results of DDH studies. The type strains of *M. fusca* and *M. malaysiensis* were closely related

Table 43.6

Characteristics differentiating validly described taxa classification in the genera *Planobispora* and *Planomonospora*

Characteristics	*Planobispora*		*Planomonospora*				
	longispora	*rosea*	*alba*	*parontospora* var. *antibiotica*	*parontosvora* var. *parontospora*	*sphaerica*	*venezuelensis*
Morphology							
Color of aerial mycelium	White	Rose	White	Pink	Pink	Pink	Pink
Number of spores in spore vesicle	2	2	1	1	1	1	1
Biochemical tests							
Esculin hydrolysis	−	+	−	−	+	−	−
Nitrate reductase	ND	ND	−	+	+	+	+
Phosphatase	+	+	+		+	+	+
Decomposition of							
Gelatin	+	+	+	+	−	+	−
Hypoxanthine	+	−	−	−	−	−	+
Tyrosine	+	+	+	+	−	+	
Utilization of							
L-Arabinose	+		+	+	−	+	−
Cellobiose	+	+	+	+	−	+	−
Citrate	ND	ND		+	+	+	−
Dextrin	ND	ND	+	+	−		−
Fructose	+	+	+	+	+	+	
Galactose	−	+	+		+	+	−
Glycerol	−	−	+	−	−	−	−
Glycogen	+	+	−	+	−	+	−
Maltose	+	+	+	+	−	+	−
Mannitol	+	+	+	+	−	+	−
Mannose	−	−	+	+	−	+	−
Rhamnose	+	−	+	−	−	+	−
Salicin	−	+	−	+	−	−	−
Succinate	ND	ND	−	+	−	+	−
Starch	+	+	+	+	−	+	−
Sucrose	−	−	+	+	−	+	−
Trehalose	−	−	+	+	−	+	−
Xylose	+	+	−	+	−	+	−
Resistance to 5 % NaCl	ND	ND	−	+	−	−	−
Growth at							
15 °C	−	−	−	+	−	−	−
45 °C	−	−	+	−	−	−	−

From Vobis (1989b) and Mertz (1994)

Symbols and abbreviation: +, positive; −, negative; and ND, not determined

(45–58 %), while other species were distantly related (21–38 %, Nakajima et al. 2003). The intraspecies DDH relatedness of *M. malaysiensis* ranged between 72 % and 98 % similarity (Nakajima et al. 2003).

About half of the descriptions of *Nonomuraea* species contain DDH data. *N. wenchangensis* and *N. coxensis* (56.5 %, Wang et al. 2011) as well as *N. coxensis* and *N. bangladeshensis* were 57.7–58.2 % (Ara et al. 2007a) forming two closely related species pairs, while *N. endophytica* and *N. candida* (41.4 %, Li et al. 2011), *N. kuesteri* and *N. turkmeniaca* (40.5 %, Kämpfer et al. 2005), as well as *N. maheshkhaliensis* and *N. kuesteri* (39.9–45.7 %, Ara et al. 2007b) were less closely related. Other

Table 43.7
Characteristics distinguishing the type strains of *Acrocarpospora* species

Characteristics	*A. corrugata* DSM 43316^T	*A. macrocephala* DSM 44705^T	*A. pleiomorpha* DSM 44706^T
Nitrate reduction	–	–	+
Starch degradation	–	+	+
Utilization of			
L(+) Arabinose	+	–	–
D(+) Mannitol	–	+	+
D(+) Raffinose	–	+	+
a-L(-) Rhamnose	–	+	+
D(+) Xylose	+	–	–

From Whitham et al. (1993) and Tamura et al. (2000)
Symbols and abbreviations: +, positive; –, negative; T, type strain; and DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen Mascheroder Weg 1B, D-38124 Braunschweig, Germany

species were even more distantly related (<40 %), e.g., *N. rhizophila* and *N. rosea* (38.5 %, Zhao et al. 2011), and *N. jiangxiensis* and other species (16.0–22.4 %, Li et al. 2012). Previously, it has been shown that some *Nonomuraea* species share high 16S rRNA gene sequence similarities (within the range 97.6–99.4 %), but low DDH values (Fischer et al. 1983; Poscher et al. 1985; Tamura et al. 2000; Kämpfer et al. 2005) and 45–48 % DDH values were reported (Stackebrandt et al. 2001) between the type strains of *N. africana*, *N. dietziae*, and *N. recticatena*, although these strains shared 98.9–99.8 % 16S rRNA gene sequence similarity. As other species of the genus shared low 16S rRNA gene sequence similarity, DDH values were not determined

The genus *Planotetraspora* was proposed by Runmao et al. (1993) and encompasses five species. Almost all of descriptions include the DDH results with moderate DDH relatedness (22.7–42 %, Tamura and Sakane 2004; Suriyachadkun et al. 2009, 2010) between the type strains.

Almost all of species of the genus *Sphaerisporangium* are closely related (40–66.6 %, Cao et al. 2009; Suriyachadkun et al. 2011). Some are more distantly related, i.e., *S. viridialbum* and *S. rubeum* (37.8 %, Suriyachadkun et al. 2011), and *S. siamense* and *S. album* (32–39 %, Duangmal et al. 2011).

The genus *Streptosporangium* was originally described by Couch (1955a), but some species were transferred to other genera (Kudo and Seino 1987; Stackebrandt et al. 1994; Tamura et al. 2000; Ara and Kudo 2007; Boondaeng et al. 2011; Ara et al. 2012). Recently described species of the genus *Streptosporangium* included DDH results with *S. canum* and *S. roseum* (64.26 %, Zhang et al. 2009) being most closely related. Other species are closely related (59.2–53.1 %), i.e., *S. canum* and *S. amethystogenes* subsp. *amethystogenes* (59.23 %, Zhang et al. 2009), *S. canum* and *S. album* (53.69 %, Zhang et al. 2009), *S.yunnanense* and *S. nondiastaticum* (56.8 %, Zhang et al. 2005), *S. yunnanense* and *S. pseudovulgare* (53.1 %, Zhang et al. 2005), *S. purpuratum* and *S. longisporum* (53.3 %, Zhang et al. 2005), and *S. anatoliense* and *S.pseudovulgare* (58.3 %, Sazak et al. 2012). *S. purpuratum* is more distantly related to other species (43.3–45.2 %, Zhang et al. 2005), as are *S. anatoliense* and *S. nondiastaticum* (43.2 %, Sazak et al. 2012) and *S. subroseum* and related species, showing 3–27 % (Zhang et al. 2002).

Data of the 16S–23S rRNA gene spacer sequences (Zhang et al. 1997, 2001) and electrophoretic mobility of ribosomal protein AT-L30 (Ochi and Miyadoh 1992; Ochi et al. 1991, 1993) are available for a few species of the genus *Streptosporangium*. 23S rRNA and 5S rRNA gene sequences were analyzed for four genera of *Streptosporangiaceae* (Wang and Zhang 2000) and *Herbidospora cretacea* K-319^T (Kudo et al. 1993), respectively. A study of the distribution of genes encoding halogenases and chitinases in the actinobacteria included some species of *Streptosporangiaceae* (Gao and Huang 2009; Kawase et al. 2004).

Genetics

Members of the family *Streptosporangiaceae* have the potential for exploitation, notably in the discovery of novel bioactive compounds, and several researchers studied genetic manipulation systems. Vectors capable of stably maintaining large segments of actinomycete DNA in *Escherichia coli* and of integrating site specifically in the *Streptomyces* genome have been developed to facilitate the manipulation of uncommon actinomycete strains, including streptosporangiae and related taxa (Donadio et al. 2002). These vectors, designated "ESAC," an abbreviation for "*E. coli-Streptomyces* artificial chromosome," are suitable for the reconstruction of gene clusters from small segments of the cloned DNA, the preparation of large insert libraries from unusual actinomycete strains, and the construction of environmental libraries. Other examples of heterologous expression of entire gene clusters in model actinomycetes have been reported (Piel et al. 2000; Tang et al. 2000; Kwon et al. 2001).

A potent antitumor antibiotic, sibiromycin, was produced by "*Streptosporangium sibiricum*" (Hurley et al. 1979), and the

Table 43.8

Specific primers developed for the identification of members of the family *Streptosporangiaceae*

Target taxa	Primer	Sequence 5′-3′	Position[a]	Reference
Streptosporangiaceae	21F	GACGAARNTGACGTGTA	407-424	Monciardini et al. (2002)
	959R	CGTTGCGTCTAATTAAGCAA	971-952	

[a]*E. coli* numbering position (Brosius et al. 1978)

biosynthesis of sibiromycin was determined based on gene cluster analysis and gene replacement techniques (Li et al. 2009b). The gene cluster coding for the biosynthesis of glycopeptide antibiotic A40926 in *Nonomuraea* strain ATCC 39727 has been isolated and characterized by Sosio et al. (2003). This glycopeptide, a member of the teichoplanin family of glycopeptides, is the precursor of dalbavancin, a second generation glycopeptide. Sosio and her colleagues also isolated the novel compound, dechloromannosyl-A40926 aglycone, following the construction of a *Nonomuraea* mutation by deleting dbr open reading frames 8–10. Recently, Marcone et al. (2010) developed the gene transfer systems of *Nonomuraea* sp. ATCC 39727 based on protoplast transformation and intergeneric conjugation from *E. coli*. Prauser (1984) reported that attempts to isolate phage from *Streptosporangiaceae* strains had been unsuccessful.

Amplification of the polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) genes from isolates are useful strategies to evaluate the secondary metabolite production. Janso and Carter (2010) amplified the PKS-1, -2, 3 and NPRS genes from the isolates and concluded that members of *Microbispora*, *Planotetraspora*, and *Sphaerisporangium* contain PKS-1, -2, -3 and NPRS genes. Similar research reported by González et al. (2005) showed that actinomycetes, including *Planobispora* and *Streptosporangium* isolated from lichen sample, contain genes coding for PKS-1, -2 and NPRS.

Specific Primer for *Streptosporangiaceae*

Monciardini and his colleagues (2002) designed a set of specific primers to recognize members of the family *Streptosporangiaceae*. They retrieved 423 16S rRNA gene sequences of different groups of the class *Actinomycetales* from the RDP-II Data base Release 8.0 and aligned the sequences within each genus. Aligned sequences were then visually compared for the identification of regions showing a high degree of conservation, for example, in members of the family *Streptosporangiaceae*, but having at their 3′-end one or more mismatches with other groups. The primers designed in this way were evaluated by probing their sequences against the whole RDP-II database using PROBE MATCH software (http://rdp.cme.msu.edu/html/index.html) to ensure that at least 90 % of the sequences belonging to members of the family *Streptosporangiaceae* were recognized with the primer. Non-target groups of sequences that could potentially yield a non-specific extension product with one primer were taken into account when choosing the second primers. The T_m of each primer was evaluated through Oligo Calculator version 3.01 (http://www.basic.nwu.edu/biotools.oligocalc.html) and primer lengths adjusted in order to mimic T_m differences within each primer set (*Table 43.8*).

Genome Analyses

The complete genome sequence of *Streptosporangium roseum* NI 9100^T has been published (Nolan et al. 2010) and analyzed for the Genomic Encyclopedia of Bacteria and Archaea project. The genome consists of a 10,341,314 bp long chromosome (INSDC ID CP001814) and a small 28,204 bp plasmid (INSDC ID CP001815) with a 70.9 % GC content. This value is only slightly higher than those determined by nuclease method (HPLC) performed on purified DNA (70 mol%). Besides 446 pseudogenes, 9,421 genes have been predicted, of which 9,501 were protein coding genes, and 80 were RNA genes. The majority of the genes (62.47 %) were assigned a putative function, 65.47 % of the genes were assigned to clusters of orthologous groups (COGs), while the remaining ones are annotated as hypothetical proteins. The distribution of genes into COGs functional categories indicates that the highest number of genes are involved in general function prediction only (974: 10.3 %) and transcription (966; 10.3 %), followed by genes coding for carbohydrate transport and metabolism (639; 6.8 %), and amino acid transport and metabolism (600; 6.4 %). Three hundred and fifteen genes (3.3 %) were found to code for secondary metabolites' biosynthesis, transport, and catabolism. A detailed listing of COG categories is given by Nolan et al. (2010). The sequence of the six 16S rRNA gene copies in the genome does not differ from each other, and their sequences are identical to the previously published sequence from DSM 43021 (X89947). However, the sequence of the JCM 3005^T version of strain NI 9100^T (U48996) differs by 24 nucleotides (1.7 %). Another genome project *Nonomuraea coxensis* DSM 45129^T (GOLD ID Gi11713) has been finished, but not published so far. The genome of *Microbispora mesophila* NBRC 14179^T has been projected (GOLD ID Gi21070).

Phenotypic Analyses

The description of the family *Streptosporangiaceae* (Goodfellow et al. 1990) emended by Ward-Rainey et al. (1997) is as given in the Abstract. The type genus is *Streptosporangium* Couch 1955a[AL].

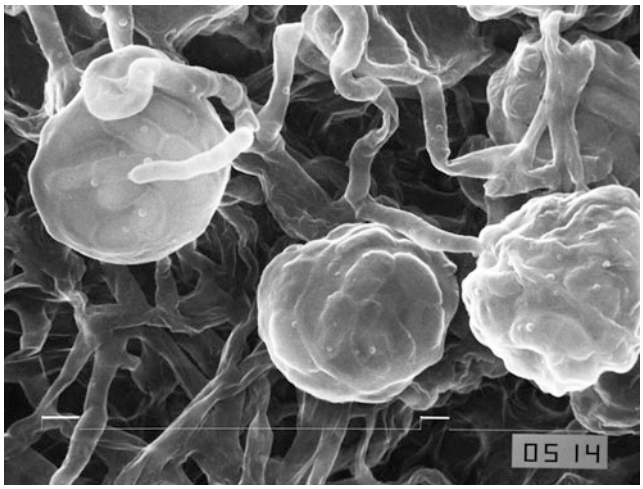
Members of the family *Streptosporangiaceae* are chemically homogeneous but morphologically diverse (◐ *Table 43.1*). However, strains that bear spore vesicles (*Acrocarpospora*, *Planobispora*, *Planomonospora*, *Planotetraspora*, and *Streptosporangium*) are closely related to organisms that have two or more spores in spore chains (*Herbidospora*, *Microbispora*, *Microtetraspora*, and *Nonomuraea*). Spore vesicles contain coiled chains of arthrospores formed by septation of an unbranched, spiral hypha within each expanded sporangiophore sheath (Vobis and Kothe 1985). Spore formation is not endogenous; hence, the term "spore vesicle" has greater precision than the original term "sporangium" (Cross 1970; Sharples et al. 1974). Studies on spore maturation have shown that spores in both spore vesicles and spore chains are formed in essentially the same way. In each case, spores are differentiated by fragmentation of a hypha within a sheath; the latter either expands to form the envelope of the spore vesicle or remains around the spore chain (Lechevalier et al. 1966; Sharples et al. 1974; Vobis and Kothe 1985).

Streptosporangium Couch 1955a, 148[AL] emend. Stackebrandt, Kroppenstedt, Jahnke, Kemmering, and Gürtler 1994, 268

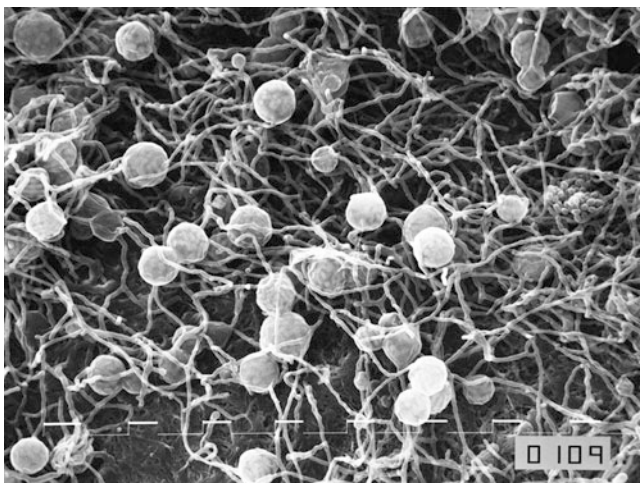
Strep.to.spo.ran'gi.um. Gr. adj. *streptos*, twisted; N.L. neut. n. *sporangium* [from Gr. n. *spora*, a seed (and in biology a spore), and Gr. n. *angeion* (Latin transliteration *angium*), vessel], sporangium; N.L. neut. n. *Streptosporangium*, spores coiled within a sporangium.

This taxon was proposed for sporangiate actinomycetes that formed nonmotile sporangiospores on abundant aerial hyphae. The genus was shown to be heterogeneous on the basis of spore and spore and spore vesicular morphology (Nonomura 1989b), electrophoretic mobility of ribosomal protein AT-L30 (Ochi and Miyadoh 1992), 16S rRNA (Kemmerling et al. 1993) and 5S rRNA (Kudo et al. 1993) gene sequences, and discontinuous distribution of chemical markers (Stackebrandt et al. 1994).

Stackebrandt and colleagues found that streptosporangiae had many chemical properties in common but could be assigned to two groups on the basis of chemical differences. Members of most species, including *S. roseum*, had a phospholipid pattern type IV and predominant proportions of MK-9 (H_2, H_4, H_6), whereas strains in the second group, which contained *S. albidum* and *S. viridogriseum*, had a MK-9 [H_4] as the predominant isoprenologue and a phospholipid pattern type II; these results were in excellent agreement with corresponding 16S rRNA gene sequence data (Kemmerling et al. 1993). Stackebrandt and colleagues proposed that *Streptosporangium albidum* Fumurai et al. (1968), *Streptosporangium viridogriseum* subsp. *kofuense* (Nonomura and Ohara 1969b), and *Streptosporangium viridogriseum* subsp. *viridogriseum* (Okuda et al. 1966) be assigned to a new taxon, the genus Kutzneria as *Kutzneria albida* comb, nov., *Kutzneria viridogrisea* comb, nov., and *Kutzneria kofuensis* comb. nov., respectively.



◘ **Fig. 43.2**
***Streptosporangium album* S16. Walls (membranes) of spore vesicles are thin (Reproduced with permission from Hayakawa; from Nonomura 1989b)**



◘ **Fig. 43.3**
***Streptosporangium vulgare* on oatmeal agar containing yeast extract. Sporangiophores are short (Reproduced with permission from Hayakawa; from Nonomura 1989b)**

Streptosporangiae characteristically form an aerial hyphae that carry, on either short or long sporophores, single or clustered spore vesicles that may be up to 40 µm in diameter (◐ *Figs. 43.2* and *43.3*). They have a wall chemotype **III** (Lechevalier and Lechevalier 1970b), that is *meso-A_2pm* (Aly type according to Schleifer and Kandler,1972) as diagnostic amino acid of peptidoglycan and a wall that lacks characteristic

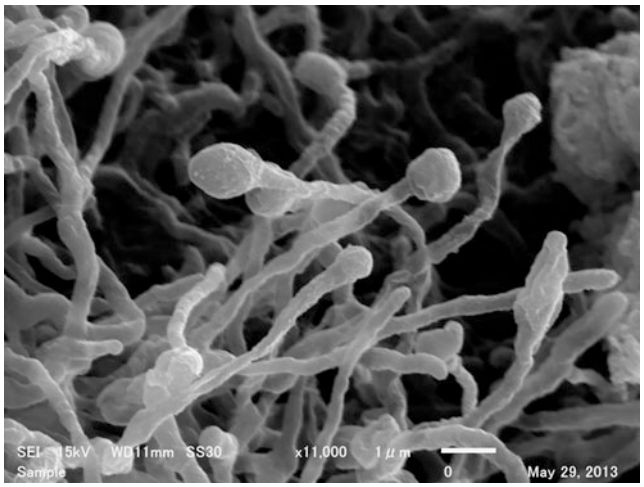


Fig. 43.4
***Acrocarpospora pleiomorpha* NBRC 16267^T on humic acid agar. Spherical or club-shaped structures are borne on the tip of the aerial mycelium (Courtesy of M. Hayakawa)**

sugars other than madurose (3-*O*-methyl-D- galactose; Lechevalier and Gerber 1970) Members of the taxon are rich in *iso*-, *anteiso*-, saturated, unsaturated, and methyl-branched fatty acids (pattern 3c; Kroppenstedt 1985; Kudo et al. 1993; Whitham et al. 1993; Stackebrandt et al. 1994), contain di- and tetrahydrogenated menaquinones with nine isoprene units as predominant isoprenologues (Kroppenstedt 1985; Kudo et al. 1993; Whitham et al. 1993; Stackebrandt et al. 1994), and have phospholipid patterns characterized by glucosamine-containing lipids with phosphatidylethanolamine, diphosphatidylglycerol, and phosphatidylinositol (Lechevalier el al., 1977; Lechevalier et al. 1981b; Kudo et al. 1993; Whitham et al. 1993; Stackebrandt et al. 1994). The G+C content of the DNA is 69-71 moI% (Tsyganov et al. 1966; Yamaguichi 1967; Farina and Bradley 1970; Stackebrandt et al. 1994). Type species: *Streptosporangium roseum* (Couch 1955a), 151^{AL}. Type strain: ATCC 12428^T = DSM 43021^T.

Acrocarpospora Tamura et al. 2000, 1170^{AL}

A.cro.car.po.spo'ra. Gr. adj. *akros*, uppermost, topmost, highest, at the top, end; Gr. n. *karpos*, fruit; Gr. fem. n. *spora*, a seed, and in biology a spore; N.L. fem. n. *Acrocarpospora*, an organism forming spores like fruits on the terminal mycelium.

This aerobic, Gram-positive, non-acid-alcohol-fast, nonmotile actinomycete forms a stable, branched substrate mycelium. Spherical and club-shaped structures, which contain coiled chains of smooth-surfaced, oval or rod-like spores (0.6–0.8 × 0.7–1.0 μm in diameter), are carried on the tips of aerial hyphae (*Fig. 43.4*). The organism grows well on oatmeal agar and at 20–30 °C. White aerial hyphae and pale yellow substrate mycelium, but no diffusible pigments, are formed on most standard growth media. Cell walls contain alanine, glutamic acid, and *meso*-A_2pm and *N*-acetylated muramic acid. The peptidoglycan is of the Alγ type. Strains contain di- and tetrahydrogenated menaquinones with nine isoprene units as predominant isoprenologues, glucose and madurose as major sugars, phosphatidylethanolamine as the diagnostic phospholipid, and *iso*- $C_{16:0j}$ 10-methyl $C_{17:0}$, $C_{17:0}$ and C_{17} as major fatty acids, but lack mycolic acids. The G+C content of the DNA is 68–69 mol%. Type species: *Acrocarpospora pleiomorpha* (Tamura et al. 2000), 1170^{AL}. Type strain: DSM 44706^T = NBRC 16267[7].

In addition to the type species, there are two additional species, *A. corrugata* and *A. macrocephala*. The former was originally classified as *Streptosporangium corrugatum* (Williams and Sharpies 1976). From the 16S rRNA *Streptosporangiaceae* gene tree (*Fig. 43.1*), the representatives of the *Acrocarpospora* species are apparently closely related to one another and to the type strains of *Herbidospora cretacea, Planotetraspora mira*, and *Planotetraspora silvatica.* However, the *Acrocarpospora* strains can be distinguished from *Herbidospora cretacea* using morphological and menaquinone data and from the genus *Planotetraspora* using morphological properties and whole-organism sugar composition.

Herbidospora Kudo et al. 1993, 319^{AL}

Her.bi.do.spo'ra. L. adj. *herbidus*, full of grass, grassy; Gr. n. *spora*, a seed and in biology a spore; N.L. fem. n. *Herbidospora*, organism forming spores like grass.

This aerobic mesophilic actinomycete forms a stable, branched substrate mycelium, but does not produce true aerial hyphae. Straight chains of nonmotile, smooth-surfaced spores (10–30 per chain) are borne at tips of sporophores branching in clusters from the vegetative mycelia. The substrate mycelia are yellow to brown on most media; distinctive exopigments are not formed. When sporulation occurs, the surface of the colony is white or brownish yellow. Thiamine is required for growth. The organism is susceptible to lysozyme. Cell walls contain *meso*-A_2pm and acetylated muramic acid but lack significant amounts of glycine. Whole-organism hydrolysates contain glucose, mannose, ribose, and a trace of madurose. Strains contain major amounts of iso-hexadecanoic, n-hexadecanoic, n-heptadecanoic, 10-methylheptadecanoic, and 2-hydroxy acids, phosphatidylethanolamine and glucosamine-containing phospholipids as diagnostic polar lipids, and major proportions of tetrahydrogenated menaquinones with ten isoprene units with hydrogenation at units III and IX (MK-10 [III, IX-H_4]), but lack mycolic acids. The G+C content of the DNA is 69–71 mol%. Type species: *Herbidospora cretacea* (Kudo et al. 1993). Type strain: DSM 44071^T = JCM 8553^T.

According to (Tamura et al. 2000) and to (Boondaeng et al. 2011), *Streptosporangium claviforme* (Petrolini et al. 1992) is a later heterotypic synonym of *Herbidospora cretacea* (Kudo et al. 1993).

Microbispora Nnomura and Ohara 1957, 307[AL]

Mi.cro.bi.spo'ra. Gr. adj. *mikros*, small; L. adv. num. *bis*, twice; Gr. n. *spora*, a seed and in biology a spore; N.L. fem. n. *Microbispora*, the small two-spored (organism).

This taxon was proposed for actinomycetes that form conspicuous aerial hyphae bearing longitudinal pairs of spores. It currently contains ten validly described species, excluding *Microbispora echinospora* (Nonomura and Ohara 1971b) and *Microbispora viridis* (Miyadoh et al. 1985), which have been reclassified as *Actinomadura rugatobispora* (Miyadoh et al. 1985, 1990) and *Actinomadura viridis* (Miyadoh et al. 1985, 1990), respectively. *Microbispora bispora* (Lechevalier 1965), which was originally described as *Thermopoly spor a bispora* (Henssen 1957), has been transferred to a new genus, *Thermobispora*, as *Thermobispora bispora* (Henssen 1957; Wang et al. 1996a). *Thermomonospora mesophilica* (Nonomura and Ohara 1971b), which forms single spores, has been reclassified as *Microbispora mesophila* (Nonomura and Ohara 1971b; Zhang et al. 1998).

Miyadoh et al. (1990) undertook a radical revision of the genus *Microbispora* in which they proposed *M. amethystogenes, M. chromogenes, M, diastatica, M. indica, M. kamatakensis*, and *M. rosea* to be assigned to a single taxon as *M. rosea* subsp. *rosea* and that *M. aerata, M. thermodiastatica*, and M. *thermorosea* be combined and recognized as *M. rosea* subsp. *aerata*. These proposals were based on DNA-DNA relatedness data, though it was acknowledged that most of the cut-off points used in the circumscription of the two taxa were below the 70 % guideline recommended for the delineation of genomic species (Wayne et al. 1987). However, *M. indica* ATCC 35926^T shared 83 % DNA relatedness with *M. rosea* JCM 3006^T; the corresponding number between the type strains of *M. diastatica* and *M. kamatakensis* was 91 %. In addition, Boondaneng et al. (2009) showed that *M. amethystogenes* was a separate genomic species from *M. rosea* subsp. *rosea based on* DNA-DNA relatedness values in reciprocal hybridization experiments. A combination of genotypic and phenotypic data supported the classification of *M. amethystogenes* as a separate species.

Microbispora species form a relatively distinct monophyletic group in the 16S rRNA gene tree, with most of the type strains forming distinct phyletic lines (*Fig. 43.1*). From the 16S rRNA gene tree, the taxon is apparently closely related to the genus *Microtetraspora* and *Planotetraspora*.

Microbisporae are aerobic, Gram-positive, nonmotile actinomycetes which typically form a conspicuous aerial mycelium bearing longitudinal pairs of spores (*Fig. 43.5*) that may be closely arranged along the aerial hyphae, giving the appearance of catkins; spores are not usually formed on the substrate mycelium. In some cases, the spores are borne at longer intervals (*Fig. 43.6*). They first appear as club-shaped initials that later become transformed into the paired spores visible under the light microscope. Spores are either sessile or on short sporophores, spherical to oval (usually 1.2–1.6 µm in diameter) with smooth surfaces. Mature spores are easily detached from the sporophores and each other when placed in water. B vitamins, particularly thiamine, are essential for growth on synthetic media.

Mesophilic and thermophilic species have been described. Mesophilic strains generally produce a pale yellow to distinct pink aerial spore mass, and the reverse side of the colonies is yellowish-brown to orange. Thermophilic strains form a white or pale yellowish-brown to pale pinkish-brown aerial spore mass; the reverse side of colonies is either pale yellowish-brown or yellow brown. Cell walls contain *N*-acetylated muramic acid and major amounts of meso-A_2pm but no characteristic sugars. Madurose is present in whole-organism hydrolysates. The organism contains tetrahydrogenated menaquinone with nine isoprene units as the predominant isoprenologue, phosphatidylcholine, and unknown glucosamine-containing components (such as diagnostic polar lipids, tuberculostearic acid, and its analogues), but not mycolic acids. The G+C content of the DNA is 71–73 mol%. Type species: *Microbispora rosea* (Nonomura and Ohara 1957), 307[AL]. Type strain: DSM 43839^T = JCM 3006T.

Microtetraspora Thiemann et al. 1986b, 296[AL]

Mi.cro.te.tra.spo'ra. Gr. adj. *mikros*, small; Gr. adj. *tetra*, four; Gr. n. *spora*, a seed and in biology a spore; N.L. fem. n. *Microtetraspora*, the small four-spored (organism).

This genus was proposed for actinomycetes that form short, sparsely branched aerial hyphae bearing chains of four spores (*Fig. 43.7*). This morphological trait was considered typical of the genus though chains of two or three spores, and more rarely five spores, have been reported. Initially, four species were recognized, the type species *Microtetraspora glauca*, *M. fusca*, *M. niveoalba*, and *M. viridis*; the latter has been reclassified as *Actinomadura viridis* (Nonomura and Ohara 1971a; Miyadoh et al. 1989). The taxon provided a temporary refuge for the *Actinomadura pusilla* group (Fischer et al. 1983; Poschner et al. 1985; Goodfellow et al. 1988; Kroppenstedt et al. 1990) until it became clear that members of this taxon and the three bona fide *Microtetraspora* species mentioned above can be distinguished using numerical taxonomic (Athalye et al. 1985), electrophoretic mobility of ribosomal AT-L30 protein (Ochi et al. 1991, 1993), and 16S rRNA gene sequence (Wang et al. 1996b) data. The *A. pusilla* group was subsequently classified in a new taxon, the genus *Nonomuraea* (Zhang et al. 1998). "*Microtetraspora tyrrkensis*" was proposed by Tomita et al. (1991) for an organism that formed hooked or spiral spore chains and other properties consistent with its assignment to the *A, pusilla* group. This organism probably belongs to the genus *Nonomuraea*, though the type strain is no longer available to test this proposition. An additional species, *M. malaysiensis*, has been described for strains isolated from a primary dipterocarp forest soil (Nakajima et al. 2003).

Microtetrasporae are aerobic, Gram-positive, non-acid-alcohol-fast, mesophilic, nonmotile actinomycetes which form stable, highly branched substrate and aerial mycelia. Spore chains, typically containing four spores, are borne exclusively

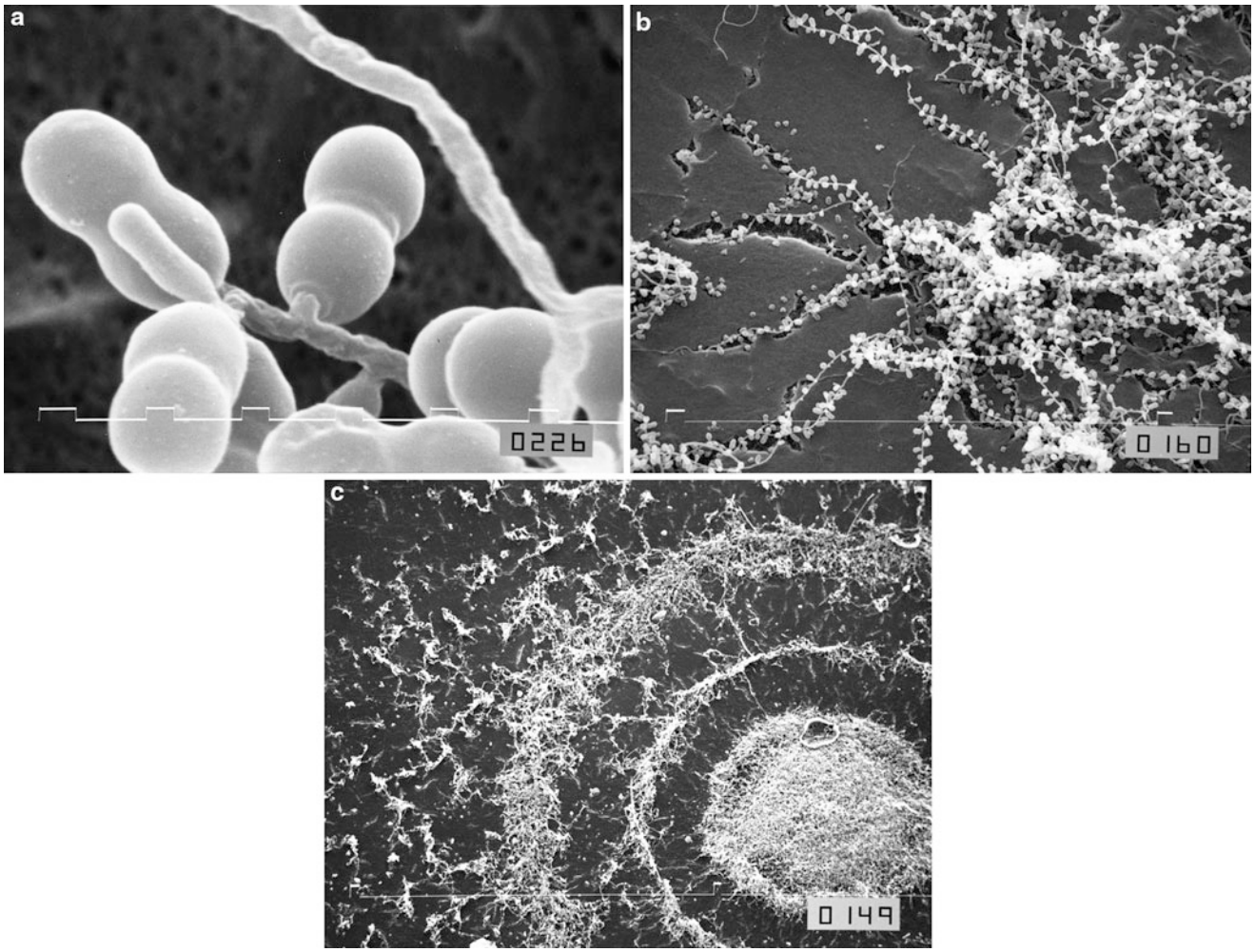


Fig. 43.5
Microbispora rosea **M20 on humic acid agar. (a) Paired spores on hyphae. (b) Spores on entire mycelium. (c) Fairy ring (Courtesy of M. Hayakawa)**

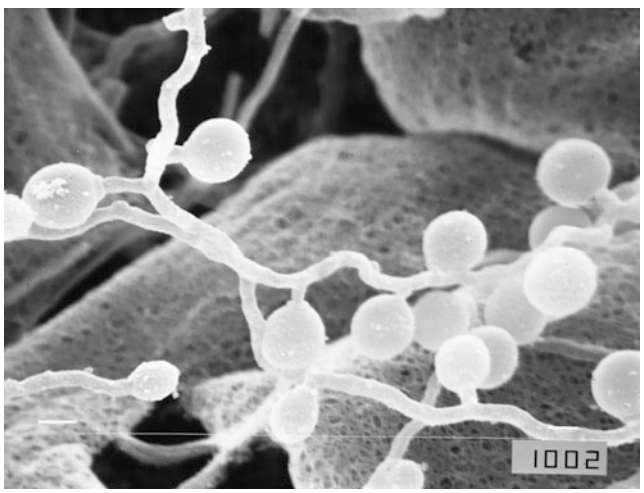


Fig. 43.6
Microbispora mesophila **T1 on humic acid-vitamin (*HV*) agar. Single spores formed on the aerial hyphae (Courtesy of M. Hayakawa)**

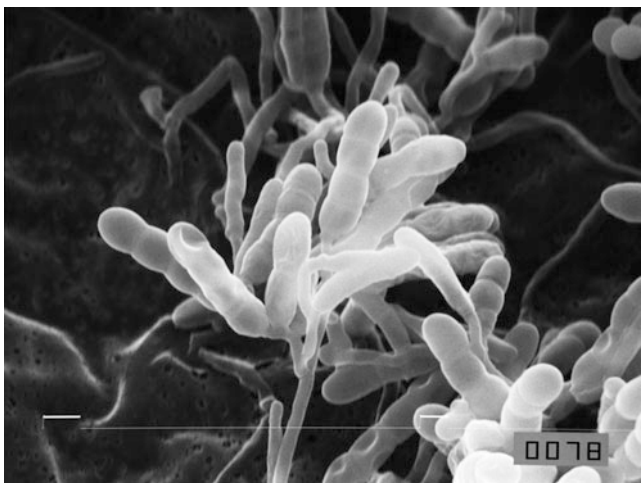


Fig. 43.7
Microtetraspora glauca **on oatmeal agar. Four spore chains formed on aerial hyphae (Reproduced with permission from Hayakawa; from Nonomura 1989b)**

on short aerial hyphae. Spores are spherical (1.2–1.5 µm in diameter) or oval to short cylindrical (1.0–1.4 to 1.2–1.7 µm in diameter) and have smooth surfaces. Some species require B vitamins for growth. The organism is chemoorganotrophic, having an oxidative type of metabolism. It grows well at 20–37 °C. Cell walls contain *N*-acetylated muramic acid and major amounts of meso-A_2pm but no characteristic sugars. Madurose is present in whole-organism hydrolysates. The organism contains major proportions of menaquinone with nine isoprene units with hydrogenation at units III and IV (MK-9 [III, IV-[H_4]), phosphatidylcholine and unknown glucosamine-containing lipids are the major polar lipids. The G+C content of the DNA is 69–71 mol%. Type species: *M. glauca* Thiemann et al. 1968b, 296^{AL}. Type strain: ATCC 23057^T = DSM 43311^T.

Nonomuraea Zhang et al. 1998, 419^{AL}

No.no.mu.ra.e'a. N.L. fem. n. *Nonomuraea*, named after Hideo Nonomura, a Japanese taxonomist of actinomycetes.

This taxon was introduced to accommodate the species assigned to the *M. pusilla* group (Fischer et al. 1983; Poschner et al. 1985; Goodfellow et al. 1988; Kroppenstedt et al. 1990). The genus encompasses aerobic, Gram-positive, non-acid-alcohol-fast strains that form extensively branched substrate and aerial hyphae. The latter bear chains of spores that may be hooked, spiral, straight, or enmeshed in pseudovesicles (Figs. 43.8 and 43.9). Spore surfaces may be folded, irregular, smooth, or warty. The growth temperature range is 20–45 °C, and some strains grow up to 55 °C. Cell walls contain *meso*-A_2pm, and madurose is present in whole-organism hydrolysates (cell wall type III/B *sensu* Lechevalier and Lechevalier 1970b). The predominant menaquinones are MK-9 [H_0, H_2, H_4], the phospholipid pattern is characterized by glucosamine-containing lipids with phosphatidylethanolamine variable, phosphatidylmethylethanolamine, diphosphatidylglycerol, and phosphatidylinositol (phospholipid type IV *sensu* Lechevalier et al. 1977), and the predominant fatty acids are 10- methyl-17-, and iso-16-branched components (pattern 3c; Kroppenstedt 1985). The G+C content of the DNA is 64–69 mol%. Type species: *Nonomuraea pusilla* (Zhang et al. 1998), 419^{AL}, Type strain: ATCC 27296^T-DSM 43357^T.

The genus *Nonomuraea* contains 27 validly described species and two subspecies, namely, *N. africana* (Preobrazhenskaya and Sveshnikova 1974; Zhang et al. 1998), *N. angiospora* (Zhukova et al. 1968; Zhang et al. 1998), *N. antimicrobica* (Qin et al. 2009), *N. bangladeshensis* Ara et al. 2007a, *N. candida* le Roes and Meyers 2008, *N. coxensis* Ara et al. 2007a, *N. dietziae* (Stackebrandt et al. 2001), *N. endophytica* (Li et al. 2011), *N. fastidiosa* (Soina et al. 1975; Zhang et al. 1998), *N. ferruginea* (Meyer 1981; Zhang et al. 1998), *N. helvata* (Nonomura and Ohara 1971c; Zhang et al. 1998), *N. kuesteri* (Kämpfer et al. 2005), *N. longicatena* (Chiba et al. 1999; Zhang et al. 1998), *N. maheshkhaliensis* Ara et al. 2007b, *N. maritima* (Xi et al. 2011), *N. polychroma* (Galatenko et al. 1981; Zhang et al. 1998), *N. pusilla* (Nonomura and Ohara 1971c; Zhang et al. 1998), *N. recticatena* (Gauze et al. 1984; Zhang et al. 1998), *N. rhizophila* (Zhao et al. 2011), *N. rosea* (Kämpfer et al. 2010), *N. roseola* (Lavrova and Preobrazhenskaya 1975; Zhang et al. 1998), *N. roseoviolacea* subsp. *carminata* (Gauze et al. 1973; Gyobu and Miyadoh 2001), *N. roseoviolacea* subsp. *roseoviolacea* (Nonomura and Ohara 1971c; Zhang et al. 1998), *N. rubra* (Sveshnikova et al. 1969; Zhang et al. 1998), *N. salmonea* (Preobrazhenskaya et al. 1975; Zhang et al. 1998), *N. spiralis* (Meyer 1981; Zhang et al. 1998)., *N. turkmeniaca* (Terekhova et al. 1982; Zhang et al. 1998), and *N. wanchangensis* (Wang et al. 2011).

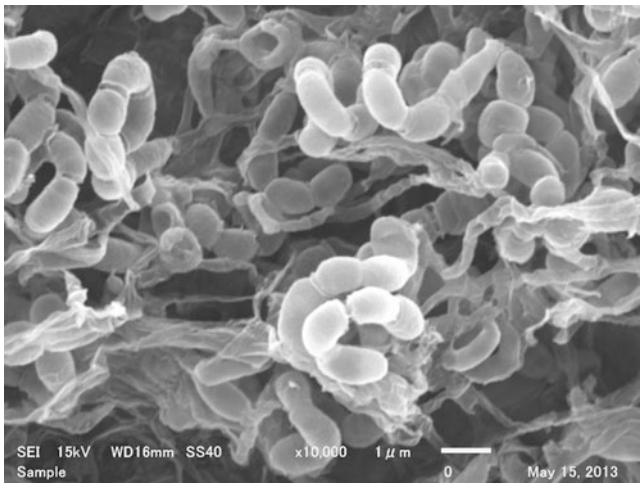


Fig. 43.8
***Nonomuraea ferruginea* NBRC 14094^T on oatmeal agar. Aerial mycelia bear spiral spore chains (Courtesy of M. Hayakawa)**

Fig. 43.9
***Nonomuraea roseola* NBRC 14685^T on oatmeal agar. Aerial mycelia bear straight spore chains (Courtesy of M. Hayakawa)**

Planobispora Thiemann and Beretta 1968a, 157[AL]

Pla.no.bi.spo'ra. Gr. n. *planos*, wanderer; L. adv. num. *bis*, twice (double); Gr. fem. n. *spora*, a seed, and in biology a spore; N.L. fem. n. *Planobispora*, a motile, double-spored organism.

This taxon encompasses aerobic, Gram-positive, non-acid-alcohol-fast, chemoorganotrophic actinomycetes that form irregular branched, occasionally septate, substrate hyphae (0.5–1.0 μm in diameter) and sparsely branched, rarely septate aerial hyphae (1 μm in diameter) (*Table 43.9*). Cylindrical to clavate spore vesicles (1.0–1.2 μm wide, 6.0–8.0 μm long), each containing a longitudinal pair of spores, are formed singly or in bundles on short ramifications of the aerial hyphae. Spores are straight or slightly curved with rounded ends (1.0–1.2 μm in length) and are motile by means of peritrichous flagella. The spores are pushed out of opposite ends of the spore vesicle, which is easily detached from the supporting hyphae; only a small percentage of spores show motility. They are only motile after being dispersed for some time and usually germinate with one or two polar germ tubes.

It is still unclear whether spores are formed endogenously (Williams and Wellington 1980) or by simple transformation of sporangeous hyphae (Bland and Couch 1981). A transverse septum or diaphragm connected to the vesicular envelope divides the two spores (Thiemann 1970; Vobis and Kothe 1985; *Fig. 43.10*). The vesicular envelope is smooth and contains fibrillar elements (Vobis and Kothe 1985) that resemble those present in *Planomonospora* (Sharpies et al. 1974). The type strains of *P. longispora* and *P. rosea* have many phenotypic properties in common; some of these distinguish them from other sporoactinomycetes with a wall chemotype III (Goodfellow and Pirouz 1982).

The substrate mycelium of planobisporae is either without distinctive color or rose-colored. The aerial mycelium, which develops only on certain agar media, is white or has a light rose tinge. Good growth occurs at pH 6.0–9.0 and temperature 28–40 °C but not 20 °C or 45 °C. Cell walls contain meso-A_2pm and madurose is the characteristic whole-organism sugar. Planobisporae contain diphosphatidylglycerol, phosphatidylethanolamine, and unknown glucosamine-containing phospholipids as diagnostic polar lipids, major amounts of straight chain, unsaturated, *iso*- and 10-methyl branched fatty acids, and tetrahydrogenated menaquinone with nine isoprene units (MK-9 [III, IV-H_4) as the predominant isoprenologue). The G+C content of the DNA is 70–71 mol%. Type species: *Planobispora longispora* (Thiemann and Beretta 1968a), 157[AL]. Type strain: DSM 43041^T = JCM 3092^T

The genus contains an additional species, namely, *Planobispora rosea* (Thiemann 1970).

Planomonospora Thiemann et al. 1967, 29[AL]

Pla.no.mo.no.spo'ra. Gr. n. *planos*, wanderer, vagabond; Gr. adj. *monos*, solitary, single; Gr. fem. n. *spora*, a seed, and in biology a spore; N.L. fem. n. *Planomonospora*, a motile, single spored organism.

This taxon was proposed for actinomycetes that form cylindrical or clavate spore vesicles, each containing a single spore, on aerial hyphae. In the type species *Planomonospora parontospora*, the spore vesicles are sessile and occur in double parallel rows on curved sporangiophores (*Fig. 43.11*) (*Table 43.9*). A single sporangiophore can bear up to 60 spore vesicles. In the other founder member of the taxon, *P. venezuelensis*, the spore vesicles are developed singly or in groups on short lateral branches forming a characteristic palm leaf pattern (Thiemann 1970). The spores may be formed endogenously (Sharpies et al. 1974), but in the *P. parontospora* spore vesicle, development begins with the growth of a sporangeous hypha inside a thin expanding sheath (Vobis 1985; Vobis and Kothe 1985). Through thickening, the sheath becomes a massive vesicular envelope. The spores, which are released through apical pores, become motile by peritrichous flagella about 30 min after being expelled. They remain motile for up to a day during which time spore germination may begin (Thiemann 1970). The type strains of *P. parontospora* and *P. venezuelensis* were assigned to a distinct duster in an extensive numerical taxonomic analysis of sporoactinomycetes with a wall chemotype III (Goodfellow and Pirouz 1982).

The genus *Planomonospora* contains five validly described taxa, namely, *Planomonospora alba* (Mertz 1994), *Planomonospora parontospora* subsp. *antibiotica* (Thiemann et al. 1967), *Planomonospora parontospora* subsp. *parontospora* (Thiemann 1967), *Planomonospora sphaerica* (Mertz 1994), and *Planomonospora venezuelensis* (Thiemann 1970). The type strains of *P. alba* and *P. sphaerica* form spore vesicles in long parallel rows, which resemble rows of bananas (Mertz 1994). *Planomonospora sphaerica* strains form large spherical bodies when grown on inorganic salt starch agar (ISP medium 4; Shirling and Gottlieb 1966).

Planomonosporae are aerobic, Gram-positive, non-acid-alcohol-fast, chemoorganotrophic actinomycetes that form branched, occasionally septate, nonfragmenting substrate hyphae (0.6–1.0 μm in diameter) and sparsely branched rarely septate aerial hyphae (0.5–1.0 μm in diameter). Cylindrical to clavate spore vesicles (1.0–1.5 μm wide, 3.5–5.5 μm long), each containing a single spore, are formed on the aerial mycelium. Spores are fusiform or cylindrical to clavate and motile by peritrichous flagella; they are 1.0–1.6 μm in diameter and 3.5–5.4 μm in length. Colonies are flat or elevated with smooth surfaces, occasionally wrinkled or slightly crustose. Substrate mycelia show a range of colors, including light orange, brown violet to light brown, and grayish yellow. Aerial mycelia are white with a rose tinge, grayish white or pink. Growth occurs at 20–50 °C.

Cell walls contain *meso*-A_2pm. Variation is found in menaquinone, sugar, and polar lipid composition. *Planomonospora alba* and *P. sphaerica* strains contain diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, and glucosamine-containing phospholipids and galactose, madurose, and xylose as whole-organism sugars (Mertz 1994), In contrast,

Table 43.9
Characteristics distinguishing the type strains of *Streptosporangium* species

	S. album DSM 43023^T	*S. amethystogenes* DSM 43179^T	*S. canum* DSM 45034^T	*S. carneum* NRRL 18437^T	*S. fragile* DSM 43847^T	*S. longisporum* DSM 43180^T	*S. nondiastaticum* DSM 43848^T	*S. oxazolinicm* JCM 17388^T	*S. pseudovulgare* DSM 4318^T	*S. purpuratum* DSM 44688^T	*S. roseum* DSM 43021^T	*S. subroseum* DSM 44662^T	*S. violaceochromogenes* DSM 43849^T	*S. viridialbum* DSM 43801^T	*S. vulgare* DSM 43802^T	*S. yunnanense* DSM 44663^T
Morphology on ISP 3																
Color of substrate mycelium																
Brown-black	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
Red-orange	–	–	+	–	–	+	+	+	+	+	+	–	–	–	+	–
Yellowish-brown to brown	+	+	–	+	–	–	+	–	+	–	+	+	+	+	+	+
Color of aerial spore mass																
Greenish-gray	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–
Pink	–	+	–	+	+	+	+	–	+	+	+	+	+	–	+	+
White	+	–	+	–	–	–	–	+	–	+	–	–	–	–	–	–
Spore vesicle size (μm)																
1–5	–	–	+	–	–	–	–	–	–	+	–	+	–	–	–	+
6–10	+	+	+	–	+	+	–	+	+	-	+	-	+	+	+	+
11–20	–	–	–	–	+	+	+	–	–	–	(+)	–	–	–	–	+
21–30	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
31–50	–	–	–	(+)	–	–	–	–	–	–	–	–	–	–	–	–
Sporangiophore size (mm)																
Short (10)	+	+	ND	–	+	+	+	ND	+	+	+	+	+	+	+	+
Long (50)	–	–	ND	+	–	–	–	ND	–	–	–	–	–	–	–	–
Spore shape																
Spherical to clavate	+	+	–	+	+	+	+	ND	+	+	+	+	+	+	+	+
Rod-like	–	–	+	–	–	–	–	ND	–	–	–	–	–	–	–	–
Soluble pigments																
Other than pale yellow-brown	–	–	–	–	+	–	–	+	–	–	+	–	+	–	–	+
B vitamins required	+	+	+	–	–	–	+	ND	+	–	+	–	–	+	+	–

Growth at																
42 °C	–	–	ND	–	+	–	+	–	+	+	–	+	–	–	–	+
50 °C	–	–	ND	–	–	–	–	–	–	+	–	–	–	–	–	–
Biochemical test																
Nitrate reduction	–	+	+	–	+	(+)	+	-	+	-	+	+	+	d	-	+
Degradation tests																
Gelatin hydrolysis	+	-	+	–	–	ND	+	(+)	+	–	+	ND	(+)	d	d	+
Iodinin production	–	+	ND	–	–	–	–	ND	–	+	–	–	–	–	–	–
Starch hydrolysis	–	+	–	–	+	+	–	+	+	+	+	–	+	+	+	+
Sole carbon source utilization (1 % w/v)																
Adonitol	+	+	ND	–	–	+	+	ND	+	ND	+	ND	ND	–	+	ND
L(+)-Arabinose	+	ND	+	–	+	+	+	–	+	–	+	+	ND	–	+	–
D(+)-Galactose	+	–	–	+	+	–	+	ND	–	–	–	+	ND	–	+	–
Glycerol	–	ND		–	–	–	–	ND	+	ND	+	ND	ND	–	+	ND
meso-Inositol	–	+	+	–	–	–	–	–	–	–	–	–	(+)	+	+	–
D(+)-Mannitol	+	ND	+	–	+	–	+	+	+	–	+	+	ND	+	–	–
L(+)- Rhamnose	–	+	+	–	+	–	–	–	–	–	–	+	(+)	+	+	–
D(+)-Turanose	+	ND	ND	ND	+	–	+	ND	+	ND	+	ND	ND	+	+	ND

From Nonomura (1989a), Mertz and Yao (1990), Whitham et al. (1993), Zhang et.al. (2002), Zhang et al. (2005), Zhang et al. (2009) and Inahashi et al. (2011)

Symbols and abbreviations: +, positive; (+),weak positive; –, negative; d, doubtful; ND, not determined; and ^{T} type strain; and DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg IB, D-38124 Braunschweig, Germany; JCM, Microbe Division/Japan Collection of Microorganisms RIKEN BioResource Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

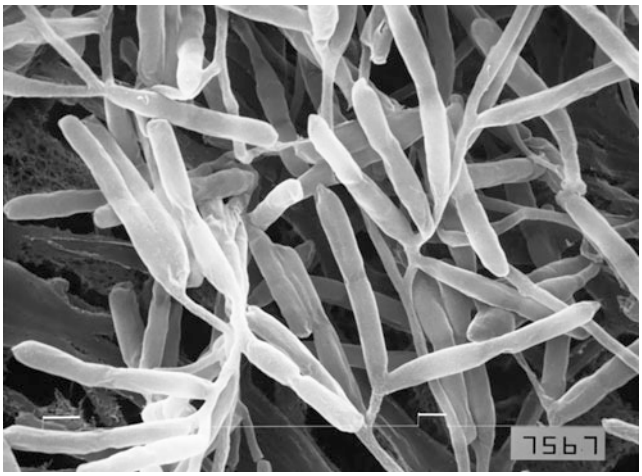


Fig. 43.10
***Planobispora longispora* on oatmeal agar containing soil extract. Longitudinal pair of spores formed in cylindrical sporangia (Courtesy of M. Hayakawa)**

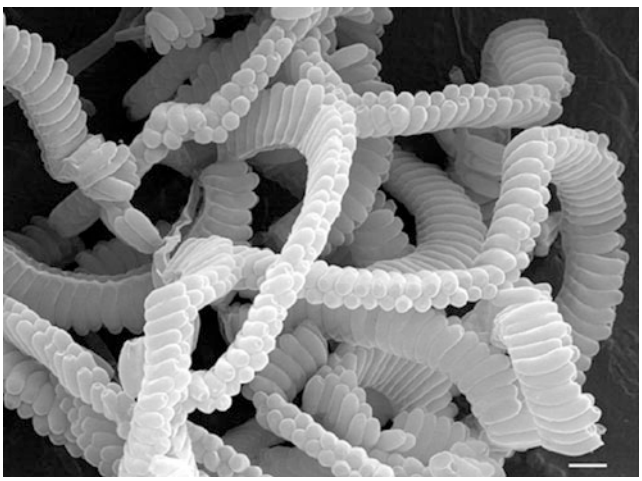


Fig. 43.11
***Planomonospora* sp. Numerous monosporous spore vesicles in double parallel rows (Reproduced with permission from Suzuki; from Suzuki et al. 2001a)**

Planomonospora parontospora and *P. venezuelensis* have madurose as the characteristic sugar (Kroppenstedt and Kutzner 1978); the diagnostic phospholipids of *P. parontospora* consist of diphosphatidylglycerol, lysophosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and unknown glucosamine-containing phospholipids (Hasegawa et al. 1979). Di- and tetrahydrogenated menaquinones with nine isoprene units are the major component in *P. parontospora*, whereas tetrahydrogenated menaquinones with eight isoprene units predominate in P. venezuelensis (Collins et al. 1984); the major component of *P. alba* and *P. sphaerica* is dehydrogenated menaquinone with nine isoprene units (Mertz 1994). Planomonosporae have complex mixtures of straight chains, unsaturated and branched fatty acids but lack mycolic acids. The G+C content of the DNA is 72 mol%. Type species: *Planomonospora parontospora* Tliiemann et al. 1967. Type strain: ATCC 23863^T = DSM 43177^T.

Planotetraspora Runmao et al. 1993, 468^{AL}

Pla.no.te.tra.spo'ra. Gr. n. *planos*, a wanderer; Gr. adj. *tetra*, four; Gr. fem. n. *spora*, a seed and in biology a spore; N.L. fem. n. *Planotetraspora*, a mobile, four-spored organism.

The genus *Planotetraspora* was proposed for an isolate that formed long cylindrical spore vesicles that contained four spores in a single row at the ends of short sporangiophores on aerial hyphae. The organism, *Planotetraspora mira*, was reported to contain *meso*-A_2pm in peptidoglycan and arabinose, galactose, mannose, ribose, and xylose in whole-organism hydrolysates. However, Kudo (2001) found that the strain contained madurose and rhamnose, but not arabinose or xylose in whole-organism hydrolysates. They also noted that it contained tetrahydrogenated menaquinone with nine isoprene units as the predominant isoprenologue and had a type IV phospholipids pattern. Stackebrandt et al. (1997) classified the genus in the family *Streptosporangiaceae*. A second species, *P. silvatica*, has been described for a strain isolated from a soil sample collected on Amami Island, Japan (Tamura and Sakane 2004).

Planotetrasporae are aerobic, Gram-positive, non-acid-fast actinomycetes which form moderate, irregularly branching, stable substrate hyphae (03–6.0 µm width) and sparsely branched, rarely septate aerial hyphae (0.2–0.4 µm in diameter). Long cylindrical spore vesicles are formed at the ends of short sporophores on aerial hyphae, with each spore vesicle (size about 2.1–2.7 µm, 0.6–0.9 µm) containing four spores in a single row. Spores are short, cylindrical, short rod-like or oval elements (0.4–1.4 µm, 0.8–1.5 µm) and may exhibit motility. They are released and become motile by means of single polar flagella when spore vesicles are immersed in water; active movement of the spores begins 30 min after they are released. In general, the vegetative mycelia are pale yellow to white. Good growth occurs at 25–30 °C. Cell walls contain alanine, glutamic acid, and *meso*-A_2pm and *N*-acetylmuramic acid. Galactose, glucose, madurose, 3-0-methylmannose, and rhamnose are found in whole-organism hydrolysates. The predominant isoprenologue is tetrahydrogenated menaquinone with nine isoprene units, phosphatidylethanolamine is the diagnostic phospholipid, and 10-methylated $C_{18:0}$ is the major cellular fatty acid. The G+C content of the DNA is 71 moI%.

Type species: *Planotetraspora mira* (Runmao et al. 1993), 468^{AL}. Type strain: DSM 44359^T = JCM 9131^T.

Sphaerisporangium Ara and Kudo 2007, 2449^{VP}

S.pha.eri.spo.ran.gium. L. n. *sphaera*, sphere; N.L. neut. n. *sporangium*, sporangia; N.L. neut. n. *Sphaerisporangium*, an organism with spherical sporangia.

The genus *Spaherisporangium* was proposed for developing spherical spore vesicle on aerial mycelium containing non-motile spores. The type strain of *Sphaerisporangium melleum* was isolated from a sandy soil collected in a forest side waterfall in Chokoria, Cox's Bazar, Bangladesh (Ara and Kudo 2007).

This taxon contains five validly described species, namely, *S. album* (Cao et al. 2009), *S. cinnabarinum* (Ara and Kudo 2007), *S. flaviroseum* (Cao et al. 2009), *S. krabiense* (Suriyachadkun et al. 2011), *S. rubeum* (Ara and Kudo 2007), and *S. viridialbum* (Ara and Kudo 2007).

Cells are Gram-positive, non-acid-fast, 0.4–0.9 μm wide, and 0.6–1.2 μm long with a smooth, wrinkled, and prominently ridged surface. The substrate hyphae are pale to brown yellow in color, and aerial hyphae are white. A non-fragmenting substrate mycelium was formed. The organisms grow well at 20–37 °C and pH 5–9. No growth occurs at 3 % NaCl. Cell walls contained *meso*- A_2pm and glucose, madurose, and mannose as the major whole-cell sugars. Tetrahydrogenated menaquinone with nine isoprene units is contained as the predominant isoprenologue. Shaerisporangiae contain phosphatidylethanolamine, ninhydrin-positive phospholipids and phosphatidylinositol mannosides as diagnostic polar lipids; major amounts of straight chain, unsaturated, *iso*- and 10-methyl branched fatty acids with small amount of saturated fatty acid, unsaturated fatty acid, anteiso- and 10 methyl fatty acids. The G+C content of the DNA is 67–72 mol%.

Type species: *Sphaerisporangium melleum* (Ara and Kudo 2007). Type strain: JCM 13064^T = DSM 44954^T.

Thermopolyspora Goodfellow et al. 2005, 1980^{VP}

Ther.mo.poly.spo.ra. Gr. n. thermos heat; Gr. adj. poly many; Gr. n. spora a seed; N.L. fem. n. *Thermopolyspora* the heat (-loving) many-spored organism.

The type strain of *Thermopolyspora flexuosa* DSM 41386^T was isolated from soil of the Pamir mountains and expressed the following properties (Goodfellow et al. 2005). Cells are aerobic, Gram-positive, non-acid-alcohol-fast, hooked, or irregular spiral chains of 4–10 warty to spiny spores (Fig. 43.12). The organisms produced 1.2–1.5-μm diameter spores and are arranged in clusters on long, moderately branched aerial hyphae on potato carrot agar. Color of aerial hyphae is light blue and produces a brown soluble pigment on the medium. Growth occurs at 40–60 °C (good growth 45–55 °C) and pH6.0–9.0. Cell wall contains meso-A2pm and *N*-acetylated muramic acid. Strains contain MK9, MK9[H_2], and MK9[H_4] as predominant isoprenologues and minor amounts of MK-9[H_6]; glucose and ribose in whole-organism hydrolysates; as diagnostic phospholipid; and saturated, unsaturated and branched chain components as major fatty acids, but lacks mycolic acids. The G+C content of the DNA is 77 mol%.

Type species: *Thermopolyspora flexuosa* (Goodfellow et al. 2005). Type strain: DSM 41386^T = NRRL B-24348^T.

Thermopolyspora flexuosa was proposed by Krassilnikov and Agre (1964) for a group of thermophilic actinomycetes that formed short chains of spores on short sporophores. The species was subsequently associated with a number of other genera including *Nocardia* (Becker et al. 1965; Lechevalier et al. 1966) *Actinomadura* (Cross and Goodfellow 1973; Lacey et al. 1978), *Micropolyspora* (Krassilnikov et al. 1968), *Microtetraspora* (Kroppenstedt et al. 1990), and latterly *Nonomuraea* (Zhang et al. 1998). Goodfellow et al. (2005) revived the genus Thermopolyspora according to the PCR product characteristic of this taxon using a set of the family-specific oligonucleotide primers (Monciardini et al. 2002).

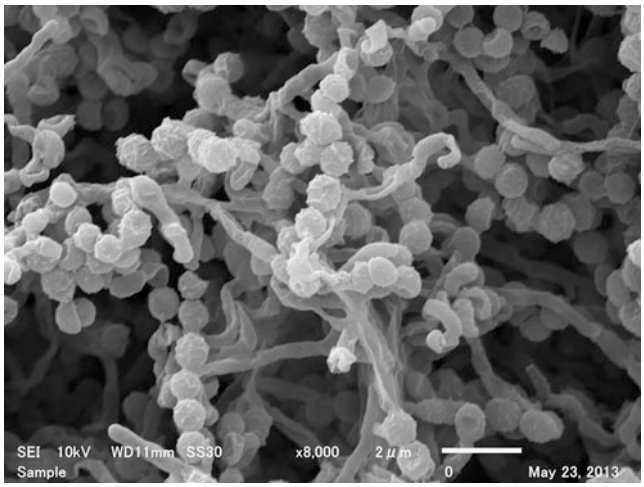


Fig. 43.12
***Thermopolyspora flexuosa* NBRC 14349^T on oatmeal agar. The spore surfaces are warty (Courtesy of M. Hayakawa)**

Sinosporangium Zhang et al. 2011, 596^{AL}

Si.no.spo.ran′gi.um. M.L. n. *sina* China; N.L. n. *sporangium* from Gr. n. *spora* a seed and, in biology, a spore and Gr. n. *angeion* (Latin transliteration *angium*) vessel, sporangium; N.L. neut. n. *Sinosporangium* an organism isolated in China, bearing sporangia.

This taxon proposed for producing globose sporangia originated from borne on aerial mycelia. The sporangia contained coiled spore chains.

The type strain of *Sinosporangium album* was isolated from a soil sample collected from Qinghai province, northwest China. Cells are Gram-positive, aerobic, nonmotile, and form elementary branching hyphae and abundant aerial mycelia with globose sporangia on ISP 4 and R2A media. The sporangia were a mean size of 2.8–3.0 μm × 3.4–4.2 μm and contained coiled spore chains. Spore surface is smooth. A cylindrical spore size is about 0.5–0.6-μm width × 0.6–1.2-μm long. Growth occur at pH 6.5–8.0 and 10–37 °C (optimum growth is 28–32 °C). Whole-cell hydrolysates contained ribose, galactose, and arabinose. Cell wall contains meso-A2pm. Phosphatidylmethylethanolamine, hydroxyl-phosphatidylethanolamine,

N-acetylglucosamine-containing phospholipids, two unknown phospholipids, and an unknown glycolipid are comprised as diagnostic phospholipids.

Strain contains saturated, *iso*- and 10-methyl branched fatty acids, and di and tetrahydrogenated menaquinone, with nine isoprene units (MK-9 [H_2, H_4]) as the predominant isoprenologue. The G+C content of the DNA is 69.4 mol%.

Genus-specific diagnostic nucleotide signature pattern of 16S rRNA is 600:638 (U-G), 658:748 (C-G), 990:1215 (U-G), 1012:1017 (G-U), and 1263: 1272 (G-C).

Type species: *Sinosporangium album* (Zhang et al. 2011), 596^{AL}.Type strain: DSM 45181^T = KCTC 19655^T

Because the phylogenetic position of the genus *Sinosporangium* has yet not been settled, this genus is included for convenience in the family *Streptosporangiaceae* (*Fig. 43.1*).

Isolation, Enrichment, and Maintenance Procedures

Isolation and Enrichment

Several strategies have been developed to facilitate the isolation, enumeration, and identification of "rare" and uncommon actinomycetes (Goodfellow and Williams 1986; Labeda and Shearer 1990; Kurtböke 1990; Hayakawa 2008). The design and application of new selective isolation procedures showed that some actinomycetes previously thought to be rare are common and widely distributed in natural habitats, as shown by procedures found to be effective for the isolation of actinomadurae (Athalye et al. 1981; Trujillo and Goodfellow 2003), actinoplanetes (Takizawa et al. 1993; Hayakawa et al. 2000), nocardiae (Yamamura et al. 2003), planomonosporae (Suzuki et al. 2001a), and planobisporae (Suzuki et al. 2001b). The isolation of "rare" and uncommon actinomycetes can be promoted by using baiting procedures and by pretreatment or enrichment of environmental samples prior to plating onto selective isolation media (Williams et al. 1984; Goodfellow and O'Donnell 1989). Baiting procedures have been successfully used to isolate planobisporae, planomonosporae, and streptosporangiae from environmental samples (*Table 43.5*). It can also be seen from this Table that a variety of pretreatment and selective media have been recommended for the isolation of members of the family *Streptosporangiaceae.* Hayakawa and Nonomura (1987a) found that media containing humic acid were more effective for the isolation of members of the family *Streptosporangiaceae* than those containing carbon and nitrogen sources such as asparagine, casein, chitin, glycerol, and starch.

The incorporation of antibiotics into isolation media is one of the most important approaches used to isolate members of the family *Streptosporangiaceae* from environmental samples (*Table 43.5*). The antifungal antibiotics cycloheximide and nystatin are routinely used to supplement media designed to be selective for actinomycetes in order to eliminate or control the growth of fungi on isolation plates (Williams and Davies 1965; Labeda and Shearer 1990; Sembiring et al. 2000). Media supplemented with antibacterial antibiotics have been used to good effect for the isolation of members of the family *Streptosporangiaceae* though it is well known that actinomycete counts, as well as those of unwanted bacteria, may be reduced (Williams and Davies 1965; Hayakawa et al. 1996).

The selectivity of isolation media is influenced not only by nutrient composition and selective inhibitors but also by the pH of media and by incubation conditions, notably temperature regimes (Williams et al. 1984; Nonomura and Hayakawa 1988; Hayakawa et al. 1996). The isolation of actinomycetes can also be influenced by the gelling agent used to prepare the isolation media. Suzuki et al. (1998, 1999, 2001a, b) showed that the incorporation of gellan gum, a polysaccharide produced by "*Pseudomonas elodea*," into selective media improves the isolation of "rare" and uncommon actinomycetes, such as members of the genera *Actinobispora*, *Planobispora*, *Planomonospora*, and *Sporichthya.* Suzuki et al. (2001a, b) isolated *Planomonospora parontospora* strains from 131 out of 1,200 soil samples (10.9 %) and *Planomonospora venezuelensis* strains from 13 of these soil samples (1.1 %). It is clear from this study that planomonosporae are widely distributed in soil albeit in relatively low numbers. In the present study, a procedure slightly modified from that of Suzuki et al. (2001a, b) was used to try and isolate members of the genera *Nonomuraea* and *Streptosporangium* from environmental samples.

Information was not provided on the selective isolation procedures used for *Acrocarpospora*, *Planotetraspora*, *Sinosporangium*, *Sphaerisporangium*, and *Thermopolyspora* (Tamura et al. 2000; Runmao et al. 1993; Zhang et al. 2011; Goodfellow et al. 2005). The type strain of *A. corrugata* (previously *Streptosporangium corrugatum*) was isolated on starch casein agar supplemented with antifungal antibiotics following inoculation with a suspension of beach sand (Williams and Sharples 1976). Similarly, *Herbidospora cretacea* strains have been isolated by plating soil suspensions onto yeast extract-starch agar (Kudo et al. 1993) and humic acid-vitamin agar (Hayakawa and Nonomura 1987b). Members of this species have been isolated on yeast extract agar supplemented with antifungal antibiotics, and plant material which had been desiccated at 28 °C for at least a week prior to being ground with a blender following the addition of sterile water; the resultant plates were incubated at 28 °C for 2 weeks (Kudo et al. 1993). The type strain of *Planotetrasopra silvatica* (Tamura and Sakane 2004) was isolated from a sample of forest soil on humic acid-vitamin agar (Hayakawa and Nonomura 1987b) using the yeast extract-sodium dodecylsulfate (SDS) method (Hayakawa and Nonomura 1989). *Acrocarpospora*, *Herbidospora*, and *Planotetraspora* strains grow well on oatmeal agar (Shirling and Gottlieb 1966). Similarly, acrocarposporae and herbidosporae show good growth on inorganic salts starch (Shirling and Gottlieb 1966) and yeast extract-starch agars (Kudo et al. 1993), respectively. The type strain of *Planotetraspora mira* grows well and sporulates on calcium malate (Runmao et al. 1993) and humic acid-vitamin agars (Hayakawa and Nonomura 1987b). The type strain of *P. silvatica* grows well on

glycerol-asparagine, tyrosine, and yeast extract-malt extract agars (Tamura and Sakane 2004). The type strain of *Sinosporangium album* grows well on R2A, ISP 4, ISP 5, Czapek solution agar, nutrient agar, and potato agar (Zhang et al. 2011). Species of the genus *Sphaerisporangium* were isolated on humic acid-vitamin agar (Hayakawa and Nonomura 1987b) or starch casein agar (Duangmal et al. 2011), modified glycerol-aspargine agar (Cao et al. 2009) supplemented with antifungal antibiotics. Janso and Carter (2010) reported the culturable diversity of endophytic actinomycetes associated with tropical, native plants. They isolated quite prevalent strains of endophytic *Sphaerisporangium* and *Planotetraspora* by using arginine vitamin agar (Nonomura and Ohara 1969a) supplemented with 3 % soil extract (Hayakawa et al. 2000) and 100-μg ml^{-1} cycloheximide and 50-μg ml^{-1} nystatin.

Dry heat treatment of air-dried soil samples and dilution plate culture with selective synthetic media are used for the preferential isolation and enumeration of some members of the family *Streptosporangiaceae*. The procedures outlined below have been developed for the selective isolation of the genera *Microbispora* and *Streptosporangium* (Nonomura and Ohara 1969a, b) and with modifications for the isolation of other actinomycete genera, notably *Microtetraspora* and *Nonomuraea* (Nonomura and Ohara 1971b, c). There is evidence (Nonomura and Hayakawa 1988) that pretreatment of soil suspensions with yeast extract (6 %, w/v) and sodium dodecyl sulfate (0.05 %, w/v) at 40 °C for 20 min, followed by dilution with water, activates actinomycete spores but kills vegetative cells of other soil bacteria in the suspensions.

This practice leads to an increase in the counts of actinomycetes on isolation plates. After soil samples are dried slowly at room temperature, passed through a 2-mm sieve, ground slightly in a mortar, spread on filter paper, and heated in a hot air oven at 120 °C for 1 h, the number of bacteria and streptomycetes is dramatically reduced, and the isolation frequency of *Microbispora*, *Microtetraspora*, and *Streptosporangium* strains is enhanced. Heated soil is incorporated directly onto isolation media, or a suspension is used to make dilution plates. Initially, arginine-vitamin (AV) and mineral glucose-asparagine plus soil extract (MGA-SE) agars were recommended for the selective isolation of microbisporae and streptosporangiae, but two additional formulations, chitin-V and humic vitamin (HV) agars (Hayakawa and Nonomura 1987a, b; Nonomura 1989b), have been developed. These media are supplemented with antifungal antibiotic(s); sometimes penicillin and polymyxin B are also used. Inoculated plates are incubated for 4–6 weeks at 30 °C (or 2–3 weeks at 50 °C) and examined using a light microscope with a long-working-distance objective. The highest counts and cleanest plates are usually obtained with HV agar.

Hayakawa et al. (1991) introduced an improved procedure for the selective isolation of streptosporangiae from soil. The method is based on the ability of streptosporangial spores to withstand dry heat and treatment with benzethonium chloride (BC) and the capacity of streptosporangiae to grow in the presence of leucomycin and nalidixic acid. Initially an air-dried soil sample is ground in a mortar and heated in a hot-air oven for an hour. Half a ml of a 10^{-1} dilution in water of the heated sample is transferred to 4.5 ml of sterile 5-mM phosphate buffer (pH 7.0) containing BC at a final concentration of 0.1 % (w/v). The resultant preparation is maintained at 30 °C for 30 min with occasional stirring, and a portion (1 ml) is then diluted with sterile tap water (1:10 or 1:15). Inocula of 0.1 ml or 0.2 ml of the dilution are then spread over the surface of plates of HV agar supplemented with leucomycin in ethanol (1 mg per liter) and nalidixic acid (20 mg per liter) and the plates are incubated at 30 °C for 3–4 weeks. Actinomycetes which appear on the plates are examined by light microscopy (600X) and assigned to genera on the basis of characteristic morphological properties. New species of the genus *Streptosporangium* and *Acrocarpospora* were isolated from soil with two criteria consisting of the growth on soil extract agar and showing small colonies (Hamaki et al. 2005).

Microbispora strains can be preferentially isolated by treating suspensions of dry heat pretreated soil samples with 1.5 % phenol at 30 °C for 30 min, diluting in water, and plating onto HV agar supplemented with nalidixic acid (20 mg per liter). *Microbispora karnatakensis* (Rao et al. 1987) was isolated by plating a suspension of soil onto inorganic salts-starch agar (Küster 1959).

Microtetraspora fusca, *M. glauca*, and *M. malaysiensis* were isolated from soil samples using methods that have not been disclosed (Thiemann et al. 1968b; Nakajima et al. 2003). However, the pretreatment procedure described above has been used to isolate several *Nonomuraea* species, including *N.* helvata, *N. pusilla*, *N. roseoviolacea*, and *N. spadix* (Nonomura and Ohara 1971c). *Nonomuraea* spores appear to be particularly resistant to dry heat at 100–120 °C, thereby allowing the slow-growing nonomuraea to develop into recognizable colonies on dilution plates. Soil dilutions are plated onto various media, including AV and MGA-SE agars, and incubated for several weeks at 28–30 °C (Nonomura and Ohara 1971b). *Microtetraspora niveoalba* was isolated from dry-heated soil on MGASE agar incubated at 40 °C for 1 month. Similarly, *M. glauca* strains have been isolated on plates of this medium incubated at 30 °C. Other *Nonomuraea* (previously *Microtetraspora*) species, such as *N. roseola* and *N. salmonea*, have been isolated from soil by Soviet investigators who supplemented media with antibiotics to improve their selectivity. Lavrova et al. (1972) added rubomycin (5, 10 or 20 μg ml^{-1}) to medium no. 2 of Gauze et al. (1957), Preobrazhenskaya et al. (1975) added bruneomycin (0.5, 1 or 2 μg ml^{-1}) or streptomycin (0.5, 1, or 2 μg ml^{-1}). The use of these antibiotics led to the growth of more nonomuraea colonies on isolation plates while reducing the number of streptomycetes. In contrast, *N. ferruginea* and *N. spiralis* were isolated by plating soil suspensions onto oatmeal agar or Gauze's no. 1 medium without addition of selective antibiotics (Meyer 1979).

Microbisporae, microtetrasporae, nonomuraea, and streptosporangiae grow well on rich media, including Bennett's (Jones 1949), glucose-yeast extract (Waksman 1950), oatmeal (ISP medium 3; Difco 0771), and yeast extract malt extract agars (ISP medium 2 [Difco 0770]; Shirling and Gottlieb 1966). Oatmeal-yeast

extract agar is recommended for the growth of mesophilic microbisporae and glycerol agar for the corresponding thermophilic strains (Nonomura 1989a). Streptosporangiae grow well and produce an abundant aerial spore mass on oatmeal-yeast extract agar (Nonomura and Ohara 1960). Good growth of vegetative and sporing aerial mycelia was obtained for *M. fusca* and *M. glauca* on Hickey and Tresner (1952) agar. *M. malaysiensis* strains grow well on yeast-malt extract agar (Nakajima et al. 2003). *Microtetraspora niveoalba* requires B vitamins for growth on synthetic media (Nonomura and Ohara 1971b).

The procedures used to isolate *Planobispora* and *Planomonospora* strains from soil were not revealed by Thiemann and his colleagues (Thiemann et al. 1967; Thiemann and Beretta 1968a). However, members of these taxa have been isolated from soil by baiting with natural substrates (Couch 1954; Bland and Couch 1981) as follows: a small amount of soil, approximately one level teaspoonful, is placed in a sterile Petri dish and flooded with sterile water (distilled water or filtered soil or charcoal water extracts may be used). Pollen and hair float are added at the water surface; various types of pollen have been employed including that from members of the genera Liquidamber, Pinus, and Sparganium (Schäfer 1973). After 1–4 weeks, examination of the water surface with a dissecting microscope (100X) and strong horizontal lighting should reveal white glistening spore vesicles formed in the air at the surface of the water by spore vesicle–forming members of the families *Micromonosporaceae* and *Streptosporangiaceae*.

The characteristic two-spored vesicles of *Planobispora* develop on long aerial hyphae growing between the baits. Similarly, sporulating aerial hyphae of *Planomonospora* strains grow on pollen grains. Single spore vesicles or bundles can be picked up with a thin needle and placed on the surface of agar media in small Petri dishes. After 2–4 weeks, the young colonies can be transferred to slant cultures. Similarly, *Planomonospora alba* strain A82600^T and *Planomonospora sphaerica* strain A15460^T were isolated by immersing a soil sample in water enriched for growth of microorganisms with motile spores, with sterile grass floating on the water surface as bait (Mertz 1994).

A multistage procedure was developed by Suzuki et al. (2001a) for the selective isolation of planobisporae from soil. Air-dried soil samples (500 mg) are heated at 90 °C for 60 min in a hot-air oven and then cooled to room temperature. Each heat treated sample is added to 2 ml of flooding solution (0.1 % skim milk [neutralized], 0.01 % Tween, 100-μg · ml^{-1} nalidixic acid in 5 mM-*N*-cyclohexyl-2-aminoethanesulfonic acid [CHES]; pH 9.0) and incubated at 35 °C for 60 min with occasional stirring to stimulate zoospore motility. After centrifugation (1,000 g) for 10 min at room temperature, 800 μl of supernatant is gently transferred to a sterile tube; 100-μl aliquots of this preparation are spread over humic acid-trace salts gellan gum medium (HSG) supplemented with cycloheximide (50 μg ml^{-1}), enoxacin (20 μg ml^{-1}), nalidixic acid (50 μg ml^{-1}), nystatin (50 μg ml^{-1}), sodium ampicillin (2 μg ml^{-1}), streptomycin sulfate (1 μg ml^{-1}), and trimethoprim (50 μg ml^{-1}). Following incubation at 32 °C for 14–21 days, planobisporae colonies growing on the HSG plates are recognized by their characteristic morphological features, as seen using a 40X long working district objective lens. Pure cultures are isolated by streaking onto HSG medium and tested for zoospore production using flooding solution containing 0.1 % skim milk in 5-mM CHES (pH 9.0).

A similar multistage procedure is available for the selective isolation of planomonosporae from soil (Suzuki et al. 2001b). Air-dried soil samples (500 mg) are heated at 100 °C for 60 min in a hot-air oven and cooled to room temperature. Each heat treated sample is added to 2 ml of sterile flooding solution (0.1 % skim milk in 5-mM *N*-cyclohexyl-2-amino-ethanesulfonic acid [CHES]; pH 9.0) and incubated at 32 °C for 90 min with occasional stirring to stimulate motility. The soil suspension is centrifuged (1,000 g) for 10 min at room temperature, incubated at 32 °C for 60 min, and 500 μl of supernatant is gently transferred to a sterile tube and 100-μl aliquots spread over HSG medium supplemented with cycloheximide (50 μg ml^{-1}), enoxacin (20 μg ml^{-1}), nalidixic acid (20 μg ml^{-1}), nystatin (50 μg ml^{-1}), sodium ampicillin (2 μg ml^{-1}), and trimethoprim (20 μg ml^{-1}). Inoculated plates are incubated at 35 °C for 14–21 days. Colonies of actinomycetes are observed directly under a phase-contrast microscope using a 40X long distance working objective lens.

Planomonosporae colonies, identified using morphological features (clavate spore vesicles containing single spores), are purified by single colony isolation on HSG plates and incubated at 35 °C for 14 days. Isolates are tested for motility with flooding solution containing 0.1 % skim milk in 5 mM CHES (pH 9.0). Isolates with motile spores can be assigned to two groups on the basis of morphological features: the *P. parontospora* group (spore vesicles arranged in double parallel rows resembling bananas) and the *P. venezuelensis* group (spore vesicles arranged in palm leaf patterns).

Planobisporae and planomonosporae grow on standard media used for cultivating streptomycetes (Waksman 1961), the first signs of visible growth appear after 3–4 days at 28–30 °C. *Planobispora longispora* produces aerial hyphae and abundant spore vesicles on calcium malate, soil extract, and yeast extract-malt extract agars (Shirling and Gottlieb 1966). Vesicular development in *P. rosea* is promoted by all media on which aerial mycelium is formed, notably soil extract and Hickey-Tresner agars. Spore vesicle development in *Planomonospora* strains is especially abundant on Bennett's, Hickey-Tresner, oatmeal, and soil extract agars (Thiemann et al. 1967; Vobis 1989b).

Maintenance

The most convenient method for short-term storage is by serial transfer from agar slants of appropriate media (see above) every 2 months (Meyer 1989). The tubes should be tightly closed with cotton plugs dipped in melted paraffin wax. Sporulated spore cultures can be stored at 5 °C and at room temperature. Lyophilization, storage in liquid nitrogen, and freezing in glycerol can be used for long-term preservation (Wellington and Williams 1978; Meyer 1989).

For lyophilization, the spore suspension or vegetative mycelium is suspended in a suitable fluid, such as serum plus

7.5 % (w/v) glucose or skimmed milk plus 7.5 % (w/v) glucose. For storage in liquid nitrogen, the microorganisms are inoculated into small test tubes containing the appropriate medium and incubated until satisfactory growth is visible. The tubes are then closed with cotton plugs dipped in melted paraffin wax and placed in a liquid nitrogen container.

Glycerol suspensions are prepared by scraping aerial growth or substrate mycelium or both from heavily inoculated plates and making heavy suspensions in 3 ml of aqueous glycerol in small (e.g., bijoux) bottles, which are stored at −20 °C or −80 °C. The frozen glycerol suspensions serve both as a practical means of long-term preservation and as convenient source of inoculum. Working inocula are obtained by thawing suspensions at room temperature prior to treating as for broth cultures. After use, glycerol suspensions are promptly frozen and stored again at −20 °C or −80 °C.

Ecology

Members of the family *Streptosporangiaceae* are usually associated with soil, but little is known about their role within this milieu. However, improvements in selective isolation procedures are beginning to cast light on the occurrence, distribution, numbers, and activity of actinomycete taxa in natural habitats (Suzuki et al. 2001a, b). Members of the family *Streptosporangiaceae* are probably involved in the primary decomposition of plant material in soils. In the course of screening for industrially important actinomycetes, several genera belonging to the family *Streptosporangiaceae* were isolated from tropical rainforests of Singapore and Vietnum (Wang et al. 1999; Muramatsu et al. 2003; Hop et al. 2011).

Streptosporangiae were associated with leaf litter (Van Brummelen and Went 1957; Potekhina 1965), as well as soil and dung (Nonomura and Ohara 1969a) until the introduction of a selective isolation procedure (Nonomura and Ohara 1969a) showed that these organisms were an integral part of the actinomycete community in soils. The number of streptosporangiae in various soils in Japan has been estimated at 104–106 colony forming units (cfu) per gram dry weight of soil (Nonomura and Ohara 1969a; Nonomura 1984). Slightly acid, humus-rich garden soils are a favorite habitat. They have also been isolated from lake sediments (Willoughby 1969a; Johnstone and Cross 1976), beach sand (Williams and Sharples 1976), and pasture and woodland soils (Whitham et al. 1993), but organisms labeled *Streptosporangium* type I from stream water (Willoughby 1969b), given their morphological properties and capacity to form motile spores, probably belong to the genus *Actinoplanes*. "*Streptosporangium bovinum*" was isolated from infected bovine hooves (Batista et al. 1963). Decades of environmental clones related to the genus *Streptosporangium* spp. were found in soil (GU556444, GU556247), gypsum-treated oil sands tailings pond (HQ092437), lake water (GQ411539-GQ411542, GQ468607,), and soil and water (GQ468603-GQ468605). On the based on BLAST search, undescribed member "*Streptosporangium koreanum*" (X89943) was related to *Streptosporangium roseum* with 99.9 % 16S rRNA gene sequence similarity. Other related strains were isolated from soil (EU119244-EU119248, FR692099, FJ261957), sediment (GU002049, KF007269, HQ157193), and surface-sterilized root of a medicinal plant (JX273661).

Few *Acrocarpospora* and *Herbidospora* strains have been isolated; hence, little is known about their distribution in natural habitats. The single representatives of *A. corrugata*, *A. macrocephala*, and *A. pleiomorpha* were isolated from beach sand (pH 7.8) at Freshfield, Lancashire, United Kingdom (Williams and Sharples 1976) and from soil samples collected from Saitama Prefecture, Japan (Tamura et al. 2000), and in Louisiana, United States (Tamura et al. 2000), respectively. Similarly, *Herbidospora cretacea* strains have been isolated from soil and plant material collected from several locations in Japan (Kudo et al. 1993). As the BLAST analysis, some strains related to *A. pleiomorpha* isolated from soil (AB193572, AB546293, AB649124) in Japan and Thailand.

Microbispora strains are common in soils. Using selective isolation procedures, counts of between 10^4 and 10^6 cfu per gram dry weight of soil have been reported from various Japanese soils (Nonomura and Ohara 1971b). Larger populations have been found in slightly acidic (pH 5–6), humus-rich garden soils (Nonomura and Ohara 1969a; Nonomura and Hayakawa 1988). Microbisporae have also been isolated from marine sediments (Weyland 1969). *Microbispora corallina* strains were isolated from soil samples collected in a deciduous dipterocarp forest in Thailand (Nakajima et al. 1999). For discovering of biocontrol agents, *Microbispora rosea* subsp. *rosea* were isolated from surface sterilized roots of Chinese cabbage, and the isolates exhibited the antagonistic activities to *Plasmodiophora brassicae* (Lee et al. 2008).

One species, *Microbispora rosea*, has been implicated in a case of pericarditis and pleuritis in a human (Louria and Gordon 1960). Most of the species previously assigned to the *Actinomadura pusilla* group, and now part of the genus *Nonomuraea*, originated from soil (Nonomura and Ohara 1971c; Meyer 1979; Galatenko et al. 1981). Microtetrasporae are common in soil, notably forest soils (Thiemann et al. 1968b; Nonomura and Ohara 1971b; Hayakawa et al. 1988; Nakajima et al. 2003). Using humic acid agar, Nonomura and Hayakawa (1988) recorded average counts of 3.6 10^4 cfu per gram dry weight soil for a number of forest soil samples collected in Japan. *Microtetraspora niveoalba* strains are particularly widely distributed albeit with counts of less than 10^3 cfu per gram dry weight of soil (Nonomura and Ohara 1971b). In contrast, *M. malaysiensis* strains have only been isolated from two locations, namely, from soil collected from below the leaf litter of mainly *Shorea* spp. in a primary lowland dipterocarp forest at Pasok, Negere Sembilan, and from a steep hill dipterocarp forest at the Virgin Jungle Reserve, Gombak, Selangor in Peninsular Malaysia (Nakajima et al. 2003). *Microtetraspora fusca* and *M. glauca* have been isolated from soil samples collected in Brazil, Italy, and Thailand. Some of the uncultured clones belonging to the genus *Microbispora* were deposited in DNA databank and their isolation sources from plant and

terrestrial environments including compost (GU188866; Xiao et al. 2011). Based on BLAST search, several *Microbispora* strains were found and their major isolated sample was soil.

Planomonospora strains have a worldwide distribution in soils of arid, temperate, and tropical regions. Thiemann (1970) isolated 37 strains of *P. parontospora* from 7 out of 454 soil samples (1.5 %) collected from Argentina, Chile, India, Peru, and Venezuela. He also isolated 7 strains of *P. venezuelensis* from 3 out of 454 soil samples (0.7 %) originating from Venezuela. Similarly, Suzuki et al. (2001b) isolated 246 *Planomonospora* strains from 137 out of 1,200 soil samples; 94 % of these isolates were from neutral to slightly alkaline soils (pH 7.0–9.0). Strains assigned to the *P. parontospora* group were recovered from 131 of these soil samples, notably from ones collected in Ecuador, Greece, and India. Strains classified in the *P. venezuelensis* group were isolated from 13 soil samples (1.1 %) collected in Bolivia, Cyprus, Egypt, Greece, India, Japan, New Caledonia, and Turkey. The single strains of *P. alba* and *P. sphaerica* were isolated from soil samples collected from The Sudan and India, respectively (Mertz 1994). *Planomonospora* strains have also been isolated from soil samples collected in Africa, Europe, and Central and North America (Vobis 1989b) and from the arid northeastern region of the Republic of South Africa (Kizuka et al. 1997). Until recently, planobisporae had rarely been isolated from soil. Several strains, including the type strains of *P. longispora* and *P. rosea*, were isolated from soil samples taken from a riverbank in Venezuela (Thiemann 1970). A few additional strains were recovered from soil samples collected from near Windhoek, Namibia (Vobis 1989a), and from arid regions of South Africa (Kizuka et al. 1997). Suzuki et al. (2001a) have shown that planobisporae are distributed over a much wider geographical area, as they isolated 119 strains from 51 soil samples (3.5 % of the samples tested) collected in Ecuador, Egypt, French Guiana, India, and Madagascar. Nearly 90 % of these strains were isolated from soil samples with pH values ranging from 7.0 to 7.9, results which suggest that *Planobispora* strains prefer neutral to alkaline environments. Suzuki and his colleagues were unable to isolate planobisporae from temperate regions in Europe, North America, and Oceania. Planotetrasporae, in contrast to planobisporae and planomonosporae, have only been isolated from two sources. The type strain of *Planotetraspora mira* was isolated from a soil sample collected in the village of Wolung, Sichuan, People's Republic of China (Runmao et al. 1993), and *P. silvatica* from a sample of forest soil originating from Amami Island, Kagoshima Prefecture, Japan (Tamura and Sakane 2004).

Sphaerisporangium and *Planotetraspora* isolates were discovered as endophytic actinomycetes from tropical plants roots collected from Papua New Guinea. Janso and Carter (2010) isolated 123 strains of endophytic actinomycetes and were derepricaqted by ribotying and 16S rRNA gene sequencing. Subsequently, 17 different genera and prevalent genera such as *Sphaerisporangium* and *Planotetraspora* were represented.

Several novel species of the genus *Nonomuraea* had been isolated from worldwide and habituated in several ecosystems such as soil, sea sediments, and plant rhizosphere (Kämpfer et al. 2010; Xi et al. 2011; Zhao et al. 2011). On the basis of BLAST search, undescribed members "*Nonomuraea rubescens*" (AY039255), "*Nonomuraea latina*" (AF277197), and "*Nonomuraea aurea*" (AY039254) were related to *Nonomuraea pusilla*, with 96.2 %, 96.2 %, and 95.2 %, respectively. Another related strains were isolated from mangrove soil (FJ261962), beach sand (EU741228), solar saltern (JN859005, Jose and Jebakumar 2012).

The studies of molecular based actinobacterial diversity by means of DGGE/clone library analysis in several environments had reported, however, only published paper described the presence of *Microbispora* in compost (Xiao et al. 2011). The survey of NCBI taxonomy browser lists culture-independent 16S rRNA gene sequences deposited for the genus *Acrocarpospora*, *Microbispora*, *Nonomuraea*, *Planomonospora*, *Planotetraspora*, *Sphaerosporangium*, and *Streptosporangium*. The entry of unnamed strains belonging to some of genera such as *Microbispora*, *Nonomuraea*, *Planotetraspora*, *Sphaerosporangium*, and *Streptosporangium* are abundant, but few entries for other genera. In near future, advances in DNA sequencing technology will provide more large information about culture-independent actinobacterial diversity in diverse ecosystems. Furthermore, diversity of culturable actinomycetes including the family *Streptosporangiaceae* is also important for understanding the several features in ecosystems. For example, Hop et al. (2011) suggested that the ratio of *Streptosporangium* to *Nonomuraea* isolates was different in latitude sites collected from Southeast Asia to Japan.

Pathogenicity: Clinical Relevance

Although "*Streptosporangium bovinum*" was reported to isolate from infected bovine hooves, there are no objective data for *Streptosporangiaceae* which has a role as clinical pathogens.

Applications

Members of the family *Streptosporangiaceae* are expected to be an increasingly rich source of commercial products, notably antibiotics and enzymes. "*Microtetraspora tyrrkensis*" produces fluvirucins active against influenza A virus (Tomita et al. 1991); *Microbispora rosea* produces deoxycephalomycin B (Okazaki and Naito 1985); *Microbispora* strain SCC 1438 produces a novel fungal antibiotic (Patel et al. 1988); *Nonomuraea roseoviolacea* produces carminomicins (Nakagawa et al. 1983, 1989); *N. rubra* produces maduromycin (Fleck et al. 1978), *N. pusilla* produces actinotiocin (Tamura et al. 1973), and *N. spiralis* produces pyralomicin (Naganawa et al. 2002). Similarly, *Streptosporangium albidum* produces aculeximycin (Murata et al. 1989); *S. pseudovulgare* produces sporamycin (Komiyama et al. 1977); *S. roseum* produces maytansin-type ansamacrolactam (Hacene et al. 1998), sporangirosamycin (Gazhal and Abl El-Aziz 1993), and thiosporamycin (Celmer et al. 1978); *S. violaceochromogenes* produces platomycins

A and B (Takasawa et al. 1975) and victomycin (Kawamoto et al. 1975), and *S. vulgare* produces sporacuracins A and B (Atsushi et al. 1975). A novel anthracycline antibiotic has been isolated from *S. fragile* (Shearer et al. 1983), an antitumor antibiotic from an organism resembling *S. pseudovulgare* (Umezawa et al. 1976) and unspecified antimicrobial agents from *Microbispora indica* and *M. karnatakensis* (Rao et al. 1987).

Microbispora rosea is an excellent source of D-xylose (glucose) isomerase (Crueger and Crueger 1982), which converts D-glucose into D-fructose. The enzyme is used to produce D-fructose on a commercial scale, and its biosynthesis, purification, and immobilization, as well as its application for the production of high fructose syrup, have been the subject of many reports and patents (Crueger and Crueger 1984). Similarly, cystathionine γ-lyase has been detected in strains of Streptosporangium (Nagasawa et al. 1984). This enzyme has been shown to catalyze the α, γ-elimination reaction of L-cystathionine and also the γ-replacement of L-homoserine in the presence of various thiol compounds (Kanzaki et al. 1986a). An efficient method based on the reaction of γ-replacement has been developed (Kanzaki et al. 1986b) for the preparation of L-cystathionine, a product that may be useful, because a deficiency of this compound has been observed in the brains of homocystinuric patients (Gerritsen and Waisman 1964). The procedure allowed the total conversion of *O*-succinyl-L-homoserine and L-cysteine into L-cystathionine. Microbispora rosea also produces exoxylanases (Kusakabe et al. 1969).

Thermophilic microbisporae synthesize a wide range of enzymes that are involved in the degradation and modification of heteropolysaccharides, notably celluloses, lignocelloloses, and hemicelluloses (Henssen and Schnepf 1967; McCarthy 1987; Crawford 1988). These enzyme systems have the potential for novel application in biotechnological processes, particularly for the enzymatic generation of fermentable sugar from agricultural residues (McCarthy et al. 1988; Zimmermann 1989).

Actinomycetes have been isolated and screened by pharmaceutical companies since the pioneering work of Selman Waksman at Rutgers State University (Waksman and Lechevalier 1953). Much of this work focused on streptomycetes as these organisms grow quickly and are easily isolated on media such as starch casein (Küster and Williams 1964) and arginine-glycerol-salts agars (El-Nakeeb and Lechevalier 1963). Many species of Streptomyces have been isolated, characterized, and named, including those that produce commercially significant antibiotics, as exemplified by actinomycin from *Streptomyces antibioticus* (Waksman and Woodruff 1941), neomycin from *Streptomyces fradiae* (Waksman and Lechevalier 1949), and streptomycin from *Streptomyces griseus* (Waksman 1961).

The discovery that members of sporoactinomycete genera other than streptomycetes were the source of potentially commercial significant antibiotics led to the introduction of procedures designed to isolate "rare" and uncommon actinomycetes (Nolan and Cross 1988; Okami and Hotta 1988; Lazzarini et al. 2000).

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44 The Family *Thermoleophilaceae*

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Abstract
Thermoleophilaceae, a family within the order *Thermoleophilales*, embraces the genus *Thermoleophilum* with two species: *Thermoleophilum album* and *T. minutum* (Reddy and Garcia-Pichel Int J Syst Evol Microbiol 59:87–94, 2009; Zarilla and Perry Arch Microbiol 137:286–290, 1984; Zarilla and Perry 1986a). The organisms included in this order have the ability to grow at elevated temperatures, with optimum growth temperatures of around 60 °C and at the expense of *n*-alkanes with 13–20 carbons; however, these organisms are unable to assimilate any other carbon and energy sources examined. The two species of the genus *Thermoleophilum* are strictly aerobic and catalase positive. These organisms possess several polyamines, but the polar lipid and fatty acids have not been examined. These organisms were isolated from hot springs and nonthermal sources.

Taxonomy, Historical and Current

Short Description of the Order, Family, and Genus

The order *Thermoleophilales* contains one family, the *Thermoleophilaceae*. The order was proposed by Reddy and Garcia-Pichel (2009) to include the only known family *Thermoleophilaceae* (Stackebrandt 2004, 2005). The family comprises one genus, *Thermoleophilum* with two species. The type species of the genus is *Thermoleophilum album*. The type strain of *Thermoleophilum album*, HS-5^T (=ATCC 35263^T), was recovered from a hot spring in Arkansas, USA (Zarilla and Perry 1984). Strains YS-3 (=ATCC 35264), NM (=ATCC 35266), and RR-D (=ATCC 35267) are additional strains of this species. The name of the genus and of the species were, however, only validated after the name of the other species of the genus, *Thermoleophilum minutum*, had been validly published in the same issue of IJSEM (Zarilla and Perry 1986a). The species *Thermoleophilum minutum* (type strain YS-4^T = ATCC 35265^T) was also isolated and described by Zarilla and Perry (1986a). It should be noted that *T. minutum* strain, PTA-1 (=ATCC 35268), was isolated from mud at ambient temperature in North Carolina, USA. The organism was briefly named "Thermomicrobium fosteri" (Merkel et al. 1978a, b; Perry 1984; Phillips and Perry 1976).

Thermoleophilales Reddy and Garcia-Pichel 2009

Thermoleophilales (Ther.mo.le.o.phi'la.les. N.L. neut. n. *Thermoleophilum* type genus of the order; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Thermoleophilales*, the *Thermoleophilum* order).

Members of the order *Thermoleophilales* are non-spore forming. The pattern of 16S rRNA signatures consists of nucleotides at positions 52 : 359 (C-G), 63 : 104 (G-C), 70 : 98, (G-C), 127 : 234, (G-C), 139 : 224 (G-C), 144 : 178 (C-G), 291 : 309 (U-A), 370 : 391 (G-C), 408 : 434 (G-C), 580 : 776 (C-G), 590 : 649 (C-G), 600 : 638 (C-G), 657 : 749, (U-A), 670 : 736 (G-C), 681 : 709 (C-G), 823 : 877 (G-C), 906 (A), 941 : 1342 (G-C), 953 : 1228 (G-C), 954 : 1226 (G-C), 955 : 1225 (U-A), 999 : 1041 (A-U), 1051 : 1207 (G-C), 1115 : 1185 (C-G), 1118 : 1155 (C-G), 1311 : 1326 (G-C), and 1410 : 1490, (A-U). The order *Thermoleophilales* belongs to the class *Actinobacteria*. The order contains the family *Thermoleophilaceae* (Reddy and Garcia-Pichel 2009).

Thermoleophilaceae Stackebrandt 2005; emend. Zhi et al. 2009

Thermoleophilaceae (Ther.mo.le.o.phi.la.ce'a.e. N.L. neut. n. *Thermoleophilum*, type genus of the family; suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Thermoleophilaceae*, the *Thermoleophilum* family).

The members of the sole family *Thermoleophilaceae* of the order *Thermoleophilales* stain Gram-negative, although transmission electron microscopy (TEM) demonstrates a series of layers typical of Gram-positive bacteria.

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_203,

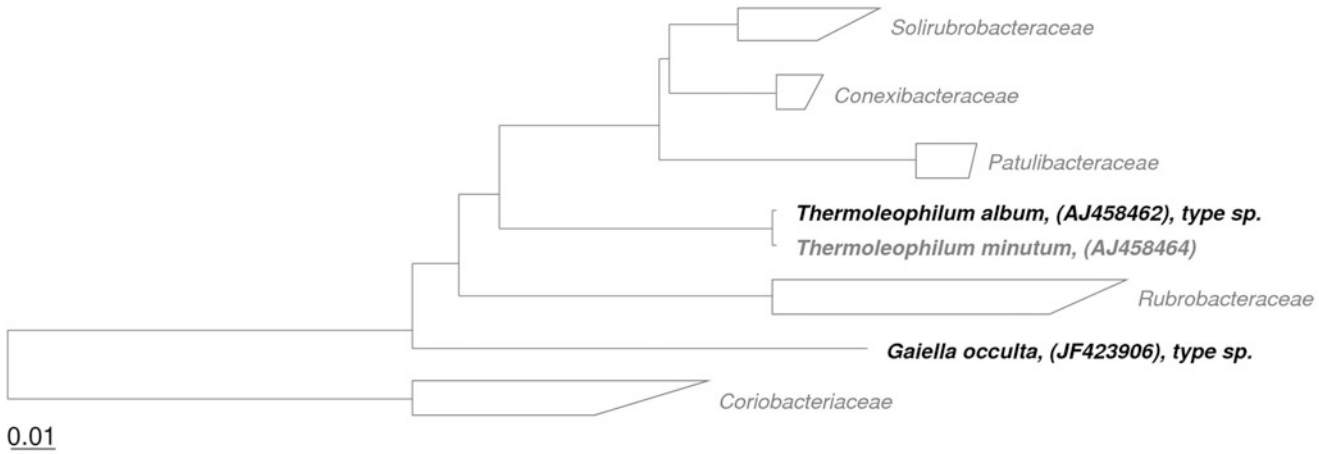

Fig. 44.1

Phylogenetic reconstruction of the family *Thermoleophilaceae* 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

The organisms form rod-shaped cells. These organisms are strictly aerobic and chemoorganotrophic and contain meso-diaminopimelic acid as the diamino acid in the peptidoglycan. The type and only genus of this family is *Thermoleophilum* (Stackebrandt 2004; Zhi et al. 2009).

Phylogenetic Structure of the Family and Its Genus

The family *Thermoleophilaceae* forms a deep branching root of the phylum Actinobacteria and is most closely related to the species of the family *Solirubrobacteraceae* (88–90 % 16S rRNA gene similarity) (Albuquerque et al. 2011) (*Fig. 44.1*). Our knowledge of the species of the genus *Thermoleophilum* remains rudimentary, but it is clear that the species *T. album* and *T. minutum* cannot be distinguished from each other on the basis of the phenotypic results available although the species can be distinguished using polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins. The phylogeny based on 16S rRNA sequence analysis of the two species also indicates that the organisms should be extremely closely related (99.9 % similarity); however, DNA-DNA hybridization, using [α-^{32}P]deoxycytidine triphosphate-labelled DNA method, produced values of 25–48 % indicating that these two organisms are indeed distinct from each other (Zarilla and Perry 1984, 1986a).

Phenotypic Analyses

The main features of *Thermoleophilum album* and *T. minutum* are listed in *Table 44.1*.

Thermoleophilum Zarilla and Perry 1986b

Thermoleophilum (Ther.mo.le.o'phi.lum. Gr. n. *therme*, heat; L. n. *oleum*, oil; N.L. neut. adj. *philum* (from Gr. neut. adj. *philon*), friend, loving; N.L. neut. n. *Thermoleophilum*, heat- and oil-loving microbe).

The two validly named species of the genus *Thermoleophilum* form short rod-shaped Gram-negative cells. Obligately thermophilic and capable of growth only on a narrow range of *n*-alkanes. Both species are able to assimilate *n*-alkanes with 13–20 carbons, but are not able to assimilate other alkanes, namely, shorter or longer *n*-alkanes, 1-alkanes from 12 to 19 carbons in length, cyclohexane and cycloheptane, alcohols with 12–18 carbons, and ketones from 13 to 19 carbons in length. Carbohydrates, organic acids, amino acids, yeast extract, peptone, or tryptone-yeast extract are not assimilated. The nitrogen source of the growth medium is ammonium chloride (Zarilla and Perry 1984). All strains contain meso-diaminopimelic acid (DAP) as the diamino acid of the peptidoglycan (Merkel et al. 1978a). A quaternary branched pentamine, 4,4-bis(3-aminopropyl)-1,8-diamino-4-azaoctane, is the major polyamine of the two species of this genus (Hamana et al. 1992). In *T. album* the major isoprenoid respiratory lipoquinone is a tetrahydrogenated menaquinone 7 [MK-7(H_4)], 2-methyl-3-VI,VII-tetrahydro-heptaprenyl-1,4-naphthoquinone (Collins et al. 1986). The G+C content of the DNA is around 70–71 mol%. It is noteworthy that the two validly named species of *Thermoleophilum* cannot be distinguished from each other on the basis of the phenotypic characteristics examined, except for polyacrylamide gel electrophoresis (PAGE) of whole-cell protein extracts; however, it is clear that the organisms represent two genomic species based on DNA-DNA hybridization and 16S rRNA gene analysis (Yakimov et al. 2003; Zarilla and Perry 1984, 1986a).

Table 44.1

Phenotypic and chemotaxonomic characteristics of members of the genus *Thermoleophilum*[a, b, c, d]

	T. album HS-5^T	*T minutum* YS-4^T
Morphology	Rods	Rods
Cell size (μm)	0.4 × 0.9	0.4 × 1.5
Pigmentation	Nonpigmented	Nonpigmented
Gram stain	Negative	Negative
Motility	–	–
Temperature for growth (°C)		
Range	45–70	45–70
Optimum	60	60
pH for growth		
Range	6.5–7.5	6.0–7.0
Optimum	7.0	6.8
Metabolism	Aerobic	Aerobic
Presence of		
Catalase	+	+
Carbon source		
n-alkanes from 13 to 20 carbons	+	+
1-alkanes from 12 to 19 carbons in length	–	–
Cyclohexane	–	–
Cycloheptane	–	–
Alcohols with 12–18 carbons	–	–
Ketones from 13 to 19 carbons in length	–	–
Arabinose	–	–
Cellobiose	–	–
Fructose	–	–
Galactose	–	–
Glucose	–	–
Lactose	–	–
Maltose	–	–
Mannose	–	–
Melibiose	–	–
Rhamnose	–	–
Ribose	–	–
Sorbose	–	–
Sucrose	–	–
Trehalose	–	–
Xylose	–	–
Glycerol	–	–
Mannitol	–	–
Sorbitol	–	–
Acetate	–	–
Butyrate	–	–
Propionate	–	–
Citrate	–	–
Pyruvate	–	–
Succinate	–	–
Acetone	–	–
Nutrient broth	–	–
Peptone	–	–
Yeast extract	–	–
Tryptone-yeast extract	–	–
Alanine	–	–
Glutamate	–	–
Nitrogen source		
n-alkanes from 13 to 20 carbons	+	+
Ammonium chloride	+	+
Alanine	–	–
Glutamate	–	–
Sensitivity to		
Chlortetracycline	+	+
Streptomycin	+	+
Kanamycin	+	+
Erythromycin	+	+
Neomycin	+	+
Chloramphenicol	+	+
Penicillin	+	+
Novobiocin	+	+
Diagnostic peptidoglycan amino acids[e]	*meso*-Dpm	*meso*-Dpm
Major polyamine	Quaternary branched pentamine, 4,4-bis (3-aminopropyl)-1,8-diamino-4-azaoctane	Quaternary branched pentamine, 4,4-bis (3-aminopropyl)-1,8-diamino-4-azaoctane
Major respiratory lipoquinone[f]	MK-7(H_4)	nd
G+C content (mol%)	70.4	70.0

Symbols: + positive, – negative

[a]Collins et al. 1986

[b]Hamana et al. 1992

[c]Zarilla and Perry 1984

[d]Zarilla and Perry 1986a

[e]*meso-Dpm meso*-diaminopimelic acid

[f]*MK* menaquinone

Isolation, Enrichment, and Maintenance Procedures

Thermoleophilum album was isolated from soil and mud samples (Zarilla and Perry 1984). The cardinal growth temperatures of the strains indicate that they possibly originate from thermal environments but several isolates were recovered from nonthermal sources. The organisms were isolated by enrichment culture techniques using mud samples taken from both thermal and nonthermal environments. Samples were obtained in the USA from thermal environments, namely, Arkansas (Hot Springs, strain HS-5^T), New Mexico (Faywood Hot Springs, strain NM), and Wyoming (Yellowstone National Park, strain YS-3) and from mud at ambient temperature at North Carolina (Roanoke Rapids, strain RR-D). The substrate for enrichment was *n*-heptadecane added at 0.1 % (v/v) to a mineral salts medium of the following composition: NH_4Cl, 1 g; $NaNO_3$, 1 g; $MgSO_4.7H_2O$, 0.2 g; $FeSO_4.7H_2O$, 1 g; Na_2HPO_4, 0.21 g; NaH_2PO_4, 0.09 g; KCl, 0.04 g; $CaCl_2$, 0.015 g; $CuSO_4.5H_2O$, 2 µg; H_3BO_3, 10 µg; $MnSO_4.5H_2O$, 10 µg; $ZnSO_4.7H_2O$, 70 µg; MoO_3, 10 µg; and deionized water 1 L. After incubation at 60 °C and repeated transfer, the organism was obtained in axenic culture by streaking on a mineral salts agar medium. The *n*-heptadecane substrate was introduced by inverting the inoculated plate and placing a drop of the hydrocarbon on the inside of the cover which vaporizes and infiltrates the inverted plate.

Thermoleophilum minutum was isolated from a hot spring in Yellowstone National Park (Wyoming, strain YS-4^T) and from mud at ambient temperature in North Carolina (Beaufort, strain PTA-1) (Zarilla and Perry 1984). The organisms were isolated using the same enrichment culture techniques used to isolate *Thermoleophilum album*.

Ecology

Habitat

The thermophilic nature of the cultivated organisms of the genus *Thermoleophilum* with an optimum growth temperature of around 60 °C and the isolation of these organisms from thermal environments argue for the colonization of hot springs with neutral pH rather than soil or mud with low temperatures. The isolation of these organisms from mud or soil environments is difficult to explain unless these organisms originate from nearly thermal areas or the soil is solar heated. The origin of long-chain *n*-alkanes is also difficult to envision in thermal springs, although alkane-utilizing bacteria have been isolated from mineral water aquifers with extremely low levels of organic nutrients (Leandro et al. 2012). The assimilation of alkanes can be viewed as a selective advantage for these organisms even when their levels in the environment are extremely low.

It is interesting to note that environclones closely related to the strains of *Thermoleophilum* have not been encountered. The most closely related 16S rRNA gene clone has a sequence of similarity of 93 % (JX240741), and the next most closely related environmental sequence has a similarity of 92 % (JF706670). The first was obtained from the Gulf of Khambhat, India, while the second sequence came from the Atacama, Chile.

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45 The Family *Tsukamurellaceae*

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Abstract
According to Bergey's road map of the Actinobacteria, the six families *Corynebacteriaceae*, *Dietziaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Segniliparaceae*, and *Tsukamurellacea* were displaced from the order "*Actinomycetales*" and assigned to the order *Corynebacteriales*. The family *Tsukamurellaceae* comprises solely the genus *Tsukamurella* which consists of 11 species. Representatives of these taxa share very high 16S rRNA gene sequence similarity values with exception of the type strain of *T. paurometabola* which is moderately related to *T. spongiae* and *T. soli*. Species delineation within the genus *Tsukamurella* is mainly based on DNA:DNA hybridization studies.

Taxonomy: Historical and Current

Short Description of the Family and the Genus *Tsukamurella*

Tsukamurellaceae Rainey, Ward-Rainey and Stackebrandt 1997, 486[VP], Emend. Zhi, Li, and Stackebrandt 2009, 595[VP]

Tsu.ka.mu.rel.la.ce'a.e. N.L. fem.n. *Tsukamurella* the type genus of the family; -aceae ending to denote the family; N.L. fem. pl. n. *Tsukamurellaceae*, the *Tsukamurella* family.

The family *Tsukamurellaceae* was proposed by Rainey et al. (1997) within the hierarchical classification system of the *Actinobacteria* and the family description was mainly based upon the phylogenetic position and the presence of defined 16S rRNA gene sequence signatures nucleotides. Rainey et al. defined the following 16S rRNA signatures for members of the genus *Tsukamurella* (Collins et al. 1988), the only genus within the family: at positions 70-98 (U-A), 293-304 (G-(A-U), 843 (C), 1007-1022 (G-U), and 1122-1151 (A-U). In 2009, the emended description of the family was published by Zhi et al. by extending the signature nucleotides considering all validly described species of the genus *Tsukamurella* published at that time. The pattern was specified for the following positions: 128:233 (G–C), 250 (U), 316:337 (C–G), 418:425 (C–G), 580:761 (C–G), 599:639 (C–G), 987:1218 (G–C), 1000:1040 (A–C), and 1059:1198 (C–G).

Type genus: *Tsukamurella* Collins et al. 1988, 387[VP].

Tsu.ka.mu.rel.la N.L. fem. dim n. *Tsukamurella* named in honor of Michio Tsukamura, a Japanese microbiologist.

Type species: *Tsukamurella paurometabola* corrig. (Steinhaus, 1941).

The type strain of the species was isolated from the mycetome and ovaries of *Cimex lectularis* (bedbug) by Edward

E. Rosenberg et al. (eds.), *The Prokaryotes – Actinobacteria*, DOI 10.1007/978-3-642-30138-4_189,

Table 45.1

Overview of DNA:DNA hybridization studies performed by the different authors. Level of DNA:DNA relatedness is indicated in %. The strain under question which was compared against the phylogenetic neighbors is indicated by X

Tsukamurella	Nam et al. (%)	Park et al. (%)	Yassin et al. (%)	Yassin et al. (%)	Olson et al. (%)	Olson et al. (%)	Seong et al. (%)
paurometabola	X	11.9	44	53.4			
strandjordii	56	7.9			44	41	
inchonensis	33	8.6	56.3	53.5			58.6
pulmonis	28	60.6	X	54.7	48	X	54.1
tyrosinosolvens	28	62.7		X			55.7
spumae	18	12.7					
pseudospumae	20	58.7					
carboxydivorans		X					
spongiae					X		
sunchonensis							X

A. Steinberg in 1941 and was first placed into the genus *Corynebacterium* as "*Corynebacterium paurometabolum*." Analysis of *Corynebacterium paurometabolum* revealed that the strain possesses meso-diaminopimelic acid and arabinogalactan in their cell walls, but differed from the genus *Corynebacterium* by the presence of long, highly unsaturated mycolic acids, with 58–76 carbon atoms (Collins and Jones 1982). In contrast, members of *Corynebacterium* are characterized by short chain mycolic acids with 22–36 carbon atoms only.

Long, highly unsaturated mycolic acids were also detected in strain *Rhodococcus aurantiacum*, first described as *Gordonia aurantia* (Tsukamura and Mizuno 1971) and later on transferred to the genus *Rhodococcus* (Tsukamura 1974; Tsukamura and Yano 1985). Based on 16S rRNA gene sequence analysis, both strains, *R. aurantiacum* and *C. paurometabolum*, were unified in a single species and described as *Tsukamurella paurometabola* (Collins et al. 1988).

Members of the genus *Tsukamurella* are aerobic, weakly up to partially acid fast, non-motile, non-spore-forming Gram-positive bacteria. Chemo-organotrophic, catalase activity positive. Mostly characterized by straight to curved rods that occur singly, in pairs or filaments. Coccobacillary forms may also occur. Rough, dry, flat, or folded colonies are developed; sometimes edges are irregular or rhizoidal. Colonies are whitish cream colored or yellow-orange to red. Aerial hyphae are not present. The diagnostic amino acid of the type A1γ peptidoglycan is meso-diaminopimelic acid. Cell wall sugars are arabinose, galactose, but ribose and glucose can also be present. Polar lipids are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylinositol mannosides (PIM). The predominant menaquinone is unsaturated with nine isoprene units (MK-9) for most species. MK-10, MK-8, and MK-7 may also be detectable. Mycolic acids have 58–78 carbon atoms and 1–7 double bonds, except for *T. carboxydivorans* which may have 81–95 carbon atoms. The fatty acid esters released on pyrolysis of mycolic acids have 20–22 carbon atoms. The long-chain cellular fatty acids are predominantly of the straight-chain saturated, monounsaturated, and 10-methyl branched types (*Table 45.3*). Phylogenetically, the genus is placed into the family *Tsukamurellaceae* which is in the order *Corynebacteriales*. According to Bergey's road map of the Actinobacteria, the six families *Corynebacteriaceae*, *Dietziaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Segniliparaceae*, and *Tsukamurellacea* were displaced from the order "*Actinomycetales*" and assigned to the order *Corynebacteriales*. The order *Actinomycetales* is now restricted to members of the family *Actinomycetaceae* only. DNA G+C content: 67–78 mol %. Isolated from various aquatic and terrestrial habitats as well as from clinical specimen.

Phylogenetic Structure and Molecular Analyses

At present 11 species with validly published names are included in the genus *Tsukamurella*. Representatives of these taxa share very high 16S rRNA gene sequence similarity values between 99.2 % and 99.7 %, with the exception of the type strains of the two species *T. spongiae* and *T. soli* (*Fig. 45.1*). Both strains are remotely related to *T. paurometabola*, showing similarity values of 98.4 % and 96.5 %, respectively. The 16S rRNA gene sequence of *T. soli* differs at more than 50 nucleotide positions as compared to the 16S rRNA gene sequence derived from whole genome sequencing of the *T. paurometabola* type strain.

Due to the very high relationship of the 16S rRNA gene sequences, species delineation within the genus *Tsukamurella* was mostly done by DNA:DNA hybridization (DDH). DDH studies have not been performed between all of the species published and was excluded for *T. soli*, because of the low similarity value of the 16S rRNA gene sequence. It is noteworthy that the name *T. sunchonensis* became validly published in 2008, even though the species description was

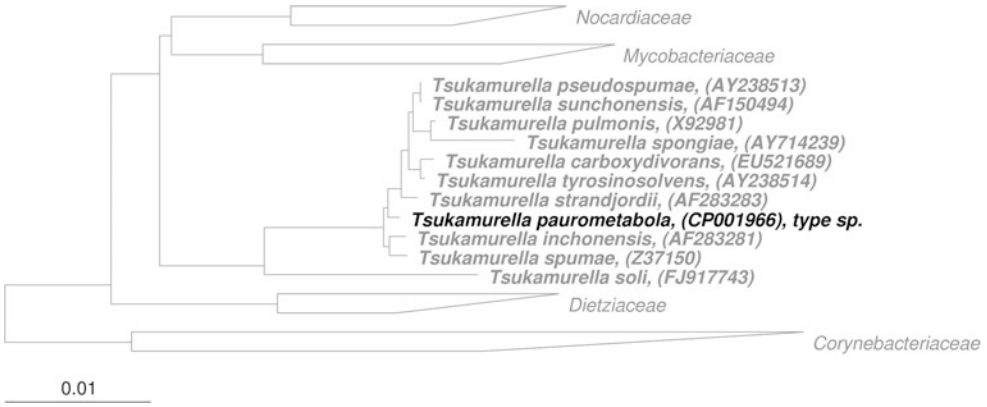

Fig. 45.1

Phylogenetic reconstruction of the family *Tsukamurellaceae* 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. *Scale bar* indicates estimated sequence divergence

already published in 2003. As a consequence, the close relationship between *T. sunchonensis* and *T. pseudospumae* was overlooked. The 16S rRNA gene sequences of both type strains are identical, but the DNA:DNA relatedness of both strains has not yet been analyzed.

Three different techniques were applied for analysis of DNA:DNA relatedness: (1) spectrophotometrically, according to De Ley et al. 1970 (Yassin et al. 1995, 1996, 1997; Olson et al. 2007); (2) by the fluorometric microplate method (Nam et al. 2003) and (3) by dot blot hybridization (Seong et. al. 2003; Park et al. 2009). DDH studies have been performed most extensively among *T. paurometabola* DSM 20162^T and other closely related type strains of the genus. The highest DNA binding rate of 56 % was reported with strain *T. strandjordii* BAA-173^T, while all other values varied between 33 % and 11.9 % with its phylogenetic neighbors *T. inchonensis* IMMIB D-771^T, *T. pulmonis* IMMIB D13-21^T, *T. tyrosinosolvens* IMMB 13-97^T, *T. spumae* N1171^T, *T. pseudospumae* N1176^T, and *T. carboxydivorans* Y2^T. Additional data are available for other type strain combinations; the values are also summarized in *Table 45.1*. (i) according to Yassin et al. (1995, 1996, 1997): *T. pulmonis* IMMIB D13-21^T against *T. paurometabola* DSM 20162^T and *T. inchonensis* IMMIB D-771^T (44 % and 56.3 %); *T. tyrosinosolvens* IMMB 13-97^T against *T. paurometabola* DSM 20162^T, *T. inchonensis* IMMIB D-771^T, and *T. pulmonis* IMMIB D13-21^T (53.4 %, 53.5 %, and 54.7 %); (ii) *T. spongiae* K-362^T against *T. pulmonis* IMMIB D13-21^T and *T. strandjordii* BAA-173^T (48 % and 44 %) and *T. pulmonis* IMMIB D13-21^T against *T. strandjordii* BAA-173^T = 41 % (Olson et al. 2007); (iii) T. sunchonensis SCNU5^T against *T. pulmonis* IMMIB D13-21^T, *T. tyrosinosolvens* IMMB 13-97^T and *T. inchonensis* IMMIB D-771^T = 54.1 %, 55.7 % and 58.6 % (Seong et al. 2003); (iv) according to Park et al. 2009 *T. carboxydivorans* Y2^T against *T. inchonensis* IMMIB D-771^T, *T. pulmonis* IMMIB D13-21^T, *T. tyrosinosolvens* IMMB 13-97^T, *T. strandjordii* BAA-173^T, *T. spumae* N1171^T, and *T. pseudospumae* N1176^T (8.6, 60.6, 62.7, 7.9, 12.7, and 58.7).

Genome Analyses

Strain *Tsukamurella paurometabola* DSM 20162^T is the only strain of the genus from which a finished genome sequence has been generated (INSDC ID CP001966) and been published in *Standards in Genomic Sciences* (Munk et al. 2011). The genome was analyzed in course of the Genomic Encyclopedia of Bacteria and Archaea project. The 4,479,724 bp long genome also contains a 99,806 bp long plasmid, designated as pTpau 01, both with a GC content of 68.4 %. Besides 93 pseudogenes, 4,391 genes were predicted, of which 4,335 were protein-coding genes, and 56 were RNA genes: 68.7 % of the genes could be assigned to a putative function, and 68.9 % of the genes were assigned to clusters of orthologous groups (COG's). The distribution of genes into COG's functional categories shows that the highest numbers of genes are involved in transcription processes (310; 9.2 %), followed by genes coding for amino acid transport and metabolism (274; 8.1 %), lipid transport and metabolism (231; 6.8 %), carbohydrate transport and metabolism (220; 6.5 %), and energy production and conversion (217; 6.4 %). Interestingly, 172 genes (5.1 %) were found to be involved in secondary metabolite biosynthesis, transport, and catabolism. A detailed listening is given by Munk et al. (2011), but is also accessible via the Integrated Microbial Genomes platform (Markowitz et al. 2009).

The sequence of plasmid pTpau01 consists of 92 genes, but the proteins encoded from those are mainly hypothetical proteins with unknown or uncertain function. Several transposases

could be predicted as well as a putative recombinase, an abortive infection protein, a TrwC relaxase (a putative TraA-like conjugal transfer protein and CopG (RepA) responsible for regulation of the plasmid copy number). Further a putative permease of the Dif318 family has been predicted, whose function is not really known, but some proteins within this family are encoded by genes involved in arsenate/arsenite resistance. The prediction of all genes located on the plasmid is given in detail within the IMG platform (Markowitz et al. 2009).

Recently the draft genome sequence of *Tsukamurella* strain 1534 has been published by Oulmi et al. (2012). In contrast to *Tsukamurella paurometabola*, strain 1534 was originally isolated from a human sputum specimen. Four thousand and four hundred putative proteins have been predicted for strain 1534, and 89.4 % could be assigned to cluster of orthologous groups. Oulmi et al. (2012) reported that the genome of strain 1534 shared 3,037 orthologs with *Tsukamurella paurometabola* and 2 ribosomal operons have been detected for both strains.

Phages

The first phage TPA1, able to lyse *Tsukamurella paurometabola*, was isolated from activated sludge by Thomas et al. (2002). Later on, Petrovski et al. (2011) showed that this phage type could successfully be isolated over a period of more than 9 years from activated sludge plants in Australia. The authors characterized two of these phages (TPA2 and TPA3) in greater detail and found that both were identical. TPA1 and TPA2 were selected for genome sequencing and analysis revealed that both sequences were also identical, even though both phages have been isolated from different activated sludge plants. For this reason, only the complete genome sequence of phage TPA2 (NC_015210) was published. TPA2 is a polyvalent *Siphoviridae* family phage and its genome sequence contains 61.44 kb, with a GC content of 69.1 %. The NCBI database listed 79 genes for phage TPA2, but most of the proteins encoded are hypothetical. Only 30 % of the TPA2 genome is related to other phages at the DNA level. As shown by Petrovski et al. (2011), TPA2 displays a broad host range within the genus *Tsukamurella* and lyses not only *T. paurometabola*, but also most of the other species of the genus with the exception of *T. inchonensis*. On the other hand, TPA2 seems to be genus specific and was not able to lyze strains of closely related genera, like *Dietzia*, *Gordonia*, or *Rhodococcus*. Also *Mycobacteria* were not affected even though the gene organization of TPA2 was found to be similar to that of the *Mycobacterium* phage Rosebush (Petrovski et al. 2011).

The draft genome of strain *Tsukamurella* strain 1534 also contains one genetic element of 49.4 kb in size, which was identified as potential prophage, named TPA4 (Oulmi et al. 2012). Twenty-five of the 59 ORF detected encode proteins which share high identity with proteins found in the Mycobacteriophage Giles, but actually only two proteins showed high identity to the TPA2 proteins.

Phenotypic Analyses

Phenotypic properties that distinguish *Tsukamurella* species from another are indicated in ❯ *Table 45.2*. Characteristics specific for the genus are described above.

Tsukamurella paurometabola Corrig. (Steinhaus, 1941). Collins, Smida, Dorsch and Stackebrandt 1988, 387^{VP}

pau.ro.me.ta'bo.la. Gr. adj. *pauros*, little; gr. adj. *metabolos*, changeable; N.L. fem. adj. *paurometabola* little changeable.

Gram-positive, strictly aerobic slightly curved rods, but coccobacillary forms occur. Cells are 0.5–0.8 µm in diameter and 1.0–5 µm in length. Colonies are whitish to cream, small and have sometimes rhizoidal edges. Metachromic granules may be formed. Most strains are able to grow between 10 °C and 37 °C. Does not survive heat shocks at 60 °C for 15 min. Some strains may produce acid from galactose, glucose, glycerol, inositol, mannose, sucrose, and trehalose, additional properties are shown in ❯ *Table 45.2*. Acetamide and nicotinamide can be used as sole nitrogen sources, but not benzamide. Resistant to ethambutol (5 µg/mL), 5-fluorouracil (20 µg/mL), mitomycin C (10 µg/mL), and piric acid (0.2 %, w/v). Composition of the whole cellular fatty acids is listed in ❯ *Table 45.3*. Polar lipid composition is DPG, PI, PE, and PIM. The predominant menaquinone is MK-9, whereas minor amounts of MK-7, MK-8, and MK-10 are detectable. The type strain was originally deposited as DSM 20162 and ATCC 8368. Accession number of the genome sequence is CP001966.

Tsukamurella carboxydivorans Park, Kim, Park and Kim 2009, 1543^{VP}

car.bo.xy.di.vor'ans. N.L. neut. n. *carboxydum* carbon monoxide; L. part. adj. *vorans* devouring, digesting; N.L. part. adj. *carboxydivorans* digesting carbon monoxide.

Gram-positive, strictly aerobic, non-motile actinomycete. Optimal growth occurs at 30 °C, tolerates up to 6 % (w/v) NaCl. Colonies on ISP2 medium are cream to yellow. Substrates which can be used as sole carbon source are indicated in ❯ *Table 45.2*. Able to grow at lower concentrations of carbon dioxide (400 p.p.m CO). In contrast to other strains of the genus, mycolic acids have 81–95 carbon atoms. Cell wall sugars are galactose and arabinose. Whole fatty acid profile is given in ❯ *Table 45.3*. No data are available concerning the polar lipid profile or the menaquinone composition. Type strain is strain Y2^{T}.

Table 45.2

Phenotypic properties and other characteristics of type strains of *Tsukumarella*[a]

Characteristic	*T. caboxy-divorans*	*T. inchonensis*	*T. paurometabola*	*T. pseudospumae*	*T. pulmonis*	*T. soli*	*T. spongiae*	*T. spumae*[b]	*T. strandjordii*	*T. sunchonensis*	*T. tyrosinosolvens*
Pigmentation	White, cream	Cream to yellow	White, cream	Orange to red	White, cream	White	White, cream	Orange to red	White, cream	Orange	White, cream
Growth at 10 °C	−	−	+	+	−	+	−	v	−	−	−
Aesculin hydrolysis	−	+	+	+	+	+	nd	−	−	+	+
Urea hydrolysis	+	+	+	−	+	+	nd	v	+	−	(−)
Hypoxanthine degradation	+	+	−	+	+	−	nd	+	−	+	+
Tyrosine degradation	+	−	−	+	−	+	nd	+	−	+	+
Assimilation of											
D-Arabinose	+	+	−	+	(+)	−	−	v	−	+	+
L-Arabinose	+	−	−	+	(+)	−	+	+	−	−	(+)
D-Arabitol	+	−	−	+	+	+	+	+	+	nd	+
Cellobiose	+	(−)	−	−	(+)	−	+	−	−	nd	(+)
Dulcitol	+	−	−	−	+	−	−	+	−	−	+
meso-Erythritol	+	−	−	−	+	−	−	v	−	−	+
D-Fructose	+	(−)	+	+	+	+	+	+	(−)	+	+
Maltose	+	+	−	+	(+)	−	−	+	(−)	(+)	+
D-Mannitol	+	+	+	−	(−)	+	+	+	+	+	+
Melezitose	+	+	−	+	−	−	+	+	(−)	+	+
Melibiose	+	+	+	−	+	−	+	+	+	−	+
D-Ribose	+	+	+	+	+	+	+	+	−	nd	+
D-Salicin	+	+	+	−	+	+	+	−	+	nd	+
D-Sorbitol	+	+	(−)	−	(−)	+	+	+	+	nd	+
G+C content of DNA (%)	77	72	67–68	nd	69.8	70	74.6	nd	nd	68.1	69−74

Strains: 1, *T. carboxydivorans* Y2T (Data from Park et al. 2009); 2, *T. inchonensis* IMMIB D-771T (Yassin et al. 1995; Nam et al. 2003); 3, T. paurometabola DSM 20162T (Collins et al. 1988; Yassin et al. 1995; Olson et al. 2007); 4, *T. pseudospumae* N1176T (Nam et al. 2003, 2004); 5, *T. pulmonis* IMMIB D-1321T (Yassin et al. 1996; Park et al. 2009); 6, *T. soli* JS18-1T (Weon et al. 2010); 7, *T. spongiae* K362T (Olson et al. 2007); 8, *T. spumae* N1171T (Nam et al. 2003); 9, *T. strandjordii* DSM 44573T (Kattar et al. 2001; Park et al. 2009); 10, *T. sunchonensis* SCNU5T (Seong et al. 2003); 11, *T. tyrosinosolvens* DSM 44344T (Yassin et al. 1997; Park et al. 2009)

+ positive, − negative, *nd* not determined

[a]Some characteristics may differ from the original description of the strain, due to variation within the methods used for biochemical testing and are indicated in brackets

[b]According to Nam et al. (2003), properties may differ between strains of the species. Relevant characteristics are indicated as variable (v)

Table 45.3

Comparative analysis of whole cellular fatty acid compositions (%) of the species within the genus *Tsukamurella*. Summed features are groups of two or three fatty acids that cannot be separated by the MIDI system. Summed feature 3: C16:1v7c and/or iso-C15:0 2-OH; summed feature 4: iso-C17:1 I and/or anteiso-C17:1 B; summed feature 5: C18:2w6,9c and/or anteiso-C18:0; summed feature 6: C19:1v9c and/or C19:1v11c

Fatty acid	*T. carboxydivorans*	*T. inchonensis*	*T. paurometabola*[a]	*T. pulmonis*[a]	*T. pseudospumae*	*T. soli*	*T. spongiae*	*T. spumae*	*T. strandjordii*[b]	*T. sunchonensis*	*T. tyrosinosolvens*[b]
C10:0	–	–	–	–	–	–	–	–	1.1	–	–
C14:0	4.4	4.8	3.8 (3.2)	4.1 (2.2)	4.4	5.1	3.8	10.3	5.6 (4.9)	2.8	4.1
C15:0	–	–	–	– (1.1)	1.1	–	–	–	–	1.1	–
C16:1w9c	–	–	–	1.5 (1.8)	0.9	–	–	1.8	2.1	–	–
C16:0	32.4	38.9	36.9 (25.4)	33.7 (29.7)	34.1	28.1	27.7	26.5	27.1 (40.3)	38.7	35.1 (42.9)
C17:1w8c	–	–	–	–	–	3.7	–	–	–	–	–
C17:0	–	1	1.6 (1.3)	1.4 (2.1)	0.7	3.7	–	–	–	–	–
C17:0 10-methyl	–	–	–	–	–	1	–	–	–	–	–
C18:1w9c	29.3	24.8	26.4 (23.5)	30.9 (33.5)	33.9	24.7	40.7	26.9	30.2 (23.7)	32.6	32.7 (29.6)
C18:0	1.3	2.8	3.3 (2.4)	5 (2.0)	5.7	1.1	3	–	2.2	3	1.6
C18:0 10 methyl	9.3	16.7	16.5 (17.9)	10.1 (9.3)	–	12.9	1.5	17.5	5.6 (16.3)	11.4	12.0 (13.2)
C20:1w9c	1.3	1.6	1.7 (6.0)	5 (3.3)	–	–	8.5	–	1.4	–	1.5
C20:1w7c	–	–	–	–	–	–	–	–	–	–	–
C20:0	–	–	–	–	–	–	1.1	–	–	–	–
C21:1w9c	–	–	–	–	–	2.6	–	–	–	–	–
Summed features											
3	16	7.1	9.2 (16.7)	6.3 (10.7)	19.5	10.6	10.3	15.7	23.1 (7.8)	–	12
4	3.3	–	–	–	–	–	–	–	–	–	–
5	1	–	–	–	–	–	–	–	–	–	–
6	–	1	–	–	–	–	–	–	–	–	–

Strains: *T. carboxydivorans* Y2^T (Weon et al. 2010); *T. inchonensis* IMMIB D-771^T (Kattar et al. 2001); *T. paurometabola* DSM 20162^T (Kattar et al. 2001); *T. pseudospumae* N1176^T (Nam et al. 2003); *T. pulmonis* IMMIB D-1321^T (Kattar et al. 2001); *T. soli* JS18-1^T(Weong et al. 2010); *T. spongiae* K362^T (Olson et al. 2007); *T. spumae* N1171^T (Nam et al. 2003); *T. strandjordii* DSM 44573^T (Weong et a. 2010); *T. sunchonensis* SCNU5^T (Seong et al. 2003); *T. tyrosinosolvens* DSM 44344^T (Weon et al. 2010)

–, Less than 1 % or not detected

[a]Profile published by Nam et al. (2003) are indicated in brackets

[b]Data published by Kattar et al. (2001) are given in brackets

Tsukamurella inchonensis Yassin, Rainey, Brezezinka, Burghardt, Lee and Schaal, 1995, 526^{VP}

in. cho. nen' sis. M. L. adj, *inchonensis*, referring to Inchon, the city in South Korea where the type strain was isolated.

Gram-positive, aerobic bacilli. Cells are rod-shaped and do not form spores, or aerial hyphae.

Growth on Loewenstein–Jensen medium occurs at 24 °C and 45 °C. Colonies are rough, saffron yellow to orange. Positive for the following enzyme activities: acetamidase, allantoinase, thermostable catalase, *P*-glucosidase, *P*-galactosidase, nicotinamidase, pyrazinamidase, urease, and phosphatase, but negative for arylsulfatase, benzamidase, nitrate reductase, and succinamidase activity. In addition to the substrates listed in *Table 45.2*, glucose, galactose, trehalose, sucrose, inositol, acetate, citrate, gluconate, lactate, isoamyl alcohol, 2,3 butandiol, 1,2 propandiol, and paraffin can be utilized as carbon source (Yassin et al. 1995). Resistant to streptomycin, isoniazid, ethambutol, rifampicin, *p*-aminosalicylic acid, protionamide, capreomycin, cycloserine, clindamycin, erythromycin, tetracycline, and cotrimoxazole. The cellular fatty acids are listed in *Table 45.3*. The predominant menaquinone is MK-9, but minor amounts of MK-8 and MK-10 are also detectable. Type strain: IMMIB D-771^T.

Tsukamurella pseudospumae Nam, Kim, Chun and Goodfellow, 2004, 1211[VP]

pseu.do.spu'mae. Gr. adj. *pseudes* false; L. gen. n. *spumae* of foam and specific epithet of a bacterial species; N.L. n. *pseudospumae* the false spumae, referring to the close relationship to *Tsukamurella spumae*.

Aerobic, Gram-positive, non-motile actinomycete. Does not forms spores and cells are straight to slightly curved rods. Long filaments can be formed. Grows between 10 °C and 37 °C, but not at 45 °C. Colonies on glucose-yeast extract are yellow-orange to red-orange with irregular margins. D-galactose, D-gentiobiose, D-glucose, meso-inositol, D-lactose, D-mannose, L-rhamnose, D-sucrose, D-trehalose, D-turanose, D-xylitol (1 %, w/v), amyl alcohol, butane-2,3-diol (1 %, v/v), sodium citrate, and sodium pyruvate (0.1 %, w/v) are used as sole sources of carbon. Additional phenotypic properties are shown in ➤ *Table 45.2*. Further L-phenylalanine, L-asparagine, and L-serine can be used as sole nitrogen source. Cell wall sugars are galactose and arabinose. The fatty acid composition of the type strain is shown in ➤ *Table 45.3*. No data are available concerning the polar lipid profile or the menaquinone composition. Resistant to 5-fluorouracil (20 μg/mL), bekamycin and oleandomycin (64 μg/mL), gentamicin and neomycin sulfate (32 μg/mL), clindamycin (2 μg/mL), tetracycline (10 μg/mL), vancomycin (4 μg/mL). Susceptible to chlortetracycline, ciproflaxin, and penicillin G. Type strain: N1176[T].

Tsukamurella pulmonis Yassin, Rainey, Brzezinka, Burghardt, Rifai, Seifert, Feldmann, and Schaal, 1996, 434[VP]

pul. mo' nis. L. gen. masc. n. *pulmonis*, of the lung, referring to the organ from which the bacterium was isolated.

Cells are Gram-positive, aerobic rods. Long rods may fragment at a later stage of growth into independent rods. Does not form spores or aerial hyphae. Colonies are rough and cream colored on ISP2 medium. Growth occurs between 24 °C and 37 °C. Contradictorily data are available concerning the ability to utilize various carbon sources as given by Yassin et al. (1996) and Nam et al. (2003). Differences are indicated in ➤ *Table 45.2* and marked with brackets. Cell wall sugars are arabinose and galactose. MK-9 is the predominant menaquinone, but MK-8 and MK-10 are also detectable in minor amounts. Polar lipids are DPG, PI, PIM with significant amounts of PE. Cellular fatty acids are indicated in ➤ *Table 45.3*. Resistant to streptomycin, ethambutol, rifampicin, capreomycin, and cycloserine. Type strain IMMIB D-1321[T].

Tsukamurella soli Weon, Yoo, Anandham, Schumann, Kroppenstedt, Kwon and Stackebrandt, 2010, 1670[VP]

so'li. L. neut. gen. n. *soli* of soil, the source of the type strain.

Aerobic, Gram-positive rods with 0.9–1.0 μm in diameter and 2.3–6,9 μm in length. Colonies are pearly white on R2A agar and ivory to cream colored on ISP2 medium. Colonies are dry with an irregular margin. Growth occurs between 10 °C and35 °C, tolerates up to 3 % (w/v) NaCl. In addition to the substrates listed in ➤ *Table 45.2*, D-glucose, D-mannitol, D-ribose, sucrose, inositol, *N*-acetylglucosamine, trisodium citrate, acetate, and lactic acid can be assimilated. The following enzyme activities are positive within the apiZYM test: alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and β-glucosidase. Cell wall sugars are arabinose, ribose, and glucose. Polar lipids are DPG, PE, and PI. The predominant menaquinone is MK-9, minor amounts of MK-8 and MK-10 are detectable in addition. Whole cellular fatty acids composition is shown in ➤ *Table 45.3*. Type strain JS18-1[T].

Tsukamurella spongiae Olson, Harmody, Bejand, McCarthy 2007, 1480[VP]

spon'gi.ae. L. gen. n. *spongiae* of a sponge, referring to the source of isolation, a deep-water sponge.

Aerobic, Gram-positive, non-motile actinomycete. Cells are straight to slightly curved rods. Growth occurs between 25 °C and 37 °C, but not at 45 °C. In general, the colonies are dry, cream to yellow with irregular margins. Phenotypic properties are listed in ➤ *Table 45.2*. Utilizes D-galactose, D-mannose, rhamnose, D-sucrose, and D-trehalose in addition. Halotolerant (up to 4 % (w/v) NaCl), but growth is much better in media with lower NaCl concentrations. The fatty acid composition is shown in ➤ *Table 45.3*. Contains mycolic acids with 58–75 carbon atoms. No data are available concerning the polar lipid composition or the menaquinone profile. Type strain: K362[T].

Tsukamurella spumae Nam, Chun, Kim, Kim, Zakrzewska-Czerwinska, Goodfellow, 2003, 373[VP]

spu'mae, L.gen.n. *spumae* of foam denoting the presence of the organism in the foam of activated sewage sludge plants.

Aerobic, Gram-positive, non-motile actinomycete. Cells are straight to slightly curved rods, and may form longer filaments. Aerial hyphae are absent. Colonies are <5 mm on glucose-yeast extract agar, orange to red with irregular margin. Growth occurs between 25 °C and 37 °C, but the strains are not able to grow at 45 °C. Some strains grow at 10 °C, but not the type strain. Phenotypic properties are shown in ➤ *Table 45.2*. Amyl alcohol, butane-2,3-diol (1 %, v/v), sodium citrate, and sodium pyruvate (0.1 %, w/v) are used as sole sources of carbon in addition. Further L-phenylalanine, L-asparagine, and L-serine can be used as sole nitrogen source. Some strains assimilate D-arabinose, and *meso*-erythritol or hydrolyze urea. Is not able to hydrolyze aesculin. Resistant to clindamycin, and cotrimazole, but susceptible to tetracycline. Whole cellular fatty acids are indicated in ➤ *Table 45.3*. Predominant menaquinone is MK-9; minor

amounts of MK-8 and MK-10 are also detectable. No data are available concerning the polar lipid composition. Type strain N1171^T.

Tsukamurella strandjordii corrig. Kattar, Cookson, laDonna, Carlson, Stiglich, Schwartz, Nguyen, Daza, Wallis, Yarfitz, and Coyle 2001, 1474^{VP}

strandjordii N.L. gen. masc. n., of Strandjord, named in honor of Paul Strandjord, founder and chair of the Department of Laboratory Medicine, University of Washington, from 1969 to 1994.

Aerobic, Gram-positive rods. Colonies are rough, tan to yellow in color. Growth occurs between 28 °C and 35 °C, but not at 42 °C. Aerial hyphae are absent. Tolerates NaCl up to 5 % (w/v). In addition to the substrates listed in ❯ *Table 45.2*, galactose, D-glucose, D-mannose, saccharose, trehalose, D-turanose, L-fucose, gluconate, inositol, L-arabitol, arbutine, salicin, and *N*-acetylglucosamine can be utilized as carbon source. Utilization of D-fructose, maltose, and melezitose is indicated as positive by Kattar et al. (2001), but given as negative in the study of Weon et al. (2010). Resistant to erythromycin, cefatoxime, cefoxitin, azithromycin, and tobramycin, but susceptible to ciprofloxacin, imipenem, amikacin, clarithromycin, and trimethoprim-sulfamethoxazole. Cellular fatty acids are shown in ❯ *Table 45.3*. No data are available concerning the polar lipid and menaquinone composition. Type strain ATCC BAA-173^T.

Tsukamurella sunchonensis Seong, Kim, Baik, Choi, Kim, Kim and Goodfellow 2003, 88^{VP}

sun.chon.ensis. M.L. n. *sunchon* Sunchon, a city in Korea; M.L. adj. *sunchonensis* of Sunchon, Korea; referring to the place where the organism was first isolated.

Aerobic, Gram-positive long rods. Spores and aerial hyphae are absent. Growth occurs between 20 °C and 37 °C, no growth at 10 °C or 45 °C. Colonies are rough, crumbly, and yellow to orange. Utilizes sucrose, galactose, and inositol in addition to the substrates listed in ❯ *Table 45.2*. Cellular fatty acids are indicated in ❯ *Table 45.3*. The major isoprenoid quinone is MK-9. Cell wall sugars are galactose and arabinose. No data are available describing the polar lipid profile. Resistant to bacitracin and penicillin G, but susceptible to streptomycin, gentamicin, tobramycin, vancomycin, and rifampicin. Type strain SCNU5^T.

Tsukamurella tyrosinosolvens Yassin, Rainey, Burghardt, Brzezinka, Schmitt, Seifert, Zimmermann, Mauch, Gierth, Lux and Schaal 1997, 612^{VP}

ty. ro. si. no. sol' vens. Gr. masc. n. *tyros*, cheese; *tyrosine*, an amino acid; L. pres. part. *solvens*, dissolving; M. L. adj. *tyrosinosolvens*, tyrosine dissolving, referring to the hydrolysis of tyrosine which is characteristic of this species.

Aerobic, Gram-positive rods. Elongated cells with septum formation are detectable. Spores and aerial hyphae absent. Colonies are whitish to yellow in color. Growth occurs between 24 °C and 37 °C, but not at 45 °C. Phenotypic properties are indicated in ❯ *Table 45.2*. Utilizes also D-glucose, galactose, sucrose, trehalose, myo-inositol, acetate, benzoate, citrate, gluconate, adipate, isoamylalcohol, 2,3 butandiol, 1,2-propandiol, and paraffin as sole carbon source. Contradictorily data are available for utilization of L-arabinose and cellobiose. Tolerates up to 5 % NaCl. Cellular fatty acids are shown in ❯ *Table 45.3*. MK-9 is the major menaquinone, but minor amounts of MK-8 and MK-10 are detectable in addition. Polar lipids are PE, PI, PIM and DPG. Resistant to streptomycin, ethambutol, rifampicin, capreomycin and cycloserine, clindamycin, cotrimoxazole, erythromycin, and tetracycline. Type strain IMMIBD-1397^T.

Isolation, Enrichment, and Maintenance Procedures

Isolation and Enrichment

Tsukamurella paurometabola DSM 20216^T(Collins et al. 1988) was isolated from the mycetome and ovaries of the bed bug, *Cinmex lectularius* L., but additional strains were isolated from human sputum (Collins et al. 1988). The strain has originally been isolated on a semisolid medium containing proteose peptone, rabbit serum, gelatin, minced rabbit kidney, and carbohydrates (Goodfellow 2011). The strain is able to grow on complex media like nutrient agar, CASO agar incubated at 30 °C.

Tsukamurella inchonensis IMMIB D-771T (Yassin et al. 1995) was isolated from a blood culture of a patient who had ingested hydrochloric acid. The isolate was routinely cultured on Loewenstein-Jensen medium at various temperatures (24 °C, 31 °C, 37 °C and 45 °C).

Tsukamurella pulmonis IMMIB D-1321^T (Yassin et al. 1996) was isolated from sputum of a 92-year-old woman who suffered from pulmonary tuberculosis and possibly an additional mycobacterial infection. The strain was isolated from the sputum after decontamination with *N*-acetyl-L-cysteine on Loewenstein-Jensen medium, but strain IMMIB D-1321^{T}also grew well on International Streptomyces Project [ISP] medium 2), and ISP medium 3 (Shirling and Gottlieb 1966) incubated at 24–37 °C.

Tsukamurella tyrosinosolvens IMMIB D-1397^T (Yassin et al. 1997) was isolated from a 62-year-old woman who had a cardiac pacemaker implant. The strain was isolated directly from the blood culture by plating aliquots of a set of different media (blood agar, chocolate agar, brain heart infusion (BHI) agar, and Muller-Hinton agar). ISP 2 agar was also used for characterization of the colony morphology of the strain. Growth occurred after incubation at 24–37 °C.

Tsukamurella strandjordii ATCC BAA-173^T (Kattar et al. 2001) was isolated from blood cultures of a 5-year-old girl with acute myelogenous leukemia. Middlebrook 7H11 agar

was routinely used for isolation of the strain, which also is able to grow on nutrient agar, trypticase soy agar, or MacConkey agar incubated at 28–35 °C.

Tsukamurella spumae N1171^T (Nam et al. 2003) was isolated from activated sludge foam, Stoke Bardolph Water Reclamation Works, near Nottingham, UK. The strain was isolated on glucose-yeast extract agar (GYEA, Gordon and Mihm 1962) containing (g per liter) glucose 10, yeast extract 10, and agar 15 (final pH 7.2). Unlike the other isolates, the type strain (N1171^T) does not grow at 10 °C, but at 25–37 °C.

Tsukamurella pseudospumae N1176^T (Nam et al. 2004) was isolated similarly from activated sludge foam collected from Stoke Bardolph Water Reclamation Works near Nottingham, UK. Isolation was done on glucose-yeast extract agar (GYEA, Gordon and Mihm 1962), incubated at room temperature.

Tsukamurella spongiae K362^T (Olson et al. 2007) was isolated from a deep-water marine sponge collected off the coast of Curacao (the Netherlands Antilles), at a depth of 220 m. A small section of the sponge was homogenized, subsequently heat-treated (70 ° C for 15 min) and plated onto maltose-seawater agar (Olson et al. 2000). Growth occurred on marine agar, nutrient agar, or brain-heart infusion agar (BHI) after 24–48 h incubation at 25 °C.

Tsukamurella sunchonensis SCNU5^T (Seong et al. 2008) was isolated from foam collected from a full-scale activated sludge plant in Sunchon, Korea. Strain SCNU5^T was grown on glucose-yeast extract agar (GYEA), yeast extract-malt extract agar (ISP medium 2), oatmeal agar (ISP medium 3), and inorganic salts-starch casein agar (ISP medium 4, Shirling and Gottlieb, 1966) incubated at 30 °C for up to 7 days.

Tsukumarella carboxydivorans Y2^T (Park et al. 2009) was isolated from a subsurface soil sample collected from the side of a busy road at Yonsei University, Seoul, Korea. For enrichment, 20 g of soil was transferred into 500-mL flasks and incubated under 400 p.p.m. CO in air for 2 weeks at 30 °C. Two grams of the enrichment was transferred to a new 500-mL flask containing 200 mL of a standard liquid mineral medium (Park et al. 2008), supplemented with 400 p.p.m. CO in air and 0.005 % (w/v) yeast extract and incubated for 2 weeks at 30 °C on a shaker at 200 r.p.m.

One milliliter aliquots were then spread plated onto solid mineral medium supplemented with 0.2 % (w/v) glucose and incubated for 2 weeks at 30 °C under 400 p.p.m. CO in air (Hardy and King 2001). Even though the strain was isolated under low CO concentration, it was also able to grow in the mineral medium at a high concentration of CO (300,000 p.p.m.). Subculturing on tryptone soya agar (TSA) is possible.

Tsukamurella soli JS18-1^T (Weon et al. 2010) was isolated from a soil sample collected from Halla mountain, Jeju island, Republic of Korea, by the spread-plate technique on R2A agar after incubation at 30 °C for 7 days. Composition of R2A agar (g per liter) is glucose 0.5, soluble starch 0.5, casamino acids 0.5, yeast extract 0.5, peptone 0.5, sodium pyruvate 0.3, $MgSO_4 \cdot 7H_2O$ 0.05, KH_2PO_4 0.3, and agar 15 (final pH 7.2).

Maintenance

Standard procedures can be applied for members of the genus *Tsukamurella*. Serial transfer of subcultures grown on glucose-yeast extract agar slants (every 6–8 weeks) is possible. Strains can also be achieved in glycerol stocks (50 % (v/v) stored at −20 °C or for better survivability at −80 °C. For long-term storage, freeze-drying or storage in liquid nitrogen should be used. Detailed protocols are given within the Cabri guidelines, accessible at www.cabri.org.

Ecology and Pathogenicity

Tsukamurellae have been isolated from various environmental habitats as well as from clinical specimen. *T. paurometabola* strains were isolated from mycetomes and ovaries of the bed bug (*Cimex lectularius*), as well as from activated sludge. Strains of the three species *T. spumae*, *T. pseudospumae*, and *T. sunchonensis* have been isolated from activated sludge plants too (Seong et al. 2003; Nam et al. 2004). *T. spongiae* was isolated from a deep water sponge in the Netherlands Antilles (Olson et al. 2007), *T. carboxidivorans* from a subsurface soil samples (10 cm depth) collected from the side of a busy road at Yonsei University, Seoul, Korea, and *T. soli* also from a soil sample, but collected from Halla mountain, Jeju island, Republic of Korea (Weong et al 2010).

All other type strains have been isolated from clinical specimen: *T. inchonensis*, blood culture of a patient who had ingested hydrochloric acid (Yassin et al. 1995); *T. pulmonis*, sputum of a patient with pulmonary tuberculosis (Yassin et al. 1995); *T. tyrosinosolvens*, blood culture of a patient with a cardiac pacemaker implant (Yassin et al. 1997); *T. strandjordii*, blood culture from a 5-year-old girl with acute myelogenous leukemia (Kattar et al. 2001). With exception of *T. strandjordii*, these species are allocated into Risk group 2 and are generally listed as opportunistic pathogens. *T. inchonensis* and *T. pulmonis* were able to cause catheter-related bacteremia and pulmonary diseases (Yassin et al. 1995, 1996; Maertens et al. 1998; Schwartz et al. 2002), or catheter-related bloodstream infections (*T. inchonensis*; Seo et al. 2012). *T. pulmonis* and *T. tyrosinosolvens* strains were also involved in ophthalmologic infections like keratitis or conjunctivitis (Woo et al. 2003, 2009).

The human body is not the only habitat from which these clinical strains could be isolated from. Several strains have been isolated from soil and rhizosphere in addition: *T. strandjordii*, *T. tyrosinosolvens* from arsenic-resistant soil or metal tolerant rhizosphere soil (Achour et al. 2007; Becerra-Castro et al. 2012), and *T. pulmonis* from potato geocaulosphere (Weinert et al. 2010). 16S rRNA gene sequences of cultivable isolates and uncultured clones related to *Tsukamurella* sp. have been detected in several diversity studies analyzing (i) Challenger Deep sediment samples from the Mariana Trench in the Pacific Ocean (Pathom-aree et al. 2006); (ii) actinobacteria from medical plants of tropical rain forests in Xishuangbanna, China (Qin et al. 2009); (iii) the bacterial diversity from

Roopkund Glacier, India (Pradhan et al. 2010); (iv) the rhizosphere community of *Arachis hypogaea* (Haldar et al. 2011); (v) the prevalent microbiota of human forehead skin compared to forearm skin (Staudinger et al. 2011); (vi) root endophytic actinobacteria in native herbaceous plants from Korea (Kim et al. 2012); (vii) cholesterol- and deoxycholate-degrading bacteria from soil samples (Merino et al. 2013).

Application

Production of secondary metabolites by bacteria often depends on the culture conditions applied for a specific strain. Onaka et al. (2011) reported that mycolic acid containing bacteria, like *Tsukamurella*, were able to induce the biosynthesis of natural products in *Streptomyces* sp. The production of a red pigment by strain *Streptomyces lividans* TK23 was induced in coculture with strain *Tsukamurella pulmonis* TP-B0596, and Onaka et al. postulated that the mycolic acid localized within the outer cell layer may be responsible for direct interaction with the *Streptomyces* strains.

Strain *Tsukamurella paurometabola* C-924 has been used as nematocidic agent for biocontrol of nematodes. A bionematicide product of the strain is available as HerberNem™. The mechanism of action has been explained by effects of desulfurase and chitinase activities on nematodes and nematodes eggs (Mena et al. 2002).

Tsukamurella paurometabola was also considered to be one of the causal agents of foaming in activated sludge plants (Goodfellow et al. 1996) and the application of *Tsukamurella* specific bacteriophages (TPA2) as biocontrol agent (anti-foaming) offers the possibility to stabilize foams in activated sludge plants (Petrovski et al. 2011).

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